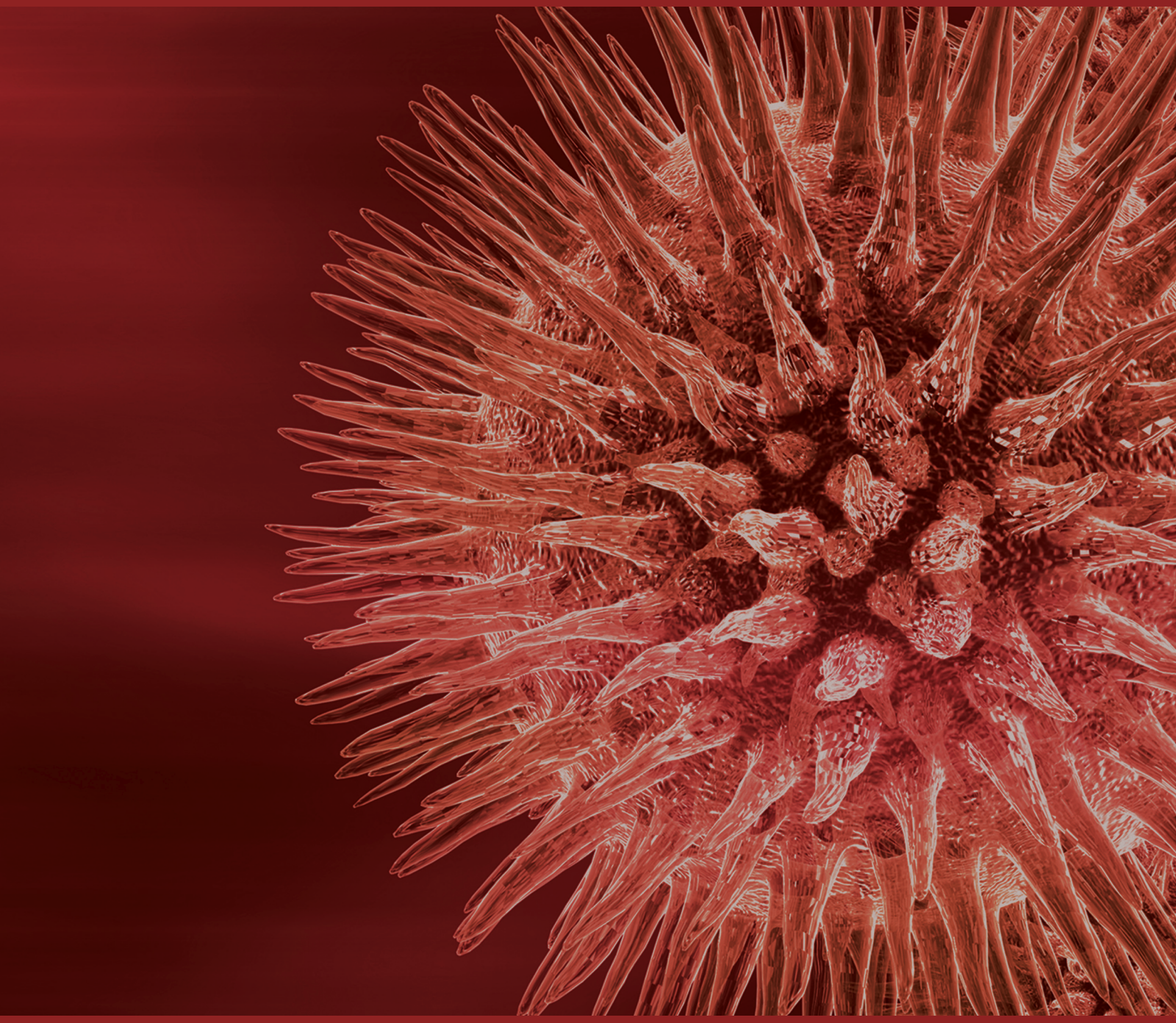
BioMed Research International

Genetic Control of Immune Response and Susceptibility to Infectious Diseases

Guest Editors: Enrique Medina-Acosta, Helder I. Nakaya, Alessandra Pontillo, and Regina Célia de Souza Campos Fernandes



Genetic Control of Immune Response and Susceptibility to Infectious Diseases

BioMed Research International

Genetic Control of Immune Response and Susceptibility to Infectious Diseases

Guest Editors: Enrique Medina-Acosta,
Helder I. Nakaya, Alessandra Pontillo,
and Regina Célia de Souza Campos Fernandes

Contents

Hindawi Publishing Corporation
BioMed Research International
Volume 2014, Article ID 796073, 3 pages
http://dx.doi.org/10.1155/2014/796073

Editorial

Genetic Control of Immune Response and Susceptibility to Infectious Diseases

Enrique Medina-Acosta,[1] Helder Takashi Imoto Nakaya,[2] Alessandra Pontillo,[3] and Regina Célia de Souza Campos Fernandes[4]

[1] *Department of Biotechnology, Center for Biosciences and Biotechnology, State University of Norte Fluminense Darcy Ribeiro, 28013-602 Campos dos Goytacazes, RJ, Brazil*
[2] *Emory Vaccine Center, Yerkes National, Primate Research Center, Emory University, Atlanta, GA 30329, USA*
[3] *Laboratory of Immunogenetics, Department of Immunology, Institute of Biomedical Sciences, University of São Paulo, 05508-900 São Paulo, SP, Brazil*
[4] *Department of Pediatrics, School of Medicine of Campos, Álvaro Alvim School Hospital, 28035-211 Campos dos Goytacazes, RJ, Brazil*

Correspondence should be addressed to Enrique Medina-Acosta; quique@uenf.br

Received 20 November 2013; Accepted 20 November 2013; Published 13 February 2014

Both genetic and nongenetic variables are known to impact, in magnitude and breadth, immune responses to infectious disease agents. Discovery and validation of genetic determinants in hosts and pathogens are crucial to better understand the basis of susceptibility to and control of infectious diseases. The interplay of these conjoined yet opposed multiple, varying factors results in an impressive dynamic phenotypically diversity in hosts. The precise mechanisms that underlie the observed interindividual variation in control of, resistance, and/or susceptibility to infectious diseases are not completely understood.

This special issue gathers three original research papers and five reviews that aimed at stimulating future research efforts towards medical and veterinary applications of genetic variation with well-defined genetic roles in host immune response and susceptibility to infectious diseases. Overall, this area of research has been dominated by population genetics on candidate genes through small scale interindividual susceptibility association studies. The observed interindividual variation is due, in part, to risk-modifying polymorphisms (rare and common) in agonist and antagonist genes of the innate and adaptive immune responses. There is robust evidence for association with control to or decrease susceptibility to infectious agents or disease progression for some host gene variants. The perspective is that the field will benefit from both small-scale replica and genomewide association studies (GWAS), firstly to validate the statistical strength of observed associations in different population backgrounds and secondly to discover significant associations with newly investigated variants with translational potential into practical tests.

A. V. Marangon et al. looked into the association of HLA class I, HLA class II, and *KIR* genes as well as single nucleotide polymorphisms (SNP) in cytokine genes (*IFNG*, *IL6*, *IL10*, *TGFB1*, *and TNF*) with risk for human-papillomavirus-(HPV-) related cervical disease in two groups of subjects: 79 unrelated admixed Brazilian women who tested positive for carcinogenic high risk HPV and presented with cervical intraepithelial neoplasia grade 3 (CIN3), and 150 HPV-negative women from the same ethnicity. The most significant findings of this study are (i) none of the fourteen *KIR* genes, the *KIR* pseudogene, HLA class I/II alleles (or haplotype combination thereof) was significantly associated with HPV-related cervical disease; (ii) the HLA-A*02-B*51 haplotype was associated with resistance to HPV-related cervical disease; (iii) the *IFNG* +874A/A genotype was associated with susceptibility to HPV infection.

Z.-Q. Feng et al. tested whether the retinoic acid-induced gene I (RIGI)-like receptors (RLRs)-viral detection and antiviral response pathways against RNA viruses are involved

in resistance to Marek's disease virus (MDV), a DNA virus, in chickens, and whether resistance to infection is affected by the genetic background of chickens. The authors experimentally infected two-chicken breeds (economic line-AA broilers and native Erlang mountainous chickens) to comparatively monitor patterns of RNA expression of melanoma differentiation associated gene 5 (*MDA-5*), interferon regulatory transcription factor 3 (*IRF-3*), *IFN-α,* and *IFN-β* by real-time PCR on total cDNAs from various tissues. The most significant findings of this study are (i) the Erlang mountainous chicken breed, but not the economic line-AA breed, survived MDV infection with slight increased control of viral loads; (ii) RNA expression for *MDA-5*, *IRF-3*, *IFN-α*, and *IFN-β* increased to different magnitudes following infection in either breed, but opposing magnitudes were observed in different tissues. The authors' perspective is that RLR-mediated antiviral pathway is involved in detection and response against MDV.

A. C. Leandro et al. interrogated *IFNG*+874*T/A and *NOS2A*-954*G/C SNPs in a case-control ($n = 172/n = 179$) study to estimate their role on susceptibility to tuberculosis and to determine whether those variants impact the levels of nitrite and nitric oxide radical in serum. The most significant finding of this study is that neither allele variant nor a combination thereof was significantly associated with either risk of developing tuberculosis or differences in secretion levels of nitric oxide radicals in the admixed Brazilian population subset studied.

C. M. Ayo et al. lent a comprehensive overview about the associations between polymorphisms in HLA class I/II, *KIR*, cytokine (*CCL2*, *CXCL9*, *IFNG*, *IL1B*, *IL12B*, *IL4*, *IL6*, *IL10*, *LTA*, *TGFB1, and TNF*), cytokine receptor (*CCR5*, *IL4R*) genes and the splicing factor *BAT1* (DDX39B) gene and the occurrence, severity, and clinical forms of Chagas disease (American trypanosomiasis). A commendable contribution of this review is the reporting of statistical significance (*P* values) of the associations from the various studies observed between HLA alleles and haplotypes and the different clinical forms of the disease (chronic Chagas cardiomyopathy, digestive form, and mixed and combinations thereof). Despite the many linkage and association studies carried out to discover host genetic variants involved in immunopathogenesis of Chagas disease, causality of susceptibility remains a challenge.

L. R. Jarduli et al. contributed with a resourceful review about the roles of HLA, *KIR,* and *MICA* genes as well as common polymorphisms in pro- and anti-inflammatory cytokine (*IFNG*, *IL1B*, *IL12B*, *IL4*, *IL6*, *IL10*, *TNF*, *and LTA*) and cytokine receptor (*IFNGR1*, *IL12RB1, and IL12RB2*), *BAT1* and the *BTNL2* (butyrophilin-like 2 HLA class II associated) gene in resistance or susceptibility to infection by *Mycobacterium leprae* and the clinical course and varying manifestations of Leprosy. A praiseworthy aspect of this review is the scope by clinical forms of the disease (multibacillary, lepromatous, borderline, tuberculoid, and erythema nodosum leprosum), with the inclusion of statistical significance (*P* values) for the associations observed in twin studies, segregation analyses, family-based linkage and association studies, candidate gene association studies, and, most recently, GWAS. Interestingly, some HLA and *KIR* gene combinations rendered favorable or unfavorable interactions, with opposing (activation or inhibition) effects on NK cells and infected host cells, which underscores the interplay of host genetic factors in pathogenesis.

F. Celsi et al. bestowed an enlightened account of the gene expression regulatory plans devised by human immunodeficiency virus type 1 (HIV-1) to escape the immune response against infected cells, given emphasis on the interplay of classical class I HLA-C and nonclassical class I HLA-G alleles and genotypes associated with effective control of viral replication and with slow progression to AIDS. The realization that genetic background differences (i.e., ethnicity) in HLA-G (i.e., HLA-G*0105N) allele distribution may influence the outcome(s) (protection/susceptibility) of association studies urgently calls for replica studies. The authors further assessed the newly discovered small noncoding RNAs (miRNAs) encrypted in the host genome for downregulating HLA-C and HLA-G gene expression through RNA interference at the 3′ untranslated regions (UTR). The possible effects of SNPs at HLA-C and HLA-G 3′ UTRs on miRNA binding were discussed as well as the prospects of using miRNA expression signatures as a biomarker of disease progression. The authors' perspective is that estimating the impact of such regulatory mechanisms on HIV-1 infection, replication, and progression to AIDS will uncover novel therapeutic targets. The prospects of existence of counteracting miRNA encrypted (putatively) in the HIV-1 genome pave the way for unprecedented approaches.

V. C. Vieira and M. A. Soares imparted a fresh appraisal of the DNA and/or RNA sequence editing dependent or independent roles of the human APOBEC family of cytidine deaminases in innate immune inhibition of viral infections (including EBV, HBV, HCV, HIV-1, HPV, HSV-1, and HTLV) as well as of the counteracting viral strategies ensued. A praiseworthy aspect of this review is the comprehensive and insightful comparison of studies on polymorphisms in *APOBEC3* genes and their associations with susceptibility to viral infections. The authors' perspective is that enhancing APOBEC3 activity constitutes a novel approach for antiviral drug development, notwithstanding the realization that APOBEC3-mediated hypermutating viral DNA/RNA may increase viral genetic diversification and, thus, emergence of viral escape and/or drug-resistant variants.

J. Malogajski et al. summated the evidence for high translational potential of basic host genomic and genetic studies for three sexually transmitted agents (HIV-1, *Chlamydia trachomatis*, and HPV) into applications of public health and care including diagnosis, treatment, and prevention of diseases. The authors asserted that there is considerable lagging between discovery of functional genetic polymorphisms and effective introduction of practical applications, which points to fertile research grounds in the areas of pharmacogenomics and personalized medicine. In fact, no market-ready application involving genetic/genomic tests for the diseases caused by these infectious agents is, at present, available. The prospects of introducing routine screening tests for epigenetic (DNA methylation) markers, known to be associated with high grade cervical intraepithelial neoplasia and cervical cancer, meet the expectation of significantly

reducing the number of unnecessary referrals to gynecologists. Similarly, bringing in *TLR4* SNP typing and *TLR2* haplotyping for identification of variants or haplotypes linked to either increased risk of tubal pathology or protection from tubal pathology, respectively, is expected to provide for a more accurate diagnosis of *Chlamydia trachomatis* causing subfertility.

We expect that the papers in this special issue will hasten perceptiveness towards discovery and application of genetic variants in biotechnology, diagnostics, and treatment of infectious diseases of both man and animals.

Enrique Medina-Acosta
Helder Takashi Imoto Nakaya
Alessandra Pontillo
Regina Célia de Souza Campos Fernandes

Hindawi Publishing Corporation
BioMed Research International
Volume 2013, Article ID 284729, 13 pages
http://dx.doi.org/10.1155/2013/284729

Review Article

Genetic Susceptibility to Chagas Disease: An Overview about the Infection and about the Association between Disease and the Immune Response Genes

Christiane Maria Ayo,[1] Márcia Machado de Oliveira Dalalio,[2] Jeane Eliete Laguila Visentainer,[1,2] Pâmela Guimarães Reis,[1] Emília Ângela Sippert,[1] Luciana Ribeiro Jarduli,[1] Hugo Vicentin Alves,[1] and Ana Maria Sell[1,2,3]

[1] *Program of Biosciences Applied to Pharmacy, Department of Clinical Analysis and Biomedicine, Maringa State University, Avenida Colombo 5790, 87020900 Maringa, PR, Brazil*
[2] *Basic Health Sciences Department, Maringa State University, Avenida Colombo 5790, 87020900 Maringa, PR, Brazil*
[3] *Departamento de Ciências Básicas da Saúde, Universidade Estadual de Maringá, Avenida Colombo 5790, 87020900 Maringa, PR, Brazil*

Correspondence should be addressed to Ana Maria Sell; anamsell@gmail.com

Received 29 March 2013; Revised 9 May 2013; Accepted 31 May 2013

Academic Editor: Enrique Medina-Acosta

Chagas disease, which is caused by the flagellate parasite *Trypanosoma cruzi*, affects 8–10 million people in Latin America. The disease is endemic and is characterised by acute and chronic phases that develop in the indeterminate, cardiac, and/or gastrointestinal forms. The immune response during human *T. cruzi* infection is not completely understood, despite its role in driving the development of distinct clinical manifestations of chronic infection. Polymorphisms in genes involved in the innate and specific immune response are being widely studied in order to clarify their possible role in the occurrence or severity of disease. Here we review the role of classic and nonclassic MHC, *KIR*, and cytokine host genetic factors on the infection by *T. cruzi* and the clinical course of Chagas disease.

1. Introduction

1.1. General Description of Chagas Disease. Chagas disease, or American trypanosomiasis, is an infection caused by the haemoflagellate protozoan *Trypanosoma cruzi*. It is one of the most important public health problems in Latin America and was first described by Carlos Justiniano Ribeiro das Chagas, a Brazilian physician and scientist [1]. The disease is endemic and is characterised by acute and chronic phases, which develop in the indeterminate, cardiac, and/or gastrointestinal forms. Their course of evolution may be influenced by both genetic and biological variability of the parasite and host [2, 3].

The World Health Organization estimates that approximately 10 million individuals are currently infected with *T. cruzi* with potential for developing cardiac or gut pathology normally associated with chronic Chagas disease [4] and a large fraction of them will die prematurely, usually from cardiac complications [5].

1.2. Origin and Discovery of Chagas Disease. The presence of *T. cruzi* is quite old, totalling about 100 million years. The historic evolution of the trypanosomes began from primitive aquatic invertebrates, and later in the digestive tract of vertebrates such as fish, amphibians, and reptiles. After that, haematophagous insect predators were able to transmit the parasite to different hosts that served as a food source, small to medium wild marsupial mammals, setting the enzootic cycle in the Americas. Thereafter, the cycle expanded to other mammals due to the behaviour of triatomines [6, 7].

The domestic cycle only settled down much later. The spread of disease is due to settlement and concentration of

human populations in pre-Columbian times [8]. However, the establishment of Chagas disease itself as a zoonosis occurred 200–300 years ago, as a result of deforestation caused by the expansion of agriculture and livestock when humans approached the natural invertebrate niches [9].

There are indications that human infection with *T. cruzi* has occurred since at least nine thousand BC years in populations of the Andean countries; it was possible to identify molecular remnants of *T. cruzi* in mummies of these era and region [10], and Peruvian ceramics dating from the thirteenth to sixteenth centuries revealed possible representations of Chagas disease, including a head with unilateral ocular oedema, identical to the Romaña signal that often characterises the context of acute infection [11].

Charles Darwin observed the behaviour of the triatomine insect transmitter during his passage through Argentina and wrote in his diary "The Voyage of the Beagle" that he had been bitten by the same insect while visiting Chile in 1835. The presence of gastric symptoms and his final death caused by heart problems in 1882 was suggested to be due to Chagas disease [12].

The disease was described for the first time in 1907 when Carlos Chagas described the trypanosome, the transmission insect, and the syndrome that characterised a new tropical parasitic disease [1, 13].

1.3. Epidemiology. It is estimated that 10 million people are infected with *T. cruzi* worldwide, mostly in Latin America [4], and about 100 million people are at risk of the disease in the Americas, with a total estimated incidence of 800,000 new cases per year [14].

Chagas disease was characterised as a neglected disease of poor and rural populations, but the progressive urbanisation, especially since the 1940, has made the disease an urban problem of medical and social importance. The disease has also spread from Latin America to nonendemic countries with the movements of people from endemic to nonendemic countries including North America, Western Pacific regions (mainly Australia and Japan), and Europe. It is estimated that today there are over 300,000 individuals infected with *T. cruzi* in the United States, over 5,500 in Canada, over 80,000 in Europe and the Western Pacific region, more than 3,000 in Japan, and more than 1,500 in Australia [15–18]. Thus, the prevalence, incidence, and mortality associated with Chagas disease showed considerable variations in recent decades, mainly due to the impact of control programs, migration of rural and urban populations, and socioeconomic changes [14]. Although the estimates of prevalence of infection are gradually decreasing, the disease still exists.

1.4. Transmission of Chagas Infection. The transmission of Chagas infection can be divided into primary and secondary mechanisms: the main mechanisms include transmission through insect vectors, by blood transfusion, contaminated food, and congenital transmission. Secondary mechanism transmissions may occur by laboratory accidents, organ transplants, sexual transmission, wounds in contact with contaminated sperm or menstrual fluid, and hypothetically by inoculation by criminal or deliberate contamination of food with the parasite [9].

The disease's reservoir lies in 100 different mammal species of wild animals. It is transmitted by several dozens of insect species belonging to the family Reduviidae, subfamily Triatominae. These insects hide in wild animals' nests or lairs and extract their blood meals (wild cycle). In the insect vector, the trypanosome undergoes several and successive developmental stages, terminating as a flagellated form that stays in the vector's rectum. At night, humans are bitten by these insects, usually in the facial area (domestic cycle). Ingestion of the blood meal causes the vector to defecate. After awakening, the victim usually rubs the bite area and pushes the stool with the trypanosome into the wound or onto the conjunctiva. After the *T. cruzi* accesses the victim's blood, this initiates the acute phase of the disease. Widely distributed via the blood stream, the trypanosome sheds its flagellum and penetrates tissue cells. They proliferate by binary fission within the cells (especially myocardium and meninges) [19].

Transfusion infection is the second most important epidemiological mechanism in the transmission of Chagas disease [19]. In 1960, the World Health Organization (WHO) estimated seven million cases per year due to blood transfusions in Latin America and this finding helped change policy and practice [14, 15]. Congenital transmission is transplacental and seems to depend on factors related to the parasite and the host; trypomastigotes penetrate the placenta through the chorionic epithelium and trophoblastic Hofbauer cells (macrophages placenta) where they transform into amastigotes [20]. Oral transmission of Chagas disease can occur, especially associated with contamination of breast milk (congenitally), fruit juices, and vegetables contaminated by infected wild vectors [21].

Other forms of exceptional transmission can occur. Accidents involve researchers and laboratory technicians working with the parasite through the blood of infected people and animals, culture media, or vector. The transmission by coitus has never been proven in humans; there are only reports of trypomastigotes in the blood of menstruation chagasic women. The presence of trypomastigotes was found in sperm from experimentally infected mice, and infection was also demonstrated after depositing *T. cruzi* in the vagina of rats [19].

1.5. Clinical Manifestations and Diagnosis. Human *T. cruzi* infection evolves from a usually oligosymptomatic acute phase to a chronic disease. The biological and genetic variability of the parasite and of the host may influence the course of disease progression [3, 22].

The early or acute phase of infection is characterised by high parasitaemia or trypomastigote circulating forms in the blood for two to four months [23]. During this period, the mortality ranges from 5% to 10% due to episodes of myocarditis and meningocefalite [24, 25]. The clinical signs associated with infection are a local inflammatory reaction with formation of strong swelling in the region of entry of the parasite (chagoma or Romaña sign), fever, splenomegaly, and cardiac arrhythmia [26]. The presence of circulating parasites can be detected by xenodiagnosis, haemoculture, [27]

and molecular characterisation of the parasite's DNA by the polymerase chain reaction (PCR) [28]. During the acute phase, the majority of infected individuals develop a humoral and cellular immune response responsible for the decrease of parasites in the blood.

After that, the patients progress to the chronic asymptomatic stage that affects most individuals (50 to 60%); this condition characterises the indeterminate clinical form (IND) of the disease, and it may remain for long periods of time [23, 27]. About 20% to 30% develop cardiomyopathy that reflects a myocardium progressively damaged by extensive chronic inflammation and fibrosis and, in terminal phases, usually presents as dilated cardiomyopathy. Chronic Chagas cardiomyopathy (CCC) is the most relevant clinical manifestation causing death from heart failure in endemic countries and accounts for a significant burden of ischaemic and inflammatory heart diseases in the USA and Europe due to "globalisation" of Chagas disease. Eight to 10% has the digestive form, characterised by dilation of the oesophagus or colon (megaoesophagus and megacolon). Some patients have associated cardiac and digestive manifestations, known as the mixed or cardiodigestive form [17, 29, 30].

The transition from acute to chronic phase is accompanied by a marked decrease in parasitaemia, due to the mounting of a relatively effective immune response, which keeps parasite frequency at below detectable levels in the host. To diagnose the disease, regardless of stage, the serological test is used by detecting antibodies specific to the parasite: IgM (acute phase) and IgG (indeterminate and chronic phase) [31]. Conventional serological tests include primarily immunofluorescence assays (IFA), enzyme-linked immunosorbent assays (ELISA), and indirect haemagglutination assays (IHA). These tests were summarised by Afonso et al. [32]. Changes in the chronic phase can be revealed by electrocardiogram clinical diagnosis, X-rays, and ultrasound [27, 33].

1.6. Host Immune Response. There is a consensus that during *T. cruzi* infection the host immune system induces complex processes to ensure the control of parasite growth. The immune response is crucial for protection against disease; however, immunological imbalances can lead to heart and digestive tract lesions in chagasic patients. Several studies have evaluated the innate, cellular, and humoral immune responses in chagasic patients in an attempt to correlate immunological findings with clinical forms of Chagas disease. However, in all clinical forms of Chagas disease the involvement of cell-mediated immunity is undoubtedly of major importance [34–36].

It is also accepted that *T. cruzi* induces a strong activation of the immune system during acute infection and that the different immunological mechanisms triggered during the early indeterminate stages of the infection may represent an essential component of the immune activity observed during ongoing, clinically distinct chronic infection. After invasion of infectious metacyclic trypomastigotes in the mammalian host, *T. cruzi* infects a variety of cell types such as macrophages and fibroblasts. Fibroblasts are numerous in the extracellular matrix of the skin and are refractory to apoptosis [37] unlike macrophages and cardiomyocytes. Infected macrophages initiate the molecular interactions that mobilise the innate immune response of the host by secretion of proinflammatory cytokines like tumour necrosis factor alpha (TNF-α) and interleukin- (IL-) 12 [38]. These cytokines activate Natural Killer (NK) cells to produce interferon-gamma (IFN-γ) that acts directly on macrophages, activating them for antimicrobial activity. Activated macrophages produce nitric oxide (NO) via NO synthase (iNOS, NOS2), potent against *T. cruzi*. Furthermore, the regulatory cytokines—IL-4, IL-10, and transforming growth factor beta (TGF-β)—inhibit the production of NO, and trypanocidal activity of activated macrophages is responsible for disabling the control of lethal inflammatory effects of type 1 cytokines produced during infection [39].

Uncontrolled activation of NK cells and macrophages can lead to tissue damage. According to Vitelli-Avelar et al. [40], there is a mixed profile of cytokine production, and high levels of IFN-γ, TNF-α, and IL-4 favour the generation of inflammatory mechanisms. This intense inflammatory process during the initial infection is essential to confine the aetiologic agent in the intracellular site (limiting infection and symptoms) and prevent tissue damage but can be determinant to immunopathology.

The lesions of the acute phase of the disease are characterised by the presence of localised inflammatory reactions, with a predominance of mononuclear cells at the foci of the pseudocyst ruptures, occasionally with the formation of granulomas located mainly in muscle and cardiac tissue [17].

There is a robust immune response displayed in the IND despite the complete lack of clinical disease. Many studies concerning the cellular immune response in the IND have been performed. Peripheral blood cells from these patients proliferate when stimulated with antiepimastigote antibodies [35]. Analysis of the expression of activation markers by T-cells showed that IND patients have a high frequency of $CD4^+$ and $CD8^+$ T-cells expressing HLA-DR and CD45RO [33]. Moreover, the vast majority of these T-cells do not express the costimulatory molecule CD28 [35] which suggested that this subpopulation displayed down-modulatory activity, due to intrinsic regulation via CTLA-4. In mice, deficiency in either class I- or class II-restricted T-cell populations was observed as a striking similarity in their mortality rate [41]. Given that $CD8^+$ T-cells seem to be the best candidate for tissue destruction, it is possible that this regulatory mechanism, working in tandem with others, helps prevent pathology in IND [42].

Despite it being considered for decades that the adaptive immune response is the most important protective mechanism during chronic infection, recent studies have suggested the importance of the innate response as a regulatory mechanism for controlling morbidity during disease. Monocytes from IND patients, *in vitro*, lead to a high expression of IL-10, consistent with a modulatory response. CD3-$D16^+CD56^+$ and $CD3^-$ $CD16^+$ $CD56^{dim}$ NK cells counts are particularly high, suggesting a protective role of this cell subpopulation. $CD56^{dim}$ NK cells had more cytotoxic activity and can contribute to the control of parasitism. Added to this, asymptomatic patients exhibited Treg cells ($CD4^+CD25^{high}$), NKT

regulatory cells ($CD3^-CD56^+CD16^+$), and macrophage-like regulatory cells ($CD14^+CD16^+$) that encourage the establishment and maintenance of the IND form [39, 43].

CCC is associated with the presence of an intense inflammatory infiltrate in the myocardium, especially at sites where *T. cruzi* antigens are observed. The infiltrate was composed especially by $CD8^+$ T-cells. IL-7 and IL-15 are critical for maintenance of these cells and their activation state in the heart tissue of CCC [44]. $CD8^+$ T-cells are also found in the circulation. Similar to what was described in IND patients, $CD8^+$ and $CD4^+$ cells present high expression of HLA-DR and lower expression of CD28; however, here, $CD4^+$ cells were correlated with the expression of TNF-alpha [35, 36] as well as IFN-γ. The latter cytokine is higher in CCC than IND patients and may be a key factor in the development of severe cardiomyopathy [45]. Monocytes from cardiac patients also produce TNF-α and IL-10. Although cells from CCC patients are able to produce IL-10, the ratio of this cytokine seems to be lower in cardiac patients [45].

During the *T. cruzi*-cardiomyocyte interaction, the parasite has control of the host cell gene expression, including expression of genes related to immune response, inflammation, cytoskeletal organisation, cell-cell and cell-matrix interactions, apoptosis, cell cycle, and oxidative stress [46]. The intense production of cytokines, chemokines, and nitric oxide that are essential elements of the defensive reaction in cardiac tissue can also result in cardiac hypertrophy. The activation of the host cell apoptotic machinery by pathogens is an offensive strategy to eliminate the host's immune response [47].

Megaoesophagus and megacolon are the major causes of morbidity in the digestive clinical form of chronic Chagas disease. Inflammatory infiltrates and fibrosis are found associated with lesions of muscle cells and of the intramural nervous system. They are composed mainly of $CD3^+CD4^+$ T lymphocytes, $CD20^+$ B lymphocytes, $CD57^+$ NK cells, and $CD68^+$ macrophage-like cells [48]. Corrêa-Oliveira et al. [45] observed that patients with the gastrointestinal form of Chagas disease demonstrated a significant decrease in the absolute number of $CD3^+$ T-cells as well as in $CD19^+$ B lymphocytes, and an inversion of the CD4/CD8 ratio, contrasting with results from CCC where the ratio of these cells is normal. Chagasic patients with megacolon present increased numbers of eosinophils and mononuclear cells [49]. These cells are associated with inflammatory processes and can contribute to tissue injury through the secretion of cytokines such as IL-1, TNF-α, and IL-6, which activate the cytotoxic process [50].

1.7. Pathogenesis in Chagas Disease. Based on the relationship of parasite and host interaction, the mechanisms of pathogenesis in human Chagas disease can be based on two main hypotheses. The first defends the pivotal role of parasite's persistence in the host as a major cause of pathology, while the other postulates that an immune response against self-antigens is responsible for the tissue damage observed in affected organs of chagasic individuals.

T. cruzi exhibits multiple strategies to ensure its establishment and persistence in the host. Although this parasite has the ability to infect different organs, heart impairment is the most frequent clinical manifestation of the disease. Calvet et al. [47] reviewed the current understanding of molecules involved in *T. cruzi*-cardiomyocyte recognition, the mechanism of invasion, and the effect of intracellular development of *T. cruzi* on the structural organisation and molecular response of the target cell. The nature of the myocardial changes in the chronic stage has been considered by some to be an autoimmune phenomenon based on antigenic mimicry in the form of an antibody targeting *T. cruzi* polypeptides [51]. More recently, however, persistence of the parasite in the tissues has been demonstrated [52].

Although the theories are controversial, autoreactivity and parasite persistence theories are not mutually exclusive. The variation in pathological manifestation has been reported and was related to differences in host immune response, such as the ability to control parasitaemia, the strength of inflammatory reactions, and the induction of autoimmune-like responses [53].

2. Genetic Factors and Their Influence on Chagas Disease

The advance in knowledge about infection and disease has changed the concept of infectious diseases, and the genetic markers play an important role in this area [54]. The spectrum of expression of Chagas disease brings strong evidence of the influence of the genetic factors on its clinical course [55].

The polymorphisms of the genes involved in the innate and specific immune response are being widely studied in order to clarify their possible role in the occurrence or severity of disease. To identify possible host genetic factors that may influence the clinical course of Chagas disease, the role of classic and nonclassic major histocompatibility complex (MHC) genes, killer cell immunoglobulin-like receptor (*KIR*) genes and cytokine genes, that are involved in the immune response will be addressed.

2.1. Strategy for Screening and Selecting Studies. This review about genetic factors and their influence on Chagas disease selected original articles carried out on humans that were found in the databases of PubMed (US National Library of Medicine), LILACs (Latin American and Caribbean Center on Information in Health Sciences), and Google Scholar. The research period covered included the limit of databases until March 2013. There was no restriction regarding language. In the PubMed database MeSH (Medical Subject Heading Terms) were used, and in the LILACS descriptors were used. In order to retrieve articles of interest, free terms were used in the LILACS and Google Scholar. The MeSH terms, descriptors, and free terms were organized according to thematic groups: (i) HLA and Chagas disease ("Chagas Disease/genetics" OR "Chagas Disease/immunology" AND "HLA Antigens/genetics" OR "HLA antigens/immunology"); (ii) MIC and Chagas disease ("Chagas disease/genetics" AND "MHC class I-related chain A"); (iii) *KIR* genes and Chagas disease ("Chagas disease/genetics" AND "Receptors, *KIR*/genetics"); (iv) Cytokine genes and Chagas disease

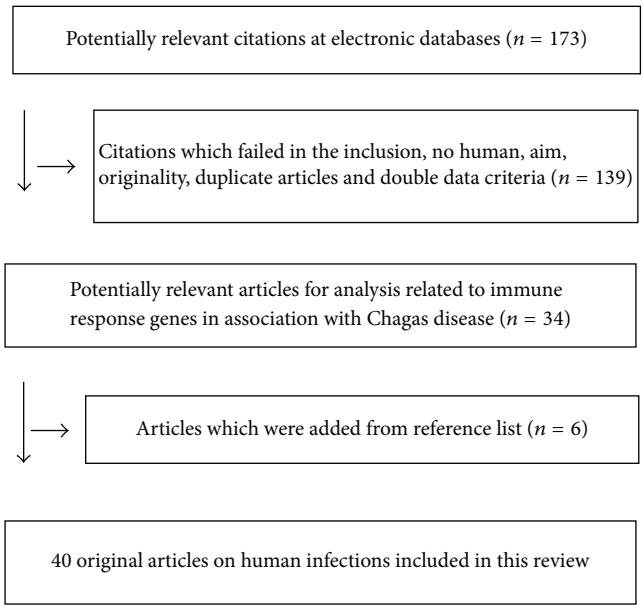


Figure 1: Flow chart of the study for review.

("Chagas disease/genetics" AND "cytokines/genetics" OR "Chemokines/genetics" OR "Receptors, cytokine/genetics." The immune response genes, as HLA, *KIR*, MIC, and cytokines, and their association with Chagas disease and its clinical forms in the American Latin population were presented. The results were summarized in Figure 1 and the selected studies are presented in Tables 1 and 2.

2.2. HLA and Chagas Disease. To identify possible host genetic factors that may influence the clinical course of Chagas disease, the molecules and genes in the region of the human leucocyte antigen (HLA) have been analysed in patients presenting with differing clinical symptoms.

The highly polymorphic HLA class I (A, B, and C) and II (DR, DQ, and DP) molecules determine the efficiency of presentation of the *T. cruzi* epitopes to $CD8^+$ and $CD4^+$ T-cells, respectively. The type of the presentation could affect the clinical course of diseases because patients may respond differently to the same antigen, depending on their HLA repertory [56].

HLA alleles and haplotypes associated with Chagas disease are summarized in Table 1.

Several HLA alleles and haplotypes have been reported to be associated with Chagas disease. In Venezuela, a study comparing class II allele frequencies between patients and controls identified a decreased frequency of *DRB1*14* and *DQB1*03:03* in patients, suggesting independent protective effects to chronic infection in this population. In this same study, a higher frequency of *DRB1*01*, *DRB1*08*, and *DQB1*05:01* and a decreased frequency of *DRB1*15:01* were found in patients with arrhythmia and congestive heart failure [57]. In an endemic area of central Venezuela, a higher frequency of the *HLA-DPB1*04:01* allele and *DPB1*04:01-HLA-DPB1*23:01* or *DPB1*04:01-DPB1*39:01* haplotype was found in patients with cardiac manifestations [58]. Susceptibility between *HLA-C*03* and CCC Venezuelan was confirmed [59].

In Chile, HLA-B40 antigen in the presence of Cw3 was significantly lower in subjects with CCC [60] and was found in higher expression in patients without evidence of heart disease in Santiago [61].

An increase of HLA-A31, B39, and DR8 and a decrease of HLA-DR4, DR5, DQ1, and DQ3 were observed in several Latin American mestizos from different countries and with CCC [62].

A study in south-eastern Brazil showed that *HLA-A*30* was involved in susceptibility to Chagas disease, whereas *HLA-DQB1*06* was related to protection, regardless of the clinical form of the disease [63], and HLA-DR2 was associated with susceptibility to chronic Chagas disease in a south Brazilian population [64]. However, in another study, the polymorphism of HLA-DR and -DQ does not influence the susceptibility to different clinical forms of Chagas disease or the progression to severe Chagas' cardiomyopathy [65].

The haplotype *HLA-DRB1*14-DQB1*03:01* was involved in resistance to infection with *T. cruzi* in a rural mestizo population of southern Peru [66].

In the Mexican population, HLA-DR4 and HLA-B39 were associated with the infection by the *T. Cruzi* whereas HLA-DR16 was a marker of susceptibility to damage to the heart and HLA-A68 was related with protection to development of CCC [67].

In Argentina, the class II allele *HLA-DRB1*04:09* and *DRB1*15:03* was significantly more prevalent in Chagas disease and *DRB1*11:03* allele was associated with disease resistance. Increased frequency of *DRB1*15:03* allele was found among CCC suggesting susceptibility [68, 69].

In Bolivia, the frequencies of *HLA-DRB1*01* and *HLA-B*14:02* were significantly lower in patients suffering from megacolon, as well as in those with ECG alteration and/or megacolon, compared with a group of IND patients. The *DRB1*01:02*, *B*14:02*, and *MICA*011* alleles were in strong linkage disequilibrium, and the *HLA-DRB1*01-B*14-MICA*011* haplotype was associated with resistance against chronic Chagas disease [70].

These different results between the HLA allele and haplotypes and Chagas disease could be caused by variability of HLA allele's distribution in different ethnic groups; the typing test (serological or molecular techniques); the methods of statistical analyses (simple chi-square test and logistic or linear regression) and interpretation (P value or P_c value that applies the Bonferroni correction for multiple comparisons); the selection of the patients and the clinical form; the numbers of individuals; linkage disequilibrium; and biological variability of the parasite. Nevertheless, genetic factors related to the HLA system reflect an important role in susceptibility or protection to Chagas disease and its clinical forms.

2.3. MIC and Chagas Disease. The HLA region contains not only classical HLA genes but also a wide variety of immunologically relevant genes, such as nonclassical class I genes (*MICA*, *MICB*; major histocompatibility complex class

Table 1: Alleles and haplotypes HLA associated with Chagas disease.

HLA class I alleles and haplotypes	HLA class II alleles and haplotypes	Population	Clinical forms	Association	Reference
	[a]*DRB1* 01, DQB1* 03:03*	**Venezuela**	Chronic phase	Protection	[57]
	[a]*DRB1* 15:01*		CCC	Protection	[57]
	[a]*DRB1* 08*		CCC	Susceptibility	[57]
	[a]*DRB1* 01, DQB1* 05:01*		CCC	Susceptibility	[57, 58]
	[a]*DPB1* 04:01*[a]*DPB1* 04:01–* 23:01* [a]*DPB1* 04:01–* 39:01* haplotypes		CCC	Susceptibility	[58]
[b]*C* 03*			CCC	Susceptibility	[59]
[a]HLA-B40-Cw3 haplotype		**Chile**	CCC	Protection	[60, 61]
[b]*HLA-A* 30*		**Brazil**	All clinical form	Susceptibility	[63]
	[b]*DQB1* 06*		All clinical form	Protection	[63]
	[b]DR2		Chronic phase	Susceptibility	[64]
				No association	[65]
	[b]*DRB1* 14-DQB1* 03:01* haplotype	**Peru**	Infection	Protection	[66]
[b]HLA-B39	[b]HLA-DR4	**Mexico**	Infection	Susceptibility	[67]
[b]HLA-B35	[b]HLA-DR16		CCC	Susceptibility	
[b]HLA-A68			CCC	Protection	
	[b]*DRB1* 04:09* and ** 15:03*	**Argentina**	Infection	Susceptibility	[68, 69]
	[b]*DRB1* 11:03*		Infection	Protection	[69]
	[b]*DRB1* 15:03*		CCC	Susceptibility	[69]
[b]*HLA-B* 14:02*	[b]*HLA-DRB1* 01*	**Bolivian**	DG or mixed	Protection	[70]
	[b]*HLA-DRB1* 01-B* 14-MICA* 011* haplotype		Infection	Protection	[70]
[b]HLA-B35-*MICA-A5* haplotype		**Guatemala**	CCC	Susceptibility	[79]
[b]A31 and B39	[b]DR8	**Latin American mestizos**	CCC	Susceptibility	[62]
	[b]DR4, DR5, DQ1, DQ3		CCC	Protection	

[a] P value ≤ 0.05 or [b] P_c value ≤ 0.05.
CCC: chronic Chagas cardiomyopathy; DG: digestive form; mixed: CCC and DG or CCC + DG.

I chain-related genes A and B) that may be involved in disease pathogenesis.

The MICA molecules are recognised by lymphocytes T$\gamma\delta$, lymphocytes T$\alpha\beta$ CD8+, and NK cells via their receptors NKG2D, present on their surfaces, in association with the DAP10 molecule, an adapter protein membrane [71, 72]. This complex, NKG2D-MICA, activates the phosphorylation of tyrosine residues of the molecule DAP10, triggering a cascade of cell signalling that ends with the process of cell lysis of the target cells [72]. A study by Steinle et al. [73] showed that changing a single amino acid—a methionine for a valine at position 129 of the α2 domain of the heavy chain—categorises the MICA as strong (MICA-129met) and weak (MICA-129val) ligands of NKG2D affecting the activation of NK cells.

MICA and MICB are weakly expressed on healthy cells, but their expression is induced in response to cellular stress in many cell types, including epithelial cells, fibroblasts, keratinocytes, endothelial cells, and monocytes [74–77].

The polymorphism of MICA may be involved in susceptibility to various diseases, but it has been suggested that this association may be secondary, due to the strong linkage disequilibrium with HLA-B alleles. Groh et al. [78] found that *MICA* 011*, which was closely linked to *HLA-B* 14* and *DRB1* 01*, might stimulate T$\gamma\delta$ cells in the gut mucosa, a phenomenon that could relate to megacolon. In Chagas disease, the same *HLA-DRB1* 01-B* 14-MICA* 011* haplotype was associated with resistance against the chronic form [70]. *MICA-A5* and *HLA-B35* synergistically enhanced susceptibility to CCC [79].

2.4. Association of KIR Genes and Their HLA Ligands with Chagas Disease. Other receptors of NK cells that recognise HLA class I molecules present on the target cells included *KIR* (killer cell immunoglobulin-like receptor) [80, 81]. *KIR* receptors are glycoproteins that belong to the immunoglobulin superfamily, and which are also found in some subpopulations of T-cells [82]. *KIR* genes are clustered in the 19q13.4 region and are characterised by both allelic (high numbers of variants) and haplotypic (different numbers of genes for inhibitory and activating receptors on individual chromosomes) polymorphisms. The specific KIR-HLA combinations may regulate NK cell-mediated immunity against infectious

Table 2: Polymorphisms in cytokines genes and their association with Chagas disease.

Gene/allele/genotype	Population	Clinical form	Association	Reference
TNFA −308, −244, −236 and *TNFB*	Peru	Infection and CCC	No association	[106]
[b] *TNF* −308*A*	Mexico	Infection and CCC	Susceptibility	[108]
TNF −308*A*	Brazil	CCC	No association	[107]
[a] *TNF* −238*A*	Brazil	Infection	Susceptibility	[109]
[a] *TNF −1031C* and *−308A*	Colombia	CCC	Susceptibility	[110]
[a] *TNFA −1031TT* and *−308GG*	Colombia	CCC	Protection	[110]
[a] *TNFa2, TNFa7, TNFa8, TNFb2, TNFb4, TNFd5, TNFd7, TNFe2*	Brazil	CCC, DG, or mixed	Protection	[111]
[a] *TNFb7* and *TNFd3*	Brazil	CCC and mixed	Susceptibility	[111]
[a] *TNFa2*	Brazil	CCC	Susceptibility	[112]
[b] *LTA +80C* and *LTA +252G*	Brazil	CCC	Susceptibility	[113]
[b] *LTA +80A +252A* haplotype	Brazil	CCC	Protection	[113]
IL6 −174GC	Peru/Colombia	Infection	No association	[114]
[b] *IL −1RN.4CC*	Mexico	CCC	Susceptibility	[115]
[b] *IL1B +5810G* allele and *IL1B −31 +395 CT* genotype	Colombia	CCC	Susceptibility	[116]
[a] *IL10 −1082A* and *−1082AA*	Brazil	CCC	Susceptibility	[119]
IL10	Colombia	Infection	No association	[120]
[a] *IFNG +874A* and *+874AA*	Colombia	Infection	Susceptibility	[122]
[a] *IL4 −590T*	Bolivia	Infection	Protection	[124]
[a] *IL4RA +148AA*		CCC	Susceptibility	[120]
[a] *TGFB110C* and *CC*	Peru/Colombia	Infection	Susceptibility	[126]
[b] *IL12B 3′ UTR C* and *CC*	Colombia	CCC	Susceptibility	[127]
[a] *CXCL9CC* and *CXCL10GG*	Brazil	CCC	Protection	[129]
CCR5CC		CCC	Susceptibility	[129]
[a,b] *CCR5 −2554T, −2733G, 59029G, 59029AG, 59029GG*	Peru/Colombia/Venezuela	CCC	Susceptibility	[130–132]
[a] *CCL2 −2518A* and *AA*	Brazil	CCC	Susceptibility	[133]
[a] *BAT1 22C 348C*	Brazil	CCC	Susceptibility	[134]

[a] P value ≤ 0.05; [b] P_c value ≤ 0.05.
CCC: chronic Chagas cardiomyopathy; DG: digestive form; mixed: CCC and DG or CCC + DG.

pathogens and contribute to diverse susceptibility to diseases and other clinical situations.

Several studies have shown the participation of *KIR* genes and their ligands in infectious diseases [83–89]; autoimmune or inflammatory diseases [90–92], cancer [93–95], and in the success of transplantation [96]. However, there are no data available on the role of *KIR* genes in the immunopathogenesis of Chagas disease.

2.5. Association of Polymorphisms in Cytokine Genes with Chagas Disease. Immunomodulatory cytokines secreted by T-cells and macrophages are molecules that act as mediators of inflammation and immune response. These molecules are key components in the pathogenesis of many diseases including infectious diseases, cancer, metabolic disorders, autoimmunity, and inflammatory conditions. The cytokines are important for parasitic control and are involved in the genesis of lesions [97].

It is known that the production of some cytokines is under genetic control and is influenced by polymorphisms in several cytokine genes. SNPs or microsatellites mainly located in regulatory regions may affect gene transcription and cause interindividual variations. Some of these polymorphisms influence the level of cytokine production, which can confer flexibility in the immune response [98–101]. Thus, the presence of certain alleles may influence the course of bacterial and viral infections [102, 103] and confer susceptibility or resistance to autoimmune disease [104]. SNPs in cytokine genes have been described as important genetic factors in the occurrence of different clinical forms of Chagas disease. The

genetic susceptibility of single nucleotide polymorphisms in cytokine genes to human Chagas disease was reviewed [105]. The cytokines polymorphisms associated with Chagas disease are summarized in Table 2.

Proinflammatory cytokines play a key role in the development of CCC. Aiming to investigate the influence of the *TNF* polymorphisms on Chagas disease, *TNFA* (positions −308 rs1800629, −244 rs673, −238 rs361525, and −1031 rs1799964) and *TNFB* genotypes have been interrogated. No significant differences were observed with these alleles or haplotypes between patients and controls in a Peruvian population [106]. *TNF-308A* also was not associated with CCC in Brazilian patients [107]. However, the same SNP could be directly involved in the genetic susceptibility of the chronic phase and CCC in a Mexican population [108]. Another study, also in Brazilian patients, suggested that allele *TNF-238A*, which correlates with production of significantly higher levels of TNF-α, could influence the susceptibility to infection [109]. The *TNF-1031C and -308A* alleles were significantly associated with the development of CCC whereas the *TNF-1031TT* and *-308 GG* genotypes were associated with lower risk to develop CCC, in a Colombian population [110]. Regarding the *TNF* gene microsatellite, ten alleles were associated with Chagas disease in the Brazilian population: eight of them correlate with susceptibility (*TNFa2*, *TNFa7*, *TNFa8*, *TNFb2*, *TNFb4*, *TNFd5*, *TNFd7*, and *TNFe2*) and two with protection (*TNFb7* and *TNFd3*) against the development of the disease [111]. Previously, the occurrence of the *TNFa2* microsatellite was correlated with reduced survival in severe cardiomyopathy [112]. Despite the controversial results, these data suggest the involvement of *TNF* in the course of Chagas disease.

Clinical, genetic, and epidemiological studies have linked lymphotoxin-α (LTA), a proinflammatory cytokine, to coronary artery disease and myocardial infarction. In Brazilian CCC patients, homozygosity with respect to the *LTA+80C* (rs2239704) and *LTA+252G* (rs909253) alleles was significantly more frequent and was associated with susceptibility, and the haplotype *LTA+80A+252A* was associated with protection against CCC. Furthermore, homozygosity for the *LTA+80A* allele correlated with the lowest levels of plasmatic TNF-α [113].

It appears that *IL6* gene polymorphism does not contribute to susceptibility in the clinical manifestations of Chagas disease. In a study in two independent populations of Colombia and Peru, no difference was observed for *IL6-174GC* (rs1800795) between Chagas disease and controls, or between asymptomatic patients and CCC [114].

Some *IL1B* alleles and haplotypes have been associated with susceptibility to inflammatory, autoimmune and infectious diseases. The *IL1B-511*(rs16944), *IL1F10.3* (rs3811058), *IL1RN.4* (rs419598), *IL1RN 6/1* (rs315952), and *IL-1RN 6/2* (rs315951) polymorphisms were analysed in Chagas disease and *IL-1RN.4CC* genotype was clearly associated with *T. cruzi* infection and CCC development [115]. The *IL1B+5810G* (rs1143633) allele and the haplotype *IL1B-31* (rs1143627) *+3954* (rs1143634) *CT* were associated with an increased risk of CCC [116]. Therefore, these studies suggest that *IL1* gene cluster polymorphisms may play a relevant role in the susceptibility to development of CCC and that the effect of *IL1B* gene on chagasic cardiomyopathy predisposition is dose dependent [116].

IL-10 and INF-γ production by T cells promotes *T. cruzi* control and protects against fatal acute myocarditis [115–118]. The SNP *IL10-1082* (rs1800896) *A* allele, which is associated with a lower expression of IL-10, had higher frequency in CCC Brazilian patients when compared to the asymptomatic group [119]. However, in Colombian patients no differences in allele frequency and haplotype of the *IL10* gene were observed in symptomatic and asymptomatic patients [120]. Linkage disequilibrium analysis on microsatellite loci suggests epistasis between MHC and IL-10 on Chagas disease susceptibility/resistance [121]. The frequency of the *IFNG+874AA* (rs62559044), which is associated with reduced production of INF-γ, was increased in the Colombian patients suggesting that this SNP may be involved in susceptibility but not in the progression of Chagas disease [122].

IL-4 is described as a prototypical anti-inflammatory cytokine. It can modulate the host and parasite survivals that depend on a fine balance between Th1 responses: on one hand it will control parasitism and, on the other hand, enhance heart inflammation throughout the course of the infection [123]. In a Bolivian population, SNP *IL4-590* (rs2243250) *T* allele was associated with protection against *T. cruzi* infection [124], although in another study only the *IL4RA* (IL-4 receptor-α; rs147781) *+I48AA* genotype showed a weak association with the development of CCC [120].

TGF-β plays a pivotal role in Chagas disease, not only in the development of chagasic cardiomyopathy, but also in many stages of the *T. cruzi* life cycle and survival in the host cell environment through regulation of (i) parasite invasion of heart cells, (ii) an intracellular parasite cycle, (iii) inflammation and immune response, (iv) heart fibrosis and remodelling, and (v) gap junction modulation and heart conduction [125]. Several SNPs in the *TGFB1* gene that may affect cytokine production have been described. A significant difference in the distribution of the *TGFB1 10* (rs1982073) *T* and *C* alleles between patients and healthy controls was observed in the Peruvian and Colombian populations: *TGFB1 10CC*, the high producer genotype, was increased in patients of both populations [126].

The IL-12 family of cytokines can influence Th17 and the production of IL-17 and INF-γ. The SNPs rs3212227 in the 3′UTR of the gene *IL12B* were investigated, and *IL12B 3′UTR C* allele and *CC* genotype were significantly increased among CCC patients when compared to asymptomatic individuals [127].

MIF (*macrophage migration inhibitory factor*) *–173C* allele and *CC* genotype were related to the major risk of Chagas disease in the Colombian and Peruvian populations [128].

Some chemokines also had been associated with the development of CCC. *CXCL9 CC* (rs10336) and *CXCL10 GG* (rs3921) genotypes were less frequent and *CCR5 CC* (rs1799988) was more frequent in patients with left ventricular dysfunction, compared with patients without this dysfunction and may indicate that CXCL9 and CXCL10 are master regulators of myocardial inflammatory cell migration,

perhaps affecting clinical progression to the life-threatening form of CCC [129]. Some SNPs of *CCR5* gene were associated with the development of severe cardiomyopathy in an endemic area of Colombia, Peru, and Venezuela [130–132].

In the Brazilian population, *BAT1* (HLA-B associated transcript 1) *C* allele and *CC* genotype, at positions 22 C/G and −348 C/T, were associated with risk of cardiomyopathy [133]. In the same population, *MCP-1* (monocyte chemoattractant protein-1) gene, *CCL2-2518A* allele (rs1024611), and the *CCL2-2518 AA* genotype correlate with susceptibility to chronic cardiomyopathy [134]. *BAT1*, *MCP-1*, and *LTA* may be involved in the pathogenesis of cardiac Chagas disease.

Despite the controversial results, these data suggest the involvement of cytokines in the course of Chagas disease. The balance between T_H1/T_H2/T regulatory cytokines has different and opposing influences on the likelihood of infection with *T. cruzi* and on the clinical course of the Chagas disease.

3. Concluding Remarks

Many genetic linkages and association studies have attempted to identify genetic variations that are involved in immunopathogenesis of Chagas disease. However, causal genetic variants underlying susceptibility remain unknown due to complexity of parasite and host. Susceptibility/resistance to Chagas disease involves multiple genetic variants functioning jointly, each with small or moderate effects. Immunological mechanisms protect against the disease but contribute to aggression and tissue damage. Genome wide association studies (GWAS) and next generation sequencing (NGS), at present lacking in Chagas disease, may help identifying novel genetic polymorphisms with genome scale associations.

The impact of pathogens on host cell functions, as well as genetic markers that were significantly associated with disease, was found on schistosomiasis [135], ascariasis [136], leprosy [137], tuberculosis [138, 139], malaria [140], dengue [141], hepatitis [142, 143], and HIV-1 [144–147]. In Chagas disease, the *2590T* allele (rs2243250) polymorphism in the promoter region of *IL4* gene is a marker for *IL4* haplotypes likely associated with protection against *T. cruzi* infection [124].

The identification of the specific genes influencing *T. cruzi* infection and Chagas disease through genome scans techniques could offer a particular opportunity for mapping genes of susceptibility or resistance. Further studies are necessary to clarify the relationship between genotype and development of disease and its clinical outcomes.

The characterisation of the susceptibility genes and their variants has important implications, not only for a better understanding of disease pathogenesis, but also for the control and development of new therapeutic strategies for infectious diseases.

References

[1] C. Chagas, "Nova tripanozomiaze humana. Estudos sobre a morfologia e o ciclo evolutivo do *Schizotrypanum cruzi* n. gen., n. sp. *Agente etiológico* Agente etiológico de uma nova entidade mórbida para o homem," *Memórias do Instituto Oswaldo Cruz*, vol. 1, pp. 159–218, 1909.

[2] A. M. Macedo and S. D. J. Pena, "Genetic variability of *Trypanosoma cruzi*: implications for the pathogenesis of Chagas disease," *Parasitology Today*, vol. 14, no. 3, pp. 119–124, 1998.

[3] L. O. Andrade, C. R. S. Machado, E. Chiari, S. D. J. Pena, and A. M. Macedo, "Differential tissue distribution of diverse clones of *Trypanosoma cruzi* in infected mice," *Molecular and Biochemical Parasitology*, vol. 100, no. 2, pp. 163–172, 1999.

[4] World Health Organization (2012) Chagas disease (American trypanosomiasis): Fact Sheet No 340.

[5] L. V. Kirchhoff, "Changing epidemiology and approaches to therapy for Chagas disease," *Current Infectious Disease Reports*, vol. 5, no. 1, pp. 59–65, 2003.

[6] C. Hoare, "The trypanosomes of mammals," *Journal of Small Animal Practice*, vol. 13, pp. 671–672, 1972.

[7] C. J. Schofield, "Overview: evolution of the triatominae," in *Proceedings of the 2nd International Workshop on Population Genetics and Control of Triatominae*, C. J. Schofield and C. Ponce, Eds., INDRE, Tegucigalpa, Honduras, 1998.

[8] F. Rothhammer, M. J. Allison, L. Nunez, V. Standen, and B. Arriaza, "Chagas disease in pre-Columbian South America," *American Journal of Physical Anthropology*, vol. 68, no. 4, pp. 495–498, 1985.

[9] J. R. Coura, "Chagas disease: what is known and what is needed—a background article," *Memorias do Instituto Oswaldo Cruz*, vol. 102, no. 1, pp. 113–122, 2007.

[10] A. C. Aufderheide, W. Salo, M. Madden et al., "A 9,000-year record of Chagas disease," *Proceedings of the National Academy of Sciences of USA*, vol. 101, no. 7, pp. 2034–2039, 2004.

[11] Y. Carlier, J. C. P. Dias, A. O. Luquetti, M. Honteberye, F. Torrico, and C. Truyens, "Trypanosomiase américaine ou maladie de Chagas," in *Encyclopedie Médico Chirurgicale*, Elsevier, Paris, Frence, 2002.

[12] W. B. Bean, "The illness of Charles Darwin," *The American Journal of Medicine*, vol. 65, no. 4, pp. 572–574, 1978.

[13] F. Köberle, "50 Years of Chagas' disease," *Munchener Medizinische Wochenschrift*, vol. 99, pp. 1193–1198, 1957.

[14] A. Moncayo and M. I. Ortiz Yanine, "An update on Chagas disease (human American trypanosomiasis)," *Annals of Tropical Medicine and Parasitology*, vol. 100, no. 8, pp. 663–677, 2006.

[15] G. A. Schmunis, "Epidemiology of Chagas disease in non-endemic countries: the role of international migration," *Memorias do Instituto Oswaldo Cruz*, vol. 102, no. 1, pp. 75–85, 2007.

[16] G. A. Schmunis and Z. E. Yadon, "Chagas disease: a Latin American health problem becoming a world health problem," *Acta Tropica*, vol. 115, no. 1-2, pp. 14–21, 2010.

[17] J. R. Coura and P. A. Vinãs, "Chagas disease: a new worldwide challenge," *Nature*, vol. 465, no. 7301, pp. S6–S7, 2010.

[18] A. F. Henao-Martínez, D. A. Schwart, and I. V. Yang, "Chagasic cardiomyopathy, from acute to chronic: is this mediated by host susceptibility factors?" *Transactions of the Royal Society of Tropical Medicine and Hygiene*, vol. 106, no. 9, pp. 521—527, 2012.

[19] D. P. Neves, A. L. Melo, and O. Genaro, Eds., *Parasitologia Humana*, Atheneu, São Paulo, Brazil, 2005.

[20] D. Born, R. E. S. Achá, and M. Ferraz, "Pregnancy and Chagas' disease," *Journal of the American College of Cardiology*, vol. 31, p. 421, 1998.

[21] E. L. P. Camandaroba, C. M. Pinheiro Lima, and S. G. Andrade, "Oral transmission of Chagas disease: importance of *Trypanosoma cruzi* biodeme in the intragastric experimental infection," *Revista do Instituto de Medicina Tropical de Sao Paulo*, vol. 44, no. 2, pp. 97–103, 2002.

[22] A. M. Macedo and S. D. J. Pena, "Genetic variability of *Trypanosoma cruzi*: implications for the pathogenesis of Chagas disease," *Parasitology Today*, vol. 14, no. 3, pp. 119–124, 1998.

[23] A. Moncayo, "Chagas disease: current epidemiological irends after the interruption of vectorial and transfusional transmission in the southern cone countries," *Memorias do Instituto Oswaldo Cruz*, vol. 98, no. 5, pp. 577–591, 2003.

[24] L. J. Silva, *A evolução da doença de Chagas no Estado de São Paulo*, Editora Hucitec, São Paulo, Brazil, 1999.

[25] W. O. Dutra and K. J. Gollob, "Current concepts in immunoregulation and pathology of human Chagas disease," *Current Opinion in Infectious Diseases*, vol. 21, no. 3, pp. 287–292, 2008.

[26] M. P. Barrett, R. J. S. Burchmore, A. Stich et al., "The trypanosomiases," *The Lancet*, vol. 362, no. 9394, pp. 1469–1480, 2003.

[27] Z. Brener, "Pathogenesis and immunopathology of chronic Chagas disease," *Memórias do Instituto Oswaldo Cruz*, vol. 82, pp. 205–213, 1987.

[28] F. Guhl, C. Jaramillo, J. C. Carranza, and G. A. Vallejo, "Molecular characterization and diagnosis of *Trypanosoma cruzi* and *T. rangeli*," *Archives of Medical Research*, vol. 33, no. 4, pp. 362–370, 2002.

[29] A. Moncayo and A. C. Silveira, "Current epidemiological trends for Chagas disease in Latin America and future challenges in epidemiology, surveillance and health policy," *Memorias do Instituto Oswaldo Cruz*, vol. 104, no. 1, pp. 17–30, 2009.

[30] Y. Moolani, G. Bukhman, and P. J. Hotez, "Neglected tropical diseases as hidden causes of cardiovascular disease," *PLOS Neglected Tropical Diseases*, vol. 6, no. 6, article e1499, 2012.

[31] E. S. Umezawa, A. O. Luquetti, G. P. Levitus et al., "Serodiagnosis of chronic and acute Chagas' disease with *Trypanosoma cruzi* recombinant proteins: results of A Collaborative Study in six Latin American countries," *Journal of Clinical Microbiology*, vol. 42, no. 1, pp. 449–452, 2004.

[32] A. M. Afonso, M. H. Ebell, and R. L. Tarleton, "A systematic review of high quality diagnostic tests for Chagas disease," *PLOS Neglected Tropical Diseases*, vol. 6, no. 11, article e1881, 2012.

[33] E. Dias, F. S. Laranja, A. Miranda, and G. Nobrega, "Chagas' disease; a clinical, epidemiologic, and pathologic study," *Circulation*, vol. 14, no. 6, pp. 1035–1060, 1956.

[34] W. O. Dutra, O. A. Martins-Filho, and J. R. Cançado, "Activated T and B lymphocytes in peripheral blood of patients with Chagas disease," *International Immunology*, vol. 6, pp. 499–506, 1994.

[35] W. O. Dutra, O. A. Martins-Filho, J. R. Cançado et al., "Chagasic patients lack CD28 expression on many of their circulating T lymphocytes," *Scandinavian Journal of Immunology*, vol. 43, no. 1, pp. 88–93, 1996.

[36] W. O. Dutra, D. G. Colley, J. C. Pinto-Dias et al., "Self and nonself stimulatory molecules induce preferential expansion of $CD5^+$B cells or activated T cells of chagasic patients, respectively," *Scandinavian Journal of Immunology*, vol. 51, no. 1, pp. 91–97, 2000.

[37] R. K. Clark and R. E. Kuhn, "Trypanosoma cruzi does not induce apoptosis in murine fibroblasts," *Parasitology*, vol. 118, no. 2, pp. 167–175, 1999.

[38] R. L. Tarleton, "*Trypanosoma cruzi*-induced suppression of IL-2 production. II. Evidence for a role for suppressor cells," *Journal of Immunology*, vol. 140, no. 8, pp. 2769–2773, 1988.

[39] D. B. Rocha Rodrigues, M. A. dos Reis, A. Romano et al., "In situ expression of regulatory cytokines by heart inflammatory cells in Chagas' disease patients with heart failure," *Clinical and Developmental Immunology*, vol. 2012, Article ID 361730, 7 pages, 2012.

[40] D. M. Vitelli-Avelar, R. Sathler-Avelar, R. L. Massara et al., "Are increased frequency of macrophage-like and natural killer (NK) cells, together with high levels of NKT and $CD4^+CD25^{high}$ T cells balancing activated $CD8^+$ T cells, the key to control Chagas' disease morbidity?" *Clinical and Experimental Immunology*, vol. 145, no. 1, pp. 81–92, 2006.

[41] R. L. Tarleton, M. J. Grusby, M. Postan, and L. H. Glimcher, "Trypanosoma cruzi infection in MHC-deficient mice: further evidence for the role of both class I- and class II-restricted T cells in immune resistance and disease," *International Immunology*, vol. 8, no. 1, pp. 13–22, 1996.

[42] W. O. Dutra, C. A. S. Menezes, F. N. A. Villani et al., "Cellular and genetic mechanisms involved in the generation of protective and pathogenic immune responses in human Chagas disease," *Memorias do Instituto Oswaldo Cruz*, vol. 104, supplement 1, pp. 208–218, 2009.

[43] D. M. Vitelli-Avelar, R. Sathler-Avelar, J. C. P. Dias et al., "Chagasic patients with indeterminate clinical form of the disease have high frequencies of circulating $CD3^+CD16^-CD56^+$ natural killer T cells and $CD4^+CD25^{High}$ regulatory T lymphocytes," *Scandinavian Journal of Immunology*, vol. 62, no. 3, pp. 297–308, 2005.

[44] S. G. Fonseca, M. M. Reis, V. Coelho et al., "Locally produced survival cytokines IL-15 and IL-7 may be associated to the predominance of $CD8^+$ T cells at heart lesions of human chronic chagas disease cardiomyopathy," *Scandinavian Journal of Immunology*, vol. 66, no. 2-3, pp. 362–371, 2007.

[45] R. Corrêa-Oliveira, J. A. S. Gomes, E. M. Lemos et al., "The role of the immune response on the development of severe clinical forms of human Chagas disease," *Memórias do Instituto Oswaldo Cruz*, vol. 9, supplement 1, pp. 253–255, 1999.

[46] R. C. Goldenberg, D. A. Iacobas, S. Iacobas et al., "Transcriptomic alterations in *Trypanosoma cruzi*-infected cardiac myocytes," *Microbes and Infection*, vol. 11, no. 14-15, pp. 1140–1149, 2009.

[47] C. M. Calvet, T. G. Melo, L. R. Garzoni et al., "Current understanding of the *Trypanosoma cruzi*-cardiomyocyte interaction," *Frontiers in Immunology*, vol. 3, p. 327, 2012.

[48] D. d'Avila Reis, E. M. Lemos, G. C. Silva et al., "Phenotypic characterization of the inflammatory cells in chagasic megaoesophagus," *Transactions of the Royal Society of Tropical Medicine and Hygienee*, vol. 95, pp. 177–178, 2001.

[49] A. B. Da Silveira, S. J. Adad, R. Correa-Oliveira, J. B. Furness, and D. d'Avila Reis, "Morphometric study of eosinophils, mast cells, macrophages and fibrosis in the colon of chronic chagasic patients with and without megacolon," *Parasitology*, vol. 134, no. 6, pp. 789–796, 2007.

[50] G. M. Cardoso, M. J. Morato, J. A. Gomes et al., "Comparative analysis of cell phenotypes in different severe clinical forms of Chagas' disease," *Frontiers in Bioscience*, vol. 11, no. 1, pp. 1158–1163, 2006.

[51] V. Michailowsky, K. Luhrs, M. O. Rocha, D. Fouts, R. T. Gazzinelli, and J. E. Manning, "Humoral and cellular immune responses to *Trypanosoma cruzi* Trypanosoma cruzi-derived paraflagellar rod proteins in patients with Chagas' disease," *Infection and Immunity*, vol. 71, no. 6, pp. 3165–3171, 2003.

[52] A. R. Vago, L. O. Andrade, A. A. Leite et al., "Genetic characterization of *Trypanosoma cruzi* directly from tissues of patients with chronic chagas disease: differential distribution of genetic

types into diverse organs," *American Journal of Pathology*, vol. 156, no. 5, pp. 1805–1809, 2000.

[53] W. O. Dutra, C. A. S. Menezes, F. N. A. Villani et al., "Cellular and genetic mechanisms involved in the generation of protective and pathogenic immune responses in human Chagas disease," *Memorias do Instituto Oswaldo Cruz*, vol. 104, supplement 1, pp. 208–218, 2009.

[54] A. Schriefer and E. M. Carvalho, "Biomarcadores em medicina," *Gazeta Médica da Bahia*, vol. 78, pp. 47–51, 2008.

[55] FIOCRUZ. Polimorfismos genéticos e suscetibilidade a cardiopatia Chagásica, http://www.fiocruz.br/chagas/cgi/cgilua.exe/sys/start.htm?sid=96.

[56] D. G. Mack, J. J. Johnson, F. Roberts et al., "HLA-class II genes modify outcome of *Toxoplasma gondii* infection," *International Journal for Parasitology*, vol. 29, no. 9, pp. 1351–1358, 1999.

[57] M. T. Fernandez-Mestre, Z. Layrisse, S. Montagnani et al., "Influence of the HLA class II polymorphism in chronic Chagas' disease," *Parasite Immunology*, vol. 20, no. 4, pp. 197–203, 1998.

[58] I. A. Colorado, H. Acquatella, F. Catalioti, M. T. Fernandez, and Z. Layrisse, "HLA class II DRB1, DQB11, DPB1 polymorphism and cardiomyopathy due to *Trypanosoma cruzi* chronic infection," *Human Immunology*, vol. 61, no. 3, pp. 320–325, 2000.

[59] Z. Layrisse, M. T. Fernandez, S. Montagnani et al., "HLA-C*03 is a risk factor for cardiomyopathy in Chagas disease," *Human Immunology*, vol. 61, no. 9, pp. 925–929, 2000.

[60] E. Llop, F. Rothhammer, M. Acuña, and W. Apt, "HLA antigens in cardiomyopathic Chilean chagasics," *American Journal of Human Genetics*, vol. 43, no. 5, pp. 770–773, 1988.

[61] E. Llop, F. Rothhammer, M. Acuña, W. Apt, and A. Arribada, "HLA antigens in Chagas cardiomyopathy: new evidence based on a case-control study," *Revista Medica De Chile*, vol. 119, no. 6, pp. 633–636, 1991.

[62] G. M. Sierp and E. D. Albert, "Analysis of the HLA data," in *Proceedings of the 5th Latin American Histocompatibility Workshop*, C. Gorodezky, G. Sierp, and E. Albert, Eds., Immunogenetics Laboratory, 1992.

[63] N. H. Deghaide, R. O. Dantas, and E. A. Donadi, "HLA class I and II profiles of patients presenting with Chagas' disease," *Digestive Diseases and Sciences*, vol. 43, no. 2, pp. 246–252, 1998.

[64] M. M. De Oliveira Dalálio, J. E. Laguila Visentainer, R. A. Moliterno, A. M. Sell, and M. L. Petzel-Erler, "Association of HLA-DR2 with chronic chagasic cardiopathy in a population at Paraná Northeast region, Brazil," *Acta Scientiarum*, vol. 24, pp. 727–730, 2002.

[65] K. C. Faé, S. A. Drigo, E. Cunha-Neto et al., "HLA and β-myosin heavy chain do not influence susceptibility to Chagas' disease cardiomyopathy," *Microbes and Infection*, vol. 2, no. 7, pp. 745–751, 2000.

[66] A. Nieto, Y. Beraún, M. D. Callado et al., "HLA haplotypes are associated with differential susceptibility to *Trypanosoma cruzi* infection," *Tissue Antigens*, vol. 55, no. 3, pp. 195–198, 2000.

[67] D. Cruz-Robles, P. A. Reyes, V. M. Monteón-Padilla, A. R. Ortiz-Muñiz, and G. Vargas-Alarcón, "MHC class I and class II genes in mexican patients with Chagas disease," *Human Immunology*, vol. 65, no. 1, pp. 60–65, 2004.

[68] S. G. Borrás, C. Diez, C. Cotorruelo et al., "HLA class II DRB1 polymorphism in Argentinians undergoing chronic *Trypanosoma cruzi* infection," *Annals of Clinical Biochemistry*, vol. 43, part 3, pp. 214–216, 2006.

[69] S. G. Borrs, L. Racca, C. Cotorruelo, C. Biondi, J. Beloscar, and A. Racca, "Distribution of HLA-DRB1 alleles in argentinean patients with chagas' disease cardiomyopathy," *Immunological Investigations*, vol. 38, no. 3-4, pp. 268–275, 2009.

[70] F. del Puerto, J. E. Nishizawa, M. Kikuchi et al., "Protective human leucocyte antigen haplotype, HLA-DRB1*01-B*14, against chronic Chagas disease in Bolivia," *PLoS Neglected Tropical Diseases*, vol. 6, no. 3, article e1587, 2012.

[71] S. Bauer, V. Groh, J. Wu et al., "Activation of NK cells and T cells by NKG2D, a receptor for stress-inducible MICA," *Science*, vol. 285, no. 5428, pp. 727–729, 1999.

[72] D. M. Pardoll, "Immunology: stress, NK receptors, and immune surveillance," *Science*, vol. 294, no. 5542, pp. 534–536, 2001.

[73] A. Steinle, P. Li, D. L. Morris et al., "Interactions of human NKG2D with its ligands MICA, MICB, and homologs of the mouse RAE-1 protein family," *Immunogenetics*, vol. 53, no. 4, pp. 279–287, 2001.

[74] S. Bahram, M. Bresnahan, D. E. Geraghty, and T. Spies, "A second lineage of mammalian major histocompatibility complex class I genes," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 91, no. 14, pp. 6259–6263, 1994.

[75] H. A. F. Stephens, "MICA and MICB genes: can the enigma of their polymorphism be resolved?" *Trends in Immunology*, vol. 22, no. 7, pp. 378–385, 2001.

[76] N. W. Zwirner, M. A. Fernández-Viña, and P. Stastny, "MICA, a new polymorphic HLA-related antigen, is expressed mainly by keratinocytes, endothelial cells, and monocytes," *Immunogenetics*, vol. 47, no. 2, pp. 139–148, 1998.

[77] N. W. Zwirner, K. Dole, and P. Stastny, "Differential surface expression of MICA by endothelial cells, fibroblasts, keratinocytes, and monocytes," *Human Immunology*, vol. 60, no. 4, pp. 323–330, 1999.

[78] V. Groh, A. Steinle, S. Bauer, and T. Spies, "Recognition of stress-induced MHC molecules by intestinal epithelial $\gamma\delta$ T cells," *Science*, vol. 279, no. 5357, pp. 1737–1740, 1998.

[79] K. Aida, S. Juarez, M. Kikuchi et al., "HLA-B35 and MICA-A5 synergistically enhanced susceptibility to chagas heart disease," *MHC*, vol. 7, pp. 63–70, 2000.

[80] C. Vilches and P. Parham, "KIR: diverse, rapidly evolving receptors of innate and adaptive immunity," *Annual Review of Immunology*, vol. 20, pp. 217–251, 2002.

[81] R. J. Boyton and D. M. Altmann, "Natural killer cells, killer immunoglobulin-like receptors and human leucocyte antigen class I in disease," *Clinical and Experimental Immunology*, vol. 149, no. 1, pp. 1–8, 2007.

[82] S. Rajagopalan and E. O. Long, "Understanding how combinations of HLA and KIR genes influence disease," *Journal of Experimental Medicine*, vol. 201, no. 7, pp. 1025–1029, 2005.

[83] M. P. Martin, X. Gao, J.-H. Lee et al., "Epistatic interaction between KIR3DS1 and HLA-B delays the progression to AIDS," *Nature Genetics*, vol. 31, no. 4, pp. 429–434, 2002.

[84] A. Méndez, H. Granda, A. Meenagh et al., "Study of KIR genes in tuberculosis patients," *Tissue Antigens*, vol. 68, no. 5, pp. 386–389, 2006.

[85] D. S. Franceschi, P. S. Mazini, C. C. Rudnick et al., "Association between killer-cell immunoglobulin-like receptor genotypes and leprosy in Brazil," *Tissue Antigens*, vol. 72, no. 5, pp. 478–482, 2008.

[86] A. V. Marangon, G. F. Silva, C. F. De Moraes et al., "KIR genes and their human leukocyte antigen ligands in the progression to cirrhosis in patients with chronic hepatitis C," *Human Immunology*, vol. 72, no. 11, pp. 1074–1078, 2011.

[87] G. Alter, D. Heckerman, A. Schneidewind et al., "HIV-1 adaptation to NK-cell-mediated immune pressure," *Nature*, vol. 476, no. 7358, pp. 96–100, 2011.

[88] L.-M. Yindom, R. Forbes, P. Aka et al., "Killer-cell immunoglobulin-like receptors and malaria caused by *Plasmodium falciparum* in the Gambia," *Tissue Antigens*, vol. 79, no. 2, pp. 104–113, 2012.

[89] C. Lu, X. L. Bai, Y. J. Shen et al., "Potential implication of activating killer cell immunoglobulin-like receptor and HLA in onset of pulmonary tuberculosis," *Scandinavian Journal of Immunology*, vol. 76, no. 5, pp. 491–496, 2012.

[90] J.-H. Yen, B. E. Moore, T. Nakajima et al., "Major histocompatibility complex class I-recognizing receptors are disease risk genes in rheumatoid arthritis," *Journal of Experimental Medicine*, vol. 193, no. 10, pp. 1159–1167, 2001.

[91] S. Ramírezs-De los Santos, P. E. Sánchez-Hernández, J. F. Muñoz-Valle et al., "Associations of killer cell immunoglobulin-like receptor genes with rheumatoid arthritis," *Disease Markers*, vol. 33, no. 4, pp. 201–206, 2012.

[92] P. Kuśnierczyk, "Killer cell immunoglobulin-like receptor gene associations with autoimmune and allergic diseases, recurrent spontaneous abortion, and neoplasms," *Frontiers in Immunology*, 2013.

[93] M. Carrington and P. Norman, "The KIR gene cluster," 2003, http://www.ncbi.nlm.nih.gov/books/bookres.fcgi/mono_003/ch1d1.pdf.

[94] E. Ashouri, M. H. Dabbaghmanesh, S. Rowhanirad, M. Bakhshayeshkaram, G. R. Omrani, and A. Ghaderi, "Activating KIR2DS5 receptor is a risk for thyroid cancer," *Human Immunology*, vol. 73, no. 10, pp. 1017–1022, 2012.

[95] A. Wiśniewski, R. Jankowska, E. Passowicz-Muszyńska et al., "KIR2DL2/S2 and HLA-C C1C1 genotype is associated with better response to treatment and prolonged survival of patients with non-small cell lung cancer in a polish caucasian population," *Human Immunology*, vol. 73, no. 9, pp. 927–31, 2012.

[96] D. S. Franceschi, C. A. De Souza, and F. J. Aranha, "Importance of killer immunoglobulin-like receptors in allogeneic hematopoietic stem cell transplantation," *Revista Brasileira de Hematologia e Hemoterapia*, vol. 33, no. 2, pp. 126–130, 2011.

[97] E. D. Cox, S. C. Hoffmann, B. S. Dimercurio et al., "Cytokine polymorphic analyses indicate ethnic differences in the allelic distribution of interleukin-2 and interleukin-6," *Transplantation*, vol. 72, no. 4, pp. 720–726, 2001.

[98] A. G. Wilson, J. A. Symons, T. L. Mcdowell, H. O. Mcdevitt, and G. W. Duff, "Effects of a polymorphism in the human tumor necrosis factor α promoter on transcriptional activation," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 94, no. 7, pp. 3195–3199, 1997.

[99] V. Pravica, A. Asderakis, C. Perrey, A. Hajeer, P. J. Sinnott, and I. V. Hutchinson, "In vitro production of IFN-γ correlates with CA repeat polymorphism in the human IFN-γ gene," *European Journal of Immunogenetics*, vol. 26, no. 1, pp. 1–3, 1999.

[100] M. R. Awad, A. El-Gamel, P. Hasleton, D. M. Turner, P. J. Sinnott, and I. V. Hutchinson, "Genotypic variation in the transforming growth factor-β1 gene: Association with transforming growth factor-β1 production, fibrotic lung disease, and graft fibrosis after lung transplantation," *Transplantation*, vol. 66, no. 8, pp. 1014–1020, 1998.

[101] D. M. Turner, D. M. Williams, D. Sankaran, M. Lazarus, P. J. Sinnott, and I. V. Hutchinson, "An investigation of polymorphism in the interleukin-10 gene promoter," *European Journal of Immunogenetics*, vol. 24, no. 1, pp. 1–8, 1997.

[102] D. S. Franceschi, P. S. Mazini, C. C. C. Rudnick et al., "Influence of TNF and IL10 gene polymorphisms in the immunopathogenesis of leprosy in the south of Brazil," *International Journal of Infectious Diseases*, vol. 13, no. 4, pp. 493–498, 2009.

[103] S. T. Moreira, D. M. Cardoso, J. E. Visentainer, U. J. V. Fonzar, and R. A. Moliterno, "The possible protective role of the IL6^{-174}GC genotype in dengue fever," *The Open Tropical Medicine Journal*, vol. 1, pp. 87–91, 2008.

[104] J. Bidwell, L. Keen, G. Gallagher et al., "Cytokine gene polymorphism in human disease: On-line databases," *Genes and Immunity*, vol. 1, no. 1, pp. 3–19, 1999.

[105] R. H. Vasconcelos, S. M. Montenegro, E. A. Azevedo, Y. M. Gomes, and C. N. Morais, "Genetic susceptibility to chronic Chagas disease: an overview of single nucleotide polymorphisms of cytokine genes," *Cytokine*, vol. 59, no. 2, pp. 203–208, 2012.

[106] Y. Beraún, A. Nieto, M. D. Collado, A. González, and J. Martín, "Polymorphisms at tumor necrosis factor (TNF) loci are not associated with Chagas' disease," *Tissue Antigens*, vol. 52, no. 1, pp. 81–83, 1998.

[107] S. A. Drigo, E. Cunha-Neto, B. Ianni et al., "Lack of association of tumor necrosis factor-α polymorphisms with Chagas disease in Brazilian patients," *Immunology Letters*, vol. 108, no. 1, pp. 109–111, 2007.

[108] J. M. Rodríguez-Pérez, D. Cruz-Robles, G. Hernández-Pacheco et al., "Tumor necrosis factor-alpha promoter polymorphism in Mexican patients with Chagas' disease," *Immunology Letters*, vol. 98, no. 1, pp. 97–102, 2005.

[109] C. W. Pissetti, D. Correia, R. F. De Oliveira et al., "Genetic and functional role of TNF-alpha in the development *Trypanosoma cruzi* infection," *PLoS Neglected Tropical Diseases*, vol. 5, no. 3, article e976, 2011.

[110] L. Criado, O. Flórez, J. Martín, and C. I. González, "Genetic polymorphisms in TNFA/TNFR2 genes and Chagas disease in a Colombian endemic population," *Cytokine*, vol. 57, no. 3, pp. 398–401, 2012.

[111] V. Campelo, R. O. Dantas, R. T. Simões et al., "NF microsatellite alleles in Brazilian Chagasic patients," *Digestive Diseases and Sciences*, vol. 12, pp. 3334–3349, 2007.

[112] S. A. Drigo, E. Cunha-Neto, B. Ianni et al., "TNF gene polymorphisms are associated with reduced survival in severe Chagas' disease cardiomyopathy patients," *Microbes and Infection*, vol. 8, no. 3, pp. 598–603, 2006.

[113] R. Ramasawmy, K. C. Fae, E. Cunha-Neto et al., "Polymorphisms in the gene for lymphotoxin-α predispose to chronic chagas cardiomyopathy," *Journal of Infectious Diseases*, vol. 196, no. 12, pp. 1836–1843, 2007.

[114] O. A. Torres, J. E. Calzada, Y. Beraún et al., "Lack of association between IL-6 -174G/C gene polymorphism and Chagas disease," *Tissue Antigens*, vol. 76, no. 2, pp. 131–134, 2010.

[115] D. Cruz-Robles, J. P. Chávez-González, M. M. Cavazos-Quero, O. Pérez-Méndez, P. A. Reyes, and G. Vargas-Alarcón, "Association between IL-1B and IL-1RN gene polymorphisms and Chagas' disease development susceptibility," *Immunological Investigations*, vol. 38, no. 3-4, pp. 231–239, 2009.

[116] O. Flórez, G. Zafra, C. Morillo, J. Martín, and C. I. González, "Interleukin-1 gene cluster polymorphism in Chagas disease in a Colombian Case-Control Study," *Human Immunology*, vol. 67, no. 9, pp. 741–748, 2006.

[117] D. A. D'Ávila, P. M. M. Guedes, A. M. Castro, E. D. Gontijo, E. Chiari, and L. M. C. Galvão, "Immunological imbalance

between IFN-γ and IL-10 levels in the sera of patients with the cardiac form of Chagas disease," *Memorias do Instituto Oswaldo Cruz*, vol. 104, no. 1, pp. 100–105, 2009.

[118] E. Roffê, A. G. Rothfuchs, H. C. Santiago et al., "IL-10 limits parasite burden and protects against fatal myocarditis in a mouse model of *Trypanosoma cruzi* infection," *Journal of Immunology*, vol. 188, no. 2, pp. 649–660, 2012.

[119] G. C. Costa, M. O. Costa Rocha, P. R. Moreira et al., "Functional IL-10 gene polymorphism is associated with Chagas disease cardiomyopathy," *Journal of Infectious Diseases*, vol. 199, no. 3, pp. 451–454, 2009.

[120] O. Flórez, J. Martín, C. I. González et al., "Interleukin 4, interleukin 4 receptor-α and interleukin 10 gene polymorphisms in Chagas disease," *Parasite Immunology*, vol. 33, no. 9, pp. 506–511, 2011.

[121] M. Moreno, E. L. Silva, L. E. Ramírez, L. G. Palacio, D. Rivera, and M. Arcos-Burgos, "Chagas' disease susceptibility/resistance: linkage disequilibrium analysis suggest epistasis between major histocompatibility complex and interleukin-10," *Tissue Antigens*, vol. 64, no. 1, pp. 18–24, 2004.

[122] O. A. Torres, J. E. Calzada, Y. Beraún et al., "Role of the IFNG +874T/A polymorphism in Chagas disease in a Colombian population," *Infection, Genetics and Evolution*, vol. 10, no. 5, pp. 682–685, 2010.

[123] M. B. P. Soares, K. N. Silva-Mota, R. S. Lima, M. C. Bellintani, L. Pontes-de-Carvalho, and R. Ribeiro-dos-Santos, "Modulation of chagasic cardiomyopathy by interleukin-4: dissociation between inflammation and tissue parasitism," *American Journal of Pathology*, vol. 159, no. 2, pp. 703–709, 2001.

[124] L. E. Alvarado Arnez, E. N. Venegas, C. Ober, and E. E. Thompson, "Sequence variation in the IL4 gene and resistance to *Trypanosoma cruzi* infection in Bolivians," *Journal of Allergy and Clinical Immunology*, vol. 127, no. 1, pp. 279–282, 2011.

[125] T. C. Araújo-Jorge, M. C. Waghabi, S. Bailly et al., "The TGF-β pathway as an emerging target for Chagas disease therapy," *Clinical Pharmacology & Therapeutics*, vol. 92, no. 5, pp. 613–621, 2012.

[126] J. E. Calzada, Y. Beraún, C. I. González, and J. Martín, "Transforming growth factor beta 1 (TGFβ1) gene polymorphisms and Chagas disease susceptibility in Peruvian and Colombian patients," *Cytokine*, vol. 45, no. 3, pp. 149–153, 2009.

[127] G. Zafra, C. Morillo, J. Martín, A. González, and C. I. González, "Polymorphism in the 3′ UTR of the IL12B gene is associated with Chagas' disease cardiomyopathy," *Microbes and Infection*, vol. 9, no. 9, pp. 1049–1052, 2007.

[128] O. A. Torres, J. E. Calzada, Y. Beraún et al., "Association of the macrophage migration inhibitory factor -173G/C polymorphism with Chagas disease," *Human Immunology*, vol. 70, no. 7, pp. 543–546, 2009.

[129] L. G. Nogueira, R. H. B. Santos, B. M. Ianni et al., "Myocardial chemokine expression and intensity of myocarditis in Chagas cardiomyopathy are controlled by polymorphisms in CXCL9 and CXCL10," *PLOS Neglected Tropical Diseases*, vol. 6, no. 10, article e1867, 2012.

[130] O. Flórez, J. Javier Martín, and C. I. González, "Genetic variants in the chemokines and chemokine receptors in Chagas disease," *Human Immunology*, vol. 73, pp. 852–858, 2012.

[131] J. E. Calzada, A. Nieto, Y. Beraún, and J. Martín, "Chemokine receptor CCR5 polymorphisms and Chagas' disease cardiomyopathy," *Tissue Antigens*, vol. 58, no. 3, pp. 154–158, 2001.

[132] M. T. Fernández-Mestre, S. Montagnani, and Z. Layrisse, "Is the CCR5-59029-G/G genotype a protective factor for cardiomyopathy in Chagas disease?" *Human Immunology*, vol. 65, no. 7, pp. 725–728, 2004.

[133] R. Ramasawmy, E. Cunha-Neto, K. C. Faé et al., "The monocyte chemoattractant protein-1 gene polymorphism is associated with cardiomyopathy in human Chagas disease," *Clinical Infectious Diseases*, vol. 43, no. 3, pp. 305–311, 2006.

[134] R. Ramasawmy, E. Cunha-Neto, K. C. Faé et al., "BAT1, a putative anti-inflammatory gene, is associated with chronic chagas cardiomyopathy," *Journal of Infectious Diseases*, vol. 193, no. 10, pp. 1394–1399, 2006.

[135] S. Williams-Blangero, J. L. Vandeberg, J. Subedi et al., "Genes on chromosomes 1 and 13 have significant effects on Ascaris infection," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 99, no. 8, pp. 5533–5538, 2002.

[136] S. Marquet, L. Abel, D. Hillaire et al., "Genetic localization of a locus controlling the intensity of infection by *Schistosoma mansoni* on chromosome 5q31-q33," *Nature Genetics*, vol. 14, no. 2, pp. 181–184, 1996.

[137] F. R. Zhang, W. Huang, S. M. Chen et al., "Genomewide association study of leprosy," *The New England Journal of Medicine*, vol. 361, no. 27, pp. 2609–2618, 2009.

[138] T. Thye, F. O. Vannberg, S. H. Wong et al., "Genome-wide association analyses identifies a susceptibility locus for tuberculosis on chromosome 18q11.2," *Nature Genetics*, vol. 42, no. 9, pp. 739–741, 2010.

[139] S. Mahasirimongkol, H. Yanai, T. Mushiroda et al., "Genome-wide association studies of tuberculosis in Asians identify distinct at-risk locus for young tuberculosis," *Journal of Human Genetics*, vol. 57, no. 6, pp. 363–367, 2012.

[140] M. Jallow, Y. Y. Teo, K. S. Small et al., "Genome-wide and fine-resolution association analysis of malaria in West Africa," *Nature Genetics*, vol. 41, no. 6, pp. 657–665, 2009.

[141] C. C. Khor, T. N. B. Chau, J. Pang et al., "Genome-wide association study identifies susceptibility loci for dengue shock syndrome at MICB and PLCE1," *Nature Genetics*, vol. 43, no. 11, pp. 1139–1141, 2011.

[142] A. Rauch, Z. Kutalik, P. Descombes et al., "Genetic variation in IL28B Is associated with chronic hepatitis C and treatment failure: a genome-wide association study," *Gastroenterology*, vol. 138, no. 4, pp. 1338–1345, 2010.

[143] Y. Kamatani, S. Wattanapokayakit, H. Ochi et al., "A genome-wide association study identifies variants in the HLA-DP locus associated with chronic hepatitis B in Asians," *Nature Genetics*, vol. 41, no. 5, pp. 591–595, 2009.

[144] J. Fellay, K. V. Shianna, D. Ge et al., "A whole-genome association study of major determinants for host control of HIV-1," *Science*, vol. 317, no. 5840, pp. 944–947, 2007.

[145] J. Fellay, D. Ge, K. V. Shianna et al., "Common genetic variation and the control of HIV-1 in humans," *PLOS Genetics*, vol. 5, no. 12, article e1000791, 2009.

[146] K. Pelak, D. B. Goldstein, and N. M. Walley, "Host determinants of HIV-1 control in African Americans," *Journal of Infectious Diseases*, vol. 201, no. 8, pp. 1141–1149, 2010.

[147] S. Limou, S. Le Clerc, C. Coulonges et al., "Genomewide association study of an AIDS-nonprogression cohort emphasizes the role played by HLA genes (ANRS genomewide association study 02)," *Journal of Infectious Diseases*, vol. 199, no. 3, pp. 419–426, 2009.

Hindawi Publishing Corporation
BioMed Research International
Volume 2013, Article ID 901740, 7 pages
http://dx.doi.org/10.1155/2013/901740

Clinical Study

No Association of *IFNG+874T/A* SNP and *NOS2A-954G/C* SNP Variants with Nitric Oxide Radical Serum Levels or Susceptibility to Tuberculosis in a Brazilian Population Subset

Ana Cristina C. S. Leandro,[1,2] Márcia Andrade Rocha,[1] Andreia Lamoglia-Souza,[1] John L. VandeBerg,[2] Valeria Cavalcanti Rolla,[3] and Maria da Gloria Bonecini-Almeida[1]

[1] *Immunology and Immunogenetics Laboratory, Evandro Chagas Clinical Research Institute, Oswaldo Cruz Foundation, Avenida Brasil 4365, Manguinhos, 21045-900 Rio de Janeiro, RJ, Brazil*
[2] *Department of Genetics and Southwest National Primate Research Center, Texas Biomedical Research Institute, 7620 NW Loop 410, 78227-5301 San Antonio, TX, USA*
[3] *Tuberculosis Clinical Laboratory, Evandro Chagas Clinical Research Institute, Oswaldo Cruz Foundation, Avenida Brasil 4365, Manguinhos, 21045-900 Rio de Janeiro, RJ, Brazil*

Correspondence should be addressed to Maria da Gloria Bonecini-Almeida; gloria.bonecini@ipec.fiocruz.br

Received 1 April 2013; Revised 5 June 2013; Accepted 5 July 2013

Academic Editor: Helder I. Nakaya

Tuberculosis (TB) is one of the most common infectious diseases in the world. *Mycobacterium tuberculosis* infection leads to pulmonary active disease in approximately 5–10% of exposed individuals. Both bacteria- and host-related characteristics influence latent infection and disease. Host genetic predisposition to develop TB may involve multiple genes and their polymorphisms. It was reported previously that interferon gamma (IFN-γ) and nitric oxide synthase 2 (NOS2) are expressed on alveolar macrophages from TB patients and are responsible for bacilli control; thus, we aimed this study at genotyping single nucleotide polymorphisms *IFNG+874T/A* SNP and *NOS2A-954G/C* SNP to estimate their role on TB susceptibility and determine whether these polymorphisms influence serum nitrite and NO_x^- production. This case-control study enrolled 172 TB patients and 179 healthy controls. Neither polymorphism was associated with susceptibility to TB. *NOS2A-954G/C* SNP was not associated with serum levels of nitrite and NO_x^-. These results indicate that variants of *IFNG+874T/A* SNP and *NOS2A-954G/C* SNP do not influence TB susceptibility or the secretion of nitric oxide radicals in the study population.

1. Introduction

Tuberculosis (TB) has been declared as a major global healthy threat by the World Health Organization since 1993. Pulmonary TB is highly prevalent in Brazil [1], mainly, in Rio de Janeiro, where 11,155 new cases were reported in 2011 [2]. Host factors play a major role in determining risk for active TB. Among them, IFN-γ production is critical in the intracellular control of *M. tuberculosis* infection, as previously demonstrated *in vitro* [3, 4] in experimental infection [5, 6]. In addition, interferon gamma (IFN-γ) induces apoptosis in mycobacteria-infected macrophages in a nitric oxide (NO) dependent environment [7, 8]. Polymorphism in the first intron of human *IFNG* gene is associated with higher *in vitro* production of this cytokine and is correlated with a gene dosage effect in the presence of the *IFNG+874T* allele [9]. This common polymorphism is associated with TB susceptibility in African, Turkish, Tunisian, and Central West Brazilian populations [10–13], but not in African-Americans, or people of Iranian, Hispanic, or Chinese origin [14–17].

NO is a free radical and second messenger that has been shown to be important in the development of several diseases, including TB. NO plays a major role in the pulmonary host-defense mechanism in response to infections and is implicated in bacteriostatic and bactericidal processes. NO is vital for macrophage function and granuloma formation

in the immune response and kills *M. tuberculosis in vitro* [18]. NO production and NOS2 expression in rat alveolar macrophages are upregulated in response to heat-killed *M. tuberculosis* [19]. However, the role of NO in killing or limiting the growth of *M. tuberculosis* in humans is still unclear. It has been proposed that NO produced by TB-infected human macrophages and by epithelial cells exhibits antimycobacterial behavior against *M. tuberculosis* [20]. It was previously reported that the alveolar macrophages from active TB patients express inducible nitric oxide synthase (iNOS/NOS2) and may control mycobacteria growth *in vivo* [21]. Thus, the *NOS2A-954G/C* SNP may represent a pivotal protective locus against TB. Investigation of this possibility is hampered by difficulty in estimating the production of NO *in vivo* mainly in lung tissues, but genetic analysis provides a potential means of examining the relation between *NOS2A* expression and disease outcome. Given the biological and genetic validity of the role of NOS2 in the immune system, SNPs have been reported in many populations worldwide [22–24]. The *NOS2A-954G/C* SNP variant was originally reported in a malaria endemic area in Africa [23], suggesting that this mutation might have originated as a consequence of selective pressure of *Plasmodium* infection. In a Mexican admixed population, this functional SNP was not associated with TB [25] and no further reports have associated it with NO radical levels.

In this present case-control study, we investigated the influence of the *IFNG+874T/A* (rs2430561) and *NOS2A-954G/C* (rs1800482) SNPs on TB susceptibility in a highly exposed and admixed population of TB patients. We also conducted functional studies to determine whether the modulation of nitric oxide radical secretion varies according to *NOS2A-954G/C* or *IFNG/NOS2A* combined genotypes.

2. Materials and Methods

2.1. Study Population. Patients and control groups were recruited from Evandro Chagas Clinical Research Institute at Fiocruz and from Municipal Health Centers, Rio de Janeiro, Brazil. All volunteers included in this study lived in the metropolitan area of Rio de Janeiro City (RJ, Brazil), were older than 18 years, and provided written informed consent. Cases and control groups were matched by age, socioeconomic class and area of residence. Control groups individuals were excluded if they had a history of prior antituberculosis therapy, signs and symptoms of suggestive active TB. The diagnostic criteria for TB were defined as the presence of a positive smear for acid fast bacilli [1] and/or culture positivity for *M. tuberculosis* in a sample from sputum and/or bronchial lavage and/or other clinical specimens according to [26]. HIV-infected people and those taking immunosuppressant drugs were excluded from participation in the study. The protocol was approved by the Research Ethics Committees in Brazil (IPEC REC ref. 0008.0.009.000-04) and Rio de Janeiro Municipal Health Centre (REC ref. S/CRH/DRH/DIC3). Ethnic background was determined for each case and control volunteers by self-identification. We recognize the inherent inaccuracy and potential bias in dichotomous self-assessment of ethnic origin, in an admixed population, but self-assessment might nevertheless lead to statistically significant differences between the two groups. All TB patients and control groups were negative for HIV 1/2 infection (following standard diagnosis from The Brazilian Ministry of Health). Tuberculin skin test (TST) response to 5UT RT-23 (Statens Serum Institute, Denmark) was performed, and the skin test response was measured at the diameter of induration 72 h after the injection. Positive results were obtained when induration was ≥10 mm. Control group was classified into those who were naturally infected with *M. tuberculosis* (latency) and those who were uninfected (TST < 10 mm). BCG vaccination status was determined by the presence of the scar tissue. Blood samples were taken after informed consent was obtained from each subject. Patients and controls from the same family were not enrolled in the study.

2.2. Genotyping of IFNG+874T/A and NOS2A-954G/C Gene Polymorphisms. Genomic DNA was extracted from fresh or frozen EDTA blood using a DNA purification kit (QIamp DNA mini Kit, Qiagen, USA) according to the manufacturer's instructions. The *IFNG+874T/A* SNP was detected by amplification refractory mutational system (ARMS-PCR) [9]. The *NOS2A-954G/C* SNP was detected by restriction fragment length polymorphism (RFLP) [23]. Amplifications were performed in a 9700 Thermocycler (96-Well GeneAmp PCR System 9700, Applied Biosystems, USA) using 2.5 UI and 1.5 UI for *IFNG* and *NOS2A* of Taq DNA polymerase, respectively (GoTaq flexi DNA polymerase, Promega, USA). Cycling PCR conditions for *IFNG+874T/A* were 3 minutes at 95°C followed by 10 cycles at 95°C for 15 s, 65°C for 50 s, and 72°C for 40 s; 20 cycles at 95°C for 20 s, 55°C for 50 s, and 72°C for 50 s; 72°C for 7 minutes and 4°C until use. Cycling PCR conditions for *NOS2A-954G/C* were 3 minutes at 95°C followed by 30 cycles at 94°C for 10 s, 60°C for 30 s, and 72°C for 30 s; 72°C for 7 minutes and 4°C until use. The amplified products were evaluated by electrophoresis on a 1.5% (*IFNG*) and 2.5% (*NOS2A*) agarose gel containing ethidium bromide (0.5 μg/mL).

2.3. Detection of Serum Nitric Oxide Radicals Concentration in TB Patients. The two primary stable nonvolatile breakdown products of NO are nitrite (NO_2^-) and nitrate (NO_3^-). First, the serum NO_2^- levels were determined using a commercial ready to use Griess reaction kit (Promega) according to the manufacturer's instruction. Briefly, serum samples were mixed with sulfanilamide solution following incubation at room temperature. N-1-Naphthylethylenediamine dihydrochloride was added, and absorbance was measured in a plate reader with a filter of 420 nm. Values were plotted in accordance with a $NaNO_2$ standard curve (0.8–100 mM). Further analysis was performed to determine the total levels of NO_2^- by reduced NO_3^-, in a Vanadium III reduction assay, following a protocol previously described by Miranda et al. [27]. Vanadium III (Sigma, 400 mg in 50 mL of 0.1 N HCl) was added to each well, incubated for 90 minutes at 37°C, and read at 540 nm to determine the total amount of NO_2^- + NO_3^- (after NO_3^- reduction to NO_2^-), which was named NO_x^-.

2.4. Statistical Analysis. Deviation from Hardy-Weinberg equilibrium for the genetic variants was assessed by the chi-square test (χ^2) in both case and control groups. We used the χ^2 test to compare the differences in each genotype, allele, and combined genotype of *IFNG+874T/A* SNP and *NOS2A-954G/C* SNP frequency. Additionally, unconditional univariate and multivariate logistic regression analyses were used to examine the associations between the selected SNPs and tuberculosis risk by estimating odds ratios (ORs) and 95% confidence intervals (CIs) with and without adjustment for gender, ethnicity, TST status, and previous BCG vaccination between TB and control groups. All statistical tests were two-sided, a P value of ≤0.05 was considered significant, and analyses were performed using Epi Info 6 (Version 6.04, July 1996, CDC, Atlanta, GA, USA), SNPStats (http://bioinfo.iconcologia.net/SNPstats), and SPSS (Version 16, September 2007). Additionally, the distributions of *IFNG+874T/A* and *NOS2A-954G/C* SNPs were compared among patients and in whom TST was positive in the control group by the χ^2 or Fisher's exact test. Subgroup analyses for genotype, allele, and combined genotype associations to tuberculosis were also conducted among TST-positive individuals. The analysis of the skin test positive group was planned because it was thought that this would represent people with probable latent TB infection. ANOVA test was used to compare the nitrite and NO_x^- levels in association with *NOS2A-954G/C* SNP and combined *IFNG+874T/A/NOS2A-954G/C* SNPs genotypes with the level of significance set at $P < 0.05$.

3. Results

3.1. Study Population. TB patients and control group were enrolled consecutively and included 105 males (61.0%) and 67 females (39.0%) with a mean age of 36.9 ± 12.7 years (mean ± standard deviation) in the TB group, and 63 males (35.2%) and 116 females (64.8%) with a mean age of 35.1 ± 11.5 years in the control group. Age was not significantly different between the groups. Each volunteer defined his or her own ethnic group as White (Caucasian) or non-White (Afro-descendants). No Indians or people with Asian background were identified in the included subjects. In the TB group, 136 of 172 (79.1%) individuals defined their ethnic group as White (52, 30.3%) or non-White (84, 48.8%), and among control group, 78 (43.6%) and 82 (45.8%) of 160 were White or non-White, respectively. TST ranged from 0 to 35 (14.9 ± 10.3) mm and from 0 to 57 (12.1 ± 12.2) mm in TB and control groups, respectively. TST-positive (≥10 mm) reaction was identified in 98 of 155 (54.7%) tested subjects from control group, confirming the highest *M. tuberculosis* exposure in Rio de Janeiro. No statistical significance in relation to TST positivity and ethnicity or gender was observed in either TB patients or control groups (Table 1).

Table 1: Clinical data from Brazilian tuberculosis patients and control group.

	Tuberculosis patients n = 172 (%)	Control group n = 179 (%)
Sex		
M	105 (61.0)	63 (35.2)
F	67 (39.0)	116 (64.8)
Age (years)	36.9 ± 12.7	35.1 ± 11.5
Skin color		
White	52 (30.3)	78 (43.6)
Non-White	84 (48.8)	82 (45.8)
Missing	36 (20.9)	19 (10.6)
BCG vaccine		
Yes	104 (60.5)	149 (83.2)
No	16 (9.3)	6 (3.3)
Missing	52 (30.2)	24 (13.5)
TST		
Positive	60 (34.9)	98 (54.7)
Negative	24 (13.9)	57 (31.8)
Missing	88 (51.2)	24 (13.5)
Diameter (mm)	(14.9 ± 10.3)	(12.1 ± 12.2)

TST: tuberculin skin test.

3.2. IFNG+874T/A SNP Distribution Is Not Associated with Tuberculosis. The genotype distribution of *IFNG+874T/A* SNP was in Hardy-Weinberg equilibrium ($P > 0.05$) in both TB and control groups. TB patients and control group had very similar genotype and allele distributions ($P > 0.05$) (Table 2). No statistical difference in genotypes was observed between TB patients and control subgroup who were TST positive (Table 2). The ability to respond to TST did not correlate with *IFNG+874T/A* SNP genotypes in either TB patients or control group. There is no statistical difference in *IFNG+874T/A* SNP genotypes between TB patients and control groups from univariate or multivariate analysis regarding ethnicity, age, and BCG vaccination status.

3.3. NOS2-954G/C SNP Distribution Is Not Associated with Tuberculosis. The genotype distributions of *NOS2A-954G/C* SNP were in Hardy-Weinberg equilibrium ($P > 0.05$) in both TB and control groups. No association was seen in the genotypes and alleles frequencies between TB patients and control groups and from those positives TST (control subgroup) (Table 2). When univariate analysis was performed by age, gender, ethnicity, and BCG vaccination, no statistical difference was observed ($P > 0.05$) (Table 2).

3.4. Serum Nitrite and NO_x^- Levels Are Not Associated with NOS2-954G/C Polymorphism in Tuberculosis Patients. The Griess reaction was performed in 75 TB patients and 78 control group, in proportion to sample sizes of the two groups. The mean serum concentration of nitrite and NO_x^- exhibited no statistical difference between TB patients (23.68 ± 15.74 μM and 32.61 ± 16.97 μM) and control group (23.99 ± 17.29 μM and 34.71 ± 19.39 μM), respectively. It was investigated whether serum levels of nitrite and NO_x^- could vary according to *NOS2A-954G/C* SNP genotypes in TB patients

Table 2: Distribution of genotypes and alleles frequencies of the *IFNG+874T/A* and *NOS2A-954G/C* SNPs in tuberculosis patients and control groups.

Genotype/allele	Tuberculosis patients $n = 172$ (%)	Control group $n = 179$ (%)	P^1	OR	TB latent infection (TST+) $n = 98$ (%)	P^2	OR
A/A	72 (41.8)	62 (34.6)		1.00	38 (38.8)		1.00
A/T	78 (45.3)	91 (50.8)	0.21	0.74	48 (49.0)	0.76	0.86
T/T	22 (12.8)	26 (14.6)		0.73	12 (12.2)		0.77
T/T+A/T versus *A/A*	100 (58.1)	117 (65.3)	0.16	0.74 (0.47–1.16)	60 (61.2)	0.61	0.88 (0.51–1.51)
T/T versus *A/A+A/T*	150 (87.2)	153 (85.4)	0.63	0.86 (0.45–1.66)	86 (87.8)	0.89	1.05 (0.47–2.38)
Alleles							
A	222 (64.5)	215 (60.1)	0.22	1.21 (0.88–1.66)	124 (63.3)	0.76	1.06 (0.72–1.55)
T	122 (35.5)	143 (39.9)			72 (36.7)		
G/G	152 (88.4)	160 (89.4)		1.00	84 (85.7)		1.00
G/C	19 (11.0)	18 (10.0)	0.77	1.11	13 (14.3)	0.78	0.81
C/C	1 (0.6)	1 (0.6)		1.05	0		NA
C/C versus *G/C+G/G*	171 (99.4)	178 (99.4)	0.97	1.04 (0.0–38.36)	97 (100)	0.45	—
G/G versus *C/C+G/C*	20 (11.6)	19 (10.6)	0.76	0.90 (0.44–1.84)	13 (13.3)	0.67	1.18 (0.52–2.63)
Alleles							
G	323 (93.9)	338 (94.4)	0.76	0.91	183 (93.4)	0.80	1.09
C	21 (6.1)	20 (5.6)		(0.46–1.66)	13 (6.6)		(0.50–2.35)

P value considered $P \leq 0.05$; OR, odds ratio
P^1 value from TB patients and control group. P^2 value from TB patients and control TST-positive subgroup. TST+: tuberculin skin test positive.

and control group. No statistical association of serum nitrite (23.77 ± 15.59 versus 23.49 ± 17.39 and 23.13 ± 17.78 versus 27.40 ± 17.08, in TB patients and control group, resp.) or NO_x^- levels (32.40 ± 16.6 versus 34.22 ± 19.59 and 34.34 ± 20.99 versus 38.12 ± 18.67, in TB patients and control group, resp.) was observed with *GG* and *GC* genotypes of *NOS2A-954G/C* SNP (Figure 1) even in the TST-positive control subgroup (data not shown) was found. These results suggested that the low *NOS2A-954G/G* SNP or moderate *NOS2A-954G/C* SNP nitric oxide producers were not associated with the modulation of nitrite and NO_x^- radical production in either TB patients or control group. The frequency of higher nitric oxide radical producers *NOS2A-954C/C* SNP was rare in our population.

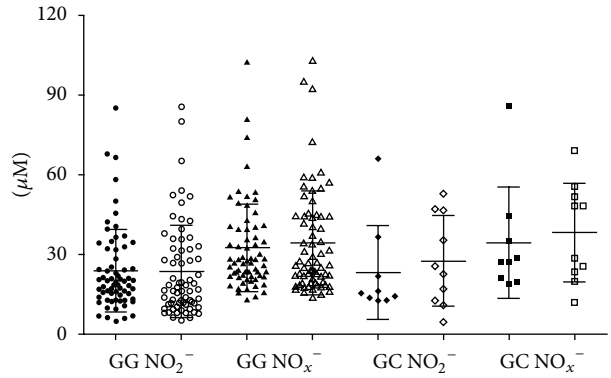


Figure 1: Comparative analysis of serum nitrite (NO_2^-) and NO_x^- (NO_2^- + NO_3^-) concentrations and *NOS2A-954G/C* genotype association in tuberculosis patients (black symbols) and control group (open symbols) ($P > 0.05$).

3.5. Combined Genotype of IFNG+874T/A and NOS2A-954C/G SNPs Is Not Associated with Either Tuberculosis or Nitrogen Radicals. To determine whether the combination of SNPs in these two genes was associated with susceptibility to TB, polymorphisms association was evaluated between pulmonary TB and control groups. The rare *NOS2A-954C/C* SNP was not taken into account in assessing the combined genotypes with the *IFNG+874T/A* SNP. No statistical significance was seen between TB and control groups *in IFNG+874T/A* and *NOS2A-954G/C* SNPs genotypic distributions (Table 3) when they were compared. In both groups, the most prevalent combined genotypes were *AT/GG* (40.7% and 44.7%) and *AA/GG* (36.0% and 31.3%) in TB and control groups, respectively, with no statistical difference. The secretion of reactive nitrogen radicals was not related with the combined genotypes when TB and control groups were compared. The most frequent combined genotype *AT/GG* in TB (23.63 ± 1.60 and 32.35 ± 1.63) and control groups (24.46 ± 1.90 and 34.83 ± 2.21) did not induce different serum levels of nitrite and NO_x^-. These results demonstrated in our population that the combined genotypes profiles of *IFNG+874T/A* SNP (low *IFNG+874AA*, moderate *IFNG+874AT,* or high *IFNG+874TT* producers) and *NOS2A-954G/C* SNP (low *NOS2A-954GG* or moderate *NOS2A-954GC* producers) are not associated with the modulation of the production of

Table 3: Combined genotype analysis of *IFNG+874T/A* and *NOS2A-954G/C* SNPs in tuberculosis patients and control groups.

Combined genotypes	Tuberculosis patients $n = 172$ (%)	Control groups					
		$n = 179$ (%)	P^1	OR	TST+ $n = 98$ (%)	P^2	OR
AA/GG	62 (36.0)	56 (31.3)		1.00	33 (33.7)		1.00
AA/GC	9 (5.2)	5 (2.8)		1.63	4 (4.1)		1.20
AA/CC	1 (0.6)	1 (0.6)		0.90	1 (1.0)		0.53
AT/GG	70 (40.7)	80 (44.7)	0.40	0.79	39 (39.8)	0.79	0.96
AT/GC	8 (4.7)	11 (6.1)		0.66	9 (9.2)		0.47
TT/GG	20 (11.6)	24 (13.4)		0.75	12 (12.2)		0.89
TT/GC	2 (1.2)	2 (1.1)		0.90	0 (0)		ND

The *AA/GG* combined genotype was used as reference in a 7×2 χ^2 for trend table. The combined genotypes *TT/GG* and *AT/GG* were not present.
P-value considered $P \leq 0.05$; P^1-value comparing TB patients and control group.
P^2-value comparing TB patients and TST+ control subgroup.
TST+: tuberculin skin test positive; OR: odds ratio; ND: not determined .

nitrite and NO_x^- radicals in both TB patients and control group or in TST-positive control subgroup.

4. Discussion

IFN-γ mediated immune activation has an important role in immunity to intracellular pathogens. IFN-γ is critical to macrophage activation, and measurable levels are lower in patients with active TB than in control group [28, 29], but they are not correlated with protection [30]. It has been more formally suggested that IFN-γ activity is a continuous, genetically controlled trait with genetic variability in both production and responsiveness to IFN-γ [31], although there is little evidence to support a role for variability in IFN-γ production. For the *IFNG* gene, there are two intronic SNPs that contribute to its expression phenotypes: *+874T/A* SNP and *+2109G/A* SNP [32]. The *AA* genotype of *IFNG+874T/A* SNP is thought to confer a low-secretor phenotype of IFN-γ. Conversely, the *TT* genotype of *IFNG+874T/A* SNP is thought to confer a high-secretor phenotype [33, 34]. Some controversy exists concerning association of *IFNG* gene polymorphisms and susceptibility to pulmonary TB [14, 16, 35–37]. Our results showed no evidence of an association between *IFNG* gene polymorphism and susceptibility to TB. The *AA* genotype frequency of *IFNG+874* in the control group (35.2%) was a little higher than that in the Sicilian (26%) [32], Spanish (28%) [30], and Indian populations (11%) [38] but was lower than that in South African (47%) [35], Hong Kong Chinese (46%) [36], and South Korean populations (74%) [16]. Reports describing the *T* allele frequency (*IFNG+874T/A* SNP) revealed that there are ethnic differences at this allele distribution. It has been reported that the *T* allele frequency is significantly lower in a Japanese population (9%) [11] than in a South African population (32%) [10] and in an Italian Caucasian population (47%) [39]. Rossouw et al. [35] reported a significantly lower *T* allele frequency *of INFG+874T/A* in patients with *M. tuberculosis* infection than that in control groups in a South African population with a high annual incidence of TB.

Lio et al. [40] and Vallinoto et al. [41] found that the *TT* genotype was relatively rare in TB patients from Sicilian and Brazilian populations. However, a study conducted in a Central West Brazilian population by Amim et al. [12] showed different results from our study; in that population, the *AA* genotype of *IFNG+874T/A* SNP was associated with TB susceptibility. Amim et al. [12] compared two different populations in their study: one from Rio de Janeiro (patients) and the other from Central West of Region Brazil (control group), which can be characterized as bias because Brazil was colonized by several ethnic groups that migrated from different countries into different regions of Brazil. During the last 500 years these populations became mixed with native Indians, and each Brazilian region has its own admixes ethnic group descendants. Our patients and control group were enrolled from Rio de Janeiro City, which is composed of migratory populations from all over the country. Thus, the population in this city has a genetic background that represents a mix of all regions of the country.

There is convincing evidence that NO and related reactive nitrogen intermediate (RNI) can kill and/or inhibit intracellular pathogens such as mycobacteria. IFN-γ knockout mice which are not capable of producing NO and RNI in response to the bacilli develop tuberculosis quickly, suggesting a role for NO and RNI in the defense mechanism against *M. tuberculosis* [42]. In contrast to murine models of TB, there is greater controversy about the role of NO in killing or limiting the growth of *M. tuberculosis* in humans. Nevertheless, alveolar macrophages from TB patients express higher amounts of NOS2 compared to control group [22], demonstrating a possible role in affecting bacilli growth. Several SNPs have been described in this gene [24] given the importance of this gene in the immune response to TB. Our study verified the influence of *NOS2A-954G/C* SNP on the risk of developing TB in a Brazilian population from a highly endemic area. We did not observe a statistical difference between the *NOS2A-954G/C* SNP genotype in our TB patients and control group. The frequency of *NOS2A-954G/C* SNP may differ among populations. The *C* allele of *NOS2A-954G/C* SNP has been shown to be absent from Caucasian populations [43] and in Peruvian population [44] and in low frequency in Asia [23]. However, the *C* allele has high frequency in African populations [23]. A single description of *NOS2A-954G/C*

SNP genotype study in Brazilian population, studying gastric cancer, showed different frequencies for *GG*, *GC,* and *CC* genotypes in *NOS2A-954G/C* SNP (64.77%, 28.69%, and 6.54%, resp.) [45] compared with our study. In Mexicans, this SNP was not associated with TB and *C* allele frequency was 5% [25].

The involvement of *NOS2A* SNPs has been studied in different pathologies and still has controversy results. In order to assess the nitric oxide radical levels and to compare them with *NOS2A-954G/C* SNP and combined genotypes of *IFNG+874T/A* SNP/*NOS2A-954G/C* SNP in TB patients and control groups, the secretions of nitrite and NO_x^- radicals were analyzed. No association was identified between TB and control groups or between different genotype profiles or combined genotypes regarding nitrite and NO_x^- production. Our results indicate that in our admixed population the association between disease development and *IFNG+874T/A* and *NOS2A-954G/C* SNPs does not exert selective pressure on *M. tuberculosis* via immune response surveillance, as shown by the absence of correlation of active TB with these SNPs profiles but can lead to different approaches to evaluate the tuberculosis immunopathology genetics background in the near future. Tuberculosis is a multifactorial disease and the pulmonary milieu involving the immune response and bacilli interaction in different genetic background and environment factors should be addressed to answer the question whether *M. tuberculosis* growth is controlled by other interaction candidate genes and/or several risk factors.

Acknowledgments

This work was supported by funds provided by the PAPES V-Fiocruz/CNPq and IPEC/Fiocruz. A. C. C. S. Leandro and M. A. Rocha received fellowships from CAPES/Brazil.

References

[1] WHO, "Global tuberculosis report 2012," World Health Organization, Geneva, Switzerland, 2010 (WHO/HTM/TB/2012.6), http://www.who.int/tb/publications/global_report/en/index.html.

[2] *Sistema Nacional de Vigilância em Saúde*, Relatório de Situação, Rio de Janeiro, Brazil, 2nd edition, 2011, http://bvsms.saude.gov.br/bvs/publicacoes/sistema_nacional_vigilancia_saude_rj_5ed.pdf.

[3] M. G. Bonecini-Almeida, S. Chitale, I. Boutsikakis et al., "Induction of in vitro human macrophage anti-*Mycobacterium tuberculosis* activity: requirement for IFN-γ and primed lymphocytes," *Journal of Immunology*, vol. 160, no. 9, pp. 4490–4499, 1998.

[4] S. C. Cowley and K. L. Elkins, "$CD4^+$ T cells mediate IFN-γ-independent control of *Mycobacterium tuberculosis* infection both in vitro and in vivo," *Journal of Immunology*, vol. 171, no. 9, pp. 4689–4699, 2003.

[5] A. M. Cooper, D. K. Dalton, T. A. Stewart, J. P. Griffin, D. G. Russell, and I. M. Orme, "Disseminated tuberculosis in interferon γ gene-disrupted mice," *Journal of Experimental Medicine*, vol. 178, no. 6, pp. 2243–2247, 1993.

[6] A. M. Green, R. Difazio, and J. L. Flynn, "IFN-γ from CD4 T cells is essential for host survival and enhances CD8 T cell function during *Mycobacterium tuberculosis* infection," *Journal of Immunology*, vol. 190, no. 1, pp. 270–277, 2013.

[7] O. J. Kwon, "The role of nitric oxide in the immune response of tuberculosis," *Journal of Korean Medical Science*, vol. 12, no. 6, pp. 481–487, 1997.

[8] B. M. Saunders, S. L. Fernando, R. Sluyter, W. J. Britton, and J. S. Wiley, "A loss-of-function polymorphism in the human P2X7 receptor abolishes ATP-mediated killing of mycobacteria," *Journal of Immunology*, vol. 171, no. 10, pp. 5442–5446, 2003.

[9] V. Pravica, C. Perrey, A. Stevens, J. H. Lee, and I. V. Hutchinson, "A single nucleotide polymorphism in the first intron of the human IFN-γ gene: absolute correlation with a polymorphic CA microsatellite marker of high IFN-γ production," *Human Immunology*, vol. 61, no. 9, pp. 863–866, 2000.

[10] G. S. Cooke, S. J. Campbell, J. Sillah et al., "Polymorphism within the interferon-γ/receptor complex is associated with pulmonary tuberculosis," *The American Journal of Respiratory and Critical Care Medicine*, vol. 174, no. 3, pp. 339–343, 2006.

[11] N. Sallakci, M. Coskun, Z. Berber et al., "Interferon-γ gene+874T-A polymorphism is associated with tuberculosis and gamma interferon response," *Tuberculosis*, vol. 87, no. 3, pp. 225–230, 2007.

[12] L. H. L. V. Amim, A. G. Pacheco, J. Fonseca-Costa et al., "Role of IFN-γ +874 T/A single nucleotide polymorphism in the tuberculosis outcome among Brazilians subjects," *Molecular Biology Reports*, vol. 35, no. 4, pp. 563–566, 2008.

[13] W. B. Selma, H. Harizi, I. Bougmiza et al., "Interferon gamma +874T/a polymorphism is associated with susceptibility to active pulmonary tuberculosis development in Tunisian patients," *DNA and Cell Biology*, vol. 30, no. 6, pp. 379–387, 2011.

[14] S. M. Mirsaeidi, M. Houshmand, P. Tabarsi et al., "Lack of association between interferon-gamma receptor-1 polymorphism and pulmonary TB in Iranian population sample," *Journal of Infection*, vol. 52, no. 5, pp. 374–377, 2006.

[15] A. Moran, X. Ma, R. A. Reich, and E. A. Graviss, "No association between the +874T/A single nucleotide polymorphism in the IFN-γ gene and susceptibility to TB," *International Journal of Tuberculosis and Lung Disease*, vol. 11, no. 1, pp. 113–115, 2007.

[16] J. H. Hwang, E. J. Kim, W. J. Koh et al., "Polymorphisms of interferon-γ and interferon-γ receptor 1 genes and non-tuberculous mycobacterial lung diseases," *Tuberculosis*, vol. 87, no. 2, pp. 166–171, 2007.

[17] F. Wu, Y. Qu, Y. Tang, D. Cao, P. Sun, and Z. Xia, "Lack of association between cytokine gene polymorphisms and silicosis and pulmonary tuberculosis in Chinese iron miners," *Journal of Occupational Health*, vol. 50, no. 6, pp. 445–454, 2008.

[18] L. M. Gómez, J. Anaya, J. R. Vilchez et al., "A polymorphism in the inducible nitric oxide synthase gene is associated with tuberculosis," *Tuberculosis*, vol. 87, no. 4, pp. 288–294, 2007.

[19] S. S. Greenberg, J. Xie, J. Kolls, C. Mason, and P. Didier, "Rapid induction of mRNA for nitric oxide synthase II in rat alveolar macrophages by intratracheal administration of *Mycobacterium tuberculosis* and *Mycobacterium avium*," *Proceedings of the Society for Experimental Biology and Medicine*, vol. 209, no. 1, pp. 46–53, 1995.

[20] K. A. Rockett, R. Brookes, I. Udalova, V. Vidal, A. V. S. Hill, and D. Kwiatkowski, "1,25-Dihydroxyvitamin D3 induces nitric oxide synthase and suppresses growth of *Mycobacterium tuberculosis* in a human macrophage-like cell line," *Infection and Immunity*, vol. 66, no. 11, pp. 5314–5321, 1998.

[21] S. Nicholson, M. D. G. Bonecini-Almeida, J. R. L. e Silva et al., "Inducible nitric oxide synthase in pulmonary alveolar

macrophages from patients with tuberculosis," *Journal of Experimental Medicine*, vol. 183, no. 5, pp. 2293–2302, 1996.

[22] M. C. Levesque, M. R. Hobbs, N. M. Anstey et al., "Nitric oxide synthase type 2 promoter polymorphisms, nitric oxide production, and disease severity in Tanzanian children with malaria," *Journal of Infectious Diseases*, vol. 180, no. 6, pp. 1994–2002, 1999.

[23] J. F. Kun, B. Mordmüller, D. J. Perkins et al., "Nitric oxide synthase 2Lambaréné (G-954C), increased nitric oxide production, and protection against malaria," *Journal of Infectious Diseases*, vol. 184, no. 3, pp. 330–336, 2001.

[24] S. E. Jamieson, E. N. Miller, G. F. Black et al., "Evidence for a cluster of genes on chromosome 17q11-q21 controlling susceptibility to tubercolosis and leprosy in Brazilians," *Genes and Immunity*, vol. 5, no. 1, pp. 46–57, 2004.

[25] P. O. Flores-Villanueva, J. A. Ruiz-Morales, C. H. Song et al., "A functional promoter polymorphism in monocyte chemoattractant protein-1 is associated with increased susceptibility to pulmonary tuberculosis," *Journal of Experimental Medicine*, vol. 202, no. 12, pp. 1649–1658, 2005.

[26] *Manual de recomendações para o controle da tuberculose no Brasil*, Ministério da Saúde, Secretaria de Vigilância Sanitária, Programa Nacional de Controle da Tuberculose, 2010, http://portal.saude.gov.br/portal/arquivos/pdf/manual_tuberculose.pdf.

[27] K. M. Miranda, M. G. Espey, and D. A. Wink, "A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite," *Nitric Oxide—Biology and Chemistry*, vol. 5, no. 1, pp. 62–71, 2001.

[28] F. O. Sanchez, J. I. Rodriguez, G. Agudelo, and L. F. Garcia, "Immune responsiveness and lymphokine production in patients with tuberculosis and healthy controls," *Infection and Immunity*, vol. 62, no. 12, pp. 5673–5678, 1994.

[29] M. Zhang, Y. Lin, D. V. Iyer, J. Gong, J. S. Abrams, and P. F. Barnes, "T-cell cytokine responses in human infection with *Mycobacterium tuberculosis*," *Infection and Immunity*, vol. 63, no. 8, pp. 3231–3234, 1995.

[30] D. López-Maderuelo, F. Arnalich, R. Serantes et al., "Interferon-γ and interleukin-10 gene polymorphisms in pulmonary tuberculosis," *The American Journal of Respiratory and Critical Care Medicine*, vol. 167, no. 7, pp. 970–975, 2003.

[31] A. O'Garra, P. S. Redford, F. W. McNab, C. I. Bloom, R. J. Wilkinson, and M. P. Berry, "The immune response in tuberculosis," *Annual Review of Immunology*, vol. 31, pp. 475–527, 2013.

[32] S. Dupuis, R. Döffinger, C. Picard et al., "Human interferon-γ-mediated immunity is a genetically controlled continuous trait that determines the outcome of mycobacterial invasion," *Immunological Reviews*, vol. 178, pp. 129–137, 2000.

[33] V. Pravica, A. Asderakis, C. Perrey, A. Hajeer, P. J. Sinnott, and I. V. Hutchinson, "In vitro production of IFN-γ correlates with CA repeat polymorphism in the human IFN-γ gene," *European Journal of Immunogenetics*, vol. 26, no. 1, pp. 1–3, 1999.

[34] S. Henri, F. Stefani, D. Parzy, C. Eboumbou, A. Dessein, and C. Chevillard, "Description of three new polymorphisms in the intronic and 3′ UTR regions of the human interferon gamma gene," *Genes and Immunity*, vol. 3, no. 1, pp. 1–4, 2002.

[35] M. Rossouw, H. J. Nel, G. S. Cooke, P. D. van Helden, and E. G. Hoal, "Association between tuberculosis and a polymorphic NFκB binding site in the interferon γ gene," *The Lancet*, vol. 361, no. 9372, pp. 1871–1872, 2003.

[36] H. W. Tso, W. K. Ip, W. P. Chong, C. M. Tam, A. K. S. Chiang, and Y. L. Lau, "Association of interferon gamma and interleukin 10 genes with tuberculosis in Hong Kong Chinese," *Genes and Immunity*, vol. 6, no. 4, pp. 358–363, 2005.

[37] M. I. Henao, C. Montes, S. C. París, and L. F. García, "Cytokine gene polymorphisms in Colombian patients with different clinical presentations of tuberculosis," *Tuberculosis*, vol. 86, no. 1, pp. 11–19, 2006.

[38] A. Abhimanyu, I. R. Mangangcha, P. Jha et al., "Differential serum cytokine levels are associated with cytokine gene polymorphisms in north Indians with active pulmonary tuberculosis," *Infection, Genetics and Evolution*, vol. 11, no. 5, pp. 1015–1022, 2011.

[39] F. Poli, A. Nocco, S. Berra et al., "Allele frequencies of polymorphisms of TNFA, IL-6, IL-10 and IFNG in an Italian Caucasian population," *European Journal of Immunogenetics*, vol. 29, no. 3, pp. 237–240, 2002.

[40] D. Lio, V. Marino, A. Serauto et al., "Genotype frequencies of the +874T → A single nucleotide polymorphism in the first intron of the interferon-γ gene in a sample of Sicilian patients affected by tuberculosis," *European Journal of Immunogenetics*, vol. 29, no. 5, pp. 371–374, 2002.

[41] A. C. R. Vallinoto, E. S. Graça, M. S. Araújo et al., "IFNG +874T/A polymorphism and cytokine plasma levels are associated with susceptibility to *Mycobacterium tuberculosis* infection and clinical manifestation of tuberculosis," *Human Immunology*, vol. 71, no. 7, pp. 692–696, 2010.

[42] D. K. Dalton, S. Pitts-Meek, S. Keshav, I. S. Figari, A. Bradley, and T. A. Stewart, "Multiple defects of immune cell function in mice with disrupted interferon-γ genes," *Science*, vol. 259, no. 5102, pp. 1739–1742, 1993.

[43] J. F. J. Kun, B. Mordmüller, B. Lell, L. G. Lehman, D. Luckner, and P. G. Kremsner, "Polymorphism in promoter region of inducible nitric oxide synthase gene and protection against malaria," *The Lancet*, vol. 351, no. 9098, pp. 265–266, 1998.

[44] J. Martín, J. E. Calzada, and A. Nieto, "Inducible nitric oxide synthase (NOS2) gene polymorphism and parasitic diseases," *The Lancet*, vol. 353, no. 9146, p. 72, 1999.

[45] Y. C. Jorge, M. C. Duarte, and A. E. Silva, "Gastric cancer is associated with NOS2-954G/C polymorphism and environmental factors in a Brazilian population," *BMC Gastroenterology*, vol. 10, article 64, 2010.

Hindawi Publishing Corporation
BioMed Research International
Volume 2013, Article ID 989837, 17 pages
http://dx.doi.org/10.1155/2013/989837

Review Article

Role of *HLA*, *KIR*, *MICA*, and Cytokines Genes in Leprosy

Luciana Ribeiro Jarduli,[1] Ana Maria Sell,[2] Pâmela Guimarães Reis,[1] Emília Ângela Sippert,[1] Christiane Maria Ayo,[1] Priscila Saamara Mazini,[1] Hugo Vicentin Alves,[1] Jorge Juarez Vieira Teixeira,[1] and Jeane Eliete Laguila Visentainer[2]

[1] *Program of Biosciences Applied to Pharmacy, Department of Clinical Analysis and Biomedicine, Maringa State University, Avenida Colombo 5790, 87020-900 Maringá, PR, Brazil*
[2] *Basic Health Sciences Department, Maringa State University, Avenida Colombo 5790, 87020-900 Maringá, PR, Brazil*

Correspondence should be addressed to Jeane Eliete Laguila Visentainer; jelvisentainer@gmail.com

Received 20 March 2013; Revised 16 May 2013; Accepted 5 June 2013

Academic Editor: Enrique Medina-Acosta

Many genes including *HLA*, *KIR*, and *MICA* genes, as well as polymorphisms in cytokines have been investigated for their role in infectious disease. *HLA* alleles may influence not only susceptibility or resistance to leprosy, but also the course of the disease. Some combinations of *HLA* and *KIR* may result in negative as well as positive interactions between NK cells and infected host cells with *M. leprae*, resulting in activation or inhibition of NK cells and, consequently, in death of bacillus. In addition, studies have demonstrated the influence of *MICA* genes in the pathogenesis of leprosy. Specifically, they may play a role in the interaction between NK cells and infected cells. Finally, pro- and anti-inflammatory cytokines have been influencing the clinical course of leprosy. Data from a wide variety of sources support the existence of genetic factors influencing the leprosy pathogenesis. These sources include twin studies, segregation analyses, family-based linkage and association studies, candidate gene association studies, and, most recently, genome-wide association studies (GWAS). The purpose of this brief review was to highlight the importance of some immune response genes and their correlation with the clinical forms of leprosy, as well as their implications for disease resistance and susceptibility.

1. Overview of Leprosy

Leprosy is a chronic infectious disease of slow evolution caused by *Mycobacterium leprae*, which primarily affects the skin and peripheral nerves and may manifest in different clinical forms. There is strong evidence for a genetic basis for host disease per se susceptibility to and its subtypes [1].

Currently, Brazil is the second in the world in absolute number of cases of leprosy [2]. Patients with leprosy can show a broad spectrum of clinical symptoms. The tuberculoid form (TT) of leprosy consists of well-defined lesions, few bacilli, and vigorous cell-mediated immunity (CMI). On the other hand, lepromatous leprosy (LL) presents as many skin lesions with uncontrolled proliferation of leprosy bacilli and inefficient CMI. Borderline leprosy manifests clinically and immunologically with characteristics between the poles of the spectrum of leprosy and may be classified as 3 subtypes: borderline lepromatous (BL), borderline borderline (BB), and borderline tuberculoid (BT) [3].

Most individuals develop sufficient immunity against *M. leprae* with no signs of clinical disease. However, in a small proportion of exposed individuals, leprosy can manifest in an array of clinical forms, ranging from the localized tuberculoid to the systemic lepromatous disease. Typically, Th1- and Th2-type immune responses are initiated against the pathogen [4]. Evidence suggests that the incidence of infection in the population is probably much higher than the incidence of clinical leprosy, because a small proportion (about 5%) of infected individuals develop clinical symptoms and the rest can develop subclinical infections or heal spontaneously. This may be due in part to environmental factors such as nutrition, genetic differences, or bacterial [5].

The clinical and pathological spectrum of leprosy can be explained by genetic differences in host resistance. While some loci affect intrinsic susceptibility to LD, others modify the clinical form of the disease [6]. This review may help to clarify the mechanisms immunopathogenics of *M. leprae*. Studies of immune response genes in patients with leprosy can be used as a research tool in assisting genetic characterization of leprosy patients, thus allowing the determination of a possible association between these gene combinations and the development of leprosy and its clinical forms.

Leprosy has long been considered a complex disease. In the past few years, several studies have attempted to characterize genes associated with leprosy, as well as their contribution to the development of the various clinical forms. Immune response genes have been associated with pathogenesis of different forms of leprosy. This review discusses the role of the human leukocyte antigen (*HLA*), Killer cell immunoglobulin-like receptors (*KIRs*), and MHC class I chain-related (*MIC*) genes, as well as polymorphisms of cytokines, in leprosy and their implications for resistance and susceptibility to the disease.

2. Strategy for Screening and Selecting Studies

This review about host genetic polymorphism studies, as well as the current status of genome-wide association studies and their influence on leprosy selected original articles carried out on humans that were found in the databases of PubMed (U.S. National Library of Medicine), LILACs (Latin American and Caribbean Center on Information in Health Sciences), and Google Scholar. The research period covered included the limit of databases until March 2013. There was no restriction regarding language. In the PubMed database MeSH (Medical Subject Heading) terms were used and in the LILACs descriptors were used. In order to retrieve articles of interest, free terms were used in the LILACS and Google Scholar. The MeSH terms, descriptors, and free terms were organized according to thematic groups: (i) HLA and Leprosy ("Leprosy" OR "Leprosy, Multibacillary" OR "Leprosy, Paucibacillary" OR "Leprosy, Tuberculoid" OR "Leprosy, Lepromatous" OR "Leprosy, Borderline" AND "HLA antigens/genetics"); (ii) KIR genes and Leprosy ("Leprosy" AND "Receptors, KIR"); (iii) MIC genes and Leprosy ("Leprosy" AND "MHC class I-related chain A"); (iv) Cytokine genes and Leprosy ("Leprosy" OR "Leprosy, Multibacillary" OR "Leprosy, Paucibacillary" OR "Leprosy, Tuberculoid" OR "Leprosy, Lepromatous" OR "Leprosy, Borderline" AND "Cytokines/genetics" OR "Receptors, Cytokine/genetics" OR "Chemokines/genetics"); (v) Genome-wide association study and Leprosy ("Leprosy" AND "Genome-Wide Association Studies"). The immune response genes, as *HLA*, *KIR*, *MIC,* and cytokines, and their association with leprosy were presented.

Screening the PubMed, LILACs, and Google Scholar databases identified 326 potentially relevant citations. Of these, 260 citations were excluded after evaluating the title and the abstract, because they did not comply with the inclusion criterion, no human, aim, originality, duplicate articles and that could not be downloaded or accessed in full length from journal archives. 64 articles related to immune response genes in association with leprosy and 19 articles more which were added from reference list, adding 83 original articles on human infections included in this review were selected.

The main characteristics of the studies selected, the populations under study, the target genes, the number of individuals, and the main finding for each are shown in Tables 1, 2, and 3.

3. HLA and Leprosy

During infection caused by *M. leprae*, *HLA* alleles influence not only susceptibility and resistance to leprosy, but also the course of the disease. The main role of HLA molecules is to present peptides derived from *M. leprae* to T cells of the host [15]. An individual that has a particular combination of *HLA* alleles that are not linked to the peptide in an appropriate way, or for whom the HLA-peptide linkage does not elicit a proper lymphocyte response, will be more susceptible to infection than an individual that linked to the peptide in an appropriate way [18]. In patients whose HLA systems offer protection against the disease, these genes likely select and stimulate T cells to multiply and eliminate the agent via inflammatory cytokine production which destroy infected cells [15, 71]. Several studies have consistently reported the involvement of *HLA* alleles and haplotypes, mainly of class II genes, as important genetic factors controlling susceptibility to different forms of leprosy [71]. According to Ridley and Jopling (1966), the clinical manifestation of leprosy depends on the type of immune response that is initiated by the host and the balance between T-helper (Th)1 and Th2 responses may be partially controlled by the mechanism of antigen presentation involving HLA molecules [35, 71]. The tuberculoid (TT) form of leprosy is associated with a Th1 (cellular) immune response, characterised by the production of proinflammatory cytokines that can participate in the clearance of the bacillus. However, the lepromatous (LL) form of leprosy is associated with a Th2 (humoral) immune response, which is characterised by an immunosuppressive cytokine environment, making this type of response problematic for the host [3, 72].

4. Classical *HLA* Class I Genes

Several studies comparing *HLA* class I gene frequencies in leprosy cases and controls have found associations either with the polar forms of leprosy or with LD. Nevertheless, results have been inconsistent.

Earlier, association studies showed HLA-Aw21 as a factor of susceptibility to TT in Ethiopian patients [7], while HLA-A9 in India, HLA-A2 in Thailand and Korea as a factor of resistance to leprosy [8, 9, 13]. In leprosy patients from Iran, HLA-B35 antigen was increased, while HLA-A1 was decreased in LL patients [10]. The HLA-B40 antigen and HLA-A2-B40, HLA-A11-B40, and HLA-A24-B40 haplotypes

Table 1: Associations between HLA class I and leprosy.

Population	Study design	Sample size	Phenotype	Serotype, allele, or haplotype	Type of association	P or Pc	Ref.
Ethiopian	Case-control	20TT, 19LL, 36 controls	TT	Aw21	Susceptibility	$Pc = 0.042$	[7]
Indian	Case-control	30BT or TT, 40 controls	TT	A9	Resistance	$Pc = 0.005$	[8]
Thai	Case-control	26TT, 183 controls	TT	A2	Resistance	$0.01 < P < 0.05$	[9]
				Bw17	Susceptibility	$0.01 < P < 0.05$	
	Case-control	70LL, 183 controls	LL	B7	Susceptibility	$0.01 < P < 0.05$	
Iran	Case-control	88LD, 125 controls	LL	A1	Resistance	$P < 0.05$	[10]
				B35	Susceptibility		
Mumbai/Indian	Case-control	158LL, 150TT, 170 controls	LL	B-40	Susceptibility	$Pc = 0.0027$	[11]
			LL	Aw19	Resistance	$Pc = 0.02$	
			LL	A2-B40	Susceptibility	$P < 0.00025$	
			TT/LL	A11-B40	Susceptibility	$P < 0.00025$	
			TT/LL	A24-B40	Susceptibility	$P < 0.00025$	
Japanese	Case-control	295LL, 74TL, 110 controls	LL	B7	Susceptibility	$Pc = 0.044$	[12]
				Bw54	Resistance	$Pc = 0.016$	
Korean	Case-control	157LD, 162 controls	LD	A2	Resistance	$P = 0.03$	[13]
				A11	Susceptibility	$P = 0.03$	
				Aw33	Susceptibility	$P = 0.003$	
				Cw5	Resistance	$P = 0.001$	
Indian	Case-control	65ENL, 71LL	ENL	A11	Susceptibility	$Pc = 0.0035$	[14]
Indian	Case-control	68LL, 237 controls	LL	B60	Susceptibility	$Pc = 0.00019$	[15]
	Case-control	138LD, 237 controls	LD	B60	Susceptibility	$Pc = 0.031$	
	Case-control	20BB, 237 controls	BB	B40	Susceptibility	$Pc = 0.018$	
Southern Chinese	Case-control	50LL, 69 controls	LL	B46	Resistance	$P < 0.01$	[16]
Turkish	Case-control	80LD, 120 controls	LD	A9	Susceptibility	$Pc = 0.0004$	[17]
				A10	Susceptibility	$Pc = 0.0226$	
				Bw4	Susceptibility	$Pc = 0.00003$	
				Bw6	Susceptibility	$Pc = 0.00001$	
				Cw1	Susceptibility	$Pc = 0.0080$	
				Cw2	Susceptibility	$Pc = 0.0055$	
				A3	Resistance	$Pc = 0.0040$	
				B49	Resistance	$Pc = 0.0035$	
Southern Indian	Case-control	32LD, 67 controls	LD	$A^*02{:}06$	Susceptibility	$Pc = 0.000007$	[18]
				$A^*11{:}02$	Susceptibility	$Pc = 0.00001$	
				$B^*51{:}10$	Susceptibility	$Pc = 0.0000005$	
				$B^*18{:}01$	Susceptibility	$Pc = 0.007$	
				$C^*04{:}07$	Susceptibility	$Pc = 1.0 \times 10^{-9}$	
				$C^*07{:}03$	Susceptibility	$Pc = 0.000001$	
				$C^*04{:}11$	Resistance	$Pc = 0.001$	

Table 1: Continued.

Population	Study design	Sample size	Phenotype	Serotype, allele, or haplotype	Type of association	P or Pc	Ref.
Mumbai/Indian	Case-control	103LD, 101 controls	LD	A02	Susceptibility	$Pc = 0.0015$	[19]
				A11	Susceptibility	$Pc = 0.009$	
				A28	Resistance	$Pc = 0.0014$	
				B12	Resistance	$Pc = 0.001$	
				B15	Resistance	$Pc = 0.05$	
				B40	Susceptibility	$Pc = 7.34 \times 10^{-7}$	
				Cw7	Susceptibility	$Pc = 2.26 \times 10^{-5}$	
				Cw3	Resistance	$Pc = 0.0002$	
	Case-control	32ML, 67 controls	ML	*A*02:06*	Susceptibility	$Pc = 7.15 \times 10^{-5}$	
				*A*11:02*	Susceptibility	$Pc = 0.00001$	
				*B*18:01*	Susceptibility	$Pc = 0.007$	
				*B*51:10*	Susceptibility	$Pc = 5.29 \times 10^{-6}$	
				*C*04:07*	Susceptibility	$Pc = 5.12 \times 10^{-9}$	
				*C*04:11*	Resistance	$Pc = 0.001$	
				*C*07:03*	Susceptibility	$Pc = 1.97 \times 10^{-5}$	
	Case-control	32ML, 67 controls	ML	*A*11-B*40*	Susceptibility	$Pc = 0.002$	
Brazilian	Case-control	224LD, 446 controls	LD	*A*11*	Susceptibility	$P = 0.0345$	[20]
				*B*38*	Susceptibility	$P = 0.0402$	
				*C*12*	Susceptibility	$P = 0.01$	
				*C*16*	Resistance	$P = 0.0124$	
				*C*07*	Susceptibility	$P = 0.0211$	
	Case-control	88LL, 48TT	LL	*B*35*	Resistance	$P = 0.0156$	
				*C*04*	Resistance	$P = 0.0464$	
Vietnamese	family study	198 families	LD	*C*15:05*	Susceptibility	$P = 0.0063$	[21]
	family study	292 families	LD	*C*15:05*	Susceptibility	$P = 8.8 \times 10^{-5}$	
Indian	Case-control	364LD, 371 controls	LD	*C*15:05*	Susceptibility	$P = 3.0 \times 10^{-8}$	

MB: multibacillary leprosy; LL: lepromatous leprosy; BB: borderline borderline; TT: tuberculoid leprosy; ENL: erythema nodosum leprosum; LD: leprosy disease; ns: not significant; Pc: corrected P Value; Ref.: reference.

Table 2: Associations between *HLA* class II and leprosy.

Population	Study design	Sample size	Phenotype	Serotype, allele, or haplotype	Type of association	*P* or *Pc*	Ref.
Japanese	Case-control	295LL, 74TL, 110 controls	LL/TT	DR2	Susceptibility	$Pc < 0.008$	[12]
			LL	DRw9	Resistance	$Pc < 0.0001$	
Thai	Case-control	32TT, 32 controls	TT	DR2	Susceptibility	$Pc = 0.02$	[22]
				DQw1	Susceptibility	$Pc = 0.008$	
Korean		157LD, 162 controls	LD	DR1	Susceptibility	$P = 0.02$	[13]
				DR2	Susceptibility	$P < 0.0001$	
				DR9	Susceptibility	$P = 0.02$	
				DR4	Resistance	$P < 0.0001$	
				DRw53	Resistance	$P < 0.0001$	
				DQw1	Susceptibility	$P < 0.0001$	
				DQw3	Resistance	$P < 0.0001$	
Turkish	Case-control	23LL, 27BL, 50 controls	LL/BL	DR2	Susceptibility	$P = 0.015$	[23]
Asian Indian	Case-control	23TT, 16PTB, 19 controls	TT	*DRB1*15:02*	Susceptibility	$P < 0.05$	[24]
Indian	Case-control	138LD, 237 controls	LD	DR2	Susceptibility	$Pc = 0.00031$	[15]
				DQw1	Susceptibility	$Pc = 0.0004$	
				DQw7	Susceptibility	$Pc = 0.00031$	
	Case-control	68LL, 237 controls	LL	DR2	Susceptibility	$Pc = 0.0063$	
				DQw1	Susceptibility	$Pc = 0.02$	
	Case-control	30BL, 237 controls	BL	DR9	Susceptibility	$Pc = 0.04$	
				DQw7	Susceptibility	$Pc = 0.0006$	
Indian	Case-control	28TT, 65LL, 47 controls	LL	*DRB1*15*	Susceptibility	$P < 0.0001$	[25]
				*DRB1*15:01*	Susceptibility	$P < 0.001$	
				*DRB1*07:01*	Susceptibility	$P < 0.01$	
				*DRB5*01:01*	Susceptibility	$P < 0.001$	
				*DQB1*06:01*	Susceptibility	$P < 0.0001$	
				*DQA1*01:02*	Susceptibility	$P < 0.01$	
				*DQA1*01:03*	Susceptibility	$P < 0.01$	
				*DQA1*02:01*	Susceptibility	$P < 0.01$	
	Case-control	28TT, 65LL, 47 controls	TT	*DRB1*15*	Susceptibility	$P < 0.01$	
				*DRB1*15:02*	Susceptibility	$P < 0.05$	
				*DQB1*0601*	Susceptibility	$P < 0.01$	
				*DQB1*05:03*	Resistance	$P < 0.01$	
Indian	Case-control	39TT, 20PTB, 46 controls	TT	*DRB1*15:02* Haplotype: *DRB1*1501-DRB5*0101-DQA1*0102-DQB1*0502*	Susceptibility	$P < 0.05$	[26]
					Resistance	$P < 0.05$	
Indian	Case-control	54TT, 44 controls	TT	*DRB1*15*	Susceptibility	$Pc = 0.0063$	[27]
Japanese	Case-control	38LL/BL, 79LD, 50 controls	BL/LL	*DRB1*02*	Susceptibility	$P = 0.037$	[28]
			LD	*DRB1*12*	Resistance	$P = 0.013$	

Table 2: Continued.

Population	Study design	Sample size	Phenotype	Serotype, allele, or haplotype	Type of association	*P* or *Pc*	Ref.
Brazilian	Case-control	32TT, 147 controls	TT	DR2	Susceptibility	$Pc = 0.0132$	[29]
Japanese	Case-control	93LD, 114 controls	LD	*DRB1* 04:05*	Resistance	$Pc < 0.05$	[30]
				DQA1 03*	Resistance	$Pc < 0.05$	
				DQB1 04:01*	Resistance	$Pc < 0.05$	
Brazilian	TDT	73 families (147 sib-pairs)	LD	*DQB1* 02:01*	Resistance	$Pc = 0.008$	[31]
				DQB1 05:01*	Susceptibility	$Pc = 0.008$	
				DRB1 7*	Resistance	$Pc = 0.036$	
	TDT	73 families (147 sib-pairs)	TT	*DQB1* 02:01*	Resistance	$Pc = 0.024$	
				DQB1 05:01*	Susceptibility	$Pc = 0.024$	
Egyptian	Case-control	24LD, 30 controls	LD	DR2	Susceptibility	$P = 0.032$	[32]
				DQ1	Susceptibility	$P = 0.015$	
Southern Indian	TDT	223 ASP	TT	*DRB1* 15(2)*	Susceptibility	$P = 0.012$	[33]
				DRB1 09*	Resistance	$P = 0.004$	
Argentinean	Case-control	70LL, 112 controls	LL	*DQB1* 02:01* / *DQB1* 02:02* / *DQB1* 02:03*	Resistance	$Pc = 0.02$	[34]
	Case-control	19PB, 112 controls	TT	*DRB1* 04*	Resistance	$Pc = 0.0192$	
North Indian	Case-control	34BT/TT, 79BL/LL, 111 controls	BL/LL	*DRB1* 15:01*	Susceptibility	$P < 0.05$	[35]
Brazilian	Case-control	578LD, 691 controls	LD	*DRB1* 04*	Resistance	$Pc = 0.04076$	[36]
				DRB1 07*	Resistance	$Pc = 0.04753$	
				DRB1 10*	Susceptibility	$Pc = 0.02102$	
				DRB1 12*	Resistance	$Pc = 0.04399$	
				DRB1 15*	Susceptibility	$Pc = 0.02288$	
Euro-Brazilian	Case-control	578LD, 691 controls	LD	*DRB1* 04*/NN[c]	Resistance	$Pc = 0.01$	
				DRB1 07*/NN[c]	Resistance	$Pc = 0.01$	
Afro-Brazilian	Case-control	578LD, 691 controls	LD	*DRB1* 10*/NN[c]	Susceptibility	$Pc = 0.024$	
				DRB1 15*/NN[c]	Susceptibility	$Pc = 0.0002$	
Vietnam	TDT	194 single-case families	LD	*DRB1* 10*	Susceptibility	$Pc = 0.04$	
				DRB1 04*	Resistance	$Pc = 0.03$	
Argentinean	Case-control	71LD, 81 controls	LD	*DRB1* 14:01*	Susceptibility	$Pc = 0.0011$	[37]
				DRB1 14:06*	Susceptibility	$Pc = 0.0011$	
				DRB1 08:08*	Resistance	$Pc = 0.0006$	
				DRB1 11:03*	Resistance	$Pc = 0.0004$	
Chinese	Case-control	305LD, 527 controls	LD	*DRB1* 15*	Susceptibility	$Pc = 0.002$	[38]
				DRB1 09*	Resistance	$Pc =< 0.001$	
Brazilian	Case-control	30BL, 178 controls	BL	*DRB1* 16:01*	Susceptibility	$Pc = 0.0208$	[39]
	Case-control	63LL, 43TT	LL	*DRB1* 08*	Susceptibility	$Pc = 0.0481$	
Taiwanese	Case-control	65LD 190 controls	ML	*DRB1* 04:05*	Resistance	$Pc =< 0.0001$	[40]
Brazilian	Case-control	17LL, 77 control	LL	*DRB1* 11*	Resistance	$Pc = 0.0132$	[41]
Brazilian	Case-control	36LL, 85 control	LL	*DRB1* 16*	Susceptibility	$Pc = 0.0105$	[42]
	Case-control	20TT, 85 control	TT	*DRB1* 14*	Susceptibility	$Pc = 0.032$	

MB: multibacillary leprosy; PB: paucibacillary; LL: lepromatous leprosy; BL: borderline lepromatous; BB: borderline borderline; TT: tuberculoid leprosy; PTB: pulmonary tuberculosis; LD: leprosy disease; NN[c]: all not significantly different alleles collapsed into a unique group (i.e., *DRB1* 04*, *07*, *10*, *12*, and *15*) [36]; ASP: affected sib-pair; ns: not significant; *Pc:* corrected *P* value; Ref.: reference.

Table 3: Associations between cytokine genes and leprosy.

Population	Study design	Sample size	Phenotype	Allele, genotype, or haplotype	Type of association	P	Ref.
Brazilian	Case-control	70LL, 85BL, 55BB, 2BT, 63TT, 10IL, 15 pure neural, 92 controls	BT/TT	*TNF-308A*	Resistance	$P = 0.005$	[43]
Brazilian	Case-control	74BT	BT/TT	*TNF-308A*	Resistance	ND	[44]
Brazilian	Case-control	210MB, 90PB, 92 controls 143MB, 79PB, 62 controls	LD PB LD	*TNF-308A* *IL10-819T* *IL10-819TT*	Resistance Susceptibility Susceptibility	$P < 0.05$ $P < 0.01$ $P = 0.04$	[45]
Brazilian	Case-control	43TT, 65LL, 50BB, 9IL, 240 controls	LD LL	*TNF-308A* *IL10-1082G/-819C/-592C*	Resistance Resistance	$P = 0.02$ $P = 0.02$	[46]
Brazilian	Family study case-control	363LD 1146LD, 1036 controls	LD	*TNF-308A*	Resistance Resistance	$P < 0.04$ $P < 0.02$	[47]
Nepal	Case-control	581(LL,BL,BB), 343(BT,TT); 101 controls	LD	*TNF-308A*	Resistance	$P = 0.016$	[48]
Indian	Case-control	121LL, 107TT, 160 controls	LL	*TNF-308A*	Susceptibility	$P = 0.02$	[49]
Thai	Case-control	24MB, 13PB, 140 controls	MB LD	*TNF-308A* *TNF-308GA*	Susceptibility Susceptibility	$P = 0.04$ $P = 0.04$	[50]
French Polynesian	Family study	6 families	LD	*IL-1 beta*, *TNF-alpha (1, 2)*, and *TNF-alpha (A, G)*	ns		[51]
Brazilian	Multi case families study	76 families	LD	*TNF*1* *TNF*1/LTA*2* *TNF*2/LTA*2*	Susceptibility Susceptibility Resistance	$P = 0.0001$ $P = 0.014$ $P = 0.001$	[31]
Mexican	Case-control	62 cases, 144 controls	LL	*TNF-308G/A* *IL10-819C*	ns		[52]
Indian	Case-control	449PB, 473MB, 1670 controls	LD	*BAT1-LTA-TNF-BTNL2*	Susceptibility	$P < 0.0001$	[53]
Brazilian	Case-control Meta-analysis	374 cases, 380 controls 2702 individuals (5 studies)	LD	*IL10-819T*	Susceptibility	$P = 0.01$	[54]
Colombian	Case-control	100 cases, 100 controls	LD	*IL10-819CC* and *CT* *IL10-592CC* and *CA* *IL10-819C-592C* *IL10-1082A-819C-592C*	Susceptibility Susceptibility Susceptibility Susceptibility	$P < 0.001$ $P < 0.001$ $P < 0.001$ $P < 0.001$	[55]
Brazilian	Case-control	131PB, 166MB, 283 controls	LD	*IL10-3575A/-2849G/-2763C* *IL10-3575T/-2849A/2763C*	Resistance Susceptibility	$P = 0.005$ $P = 0.027$	[56]
Indian	Case-control	144MB, 142PB, 266 controls	LD	*IL10-3575T/-2849G/-2763C/-1082A/-819C/-592C* *IL10-3575T/-2849G/-2763C/-1082A/-819T/-592A*	Resistance Susceptibility	$P = 0.01$ $P = 0.0002$	[57]
Indian	Case-control	80 cases, 89 controls	LD	*IL12B 3′UTR 2.2*	Resistance	$P = 0.001$	[58]
Korean	Case-control	93LL, 94 controls	LL	*IL12RB1* *IFNGR1*	ns		[59]
Japanese	Case-control	130LL, 46TL, 68 controls	LL	*IL12RB2-1035G* *IL12RB2-1023G* *IL12RB2-650delG* *IL12RB2-464G* *IL12RB2-1035A/-1023A/-650G/-464A*	Susceptibility	$P < 0.001$ $P < 0.01$ $P < 0.001$ $P < 0.01$ $P < 0.039$	[60]

Table 3: Continued.

Population	Study design	Sample size	Phenotype	Allele, genotype, or haplotype	Type of association	P	Ref.
Mexican	Case-control	44LL, 51 controls	LL	*IL12 3′ UTR 1188A/C*	Susceptibility	$P < 0.05$	[61]
Mexican	Case-control	66LL, 140 controls	LL	*IL12 3′ UTR 1188A/C*	ns		[62]
Chinese	Multiple-stage genetic association	4971 cases, 5503 controls	LD	*IL18RAP/IL18R1*(rs2058660) *IL12B*(rs6871626)	Susceptibility	$P = 4.57\times10^{-19}$ $P = 3.95 \times 10^{-18}$	[63]
Brazilian	Case-control	1045 cases, 1080 controls	LD	*IFNG+ 874T*	Resistance	$P = 0.005$	[64]
Chinese	Case-control	527 cases, 583 controls	LD LD, MB MB MB PB	*IFNG+ 874T/A* *IFNG(10CA),* *IFNG(13CA)* *IFNG(15CA)* *IFNG(17CA)*	ns Susceptibility Susceptibility Susceptibility Susceptibility	 $P = 0.001$ $P = 0.026$ $P = 0.007$ $P = 0.04$	[65]
Brazilian	Case-control	108 cases, 113 controls	PB	*IFNG+874AA* *IFNG(16CA)*	Susceptibility Susceptibility	$P = 0.028$ $P = 0.019$	[66]
Brazilian	Case-control	10TT, 59BB, 27LL, 98 controls	LD	*IFNG(15CA), (16CA),* and *(17CA)*	Susceptibility	$P = 0.01$	[67]
Iranian	Case report	3 cases	LD	*IFNGR1-56T/C*	Susceptibility	ND	[68]
Chinese	Case-control	80PB, 352MB, 465 controls	LD	*IL4-590TC* *IL4-590CC* *IL4-590C*	Resistance Resistance Resistance	$P = 0.044$ $P = 0.01$ $P = 0.001$	[69]
Indian	Case-control	2447 cases, 1294 controls	LD	*IL-10* (rs1800871, rs1800872, rs1554286); *IL-10RB* (rs3171425; rs7281762); *TGFBR2* (rs2228048, rs744751); *IL-6* (rs1800797)	Susceptibility	$P < 0.05$	[70]

MB: multibacillary; PB: paucibacillary; LL: lepromatous leprosy; BL: borderline lepromatous; BT: borderline tuberculoid; TT: tuberculoid leprosy; LD: leprosy disease; ns: not significant; ND: no data; P value; Ref.: reference.

were frequent among Indian leprosy patients [11], while HLA-A11 and HLA-A33 were increased among Korean LL patients [13].

In Indian patients, an increasing frequency of HLA-A11 [14] and HLA-B60 [15] antigens have been observed in LL patients. In southern Chinese, significantly decreased HLA-B46 was found in multibacillary leprosy [16]. In a Turkish LL case-control study, HLA class I serotypes A9, A10, Bw4, Bw6, Cw1, and Cw2 were significantly overrepresented, and serotypes A3 and B49 were significantly underrepresented in the LD patients [17].

Subsequently, with the advent of molecular genotyping, *HLA* class I alleles were determined in multibacillary leprosy patients, resulting in a positive association with *HLA-A*02:06*, *A*11:02*, *B*18:01*, *B*51:10*, *C*04:07*, and *C*07:03* alleles, and a negative association with *C*04:11* [18]. The *A*11-B*40* haplotype was increased in multibacillary leprosy patients compared to controls [19].

Recent studies have shown a positive association between LD and *HLA-A*11*, *HLA-B*38*, and *HLA-C*12*, as well as a negative association with *HLA-C*16*. When groups were stratified, *HLA-B*35* and *HLA-C*04* were shown to be protective against lepromatous leprosy, while *HLA-C*07* was shown to be a susceptibility variant [20]. Further, the allele *HLA-C*15:05* was related to phenotype LD in certain populations from India and Vietnam [21]. Table 1 summarizes these findings.

5. Classical *HLA* Class II Genes

According to some studies, the main restriction determinants for *M. leprae* reside on DR, and not DP or DQ molecules [73, 74]. The HLA-DR2 molecule [12, 13, 15, 21–24, 29, 32], later identified as *DRB1*15* and *DRB1*16* variants, is primarily associated with leprosy (LD or different clinical forms) in Indian, Japanese, Brazilian, and Chinese patients [25–27, 30, 33, 35, 36, 38, 39, 42].

In Indian patients, *DRB1*15:02* was associated with TT [24, 25], whereas *DRB1*15:01* was associated with LL [25]. *DRB1*15:01* and **15:02* alleles differ from each other by a single amino acid at codon 86. Class II molecules have polymorphic pockets that accommodate the side chains of bound peptides. The codon 86 residue lies in binding pocket 1. In another Indian study, both *DRB1*15:01* and **15:02* were found to be associated with tuberculoid leprosy, [27] indicating that the residue in pocket 1 may not be involved in determining the outcome of leprosy infection. Instead, it appears that certain residues that contribute to the net charge in the putative peptide-specific binding pocket 4 may be more important [75]. It is hypothesized that net negative or neutral charges in binding pocket 4 cause poor binding of the DRB1 molecule to *M. leprae* antigens. HLA molecules with the highest affinity to peptide produce the greatest T-cell proliferation and IFN-γ response [76], and the peptide presentation by low affinity class II molecules may result in muted cell-mediated immunity [75]. Alternatively, peptide presentation by specific class II molecules may result in activation of suppressor/regulatory T-cells [77].

Studies involving HLA-DRB1 have found a link between innate and T-cell-mediated immunity [78, 79], and results obtained from a multiple sclerosis study show that the presence of a VDRE (vitamin D response elements) in the proximal promoter region of the *HLA-DRB1* gene increased gene expression and imparts 1,25-(OH)2-D3 (Vitamin D) sensitivity to the *DRB1*15:01* allele [79].

These observations point to the need to apply this possibility of association between these genetic variants and leprosy pathogenesis, since vitamin D, itself, may have a direct effect on leprosy through its receptors, VDR, or may influence leprosy through indirect effects [79].

Amino acid residues involved in the peptide binding groove of *HLA-DRB1* alleles were examined in three Nigerian ethnic groups (Bini/Igbo, Yoruba, and Efik) with leprosy. Nine positively charged motifs and 2 others with neutral charge in the peptide binding groove were detected. These motifs were more frequent in leprosy patients than was expected by chance. In contrast, 5 motifs with negative or "modified" neutral charges in the pocket were negatively associated with leprosy. Therefore, the clinical outcome of infection by *M. leprae* is largely determined by a shared epitope in *DRB1* alleles characterized by several motifs [75].

In leprosy patients from a Javanese population in Yogyakarta, Indonesia, *HLA-DRB1*02* was associated with susceptibility to LL, while *HLA-DRB1*12* was associated with resistance [28]. Risk for leprosy associated with the *DRB1*10* allele has been described in Turkish, Vietnamese, and Brazilian populations [17, 36], whereas the *HLA-DRB1*14* allele was associated with the TT group in a population from northeastern Brazil [42] and *DRB1*14:01* and *DRB1*14:06* were associated with leprosy per se in Argentinean population [37]. A protective effect on leprosy has been described for *DRB1*04* in Brazilian, Korean, Japanese, Vietnamese, Argentinean, and Taiwanese populations [13, 30, 34, 36, 40]. Associations between HLA class II and leprosy are summarized in Table 2.

The HLA complex has been studied in leprosy patients due to the direct involvement of these alleles in the immune response. In terms of both infection control and the manifestation of the different clinical forms, investigation of *HLA* genes may elucidate mechanisms of susceptibility and resistance, as well as disease course.

Even though genetic epidemiology data in leprosy involving alleles *HLA* is extensive, results should be cautiously interpreted due to the strong linkage disequilibrium across the alleles in this region, the common occurrence of weak study designs, and publication bias of positive results. Furthermore, functional data to support these associations are required.

6. *KIR* Genes and Leprosy

Killer cell immunoglobulin-like receptors (KIRs) are members of a group of regulatory molecules found on natural killer (NK) cells. These proteins are encoded by a complex of genes located in the Leukocyte Receptor Complex on chromosome 19p13.4, which has many polymorphisms that may be related to resistance to infection [80]. Known roles of NK cells include modulation on the immune system by the production

of cytokines, as well as direct elimination of infected cells [81]. KIR molecules are either activating or inhibitory to NK cells. Inhibitory molecules (KIR2DL1, 2DL2, 2DL3, 2DL4, 2DL5, 3DL1, 3DL2, and 3DL3) function via the well-documented immunoreceptor tyrosine-based inhibitory motifs (ITIMs) [81]. The phosphorylated ITIMs serve as efficient recruitment points for the cytosolic protein tyrosine phosphatases, SHP-1 and SHP-2, resulting in the dephosphorylation of substrates critical for cellular activation [81].

Activating receptors (2DS1, 2DS2, 2DS3, 2DS4, 2DS5, and 3DS1) have truncated cytoplasmic domains lacking ITIMs but possess a charged residue (ITAMs) in their transmembrane domains that mediates interaction with the DAP-12 signal transduction chain. DAP-12 is a member of the immunoglobulin super family encoded at the centromeric end of the LRC. DAP-12 activation then leads to enhanced degranulation and production of cytokines and chemokines [82]. Studies performed over the last few years have revealed extensive diversity at the *KIR* gene locus, stemming from both its polygenic and multiallelic polymorphisms [83, 84].

Biologically, NK cell reactivity against target cells is partially based on the presence of KIRs and their cognate ligands, the HLA class I molecules. Some combinations of HLAs and KIRs may result in activation or inhibition of NK cells. It seems likely that NK receptor variants may be risk factors for infectious diseases in addition to HLA variants, as has been reported in leprosy. To further elucidate the balance between inhibitory and activating KIRs in the context of disease pathogenesis, continued epidemiological analysis of KIRs and disease should be pursued [85].

There are many studies showing the influence of *KIR* genes and their ligand pairs on the role of various infectious diseases. However, to the best of our knowledge only one study has explored the role of *KIR* genes in the pathogenesis of leprosy [82].

According to Franceschi et al. [82], a significant difference between *KIR* genes in TT and LL patients has been observed. In TT patients, the frequency of *KIR2DS3* (38.1%) was significantly higher than in LL patients (18.5%), and the frequency of *KIR2DS2* showed a trend of being higher in TT patients (61.9%) compared to LL patients (43.1%). *KIR2DS3* and *KIR2DS2* are activator genes in linkage disequilibrium. Tuberculoid patients with both activator genes could develop a better NK-cell activation and then a more efficient cell-mediated immune response, with a milder manifestation of the disease. When KIR inhibitor genes and their HLA ligands were analyzed, TT patients had low frequencies of these KIRs in association with their correlated ligands, conferring a reduced NK cell inhibition and resulting in a protective mechanism against the most severe forms of the disease.

In the same study, patients with the form BB were observed to have a higher frequency of *KIR3DL2-A3/11* genes (40.0%) compared to the control group (24.6%) and LL patients (20.0%). In contrast, a reduced frequency of *KIR2DL1* with the *C2* as a ligand was found compared to TT patients (48.9% versus 76.3%) and the control group (48.9 versus 66.4%). This balance between these interactions may explain the undefined characteristics observed in BB patients.

According to Parham [86], of the family of KIR2DL molecules, KIR2DL1 with the ligand C2 is the most potent inhibitor. In the study by Franceschi et al. [82], an increased frequency of homozygous *C2*/*C2* was observed in TT patients compared to BB patients and to control group, suggesting that TT patients may be more susceptible to infection than the control group.

7. *MICA* Genes

In 1994, Bahram et al. [87] and Leelayuwat et al. [88] independently identified a new set of *loci* called MHC class I chain-related genes (*MIC*). The *MIC* family has two members, *MICA* and *MICB*, and 5 pseudogene members: *MICC*, *MICD*, *MICE*, *MICF*, and *MICG*. *MICA* is located at the centromeric end of the classical *HLA* class I region, approximately, 46.4 kb from *HLA-B* [89]. *MIC* genes encode a cell-surface glycoprotein of 383 amino acids, which is expressed in keratinocytes, fibroblasts and gastrointestinal epithelium, and several other cell types [90]. Exons 2, 3, and 4 of the gene encode three extracellular domains (α1, α2, and α3, resp.), while exon 5 encodes the transmembrane domain. Amino acid sequence alignments with classical HLA class I chains reveal between 15 and 21% homology in α1 and α2 domains but between 32% and 36% homology in the α3 domain [87].

Studies have shown that MICA works as a ligand for NK cells, $\gamma\delta$ T cells, and $\alpha\beta$ CD8+ T cells, which express a common activating NK cell receptor NKG2D [91]. NKG2D recognizes the human MICA protein in conjunction with a transmembrane signaling adaptor protein, DNAX-activation protein (DAP) [92].

Within exon 5, there is a short tandem repeat (STR) of GCT triplets in varying lengths [93]. This STR is commonly referred to as an "A" followed by the number of GCT repeats and occasionally a "1", which reflects the presence of a G insertion (e.g., *A4*, *A5*, and *A5.1*). This information about exon 5 in *MICA* may therefore be of importance as polymorphisms in the transmembrane domain were correlated with the induction of autoreactive CD8+ cytotoxic T lymphocytes [94]. In addition, a G insertion within the exon 5 STR leads to a premature stop codon, which is translated into a truncated protein with impaired function [95].

Similar to classical *HLA*, *MICA* displays a high degree of allelic polymorphism within the nonclassical *HLA* gene *loci*, which results in MICA polymorphic residues that are positioned on the outer edge of an antigen-binding cleft, unlike MHC class I molecules [96], and they may have a role in the innate immune response to infection.

Since MIC expression is inducible by heat, viral infection, inflammation, and DNA damage, the molecules may be markers of stress in cells.

8. *MICA* and Leprosy

Studies linking *MIC* genes and leprosy are limited. Wang et al. [16] analyzed 69 southern Chinese leprosy patients and observed that *MICA-A5* allele showed a tendency to

be negatively associated to multibacillar leprosy but not to paucibacillar. In the same group of patients, a negative association between the *HLA-B46*/*MICA-A5* haplotype and leprosy was found, suggesting that the *HLA-B46*/*MICA-A5* haplotype is significantly associated with resistance to leprosy. On the other hand, Tosh et al. [33] provided strong evidence that truncated MICA protein encoded by the *MICA-A5.1* allele plays a role in leprosy susceptibility in South Indian families.

In the study performed in southern Brazil by Sacramento et al. [96], 223 patients with leprosy from towns in the northern and northwestern regions of the State of Paraná participated. *MICA***002*, **008*, **004*, and **009* alleles were the most frequent, totalling 74.0% of all alleles in leprosy patients and 68.5% in the control group. There was only one significant difference: the frequency of the *MICA***027* allele was higher in the control group compared to patients with LD. The alleles *MICA***010* and *MICA***027* had significant differences in multibacillary (LL, BB, and BL) patients compared to the control group. For the paucibacillary (TT and BT) group, no difference was found.

In this context, the *MICA***027* allele was associated with protection against leprosy *per se* and the multibacillary subtypes. Individuals with the *MICA***027* variant have normal expression of A5, a transmembrane domain which enables the interaction between MICA and NKG2D, activating NK cells.

Finally, these results suggest the influence of *MICA* alleles in the development of the leprosy and their clinical forms and need to be replicated.

9. Cytokines

An important factor that directs the clinical course of leprosy is the presence of proinflammatory and anti-inflammatory cytokines. Paucibacillary patients show a pattern of CMI of the Th1 type, which is characterized by the production of IFN-γ, IL-2, IL-7, IL-12, IL-15, and IL-18 in skin lesions. Conversely, multibacillary patients present a Th2 response with production of TGF-β1, IL-4, IL-5, and IL-10 in skin lesions with high antibody production, but insufficient CMI [97, 98].

SNPs are the most abundant source of genetic variation in the human genome, which can lead to differences in expression of proteins, causing structural and functional changes. Linking SNPs with the phenotypes of human diseases has great potential for direct clinical application, providing more accurate genetic markers for diagnosis and prognosis, and possibly new therapeutic targets [99]. Some SNPs in cytokine genes have been described as important genetic factors in the occurrence of different clinical forms of leprosy.

10. Cytokines and Leprosy

The gene encoding tumor necrosis factor (*TNF*) is located in the MHC region on chromosome 6. This cytokine exists in soluble and transmembrane forms [100] and is produced by cells of the immune system, tumor cells, and other cell types in response to inflammatory stimuli, infection, or stress [100]. There is ample evidence of the involvement of cytokines, especially tumor necrosis factor-alpha (TNF-α), in the immune response to leprosy. They may have a beneficial role in host defense but, if produced at high levels, cause tissue damage [101].

Studies conducted in Brazil by Santos et al. [43], Moraes et al. [44], Santos et al. [45], Franceschi et al. [46], and Cardoso et al. [47] indicated the association of *TNF-308A* (rs1800629) allele with a protective effect against the development of the disease. Vanderborght et al. [101], in a study in Rio de Janeiro, observed that patients possessing an A allele in the promoter region of *TNF-308* had a lower bacteriological index (BI), whereas the carriers of the A allele in the promoter region of *TNF-238* (rs361525) had higher BI.

A study in Nepal in 2010 by Sapkota et al. [48] showed results similar to Brazilian studies in relation to *TNF-308A* allele. However, studies conducted in an Indian and Thai populations [49, 50] showed a higher frequency of *TNF2* allele (with substitution G>A at position 308 in the *TNF* promoter region) in lepromatous and multibacillary patients, respectively, compared to the control group, indicating that this allele is associated with susceptibility to this form of disease. Nevertheless, a linkage study conducted in six French Polynesian families for Levée et al. [51] found no evidence of linkage between the loci *G1M*, *G2M*, *KM*, *IL-1* beta, *TNF-alpha* (1, 2), and *TNF-alpha* (A, G) and leprosy.

In the study multicase leprosy families from northeastern of Brazil, the combined segregation and linkage analysis to the major locus showed strong linkage to *HLA* class II and tumour necrosis factor genes. Extended transmission disequilibrium testing, using multiple affected family members, demonstrated that the common allele *TNF***1* of the −308 promoter region polymorphism showed linkage and/or association with disease per se, at a high level of significance. Two locus transmission disequilibrium testing suggested susceptibility (*TNF***1*/*LTA***2*) and protective (*TNF***2*/*LTA***2*) haplotypes in the class III region. Taken together the segregation and HLA analyses suggest the possibility of more than one susceptibility locus to leprosy in the MHC [31].

In a recent study in Mexico [52], no association was found between *TNF-308G/A* and leprosy, suggesting that other polymorphisms may be important in susceptibility to leprosy in this population. However, a study performed in a population from Northern India [53] provided further evidence for the role of variants *BAT1-LTA-TNF-BTNL2* genes in susceptibility to leprosy. According to authors, the combination of low T-cell inhibition status of BTNL2, less inhibition of TNF by BAT1, and low TNF expression may provide protection from leprosy, which may be stronger in the presence of high TNF producer allele genetic background.

Interleukin 10 (IL-10) is a cytokine produced by monocytes and activated T cells. It is deeply involved in the regulation of inflammatory and immunological reactions. Its effects do not only affect the immune system but can influence many physiological processes, including angiogenesis, tumorigenesis, and infection. Several polymorphisms have been observed in the *IL10* gene, including 6–11 CA repeats-

microsatellite polymorphisms, and three point mutations: −1082 (G/A) (rs1800896), −819 (C/T) (rs1800871), and −592 (C/A) (rs1800872) [102].

Recently, in Mexican patients, Velarde-Félix et al. [52] found no statistically significant difference in the frequency of *IL10−819C* allele in patients and controls. However, in a Brazilian population, Pereira et al. [54] had reported that the *IL10−819T* allele was associated with leprosy in both a case-control study and in a meta-analysis.

Similar results were found in another Brazilian population of Rio de Janeiro by Santos et al. [45], where the *IL10−819TT* genotype was significantly higher in patients than in healthy controls, and the frequency of the *IL10−819T* SNP was greater in paucibacillary patients compared to multibacillary or among control subjects. However, in Colombian patients, the genotypes C/C and C/T in the SNP −819 and C/C and C/A in the −592 SNP were positively associated to leprosy. The haplotypes −819C−592C and −1082A−819C−592C showed significant association and these same haplotypes in homozygosis conditions were also associated with leprosy [55].

In another study, Moraes et al. [56] observed that in patients from the same Brazilian region the haplotype *IL10−3575A/−2849G/−2763C* was associated with resistance to leprosy and development of more severe forms of the disease, and that the haplotype *IL10−3575T/−2849A/2763C* was associated with susceptibility to LD.

In a study conducted in India, Malhotra et al. [57] observed that the extended haplotype *IL10−3575T/−2849G/−2763C/−1082A/−819C/−592C* conferred resistance to leprosy *per se* and to development of more severe forms of disease, whereas the haplotype *IL10−3575T/−2849G/−2763C/−1082A/−819T/−592A* was associated with the risk of developing a more severe form of the disease. A study in a population of southern Brazil by Franceschi et al. [46] showed a lower frequency of haplotype *IL10−1082G/−819C/−592C* in patients with the lepromatous form of the disease compared to the control group. The results of these studies strongly suggest the involvement of SNPs in the promoter region of the *IL10* gene in leprosy.

IL-12 consists of two covalently linked subunits: p35 and p40. Antigen-presenting cells, specifically dendritic cells and macrophages, are the main producers of this cytokine. The effects of IL-12 are mainly controlled by the level of transcription of p40 and expression of IL-12R. IL-12 is produced quickly after infection and acts as a proinflammatory cytokine by inducing IFN-γ production and enhancing the proliferation and cytotoxicity of NK and T cells [103].

According to Morahan et al. [58], in Indian patients, subjects with leprosy were less likely to have the 3′UTR genotype associated with lower IL-12B expression. However, in Korean patients, Lee et al. [59] found no significant differences in allele frequencies of *IL12RB1* between leprosy patients and the control group [59]. Now, in relation to gene in the 5′ flanking region of *IL12RB2*, Ohyama et al. [60] determined the functional effects of these SNPs on NK-cell activity, including IFN-γ production and *IL-12RB2* gene expression. The results suggest that these SNPs in *IL12RB2* have differential effects on cellular activation of T and NK cells [60].

In Western Mexico, Alvarado-Navarro et al. [61] found that the 1188A/C polymorphism in the 3′UTR of *IL12p40* gene was associated with greater susceptibility to lepromatous leprosy, independent of the expression levels of IL-12 p40. Conversely, Jesús Salvador et al. [62] in a study with Mexican patients found no significant association between genotype and allele frequencies of the 1188A/C polymorphism and lepromatous leprosy [62].

Recently, Liu et al. [63] conducted a multiple-stage genetic association study in leprosy patients from China and discovered associations implicating *IL18RAP/IL18R1* (rs2058660) and *IL12B* (rs6871626) as susceptibility genes for leprosy.

The *IFNG* gene encodes the IFN-γ cytokine, which plays a key role in host defense against intracellular pathogens. SNPs in *IFNG* were evaluated in several epidemiological studies; the SNP *INFG+874T/A* (rs2430561), more specifically, the allele *INFG+874T* has been associated with protection against infectious diseases [104].

In patients from São Paulo and Rio de Janeiro, two independent studies conducted by Cardoso et al. [64] showed that the *INFG+874T* allele conferred protection against leprosy. Recently, in Chinese patients, Wang et al. [65] found no association between *INFG+874T/A* and leprosy. However, the variant rs3138557 in the *IFNG* gene had many CA-repeat alleles and they observed that the alleles *INFG* (10CA), INFG (13CA), and *INFG* (15CA) had a higher frequency in patients, especially in multibacillary compared to the control group (3.2 versus 0.6%; 21.3 versus 18.6%; and 21.8 versus 18.0%, resp.), and that the allele *INFG* (17CA) was more frequent in paucibacillary patients than in controls (2.8 versus 1.2%). In patients from Amazonas state, Brazil, there were no significant differences between patients and control subjects, as well as according to Ridley-Jopling classification. However, the A/A genotype and the allele *INFG* (16CA) were significantly associated with paucibacillary compared to multibacillary patients [66].

In a population of Brazilian patients, Reynard et al. [67] observed that a higher frequency of alleles *INFG* (15CA), *INFG* (16CA), and *INFG* (17CA) was positively associated with leprosy, which indicates that the *IFNG* gene polymorphism may contribute to the course of infection.

In Korean patients, no significant differences were found in allele frequencies *IFNGR1* (interferon γ receptor 1) between leprosy patients and the control group [59]. However, a case report showed that the *IFNGR1* polymorphism at position −56T/C was positively associated with an increased susceptibility to leprosy, in Iranian children of the same family [68].

Polymorphisms in the *IL4* gene influence the production of IL-4, an important anti-inflammatory cytokine generated by T-helper type 2 (Th2) cells, which have multiple roles in the immune system. Three polymorphisms in *IL4* have been described: a single base polymorphism −590T/C (rs2243250) in the promoter region, polymorphism +33C/T (rs2070874) in exon 1, and type VNTR polymorphism (variable number of tandem repeat) in intron 3. In a Chinese study, Yang et al.

[69] observed that the *IL4–590T/C* and C/C genotypes, and the −590C allele were less frequent in leprosy patients than in the control group (25 versus 29.9%; 3.9 versus 7.5%; and 16.4 versus 22.5%, resp.), suggesting that the allele *IL4–590C* is associated with resistance to leprosy in this population.

Interleukin-6 (IL-6) is a pleiotropic cytokine, produced by different cell types, such as macrophages, fibroblasts, and endothelial cells. IL-6 plays an important role in a wide range of processes, such as immune response, acute phase reactions, and hematopoiesis [105].

Recently, in a case-control study, Sousa et al. [106] observed a correlation between plasma levels of IL-6 and *IL6* genotypes in patients with Type-2 reactions in leprosy. Type-1 and Type-2 leprosy reactions are aggressive inflammatory episodes with highly variable incidence rates across populations but affect up to 50% of leprosy patients. Identification of genetic factors predictive of leprosy reactions could have a great impact on prevention strategies.

A study conducted in MassARRAY platform, carried out by Aggarwal et al. [70], in the Indian population investigated the association of 51 SNPs in anti-inflammatory cytokine and receptor genes with susceptibility to leprosy. Significant associations with leprosy were observed for 8 polymorphisms (rs1800871, rs1800872, and rs1554286 of *IL10*, rs3171425 and rs7281762 of *IL10RB*, rs2228048 and rs744751 of *TGFBR2*, and rs1800797 of *IL6*). The study revealed a greater association of these polymorphisms with the risk for leprosy than those obtained for any SNP studied individually. This provides an interesting insight on the cumulative polygenic host component that regulates leprosy pathogenesis [70]. Table 3 summarizes these findings.

Studies have been carried out in order to investigate a possible combined effect of *HLA* genes and cytokines genes in leprosy, more specifically *TNF* gene and *HLA* class II [31, 49]. However, the results are inconsistent. The first study by Roy et al. [49] did not find linkage disequilibrium between *TNF2* allele and *HLA* class II, showing that these genes appear to be independent, whereas Shaw et al. [31] showed strong linkage between *HLA* class II (*HLA-DQB1*, $P = 0.000002$; *HLA-DQA1*, $P = 0.000002$; *HLA-DRB1*, $P = 0.0000003$) and *TNF* genes (*TNF*, $P = 0.00002$; *LTA*, $P = 0.003$). More studies are needed to clarify this linkage because polymorphisms within the TNF gene, which is located close to the class II region, may lead to variability in TNF-α secretion during the leprosy infection [49]. This is significant, since in mycobacterial infections, TNF-α promotes host defense mechanisms and granuloma formation, but high concentrations of TNF-α are associated with immunopathology [49].

11. Genome-Wide and Leprosy

Finally, we will summarize findings from some important genome-wide association studies of leprosy. The first GWAS of leprosy susceptibility reported convincing associations with markers in six genetic loci: *HLA-DR-DQ* (rs602875, $P = 5.4 \times 10^{-27}$, OR = 0.67), receptor-interacting serine-threonine kinase 2 (*RIPK2*) (rs42490, $P = 1.4 \times 10^{-16}$, OR = 0.76), tumor necrosis factor [ligand] superfamily member 15 (*TNFSF15*) (rs6478108, $P = 3.4 \times 10^{-21}$, OR = 1.37), laccase (multicopper oxidoreductase) domain-containing 1 (*LACC1*; previously known as *C13*orf31) (rs3764147, $P = 3.7 \times 10^{-54}$, OR = 1.68), coiled-coil domain-containing 122 (*CCDC122*) (rs3088362, $P = 1.4 \times 10^{-31}$, OR = 1.52), and nucleotide-binding oligomerization domain-containing 2 (*NOD2*) (rs9302752, $P = 3.8 \times 10^{-40}$, OR = 1.59) [107].

Subsequently, associations between leprosy and the *HLA-DR-DQ* region, *LACC1*, *CCDC122*, and the I602S functional SNP in the Toll-like receptor 1 (*TLR1*) gene were replicated in an Indian population [108, 109] and between the *HLA-DR-DQ*, *RIPK2*, *CCDC122*, *LACC1*, and *NOD2* in Vietnam [110].

Interesting, an association between *LACC1* (previous C13orf31) and *CCDC122* and susceptibility to Crohn's disease was related [111]. However, both genes were of unknown function and should be investigated in relation to their biologic function, which will probably clear a pathogenic mechanism of both diseases.

Recently, Yang et al. [112] carried out a genome-wide single nucleotide polymorphism (SNP) based linkage analysis using 23 pedigrees, each with 3 to 7 family members affected by leprosy, in China [112]. They suggested genomewide significant evidence for linkage on chromosome 2p14, and a suggestive evidence for linkage on chr.4q22 (rs1349350), chr.8q24 (rs1618523), and chr.16q24 (rs276990), as well as a moderate evidence for a linkage locus on chromosome 6q24–26 (rs6570858), overlapping a previously reported linkage region on chromosome 6q25-26 [112].

12. Conclusion

The analysis of genetic variants in the susceptibility to infectious diseases has been a topic widely discussed. Through various studies, it is known that the environment and the virulence of the pathogen are not sufficient to explain the different immune response patterns presented in the same population against a particular pathogen. The hypothesis of the existence of a complex network of factors acting simultaneously in infectious disease is recognized, and within this context, in leprosy the host immune response is a critical factor for the onset of the disease, and the levels of this response are influenced by the interaction of different genes.

M. leprae can cause very different disease phenotypes in humans, probably due to individual variation in genetic profile and, consequently, in immune responses. Of the many reports of genes associated with leprosy, relatively few have been replicated in additional study populations. Further studies, involving a large number of genetic factors in populations from different parts of Brazil and the world, should be conducted to elucidate the interactions between these factors, which may be useful in the prognosis and clinical evolution of leprosy patients.

The purpose of this brief review was to highlight the importance of some immune response genes and their correlation with the development of clinical forms of leprosy, as well as their implications for disease resistance and susceptibility.

Disclosure

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

References

[1] G. A. Hansen, "Baccilus leprae," *Norsk Mag Laegevidenskaben*, vol. 9, pp. 1–21, 1874.

[2] World Health Organization, "Global leprosy situation," *Weekly Epidemiological Record*, vol. 85, p. 337, 2010.

[3] D. S. Ridley and W. H. Jopling, "Classification of leprosy according to immunity. A five-group system," *International Journal of Leprosy and Other Mycobacterial Diseases*, vol. 34, no. 3, pp. 255–273, 1966.

[4] D. M. Scollard, L. B. Adams, T. P. Gillis, J. L. Krahenbuhl, R. W. Truman, and D. L. Williams, "The continuing challenges of leprosy," *Clinical Microbiology Reviews*, vol. 19, no. 2, pp. 338–381, 2006.

[5] P. E. M. Fine, "Natural history of leprosy: aspects relevant to a leprosy vaccine," *International Journal of Leprosy*, vol. 51, no. 4, pp. 553–555, 1983.

[6] E. Schurr, A. Alcaïs, L. de Léséleuc, and L. Abel, "Genetic predisposition to leprosy: a major gene reveals novel pathways of immunity to Mycobacterium leprae," *Seminars in Immunology*, vol. 18, no. 6, pp. 404–410, 2006.

[7] E. Thorsby, T. Godal, and B. Myrvang, "HLA antigens and susceptibility to diseases. II. Leprosy," *Tissue Antigens*, vol. 3, no. 5, pp. 373–377, 1973.

[8] A. Dasgupta, N. K. Mehra, S. K. Ghei, and M. C. Vaidya, "Histocompatibility antigens (HLA) in leprosy," *Tissue Antigens*, vol. 5, no. 2, pp. 85–87, 1975.

[9] J. Greiner, E. Schleiermacher, and T. Smith, "The HLA system and leprosy in Thailand," *Human Genetics*, vol. 42, no. 2, pp. 201–213, 1978.

[10] N. Mohagheghpour, H. Tabatabai, and K. Mohammad, "Histocompatibility antigens in patiens with leprosy from Azarbaijan, Iran," *International Journal of Leprosy*, vol. 47, no. 4, pp. 597–600, 1979.

[11] U. M. Bale, M. M. Mehta, and N. M. Contractor, "HLA antigens in leprosy patients," *Tissue Antigens*, vol. 20, no. 2, pp. 141–143, 1982.

[12] S. Izumi, K. Sugiyama, Y. Matsumoto, and S. Ohkawa, "Analysis of the immunogenetic background of Japanese leprosy patients by the HLA system," *Vox Sanguinis*, vol. 42, no. 5, pp. 243–247, 1982.

[13] S. J. Kim, I. H. Choi, and S. Dahlberg, "HLA and leprosy in Koreans," *Tissue Antigens*, vol. 29, no. 3, pp. 146–153, 1987.

[14] J. N. Agrewala, S. K. Ghei, K. S. Sudhakar, B. K. Girdhar, and U. Sengupta, "HLA antigens and erythema nodosum leprosum (ENL)," *Tissue Antigens*, vol. 33, no. 4, pp. 486–487, 1989.

[15] R. Rani, S. A. Zaheer, and R. Mukherjee, "Do human leukocyte antigens have a role to play in differential manifestation of multibacillary leprosy: a study on multibacillary leprosy patients from North India," *Tissue Antigens*, vol. 40, no. 3, pp. 124–127, 1992.

[16] L.-M. Wang, A. Kimura, M. Satoh, and S. Mineshita, "HLA linked with leprosy in southern China; HLA-linked resistance alleles to leprosy," *International Journal of Leprosy and Other Mycobacterial Diseases*, vol. 67, no. 4, pp. 403–408, 1999.

[17] M. Koçak, M. Balci, B. Pençe, and N. Kundakçi, "Associations between human leukocyte antigens and leprosy in the Turkish population," *Clinical and Experimental Dermatology*, vol. 27, no. 3, pp. 235–239, 2002.

[18] U. Shankarkumar, K. Ghosh, S. Badakere, and D. Mohanty, "Novel HLA class I alleles. Associated with Indian leprosy patients," *Journal of Biomedicine and Biotechnology*, vol. 3, pp. 208–211, 2003.

[19] U. Shankarkumar, "HLA associations in leprosy patients from Mumbai, India," *Leprosy Review*, vol. 75, no. 1, pp. 79–85, 2004.

[20] D. S. A. Franceschi, L. T. Tsuneto, P. S. Mazini et al., "Class-I human leukocyte alleles in leprosy patients from southern Brazil," *Revista da Sociedade Brasileira de Medicina Tropical*, vol. 44, no. 5, pp. 616–620, 2011.

[21] A. Alter, N. T. Huong, M. Singh et al., "Human leukocyte antigen class I region single-nucleotide polymorphisms are associated with leprosy susceptibility in Vietnam and India," *The Journal of Infectious Diseases*, vol. 203, no. 9, pp. 1274–1281, 2011.

[22] V. Schauf, S. Ryan, and D. Scollard, "Leprosy associated with HLA-DR2 and DQw1 in the population of northern Thailand," *Tissue Antigens*, vol. 26, no. 4, pp. 243–247, 1985.

[23] M. Cem Mat, H. Yazici, F. Ozbakir, and Y. Tuzun, "The HLA association of lepromatous leprosy and borderline lepromatous leprosy in Turkey. A preliminary study," *International Journal of Dermatology*, vol. 27, no. 4, pp. 246–247, 1988.

[24] N. K. Mehra, W. Verduijn, V. Taneja, J. Drabbels, S. P. N. Singh, and M. J. Giphart, "Analysis of HLA-DR2-associated polymorphisms by oligonucleotide hybridization in an Asian Indian population," *Human Immunology*, vol. 32, no. 4, pp. 246–253, 1991.

[25] R. Rani, M. A. Fernandez-Vina, S. A. Zaheer, K. R. Beena, and P. Stastny, "Study of HLA class II alleles by PCR oligotyping in leprosy patients from North India," *Tissue Antigens*, vol. 42, no. 3, pp. 133–137, 1993.

[26] N. K. Mehra, R. Rajalingam, D. K. Mitra, V. Taneja, and M. J. Giphart, "Variants of HLA-DR2/DR51 group haplotypes and susceptibility to tuberculoid leprosy and pulmonary tuberculosis in Asian Indians," *International Journal of Leprosy*, vol. 63, no. 2, pp. 241–248, 1995.

[27] L. Zerva, B. Cizman, N. K. Mehra et al., "Arginine at position 13 or 70-71 in pocket 4 of HLA-DRB1 alleles is associated with susceptibility to tuberculoid leprosy," *The Journal of Experimental Medicine*, vol. 183, no. 3, pp. 829–836, 1996.

[28] H. Soebono, M. J. Giphart, G. M. T. Schreuder, P. R. Klatser, and R. R. P. de Vries, "Associations between HLA-DRB1 Alleles and Leprosy in an Indonesian Population," *International Journal of Leprosy and Other Mycobacterial Diseases*, vol. 65, no. 2, pp. 190–196, 1997.

[29] J. E. L. Visentainer, L. T. Tsuneto, M. F. Serra, P. R. F. Peixoto, and M. L. Petzl-Erler, "Association of leprosy with HLA-DR2 in a Southern Brazilian population," *Brazilian Journal of Medical and Biological Research*, vol. 30, no. 1, pp. 51–59, 1997.

[30] S. Joko, J. Numaga, H. Kawashima, M. Namisato, and H. Maeda, "Human leukocyte antigens in forms of leprosy among Japanese patients," *International Journal of Leprosy and Other Mycobacterial Diseases*, vol. 68, no. 1, pp. 49–56, 2000.

[31] M.-A. Shaw, I. J. Donaldson, A. Collins et al., "Association and linkage of leprosy phenotypes with HLA class II and tumour necrosis factor genes," *Genes and Immunity*, vol. 2, no. 4, pp. 196–204, 2001.

[32] A. A. Hegazy, I. A. Abdel-Hamid, E.-S. F. Ahmed, S. M. Hammad, and S. A. Hawas, "Leprosy in a high-prevalence

Egyptian village: epidemiology and risk factors," *International Journal of Dermatology*, vol. 41, no. 10, pp. 681–686, 2002.

[33] K. Tosh, M. Ravikumar, J. T. Bell, S. Meisner, A. V. S. Hill, and R. Pitchappan, "Variation in MICA and MICB genes and enhanced susceptibility to paucibacillary leprosy in South India," *Human Molecular Genetics*, vol. 15, no. 19, pp. 2880–2887, 2006.

[34] P. M. F. Motta, N. Cech, C. Fontan et al., "Role of HLA-DR and HLA-DQ alleles in multibacillary leprosy and paucibacillary leprosy in the province of Chaco (Argentina)," *Enfermedades Infecciosas y Microbiologia Clinica*, vol. 25, no. 10, pp. 627–631, 2007.

[35] M. Singh, A. Balamurugan, K. Katoch, S. K. Sharma, and N. K. Mehra, "Immunogenetics of mycobacterial infections in the North Indian population," *Tissue Antigens*, vol. 69, supplement 1, pp. 228–230, 2007.

[36] P. R. Vanderborght, A. G. Pacheco, M. E. Moraes et al., "HLA-DRB1* 04 and DRB1* 10 are associated with resistance and susceptibility, respectively, in Brazilian and Vietnamese leprosy patients," *Genes and Immunity*, vol. 8, no. 4, pp. 320–324, 2007.

[37] S. G. Borrás, C. Cotorruelo, L. Racca et al., "Association of leprosy with HLA-DRB1 in an Argentinean population," *Annals of Clinical Biochemistry*, vol. 45, no. 1, pp. 96–98, 2008.

[38] F. Zhang, H. Liu, S. Chen et al., "Evidence for an association of HLA-DRB1*15 and DRB1*09 with leprosy and the impact of DRB1*09 on disease onset in a Chinese Han population," *BMC Medical Genetics*, vol. 10, p. 133, 2009.

[39] S. A. da Silva, P. S. Mazini, P. G. Reis et al., "HLA-DR and HLA-DQ alleles in patients from the south of Brazil: markers for leprosy susceptibility and resistance," *BMC Infectious Diseases*, vol. 9, pp. 134–140, 2009.

[40] N.-K. Hsieh, C.-C. Chu, N.-S. Lee, H.-L. Lee, and M. Lin, "Association of HLA-DRB1*0405 with resistance to multibacillary leprosy in Taiwanese," *Human Immunology*, vol. 71, no. 7, pp. 712–716, 2010.

[41] R. Lavado-Valenzuela, M. José Bravo, A. P. Junqueira-Kipnis et al., "Distribution of the HLA class II frequency alleles in patients with leprosy from the mid-west of Brazil," *International Journal of Immunogenetics*, vol. 38, no. 3, pp. 255–258, 2011.

[42] R. D. G. Corrêa, D. M. Aquino, A. d. J. Caldas et al., "Association analysis of human leukocyte antigen class II, (DRB1) alleles with leprosy in individuals from São Luís, state of Maranhão, Brazil," *Memórias Instituto Oswaldo Cruz*, vol. 107, supplement 1, pp. 150–155, 2012.

[43] A. R. Santos, A. S. Almeda, P. N. Suffys et al., "Tumor necrosis factor promoter polymorphism (TNF2) seems to protect against development of severe forms of leprosy in a pilot study in Brazilian patients," *International Journal of Leprosy and Other Mycobacterial Diseases*, vol. 68, no. 3, pp. 325–327, 2000.

[44] M. O. Moraes, N. C. Duppre, P. N. Suffys et al., "Tumor necrosis factor-α promoter polymorphism TNF2 is associated with a stronger delayed-type hypersensivity reaction in the skin of borderline tuberculoid leprosy patients," *Immunogenetics*, vol. 53, no. 1, pp. 45–47, 2001.

[45] A. R. Santos, P. N. Suffys, P. R. Vanderborght et al., "Role of tumor necrosis factor-α and interleukin-10 promoter gene polymorphisms in leprosy," *The Journal of Infectious Diseases*, vol. 186, no. 11, pp. 1687–1691, 2002.

[46] D. S. A. Franceschi, P. S. Mazini, C. C. C. Rudnick et al., "Influence of TNF and IL10 gene polymorphisms in the immunopathogenesis of leprosy in the south of Brazil," *International Journal of Infectious Diseases*, vol. 13, no. 4, pp. 493–498, 2009.

[47] C. C. Cardoso, A. C. Pereira, V. N. Brito-de-Souza et al., "TNF -308G>A single nucleotide polymorphism is associated with leprosy among Brazilians: a genetic epidemiology assessment, meta-analysis, and functional study," *The Journal of Infectious Diseases*, vol. 204, no. 8, pp. 1256–1263, 2011.

[48] B. R. Sapkota, M. Macdonald, W. R. Berrington et al., "Association of TNF, MBL, and VDR polymorphisms with leprosy phenotypes," *Human Immunology*, vol. 71, no. 10, pp. 992–998, 2010.

[49] S. Roy, W. McGuire, C. G. N. Mascie-Taylor et al., "Tumor necrosis factor promoter polymorphism and susceptibility to lepromatous leprosy," *The Journal of Infectious Diseases*, vol. 176, no. 2, pp. 530–532, 1997.

[50] S. Vejbaesya, P. Mahaisavariya, P. Luangtrakool, and C. Sermduangprateep, "TNF α and NRAMP1 polymorphisms in leprosy," *Journal of the Medical Association of Thailand*, vol. 90, no. 6, pp. 1188–1192, 2007.

[51] G. Levée, E. Schurr, and J. P. Pandey, "Tumor necrosis factor-alpha, interleukin-1-beta and immunoglobulin (GM and KM) polymorphisms in leprosy. A linkage study," *Experimental and Clinical Immunogenetics*, vol. 14, no. 2, pp. 160–165, 1997.

[52] J. S. Velarde-Félix, S. Cázarez-salazar, J. J. Ríos-Tostado, A. Flores-Garcia, H. Rangel-Villalobos, and J. Murillo-Llanes, "Lack of effects of the TNF-alpha and IL-10 gene polymorphisms in Mexican patients with lepromatous leprosy," *Leprosy Review*, vol. 83, no. 1, pp. 34–39, 2012.

[53] S. Ali, R. Chopra, S. Aggarwal et al., "Association of variants in BAT1-LTA-TNF-BTNL2 genes within 6p21.3 region show graded risk to leprosy in unrelated cohorts of Indian population," *Human Genetics*, vol. 131, no. 5, pp. 703–716, 2012.

[54] A. C. Pereira, V. N. Brito-de-Souza, C. C. Cardoso et al., "Genetic, epidemiological and biological analysis of interleukin-10 promoter single-nucleotide polymorphisms suggests a definitive role for -819C/T in leprosy susceptibility," *Genes and Immunity*, vol. 10, no. 2, pp. 174–180, 2009.

[55] N. Cardona-Castro, M. Sánchez-Jiménez, W. Rojas, and G. Bedoya-Berrío, "IL-10 gene promoter polymorphisms and leprosy in a Colombian population sample," *Biomédica*, vol. 32, pp. 71–76, 2012.

[56] M. O. Moraes, A. G. Pacheco, J. J. M. Schonkeren et al., "Interleukin-10 promoter single-nucleotide polymorphisms as markers for disease susceptibility and disease severity in leprosy," *Genes and Immunity*, vol. 5, no. 7, pp. 592–595, 2004.

[57] D. Malhotra, K. Darvishi, S. Sood et al., "IL-10 promoter single nucleotide polymorphisms are significantly associated with resistance to leprosy," *Human Genetics*, vol. 118, no. 2, pp. 295–300, 2005.

[58] G. Morahan, G. Kaur, M. Singh et al., "Association of variants in the IL12B gene with leprosy and tuberculosis," *Tissue Antigens*, vol. 69, supplement 1, pp. 234–236, 2007.

[59] S.-B. Lee, B. C. Kim, S. H. Jin et al., "Missense mutations of the interleukin-12 receptor beta 1(IL12RB1) and interferon-gamma receptor 1 (IFNGR1) genes are not associated with susceptibility to lepromatous leprosy in Korea," *Immunogenetics*, vol. 55, no. 3, pp. 177–181, 2003.

[60] H. Ohyama, N. Kato-Kogoe, F. Nishimura et al., "Differential effects of polymorphisms in the 5′ flanking region of IL12RB2 on NK- and T-cell activity," *Journal of Interferon and Cytokine Research*, vol. 28, no. 9, pp. 563–569, 2008.

[61] A. Alvarado-Navarro, M. Montoya-Buelna, J. F. Muñoz-Valle, R. I. López-Roa, C. Guillén-Vargas, and M. Fafutis-Morris, "The

3′UTR 1188 A/C polymorphism in the interleukin-12p40 gene (IL-12B) is associated with lepromatous leprosy in the west of Mexico," *Immunology Letters*, vol. 118, no. 2, pp. 148–151, 2008.

[62] V.-F. Jesús Salvador, R.-M. José Guadalupe, O.-R. Luis Antonio, and R.-V. Héctor, "Lack of association between 3′ UTR 1188 A/C polymorphism in the IL-12p40 gene and lepromatous leprosy in Sinaloa, México," *International Journal of Dermatology*, vol. 51, no. 7, pp. 875–876, 2012.

[63] H. Liu, A. Irwanto, H. Tian et al., "Identification of IL18RAP/IL18R1 and IL12B as leprosy risk genes demonstrates shared pathogenesis between inflammation and infectious diseases," *American Journal of Human Genetics*, vol. 91, no. 5, pp. 935–941, 2012.

[64] C. C. Cardoso, A. C. Pereira, V. N. Brito-De-Souza et al., "IFNG +874 T>A single nucleotide polymorphism is associated with leprosy among Brazilians," *Human Genetics*, vol. 128, no. 5, pp. 481–490, 2010.

[65] D. Wang, J.-Q. Feng, Y.-Y. Li et al., "Genetic variants of the MRC1 gene and the IFNG gene are associated with leprosy in Han Chinese from Southwest China," *Human Genetics*, vol. 131, no. 7, pp. 1251–1260, 2012.

[66] G. A. V. Silva, M. P. Santos, I. Mota-Passos et al., "IFN-c +875 microsatellite polymorphism as a potential protection marker for leprosy patients from Amazonas state, Brazil," *Cytokine*, vol. 60, pp. 493–497, 2012.

[67] M. P. Reynard, D. Turner, A. P. Junqueira-Kipnis, M. R. de Souza, C. Moreno, and C. V. Navarrete, "Allele frequencies for an interferon-γ microsatellite in a population of Brazilian leprosy patients," *European Journal of Immunogenetics*, vol. 30, no. 2, pp. 149–151, 2003.

[68] A. A. Velayati, P. Farnia, S. Khalizadeh, A. M. Farahbod, M. Hasanzadh, and M. F. Sheikolslam, "Case report: interferon-gamma receptor-1 gene promoter polymorphisms and susceptibility to leprosy in children of a single family," *American Journal of Tropical Medicine and Hygiene*, vol. 84, no. 4, pp. 627–629, 2011.

[69] D. Yang, H. Song, W. Xu et al., "Interleukin 4-590T/C polymorphism and susceptibility to leprosy," *Genetic Testing and Molecular Biomarkers*, vol. 15, no. 12, pp. 877–881, 2011.

[70] S. Aggarwal, S. Ali, R. Chopra et al., "Genetic variations and interactions in anti-inflammatory cytokine pathway genes in the outcome of leprosy: a study conducted on a MassARRAY platform," *The Journal of Infectious Diseases*, vol. 204, no. 8, pp. 1264–1273, 2011.

[71] M. T. Mira, "Genetic host resistance and susceptibility to leprosy," *Microbes and Infection*, vol. 8, no. 4, pp. 1124–1131, 2006.

[72] P. R. Vanderborght and C. C. Cardoso, "Susceptibilidade genética na hanseníase," in *Estudos de Associação HLA x Doenças; II Simpósio Brasileiro*, p. 97, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, Brazil, 2009.

[73] M. Ravikumar, V. Dheenadhayalan, K. Rajaram et al., "Associations of HLA-DRB1, DQB1 and DPB1 alleles with pulmonary tuberculosis in south India," *Tubercle and Lung Disease*, vol. 79, no. 5, pp. 309–317, 1999.

[74] H. Ohyama, S. Matsushita, F. Nishimura et al., "T cell responses to major membrane protein II (MMP II) of Mycobacterium leprae are restricted by HLA-DR molecules in patients with leprosy," *Vaccine*, vol. 20, no. 3-4, pp. 475–482, 2001.

[75] G. P. Uko, L.-Y. Lu, M. A. Asuquo et al., "HLA-DRB1 leprogenic motifs in Nigerian population groups," *Clinical and Experimental Immunology*, vol. 118, no. 1, pp. 56–62, 1999.

[76] J. N. Agrewala and R. J. Wilkinson, "Influence of HLA-DR on the phenotype of $CD4^+$ T lymphocytes specific for an epitope of the 16-kDa alpha-crystallin antigen of Mycobacterium tuberculosis," *European Journal of Immunology*, vol. 29, pp. 1753–1761, 1999.

[77] T. Mutis, Y. E. Cornelisse, G. Datema, P. J. van den Elsen, T. H. M. Ottenhoff, and R. R. P. de Vries, "Definition of a human suppressor T-cell epitope," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 91, no. 20, pp. 9456–9460, 1994.

[78] W. F. C. Rigby, M. Waugh, and R. F. Graziano, "Regulation of human monocyte HLA-DR and CD4 antigen expression, and antigen presentation by 1,25-dihydroxyvitamin D3," *Blood*, vol. 76, no. 1, pp. 189–197, 1990.

[79] S. V. Ramagopalan, N. J. Maugeri, L. Handunnetthi et al., "Expression of the multiple sclerosis-associated MHC class II allele HLA-DRB1*1501 is regulated by vitamin D," *PLoS Genetics*, vol. 5, no. 2, Article ID e1000369, 2009.

[80] M. Bléry, L. Olcese, and E. Vivier, "Early signaling via inhibitory and activating NK receptors," *Human Immunology*, vol. 61, no. 1, pp. 51–64, 2000.

[81] E. O. Long, "Regulation of immune responses through inhibitory receptors," *Annual Review of Immunology*, vol. 17, pp. 875–904, 1999.

[82] D. S. A. Franceschi, P. S. Mazini, C. C. C. Rudnick et al., "Association between killer-cell immunoglobulin-like receptor genotypes and leprosy in Brazil," *Tissue Antigens*, vol. 72, no. 5, pp. 478–482, 2008.

[83] H. G. Shilling, L. A. Guethlein, N. W. Cheng et al., "Allelic polymorphism synergizes with variable gene content to individualize human KIR genotype," *Journal of Immunology*, vol. 168, no. 5, pp. 2307–2315, 2002.

[84] C. M. Gardiner, L. A. Guethlein, H. G. Shilling et al., "Different NK cell surface phenotypes defined by the DX9 antibody are due to KIR3DL1 gene polymorphism," *Journal of Immunology*, vol. 166, no. 5, pp. 2992–3001, 2001.

[85] J. Trowsdale, "Genetic and functional relationships between MHC and NK receptor genes," *Immunity*, vol. 15, no. 3, pp. 363–374, 2001.

[86] P. Parham, "MHC class I molecules and KIRS in human history, health and survival," *Nature Reviews Immunology*, vol. 5, no. 3, pp. 201–214, 2005.

[87] S. Bahram, M. Bresnahan, D. E. Geraghty, and T. Spies, "A second lineage of mammalian major histocompatibility complex class I genes," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 91, no. 14, pp. 6259–6263, 1994.

[88] C. Leelayuwat, D. C. Townend, M. A. Degli-Esposti, L. J. Abraham, and R. L. Dawkins, "A new polymorphic and multicopy MHC gene family related to nonmammalian class I," *Immunogenetics*, vol. 40, no. 5, pp. 339–351, 1994.

[89] T. Shiina, G. Tamiya, A. Oka et al., "Molecular dynamics of MHC genesis unraveled by sequence analysis of the 1,796,938-bp HLA class I region," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 96, no. 23, pp. 13282–13287, 1999.

[90] N. W. Zwirner, M. A. Fernández-Viña, and P. Stastny, "MICA, a new polymorphic HLA-related antigen, is expressed mainly by keratinocytes, endothelial cells, and monocytes," *Immunogenetics*, vol. 47, no. 2, pp. 139–148, 1998.

[91] S. Bauer, V. Groh, A. Steinle et al., "Activation of NK cells and T cells by NKG2D, a receptor for stress-inducible MICA," *Science*, vol. 285, no. 5428, pp. 727–729, 1999.

[92] J. Wu, Y. Song, A. B. H. Bakker et al., "An activating immunoreceptor complex formed by NKG2D and DAP10," *Science*, vol. 285, no. 5428, pp. 730–732, 1999.

[93] M. Gannagé, A. Buzyn, S. I. Bogiatzi et al., "Induction of NKG2D ligands by gamma radiation and tumor necrosis factor-α may participate in the tissue damage during acute graft-versus-host disease," *Transplantation*, vol. 85, no. 6, pp. 911–915, 2008.

[94] H. Yasuoka, Y. Okazaki, Y. Kawakami et al., "Autoreactive $CD8^+$ cytotoxic T lymphocytes to major histocompatibility complex class I chain-related gene A in patients with Behçet's disease," *Arthritis and Rheumatism*, vol. 50, no. 11, pp. 3658–3662, 2004.

[95] H. Suemizu, M. Radosavljevic, M. Kimura et al., "A basolateral sorting motif in the MICA cytoplasmic tail," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 99, no. 5, pp. 2971–2976, 2002.

[96] W. Sergio do Sacramento, P. S. Mazini, D. A. S. Franceschi et al., "Frequencies of MICA alleles in patients from southern Brazil with multibacillary and paucibacillary leprosy," *International Journal of Immunogenetics*, vol. 39, no. 3, pp. 210–215, 2012.

[97] T. Kasahara, J. J. Hooks, S. F. Dougherty, and J. J. Oppenheim, "Interleukin 2-mediated immune interferon (IFN-γ) production by human T cells and T cell subsets," *Journal of Immunology*, vol. 130, no. 4, pp. 1784–1789, 1983.

[98] A. Alcaïs, M. Mira, J. L. Casanova, E. Shurr, and L. Abel, "Genetic dissection of immunity in leprosy," *Current Opinion in Immunology*, vol. 17, no. 1, pp. 44–48, 2005.

[99] Y. Suh and J. Vijg, "SNP discovery in associating genetic variation with human disease phenotypes," *Mutation Research*, vol. 573, no. 1-2, pp. 41–53, 2005.

[100] B. B. Aggarwal, A. Samanta, and M. Feldmann, "TNFα," in *Cytokine Reference: A compendium of Cytokines and Other Mediators of Host Defense*, pp. 413–434, 2000.

[101] P. R. Vanderborght, H. J. Matos, A. M. Salles et al., "Single nucleotide polymorphisms (SNPs) at -238 and -308 positions in the TNFalpha promoter: clinical and bacteriological evaluation in leprosy," *International Journal of Leprosy and Other Mycobacterial Diseases*, vol. 72, no. 2, pp. 143–148, 2004.

[102] R. W. Malefyt, "IL-10," in *Cytokine Reference: A Compendium of Cytokines and Other Mediators of Host Defense*, pp. 165–185, 2000.

[103] C. Esche, M. R. Shurin, and M. T. Lotze, "IL-12," in *Cytokine Reference: A Compendium of Cytokines and Other Mediators of Host Defense*, pp. 187–201, 2000.

[104] A. Billiau and K. Vandenbroeck, "IFNG," in *Cytokine Reference: A Compendium of Cytokines and Other Mediators of Host Defense*, pp. 641–688, 2000.

[105] T. Matsuda and T. Hirano, "IL-6," in *Cytokine Reference: A compendium of Cytokines and Other Mediators of Host Defense*, pp. 537–563, 2000.

[106] L. Sousa, V. M. Fava, L. H. Sampaio et al., "Genetic and immunological evidence implicates interleukin 6 as a susceptibility gene for leprosy type 2 reaction," *The Journal of Infectious Diseases*, vol. 205, no. 9, pp. 1417–1424, 2012.

[107] F. R. Zhang, W. Huang, S. M. Chen et al., "Genomewide association study of leprosy," *The New England Journal of Medicine*, vol. 361, no. 27, pp. 2609–2618, 2009.

[108] S. H. Wong, A. V. S. Hill, and F. O. Vannberg, "Genomewide association study of leprosy," *The New England Journal of Medicine*, vol. 362, no. 15, pp. 1446–1447, 2010.

[109] S. H. Wong, S. Gochhait, D. Malhotra et al., "Leprosy and the adaptation of human toll-like receptor 1," *PLoS pathogens*, vol. 6, Article ID e1000979, 2010.

[110] V. Grant, A. Alter, N. T. Huong et al., "Crohn's disease susceptibility genes are associated with leprosy in the Vietnamese population," *The Journal Infectious Diseases*, vol. 206, no. 11, pp. 1763–1767, 2012.

[111] J. C. Barrett, S. Hansoul, D. L. Nicolae et al., "Genome-wide association defines more than 30 distinct susceptibility loci for Crohn's disease," *Nature Genetics*, vol. 40, no. 8, pp. 955–962, 2008.

[112] Q. Yang, H. Liu, H. -Q. Low et al., "Chromosome 2p14 is Linked to Susceptiblility to leprosy," *PLoS One*, vol. 7, no. 1, Article ID e29747, 2012.

Hindawi Publishing Corporation
BioMed Research International
Volume 2013, Article ID 146079, 11 pages
http://dx.doi.org/10.1155/2013/146079

Research Article

The Association of the Immune Response Genes to Human Papillomavirus-Related Cervical Disease in a Brazilian Population

Amanda Vansan Marangon,[1] Gláucia Andreia Soares Guelsin,[1] Jeane Eliete Laguila Visentainer,[2] Sueli Donizete Borelli,[2] Maria Angélica Ehara Watanabe,[3] Márcia Edilaine Lopes Consolaro,[4] Katiany Rizzieri Caleffi-Ferracioli,[4] Cristiane Conceição Chagas Rudnick,[5] and Ana Maria Sell[2]

[1] *Campinas State University, Rua Carlos Chagas 480, Barão Geraldo, 13083878 Campinas, SP, Brazil*
[2] *Basic Health Sciences Department, Maringa State University, Avenue Colombo 5790, 87020900 Maringá, PR, Brazil*
[3] *Pathology Sciences Department, Londrina State University, Rodovia Celso Garcia Cid (PR 445), 86051-970 Londrina, PR, Brazil*
[4] *Department of Clinical Analysis and Biomedicine, Maringa State University, Avenue Colombo 5790, 87020900 Maringá, PR, Brazil*
[5] *Program of Biosciences Applied to Pharmacy, Department of Clinical Analysis and Biomedicine, Maringa State University, Avenue Colombo 5790, 87020900 Maringá, PR, Brazil*

Correspondence should be addressed to Ana Maria Sell; anamsell@gmail.com

Received 29 March 2013; Revised 5 June 2013; Accepted 15 June 2013

Academic Editor: Enrique Medina-Acosta

The genetic variability of the host contributes to the risk of human papillomavirus (HPV)-related cervical disease. Immune response genes to HPV must be investigated to define patients with the highest risk of developing malignant disease. The aim of this study was to investigate the association of polymorphic immune response genes, namely *KIR*, HLA class I and II, and single-nucleotide polymorphisms (SNPs) of cytokines with HPV-related cervical disease. We selected 79 non-related, admixed Brazilian women from the state of Paraná, southern region of Brazil, who were infected with high carcinogenic risk HPV and present cervical intraepithelial neoplasia grade 3 (CIN3), and 150 HPV-negative women from the same region matched for ethnicity. *KIR* genes were genotyped using an in-house PCR-SSP. HLA alleles were typed using a reverse sequence-specific oligonucleotide technique. SNPs of *TNF −308G>A, IL6 −174G>C, IFNG +874T>A, TGFB1 +869T>C +915G>C,* and *IL10 −592C>A −819C>T −1082G>A* were evaluated using PCR-SSP. The *KIR* genes were not associated with HPV, although some pairs of i(inhibitory)KIR-ligands occurred more frequently in patients, supporting a role for NK in detrimental chronic inflammatory and carcinogenesis. Some HLA haplotypes were associated with HPV. The associations of *INFG* and *IL10* SNPs potentially reflect impaired or invalid responses in advanced lesions.

1. Introduction

Human papillomavirus (HPV) infections occur frequently in healthy individuals, and high carcinogenic risk (HR) HPV types are a major causal factor for cervical cancer. Persistent infection with one among approximately 15 genotypes of carcinogenic HPV causes almost all cases of cervical cancer; type 16 and HPV-18 account for more than 70% of the cervical cancers detected worldwide. Despite being considered a preventable disease, cervical cancer remains the second most common malignancy among women worldwide, with a higher incidence in underdeveloped countries [1, 2].

The major mechanisms by which HPV contribute to neoplastic initiation and progression involve the activity of two viral oncoproteins, E6 and E7, which interfere with the critical cell cycle tumor suppressive proteins p53 and retinoblastoma (Rb). However, HPV infection alone is not sufficient to induce

malignant transformation. The multistep process of tumor formation requires the contribution of other significant cofactors, such as individual genetic variations, intratypic HPV variability, and environmental factors [1, 2]. The genetic variability of the host also plays a role in the risk of developing cervical cancer, especially variability of genes that control the immune response. These highly polymorphic genes are important risk determinants of HPV persistence and disease progression.

The innate immune system comprises the first line of defense following HPV infection. It provides nonspecific protection and enhances the adaptive immune response [3]. Inflammatory cell infiltration occurs in response to HPV tissue damage, with infiltrates consisting initially of neutrophils followed by macrophages and T lymphocytes cells. NK and NKT cells contribute to antiviral innate immune responses. NK cell activation depends on type 1 interferon and proinflammatory cytokines such as IL-12 and IL-18; these cells are able to detect decreased expression of HLA class I in infected and transformed cells [4]. Most cervical HPV infections are cleared or suppressed via cell-mediated immunity: CD4+ and CD8+ T cells are the major effector cells [4], and the Th1 response is associated with clearance of the HPV infection and regression of the cervical cancer [5]. Th2 responses are associated with cervical carcinogenesis [6].

To define patients with the highest risk of developing malignant diseases, the interaction between the host immune response and HPV infection must be investigated. The goal of the present study was to investigate the association of the polymorphic immune response genes, namely, the *KIR* genes, HLA classes I and II, and SNPs of cytokines, with HPV infection in Brazilian women.

The *KIR* locus comprises an approximately 150 kb region located on chromosome 19q13.4, which encodes a group of inhibitory and activating KIR molecules. KIRs are key receptors of human natural killer (NK) cells, a subset of lymphocytes that trigger early innate immune response against infection and tumors [7].

The major histocompatibility complex (MHC), also known as the human leukocyte antigen (HLA) complex, located on chromosome 6p21.3, is the most polymorphic genetic system in mammalians and has been studied with regard to a wide variety of diseases of distinct etiology. The fundamental role of the different molecules within the MHC is antigen processing and presentation to the T-cell receptor (TCR), which is crucial for the cell interactions in cell-mediated immunity [8].

Polymorphisms of regulating regions of cytokine genes have been correlated with its production and can confer flexibility in the immune response to the viral infections and cancer biology. Five independent regions were investigated: chromosome 1: *IL10* region [9], chromosome 6: tumor necrosis factor (*TNF)* [10], chromosome 7: interleukin-6 *(IL6)* [11], chromosome 12: interferon-gamma (*IFNG*) [12], and chromosome 19: transforming growth factor-beta (*TGFB1*) region [13].

2. Materials and Methods

2.1. Patients and Samples. Patients comprised 79 nonrelated, admixed Brazilian women from the state of Paraná in the southern region of Brazil, who were infected with HR-HPV and present cervical intraepithelial neoplasia (CIN) grade 3 (CIN3) and 150 women from the same region matched for ethnicity who were HPV-negative/normal cytology. The study protocol was approved by the ethics committee, and all selected patients signed the free and informed consent form.

In the Paraná state, the degree of the European ancestry is high (80.6%), with a small but significant contribution of African (12.5%) and Amerindian (7.0%) genes according to Probst et al. [14], and the studied populations were considered admixed. The risk of population stratification bias, due to differences in ethnic background between patients and controls, and variations of allele frequencies, according to ethnic background, were minimized by matching patients with control individuals of the same ethnic background, mean age, gender rates, and residence in the same geographical areas.

The patients were diagnosed with high-grade squamous intraepithelial lesion (HSIL) by cytologic smears, CIN3 by histopathology, and also with HR-HPV.

2.1.1. Cytology and Histopathology. The cervical and endocervical material was collected with the aid of an Ayre spatula and a cytobrush for cervical smears and for PCR amplification (suspended in 1 mL of 0.9% NaCl solution and stored at −20°C until analysis). The cytological smears were evaluated and reported according to the Bethesda system as atypical squamous cells of undetermined significance (ASC-US); atypical squamous cells of undetermined significance, which cannot exclude a high-grade squamous intraepithelial lesion (ASC-H); low-grade squamous intraepithelial lesion (LSIL); high-grade squamous intraepithelial lesion (HSIL); *in situ* or invasive adenocarcinoma (ISCC); or invasive squamous cell carcinoma (SCC).

The cytological criteria for HSIL diagnosis adopted were squamous cells, either isolated or present in small fragments with fewer than ten cells. The cells were the length of the metaplastic cells, showing an increase in the proportion in the nuclear area. The nuclear irregularities, including hyperchromasia, chromatic clustering, irregularity, thickening, or multinucleation, were also used as important cytological criteria [15]. The histopathology findings of biopsy samples were classified as CIN grades I, II, or III, microinvasive or invasive squamous cell carcinoma, or *in situ* or invasive adenocarcinoma. The histological criteria for CIN were the failure of maturation of the squamous epithelium, nuclear hyperchromasia, and an increased nucleus/cytoplasm ratio. The intensity of these criteria was used to stage the degree of CIN or carcinomas [16]. The HSIL cytological cases included in the present study were confirmed as CIN III by histopatnology.

2.1.2. HPV Molecular Detection. For HPV molecular detection, genomic DNA was extracted using DNAzol (Invitrogen, Carlsbad, CA, USA). HPV polymerase chain reaction (PCR) amplification for HPV was carried out using

Table 1: Cytokine gene SNPs interrogated in this study.

Cytokine gene	Gene chromosome location	SNP designation in the kit	dbSNP-ID	Location
TNF	6p21.3	−308 G/A	rs1800629	Promoter
IFNG	12q14	+874 T/A	rs2430561	Intron
IL6	7p21	−174 G/C	rs1800795	Promoter
IL10	1q31-q32	−1082 A/G	rs1800896	Promoter
		−819 C/T	rs1800871	Promoter
		−592 C/A	rs1800872	Promoter
TGFB1	19q13.1	−509 T/C (or 869 T/C)	rs1800469	Promoter
		+915 G/C	rs1800471	Exon 1

MY09 (5′-CGTCCMAARGGAWACTGATC-3′)/MY11(5′-GCMCAGGGWCATAAYAATGG-3′), and the PCR product was electrophoresed on a 1.5% agarose gel, stained with 1 μg/mL ethidium bromide, and photodocumented under UV light (approximately 450 bp). Coamplification of the human beta-globin gene (approximately 268 bp) was performed as an internal control, using primers GH20 (5′-GAAGAGCCAAGGACAGGTAC-3′) and PC04 (5′-CAA CTTCATCCACG TTCACC-3′) under the same conditions as the HPV PCR. Two types of controls were also included in each reaction series: "no DNA" (negative control) and "HPV-positive DNA" (positive control) [17].

Genotyping was carried out using PCR-based restriction fragment length polymorphism analysis using *Hpy*CH4V (New England Biolabs, Inc., Ipswich, MA, USA). The following HPV genotypes were determined for this genotyping method: HR (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 66, 73, and 82), UR—undetermined risk (26 and 53), and LR—low-risk (6, 11, 30, 34, 40, 42, 43, 44, 54, 55, 61, 62, 64, 67, 69, 70, 72, 74, 81, 83, 84, and 91). The genotypes were grouped according to the International Agency for Research on Cancer (IARC) based on the carcinogenic potential and evolutionary branch [18].

2.2. Genotyping of KIR, HLA, and Cytokine Genes. Genomic DNA samples were extracted from 150 μL of the buffy coat obtained from 5 mL of EDTA anticoagulant peripheral blood using the EZ-DNA Kit (Biological Industries, Beit Haemek, Israel). The DNA concentration was then determined using a Qubit fluorometer (Life Technologies Corporation, Eugene, Oregon, USA).

All genotyping methods were validated using previously typed and tested reference samples. Positive and negative controls were included in all genotyping method.

2.2.1. KIR Genes Genotyping. Fourteen *KIR* genes and one pseudogene (*KIR2DL1, KIR2DL2, KIR2DL3, KIR2DL4, KIR2DL5, KIR2DS1, KIR2DS2, KIR2DS3, KIR2DS4, KIR2DS5, KIR3DL1, KIR3DL2, KIR3DL3, KIR3DS1,* and *KIR2DP1*) were studied using an in-house polymerase chain reaction using the sequence-specific primer method (PCR-SSP) according to Martin et al. [19] and adapted by Rudnick et al. [20]. Primers were synthesized by Invitrogen (Life Technologies Corporation, Grand Island, NY, USA), and the amplified products were visualized by 2% agarose gel electrophoresis.

2.2.2. HLA Classes I and II Typing. HLA classes I and II allele typing was conducted using the reverse sequence-specific oligonucleotide technique (rSSO; One Lambda Inc., Canoga Park, CA, USA) with Luminex xMap technology (Luminex Corporation, Austin, USA). HLA groups 1 (C1) and 2 (C2) of HLA-C and group Bw4 of HLA-B were defined according to Carrington and Norman [21] and Petersdorf [22].

2.2.3. Genotyping of SNPs in Cytokine Genes. Sequence-specific primer PCR (PCR-SSP; One Lambda Cytokine Genotyping Primer Pack, One Lambda, CA, USA) was performed to genotype the following SNPs: *TNF −308G>A* (rs1800629), *IFNG +874T>A* (rs2430561), *IL6 −174G>C* (rs1800795), *IL10 −1082G>A* (rs1800896), *IL10 −819C>T* (rs1800871), *IL10 −592C>A* (rs1800872), *TGFB −509T>C* (rs1800469), and *TGFB1 +915G>C* (rs1800471) (Table 1), according to the manufacturer's instructions.

2.3. Statistical Analyses. Allele, genotype, and haplotype frequencies of *KIR*, HLA, and cytokines were calculated by direct counting. Fisher's exact test and the chi-square test with Yates' correction were used for statistical comparisons. $P \leq 0.05$ were considered significant, and P values were adjusted by means of the Bonferroni correction to enable multiple comparisons. The odds ratio (OR) was calculated based on the cross product ratio and the exact 95% confidence intervals (CI) using the SISA statistical package (http://www.quantitativeskills.com/sisa/index.htm). Hardy-Weinberg equilibrium [23] was determined by calculating the expected genotype frequencies and comparing them to the observed values using Arlequin software version 3.1 (http://cmpg.unibe.ch/software/arlequin3/).

3. Results

The distributions of allele frequency ratios for all analyzed genes and for *KIR* haplotype frequencies were in Hardy-Weinberg equilibrium.

There were no significant differences between *KIR* genes frequencies in patients and controls (Table 2), and the frequency distribution was similar to that reported in another study of the same region [24].

There was no relationship in the frequencies of ligands (C1, C2, Bw4, and HLA-A3/11) and in the combination of KIR-HLA ligands with the HPV disease (Table 3).

Table 2: Frequencies of *KIR* genes in HPV patients and controls.

KIR genes	HPV patients $N = 71$		Controls $N = 118$	
	n	%	*n*	%
2DL1	68	95.8	116	98.3
2DL2	35	49.3	55	46.6
2DL3	63	88.7	104	88.1
2DL5	43	60.6	62	52.5
2DP1	68	95.8	115	97.4
2DS1	30	42.2	50	42.4
2DS2	37	52.1	57	48.3
2DS3	26	36.6	36	30.5
2DS4	65	91.5	108	91.5
2DS5	28	39.4	40	33.9
3DL1	65	91.5	109	92.4
3DS1	31	43.6	48	40.7
2DL4, 3DL2, 3DL3, and *3DP1*	71	100	118	100

KIR gene frequencies were similar in both the groups ($P \geq 0.05$).

Table 3: Distribution of KIR and HLA ligands in HPV patients and controls.

KIR and HLA ligands	HPV patients		Controls	
	n	%	*n*	%
2DL1-C2	46	67.65	78	67.24
2DL1 without C2	22	32.35	38	32.75
2DL2-C1	28	80.0	43	78.18
2DL2 without C1	7	20.0	12	21.81
2DL3-C1	54	85.71	87	83.65
2DL3 without C1	9	14.29	17	16.34
3DL1-Bw4	44	67.69	73	66.97
3DL1 without Bw4	21	32.31	36	33.02
2DS1-C2	22	73.33	30	60.00
2DS1 without C2	8	26.67	20	40.00
2DS2-C1	30	81.08	45	78.94
2DS2 without C1	7	18.92	12	21.05
3DS1-Bw4	21	67.74	30	62.50
3DS1 without Bw4	10	32.26	18	37.50

Bw4: HLA-A∗23, 24, and 32; HLA-B∗08, 13, 27, 37, 44, 51, 52, 53, 57, and 58.
Group C1: HLA-C∗01, 03, 07, 08, 12, 14, and 16.
Group C2: HLA-C∗02, 04, 05, 06, 07, 15, 17, and 18.
Difference was not observed ($P \geq 0.05$).

The number and type of inhibitory KIR-HLA pairs were evaluated (Table 4), and there was a greater frequency in the patients of three pairs (38.0%) and two pairs (36.6%), followed by one pair (14.1%) and four pairs (11.2%). In the controls, two pairs (46.6%) were the most frequent, followed by three (32.2%), one (11.0%), and four (10.2%) pairs. The pairs KIR3DL1-Bw4 and KIR3DL2-HLA-A3/11 were not detected in either group. Significant difference was observed between patients and controls with respect to the three pairs KIR2DL2/3-C1, KIR3DL1-Bw4, and KIR3DL2-A3/11, which were more frequent in the patients.

Twenty-six KIR haplotypes were observed in HPV patients, 10 of them were not found in controls, and, otherwise, 13 others haplotypes were present only in controls (Figure 1). There were no differences between patients and controls.

No differences were observed in the distribution of HLA-A, B, and DRB1 allele groups between patients and controls (Table 5).

Only the *HLA-A∗02-HLA-B∗51* haplotype showed a reduced frequency in HPV patients (0.006 versus 0.052, $P = 0.0065$, OR = 0.1186, and 95% CI = 0.015–0.8717) than in controls; the other six haplotypes were more frequent in the patients, but a large CI was obtained due to the small number of patients and controls (Table 6).

The cytokine allele frequencies did not differ between HPV patients and controls (Table 7), and the frequencies distribution were consistent with the results of a previous study of the same region [25].

Based on the genotypes, the phenotypes of cytokines production level were inferred (low, intermediate, or high). There were significant differences for the low producer phenotypes of INF-γ, defined by genotype AA [12], which had an increased frequency in patients (35.90 versus 29.59; $P = 0.0221$; OR = 1.81; 95% CI = 1.18–4.60), and for an intermediate producer of IL-10 (32.05% versus 48.00%; $P = 0.0462$; OR = 0.1607; 95% CI = 0.28–0.95) defined by the haplotype GCC/ACC and GCC/ATA (Table 8). The GCC/GCC genotype (high producer phenotype of IL-10) [9] was more frequent among patients (20.5%) compared with controls (12%), although this difference was not significant.

4. Discussion

It is widely accepted that cofactors, including endogenous hormones and genetic factors, such as HLA and other genes related to the host immune response, may have important roles in the development of HPV-cervical lesions [26]. Inherited genetic polymorphisms within immune response genes have been shown to be associated with an increased risk of invasive cervical cancer and its immediate precursor, cervical intraepithelial neoplasia grade 3 [27]. An inappropriate innate and specific immune response may increase the risk of lesions and disease progression.

4.1. KIR and Their HLA Ligands. NK cells play an important role in innate immunity against infected and transformed cells as part of the immune surveillance process. *KIR* genes encode molecules that convey either inhibitory or activating signals (iKIR and aKIR) to NK cells and to a subset of CD8+ T cells. Binding of iKIR (designated 2DL and 3DL) to specific HLA allotypes has been clearly demonstrated and correlated to the ability to inhibit NK cytolysis of target cells bearing these HLA molecules. These interactions are remarkably complex, and synergistic relationship between these polymorphic loci may regulate NK cell-mediated immunity against viral infections [28].

No relationship was found between KIR genes and HPV-related cervical disease in Brazilian patients, consistent with

Table 4: Combinations of inhibitory KIR-HLA pairs and their frequencies in HPV and control Brazilian women from Paraná, Southern Brazil.

Number of pairs	KIR-HLA	HPV patients *n* (%)	Control *n* (%)
1 pair	2DL2/3-C1	3 (30.0)	7 (61.5)
	2DL1-C2	7 (70.0)	4 (38.5)
2 pairs	2DL2/3-C1, 3DL1-Bw4	12 (46.2)	16 (34.5)
	2DL2/3-C1, 2DL1-C2	7 (26.9)	14 (29.0)
	2DL1-C2, 3DL1-Bw4	4 (15.4)	11 (23.6)
	2DL1-C2, 3DL2-A3/11	2 (7.7)	4 (9.1)
	2DL2/3-C1, 3DL2-A3/11	1 (3.9)	2 (3.7)
3 pairs	2DL1-C2, 2DL2/3-C1, 3DL1-Bw4	14 (51.9)	25 (76.3)
	2DL2/3-C1, 3DL1-Bw4, 3DL2-A3/11[a]	7 (25.9)	2 (5.3)
	2DL1-C2, 2DL2/3-C1, 3DL2-A3/11	6 (23.0)	5 (15.8)
	2DL1-C2, 3DL1-Bw4, 3DL2-A3/11	0 (0)	1 (2.6)
4 pairs	2DL1-C2, 2DL2/3-C1, 3DL1-Bw4, 3DL2-A3/11	8 (100)	12 (100)

[a] $P = 0.025$; OR = 3.42; 95% CI = 2.45–18.22.

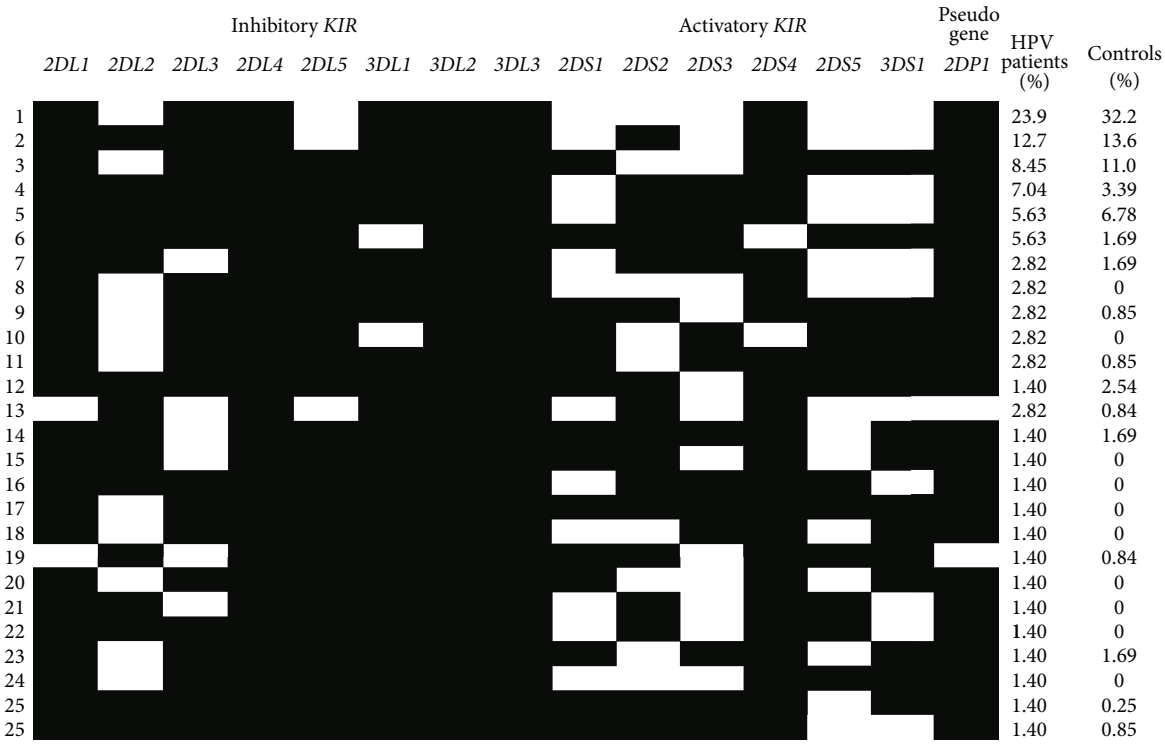


Figure 1: *KIR* genotypes frequencies in HPV patients and controls from Paraná, Southern Brazil.

the findings of Song et al. in Korean patients [29]. Although not significant, *KIR2DS3, KIR2DS5,* and *KIR2DL5* were more frequent in the patient group. *KIR2DL5*, an inhibitory receptor, possesses a combination of genetic, structural, and functional features that make it unique among the KIR [30] and potentially contribute to HPV pathogenesis. *KIR3DS1* can induce a persistent, weak inflammatory reaction to HPV that results in continuous tissue injury, similar to HBV-susceptible genes [31]. Carrington et al. [32] found that the presence of the activating *KIR3DS1* is related to an increased risk of neoplasia, particularly in the absence of protective inhibitory KIR-HLA. In contrast, Arnheim et al. [33] indicated that the inhibitory allele *KIR3DL1* is associated with increased risk of CIN.

The frequencies of the ligand groups (C1, C2 group, Bw4, and HLA-A3/11) did not differ between patients and controls (data not shown). However, Madeleine et al. [34] demonstrated an association between HLA-C subtypes and squamous cell cervical cancer, and Martin et al. [27] showed that C1 (asparagine at position 80) is over represented in

Table 5: HLA allele frequencies in HPV patients and control groups.

HLA-A allele types			HLA-B allele types			HLA-DRB1 allele types		
	HPV patients	Controls		HPV patients	Controls		HPV patients	Controls
	$N = 156$	$n = 300$		$n = 156$	$n = 300$		$n = 156$	$n = 300$
	n (f%)	n (f%)		n (f%)	N (f%)		n (f%)	n (f%)
A∗01	14 (9.0)	31 (10.3)	**B∗07**	8 (5.1)	17 (5.7)	**DRB1∗01**	20 (12.8)	51 (17.0)
A∗02	37 (23.7)	67 (22.3)	**B∗08**	11 (7.0)	15 (5)	**DRB1∗03**	17 (10.9)	23 (7.7)
A∗03	21 (13.5)	29 (9.7)	**B∗13**	3 (1.9)	6 (2)	**DRB1∗04**	8 (5.1)	25 (8.3)
A∗11	11 (7.0)	21 (7.0)	**B∗14**	9 (5.8)	14 (4.7)	**DRB1∗07**	21 (13.5)	33 (11.0)
A∗23	7 (4.5)	13 (4.3)	**B∗15**	22 (14.1)	26 (8.7)	**DRB1∗08**	10 (6.4)	17 (5.7)
A∗24	20 (12.8)	31 (10.3)	**B∗18**	6 (3.8)	21 (7)	**DRB1∗09**	3 (19.0)	4 (1.3)
A∗25	2 (1.2)	7 (2.3)	**B∗27**	6 (3.8)	10 (3.3)	**DRB1∗10**	6 (3.8)	7 (2.3)
A∗26	2 (1.2)	13 (4.3)	**B∗35**	18 (11.5)	39 (13)	**DRB1∗11**	27 (17.3)	34 (11.3)
A∗29	8 (5.1)	14 (4.7)	**B∗37**	2 (1.3)	4 (1.3)	**DRB1∗12**	4 (2.6)	6 (2.0)
A∗30	12 (7.7)	15 (5.0)	**B∗38**	2 (1.3)	3 (1)	**DRB1∗13**	17 (10.9)	47 (15.7)
A∗31	6 (3.8)	13 (4.3)	**B∗39**	5 (3.2)	10 (3.3)	**DRB1∗14**	8 (5.1)	14 (4.7)
A∗32	2 (1.3)	9 (3.0)	**B∗40**	5 (3.2)	15 (5)	**DRB1∗15**	8 (5.1)	25 (8.3)
A∗33	3 (1.9)	14 (4.7)	**B∗41**	1 (0.6)	6 (2)	**DRB1∗16**	7 (4.5)	14 (4.7)
A∗34	1 (0.6)	1 (0.3)	**B∗42**	1 (0.6)	10 (0.3)			
A∗66	1 (0.6)	3 (1.0)	**B∗44**	21 (13.5)	34 (11.3)			
A∗68	9 (5.8)	16 (5.3)	**B∗45**	4 (2.6)	5 (1.7)			
			B∗48	1 (0.6)	1 (0.3)			
			B∗49	4 (2.6)	9 (3.0)			
			B∗50	2 (1.3)	8 (2.7)			
			B∗51	12 (7.7)	25 (8.3)			
			B∗52	4 (2.6)	5 (1.7)			
			B∗53	1 (0.6)	10 (3.3)			
			B∗55	1 (0.6)	3 (1.0)			
			B∗57	4 (2.6)	9 (3.0)			
			B∗58	3 (1.9)	4 (1.3)			

N: number of alleles; n: number of individuals; f%: alleles frequencies.
Difference was not observed between both groups ($P \geq 0.05$).

Table 6: HLA haplotype frequencies with significant differences between HPV patients and controls.

Haplotypes	Patients n (hf)	Controls n (hf)	P	OR	CI
HLA-A∗02–HLA-B∗51	1 (0.006)	16 (0.052)	0.006	0.1186	0.015–0.8717
HLA-A∗03–HLA-DRB1∗11	7 (0.043)	1 (0.003)	0.002	150.828	1.712–115.230
HLA-B∗14–HLA-DRB1∗13	5 (0.032)	1 (0.003)	0.017	108.906	1.146–85.504
HLA-B∗15–HLA-DRB1∗07	5 (0.029)	1 (0.003)	0.017	108.906	1.146–85.505
HLA-B∗15–HLA-DRB1∗11	7 (0.048)	1 (0.003)	0.002	150.828	1.712–115.230
HLA-B∗44–HLA-DRB1∗01	6 (0.036)	2 (0.005)	0.018	49.028	1.188–29.885
HLA-B∗44–HLA-DRB1∗11	7 (0.048)	1 (0.003)	0.002	150.828	1.712–115.230

n: haplotype numbers; hf: haplotype frequencies (%); P: P value; OR: odds ratio; CI (95%): 95% confidence interval.

women with cervical cancer. In Korean women, HLA-C is associated with HPV-cervical disease: HLA-C∗03:03 confers susceptibility whereas HLA-C∗01 has a protective effect [29].

In the present work, KIR2DL1-C2 was more frequent in patients (70%) than in controls (38.5%). The strength of NK inhibition varies according to the receptor and the ligand: KIR2DL1-C2 provides a stronger inhibition than other iKIR-HLA [32, 35]. The reduced resistance to viral infections among KIR2DL1-C2-positive individuals may result from the increased inhibition of NK cells. There were significant differences for the three pairs KIR2DL2/3-C1, KIR3DL1-Bw4, and KIR3DL2-A3/11, which displayed an increased frequency in patients. This combination of iKIR and ligands could be associated with persistent inflammatory reactions that play a role in carcinogenesis.

4.2. HLA and Its Association with HPV and CIN. HLA class I and class II proteins are central to host immune responses to viral infections and other pathogens. They are the most polymorphic genes in the human genome, and variations in the peptide binding groove of these proteins influence antigenic specificity. Numerous studies have evaluated the

Table 7: Cytokines alleles and genotypes frequencies in HPV patients and controls.

Cytokine alleles and genotypes	HPV patients $N = 79$ $n\ (f\%)$	Controls $N = 101$ $n\ (f\%)$	Cytokine alleles and genotypes	HPV patients $N = 79$ $n\ (f\%)$	Controls $N = 100$ $n\ (f\%)$
TNF −308			*IL10 −1082*		
G	139 (88.0)	174 (86.1)	*G*	50 (32.1)	72 (36)
A	19 (12.0)	28 (13.9)	*A*	106 (68)	128 (64)
G/G	62 (78.5)	73 (72.3)	*G/G*	9 (11.5)	12 (12)
G/A	15 (19.0)	28 (27.7)	*G/A*	32 (41.0)	48 (48)
A/A	2 (2.5)	0 (0)	*A/A*	37 (47.4)	40 (40)
INFG +874			*IL10 −819*		
T	69 (44.2)	88 (44.9)	*C*	98 (62.8)	129 (64.5)
A	87 (55.8)	108 (55.1)	*T*	58 (37.2)	71 (35.5)
T/T	19 (24.4)	19 (19.4)	*C/C*	31 (39.8)	42 (42)
T/A	31 (39.7)	50 (51.0)	*C/T*	36 (46.2)	45 (45)
A/A	28 (35.9)	29 (29.6)	*T/T*	11 (14.1)	13 (13)
IL6 −174			*IL10 −592*		
G	116 (73.4)	132 (65.4)	*C*	98 (62.8)	129 (64.5)
C	42 (26.6)	70 (34.7)	*A*	58 (37.2)	71 (35.5)
G/G	44 (55.7)	45 (44.6)	*C/C*	31 (39.8)	42 (42)
G/C	28 (35.4)	42 (41.6)	*C/A*	36 (46.2)	45 (45)
C/C	7 (8.9)	14 (13.9)	*A/A*	11 (14.1)	13 (13)
TGFB1 +869			*TGFB1 +915*		
T	89 (56.3)	108 (54)	*G*	141 (89.2)	190 (95)
C	69 (43.7)	92 (46)	*C*	17 (10.7)	10 (5)
T/T	26 (32.9)	25 (25)	*G/G*	63 (79.8)	90 (90)
T/C	37 (46.8)	58 (58)	*G/C*	15 (19.0)	10 (10)
C/C	16 (20.3)	17 (17)	*C/C*	1 (1.3)	0 (0)

n: number of observed alleles and genotypes; $f\%$: allele and genotype frequencies.
Difference was not observed between both groups ($P \geq 0.05$).

association of HLA with HPV infection and the importance of HLA in the pathogenesis of cervical neoplasia [36]. Similar to other studies [37–39], there was no association between HLA specificities and HPV infection in admixed Brazilian women from the state of Paraná. However, the *HLA-A∗02-B∗51* haplotype was associated with resistance to disease. Susceptibility to HPV infection or cervical cancer and precancerous lesion development was associated with the HLA class II: HLA-DRB1 alleles [34, 39–51]; HLA-DQB1 alleles [34, 39, 46, 49–51]; HLA-DPB1 alleles [51]; and classes I and II haplotypes [30, 34, 40–42, 48, 52, 53]. Some alleles and haplotypes had a protective effect against the progression to infection and cancer [34, 38–40, 43, 46, 47, 51, 54, 55]. In general, *HLA-DQB1∗03* increases and *DRB1∗13* decreases the risk of cervical cancer. In other Brazilian populations, Maciag et al. [44] found that HLA class II polymorphism was involved in genetic susceptibility to HPV infection and cervical cancer: *DRB1∗15:03*, *DRB1∗04:05,* and *DQB1∗06:02* alleles.

A genome-wide association study of 731.422 SNPs was performed in cervical cancer patients and controls [56]. Three independent loci in MHC region were associated with cervical cancer: the first is adjacent to the MHC class I polypeptide-related sequence A gene (MICA) (rs2516448; OR = 1.42; 95% CI = 1.31 to 1.54; $P = 1.6 \times 10^{-18}$); the second is between HLA-DRB1 and HLA-DQA1 (rs9272143; OR = 0.67; 95% CI = 0.62 to 0.72; $P = 9.3 \times 10^{-24}$); and the third is at HLA-DPB2 (rs3117027; OR = 1.25; 95% CI = 1.15 to 1.35; $P = 4.9 \times 10^{-8}$). Previously reported associations of *B∗07:02* and *DRB1∗15:01-DQB1∗06:02* with susceptibility to *DRB1∗13:01-DQA1∗01:03-DQB1∗06:03* with protection against cervical cancer were confirmed.

The variable results for the association between HLA and disease could be related to the differences in the distribution of HLA in the population; the disease phases (persistence or transitory HPV infection, intraepithelial neoplasia, and cancer); and HPV types. The effects of HLA polymorphisms on cervical carcinogenesis and their biological mechanisms remain unknown. Previous findings suggest a strong link between an inefficient immune response, particularly inefficient cell-mediated and innate immunity, both of which involve classes I and II HLA alleles, and susceptibility to HPV infection. HPV infections are more prevalent and more likely to persist in immunosuppressed individuals.

Table 8: Expected phenotype frequencies according to genotypes for the cytokines TNF-α, IFN-γ, IL-6, IL-10, and TGF-β1.

Phenotypes	Genotypes	Patients ($N = 79$) n (%)	Controls ($N = 101$) n (%)
	TNF		
Low	*G/G*	62 (78.48)	73 (72.28)
High	*G/A* *A/A*	17 (21.52)	28 (27.72)
	IL6		
High	*G/G* *G/C*	72 (91.14)	87 (86.14)
Low	*C/C*	7 (8.86)	14 (13.86)
	INFG		
High	*T/T*	19 (24.36)	19 (19.39)
Intermediate	*T/A*	31 (39.74)	50 (51.02)
Low[b]	*A/A*	28 (35.90)	29 (29.59)
	IL10		
High	*GCC/GCC*	16 (20.51)	12 (12.00)
Intermediate[a]	*GCC/ACC* *GCC/ATA*	25 (32.05)	48 (48.00)
Low	*ACC/ACC* *ACC/ATA* *ATA/ATA*	37 (47.44)	40 (40.00)
	TGFB1		
High	*T/T G/G* *T/C G/G*	52 (65.82)	78 (78.00)
Intermediate	*T/C G/C* *C/C G/G* *T/T G/C*	22 (27.85)	17 (17.00)
Low	*C/C G/C* *C/C C/C* *T/T C/C* *T/C C/C*	5 (6.33)	5 (5.00)

n: number of excepted phenotypes according to genotypes.
%: frequencies.
[a] $P = 0.046$; OR = 0.1607; 95% CI = 0.276–0.947.
[b] $P = 0.022$; OR = 1.81; 95% CI = 1.178–4.604.

4.3. Cytokines and HPV. Accumulating epidemiological evidence suggests that polymorphisms in cytokine genes may be involved in the etiology of cervical carcinoma [6]. Th1 cytokines such IFN-γ and TNF-α can induce a cell-mediated immune response, whereas Th2 cytokines such as IL-6 and IL-10 induce predominantly a humoral immune response and immunomodulation of the cellular response. The Th2 cytokine profile is associated with progression to cervical cancer [57].

In the present study, we genotyped SNPs of *TNF, IFNG, IL6, IL10,* and *TGFB1* which are multifunctional cytokine that have been implicated in inflammation, immunity, and cellular organization and have been proposed to play important roles in infection and cancer biology.

Susceptibility to infection was observed in patients with the *IFNG +874A/A* genotype, which characterized the low producer phenotype of IFN-γ and was more frequent among patients compared with controls. According to Telesheva et al. [58], the outcome of HPV infection is controlled by the interferon component of the immune response: a transitory course of HPV infection is characterized by increased levels of IFN-alpha and IFN-gamma, and persistent infection is related to decreased levels of IFN-alpha.

In the current study, the *IL10 GCC/ACC* and *GCC/ATA* genotype, which characterized the intermediate producer phenotype of IL-10, were less frequent in patients, suggesting protection against disease. The IL-10 high producer phenotypes was more frequent in patients, although this increased frequency was not significant and might be related to an immunosuppressive response and development of HPV-positive cervical cancer. Serum levels of IL-10 and its expression in tumor cells are elevated in patients with cervical cancer [56]. IL-10 produced by tumor macrophages induces a regulatory phenotype in T cells and an escape mechanism of the immune response that facilitates tumor growth [59]. The SNP *IL10 −1082G>A* was not associated with susceptibility to the development of cervical cancer or HPV infection [60].

TGF-β is well known for its antiproliferative effects; however, neoplastic cells often lose their sensitivity to TGF-β. Iancu et al. [61] showed that in human cervical cancer, disruption of the TGF-β signaling pathway might contribute to the malignant progression of cervical dysplasia. In the present study, SNPs of *TGFB1 +869*, *+915* were not associated with HPV infection.

TNF-α, which is secreted mainly by activated macrophages, is an extraordinarily pleiotropic cytokine that has a central role in immune homeostasis, inflammation, and host defense and could be involved in protection against HPV infection by modulating viral replication. Dysregulated TNF expression within the tumor microenvironment appears to favor malignant cell tissue invasion, migration, and ultimately metastasis [62]. Our findings are similar to those reported by Wang et al. [63], who demonstrated that there is no significant association between the *TNF −308G>A* and HPV infection or cervical cancer. However, our findings differ from those reported for the Argentina population, among whom the high producer allele *TNFA −307A* was associated with an increased risk for the development of cervical cancer [64].

IL6 encodes a cytokine that plays important roles in the risk for cervical carcinogenesis. In the present study, there was no significant association between *IL6* and HPV-related cervical disease. However, a previous report has shown a significant association between the IL6-rs2069837 SNP and an increased risk of cervical cancer [65].

5. Conclusion

The genetic variability of the host contributes to the risk of HPV-related cervical disease. *KIR* genes were not associated with HPV, although some pairs of iKIR-ligands were more frequent in patients, suggesting that NK cells play a role in detrimental chronic inflammatory conditions and

in carcinogenesis. HLA was associated with HPV and participated in the immune response, although its function in carcinogenesis remains unclear. The polymorphic *INFG* and *IL10* genes were associated with the outcome of HPV infection and might be indicative of impaired or invalid immune responses in patients with advanced stage lesions. Additional studies of the immune response to HPV are needed to better define the risk of developing malignant diseases associated with HPV infection.

Acknowledgments

The authors are grateful to all of the volunteers who participated in this study and to both Fabiano Cavalcante de Melo and Marco Antonio Braga for performing the technical procedures.

References

[1] M. Schiffman, P. E. Castle, J. Jeronimo, A. C. Rodriguez, and S. Wacholder, "Human papillomavirus and cervical cancer," *The Lancet*, vol. 370, no. 9590, pp. 890–907, 2007.

[2] D. Subramanya and P. D. Grivas, "HPV and cervical cancer: updates on an established relationship," *Postgraduate Medicine*, vol. 120, no. 4, pp. 7–13, 2008.

[3] C. D. Woodworth, "HPV innate immunity," *Frontiers in Bioscience*, vol. 7, pp. d2058–d2071, 2002.

[4] M. H. Hibma, "The immune response to papillomavirus during infection persistence and regression," *The Open Virology Journal*, vol. 6, pp. 241–248, 2012.

[5] T. Sasagawa, H. Takagi, and S. Makinoda, "Immune responses against human papillomavirus (HPV) infection and evasion of host defense in cervical cancer," *Journal of Infection and Chemotherapy*, vol. 18, no. 6, pp. 807–815, 2012.

[6] A. Sharma, M. Rajappa, A. Satyam, and M. Sharma, "Cytokines (T_H1 and T_H2) in patients with advanced cervical cancer undergoing neoadjuvant chemoradiation correlation with treatment response," *International Journal of Gynecological Cancer*, vol. 19, no. 7, pp. 1269–1275, 2009.

[7] S. Rajagopalan and E. O. Long, "Understanding how combinations of HLA and KIR genes influence disease," *Journal of Experimental Medicine*, vol. 201, no. 7, pp. 1025–1029, 2005.

[8] R. N. Germain and D. H. Margulies, "The biochemistry and cell biology and antigen processing and presentation," *Annual Review of Immunology*, vol. 11, pp. 403–450, 1993.

[9] D. M. Turner, D. M. Williams, D. Sankaran, M. Lazarus, P. J. Sinnott, and I. V. Hutchinson, "An investigation of polymorphism in the interleukin-10 gene promoter," *European Journal of Immunogenetics*, vol. 24, no. 1, pp. 1–8, 1997.

[10] K. M. Kroeger, K. S. Carville, and L. J. Abraham, "The -308 tumor necrosis factor-α promoter polymorphism effects transcription," *Molecular Immunology*, vol. 34, no. 5, pp. 391–399, 1997.

[11] D. Fishman, G. Faulds, R. Jeffey et al., "The effect of novel polymorphisms in the interleukin-6 (IL-6) gene on IL-6 transcription and plasma IL-6 levels, and an association with systemic-onset juvenile chronic arthritis," *Journal of Clinical Investigation*, vol. 102, no. 7, pp. 1369–1376, 1998.

[12] V. Pravica, A. Asderakis, C. Perrey, A. Hajeer, P. J. Sinnott, and I. V. Hutchinson, "In vitro production of IFN-γ correlates with CA repeat polymorphism in the human IFN-γ gene," *European Journal of Immunogenetics*, vol. 26, no. 1, pp. 1–3, 1999.

[13] M. R. Awad, A. El-Gamel, P. Hasleton, D. M. Turner, P. J. Sinnott, and I. V. Hutchinson, "Genotypic variation in the transforming growth factor-β1 gene: association with transforming growth factor-β1 production, fibrotic lung disease, and graft fibrosis after lung transplantation," *Transplantation*, vol. 66, no. 8, pp. 1014–1020, 1998.

[14] C. M. Probst, E. P. Bompeixe, N. F. Pereira et al., "HLA polymorphism and evaluation of European, African, and Amerindian contribution to the white and mulatto populations from Paraná, Brazil," *Human Biology*, vol. 72, no. 4, pp. 597–617, 2000.

[15] D. Solomon and R. Nayar, *Bethesda System for Cervical-Vaginal Cytology*, Revinter, Rio de Janeiro, Brazil, 2005.

[16] D. C. Szurkus and T. A. Harrison, "Loop excision for high-grade squamous intraepithelial lesion on cytology: correlation with colposcopic and histologic findings," *American Journal of Obstetrics and Gynecology*, vol. 188, no. 5, pp. 1180–1182, 2003.

[17] W. Qu, G. Jiang, Y. Cruz et al., "PCR detection of human papillomavirus: comparison between MY09/MY11 and GP5+/GP6+ primer systems," *Journal of Clinical Microbiology*, vol. 35, no. 6, pp. 1304–1310, 1997.

[18] E. Santiago, L. Camacho, M. L. Junquera, and F. Vázquez, "Full HPV typing by a single restriction enzyme," *Journal of Clinical Virology*, vol. 37, no. 1, pp. 38–46, 2006.

[19] M. P. Martin, G. Nelson, J.-H. Lee et al., "Cutting edge: susceptibility to psoriatic arthritis: influence of activating killer Ig-like receptor genes in the absence of specific HLA-C alleles," *Journal of Immunology*, vol. 169, no. 6, pp. 2818–2822, 2002.

[20] C. C. C. Rudnick, G. A. S. Guelsin, A. V. Marangon, D. S. A. Franceschi, A. M. Sell, and J. E. L. Visentainer, "Methodology optimization for KIR genotyping," *Jornal Brasileiro de Patologia e Medicina Laboratorial*, vol. 46, no. 3, pp. 215–224, 2010.

[21] M. Carrington and P. Norman, *The KIR Gene Cluster*, National Library of Medicine (US), NCBI, Bethesda, Md, USA, 2003.

[22] E. W. Petersdorf, "Risk assessment in haematopoietic stem cell transplantation: histocompatibility," *Best Practice and Research: Clinical Haematology*, vol. 20, no. 2, pp. 155–170, 2007.

[23] S. W. Guo and E. A. Thompson, "Performing the exact test of Hardy-Weinberg proportion for multiple alleles," *Biometrics*, vol. 48, no. 2, pp. 361–372, 1992.

[24] C. C. C. Rudnick, D. S. A. Franceschi, A. V. Marangon, G. A. S. Guelsin, A. M. Sell, and J. E. L. Visentainer, "Killer cell immunoglobulin-like receptor gene diversity in a Southern Brazilian population from the state of Paraná," *Human Immunology*, vol. 69, no. 12, pp. 872–876, 2008.

[25] J. E. L. Visentainer, A. M. Sell, G. C. Da Silva et al., "TNF, IFNG, IL6, IL10 and TGFB1 gene polymorphisms in South and Southeast Brazil," *International Journal of Immunogenetics*, vol. 35, no. 4-5, pp. 287–293, 2008.

[26] E. M. Burd, "Human papillomavirus and cervical cancer," *Clinical Microbiology Reviews*, vol. 16, no. 1, pp. 1–17, 2003.

[27] M. P. Martin, I. B. Borecki, Z. Zhang et al., "HLA-Cw group 1 ligands for KIR increase susceptibility to invasive cervical cancer," *Immunogenetics*, vol. 62, no. 11-12, pp. 761–765, 2010.

[28] M. Han, M. Fallena, Y. Guo, and P. Stastny, "Natural killer cell crossmatch: functional analysis of inhibitory killer immunoglobulin-like receptors and their HLA ligands," *Human Immunology*, vol. 68, no. 6, pp. 507–513, 2007.

[29] M. J. Song, C. W. Lee, J. H. Kim et al., "Association of KIR genes and HLA-C alleles with HPV-related uterine cervical disease in

Korean women," *Tissue Antigens*, vol. 81, no. 03, pp. 164–170, 2013.

[30] S.-I. Yusa, T. L. Catina, and K. S. Campbell, "KIR2DL5 can inhibit human NK cell activation via recruitment of Src homology region 2-containing protein tyrosine phosphatase-2 (SHP-2)," *Journal of Immunology*, vol. 172, no. 12, pp. 7385–7392, 2004.

[31] Z.-M. Lu, Y.-L. Jiao, Z.-L. Feng et al., "Polymorphisms of killer cell immunoglobulin-like receptor gene: possible association with susceptibility to or clearance of hepatitis B virus infection in Chinese Han population," *Croatian Medical Journal*, vol. 48, no. 6, pp. 800–806, 2007.

[32] M. Carrington, S. Wang, M. P. Martin et al., "Hierarchy of resistance to cervical neoplasia mediated by combinations of killer immunoglobulin-like receptor and human leukocyte antigen loci," *Journal of Experimental Medicine*, vol. 201, no. 7, pp. 1069–1075, 2005.

[33] L. Arnheim, J. Dillner, and C. B. Sanjeevi, "A population-based cohort study of KIR genes and genotypes in relation to cervical intraepithelial neoplasia," *Tissue Antigens*, vol. 65, no. 3, pp. 252–259, 2005.

[34] M. M. Madeleine, B. Brumback, K. L. Cushing-Haugen et al., "Human leukocyte antigen class II and cervical cancer risk: a population-based study," *Journal of Infectious Diseases*, vol. 186, no. 11, pp. 1565–1574, 2002.

[35] P. Parham, "MHC class I molecules and KIRS in human history, health and survival," *Nature Reviews Immunology*, vol. 5, no. 3, pp. 201–214, 2005.

[36] K. Chattopadhyay, "A comprehensive review on host genetic susceptibility to human papillomavirus infection and progression to cervical cancer," *Indian Journal of Human Genetics*, vol. 17, no. 3, pp. 132–144, 2011.

[37] Y.-C. Yang, T.-Y. Chang, Y.-J. Lee et al., "HLA-DRB1 alleles and cervical squamous cell carcinoma: experimental study and meta-analysis," *Human Immunology*, vol. 67, no. 4-5, pp. 331–340, 2006.

[38] S. Ades, A. Koushik, E. Duarte-Franco et al., "Selected class I and class II HLA alleles and haplotypes and risk of high-grade cervical intraepithelial neoplasia," *International Journal of Cancer*, vol. 122, no. 12, pp. 2820–2826, 2008.

[39] A. Guzalinuer, A. Mihrinsa, S.-Q. Zhang, H. Li, N. Gulishare, and G.-Q. Zhang, "Association between HPV infection and HLA-DQB1 alleles polymorphism in the cervical carcinogenesis in Uyghur women in southern Xinjiang," *Zhonghua Zhong Liu Za Zhi*, vol. 32, no. 7, pp. 492–496, 2010.

[40] I. Kohaar, S. Hussain, N. Thakur et al., "Association between human leukocyte antigen class II alleles and human papillomavirus-mediated cervical cancer in Indian women," *Human Immunology*, vol. 70, no. 4, pp. 222–229, 2009.

[41] D. M. Hernández-Hernández, R. M. Cerda-Flores, T. Juárez-Cedillo et al., "Human leukocyte antigens I and II haplotypes associated with human papillomavirus 16-positive invasive cervical cancer in Mexican women," *International Journal of Gynecological Cancer*, vol. 19, no. 6, pp. 1099–1106, 2009.

[42] Y. Ben Othmane, E. Ghazouani, A. Mezlini et al., "HLA class II susceptibility to cervical cancer among Tunisian women," *Bull Cancer*, vol. 99, no. 9, pp. 81–86, 2012.

[43] F. A. Castro, K. Haimila, I. Sareneva et al., "Association of HLA-DRB1, interleukin-6 and cyclin D1 polymorphisms with cervical cancer in the Swedish population—a candidate gene approach," *International Journal of Cancer*, vol. 125, no. 8, pp. 1851–1858, 2009.

[44] P. C. Maciag, N. F. Schlecht, P. S. A. Souza, E. L. Franco, L. L. Villa, and M. L. Petzl-Erler, "Major histocompatibility complex class II polymorphisms and risk of cervical cancer and human papillomavirus infection in Brazilian women," *Cancer Epidemiology Biomarkers & Prevention*, vol. 9, no. 11, pp. 1183–1191, 2000.

[45] J. Cervantes, C. Lema, L. V. Hurtado et al., "HLA-DRB1*1602 allele is positively associated with HPV cervical infection in Bolivian Andean women," *Human Immunology*, vol. 64, no. 9, pp. 890–895, 2003.

[46] K. Eiguchi, S. Tatti, L. V. Alonio et al., "Association of DRB1 and DQB1 HLA class II polymorphisms in high-grade and neoplastic cervical lesions of women from Argentina," *Journal of Lower Genital Tract Disease*, vol. 12, no. 4, pp. 262–268, 2008.

[47] C. Alaez-Verson, J. Berumen-Campos, A. Munguía-Saldaña et al., "HPV-16 and HLA-DRB1 alleles are associated with cervical carcinoma in Mexican Mestizo women," *Archives of Medical Research*, vol. 42, no. 5, pp. 421–425, 2011.

[48] L.-C. Chuang, C.-Y. Hu, H.-C. Chen et al., "Associations of human leukocyte antigen class II genotypes with human papillomavirus 18 infection and cervical intraepithelial neoplasia risk," *Cancer*, vol. 118, no. 1, pp. 223–231, 2012.

[49] D. D. Dao, C. H. Sierra-Torres, S. C. Robazetti et al., "HLA-DQB1 and cervical cancer in Venezuelan women," *Gynecologic Oncology*, vol. 96, no. 2, pp. 349–354, 2005.

[50] C. Lema, A. L. Fuessel-Haws, L. R. Lewis et al., "Association between HLA-DQB1 and cervical dysplasia in Vietnamese women," *International Journal of Gynecological Cancer*, vol. 16, no. 3, pp. 1269–1277, 2006.

[51] J. Liang, A. Xu, Y. Xie, A. O. Awonuga, and Z. Lin, "Some but not all of HLA-II alleles are associated with cervical cancer in Chinese women," *Cancer Genetics and Cytogenetics*, vol. 187, no. 2, pp. 95–100, 2008.

[52] Y. Wu, B. Liu, W. Lin et al., "Human leukocyte antigen class II alleles and risk of cervical cancer in China," *Human Immunology*, vol. 68, no. 3, pp. 192–200, 2007.

[53] P. S. De Araujo Souza and L. L. Villa, "Genetic susceptibility to infection with human papillomavirus and development of cervical cancer in women in Brazil," *Mutation Research*, vol. 544, no. 2-3, pp. 375–383, 2003.

[54] K. Matsumoto, H. Maeda, A. Oki et al., "HLA class II DRB1*1302 allele protects against progression to cervical intraepithelial neoplasia grade 3: a multicenter prospective cohort study," *International Journal of Gynecological Cancer*, vol. 22, no. 3, pp. 471–478, 2012.

[55] P. K. S. Chan, J. L. K. Cheung, T.-H. Cheung et al., "HLA-DQB1 polymorphisms and risk for cervical cancer: a case-control study in a southern Chinese population," *Gynecologic Oncology*, vol. 105, no. 3, pp. 736–741, 2007.

[56] D. Chen, I. Juko-Pecirep, J. Hammer et al., "Genome-wide association study of susceptibility loci for cervical cancer," *Journal of the National Cancer Institute*, vol. 105, no. 9, pp. 624–633, 2013.

[57] Q. Feng, H. Wei, J. Morihara et al., "Th2 type inflammation promotes the gradual progression of HPV-infected cervical cells to cervical carcinoma," *Gynecologic Oncology*, vol. 127, no. 2, pp. 412–419, 2012.

[58] L. F. Telesheva, V. F. Dolgushina, O. S. Abramovskikh et al., "Cytokine status of cervical mucus in women with transitory and persistent course of papillomavirus infection," *Zhurnal Mikrobiologii, Epidemiologii, i Immunobiologii*, vol. 4, pp. 118–121, 2012.

[59] A. Bolpetti, J. S. Silva, L. L. Villa, and A. P. Lepique, "Interleukin-10 production by tumor infiltrating macrophages plays a role in Human Papillomavirus 16 tumor growth," *BMC Immunology*, vol. 11, article 27, 2010.

[60] G. Barbisan, L. O. Pérez, A. Contreras et al., "TNF-α and IL-10 promoter polymorphisms, HPV infection, and cervical cancer risk," *Tumor Biology*, vol. 33, no. 5, pp. 1549–1556, 2012.

[61] I. V. Iancu, A. Botezatu, C. D. Goia-Ruşanu et al., "TGF-beta signalling pathway factors in HPV-induced cervical lesions," *Roumanian Archives of Microbiology and Immunology*, vol. 69, no. 3, pp. 113–118, 2010.

[62] S. Mocellin and D. Nitti, "TNF and cancer: the two sides of the coin," *Frontiers in Bioscience*, vol. 13, no. 7, pp. 2774–2783, 2008.

[63] N. Wang, D. Yin, S. Zhang et al., "TNF-alpha rs1800629 polymorphism is not associated with HPV infection or cervical cancer in the Chinese population," *PLoS ONE*, vol. 7, no. 9, Article ID e44952, 2012.

[64] I. Badano, S. M. Stietz, T. G. Schurr et al., "Analysis of TNFα promoter SNPs and the risk of cervical cancer in urban populations of Posadas (Misiones, Argentina)," *Journal of Clinical Virology*, vol. 53, no. 1, pp. 54–59, 2012.

[65] T. Y. Shi, M. L. Zhu, J. He et al., "Polymorphisms of the Interleukin 6 gene contribute to cervical cancer susceptibility in Eastern Chinese women," *Human Genetics*, vol. 132, no. 3, pp. 301–312, 2013.

Hindawi Publishing Corporation
BioMed Research International
Volume 2013, Article ID 683095, 18 pages
http://dx.doi.org/10.1155/2013/683095

Review Article

The Role of Cytidine Deaminases on Innate Immune Responses against Human Viral Infections

Valdimara C. Vieira[1] and Marcelo A. Soares[1,2]

[1] *Programa de Oncovirologia, Instituto Nacional de Câncer, Rua André Cavalcanti, No. 37–4 Andar, Bairro de Fátima, 20231-050 Rio de Janeiro, RJ, Brazil*
[2] *Departamento de Genética, Universidade Federal do Rio de Janeiro, 21949-570 Rio de Janeiro, RJ, Brazil*

Correspondence should be addressed to Marcelo A. Soares; masoares@biologia.ufrj.br

Received 16 March 2013; Revised 29 May 2013; Accepted 31 May 2013

Academic Editor: Enrique Medina-Acosta

The APOBEC family of proteins comprises deaminase enzymes that edit DNA and/or RNA sequences. The APOBEC3 subgroup plays an important role on the innate immune system, acting on host defense against exogenous viruses and endogenous retroelements. The role of APOBEC3 proteins in the inhibition of viral infection was firstly described for HIV-1. However, in the past few years many studies have also shown evidence of APOBEC3 action on other viruses associated with human diseases, including HTLV, HCV, HBV, HPV, HSV-1, and EBV. APOBEC3 inhibits these viruses through a series of editing-dependent and independent mechanisms. Many viruses have evolved mechanisms to counteract APOBEC effects, and strategies that enhance APOBEC3 activity constitute a new approach for antiviral drug development. On the other hand, novel evidence that editing by APOBEC3 constitutes a source for viral genetic diversification and evolution has emerged. Furthermore, a possible role in cancer development has been shown for these host enzymes. Therefore, understanding the role of deaminases on the immune response against infectious agents, as well as their role in human disease, has become pivotal. This review summarizes the state-of-the-art knowledge of the impact of APOBEC enzymes on human viruses of distinct families and harboring disparate replication strategies.

1. Introduction

The human immune system is constantly challenged by invading pathogens, against which it acts by eliminating them or reducing their impact once infection is established. Current and emerging viruses constitute an important fraction of these pathogens that are able to develop short to life-long persistent infections, to some of which no protective vaccines are yet available. In this regard, a better understanding of the mechanisms by which innate and adaptive immunity restricts viral infections and/or modulate viral pathogenesis is urged.

The innate immune system constitutes the first line of defense against viruses, initiating an antiviral response. Viruses are recognized by this system primarily through detection of their nucleic acids, either their packaged genome or viral replication intermediates within the infected cell [1]. Toll-like receptors are good examples of the former viral sensing mechanisms, while the latter are represented by RIG-I-like or DAI and AIM2 receptors [2, 3]. These types of recognition induce the transcription of proinflammatory cytokines and type I interferons (IFNs). These, on their hand, activate the expression of hundreds of IFN-stimulated genes (ISGs) which will engage in counteracting virus replication and spread [4].

Among the ISGs, the genes encoding the family of apolipoprotein B mRNA-editing catalytic polypeptide (APOBEC) cytidine deaminases have been largely studied in the last years. Mounting evidence suggest that these enzymes are key players in restricting infections by different viruses. In this review, we summarize the state-of-the-art knowledge of the impact of APOBEC enzymes on human viruses of distinct families and harboring disparate replication strategies.

2. The APOBEC Family of Deaminases

The APOBEC family of proteins comprises a group of cytidine deaminases that are able to edit DNA and/or RNA sequences. Although it belongs to a larger superfamily of

deaminases, APOBECs are restricted to vertebrates [5]. In humans, the family comprises eleven members with distinct functions: activation-induced deaminase (AID) and APOBEC1, whose genes are located in chromosome 12; APOBEC2, whose gene is in chromosome 6; seven *APOBEC3* genes, located in chromosome 22; and APOBEC4, whose gene is located in chromosome 1 [6–10]. The members of this family are distinguished by the presence of one or two catalytic domains containing a zinc-binding deaminase motif, characterized by the conserved amino acid sequences H-X-E-$X_{(23-28)}$-P-C-$X_{(2-4)}$-C (X is any amino acid; Figure 1) [9]. The deamination mediated by these enzymes involves the hydrolytic removal of the amino group at the C4 position of a cytidine (C) or deoxycytidine (dC), generating a uridine (U) or deoxyuridine (dU) (Figure 1) [11].

APOBEC1 (A1), the first member of the family to be described, is an RNA-editing enzyme [12] but also presents the ability to edit DNA in bacterial assays [13]. It is primarily expressed in the gastrointestinal compartment and catalyzes the posttranscriptional editing of the apolipoprotein B (*apoB*) mRNA. A1 is a nucleocytoplasmic protein [14], and the *apoB* mRNA editing occurs in the nucleus in the presence of the APOBEC-1 complementation factor (ACF) [15, 16]. A1 edits this mRNA at a single base, resulting in the formation of a premature stop codon and leading to the synthesis of a truncated protein (ApoB48) [12]. As a consequence, the human gut produces two forms of ApoB, a longer (ApoB100) and a shorter (ApoB48), involved in the transport of endogenously produced cholesterol and triglycerides and the absorption and transport of exogenous dietary lipids, respectively [17]. Recently, additional mRNA targets of APOBEC1 have been described; the interaction occurs at AU-rich segments of their 3′ untranslated regions. A1 may thus play a role regulating the stability of such specific mRNAs [18, 19].

AID, which deaminates single stranded DNA [20, 21], is predominantly cytoplasmic and shuttles between the nucleus and the cytoplasm [22–25]. It is expressed in germinal center B cells and is essential for the events of class switch recombination and somatic hypermutation during the process of antibody diversification [26–28]. AID has also been reported as involved in DNA demethylation [29–31].

APOBEC2 is expressed in heart and skeletal muscles [8], and although its precise physiologic role is not established, its expression appears to be essential to muscle development [32].

The APOBEC3 group comprises seven proteins in humans: APOBEC3A, APOBEC3B, APOBEC3C, APOBEC3-DE, APOBEC3F, APOBEC3G, and APOBEC3H. A3A, A3C, and A3H present one copy of the zinc-binding domain, while the remaining harbor two copies; the N- and C-terminal domains are named CD1 and CD2, respectively (Figure 1). In those three enzymes, only CD2 is catalytically active [13, 33, 34]. A3 enzymes are capable of editing single-stranded DNA and recognize specific target sequences. A3G and A3F, for example, edit C's preferentially at C$\underline{\text{C}}$ and T$\underline{\text{C}}$ dinucleotide contexts (G$\underline{\text{G}}$ and A$\underline{\text{G}}$ in the complementary DNA strand), respectively [35–38].

A3 enzymes play an important role on the innate immune system, acting on host defense against exogenous viruses and endogenous retroelements [39–42]. Viral restriction occurs mainly by their DNA editing mechanism, but A3s also display editing-independent phenotypes [38, 43–45], as will be discussed later. They are further possibly implicated into a specific pathway of exogenous DNA clearance in human cells [46]. More recently, lines of mounting evidence have also shown that A3 enzymes insert mutations in human nuclear and mitochondrial DNA, suggesting roles in DNA catabolism [47]. On the other hand, this phenomenon may represent a possible source of mutations towards the development of cancer [47, 48].

A3 enzymes localize to the cell cytoplasm and/or nucleus, enabling the protection of both compartments through restriction of nuclear (such as the human papillomavirus, the herpes simplex virus, and non-LTR retrotransposons) or cytoplasmic (like the hepatitis B virus, retroviruses, and LTR retrotransposons) replicating elements. A3D, A3F, and A3G are known to be cytoplasmic [40, 49]; A3B localizes to the nucleus [50], while A3A, A3C, and A3H are found both in the nucleus and in the cytoplasm [40, 51]. Noteworthy, different A3H haplotypes present distinct localizations; the protein encoded by haplotype I is mainly nuclear, while the one encoded by haplotype II is predominantly cytoplasmic [51]. With respect to A3A, it has been recently reported that its endogenous version in primary $CD14^+$ monocytes and in the monocytic cell line THP-1 localizes to the cytoplasm, contrasting with the broad nucleocytoplasmic distribution observed upon A3A transfection, an observation likely explained by artificial overexpression of the enzyme [52].

In addition to presenting distinct subcellular localization, some APOBEC3 proteins also display an intracellular mode of regulation by localization into specific subcellular structures. It is known that A3G is present in two distinct molecular forms within the cell: a form of low molecular mass (LMM) and another in ribonucleoproteic complexes of high molecular mass (HMM) [53–55]. The HMM complex is enzymatically inactive and can be converted into LMM complexes, enzymatically active, through RNase digestion [53]. Besides A3G, other APOBECs like A3C, A3F, and A3H also show the ability of assembling into HMM complexes [56–58].

It has been shown that the switch between HMM and LMM can be stimulated by different cytokines [59, 60], and the predominant form varies among different cell types or distinct cell type subsets. The presence of LMM A3G has been related to a reduced susceptibility to HIV-1 infection, as is suggested as postentry restriction factor for this virus [53, 60, 61]. For example, unstimulated peripheral blood $CD4^+$ T-cell lymphocytes and monocytes, which are nonpermissive to HIV-1 infection, present LMM A3G. However, when $CD4^+$ T-cell lymphocytes are activated or the monocytes stimulated to differentiate into macrophages, they shift their A3G profile to HMM [53]. Noteworthy, the knockdown of A3G in unstimulated $CD4^+$ T cells does not turn them permissive to infection, suggesting that the presence of LMM A3G in the cells is not the unique determinant for their resistance to HIV-1 [62, 63]. Moreover, LMM A3G is preferentially packaged into HIV-1 particles [56, 64–66]. Finally, HMM

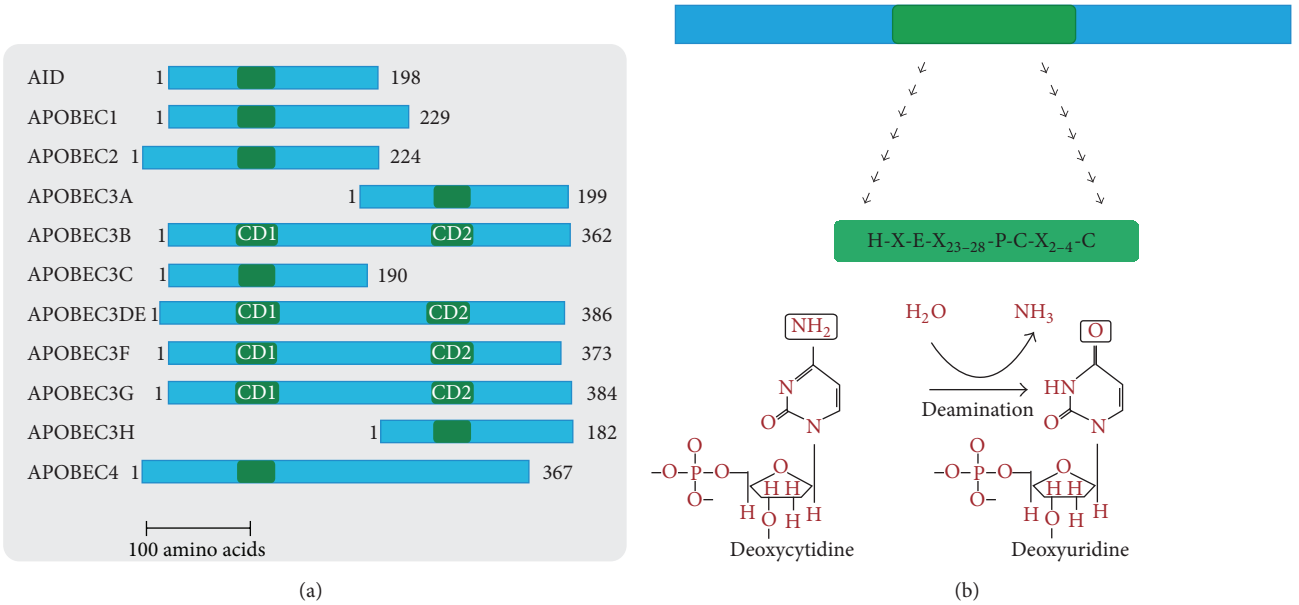


Figure 1: Structure and function of human APOBEC proteins. (a) Schematic representation of APOBEC proteins. The zinc-binding motifs, represented by the catalytic domains (CD) and present in single or double copies, are depicted in green. In the proteins that harbor two CD copies, the N- and C-terminal domains are named CD1 and CD2, respectively. APOBEC proteins are drawn to scale, and the total number of amino acids is shown to the right of each version. The scale bar represents the length of 100 amino acids. (b) The conserved amino acid sequence of the zinc-binding motif is shown; the hydrolytic deamination reaction mediated by these enzymes is shown at the bottom of the figure.

A3G is also able to interact with and sequester *Alu* RNA elements, inhibiting their transposition and evidencing the role of different A3G molecular forms in the restriction of retroelements [67].

A3G and A3F are also able to accumulate in processing bodies (P-bodies) and stress granules, where they interact with RNAs and several proteins that regulate their metabolism [68–71]. However, the functional consequences of the occurrence of A3 proteins in those structures are not yet clear [72].

APOBEC4 is expressed in testicles, and its function is still unknown [10]. Like A2, A4 does not present mutagenic activity in bacterial or yeast assays [73].

APOBEC proteins are found throughout vertebrates, with AID and APOBEC2 being ancestral members of the family and APOBEC1 and APOBEC3 being more recent, while the origins of APOBEC4 are not clear [74–77]. The APOBEC3 enzymes are exclusively found in mammals [5, 78], and their gene copy number is species-specific. While mice have only a single *APOBEC3* gene, pigs have two, sheep and cattle have three, cats have four, horses have six, and primates have at least seven *APOBEC3* genes [5, 9, 79, 80].

The evolutionary history of the *APOBEC3* genes involves expansion, divergence, selection and extinction of specific A3 copies [80]. It is believed that the genome of the mammalian ancestor encoded for at least one ancestral *APOBEC3* gene and that this gene family expanded in the different lineages as a response to changes in viral, retroviral, and retrotransposon pressure [78, 79]. Interestingly, the rapid expansion of the *APOBEC3* locus in primates is correlated with a marked reduction in retrotransposon activity, suggesting an important role in the host genome defense against retroelements [81, 82].

There is evidence that APOBEC3 proteins are able to restrict non-LTR and LTR retrotransposons, including both long interspersed nuclear elements (LINEs) and short interspersed nuclear elements (SINEs) [40, 57, 83–87]. While for some murine LTR retrotransposons, like IAP e MusD, DNA deamination was observed as part of the restriction mechanism [39, 85], the exact mechanism and the retrotransposition step targeted by APOBEC3 is unknown for non-LTR elements (reviewed in [82]).

It is interesting to note that AID and APOBEC1 from multiple species have been shown to possess activity against retroelements [86, 88–90] and exogenous viruses [89, 91–93], suggesting that these proteins may also have a role in innate immunity of some vertebrates [82, 89, 92].

3. Role of APOBEC Enzymes on Different Human Viral Infections

3.1. Human Immunodeficiency Virus (HIV). The human immunodeficiency virus (HIV) is a member of the Retroviridae family, and belongs to the *Lentivirus* genus, which characterizes viruses of slow symptomatology. As a retrovirus, HIV harbors a genome consisting of two single-stranded RNA molecules of positive polarity that undergoes a reverse transcription step (through a complementary DNA-cDNA-molecule) carried out by its encoded reverse transcriptase (reviewed in [111]). The cDNA is then integrated into the host

cell genome, from where the viral genes are transcribed by the host RNA polymerase II. In addition to the essential genes, HIV also encodes several accessory and regulatory proteins that enhance virus replication and burden, being the viral infectivity factor (Vif) among them (reviewed in [111]).

The hypermutation mediated by APOBEC enzymes in HIV type 1 (HIV-1) is well described (Figure 2). It is known that in HIV-1-infected cells, in the absence of a functional Vif, A3G molecules are incorporated into incoming virus particles. This packaging is mediated by the interaction of A3G with the nucleocapsid (NC) domain of the Gag protein [112–116] and occurs in an RNA-dependent manner [114, 115, 117–120]. After a new infection, the editing process occurs during viral reverse transcription. A3G deaminates dC residues in the negative strand of the complementary DNA (cDNA), originating dU. These nucleotides serve as templates for the incorporation of dA in the positive strand and are evidenced as G-to-A changes in the proviral DNA. The frequency of edited dC's in the viral genome can exceed 10% of the sites [38, 121]. The excessive number of changes results in loss of genetic information and production of largely defective virions in the subsequent replication cycle.

A reduction of viral reverse transcription products is also observed in the presence of A3G. It has been hypothesized that the presence of dU's in the retroviral DNA could be recognized as anomalous, leading to its degradation even before its integration into the host cell genome (Figure 2). This would occur by removal of the uracil residues by uracil-DNA glycosylases (UDGs), followed by apurinic/apyrimidinic (AP) endonuclease-mediated degradation [122, 123]. Noteworthy, it has been shown that APOBEC-mediated restriction occurs even in the absence of UDGs such as UNG2 and SMUG1 [124, 125], leaving the requirement of retroviral DNA degradation for virus restriction as an open question.

In addition to restricting viral infection through hypermutation, A3G also exert editing-independent mechanisms of restriction. These involve the interference with reverse transcription and with proviral integration by disturbing tRNA primer annealing and removal, DNA synthesis initiation and elongation and strand transfer reaction [43–45, 126–135].

Further to the direct mechanisms of viral inhibition, A3G appears to play a pivotal role in the activation of the host immune system. It has been shown that the generated pool of defective viruses encoding truncated or misfolded proteins represents an important source of viral antigens, associated with a strong activation of HIV-1-specific CD8+ cytotoxic T-cells [136]. APOBEC3 may also enhance the recognition of HIV-infected cells by natural killer cells through the activation of the DNA-damage repair response by viruses harboring uridines in their genomes [137].

HIV-1, like the majority of lentiviruses, counteracts the restriction mediated by the A3 enzymes through expression of the Vif protein [138, 139]. The main mechanism of action assigned to Vif is the induction of A3G protein degradation via proteasome. Vif simultaneously binds to A3 and to an E3 ubiquitin-ligase complex, leading to polyubiquitination of A3G and its consequent degradation [138–141]. Vif is also able to prevent A3G packaging into the virion in a degradation-independent manner and to interfere with A3G mRNA translation, thus reducing the intracellular levels of the protein (Figure 2) [142–147].

APOBEC3F (A3F) has also been associated with HIV restriction in a consistent manner. However, A3G seems to have a more important role in viral restriction of cells targeted by HIV, whereas the role of A3F seems to be dispensable for virus restriction in these cells [148, 149]. A3A, A3B, A3C, A3DE, and some A3H haplotypes have also been implicated in restriction [148, 150–157], although controversial results have been observed (reviewed in [158]).

HIV hypermutated proviral DNA sequences have been reported in several *in vivo* studies. Yet some reports have shown a correlation between hypermutation and a favorable clinical outcome, this relationship is not consensual. In a population level analysis of HIV-1 subtype B near full-length proviral sequences, hypermutation levels were associated with reduced pretreatment viremia [101]. In agreement with this, higher hypermutation levels were observed in patients with low HIV viral loads (<10,000 copies/mL for at least 3 years) in another study [159]. The presence of hypermutation was also correlated with higher $CD4^+$ T-cell counts [160]. More recently, Kourteva et al. [161] found more A3G-hypermutated sites in proviral sequences derived from HIV long-term nonprogressors (LNTP) compared to noncontrollers (NC). On the other hand, some studies could not find such associations, either in adults or in children [162–164].

A more consistent association has been observed between the APOBEC3 mRNA levels and clinical outcomes. Several studies showed a positive correlation of A3 levels with $CD4^+$ T-cell counts and a negative correlation with HIV-1 viral load [159, 161, 165, 166] or viral set point [167]. However, two studies could not find an association between A3G levels and $CD4^+$ T-cell counts or viral load [104, 168]. APOBEC3G expression has also been inversely correlated with provirus burden [161] and positively correlated with the level of G-to-A changes [159, 161, 162]. A3G has also been found significantly increased in HIV-exposed uninfected (EU) compared to healthy controls [159, 169] or infected individuals [169]. The level of expression in EU significantly decreased after one year from HIV diagnosis and subsequent treatment of their partners. This suggests that, in these individuals, exposure to HIV can trigger APOBEC3G expression in the absence of infection and that the expression decreases with cessation of exposure [159]. Interestingly, the higher expression of APOBEC3G in EU was seen not only in PBMCs but also in cervical tissues, and may be important for the susceptibility to sexually transmitted HIV infection [169].

Higher levels of APOBEC3G mRNA were observed in LTNPs when compared to HIV-uninfected subjects and progressors [165]. Accordingly, higher levels of A3G and A3F were found in LTNP when compared to noncontrollers [161], and higher levels of A3G and A3B were also found in slow progressing patients (SP) when compared to AIDS patients [166]. However, in a group of perinatally HIV-infected children, no correlation was observed between A3G/A3F expression and disease progression [164]. Paradoxically, A3G levels were higher in HIV-negative when compared with HIV

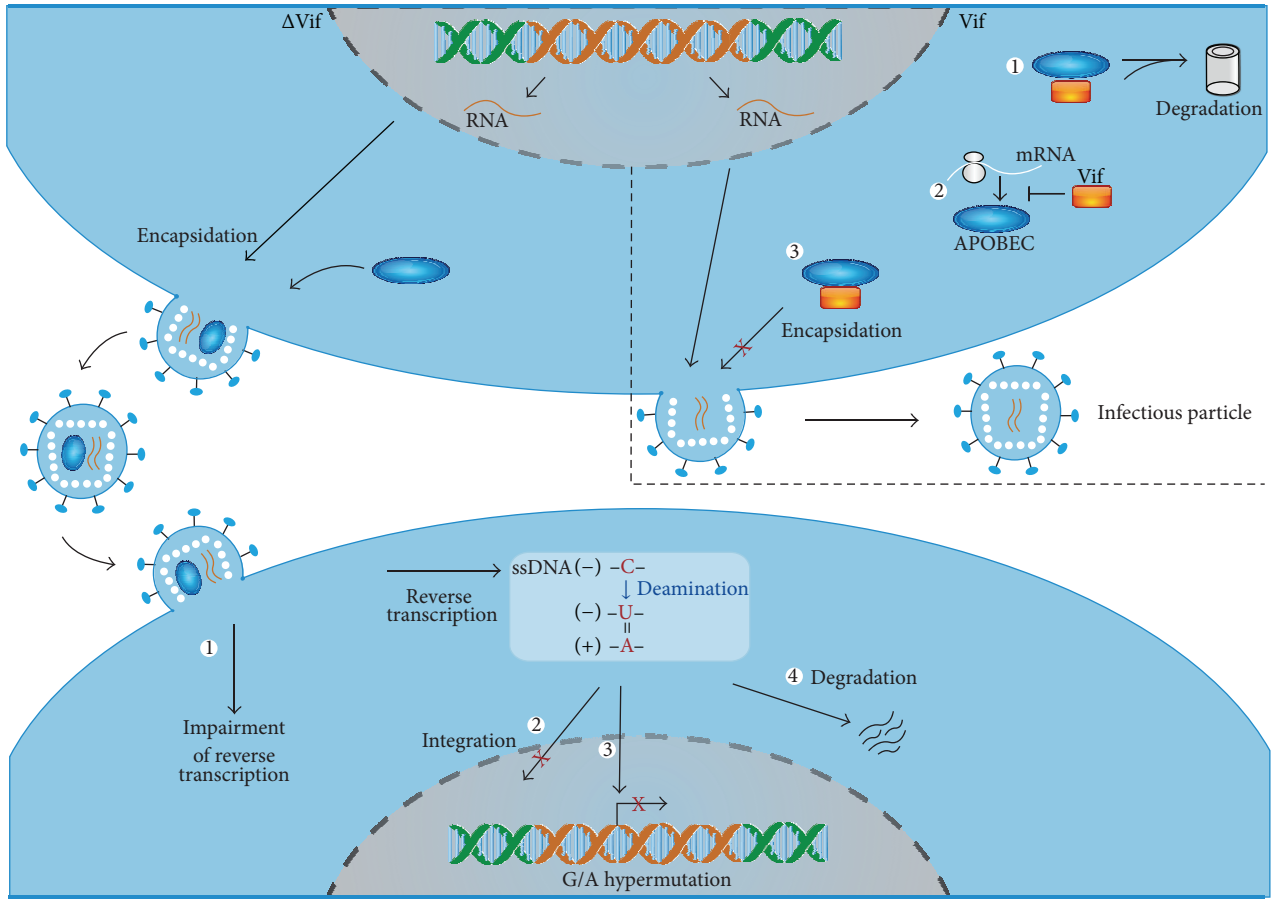


Figure 2: Mechanism of action of APOBEC3 (A3) and Vif in the HIV-1 life cycle. At the top panel, a virus-producing cell in the absence (left) or in the presence (right) of a functional Vif protein is shown. In the presence of Vif (orange rectangle), A3 (blue ellipse) is primarily targeted to proteasomal degradation (1); Vif can also block A3 mRNA translation (2) and prevent A3 packaging into the virion in a degradation-independent manner (3). In the absence of Vif, A3 molecules are packaged into incoming virus particles. After a new infection (bottom panel), A3 exerts its antiviral activity in multiple ways. A3 can interfere with reverse transcription in a deamination-independent manner (1). A3 can also interfere with proviral integration through the formation of abnormal viral DNA ends (2). In the hypermutation process, A3 mainly deaminates dC residues in the negative strand of the complementary viral DNA, originating dU, that serves as template for the incorporation of dA in the positive strand. If able to integrate, hypermutated proviruses are normally largely defective (3). Alternatively, viral DNA containing multiple dU can also be degraded before integration (4).

positive individuals [104, 166, 168], including matched pre- and postinfection samples from the same subjects. This may suggest that APOBEC3G transcription is rapidly downregulated upon HIV-1 infection [104]. In view of these confounding evidence, additional work is necessary to robustly define the role of A3 expression in the control of HIV infection and disease progression.

Due to the potential antiviral role of the A3 enzymes, therapeutic interventions have been idealized to enhance their action and lead to viral inhibition through hypermutation [170–174]. In this sense, molecules have been identified that are able to interact with A3G and counteract Vif-mediated degradation [171] or to induce Vif degradation in the presence of A3G [172]. On the other hand, it is possible that the editing mediated by the APOBEC enzymes, upon sublethal conditions (which do not drive virus extinction), acts in the diversification of the viral genome, providing source for the selection and evolution of highly fit variants. This can favor, for example, the acquisition of immune system escape mutations or drug resistance mutations [75, 175–182]. In this regard, novel therapeutic strategies based on complete A3G inhibition, eliminating this additional source of virus diversification, have also been proposed [174, 183, 184].

3.2. Human T-Cell Lymphotropic Virus (HTLV). HTLV is a complex retrovirus belonging to the *Deltaretrovirus* genus. In most cases infection by HTLV-1 is asymptomatic; however, up to 5% of the infected subjects develop adult T-cell leukemia (ATL) and another 1-2% develop HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) [185, 186].

Like HIV-1, HTLV targets mainly $CD4^+$ T lymphocytes [187], and therefore, it would be exposed to several A3 enzymes that are expressed in this cell type [188]. HTLV does not encode any product with Vif-like, A3 antagonist

function, and it is apparently incapable of inducing A3 degradation in cell culture [34, 189]. Unexpectedly, however, hyperedited HTLV sequences appear to be rare. Yet HTLV hypermutated sequences with estimated frequencies between 0.1 and 5% have been observed *in vitro*, no instance of hypermutation was reported in PBMC from HTLV-infected patients [34]. Moreover, a retrospective analysis of previously published HTLV sequences has identified a single hypermutated sequence recovered from an HTLV-1 infection of an animal model [190].

Although rare *in vivo*, HTLV hypermutated sequences were recovered from cell lines derived from ATL and HAM/TSP patients. The main context of the observed changes was GG, followed by GA and GC, suggesting the involvement of A3G and also of other A3 such as A3A and A3B in HTLV hypermutation [189]. Despite the fact that A3G is able to edit the viral genome [191, 192], some studies have shown that HTLV-1 is resistant or poorly susceptible to this enzyme [34, 189, 192, 193], in agreement with the low frequency of editing observed *in vivo*.

Two possible reasons for the low frequency of hypermutated HTLV sequences *in vivo*, when compared to HIV hypermutation, have been discussed. One of the possibilities resides in the differences of replicative strategies of these two viruses. After primary infection, HTLV presents a low level of productive replication, and the proviral genome is mainly replicated through oligoclonal expansion of infected cells, a fact that contrasts with the high rate of *de novo* cell infection seen during chronic HIV disease [185, 194–196]. Therefore, it has been suggested that the infrequent replication via reverse transcription seen in HTLV infection represents a reduction of opportunities for APOBEC3-mediated edition to occur. Moreover, a direct resistance mechanism to A3G has been described in HTLV-1. Through a *cis*-acting exclusion mechanism, elements in the C-terminal region of the HTLV-1 nucleocapsid inhibit A3G packaging to the particle, resulting in reduced efficiency of its packaging in HTLV-1 particles when compared to HIV Δvif virus-like particles [197].

Recently, an analysis of proviral genomes of 60 ATL patients and asymptomatic carriers showed that G-to-A changes are the most frequent nucleotide substitutions. These changes occurred preferentially in the target context of A3G and were involved in the generation of multiple missense substitutions. It was then suggested that HTLV-1-infected cells can take advantage of A3G activity to escape the host immune system by abrogating the expression of viral proteins [191].

3.3. Hepatitis B Virus (HBV). HBV belongs to the Hepadnaviridae family and presents a circular, partially double stranded DNA genome [198]. It replicates through reverse transcription of an intermediate pregenomic RNA. It is estimated that two billion people have been infected and more than 240 million have chronic liver infections worldwide [199]. HBV infection can cause acute and chronic liver disease, including cirrhosis and hepatocellular carcinoma [198].

A number of studies have shown that the HBV genome is susceptible to human APOBEC enzymes. In addition to A1 and AID, all A3 enzymes except for A3DE were able to edit the viral genome *in vitro* [200–206] with editing levels estimated between 10^{-2} and 10^{-5} [205]. Viral genome editing occurs preferentially in the negative DNA strand resulting in G-to-A changes in the positive strand; C-to-T changes have also been observed in the positive strand, evidencing editing of both viral DNA strands [202].

In addition to the identification of HBV sequences extensively edited by APOBEC enzymes, several studies have shown evidence of restriction to virus replication *in vitro*, by both editing-dependent and -independent mechanisms [201, 207, 208]. In this sense, a strong inhibitory effect of A3G has been described [201, 204, 207, 208], and a reduction of approximately 30-fold in the levels of viral DNA in the presence of A3G expression has been shown [204]. Turelli et al. [207] showed that A3G leads to a reduction of viral DNA and also of core-associated RNA; this effect was sustained when a catalytically-inactive A3G mutant was used. Among the possible deamination-independent mechanisms of action are the inhibition of pregenomic RNA packaging and the interference of reverse transcription [201, 207, 208]. Additional unrelated factors may account for the suppression of HBV replication in hepatocytes, including the inhibition of HBV transcription through the interaction of A3B with the heterogeneous nuclear ribonucleoprotein K (hnRNP-K), a positive regulator of HBV expression [209].

In the healthy human liver, low to moderate APOBEC expression levels are observed [9, 188, 210]. However, some of these enzymes, particularly A3G, have their expression significantly increased in primary hepatocytes and in hepatoma cell lines in response to stimulation by interferon alpha and gamma [210–212]. In agreement with those data, low hypermutation levels (from 10^{-4} to 0.6%, depending on the method used) have been described in patients with acute and chronic HBV infection [202, 213, 214]. This has led to the hypothesis that hypermutation is intrinsic to the natural response to HBV infection and that it may contribute to the noncytolytic clearance of HBV [202, 207, 210]. However, there are no current *in vitro* evidence supporting a major role of A3 on the IFN-mediated HBV inactivation in the liver [215, 216].

In contrast to the moderate levels of APOBEC expression in the healthy liver, overexpression of these enzymes is observed in cirrhotic tissues, likely resultant from the high production of cytokines associated with the chronic inflammatory response against the infection. Consequent to this increase in APOBEC expression, high levels of HBV hypermutation have been observed, reaching 35% of the sequences in some cases [206].

A potential role of APOBEC3 has been suggested on the oncogenesis of the hepatocellular carcinoma (HCC). It has been shown that some APOBEC3 enzymes are able to generate truncation mutants of the HBx viral protein, leading to a selective advantage to preneoplastic and neoplastic hepatocytes [217]. Moreover, A3B was found to be overexpressed in HCC tissues. In HepG2 cells, A3B overexpression promoted their growth and led to an upregulation of the heat

shock transcription factor-1 (HSF-1), which was also found to be upregulated in HCC [217]. HSF-1 is a regulator of the heat-shock response and is known to facilitate malignant transformation, cancer cell survival, and proliferation in model systems. Recently, it has been shown to coordinate a transcriptional program in malignancy, which differs from that induced by thermal stress. This program activates cancer-specific genes that support oncogenic events and was found to be active in breast, colon, and lung tumors [180].

3.4. Hepatitis C Virus (HCV). HCV belongs to the Flaviviridae family and presents a positive sense, single-stranded RNA genome [218]. HCV is a causative agent of acute and chronic liver diseases. Chronic HCV infection, along with chronic HBV infection and its associated liver cirrhosis, constitutes major risk factors for HCC development [219]. It has been recently shown that A3G is able to inhibit HCV replication *in vitro*. However, viral hypermutated sequences have not been found, suggesting a role of deaminase-independent mechanisms of viral inhibition. Considering that A3G targets ssDNA, such lack of hypermutation is expected for HCV, which presents exclusively RNA as genomic material during all phases of its replication. Interestingly, it has also been shown that the presence of exogenous HIV-1 Vif led to an intracellular decrease of A3G and consequently to an increase of HCV replication, suggesting the involvement of Vif in the HIV-1/HCV coinfection [220].

As previously mentioned, the APOBEC enzymes are expressed at moderate levels in the normal liver. However, HCV infection is also associated to an increase in expression of those enzymes. In patients chronically infected by HCV, a significant increase in A3G expression was seen in hepatocytes and in lymphocytes [221]. Overexpression of APOBEC has also been observed in HCV/HBV coinfection [206].

APOBEC3 also appears to play an important role on treatment with exogenous interferon alpha (IFNα) *in vivo*. Jiménez-Sousa et al. [222] analyzed the profile of gene expression in HCV chronically infected patients after 12 weeks of treatment with IFNα/ribavirin (RBV), and A3A was among the IFN-induced genes that was upregulated in early responders but not in nonresponders. In another study, a significant increase in the expression of A3G/3F was observed in CD4 T-cell lymphocytes of HIV/HCV coinfected patients during treatment with pegIFN/RBV. In that study, APOBEC3 induction was correlated with the levels of HIV hypermutation [223].

In addition to the HCV restriction phenotype during the natural course of infection and during treatment, APOBEC3 enzymes have also been suggested as a putative target in anti-HCV drug development. Treatment of HCV-infected Huh7.5 cells with two stabilizing components of APOBEC3G, which increased its intracellular levels, inhibited HCV replication [220].

3.5. Human Papillomavirus (HPV). HPV belongs to the Papillomaviridae family and presents a circular, double-stranded DNA genome. Infection by HPV is a necessary condition for the development of cervical cancer, but the evolution to invasive carcinoma only occurs in a fraction of the infected women [224].

Vartanian et al. [225] showed that both strands of HPV DNA are susceptible to APOBEC editing. In that study, nine HPV16-positive precancerous cervical and six HPV1a-positive plantar wart samples were analyzed for the presence of hypermutation. Of the samples, two HPV-16 and one HPV-1a presented edited sequences. *In vitro* A3A, A3C and A3H were shown to be able to hyperedit HPV DNA. The preferred *in vitro* dinucleotide context for these three A3 enzymes correlated with the editing contexts observed *in vivo*, suggesting that APOBEC3A, APOBEC3C, and APOBEC3H may be involved in edition of HPV *in vivo*.

3.6. Human Herpesviruses (HHV). Human APOBEC enzymes have also been shown to restrict DNA genome viruses belonging to the Herpesviridae family [226, 227]. Herpesviruses are enveloped viruses harboring a double-stranded DNA genome. They are associated with a range of different diseases and are able to establish latent infection and persist in the infected host for life [228–231].

Herpes simplex virus type 1 (HSV-1) can cause from mild infections of mucous membranes, including herpes labialis and genital infections, to life threatening infections, such as HSV encephalitis [228, 229]. Suspène et al. [226] have identified the presence of HSV hypermutated genomes in four out of eight oral lesions. Overexpression of A3C *in vitro* led to a fourfold reduction in viral titers and to a 10-fold reduction in viral infectivity. Moreover, it has been shown that not only A3C but also AID, A3A, and A3G are able to edit the HSV-1 genome *in vitro*, although the last three had no significant impact on virus replication.

In addition to APOBEC3, APOBEC1 has also been shown to restrict HSV-1 replication *in vitro* in a significant fashion, in both deamination-dependent and -independent ways. Upregulation of A1 has been observed in rat brain tissues upon HSV-1 infection, suggesting that A1 induction during encephalitis can promote restriction to HSV-1 infection [227].

Epstein-Barr virus (EBV) can cause mucocutaneous manifestations in infectious mononucleosis and is also associated with other benign and malignant conditions, including plasmablastic lymphoma, oral hairy leukoplakia, posttransplant lymphoproliferative disorders, Burkitt's lymphoma, and Hodgkin's lymphoma [231]. In order to know whether EBV genomes were also susceptible to A3 editing, Suspène et al. [226] analyzed EBV from transformed peripheral blood mononuclear cell lines, which carry EBV in a latent form. Edited EBV DNA was found in four out of five EBV cell lines studied. A3C was found to be the most abundantly expressed A3 in these cell lines.

4. Polymorphisms in APOBEC3 Genes and Susceptibility to Viral Infections

Polymorphisms in the genes encoding APOBEC proteins have been associated with the modulation in the course of some human viral infections. A deletion of approximately 29.5 kb located between exon 5 of *APOBEC3A* and exon

Table 1: Studies that investigated the association of *APOBEC* gene variants and the course of human viral infections.

A3 family member	Series description	Main findings	Reference
A3B	4,216 individuals from five HIV-1 natural history cohorts based in the United States of America	Homozygous deletion associated with increased risk for HIV-1 infection ($P = 0.24$), progression to AIDS ($P = 0.03$), and viral set point ($P = 0.04$)	[94]
A3B	724 HBV carriers and 469 healthy control subjects	*APOBEC3*B deletion homozygosity was associated with mild liver fibrosis ($P = 0.0019$) No significant association between deletion and chronic HBV infection	[95]
A3B	361 Japanese subjects: 95 HIV-1-infected patients (48 nonprogressors and 47 slow progressors) and 266 controls 453 Indian subjects: 251 HIV-1-infected patients and 202 controls	No evidence of association between the *APOBEC3B* deletion and susceptibility to HIV infection and AIDS	[96]
A3B	1,124 individuals with HCC, 510 individuals with persistent HBV infection, and 826 healthy controls. All subjects were of Han Chinese ethnicity	Higher frequency of the *APOBEC3B* deletion allele in persistent HBV carriers ($P = 0.0015$) and HCC patients ($P = 1.28 \times 10^{-11}$) compared to controls Presence of at least one deletion allele was associated with an increased risk for persistent HBV infection ($P = 0.0272$) and HCC development ($P = 1.28 \times 10^{-11}$)	[97]
A3B	179 HBV chronic carriers and 216 healthy control subjects from the Moroccan population	No significant difference in the frequency of deleted *APOBEC3B* alleles between patients with chronic hepatitis B and control subjects Subjects carrying the Del/Del genotype displayed a trend for increased susceptibility to HBV infection compared to the wild type genotype ($P = 0.07$) Carriers of the *APOBEC3B* deletion had significantly lower viral loads than patients with the wild type genotype ($P = 0.0023$)	[98]
A3G	3,073 participants enrolled in six HIV/AIDS prospective cohorts: 1,481 European Americans, 949 African Americans from five US-based cohorts, and 643 patients enrolled in the Swiss HIV cohort	For African Americans, the variant allele 186R was strongly associated with a decline of $CD4^+$ T cells ($P = 0.009$)	[99]
A3G	773 white French individuals: 327 HIV-1^+ (245 slow progressors; 82 rapid progressors) and 446 healthy control subjects of similar ethnic origin	29 polymorphisms with allele frequencies >1% were identified No significant associations were found between the polymorphisms or haplotypes and disease progression	[100]
A3G	136 adult HIV-infected patients from the Western Australian HIV cohort	22 single nucleotide polymorphisms were identified No significant association of these *APOBEC3G* genetic variants and the presence of HIV-1 hypermutation was found (although an intronic allele 6892C was marginally associated with HIV-1 hypermutation)	[101]
A3G	122 Caucasian individuals exposed to HIV enrolled in prospective cohort studies in Montreal	The C40693T variant was significantly associated with an increased risk of infection ($P = 0.03$)	[102]
A3G	560 North Indians: 50 HIV-1 exposed seronegative individuals, 190 HIV-1^+ patients, and 320 healthy controls	No H186R polymorphism of *APOBEC3G* was found among North Indians	[103]
A3G	250 South African women at high risk for HIV-1 subtype C infection	The H186R mutation and a 3′ extragenic mutation (rs35228531) were associated with high HIV viral loads ($P = 0.0097$ and $P < 0.0001$) and decreased $CD4^+$ T-cell counts ($P = 0.0081$ and $P < 0.0001$)	[104]
A3G	534 children perinatally exposed to HIV-1 (109 exposed uninfected and 425 HIV-1-infected), from a pediatric cohort of white-Hispanic ethnicity from Argentina	HIV-1 perinatal transmission and progression to AIDS were not affected by *APOBEC3G* H186R or *APOBEC3G* C40693T *APOBEC3G* C40693T was correlated with substitutions in Vif motifs involved in the interaction with APOBEC3G ($P = 0.004$)	[105]

Table 1: Continued.

A3 family member	Series description	Main findings	Reference
A3G	400 HIV-1-infected individuals naive to drug therapy from the Brazilian population	Seven *loci* were analyzed: SNP −571 (rs5757463); −199 (rs34550797); −90 (rs5750743); 119 (rs5757465); 186 (rs8177832); 197 (rs3736685); 199 (rs2294367) For the SNP −571, heterozygous (C/G) and homozygous (G/G) individuals had lower $CD4^+$ T-cell counts compared to homozygous (C/C) individuals ($P = 0.0076$)	[106]
A3G	93 perinatally infected children with white-Hispanic ethnicity, from an Argentinian pediatric cohort	The *APOBEC3G* H186R and *APOBEC3G* C40693T variants were not associated with different levels of HIV-1 editing	[107]
A3G	1,049 HIV-1-infected children from the Pediatric AIDS Clinical Trials Group (PACTG) protocols P152 and P300 (60% non-Hispanic black, 26% Hispanic, 13% non-Hispanic white, and 1% other or unknown race/ethnicity)	*APOBEC3G* H186R homozygous G/G genotype was associated with faster HIV-1 disease progression ($P = 0.01$) and central nervous system (CNS) impairment ($P = 0.02$) *APOBEC3G* F119F-C allele was associated with protection against disease progression and CNS impairment in both additive and dominant models ($P = 0.002$ and $P = 0.001$, resp.) and CNS impairment ($P = 0.02$ and $P = 0.007$, resp.)	[108]
A3G	179 HBV chronic carriers and 216 healthy control subjects from the Moroccan population	No significant difference in the frequencies of *APOBEC3G* H186R genotype between patients with chronic hepatitis B and control subjects	[98]
A3H	70 Italian HIV-exposed seronegative individuals and their HIV-1-infected sexual partners	The *APOBEC3H* haplotype I was found in a higher frequency in the exposed seronegative compared to the HIV^+ individuals ($P = 0.0056$), suggesting a protection from sexually transmitted HIV-1 infection	[109]
A3H	96 recently HIV-1-infected treatment-naïve adults	68 SNPs were analyzed Homozygous carriers of an *APOBEC3H* risk haplotype (A3Hrh) had lower GA → AA (A3F) sequence editing on proviral HIV-1 *vif* sequence ($P = 0.01$) and lower HIV-1 RNA levels ($P = 0.015$)	[110]

8 of *APOBEC3B*, which results in the complete removal of the *APOBEC3B* coding region, has been found in different human populations. Its frequency varies among ethnic groups, being more prevalent in East Asians, Amerindians, and Oceanic populations (36.9%, 57.7%, and 92.9%, resp.) but rare among Africans and Europeans (0.9% and 6%, resp.) [232]. This polymorphism has been associated with increased risk for persistent HBV infection and for the development of HBV-associated hepatocellular carcinoma. Due to the A3B ability of restricting HBV, it has been suggested that this gene deletion can result in reduced viral clearance, culminating with persistent infection [97]. In other studies, the homozygosity status for the deletion was associated with mild liver fibrosis, but not with a chronic carrier status [95], and with faster progression of liver disease [98]. Homozygous individuals for this deletion have also been reported with an increased risk for HIV acquisition, for a higher viral set point and for progression to AIDS [94], yet no effect was found on susceptibility to HIV infection and AIDS in Japanese or Indian populations [96].

Several polymorphisms in the *APOBEC3G* gene have also been described. In a study with 3,073 HIV patients from 6 different cohorts, a variant at *APOBEC3G* exon 4, H186R, was frequently found in African Americans (37%) but rare in European American (<3%) and in Europeans (5%). This polymorphism was associated with $CD4^+$ T-cell decline and with accelerated progression to AIDS-defining conditions in African Americans [99]. In another study with South African infected women, H186R was associated with higher HIV viral loads, and an extragenic mutation (rs35228531) was associated with decreased $CD4^+$ T-cell levels [104]. In a study with HIV-1-infected children from Pediatric AIDS Clinical Trials Group (PACTG) protocols P152 and P300, the H186R and F119F variants were associated with altered HIV-1-related disease progression and central nervous system impairment [108]. However, no correlation was found between H186R and disease progression in a French cohort [100]. This polymorphism has not been found in Indians [103]. Another *APOBEC3G* gene polymorphism, C40693T, was associated with an increased risk of HIV infection in a cohort of 122 Caucasian individuals highly exposed to HIV-1 [102]. Finally, the *APOBEC3G* SNP-571 (rs5757463) was associated with lower $CD4^+$ T-cell counts in homo- and heterozygosis in a group of HIV-1-infected individuals naive to drug therapy from Brazil [106].

APOBEC3H gene is also polymorphic, with seven haplotypes identified in human populations so far (named from I to VII). Of them, only three (II, V, and VII) seem to

originate a stable A3H protein, and have a higher anti-HIV-1 activity [156, 157, 233]. *APOBEC3H* haplotype II has also been reported to potently restrict HTLV-1 [189]. Some of these haplotypes have a variable frequency. Haplotype 2 is present in high frequency in African populations [233]; haplotype V is more frequently detected in African-Americans, Caribbeans, and Chinese, while haplotype VII was rarer and found only in European Caucasians [157].

A comprehensive list of studies that described the association of particular *APOBEC* gene variants with viral infections of disease outcomes can be seen in Table 1. Despite several of these so-called candidate gene analysis studies identified definite associations, particularly in the context of HIV and HBV infections, they were not confirmed in genome-wide association studies (GWAS). The latter studies point mainly to a significant role of human leukocyte antigen (HLA) alleles [234–239], suggesting that the impact of *APOBEC* variations on viral diseases is less robust and only moderate [234].

5. Concluding Remarks

Innate immunity mechanisms are the first line against invading viruses and are promising targets for preventing viral infections. Some infections are life-long once established, for example, by HIV and HSV-1, and therefore innate immunity is pivotal to avoiding the unfavorable consequences of infection of the host. The APOBEC family of cytidine deaminases plays an important role within innate immunity by deteriorating the genetic information of many viruses through hypermutation-dependent and -independent mechanisms. Polymorphisms in *APOBEC* genes that render differences in their expression and enzymatic activity also affect viral infection outcomes, and yet currently these effects appear modest and are not evidenced by GWAS. Viruses, on their hand, evolved molecular mechanisms to counteract APOBEC effects. Diminished APOBEC activity and sublethal levels of hypermutation may favor virus evolution, by generating viable variants carrying immune escape or drug resistance mutations. Conversely, the unfavorable consequences of APOBEC upregulation, particularly its recently described carcinogenic and genotoxic potential, are important caveats that will require further assessment and will pose a challenge to strategies aiming at increasing APOBEC expression to counteract viral infections. These issues will certainly warrant continuing investigation on the role and effects of cytidine deamination in infectious diseases and cancer.

Acknowledgments

V. C. Vieira is a recipient of a Ph.D. scholarship from the Brazilian Ministry of Health. This work is part of her requirements for obtaining a Ph.D. degree at the Graduate Program in Oncology of the Brazilian Cancer Institute (INCA). M. A. Soares is sponsored by grants of the Brazilian Ministry of Health, Brazilian Research Council (CNPq) and the Rio de Janeiro State Science Foundation (FAPERJ).

References

[1] A. Iwasaki, "A virological view of innate immune recognition," *Annual Review of Microbiology*, vol. 66, pp. 177–196, 2012.

[2] O. Takeuchi and S. Akira, "Innate immunity to virus infection," *Immunological Reviews*, vol. 227, no. 1, pp. 75–86, 2009.

[3] M. R. Thompson, J. J. Kaminski, E. A. Kurt-Jones, and K. A. Fitzgerald, "Pattern recognition receptors and the innate immune response to viral infection," *Viruses*, vol. 3, no. 6, pp. 920–940, 2011.

[4] D. B. Stetson and R. Medzhitov, "Type I interferons in host defense," *Immunity*, vol. 25, no. 3, pp. 373–381, 2006.

[5] S. G. Conticello, C. J. F. Thomas, S. K. Petersen-Mahrt, and M. S. Neuberger, "Evolution of the AID/APOBEC family of polynucleotide (deoxy)cytidine deaminases," *Molecular Biology and Evolution*, vol. 22, no. 2, pp. 367–377, 2005.

[6] T. Muto, M. Muramatsu, M. Taniwaki, K. Kinoshita, and T. Honjo, "Isolation, tissue distribution, and chromosomal localization of the human activation-induced cytidine deaminase (AID) gene," *Genomics*, vol. 68, no. 1, pp. 85–88, 2000.

[7] R. Espinosa III, T. Funahashi, C. Hadjiagapiou, M. M. le Beau, and N. O. Davidson, "Assignment of the gene encoding the human apolipoprotein B mRNA editing enzyme (APOBEC1) to chromosome 12p13.1," *Genomics*, vol. 24, no. 2, pp. 414–415, 1994.

[8] W. Liao, S. Hong, B. H. Chann, F. B. Rudolph, S. Clark, and L. Chan, "APOBEC-2, a cardiac- and skeletal muscle-specific member of the cytidine deaminase supergene family," *Biochemical and Biophysical Research Communications*, vol. 260, pp. 398–404, 1999.

[9] A. Jarmuz, A. Chester, J. Bayliss et al., "An anthropoid-specific locus of orphan C to U RNA-editing enzymes on chromosome 22," *Genomics*, vol. 79, no. 3, pp. 285–296, 2002.

[10] I. B. Rogozin, M. K. Basu, I. K. Jordan, Y. I. Pavlov, and E. V. Koonin, "APOBEC4, a new member of the AID/APOBEC family of polynucleotide (deoxy)cytidine deaminases predicted by computational analysis," *Cell Cycle*, vol. 4, no. 9, pp. 1281–1285, 2005.

[11] J. E. Wedekind, G. S. C. Dance, M. P. Sowden, and H. C. Smith, "Messenger RNA editing in mammals: new members of the APOBEC family seeking roles in the family business," *Trends in Genetics*, vol. 19, no. 4, pp. 207–216, 2003.

[12] B. Teng, C. F. Burant, and N. O. Davidson, "Molecular cloning of an apolipoprotein B messenger RNA editing protein," *Science*, vol. 260, no. 5115, pp. 1816–1818, 1993.

[13] R. S. Harris, S. K. Petersen-Mahrt, and M. S. Neuberger, "RNA editing enzyme APOBEC1 and some of its homologs can act as DNA mutators," *Molecular Cell*, vol. 10, no. 5, pp. 1247–1253, 2002.

[14] Y. Yang, Y. Yang, and H. C. Smith, "Multiple protein domains determine the cell type-specific nuclear distribution of the catalytic subunit required for apolipoprotein B mRNA editing," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 94, no. 24, pp. 13075–13080, 1997.

[15] P. P. Lau, W. Xiong, H.-J. Zhu, S.-H. Chen, and L. Chan, "Apolipoprotein B mRNA editing is an intranuclear event that occurs posttranscriptionally coincident with splicing and polyadenylation," *The Journal of Biological Chemistry*, vol. 266, no. 30, pp. 20550–20554, 1991.

[16] M. P. Sowden, N. Ballatori, K. L. de Mesy Jensen, L. Hamilton Reed, and H. C. Smith, "The editosome for cytidine to uridine

mRNA editing has a native complexity of 27S: identification of intracellular domains containing active and inactive editing factors," *Journal of Cell Science*, vol. 115, no. 5, pp. 1027–1039, 2002.

[17] S. Anant and N. O. Davidson, "Molecular mechanisms of apolipoprotein B mRNA editing," *Current Opinion in Lipidology*, vol. 12, no. 2, pp. 159–165, 2001.

[18] S. Anant and N. O. Davidson, "An AU-rich sequence element (UUUN[A/U]U) downstream of the edited C in apolipoprotein B mRNA is a high-affinity binding site for Apobec-1: binding of Apobec-1 to this motif in the 3' untranslated region of c-myc increases mRNA stability," *Molecular and Cellular Biology*, vol. 20, no. 6, pp. 1982–1992, 2000.

[19] B. R. Rosenberg, C. E. Hamilton, M. M. Mwangi, S. Dewell, and F. N. Papavasiliou, "Transcriptome-wide sequencing reveals numerous APOBEC1 mRNA-editing targets in transcript 3' UTRs," *Nature Structural & Molecular Biology*, vol. 18, no. 2, pp. 230–238, 2011.

[20] S. K. Dickerson, E. Market, E. Besmer, and F. N. Papavasiliou, "AID mediates hypermutation by deaminating single stranded DNA," *The Journal of Experimental Medicine*, vol. 197, no. 10, pp. 1291–1296, 2003.

[21] R. Bransteitter, P. Pham, M. D. Scharfft, and M. F. Goodman, "Activation-induced cytidine deaminase deaminates deoxycytidine on single-stranded DNA but requires the action of RNase," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 100, no. 7, pp. 4102–4107, 2003.

[22] C. Rada, J. M. Jarvis, and C. Milstein, "AID-GFP chemirec protein increases hypermutation og Ig genes with no evidence of nuclear localization," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 99, no. 10, pp. 7003–7008, 2002.

[23] S. S. Brar, M. Watson, and M. Diaz, "Activation-induced cytosine deaminase (AID) is actively exported out of the nucleus but retained by the induction of DNA breaks," *The Journal of Biological Chemistry*, vol. 279, no. 25, pp. 26395–26401, 2004.

[24] S. Ito, H. Nagaoka, R. Shinkura et al., "Activation-induced cytidine deaminase shuttles between nucleus and cytoplasm like apolipoprotein B mRNA editing catalytic polypeptide 1," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 101, no. 7, pp. 1975–1980, 2004.

[25] K. M. McBride, V. Barreto, A. R. Ramiro, P. Stavropoulos, and M. C. Nussenzweig, "Somatic hypermutation is limited by CRM1-dependent nuclear export of activation-induced deaminase," *The Journal of Experimental Medicine*, vol. 199, no. 9, pp. 1235–1244, 2004.

[26] M. Muramatsu, K. Kinoshita, S. Fagarasan, S. Yamada, Y. Shinkai, and T. Honjo, "Class switch recombination and hypermutation require activation-induced cytidine deaminase (AID), a potential RNA editing enzyme," *Cell*, vol. 102, no. 5, pp. 553–563, 2000.

[27] J. M. Di Noia and M. S. Neuberger, "Molecular mechanisms of antibody somatic hypermutation," *Annual Review of Biochemistry*, vol. 76, pp. 1–22, 2007.

[28] M. Muramatsu, V. S. Sankaranand, S. Anant et al., "Specific expression of activation-induced cytidine deaminase (AID), a novel member of the RNA-editing deaminase family in germinal center B cells," *The Journal of Biological Chemistry*, vol. 274, no. 26, pp. 18470–18476, 1999.

[29] C. Popp, W. Dean, S. Feng et al., "Genome-wide erasure of DNA methylation in mouse primordial germ cells is affected by AID deficiency," *Nature*, vol. 463, no. 7284, pp. 1101–1105, 2010.

[30] N. Bhutani, J. J. Brady, M. Damian, A. Sacco, S. Y. Corbel, and H. M. Blau, "Reprogramming towards pluripotency requires AID-dependent DNA demethylation," *Nature*, vol. 463, no. 7284, pp. 1042–1047, 2010.

[31] J. U. Guo, Y. Su, C. Zhong, G.-L. Ming, and H. Song, "Hydroxylation of 5-methylcytosine by TET1 promotes active DNA demethylation in the adult brain," *Cell*, vol. 145, no. 3, pp. 423–434, 2011.

[32] Y. Sato, H. C. Probst, R. Tatsumi, Y. Ikeuchi, M. S. Neuberger, and C. Rada, "Deficiency in APOBEC2 leads to a shift in muscle fiber type, diminished body mass, and myopathy," *The Journal of Biological Chemistry*, vol. 285, no. 10, pp. 7111–7118, 2010.

[33] J. E. Wedekind, R. Gillilan, A. Janda et al., "Nanostructures of APOBEC3G support a hierarchical assembly model of high molecular mass ribonucleoprotein particles from dimeric subunits," *The Journal of Biological Chemistry*, vol. 281, no. 50, pp. 38122–38126, 2006.

[34] F. Navarro, B. Bollman, H. Chen et al., "Complementary function of the two catalytic domains of APOBEC3G," *Virology*, vol. 333, no. 2, pp. 374–386, 2005.

[35] R. C. L. Beale, S. K. Petersen-Mahrt, I. N. Watt, R. S. Harris, C. Rada, and M. S. Neuberger, "Comparison of the differential context-dependence of DNA deamination by APOBEC enzymes: correlation with mutation spectra *in vivo*," *Journal of Molecular Biology*, vol. 337, no. 3, pp. 585–596, 2004.

[36] A. E. Armitage, A. Katzourakis, T. de Oliveira et al., "Conserved footprints of APOBEC3G on hypermutated human immunodeficiency virus type 1 and human endogenous retrovirus HERV-K(HML2) sequences," *Journal of Virology*, vol. 82, no. 17, pp. 8743–8761, 2008.

[37] K. N. Bishop, R. K. Holmes, A. M. Sheehy, N. O. Davidson, S.-J. Cho, and M. H. Malim, "Cytidine deamination of retroviral DNA by diverse APOBEC proteins," *Current Biology*, vol. 14, no. 15, pp. 1392–1396, 2004.

[38] R. S. Harris, K. N. Bishop, A. M. Sheehy et al., "DNA deamination mediates innate immunity to retroviral infection," *Cell*, vol. 113, no. 6, pp. 803–809, 2003.

[39] H. P. Bogerd, H. L. Wiegand, B. P. Doehle, K. K. Lueders, and B. R. Cullen, "APOBEC3A and APOBEC3B are potent inhibitors of LTR-retrotransposon function in human cells," *Nucleic Acids Research*, vol. 34, no. 1, pp. 89–95, 2006.

[40] M. Kinomoto, T. Kanno, M. Shimura et al., "All APOBEC3 family proteins differentially inhibit LINE-1 retrotransposition," *Nucleic Acids Research*, vol. 35, no. 9, pp. 2955–2964, 2007.

[41] F. Delebecque, R. Suspène, S. Calattini et al., "Restriction of foamy viruses by APOBEC cytidine deaminases," *Journal of Virology*, vol. 80, no. 2, pp. 605–614, 2006.

[42] Q. Yu, D. Chen, R. König, R. Mariani, D. Unutmaz, and N. R. Landau, "APOBEC3B and APOBEC3C are potent inhibitors of simian immunodeficiency virus replication," *The Journal of Biological Chemistry*, vol. 279, no. 51, pp. 53379–53386, 2004.

[43] J. L. Anderson and T. J. Hope, "APOBEC3G restricts early HIV-1 replication in the cytoplasm of target cells," *Virology*, vol. 375, no. 1, pp. 1–12, 2008.

[44] K. N. Bishop, R. K. Holmes, and M. H. Malim, "Antiviral potency of APOBEC proteins does not correlate with cytidine deamination," *Journal of Virology*, vol. 80, no. 17, pp. 8450–8458, 2006.

[45] K. N. Bishop, M. Verma, E.-Y. Kim, S. M. Wolinsky, and M. H. Malim, "APOBEC3G inhibits elongation of HIV-1 reverse transcripts," *PLoS Pathogens*, vol. 4, no. 12, Article ID e1000231, 2008.

[46] M. D. Stenglein, M. B. Burns, M. Li, J. Lengyel, and R. S. Harris, "APOBEC3 proteins mediate the clearance of foreign DNA from human cells," *Nature Structural & Molecular Biology*, vol. 17, no. 2, pp. 222–229, 2010.

[47] R. Suspène, M.-M. Aynaud, D. Guétard et al., "Somatic hypermutation of human mitochondrial and nuclear DNA by APOBEC3 cytidine deaminases, a pathway for DNA catabolism," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 108, no. 12, pp. 4858–4863, 2011.

[48] M. B. Burns, L. Lackey, M. A. Carpenter et al., "APOBEC3B is an enzymatic source of mutation in breast cancer," *Nature*, vol. 494, pp. 366–370, 2013.

[49] R. P. Bennett, V. Presnyak, J. E. Wedekind, and H. C. Smith, "Nuclear exclusion of the HIV-1 host defense factor APOBEC3G requires a novel cytoplasmic retention signal and is not dependent on RNA binding," *The Journal of Biological Chemistry*, vol. 283, no. 12, pp. 7320–7327, 2008.

[50] L. Lackey, Z. L. Demorest, A. M. Land, J. F. Hultquist, W. L. Brown, and R. S. Harris, "APOBEC3B and AID have similar nuclear import mechanisms," *Journal of Molecular Biology*, vol. 419, no. 5, pp. 301–314, 2012.

[51] M. M. H. Li and M. Emerman, "Polymorphism in human APOBEC3H affects a phenotype dominant for subcellular localization and antiviral activity," *Journal of Virology*, vol. 85, no. 16, pp. 8197–8207, 2011.

[52] A. M. Land, E. K. Law, M. A. Carpenter, L. Lackey, W. L. Brown, and R. S. Harris, "Endogenous APOBEC3A is cytoplasmic and non-genotoxic," *The Journal of Biological Chemistry*, 2013.

[53] Y.-L. Chiu, V. B. Soros, J. F. Kreisberg, K. Stopak, W. Yonemoto, and W. C. Greene, "Cellular APOBEC3G restricts HIV-1 infection in resting CD4+ T cells," *Nature*, vol. 435, no. 7038, pp. 108–114, 2005.

[54] Y.-L. Chiu and W. C. Greene, "The APOBEC3 cytidine deaminases: an innate defensive network opposing exogenous retroviruses and endogenous retroelements," *Annual Review of Immunology*, vol. 26, pp. 317–353, 2008.

[55] H. C. Smith, R. P. Bennett, A. Kizilyer, W. M. McDougall, and K. M. Prohaska, "Functions and regulation of the APOBEC family of proteins," *Seminars in Cell and Developmental Biology*, vol. 23, no. 3, pp. 258–268, 2012.

[56] X. Wang, P. T. Dolan, Y. Dang, and Y.-H. Zheng, "Biochemical differentiation of APOBEC3F and APOBEC3G proteins associated with HIV-1 life cycle," *The Journal of Biological Chemistry*, vol. 282, no. 3, pp. 1585–1594, 2007.

[57] L. Tan, P. T. N. Sarkis, T. Wang, C. Tian, and X.-F. Yu, "Sole copy of Z2-type human cytidine deaminase APOBEC3H has inhibitory activity against retrotransposons and HIV-1," *The FASEB Journal*, vol. 23, no. 1, pp. 279–287, 2009.

[58] A. M. Niewiadomska, C. Tian, L. Tan, T. Wang, P. T. N. Sarkis, and X.-F. Yu, "Differential inhibition of long interspersed element 1 by APOBEC3 does not correlate with high-molecular-mass-complex formation or P-body association," *Journal of Virology*, vol. 81, no. 17, pp. 9577–9583, 2007.

[59] J. F. Kreisberg, W. Yonemoto, and W. C. Greene, "Endogenous factors enhance HIV infection of tissue naive CD4 T cells by stimulating high molecular mass APOBEC3G complex formation," *The Journal of Experimental Medicine*, vol. 203, no. 4, pp. 865–870, 2006.

[60] K. S. Stopak, Y.-L. Chiu, J. Kropp, R. M. Grant, and W. C. Greene, "Distinct patterns of cytokine regulation of APOBEC3G expression and activity in primary lymphocytes, macrophages, and dendritic cells," *The Journal of Biological Chemistry*, vol. 282, no. 6, pp. 3539–3546, 2007.

[61] P. J. Ellery, E. Tippett, Y.-L. Chiu et al., "The CD16+ monocyte subset is more permissive to infection and preferentially harbors HIV-1 *in vivo*," *Journal of Immunology*, vol. 178, no. 10, pp. 6581–6589, 2007.

[62] M. Kamata, Y. Nagaoka, and I. S. Y. Chen, "Reassessing the role of APOBEC3G in human immunodeficiency virus type 1 infection of quiescent CD4+ T-cells," *PLoS Pathogens*, vol. 5, no. 3, Article ID e1000342, 2009.

[63] F. R. Santoni de Sio and D. Trono, "APOBEC3G-depleted resting CD4+ T cells remain refractory to HIV1 infection," *PLoS ONE*, vol. 4, no. 8, article e6571, 2009.

[64] V. B. Soros, W. Yonemoto, and W. C. Greene, "Newly synthesized APOBEC3G is incorporated into HIV virions, inhibited by HIV RNA, and subsequently activated by RNase H," *PLoS Pathogens*, vol. 3, no. 2, article e15, 2007.

[65] M. A. Khan, R. Goila-Gaur, S. Kao, E. Miyagi, R. C. Walker Jr., and K. Strebel, "Encapsidation of APOBEC3G into HIV-1 virions involves lipid raft association and does not correlate with APOBEC3G oligomerization," *Retrovirology*, vol. 6, article 1742, 2009.

[66] J. Ma, X. Li, J. Xu et al., "The cellular source for APOBEC3G's incorporation into HIV-1," *Retrovirology*, vol. 8, article 2, 2011.

[67] Y.-L. Chiu, H. E. Witkowska, S. C. Hall et al., "High-molecular-mass APOBEC3G complexes restrict Alu retrotransposition," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 103, no. 42, pp. 15588–15593, 2006.

[68] S. Gallois-Montbrun, B. Kramer, C. M. Swanson et al., "Antiviral protein APOBEC3G localizes to ribonucleoprotein complexes found in P bodies and stress granules," *Journal of Virology*, vol. 81, no. 5, pp. 2165–2178, 2007.

[69] S. Gallois-Montbrun, R. K. Holmes, C. M. Swanson et al., "Comparison of cellular ribonucleoprotein complexes associated with the APOBEC3F and APOBEC3G antiviral proteins," *Journal of Virology*, vol. 82, no. 11, pp. 5636–5642, 2008.

[70] S. L. Kozak, M. Marin, K. M. Rose, C. Bystrom, and D. Kabat, "The anti-HIV-1 editing enzyme APOBEC3G binds HIV-1 RNA and messenger RNAs that shuttle between polysomes and stress granules," *The Journal of Biological Chemistry*, vol. 281, no. 39, pp. 29105–29119, 2006.

[71] M. J. Wichroski, G. B. Robb, and T. M. Rana, "Human retroviral host restriction factors APOBEC3G and APOBEC3F localize to mRNA processing bodies," *PLoS Pathogens*, vol. 2, no. 5, article e41, 2006.

[72] P. K. Phalora, N. M. Sherer, S. M. Wolinsky, C. M. Swanson, and M. H. Malim, "HIV-1 replication and APOBEC3 antiviral activity are not regulated by P bodies," *Journal of Virology*, vol. 86, no. 21, pp. 11712–11724, 2012.

[73] A. G. Lada, C. Frahm Krick, S. G. Kozmin et al., "Mutator effects and mutation signatures of editing deaminases produced in bacteria and yeast," *Biochemistry*, vol. 76, no. 1, pp. 131–146, 2011.

[74] S. G. Conticello, "Creative deaminases, self-inflicted damage, and genome evolution," *Annals of the New York Academy of Sciences*, vol. 1267, pp. 79–85, 2012.

[75] C. Münk, B. E. O. Jensen, J. Zielonka, D. Häussinger, and C. Kamp, "Running loose or getting lost: how HIV-1 counters and capitalizes on APOBEC3-induced mutagenesis through its Vif protein," *Viruses*, vol. 4, pp. 3132–3161, 2012.

[76] F. Severi, A. Chicca, and S. G. Conticello, "Analysis of reptilian APOBEC1 suggests that RNA Editing may not be its ancestral function," *Molecular Biology and Evolution*, vol. 28, no. 3, pp. 1125–1129, 2011.

[77] R. S. Harris and M. T. Liddament, "Retroviral restriction by APOBEC proteins," *Nature Reviews Immunology*, vol. 4, no. 11, pp. 868–877, 2004.

[78] C. Münk, A. Willemsen, and I. Bravo, "An ancient history of gene duplications, fusions and losses in the evolution of APOBEC3 mutators in mammals," *BMC Evolutionary Biology*, vol. 12, article 71, 2012.

[79] R. S. LaRue, S. R. Jónsson, K. A. T. Silverstein et al., "The artiodactyl APOBEC3 innate immune repertoire shows evidence for a multi-functional domain organization that existed in the ancestor of placental mammals," *BMC Molecular Biology*, vol. 9, no. 104, pp. 1–20, 2008.

[80] C. Münk, T. Beck, J. Zielonka et al., "Functions, structure, and read-through alternative splicing of feline APOBEC3 genes," *Genome Biology*, vol. 9, no. 3, article R48, 2008.

[81] G. G. Schumann, "APOBEC3 proteins: major players in intracellular defence against LINE-1-mediated retrotransposition," *Biochemical Society Transactions*, vol. 35, no. 3, pp. 637–642, 2007.

[82] A. Koito and T. Ikeda, "Intrinsic immunity against retrotransposons by APOBEC cytidine deaminases," *Frontiers in Microbiology*, vol. 4, pp. 1–9, 2013.

[83] H. P. Bogerd, H. L. Wiegand, A. E. Hulme et al., "Cellular inhibitors of long interspersed element 1 and Alu retrotransposition," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 103, no. 23, pp. 8780–8785, 2006.

[84] A. E. Hulme, H. P. Bogerd, B. R. Cullen, and J. V. Moran, "Selective inhibition of Alu retrotransposition by APOBEC3G," *Gene*, vol. 390, no. 1-2, pp. 199–205, 2007.

[85] C. Esnault, O. Heidmann, F. Delebecque et al., "APOBEC3G cytidine deaminase inhibits retrotransposition of endogenous retroviruses," *Nature*, vol. 433, no. 7024, pp. 430–433, 2005.

[86] C. Esnault, J. Millet, O. Schwartz, and T. Heidmann, "Dual inhibitory effects of APOBEC family proteins on retrotransposition of mammalian endogenous retroviruses," *Nucleic Acids Research*, vol. 34, no. 5, pp. 1522–1531, 2006.

[87] A. J. Schumacher, D. V. Nissley, and R. S. Harris, "APOBEC3G hypermutates genomic DNA and inhibits Ty1 retrotransposition in yeast," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 28, pp. 9854–9859, 2005.

[88] D. A. Macduff, Z. L. Demorest, and R. S. Harris, "AID can restrict L1 retrotransposition suggesting a dual role in innate and adaptive immunity," *Nucleic Acids Research*, vol. 37, no. 6, pp. 1854–1867, 2009.

[89] T. Ikeda, K. H. Abd El Galil, K. Tokunaga et al., "Intrinsic restriction activity by apolipoprotein B mRNA editing enzyme APOBEC1 against the mobility of autonomous retrotransposons," *Nucleic Acids Research*, vol. 39, no. 13, pp. 5538–5554, 2011.

[90] M. Metzner, H.-M. Jäck, and M. Wabl, "LINE-1 retroelements complexed and inhibited by activation induced cytidine deaminase," *PloS One*, vol. 7, no. 11, article e49358, 2012.

[91] K. N. Bishop, R. K. Holmes, A. M. Sheehy, and M. H. Malim, "APOBEC-mediated editing of viral RNA," *Science*, vol. 305, no. 5684, article 645, 2004.

[92] T. Ikeda, T. Ohsugi, T. Kimura et al., "The antiretroviral potency of APOBEC1 deaminase from small animal species," *Nucleic Acids Research*, vol. 36, no. 21, pp. 6859–6871, 2008.

[93] V. Petit, D. Guétard, M. Renard et al., "Murine APOBEC1 is a powerful mutator of retroviral and cellular RNA *in vitro* and *in vivo*," *Journal of Molecular Biology*, vol. 385, no. 1, pp. 65–78, 2009.

[94] P. An, R. Johnson, J. Phair et al., "APOBEC3B deletion and risk of HIV-1 acquisition," *The Journal of Infectious Diseases*, vol. 200, no. 7, pp. 1054–1058, 2009.

[95] H. Abe, H. Ochi, T. Maekawa et al., "Effects of structural variations of APOBEC3A and APOBEC3B genes in chronic hepatitis B virus infection," *Hepatology Research*, vol. 39, no. 12, pp. 1159–1168, 2009.

[96] S. Itaya, T. Nakajima, G. Kaur et al., "No evidence of an association between the APOBEC3B deletion polymorphism and susceptibility to HIV infection and AIDS in Japanese and indian populations," *The Journal of Infectious Diseases*, vol. 202, no. 5, pp. 815–816, 2010.

[97] T. Zhang, J. Cai, J. Chang et al., "Evidence of associations of APOBEC3B gene deletion with susceptibility to persistent HBV infection and hepatocellular carcinoma," *Human Molecular Genetics*, vol. 22, no. 6, pp. 1262–1269, 2012.

[98] S. Ezzikouri, B. Kitab, K. Rebbani et al., "Polymorphic APOBEC3 modulates chronic hepatitis B in Moroccan population," *Journal of Viral Hepatitis*, pp. 1–9, 2012.

[99] P. An, G. Bleiber, P. Duggal et al., "APOBEC3G genetic variants and their influence on the progression to AIDS," *Journal of Virology*, vol. 78, no. 20, pp. 11070–11076, 2004.

[100] H. Do, A. Vasilescu, G. Diop et al., "Exhaustive genotyping of the CEM15 (APOBEC3G) gene and absence of association with AIDS progression in a French cohort," *The Journal of Infectious Diseases*, vol. 191, no. 2, pp. 159–163, 2005.

[101] C. Pace, J. Keller, D. Nolan et al., "Population level analysis of human immunodeficiency virus type 1 hypermutation and its relationship with APOBEC3G and vif genetic variation," *Journal of Virology*, vol. 80, no. 18, pp. 9259–9269, 2006.

[102] H. S. Valcke, N. F. Bernard, J. Bruneau, M. Alary, C. M. Tsoukas, and M. Roger, "APOBEC3G genetic variants and their association with risk of HIV infection in highly exposed Caucasians," *AIDS*, vol. 20, no. 15, pp. 1984–1986, 2006.

[103] A. Rathore, A. Chatterjee, N. Yamamoto, and T. N. Dhole, "Absence of H186R polymorphism in exon 4 of the APOBEC3G gene among north Indian individuals," *Genetic Testing*, vol. 12, no. 3, pp. 453–456, 2008.

[104] K. Reddy, C. A. Winkler, L. Werner, K. Mlisana, S. S. Abdool Karim, and T. Ndung'U, "Apobec3g expression is dysregulated in primary hiv-1 infection and polymorphic variants influence cd4+ t-cell counts and plasma viral load," *AIDS*, vol. 24, no. 2, pp. 195–204, 2010.

[105] F. A. de Maio, C. A. Rocco, P. C. Aulicino, R. Bologna, A. Mangano, and L. Sen, "Effect of HIV-1 Vif variability on progression to pediatric AIDS and its association with APOBEC3G and CUL5 polymorphisms," *Infection, Genetics and Evolution*, vol. 11, no. 6, pp. 1256–1262, 2011.

[106] M. C. Bizinoto, É. Leal, R. S. Diaz, and L. M. Janini, "Loci polymorphisms of the APOBEC3G gene in HIV type 1-infected Brazilians," *AIDS Research and Human Retroviruses*, vol. 27, no. 2, pp. 137–141, 2011.

[107] F. A. de Maio, C. A. Rocco, P. C. Aulicino, R. Bologna, A. Mangano, and L. Sen, "APOBEC3-mediated editing in

HIV type 1 from pediatric patients and its association with APOBEC3G/CUL5 polymorphisms and Vif variability," *AIDS Research and Human Retroviruses*, vol. 28, no. 6, pp. 619–627, 2012.

[108] K. K. Singh, Y. Wang, K. P. Gray et al., "Genetic variants in the host restriction factor APOBEC3G are associated with HIV-1-related disease progression and central nervous system impairment in children," *Journal of Acquired Immune Deficiency Syndromes*, vol. 62, no. 2, pp. 197–203, 2013.

[109] R. Cagliani, S. Riva, M. Fumagalli et al., "A positively selected APOBEC3H haplotype is associated with natural resistance to HIV-1 infection," *Evolution*, vol. 65, no. 11, pp. 3311–3322, 2011.

[110] P. A. Gourraud, A. Karaouni, J. M. Woo et al., "APOBEC3H haplotypes and HIV-1 pro-viral vif DNA sequence diversity in early untreated human immunodeficiency virus-1 infection," *Human Immunology*, vol. 72, no. 3, pp. 207–212, 2011.

[111] J. A. Levy, "HIV pathogenesis: 25 years of progress and persistent challenges," *AIDS*, vol. 23, no. 2, pp. 147–160, 2009.

[112] S. Cen, F. Guo, M. Niu, J. Saadatmand, J. Deflassieux, and L. Kleiman, "The interaction between HIV-1 gag and APOBEC3G," *The Journal of Biological Chemistry*, vol. 279, no. 32, pp. 33177–33184, 2004.

[113] T. M. Alce and W. Popik, "APOBEC3G is incorporated into virus-like particles by a direct interaction with HIV-1 gag nucleocapsid protein," *The Journal of Biological Chemistry*, vol. 279, no. 33, pp. 34083–34086, 2004.

[114] K. Luo, B. Liu, Z. Xiao et al., "Amino-terminal region of the human immunodeficiency virus type 1 nucleocapsid is required for human APOBEC3G packaging," *Journal of Virology*, vol. 78, no. 21, pp. 11841–11852, 2004.

[115] V. Zennou, D. Perez-Caballero, H. Göttlinger, and P. D. Bieniasz, "APOBEC3G incorporation into human immunodeficiency virus type 1 particles," *Journal of Virology*, vol. 78, no. 21, pp. 12058–12061, 2004.

[116] M. Douaisi, S. Dussart, M. Courcoul, G. Bessou, R. Vigne, and E. Decroly, "HIV-1 and MLV Gag proteins are sufficient to recruit APOBEC3G into virus-like particles," *Biochemical and Biophysical Research Communications*, vol. 321, no. 3, pp. 566–573, 2004.

[117] A. Schäfer, H. P. Bogerd, and B. R. Cullen, "Specific packaging of APOBEC3G into HIV-1 virions is mediated by the nucleocapsid domain of the gag polyprotein precursor," *Virology*, vol. 328, no. 2, pp. 163–168, 2004.

[118] E. S. Svarovskaia, H. Xu, J. L. Mbisa et al., "Human apolipoprotein B mRNA-editing enzyme-catalytic polypeptide-like 3G (APOBEC3G) is incorporated into HIV-1 virions through interactions with viral and nonviral RNAs," *The Journal of Biological Chemistry*, vol. 279, no. 34, pp. 35822–35828, 2004.

[119] A. Burnett and P. Spearman, "APOBEC3G multimers are recruited to the plasma membrane for packaging into human immunodeficiency virus type 1 virus-like particles in an RNA-dependent process requiring the NC basic linker," *Journal of Virology*, vol. 81, no. 10, pp. 5000–5013, 2007.

[120] T. Wang, W. Zhang, C. Tian et al., "Distinct viral determinants for the packaging of human cytidine deaminases APOBEC3G and APOBEC3C," *Virology*, vol. 377, no. 1, pp. 71–79, 2008.

[121] D. Lecossier, F. Bouchonnet, F. Clavel, and A. J. Hance, "Hypermutation of HIV-1 DNA in the absence of the Vif protein," *Science*, vol. 300, no. 5622, p. 1112, 2003.

[122] B. Schröfelbauer, Q. Yu, S. G. Zeitlin, and N. R. Landau, "Human immunodeficiency virus type 1 Vpr induces the degradation of the UNG and SMUG Uracil-DNA glycosylases," *Journal of Virology*, vol. 79, no. 17, pp. 10978–10987, 2005.

[123] B. Yang, K. Chen, C. Zhang, S. Huang, and H. Zhang, "Virion-associated uracil DNA glycosylase-2 and apurinic/apyrimidinic endonuclease are involved in the degradation of APOBEC3G-edited nascent HIV-1 DNA," *The Journal of Biological Chemistry*, vol. 282, no. 16, pp. 11667–11675, 2007.

[124] S. M. Kaiser and M. Emerman, "Uracil DNA glycosylase is dispensable for human immunodeficiency virus type 1 replication and does not contribute to the antiviral effects of the cytidine deaminase Apobec3G," *Journal of Virology*, vol. 80, no. 2, pp. 875–882, 2006.

[125] M.-A. Langlois and M. S. Neuberger, "Human APOBEC3G can restrict retroviral infection in avian cells and acts independently of both UNG and SMUG1," *Journal of Virology*, vol. 82, no. 9, pp. 4660–4664, 2008.

[126] R. K. Holmes, F. A. Koning, K. N. Bishop, and M. H. Malim, "APOBEC3F can inhibit the accumulation of HIV-1 reverse transcription products in the absence of hypermutation: comparisons with APOBEC3G," *The Journal of Biological Chemistry*, vol. 282, no. 4, pp. 2587–2595, 2007.

[127] Y. Iwatani, D. S. B. Chan, F. Wang et al., "Deaminase-independent inhibition of HIV-1 reverse transcription by APOBEC3G," *Nucleic Acids Research*, vol. 35, no. 21, pp. 7096–7108, 2007.

[128] E. N. C. Newman, R. K. Holmes, H. M. Craig et al., "Antiviral function of APOBEC3G can be dissociated from cytidine deaminase activity," *Current Biology*, vol. 15, no. 2, pp. 166–170, 2005.

[129] J. L. Mbisa, R. Barr, J. A. Thomas et al., "Human immunodeficiency virus type 1 cDNAs produced in the presence of APOBEC3G exhibit defects in plus-strand DNA transfer and integration," *Journal of Virology*, vol. 81, no. 13, pp. 7099–7110, 2007.

[130] K. Gillick, D. Pollpeter, P. Phalora, E. Y. Kim, S. M. Wolinsky, and M. H. Malim, "Suppression of HIV-1 infection by APOBEC3 proteins in primary human CD4+ T cells is associated with inhibition of processive reverse transcription as well as excessive cytidine deamination," *Journal of Virology*, vol. 87, no. 3, pp. 1508–1517, 2013.

[131] F. Guo, S. Cen, M. Niu, J. Saadatmand, and L. Kleiman, "Inhibition of tRNA3Lys-primed reverse transcription by human APOBEC3G during human immunodeficiency virus type 1 replication," *Journal of Virology*, vol. 80, no. 23, pp. 11710–11722, 2006.

[132] X.-Y. Li, F. Guo, L. Zhang, L. Kleiman, and S. Cen, "APOBEC3G inhibits DNA strand transfer during HIV-1 reverse transcription," *The Journal of Biological Chemistry*, vol. 282, no. 44, pp. 32065–32074, 2007.

[133] X. Wang, Z. Ao, L. Chen, G. Kobinger, J. Peng, and X. Yao, "The cellular antiviral protein APOBEC3G interacts with HIV-1 reverse transcriptase and inhibits its function during viral replication," *Journal of Virology*, vol. 86, no. 7, pp. 3777–3786, 2012.

[134] K. Luo, T. Wang, B. Liu et al., "Cytidine deaminases APOBEC3G and APOBEC3F interact with human immunodeficiency virus type 1 integrase and inhibit proviral DNA formation," *Journal of Virology*, vol. 81, no. 13, pp. 7238–7248, 2007.

[135] J. L. Mbisa, W. Bu, and V. K. Pathak, "APOBEC3F and APOBEC3G inhibit HIV-1 DNA integration by different mechanisms," *Journal of Virology*, vol. 84, no. 10, pp. 5250–5259, 2010.

[136] N. Casartelli, F. Guivel-Benhassine, R. Bouziat, S. Brandler, O. Schwartz, and A. Moris, "The antiviral factor APOBEC3G improves CTL recognition of cultured HIV-infected T cells," *The Journal of Experimental Medicine*, vol. 207, no. 1, pp. 39–49, 2010.

[137] J. M. Norman, M. Mashiba, L. A. McNamara et al., "The antiviral factor APOBEC3G enhances the recognition of HIV-infected primary T cells by natural killer cells," *Nature Immunology*, vol. 12, no. 10, pp. 975–983, 2011.

[138] A. M. Sheehy, N. C. Gaddis, and M. H. Malim, "The antiretroviral enzyme APOBEC3G is degraded by the proteasome in response to HIV-1 Vif," *Nature Medicine*, vol. 9, no. 11, pp. 1404–1407, 2003.

[139] X. Yu, Y. Yu, B. Liu et al., "Induction of APOBEC3G ubiquitination and degradation by an HIV-1 Vif-Cul5-SCF complex," *Science*, vol. 302, no. 5647, pp. 1056–1060, 2003.

[140] S. G. Conticello, R. S. Harris, and M. S. Neuberger, "The Vif protein of HIV triggers degradation of the human antiretroviral DNA deaminase APOBEC3G," *Current Biology*, vol. 13, no. 22, pp. 2009–2013, 2003.

[141] K. Shirakawa, A. Takaori-Kondo, M. Kobayashi et al., "Ubiquitination of APOBEC3 proteins by the Vif-Cullin5-ElonginB-ElonginC complex," *Virology*, vol. 344, no. 2, pp. 263–266, 2006.

[142] R. Mariani, D. Chen, B. Schröfelbauer et al., "Species-specific exclusion of APOBEC3G from HIV-1 virions by Vif," *Cell*, vol. 114, no. 1, pp. 21–31, 2003.

[143] A. Mehle, B. Strack, P. Ancuta, C. Zhang, M. McPike, and D. Gabuzda, "Vif overcomes the innate antiviral activity of APOBEC3G by promoting its degradation in the ubiquitin-proteasome pathway," *The Journal of Biological Chemistry*, vol. 279, no. 9, pp. 7792–7798, 2004.

[144] S. Opi, S. Kao, R. Goila-Gaur et al., "Human immunodeficiency virus type 1 Vif inhibits packaging and antiviral activity of a degradation-resistant APOBEC3G variant," *Journal of Virology*, vol. 81, no. 15, pp. 8236–8246, 2007.

[145] S. Kao, M. A. Khan, E. Miyagi, R. Plishka, A. Buckler-White, and K. Strebel, "The human immunodeficiency virus type 1 Vif protein reduces intracellular expression and inhibits packaging of APOBEC3G (CEM15), a cellular inhibitor of virus infectivity," *Journal of Virology*, vol. 77, no. 21, pp. 11398–11407, 2003.

[146] K. Stopak, C. de Noronha, W. Yonemoto, and W. C. Greene, "HIV-1 Vif blocks the antiviral activity of APOBEC3G by impairing both its translation and intracellular stability," *Molecular Cell*, vol. 12, no. 3, pp. 591–601, 2003.

[147] G. Mercenne, S. Bernacchi, D. Richer et al., "HIV-1 Vif binds to APOBEC3G mRNA and inhibits its translation," *Nucleic Acids Research*, vol. 38, no. 2, pp. 633–646, 2009.

[148] C. Chaipan, J. L. Smith, W. S. Hu, and V. K. Pathak, "APOBEC3G restricts HIV-1 to a greater extent than APOBEC3F and APOBEC3DE in human primary CD4+ T cells and macrophages," *Journal of Virology*, vol. 87, no. 1, pp. 444–453, 2013.

[149] L. C. F. Mulder, M. Ooms, S. Majdak et al., "Moderate influence of human APOBEC3F on HIV-1 replication in primary lymphocytes," *Journal of Virology*, vol. 84, no. 18, pp. 9613–9617, 2010.

[150] M. T. Liddament, W. L. Brown, A. J. Schumacher, and R. S. Harris, "APOBEC3F properties and hypermutation preferences indicate activity against HIV-1 *in vivo*," *Current Biology*, vol. 14, no. 15, pp. 1385–1391, 2004.

[151] H. L. Wiegand, B. P. Doehle, H. P. Bogerd, and B. R. Cullen, "A second human antiretroviral factor, APOBEC3F, is suppressed by the HIV-1 and HIV-2 Vif proteins," *The EMBO Journal*, vol. 23, no. 12, pp. 2451–2458, 2004.

[152] K. Bourara, T. J. Liegler, and R. M. Grant, "Target cell APOBEC3C can induce limited G-to-A mutation in HIV-1," *PLoS Pathogens*, vol. 3, no. 10, pp. 1477–1485, 2007.

[153] Y. Dang, M. S. Lai, X. Wang, Y. Han, R. Lampen, and Y.-H. Zheng, "Human cytidine deaminase APOBEC3H restricts HIV-1 replication," *The Journal of Biological Chemistry*, vol. 283, no. 17, pp. 11606–11614, 2008.

[154] Y. Dang, X. Wang, W. J. Esselman, and Y.-H. Zheng, "Identification of APOBEC3DE as another antiretroviral factor from the human APOBEC family," *Journal of Virology*, vol. 80, no. 21, pp. 10522–10533, 2006.

[155] B. P. Doehle, A. Schäfer, and B. R. Cullen, "Human APOBEC3B is a potent inhibitor of HIV-1 infectivity and is resistant to HIV-1 Vif," *Virology*, vol. 339, no. 2, pp. 281–288, 2005.

[156] A. Harari, M. Ooms, L. C. F. Mulder, and V. Simon, "Polymorphisms and splice variants influence the antiretroviral activity of Human APOBEC3H," *Journal of Virology*, vol. 83, no. 1, pp. 295–303, 2009.

[157] X. Wang, A. Abudu, S. Son, Y. Dang, P. J. Venta, and Y.-H. Zheng, "Analysis of human APOBEC3H haplotypes and anti-human immunodeficiency virus type 1 activity," *Journal of Virology*, vol. 85, no. 7, pp. 3142–3152, 2011.

[158] J. S. Albin and R. S. Harris, "Interactions of host APOBEC3 restriction factors with HIV-1 *in vivo*: implications for therapeutics," *Expert Reviews in Molecular Medicine*, vol. 12, no. e4, pp. 1–26, 2010.

[159] J. A. Vázquez-Pérez, C. E. Ormsby, R. Hernández-Juan, K. J. Torres, and G. Reyes-Terán, "APOBEC3G mRNA expression in exposed seronegative and early stage HIV infected individuals decreases with removal of exposure and with disease progression," *Retrovirology*, vol. 6, article 23, 2009.

[160] A. M. Land, T. B. Ball, M. Luo et al., "Human immunodeficiency virus (HIV) type 1 proviral hypermutation correlates with CD4 count in HIV-infected women from Kenya," *Journal of Virology*, vol. 82, no. 16, pp. 8172–8182, 2008.

[161] Y. Kourteva, M. de Pasquale, T. Allos, C. McMunn, and R. T. D'Aquila, "APOBEC3G expression and hypermutation are inversely associated with human immunodeficiency virus type 1 (HIV-1) burden *in vivo*," *Virology*, vol. 430, pp. 1–9, 2012.

[162] N. K. Ulenga, A. D. Sarr, D. Hamel, J.-L. Sankale, S. Mboup, and P. J. Kanki, "The level of APOBEC3G (hA3G)-related G-to-A mutations does not correlate with viral load in HIV type 1-infected individuals," *AIDS Research and Human Retroviruses*, vol. 24, no. 10, pp. 1285–1290, 2008.

[163] A. Piantadosi, D. Humes, B. Chohan, R. S. McClelland, and J. Overbaugh, "Analysis of the percentage of human immunodeficiency virus type 1 sequences that are hypermutated and markers of disease progression in a longitudinal cohort, including one individual with a partially defective vif," *Journal of Virology*, vol. 83, no. 16, pp. 7805–7814, 2009.

[164] N. D. Amoêdo, A. O. Afonso, S. M. Cunha, R. H. Oliveira, E. S. Machado, and M. A. Soares, "Expression of APOBEC3G/3F and G-to-A hypermutation levels in HIV-1-infected children with different profiles of disease progression," *PloS One*, vol. 6, no. 8, article e24118, 2011.

[165] X. Jin, A. Brooks, H. Chen, R. Bennett, R. Reichman, and H. Smith, "APOBEC3G/CEM15 (hA3G) mRNA levels associate inversely with human immunodeficiency virus viremia," *Journal of Virology*, vol. 79, no. 17, pp. 11513–11516, 2005.

[166] M. Zhao, W. Geng, Y. Jiang et al., "The associations of hA3G and hA3B mRNA levels with HIV disease progression among HIV-infected individuals of China," *Journal of Acquired Immune Deficiency Syndromes*, vol. 53, supplement 1, pp. S4–S9, 2010.

[167] N. K. Ulenga, A. D. Sarr, S. Thakore-Meloni, J.-L. Sankalé, G. Eisen, and P. J. Kanki, "Relationship between human immunodeficiency type 1 infection and expression of human APOBEC3G and APOBEC3F," *The Journal of Infectious Diseases*, vol. 198, no. 4, pp. 486–492, 2008.

[168] S.-J. Cho, H. Drechsler, R. C. Burke, M. Q. Arens, W. Powderly, and N. O. Davidson, "APOBEC3F and APOBEC3G mRNA levels do not correlate with human immunodeficiency virus type 1 plasma viremia or CD4+ T-cell count," *Journal of Virology*, vol. 80, no. 4, pp. 2069–2072, 2006.

[169] M. Biasin, L. Piacentini, S. Lo Caputo et al., "Apolipoprotein B mRNA-editing enzyme, catalytic polypeptide-like 3G: a possible role in the resistance to HIV of HIV-exposed seronegative individuals," *The Journal of Infectious Diseases*, vol. 195, no. 7, pp. 960–964, 2007.

[170] T. Ejima, M. Hirota, T. Mizukami, M. Otsuka, and M. Fujita, "An anti-HIV-1 compound that increases steady-state expression of apoplipoprotein B mRNA-editing enzyme-catalytic polypeptide-like 3G," *International Journal of Molecular Medicine*, vol. 28, no. 4, pp. 613–616, 2011.

[171] S. Cen, Z.-G. Peng, X.-Y. Li et al., "Small molecular compounds inhibit HIV-1 replication through specifically stabilizing APOBEC3G," *The Journal of Biological Chemistry*, vol. 285, no. 22, pp. 16546–16552, 2010.

[172] R. Nathans, H. Cao, N. Sharova et al., "Small-molecule inhibition of HIV-1 Vif," *Nature Biotechnology*, vol. 26, no. 10, pp. 1187–1192, 2008.

[173] Z. Xiao, E. Ehrlich, K. Luo, Y. Xiong, and X.-F. Yu, "Zinc chelation inhibits HIV Vif activity and liberates antiviral function of the cytidine deaminase APOBEC3G," *The FASEB Journal*, vol. 21, no. 1, pp. 217–222, 2007.

[174] J. F. Hultquist and R. S. Harris, "Leveraging APOBEC3 proteins to alter the HIV mutation rate and combat AIDS," *Future Virology*, vol. 4, no. 6, pp. 605–619, 2009.

[175] L. C. F. Mulder, A. Harari, and V. Simon, "Cytidine deamination induced HIV-1 drug resistance," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 105, no. 14, pp. 5501–5506, 2008.

[176] P. Jern, R. A. Russell, V. K. Pathak, and J. M. Coffin, "Likely role of APOBEC3G-mediated G-to-A mutations in HIV-1 evolution and drug resistance," *PLoS Pathogens*, vol. 5, no. 4, Article ID e1000367, 2009.

[177] N. Wood, T. Bhattacharya, B. F. Keele et al., "HIV evolution in early infection: selection pressures, patterns of insertion and deletion, and the impact of APOBEC," *PLoS Pathogens*, vol. 5, no. 5, Article ID e1000414, 2009.

[178] G. Haché, K. Shindo, J. S. Albin, and R. S. Harris, "Evolution of HIV-1 isolates that use a novel Vif-independent mechanism to resist restriction by human APOBEC3G," *Current Biology*, vol. 18, no. 11, pp. 819–824, 2008.

[179] S. K. Pillai, J. K. Wong, and J. D. Barbour, "Turning up the volume on mutational pressure: is more of a good thing always better? (A case study of HIV-1 Vif and APOBEC3)," *Retrovirology*, vol. 5, article 26, 2008.

[180] E.-Y. Kim, T. Bhattacharya, K. Kunstman et al., "Human APOBEC3G-mediated editing can promote HIV-1 sequence diversification and accelerate adaptation to selective pressure," *Journal of Virology*, vol. 84, no. 19, pp. 10402–10405, 2010.

[181] H. A. Sadler, M. D. Stenglein, R. S. Harris, and L. M. Mansky, "APOBEC3G contributes to HIV-1 variation through sublethal mutagenesis," *Journal of Virology*, vol. 84, no. 14, pp. 7396–7404, 2010.

[182] S. Fourati, I. Malet, S. Lambert et al., "E138K and M184I mutations in HIV-1 reverse transcriptase coemerge as a result of APOBEC3 editing in the absence of drug exposure," *AIDS*, vol. 26, no. 13, pp. 1619–1624, 2012.

[183] M. E. Olson, M. Li, R. S. Harris, and D. A. Harki, "Small-molecule APOBEC3G DNA cytosine deaminase inhibitors based on a 4-amino-1, 2, 4-triazole-3-thiol scaffold," *ChemMedChem*, vol. 8, pp. 112–117, 2013.

[184] M. Li, S. M. D. Shandilya, M. A. Carpenter et al., "First-in-class small molecule inhibitors of the single-strand DNA cytosine deaminase APOBEC3G," *ACS Chemical Biology*, vol. 7, no. 3, pp. 506–517, 2012.

[185] M. Matsuoka and K.-T. Jeang, "Human T-cell leukaemia virus type 1 (HTLV-1) infectivity and cellular transformation," *Nature Reviews Cancer*, vol. 7, no. 4, pp. 270–280, 2007.

[186] P. Kannian and P. L. Green, "Human T lymphotropic virus type 1 (HTLV-1): molecular biology and oncogenesis," *Viruses*, vol. 2, no. 9, pp. 2037–2077, 2010.

[187] J. H. Richardson, A. J. Edwards, J. K. Cruickshank, P. Rudge, and A. G. Dalgleish, "In vivo cellular tropism of human T-cell leukemia virus type 1," *Journal of Virology*, vol. 64, no. 11, pp. 5682–5687, 1990.

[188] E. W. Refsland, M. D. Stenglein, K. Shindo, J. S. Albin, W. L. Brown, and R. S. Harris, "Quantitative profiling of the full APOBEC3 mRNA repertoire in lymphocytes and tissues: implications for HIV-1 restriction," *Nucleic Acids Research*, vol. 38, no. 13, pp. 4274–4284, 2010.

[189] M. Ooms, A. Krikoni, A. K. Kress, V. Simon, and C. Münk, "APOBEC3A, APOBEC3B, and APOBEC3H haplotype 2 restrict human T-lymphotropic virus type 1," *Journal of Virology*, vol. 86, no. 11, pp. 6097–6108, 2012.

[190] J.-P. Vartanian, U. Plikat, M. Henry et al., "HIV genetic variation is directed and restricted by DNA precursor availability," *Journal of Molecular Biology*, vol. 270, no. 2, pp. 139–151, 1997.

[191] J. Fan, M. Guangyong, K. Nosaka et al., "APOBEC3G generates nonsense mutations in human T-cell leukemia virus type 1 proviral genomes *in vivo*," *Journal of Virology*, vol. 84, no. 14, pp. 7278–7287, 2010.

[192] R. Mahieux, R. Suspène, F. Delebecque et al., "Extensive editing of a small fraction of human T-cell leukemia virus type 1 genomes by four APOBEC3 cytidine deaminases," *Journal of General Virology*, vol. 86, no. 9, pp. 2489–2494, 2005.

[193] A. Sasada, A. Takaori-Kondo, K. Shirakawa et al., "APOBEC3G targets human T-cell leukemia virus type 1," *Retrovirology*, vol. 2, pp. 1–10, 2005.

[194] E. Wattel, J.-P. Vartanian, C. Pannetier, and S. Wain-Hobson, "Clonal expansion of human T-cell leukemia virus type I-infected cells in asymptomatic and symptomatic carriers without malignancy," *Journal of Virology*, vol. 69, no. 5, pp. 2863–2868, 1995.

[195] M. Cavrois, S. Wain-Hobson, A. Gessain, Y. Plumelle, and E. Wattel, "Adult T-cell leukemia/lymphoma on a background of clonally expanding human T-cell leukemia virus type-1-positive cells," *Blood*, vol. 88, no. 12, pp. 4646–4650, 1996.

[196] M. Cavrois, I. Leclercq, O. Gout, A. Gessain, S. Wain-Hobson, and E. Wattel, "Persistent oligoclonal expansion of human T-cell leukemia virus type 1-infected circulating cells in patients with

Tropical spastic paraparesis/HTLV-1 associated myelopathy," *Oncogene*, vol. 17, no. 1, pp. 77–82, 1998.

[197] D. Derse, S. A. Hill, G. Princler, P. Lloyd, and G. Heidecker, "Resistance of human T cell leukemia virus type 1 to APOBEC3G restriction is mediated by elements in nucleocapsid," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, no. 8, pp. 2915–2920, 2007.

[198] J.-H. Kao and D.-S. Chen, "Global control of hepatitis B virus infection," *Lancet Infectious Diseases*, vol. 2, no. 7, pp. 395–403, 2002.

[199] WHO, "HBV Fact Sheet no. 204," http://www.who.int/mediacentre/factsheets/fs204/en/.

[200] M. C. Gonzalez, R. Suspène, M. Henry, D. Guétard, S. Wain-Hobson, and J.-P. Vartanian, "Human APOBEC1 cytidine deaminase edits HBV DNA," *Retrovirology*, vol. 6, article 96, 2009.

[201] C. Rösler, J. Köck, M. Kann et al., "APOBEC-mediated interference with hepadnavirus production," *Hepatology*, vol. 42, no. 2, pp. 301–309, 2005.

[202] R. Suspène, D. Guétard, M. Henry, P. Sommer, S. Wain-Hobson, and J.-P. Vartanian, "Extensive editing of both hepatitis B virus DNA strands by APOBEC3 cytidine deaminases *in vitro* and *in vivo*," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 23, pp. 8321–8326, 2005.

[203] T. F. Baumert, C. Rösler, M. H. Malim, and F. von Weizsäcker, "Hepatitis B virus DNA is subject to extensive editing by the human deaminase AP0BEC3C," *Hepatology*, vol. 46, no. 3, pp. 682–689, 2007.

[204] J. Köck and H. E. Blum, "Hypermutation of hepatitis B virus genomes by APOBEC3G, APOBEC3C and APOBEC3H," *Journal of General Virology*, vol. 89, no. 5, pp. 1184–1191, 2008.

[205] M. Henry, D. Guétard, R. Suspène, C. Rusniok, S. Wain-Hobson, and J.-P. Vartanian, "Genetic editing of HBV DNA by monodomain human APOBEC3 cytidine deaminases and the recombinant nature of APOBEC3G," *PLoS ONE*, vol. 4, no. 1, article e4277, 2009.

[206] J.-P. Vartanian, M. Henry, A. Marchio et al., "Massive APOBEC3 editing of hepatitis B viral DNA in cirrhosis," *PLoS Pathogens*, vol. 6, no. 5, Article ID e1000928, 2010.

[207] P. Turelli, B. Mangeat, S. Jost, S. Vianin, and D. Trono, "Inhibition of Hepatitis B virus replication by APOBEC3G," *Science*, vol. 303, no. 5665, article 1829, 2004.

[208] D. H. Nguyen, S. Gummuluru, and J. Hu, "Deamination-independent inhibition of hepatitis B virus reverse transcription by APOBEC3G," *Journal of Virology*, vol. 81, no. 9, pp. 4465–4472, 2007.

[209] W. Zhang, X. Zhang, C. Tian et al., "Cytidine deaminase APOBEC3B interacts with heterogeneous nuclear ribonucleoprotein K and suppresses hepatitis B virus expression," *Cellular Microbiology*, vol. 10, no. 1, pp. 112–121, 2008.

[210] M. Bonvin, F. Achermann, I. Greeve et al., "Interferon-inducible expression of APOBEC3 editing enzymes in human hepatocytes and inhibition of hepatitis B virus replication," *Hepatology*, vol. 43, no. 6, pp. 1364–1374, 2006.

[211] Y. Tanaka, H. Marusawa, H. Seno et al., "Anti-viral protein APOBEC3G is induced by interferon-α stimulation in human hepatocytes," *Biochemical and Biophysical Research Communications*, vol. 341, no. 2, pp. 314–319, 2006.

[212] C. Noguchi, N. Hiraga, N. Mori et al., "Dual effect of APOBEC3G on Hepatitis B virus," *Journal of General Virology*, vol. 88, no. 2, pp. 432–440, 2007.

[213] S. Günther, G. Sommer, U. Plikat et al., "Naturally occurring hepatitis B virus genomes bearing the hallmarks of retroviral G → A hypermutation," *Virology*, vol. 235, no. 1, pp. 104–108, 1997.

[214] S. Margeridon-Thermet, N. S. Shulman, A. Ahmed et al., "Ultra-deep pyrosequencing of hepatitis b virus quasispecies from nucleoside and nucleotide reverse-transcriptase inhibitor (NRTI)-treated patients and NRTI-naive patients," *The Journal of Infectious Diseases*, vol. 199, no. 9, pp. 1275–1285, 2009.

[215] S. Proto, J. A. Taylor, S. Chokshi, N. Navaratnam, and N. V. Naoumov, "APOBEC and iNOS are not the main intracellular effectors of IFN-γ-mediated inactivation of Hepatitis B virus replication," *Antiviral Research*, vol. 78, no. 3, pp. 260–267, 2008.

[216] S. Jost, P. Turelli, B. Mangeat, U. Protzer, and D. Trono, "Induction of antiviral cytidine deaminases does not explain the inhibition of hepatitis B virus replication by interferons," *Journal of Virology*, vol. 81, no. 19, pp. 10588–10596, 2007.

[217] R. Xu, X. Zhang, W. Zhang, Y. Fang, S. Zheng, and X.-F. Yu, "Association of human APOBEC3 cytidine deaminases with the generation of hepatitis virus B x antigen mutants and hepatocellular carcinoma," *Hepatology*, vol. 46, no. 6, pp. 1810–1820, 2007.

[218] D. Moradpour, F. Penin, and C. M. Rice, "Replication of hepatitis C virus," *Nature Reviews Microbiology*, vol. 5, no. 6, pp. 453–463, 2007.

[219] M. Levrero, "Viral hepatitis and liver cancer: the case of hepatitis C," *Oncogene*, vol. 25, no. 27, pp. 3834–3847, 2006.

[220] Z.-G. Peng, Z.-Y. Zhao, Y.-P. Li et al., "Host apolipoprotein b messenger RNA-editing enzyme catalytic polypeptide-like 3G is an innate defensive factor and drug target against hepatitis C virus," *Hepatology*, vol. 53, no. 4, pp. 1080–1089, 2011.

[221] Y. Komohara, H. Yano, S. Shichijo, K. Shimotohno, K. Itoh, and A. Yamada, "High expression of APOBEC3G in patients infected with hepatitis C virus," *Journal of Molecular Histology*, vol. 37, no. 8-9, pp. 327–332, 2006.

[222] M. Á. Jiménez-Sousa, R. Almansa, C. de la Fuente et al., "Gene expression profiling in the first twelve weeks of treatment in chronic hepatitis C patients," *Enfermedades Infecciosas y Microbiologia Clinica*, vol. 29, no. 8, pp. 573–580, 2011.

[223] S. K. Pillai, M. Abdel-Mohsen, J. Guatelli et al., "Role of retroviral restriction factors in the interferon-α-mediated suppression of HIV-1 *in vivo*," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 109, no. 8, pp. 3035–3040, 2012.

[224] N. Muñoz, X. Castellsagué, A. B. de González, and L. Gissmann, "HPV in the etiology of human cancer," *Vaccine*, vol. 24, supplement 3, pp. S3/1–S3/10, 2006.

[225] J.-P. Vartanian, D. Guétard, M. Henry, and S. Wain-Hobson, "Evidence for editing of human papillomavirus DNA by APOBEC3 in benign and precancerous lesions," *Science*, vol. 320, no. 5873, pp. 230–233, 2008.

[226] R. Suspène, M.-M. Aynaud, S. Koch et al., "Genetic editing of herpes simplex virus 1 and epstein-barr herpesvirus genomes by Human APOBEC3 cytidine deaminases in culture and *in vivo*," *Journal of Virology*, vol. 85, no. 15, pp. 7594–7602, 2011.

[227] P. Gee, Y. Ando, H. Kitayama et al., "APOBEC1-mediated editing and attenuation of herpes simplex virus 1 DNA indicate that neurons have an antiviral role during herpes simplex encephalitis," *Journal of Virology*, vol. 85, no. 19, pp. 9726–9736, 2011.

[228] R. J. Whitley, D. W. Kimberlin, and B. Roizman, "Herpes simplex viruses," *Clinical Infectious Diseases*, vol. 26, no. 3, pp. 541–555, 1998.

[229] R. J. Whitley and B. Roizman, "Herpes simplex virus infections," *The Lancet*, vol. 357, no. 9267, pp. 1513–1518, 2001.

[230] C. Chisholm and L. Lopez, "Cutaneous infections caused by herpesviridae: a review," *Archives of Pathology and Laboratory Medicine*, vol. 135, no. 10, pp. 1357–1362, 2011.

[231] N. Mendoza, M. Diamantis, A. Arora et al., "Mucocutaneous manifestations of Epstein-Barr virus infection," *American Journal of Clinical Dermatology*, vol. 9, no. 5, pp. 295–305, 2008.

[232] J. M. Kidd, T. L. Newman, E. Tuzun, R. Kaul, and E. E. Eichler, "Population stratification of a common APOBEC gene deletion polymorphism," *PLoS Genetics*, vol. 3, no. 4, article e63, 2007.

[233] M. OhAinle, J. A. Kerns, M. M. H. Li, H. S. Malik, and M. Emerman, "Antiretroelement activity of APOBEC3H was lost twice in recent human evolution," *Cell Host & Microbe*, vol. 4, no. 3, pp. 249–259, 2008.

[234] P. An and C. A. Winkler, "Host genes associated with HIV/AIDS: advances in gene discovery," *Trends in Genetics*, vol. 26, no. 3, pp. 119–131, 2010.

[235] J. R. Lingappa, S. Petrovski, E. Kahle et al., "Genomewide association study for determinants of HIV-1 acquisition and viral set point in HIV-1 serodiscordant couples with quantified virus exposure," *PLoS ONE*, vol. 6, no. 12, article e28632, 2011.

[236] D. van Manen, A. B. van Wout, and H. Schuitemaker, "Genome-wide association studies on HIV susceptibility, pathogenesis and pharmacogenomics," *Retrovirology*, vol. 9, no. 70, pp. 1–8, 2012.

[237] Y. Kamatani, S. Wattanapokayakit, H. Ochi et al., "A genome-wide association study identifies variants in the HLA-DP locus associated with chronic hepatitis B in Asians," *Nature Genetics*, vol. 41, no. 5, pp. 591–595, 2009.

[238] H. Zhang, Y. Zhai, Z. Hu et al., "Genome-wide association study identifies 1p36.22 as a new susceptibility locus for hepatocellular carcinoma in chronic hepatitis B virus carriers," *Nature Genetics*, vol. 42, no. 9, pp. 755–758, 2010.

[239] S. Li, J. Qian, Y. Yang et al., "GWAS identifies novel susceptibility loci on 6p21. 32 and 21q21. 3 for hepatocellular carcinoma in chronic hepatitis B virus carriers," *PLoS Genetics*, vol. 8, no. 7, pp. 1–8, 2012.

Hindawi Publishing Corporation
BioMed Research International
Volume 2013, Article ID 693643, 13 pages
http://dx.doi.org/10.1155/2013/693643

Review Article

HLA-G/C, miRNAs, and Their Role in HIV Infection and Replication

Fulvio Celsi,[1] Eulalia Catamo,[2] Giulio Kleiner,[1] Paola Maura Tricarico,[2] Josef Vuch,[2] and Sergio Crovella[1,2]

[1] *Institute for Maternal and Child Health, IRCCS Burlo Garofolo, 34137 Trieste, Italy*
[2] *University of Trieste, 34128 Trieste, Italy*

Correspondence should be addressed to Fulvio Celsi; celsi@burlo.trieste.it

Received 21 March 2013; Revised 3 May 2013; Accepted 7 May 2013

Academic Editor: Enrique Medina-Acosta

In recent years, a number of different mechanisms regulating gene expressions, either in normal or in pathological conditions, have been discovered. This review aims to highlight some of the regulatory pathways involved during the HIV-1 infection and disease progression, focusing on the novel discovered microRNAs (miRNAs) and their relation with immune system's agents. Human leukocyte antigen (HLA) family of proteins plays a key role because it is a crucial modulator of the immune response; here we will examine recent findings, centering especially on HLA-C and -G, novel players lately discovered to engage in modulation of immune system. We hope to provide novel perspectives useful to find out original therapeutic roads against HIV-1 infection and AIDS progression.

1. Introduction

Gene expression is a tightly regulated mechanism, in a cell- as well as time-specific manner, and numerous different pathways exist to regulate this activity. Human immunodeficiency virus (HIV) infection is able to perturb and alter gene expression through several mechanisms that can, lastly, cause acquired immunodeficiency syndrome (AIDS). In this review, we will focus on novel mechanisms of gene expression regulation, centering on recently discovered players in the interaction between HIV and immune system. Major histocompatibility complex class I molecules (MHC-I) are necessary for an efficient host immune response to HIV-1 infection, as detailed below. A subset of MHC-1 allotypes are associated with effective control of viral replication and slow disease progression. In a series of recent genome-wide association studies, a relevant association between some HLA-C single-nucleotide polymorphisms (SNPs) and HIV-1 infection has been found [1–4], and similar researches have been done also for HLA-G, as detailed below [5, 6].

1.1. HIV Infection and Replication. The human immunodeficiency virus (HIV) is an RNA virus included in the genus *Lentivirus*, family Retroviridae, and it is the cause of the AIDS. This virus is formed of a diploid single strand RNA genome enclosed in a truncated cone capsid with a phospholipidic bilayer envelope, containing the proteins that allow the virus entry into the cells. The HIV-1 genome consists of three sequences, *gag*, *pol,* and *env*. *Gag* codes for the proteins, p6 of the viral capsid, p7 of the nucleocapsid, and p17 of the matrix. *Pol* codes for the replicative enzymes required for the biological cycle and replication, as reverse transcriptase, protease, and so forth. Finally, *env* codes for gp160, which is the precursor of the viral envelope proteins, gp120 and gp41. Furthermore, the HIV-1 genome present 6 genes encoding proteins that regulate the life cycle of the virus [7], as *tat* and *rev*, essential for replication, *vif*, *vpu,* and *vpr*, which regulate the ability of replication and assembly of the new viral particles, *nef*, which reduces the expression of CD4 on the host cell and promotes virus release [8]. The HIV-1 infection is mediated by interaction between the proteins of the viral

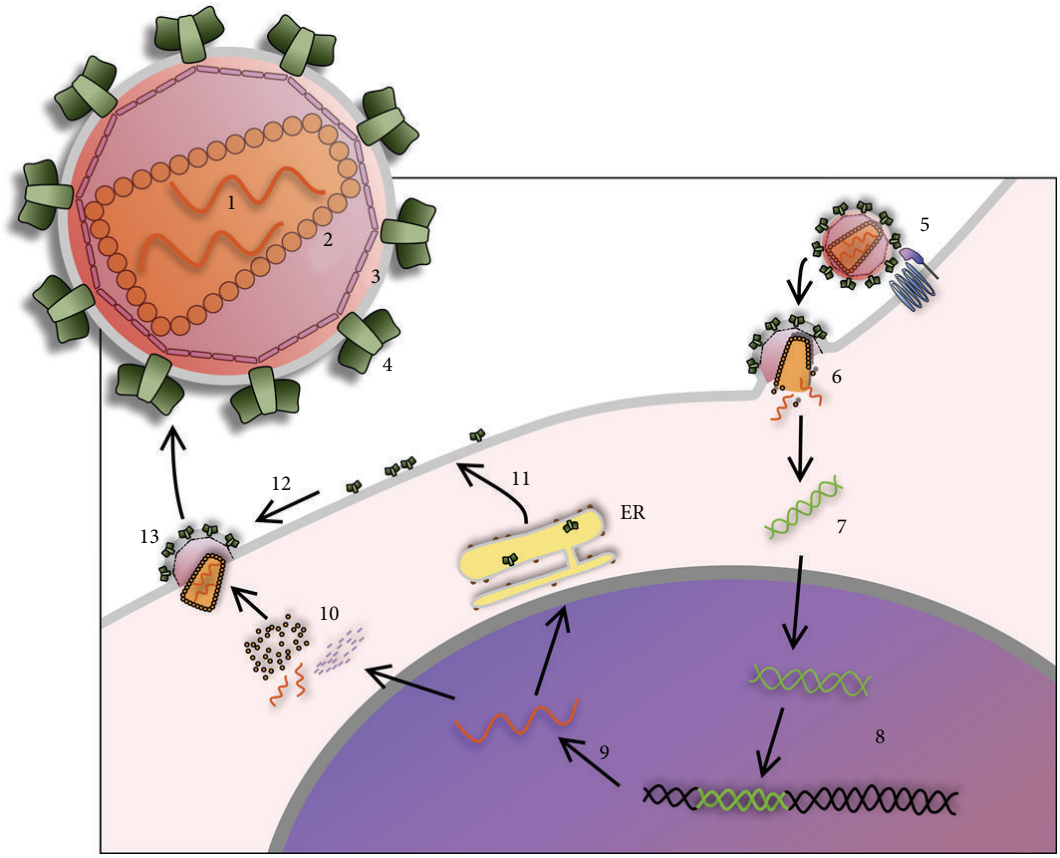


Figure 1: HIV virus is formed by a diploid single strand RNA genome (1) enclosed in a truncated cone capsid (2) with a phospholipidic bilayer envelope (3), containing the proteins that allow the virus entry into the cells (4). The HIV-1 infection is mediated by interaction between the proteins of the viral envelope, leukocyte receptor, and coreceptor (5). This interaction causes the membranes fusion and the uncoating of the virion core (6). The viral RNA is reverse transcribed in DNA (7) which enters in the nucleus where the integrase enzyme catalyzes the insertion of the viral genome into the genome of the host cell (8). The expression of integrated viral genome is controlled by the RNA-binding proteins *tat* and *rev*. A set of RNAs are transported from the nucleus to the cytoplasm, where they can be translated or packaged (9). The new core proteins localize near the cell membrane (10), while the envelope mRNA is translated at the endoplasmic reticulum (ER) and subsequently the envelope proteins are placed on the cell membrane (11). Finally, the capsid proteins are assembled with the viral genomic RNA (12), and an immature virion begins to bud from cell surface (13).

envelope, gp120 and gp41, and the receptor of T lymphocyte, CD4. This interaction causes a conformational change of gp120 that binds the coreceptors CXCR4 and CCR5, present on the surface of T lymphocyte [9]. A hydrophobic region of gp41, called "fusion peptide," penetrates the membrane of the target cell and causes the fusion between plasmalemmas. The virion core is then uncoated to expose a viral nucleoprotein complex, and then the RNA of this complex is reverse transcribed in DNA. The viral DNA thus enters in the nucleus together with the integrase enzyme, which catalyzes the insertion of the viral genome into the genome of the host cell (see Figure 1).

The expression of integrated viral genome is controlled by the RNA-binding proteins, *tat* and *rev*, able to orchestrate complex interactions with the cellular transcription, RNA splicing, and RNA transport machinery. HIV latency arises when levels of the regulatory protein *tat* fall to below threshold levels [10].

A set of RNAs, either spliced or full genome length, is transported from the nucleus to the cytoplasm, where RNAs can be translated or packaged. The new core proteins localize near the cell membrane, while the envelope (*env*) mRNA is translated at the endoplasmic reticulum (ER), and subsequently the envelope proteins are placed on the cell membrane. Finally, the capsid proteins are assembled with the viral genomic RNA, and an immature virion begins to bud from cell surface. In this process the virions are also coated with *env*. Before having the ability to infect another cell, the virion undergoes a morphological change known as maturation that includes proteolytic processing of the Gag and Gag-Pol polyproteins by viral enzyme PR and a less well defined function of Vif [8].

1.2. Small Noncoding RNA. The human genome is constituted by protein-coding sequences for only two percent while the remaining vast majority of it, previously considered as "junk DNA," could be transcribed and it is now named as non-coding RNA (ncRNA). These different RNAs could be responsible for some of the complex differences between

humans and other primates, since protein-coding components are extremely similar even in these different species [11]. The ncRNAs present in the cell are the already well-known transfer RNA (tRNA) and ribosomal RNA (rRNA) together with the less-known small nucleolar RNA (snoRNA), long noncoding RNA (lncRNA), piwi-interacting RNA (piRNA), and microRNA (miRNA). SnoRNAs are small molecules of RNA found in the nucleolus: they are responsible for guiding a series of site-specific posttranscriptional modifications in tRNA and rRNA, such as conversion of the nucleoside uridine to pseudouridine [11]. The so-called "long" ncRNAs are over 200 nucleotides in length (sometimes even more than 15 kb) and, in the majority of cases, do not share sequence homology between each other. These longer transcripts are spliced, capped, and polyadenylated, and they are involved in the regulation of the gene expression [11, 12]. PiRNAs are the largest class of small ncRNAs expressed in vertebrates; generally ranging from 25 to 33 nucleotides in length, piRNAs play a key role during spermatogenesis in defending germline cells against transposons by selectively silencing them [11, 13].

Last but not least miRNAs, 22 nt endogenous RNAs, are known to play important regulatory roles in eukaryotic cells by targeting mRNAs for cleavage or translational repression. The first step in the miRNA biogenesis is the transcription by (usually) polymerase II of a long sequence called "primary-miRNA" (pri-miRNA), subsequently cleaved in the nucleus to liberate a 60–70 nt stem loop intermediate, known as the miRNA precursor (pre-miRNA). An RNase III endonuclease, called Drosha, cleaves the RNA duplex with a staggered cut, and thus the base of the pre-miRNA stem loop has a $5'$ phosphate and 2 nt $3'$ overhang. The next step is the pre-miRNA active transport from the nucleus to the cytoplasm by Ran-GTP and the export receptor Exportin-5. In the cytoplasm, the Dicer enzyme (another RNase III endonuclease) cuts both strands of the duplex and leaves the $5'$ phosphate and 2 nt $3'$ producing an imperfect duplex. These strands produced by Dicer are incorporated as single-stranded RNAs into a ribonucleoprotein complex, known as the RNA-induced silencing complex (RISC). The RISC identifies target sequences based on Watson-Crick complementarity between the $3'$ miRNA and the mRNA.

If the annealing between the sequence loaded on the RISC complex and the target is perfect, then Argonaute protein (specifically Ago2, one of the component of RISC complex) cleaves the RNA duplex approximately in the middle, producing two "naked" RNA sequences (one without the poly-A signal, the other without the "cap") that are subsequently degraded. If the annealing between the miRNA sequence and the target sequence is not perfect, the Ago2 RNase activity is not present, nonetheless the mRNA translation is inhibited (through not yet clear mechanisms), and the target mRNA is subsequently degraded [11, 14].

1.3. Human Leukocyte Antigen. The major histocompatibility complex-1 (MHC-1), also known as human leukocyte antigen-1 (HLA-1), plays a central role in both adaptive and innate immunity; its locus, mapping at chromosome 6 in the human genome, comprises two subgroups, the classical class I molecules (HLA-1a) and the nonclassical molecules (HLA-1b). The highly polymorphic HLA-1a family includes HLA-A, -B, and -C, widely expressed in most tissues, where they expose on the cell surfaces the antigenic peptides. In contrast, the nonclassical HLA-1b family includes HLA-E, -F, and -G: they are less polymorphic and often exhibit a relatively restricted tissue distribution [15].

HLA-1a and -1b molecules consist, respectively, of a 44 kDa and 41 kDa heavy α-chain (divided in 3 subdomains: α1, α2, and α3) noncovalently associated with a light β-chain, the β_2-microglobulin (β_2M), and a short antigen peptide derived from the degradation of intracellular proteins [16]. In particular, HLA-1a molecules are membrane-bound proteins, while HLA-1b molecules have membrane-bound isoforms and soluble isoforms [17].

MHC-1 molecules are assembled in the lumen of the endoplasmic reticulum (ER) with the help of molecular chaperones before being loaded with the immunological-relevant peptides. These peptides are of cellular or viral origin, and they are produced in the cytosol by proteasome. Transporter associated with antigen processing (TAP) pumps the peptides into the ER lumen where they are bounded to MHC-1 thanks to the adaptor protein tapasin. After leaving the ER, the complexes egress through the golgi to the plasma membrane where it can remain or can be released in a soluble form to extracellular space (see Figure 2). In this way, MHC-1 can interact with the T cells and the natural killer cells (NK) [18].

HLA genes are extremely polymorphic; for this reason antigen-presenting cells can differently present a specific antigenic peptide to the immune system. Therefore, HLA genes have also been studied as candidate genes able to modify host's genetic background during infectious diseases progression [19].

1.3.1. HLA-C. HLA-C is naturally expressed on the cell surface at levels approximately 10-fold less than most HLA-A and HLA-B allotypes, and fewer alleles of this protein have been identified, as compared to HLA-A or -B [20]. HLA-C plays a dual role: it presents antigen to cytotoxic T lymphocytes (CTLs), albeit less efficiently than either HLA-A or -B [21], while it results more efficient in inhibiting natural killer (NK) cell lysis via its interaction with inhibitory killer cell immunoglobulin-like receptor (KIR) [22].

Many viruses, such as HCV [23] and HIV [24], use this inhibitory capacity to facilitate their infections in host organism. Indeed, viruses take advantage of several regulatory mechanisms in order to modify levels and distribution of HLA-1a. In all cases, protection from attack by cytotoxic T lymphocytes (CTL) is primarily mediated by down-regulation of HLA-A and -B, but not HLA-C. In this way, the presence of HLA-C may allow inhibition of NK cells expressing KIRs [25, 26]. However, high HLA-C expression levels could damage viral infections due to the increase of the antigen presentation to CTL. For these reasons, the HLA-C levels are finely tuned during viral infections [27].

1.3.2. HLA-G. HLA-G proteins can be expressed as seven distinct isoforms, by means of an alternative splicing from

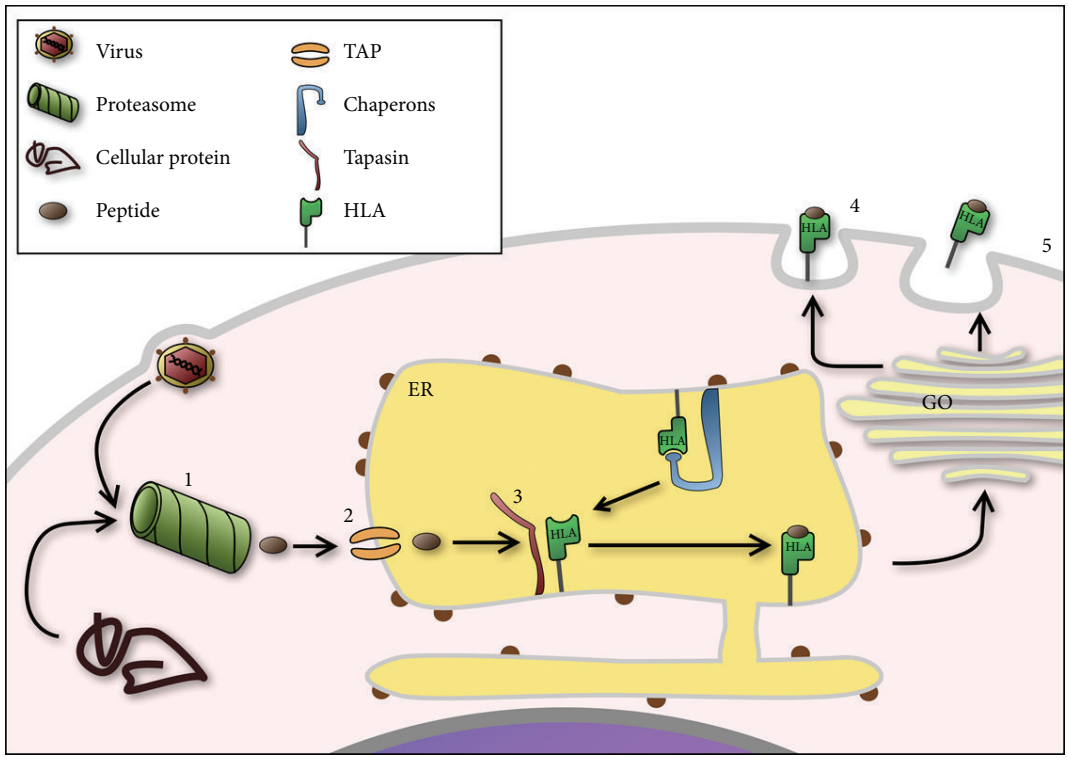


FIGURE 2: MHC1 are assembled in the lumen of the endoplasmatic reticulum where interaction with target peptides is mediated by chaperons. These peptides, cellular or viral derived, are produced in the cytosol by proteasome (1) and are transported into the ER lumen by TAP pump (2). Subsequently, the chaperon tapasin interacts with TAP, promoting the bond between peptides and HLA (3). The complex HLA peptide egresses through the golgi complex to the plasma membrane where it can remain (4) or can be released in soluble form to the extracellular space (5). ER: endoplasmic reticulum. GO: golgi apparatus.

a single primary transcript. Four isoforms are membrane-bound proteins (HLA-G1, -G2, -G3, and -G4), and the other three isoforms are soluble proteins (HLA-G5, -G6, and G7) [17]. HLA-G molecules are not ubiquitously expressed, albeit they are detected under physiological conditions in several tissues, such as placental trophoblast cells, thymus, cornea, nail matrix, pancreas, and some cells of the immune system as monocytes [28, 29]; however, in nonphysiological conditions, (i.e., in tumors) HLA-G molecules are overexpressed in aberrant patterns [30].

HLA-G is an important immunomodulatory molecule, and it plays a fundamental role in maternal-fetal tolerance, transplantation, and in tumor progression [31]. HLA-G molecules can inhibit natural killer (NK) cells lysis and cytotoxic activity of $CD8^+$ T cells by interaction with inhibitory receptors KIR, LILRB1, and LILRB2 [32]. The inhibition of both these classes of inflammatory cells creates an anti-inflammatory environment, due to a release of cytokines, such as interleukin 10 (IL-10), with anti-inflammatory properties that are able to upregulate HLA-G, and those in turn increases IL-10 secretion, thus creating a positive-feedback loop [28]. This immunosuppressive capacity of HLA-G is exploited by many viruses, which have developed multiple strategies for subverting host immune defenses. In fact, it has been reported that HLA-G expression in cells is also upregulated following infection with human cytomegalovirus (CMV) and HIV [33, 34].

2. HLA and HIV

During viral infection, some viruses undergo degradation by cellular proteasome complex, and the cytosolic antigenic peptides are carried into the ER. In this organelle, the peptides are captured by HLA I molecules and then exposed on the cell surface, triggering the cytotoxic activity of the circulating $CD8^+$ T lymphocytes, as described previously.

The HIV has devised different ways to evade the immune response, including a Nef-dependent mechanism that downregulates the HLA I expression, thus avoiding the recognition of the infected cells by $CD8^+$ T lymphocytes [25]. Selectively, Nef alters the expression of HLA-A and -B by recognition of a sequence (Y_{320}SQAASS) present on the cytoplasmic tail of these HLA molecules [35, 36] accelerating their endocytosis from the plasma membrane [37–39] and blocking the transport of newly synthesized MHC class I molecules to the cell surface [40]. Nef maintains the expression of HLA-C [41] -G, and -E unchanged [42, 43], in order to inhibit the innate response of the natural killer cells (NK) [41] (see also Figure 3).

Furthermore, the gp41 protein of the viral envelope upregulates the synthesis of IL-10 by monocytes [44]; in turn, as mentioned before, this cytokine increases the expression of HLA-G molecules [28] to control immune response and facilitate infection [45].

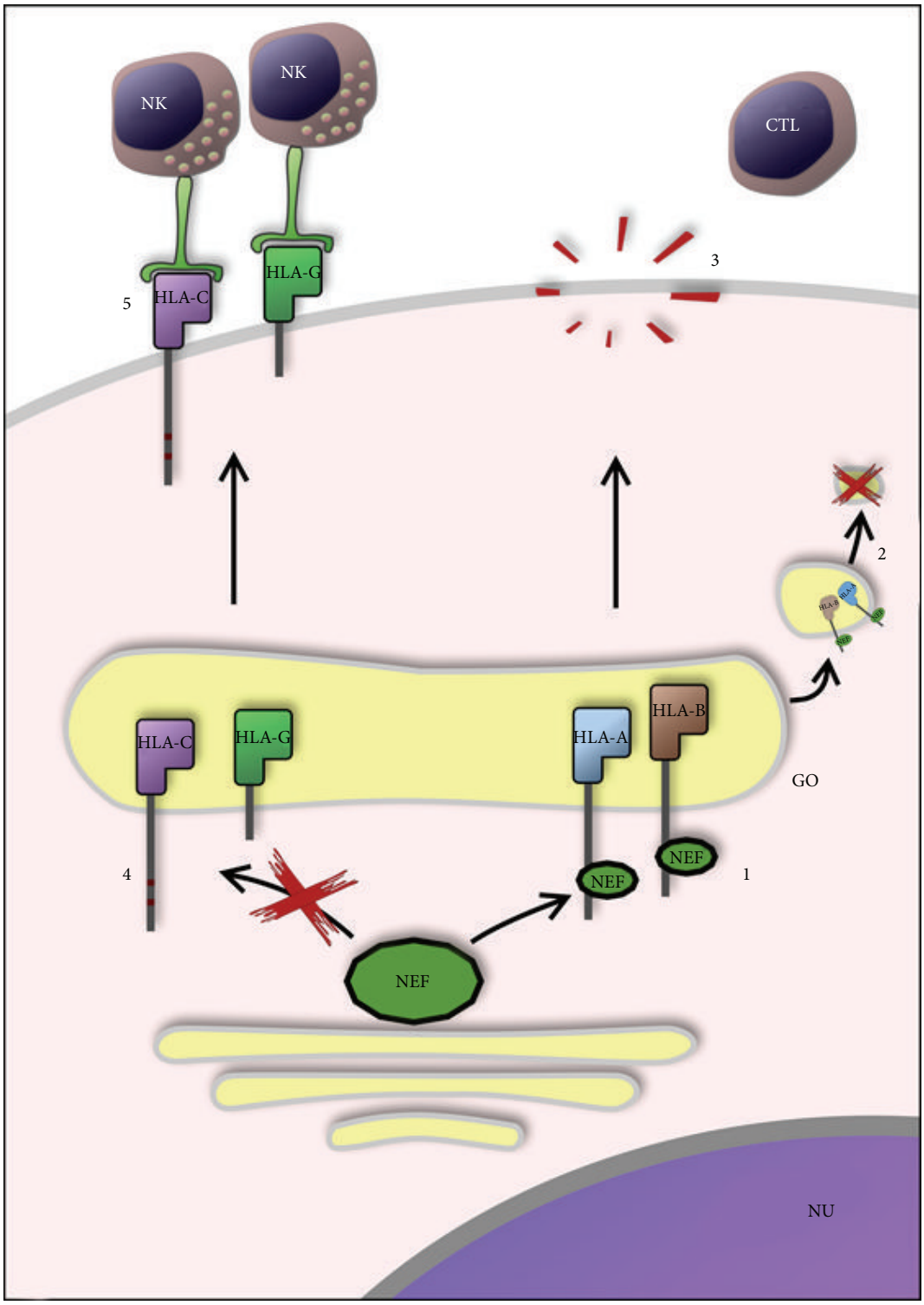


FIGURE 3: The HIV-1 has developed a nef-dependent mechanism to escape the immune system attack to infected cells. Nef alters the expression of HLA-A and -B by recognition of a sequence (Y_{320}SQAASS) in the cytoplasmic tail (1), blocking the transport of newly synthesized HLA-A and -B (2), in order to avoid the recognition by CTL (3) while Nef maintains the expression of HLA-C (C_{320}SQAASS) and -G (short cytoplasmic tail) unchanged (4) in order to inhibit the innate response of the NK (5). NU: nucleus; GO: golgi apparatus.

2.1. HLA-G and HIV. Numerous studies have been conducted, aimed at observing the expression of the molecule HLA-G in the early stage of infection by HIV and its progression. In 2004, Derrien and colleagues demonstrated that during HIV-1 infection the HLA-G1 isoform was downregulated by a Vpu-dependent mechanism, which recognizes a double lysine residues in 4 and 5 positions of the C terminus [46]. The HLA-G1 isoform has the major ability to present viral peptides to $CD8^+$ T lymphocytes [47]; therefore, the recognition of HIV-1 infected cells by $CD8^+$ T lymphocytes could depend on the expression of HLA-G1 [46].

In contrast, in 2002, Lozano and collegues observed high surface expression of HLA-G on monocytes and on some T lymphocytes in HIV positive patients with or without antiretroviral treatment. They hypothesized that the high expression of HLA-G was an indirect induction by infection of HIV-1, related to the pathogenesis of the infection, considering that HLA-G expression occurs in a very high proportion of monocytes, which are unlikely to be infected [48]. Another possible explanation, however, is the possibility that this increase is caused indirectly as a consequence to high levels of cytokines, as IL-10 [28].

Donaghy et al. observed in 2007 high circulating levels of soluble HLA-G (sHLA-G) in HIV-1-infected individuals, and they hypothesized that these levels could depend on the release of the membrane-bound moiety and thus participate to the pathogenesis of HIV by inducing tolerance [49]. In agreement with the findings by Donaghy and collegues, a subsequent work by Murdaca et al. [50] showed elevated serum levels of sHLA-G in HIV-1-positive individuals. These results were correlated with parameters of immunological and virological response to antiretroviral treatment, and the authors found decreased levels of sHLA-G in patients in which the replication of HIV-1 was suppressed during Highly Active Antiretroviral Therapy (HAART) treatment, while the sHLA-G levels remained elevated in patients in which were present high levels of HIV-RNA after 36 months from antiretroviral therapies. These elevated serum levels of sHLA-G may depend on the increased production of cytokines during the HIV-1 infection, contribute to the immunosuppressive state of the HIV-1-positive individuals, and facilitate their progression to AIDS [50].

Lajoie et al. in 2009 performed a longitudinal study evaluating sHLA-G plasma levels in HIV-1-infected patients with different rates of clinical progression to determine whether sHLA-G expression was associated with HIV-1 infection expansion. The authors observed elevated levels of sHLA-G in the early stages of HIV-1 infection, whereas in the chronic stage, in untreated normal progressor, and in long-term progressor the sHLA-G levels were restored to normality, as a result of the immune system's ability to control the HIV-1 infection. Conversely, the levels of sHLA-G remained high in rapid progressor HIV-1-positive patients. Once again, these data were justified by the high concentration of IL-10 in rapid progressor patients with respect to HIV-1-negative individuals. Another explanation could be the elevated blood levels of mature plasmacytoid dendritic cells, major producers of sHLA-G [51], during the chronic stages of the infection in rapid progressor individuals with respect to HIV-negative subjects [52].

Although myeloid dendritic cells are the major producers of HLA-G, together with plasmacytoid dendritic cells [51], Hazenberg et al. in 2010 demonstrated that elevated levels of sHLA-G mediate the dysfunction of the myeloid dendritic cells through the binding to the myelomonocytic MHC-I receptor ILT4 during the progression of the HIV-1 infection. Therefore, the authors hypothesized that elevated levels of HLA-G may inhibit the antigen-presenting properties of dendritic cells, while they enhance the capacity of the dendritic cells to secrete proinflammatory cytokines, as IL-12p70, determining an important proinflammatory environment for the HIV-1 immunopathogenesis [53].

Lajoie et al. in 2010 observed lower plasma levels of sHLA-G in HIV-1-positive Beninese commercial sex workers, and they hypothesized that the difference of sHLA-G levels could be genetically determined [52]. In agreement with this hypothesis, Turk and coworkers in 2013 reported that the HLA-G*01:01:01 genotype was significantly associated with HIV-1-resistant women, while the HLA-G*01:04:04 genotype was significantly associated with susceptibility to HIV-1 infection [54].

Matte et al. in 2004 demonstrated a highly significant association between HLA-G*0105N allele (null allele) and protection from HIV-1 infection. These authors proposed that this allele, not encoding a functional soluble or membrane-bound HLA-G1 isoform [55], could facilitate the natural killer cells to destroy HIV-1-infected cells [45].

Conversely, Segat and colleagues in 2010 showed a significant increase of HLA-G*0105N allele in HIV-1-positive women patients with respect to HIV-1-negative women controls. They suggested that the different results obtained with respect to the study made by Matte et al. were imputable to the different ethnicity of the studied population. Moreover, they hypothesized that the association between HLA-G*0105N and the increased risk of HIV-1 infection was caused by the capacity of the null allele, when present, to increase the production of other HLA-G isoforms, thus compensating the absence of HLA-G1 and -G5 [56] and inhibiting the NK lysis [5].

HLA-G mRNA transcription and translation are tightly regulated processes, giving the immunosuppressive property of this molecule. Some recent works have discovered that HLA-G 3′UTR mRNA has some binding site for miRNAs. Castelli and coauthors in 2009 have made an extensive *in silico* analysis of the HLA-G 3′UTR region, seeking for putative miRNA binding site. They found that different miRNAs bind to this region and moreover the vast majority of these sequences encompass eight highly polymorphic sites [57]. One of them above all, the C/G polymorphism at position +3142, putative binding site for at least three miRNAs, hsa-mir-148a, hsa-mir-148b, and hsa-mir152, has been subsequently confirmed to be the binding site for mir152, and this binding reduces HLA-G expression [58]. However, the effect of this polymorphism in miRNA binding has been recently questioned by Manaster and colleagues. They confirmed previous results, finding that mir-152 and mir-148a bound to HLA-G 3′UTR and downregulate its mRNA, but they also found that the C/G polymorphism at +3142 has no effect on miRNAs binding and efficacy [59]. Interestingly, they found

in placenta low levels of these miRNA, and they suggest that this could be a regulation mechanism, allowing HLA-G expression only where needed and not in other district, where the immunosuppressive activity of this molecule could be detrimental. In an interesting parallel, however, the hsa-mir-148a has been proposed to bind HLA-C 3′UTR, and a polymorphism in the binding site for this miRNA, which increase the binding strength, has been associated with poor HIV-1 infection control (see the following section). Thus, the connection between miRNA, HLA-G expression, and HIV-1 needs to be further explored because it can reveal novel information about HIV-1 control of the immune system.

2.2. HLA-C and HIV. The mechanisms that regulate HLA-C expression and the link between this molecule and HIV infection are not yet completely understood. HLA-C can present antigens to CTL, and it is able to inhibit NK cell lysis, but for some reason it is normally expressed on the cell surface at levels approximately 10-fold less than most HLA-A and HLA-B allotypes [60]. This observation could be explained by a new study, focused on a new miRNA targeting sequences identified on HLA-C gene and regulating the surface expression of the protein (see the following paragraphs) [61]. Another possible explanation for this low level of expression comes from a study focused on the low level of affinity between β2M and the HLA-C heavy chains, which are then accumulated in the ER and ultimately degraded [62].

HIV-1 also is able to regulate via Nef the expression of MHC-I molecules. However, since the removal of class I molecules from the surface of virus-infected cell may result in attacks by NK cells, HIV-1 significantly downregulates HLA-A and HLA-B [25], recognized by the majority of CTL, but not HLA-C and HLA-E, thus maintaining their inhibitory role on NK. This selective regulatory activity allows viruses to counteract at the same moment both innate and adaptive immune responses. This mechanism of class I downregulation is a bypass of the immune response, even if it does not ensure to the virus an unfailing escape from the immune system [41, 63].

Indeed, even the host can count on several processes aimed at protecting itself from viral infection. In the last years several studies focused on HLA-C in affecting HIV disease progression. One single-nucleotide polymorphism (SNP) 35 kb upstream of HLA-C locus (-35C/T) has been shown to be crucial for the HIV progression [3, 64] and the surface expression of HLA-C, with higher surface expression associated with slower disease progression [65]. This two aspects resulted to be strictly linked by functional studies demonstrating that -35C allele leads to higher HLA-C cell surface expression and that AIDS and viremia progress slower in individuals with high-expressing HLA-C alleles compared to individuals with low HLA-C expressing alleles (-35T) [27, 65]. Although the mechanism underlying this changing expression is still unknown, the augmented expression of surface HLA-C mediated by the genetic variant -35C may bypass the regulatory activity of Nef, thus resulting in being particularly protective because HIV-1 does not affect HLA-C, as detailed previously [63]. Moreover, the protective effects of the allele -35C could be exerted through a more effective antigen presentation to CTL or an enhancement of NK cell activity driven by the high levels of HLA-C on the infected-cells surface [27]. Substantially, the -35CC genotype (i.e., the presence of C allele at both strands in -35 position) may improve control of HIV/AIDS thanks to an augmented HLA-C surface expression resulting in an enhanced antigen presentation.

However, this protective effect could be not so strong since there are evidences showing that the -35T SNP does not lead to an unequivocal correlation with low HLA-C expression levels because there is an overlap in the distribution of HLA-C surface expression between the different genotypes; moreover, some individuals with the -35CC alleles exhibit high viral loads. The authors thus proposed that the amount of HLA-C, expressed on the cell-infected surface, could represent the protective mechanism itself, rather than the -35C/T genotype [63]. This suggestion means that the -35C/T SNP could not be so fundamental in the control of the HIV progression. Indeed, control of HLA-C surface expression has been correlated with the presence of a microRNA binding site that affects HLA-C expression and the control of HIV disease [61]. It has been suggested that the -35 SNP could not be the real cause for differential HLA-C expression, but rather the marker of another polymorphism, able to directly affect levels of surface HLA-C. In fact, linkage disequilibrium between the -35 SNP and a single nucleotide insertion/deletion at the 3′UTR of the HLA-C gene, directly into the binding site of microRNA hsa-miR-148, has been reported. The 263del/ins SNP associates strongly with the control of HIV-1 replication, since the 263insG increases the stability of the binding with the miR-148a/miR-148b, leading to a low surface expression of HLA-C. On the opposite, 263del disrupts the binding site, leading to a high surface expression of HLA-C (see also Figure 4). The authors found that 263insG is in linkage disequilibrium with -35T allele. These data suggest that 263ins/del could be the causal variant for differential HLA-C expression and subsequently for AIDS/HIV progression.

3. miRNA and HIV

3.1. HIV Regulates Cellular miRNA. HIV-1 infection could change the intracellular miRNA milieu, as many different viruses do. One of the important characteristics of *Tat* protein, encoded by the viral genome, is its silencing RNA (siRNA) suppressor (SRS) activity. Some reports say that *Tat* interacts directly with Dicer, thus blocking its ability to process miRNA precursors [66]. Others affirm that *Tat* would sequester miRNA duplexes, through a nonsequence-specific mechanism, thus effectively blocking the RISC pathway [67]. This SRS activity is a common mechanism, shared by other viruses (i.e., influenza virus, Ebola virus, and others) to effectively suppress one of main cellular defense from infections (for a review see [68]).

Apart from this SRS mechanism, HIV-1 infection could change intracellular miRNA response in other ways. In *ex vivo* experiments using lymphocytes, Hayes et al. (2011) found changes in expression of 145 miRNA [69] (and only 22 could

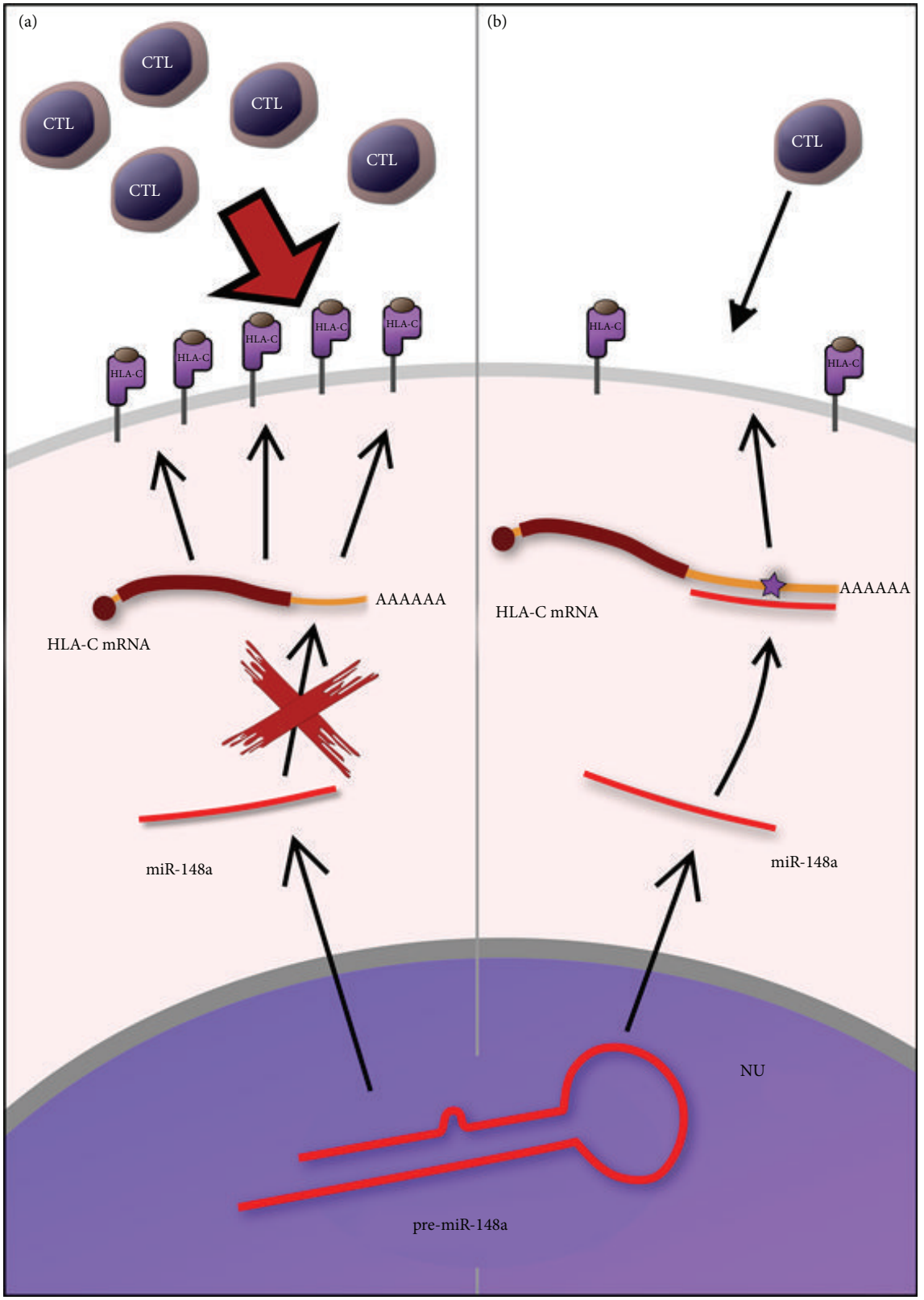


Figure 4: The 263del/ins SNP associates strongly with surface expression of HLA-C. (a) 263del disrupts the binding site of miR-148a not altering the expression of HLA-C. (b) 263insG increases the stability of the binding with the miR-148a reducing surface expression of HLA-C and the recognition by CTL.

be explained by *Tat* SRS activity), and in a subsequent work Sun et al. (2012) found reduced expression of several cellular miRNAs. miR29a, miR29b, and miR29c appear reduced in both these works, thus suggesting a pivotal role for these miRNAs in HIV-1 infection [70]. These studies made in *ex vivo* settings maybe do not correctly depict the real *in vivo* situation. Recent studies, however, start to delineate the *in vivo* situation, a more challenging condition because in infected individual only a small fraction of $CD4^+$ T cells are HIV-1 positive (estimated to be 1 in 10000 in blood and 1 in 100 in lymph nodes) [71]. In a seminal work in 2008, Houzet et al. profile miRNA from PBMCs in 36 individuals, classified as patients with high $CD4^+$ T cell count and low viral load (class I), high $CD4^+$ T cell count and high viral load (class II), low $CD4^+$ T cell count and low viral load (class III), and low $CD4^+$ T cell count and high viral load (class IV). They found miRNA profiles specific for those different four classes of patients [72]. In a subsequent study, Witwer and coworkers analyzed miRNA profiles from healthy individuals, elite HIV-1 controllers, and untreated viremic HIV-1 patients. Their results confirm and expand Houzet's work, especially data on viremic patients, because they show downregulation of miRNA 150 e miRNA 29 families (two crucial miRNAs in other pathways, as shown below). Moreover, they correlate miRNA changes with clinical parameters, that is, $CD4^+$ T cell counts and viral loads, and they found that miRNA expression could be used as a biomarker for HIV-1 disease progression [73].

A crucial point to understand how HIV-1 changes miRNA cellular milieu is to define the differences between cells prone to HIV-1 infection, that is, activated $CD4^+$ T cells, and cells that are less liable to this infection, that is, resting $CD4^+$ T cells and monocytes. A groundbreaking work in this field is the one done by Huang and coworkers in 2007. They found that miR-28, miR-125b, miR-150, miR-223, and miR-382 are enriched in resting $CD4^+$ T cells. Those miRNAs have binding site at the 3′UTR HIV-1 sequence and thus, upon binding, repress viral RNA expression. According to the authors, this mechanism could explain HIV-1 latency in resting $CD4^+$ T cells [74]. Another important miRNA in regulating HIV-1 expression is miR-29a, that has been found to be highly expressed in resting $CD4^+$ T cells and, more importantly, to be able to target both the 3′ end of HIV-1 transcript and the HIV-1 encoded protein Nef, thus interfering with HIV-1 expression and replication [75]. Moreover a recent report confirms these previous results, using resting and activated $CD4^+$ T cells. Chiang and coworkers identify miR-27b, miR-29b, miR-150, and miR-223 as being significantly downregulated upon $CD4^+$ T cell activation. MiR27b binds to, among others target, cyclin T1 3′UTR. This protein is crucial for HIV-1 replication process, as the viral *Tat* recruits and binds it to promote viral genome transcription. The authors thus suggest that miR27b could directly regulate HIV-1 transcription, while the other miRNAs could be indirectly regulating cyclin T1 [76]. On the contrary, a very recent article from the same authors [77] shows how in activated $CD4^+$ T cells miR-132 is upregulated and potentiates viral replication, probably through downregulation of MeCP2 (methyl-CpG binding protein 2, a transcriptional regulatory protein). In the same cell system ($CD4^+$ T cells), Chang and coworkers have profiled the expression of cellular miRNAs and some sncRNA after HIV infection using next generation sequencing [78]. The authors used different time points after HIV-1 infection, and they found a specific miRNAs signature in the early phase of infection. Moreover, they characterized the changes in the transcriptional landscape of infected cells, due to the differential miRNA expression. Their work marks the start of a new phase in analyzing the interactions between HIV-1 and cellular sncRNAs.

Another class of cells that are less permissive towards HIV-1 infection is peripheral blood monocytes. When differentiating into macrophages, those cells appear to downregulate expression of several miRNA, and among them, one (miR198) was identified as negative regulator of cyclin T1, a cell-cycle regulator protein. Sung and Rice in 2009 suggested that regulation of this protein could influence HIV-1 proviral expression and infection, being a crucial *Tat* cofactor. [79]. However a recent study, reviewing the literature and testing different miRNAs expression models in the monocyte/macrophage system, has found little or no expression of miR198 in primary monocytes, thus questioning the role of this miRNA in HIV-1 infection mechanisms [80]. Subsequently another report identifies overexpression of the "so-called" "anti-HIV" miRNA found in resting $CD4^+$ T cells as well as in monocytes, and the authors suggest that this could be a leading mechanism in monocyte resistance [81]. Although also this report has been subsequently questioned [82], due to the use of combined miRNA inhibitors which could affect different genes, its importance remains pivotal, considering that the data were previously validated in previous studies and in a different model ($CD4^+$ T cells). However, when considering the work of Specht et al. [63], we should be aware that it has not been confirmed or replicated, and thus it awaits further confirmations.

3.2. miRNA Encoded by HIV-1. At current time, no general agreement is present in the field about the possibility that HIV-1 genome encodes functional miRNAs and how (if this would be the case) this fits into the existing paradigm of miRNA-based gene regulation. One of first evidences for sncRNAs encoded in HIV-1 sequences was found by Bennasser and coauthors in 2004 [83], where they hypothesized the presence of a TAR hairpin miRNA-like in HIV-1 genome. Following this paper, another confirmation from the same authors has been made in 2005 [84] where they demonstrated that in HIV-1 genome 19-bp sequences perfectly duplex and theoretically able form stem-loop structures are present. Another group independently demonstrated the presence and the activity of a miRNA-like sequence on the *nef* gene encoded by HIV-1 [85, 86]. Furthermore a recent paper reports the ability of TAR miRNA to downregulate excision repair cross-complementing rodent repair deficiency, complementation group 1 (ERCC1), and immediate early response 3 (IER3) proteins, thus blocking apoptosis in infected cells [87], so a possible function of this miRNA has been in part clarified.

However these results have been contested by Lin and Cullen in an extensive work. They used different cell lines and PBMCs from HIV-1-positive individuals assessing the presence or the absence of sncRNA with small RNA cloning strategy: almost no miRNA or siRNA in HIV-1-infected cells has been found [88]. On the contrary, some recent reports suggest the existence of HIV-1-encoded miRNA. Yeung and coworker using pyrosequencing analyzed HIV-1 latently infected human monocyte and T-cell lines, and they found numerous small ncRNAs encoded by HIV-1 [89]. A more recent study used deep sequencing to evaluate the presence of HIV-1-derived miRNA in infected cells. They found on the "plus" strand of HIV-1 genome two putative viral miRNA: one (vmiRNA-43/9175) already found in a previous work [87] in the TAR region and a new one (vmiRNA-2413) in the pol gene; moreover, they identified possible viral miRNA also on the "minus" strand. Specifically, the authors found that the 3′ end of HIV-1 genome could produce different small antisense RNA, and that they can form dsRNA with the sense transcript, inhibiting viral production through activation of the siRNA machinery. *Tat* protein has a potent SRS activity (see previous section), and probably it helps overcoming the mechanism just described that could be started by insertion of the viral genome with an upstream promoter near the 3′ ends. More interestingly, this mechanism could in theory be exploited to block HIV-1 replication, thus opening possible novel therapeutic opportunities.

Nevertheless a very recent paper by Cullen and coauthors demonstrates instead that HIV-1 genome is neither sensitive to cellular miRNA or expressing its own miRNA. The authors using deep sequencing failed to find any putative miRNA encoded by HIV-1 in different cell-infected lines, and moreover, they did not find any cellular miRNA binding to HIV-1 genome, although multiple putative binding sequences are present [90]. We can then conclude that the existence of viral encoded miRNA is deeply controversial, needing more work to confirm its existence; if the existence confirmed, then new opportunities to conceive original therapeutic strategies to fight HIV-1 based on miRNA could arise.

4. Conclusion and Future Perspective

In this review we have focused on recently discovered mechanisms whereby the HIV-1 virus escapes the immune response, also by way of interactions with novel partners, i.d. HLA-C, and HLA-G.

Our knowledge about these interactions and about the role of the RNA interference mechanism in HIV-1 infection and progression is still at a nascent stage; one main question remains if whatever HIV-1 encoded miRNA exists and which role it could possibly have in the disease progression. We are wondering if

(a) those putative virus-encoded miRNA are linked in some yet unknown way to the HLA-C and HLA-G roles in HIV-1 infection,

(b) those miRNA could exert another completely different role.

If the first hypothesis (a) is true, we will confirm the existence and the functionality of HIV-1-encoded miRNA; the recent studies reported and discussed in this review seem to provide solid proofs on this possibility.

Another interesting point that we would like to raise is related to the 3′ antisense transcript from the HIV-1 genome: is this long, ncRNA a precursor of different and physiologically active miRNAs and which role may they have in the disease progress? Until now no definitive study has been able to answer this question, but we do think that it is an issue of great interest in HIV-1-infection biology, and future works will be surely consider this point.

When considering HLA-G, another question remains unanswered: is HLA-G expression upregulated in response to IL-10 increase or simply in correlation to infective state? Answering this question could be quite important not only in AIDS but also in relation to other pathologies, and simple experiments could solve this puzzle: in analogy on what has been done in tumors [91], it will be possible to examine the transcriptional response of HLA-G during stimulation with IL-10 in HIV-1 infected cells.

The identification of novel mechanisms involved in HLA-G and HLA-C gene expression regulation trough miRNAs, able to influence HIV-infection susceptibility, virus replication, and disease progression, opens a new era for genetic hunters looking for variations in host genome capable to modulate the response to the virus, possibly helping to discover new strategies to fight HIV. The interactions within host and virus genome are probably more complex than what is known up to date, and the tuning of gene expression regulation through miRNAs represents a new genomic universe to be investigated.

Abbreviations

ncRNA: Non-coding RNA
tRNA: Transfer RNA
rRNA: Ribosomal RNA
snoRNA: Small nucleolar RNA
piRNA: Piwi-interacting RNA
miRNA: MicroRNA
MHC: Major histocompatibility complex
HLA: Human leukocyte antigen
KIR: Killer cell immunoglobulin-like receptor
LILRB1-2: Leukocyte immunoglobulin-like receptor
NK: Natural killer
CTL: Cytotoxic T lymphocytes
ERCC1: Excision repair cross-complementing rodent repair deficiency, complementation group 1
IER3: Immediate early response 3.

References

[1] R. Apps, Y. Qi, J. M. Carlson et al., "Influence of HLA-C expression level on HIV control," *Science*, vol. 340, no. 6128, pp. 87–91, 2013.

[2] S. Buranapraditkun, U. Hempel, P. Pitakpolrat et al., "A novel immunodominant $CD8^+$ T cell response restricted by a common HLA-C allele targets a conserved region of Gag HIV-1

clade CRF01_AE infected thais," *PLoS ONE*, vol. 6, no. 8, Article ID e23603, 2011.

[3] J. Fellay, K. V. Shianna, D. Ge et al., "A whole-genome association study of major determinants for host control of HIV-1," *Science*, vol. 317, no. 5840, pp. 944–947, 2007.

[4] E. Trachtenberg, T. Bhattacharya, M. Ladner, J. Phair, H. Erlich, and S. Wolinsky, "The HLA-B/-C haplotype block contains major determinants for host control of HIV," *Genes and Immunity*, vol. 10, no. 8, pp. 673–677, 2009.

[5] L. Segat, E. Catamo, A. Fabris et al., "HLA-G*0105N allele is associated with augmented risk for HIV infection in white female patients," *AIDS*, vol. 24, no. 12, pp. 1961–1964, 2010.

[6] M. H. Larsen, R. Zinyama, P. Kallestrup et al., "HLA-G 3′ untranslated region 14-base pair deletion: association with poor survival in an HIV-1-infected zimbabwean population," *The Journal of Infectious Diseases*, vol. 207, no. 6, pp. 903–906, 2013.

[7] B. R. Cullen, "HIV-1 auxiliary proteins: making connections in a dying cell," *Cell*, vol. 93, no. 5, pp. 685–692, 1998.

[8] A. D. Frankel and J. A. T. Young, "HIV-1: fifteen proteins and an RNA," *Annual Review of Biochemistry*, vol. 67, pp. 1–25, 1998.

[9] D. R. Littman, "Chemokine receptors: keys to AIDS pathogenesis?" *Cell*, vol. 93, no. 5, pp. 677–680, 1998.

[10] J. Karn and C. M. Stoltzfus, "Transcriptional and posttranscriptional regulation of HIV-1 gene expression," *Cold Spring Harbor Perspectives in Medicine*, vol. 2, no. 2, 2012.

[11] M. W. Wright and E. A. Bruford, "Naming "junk": human non-protein coding RNA (ncRNA) gene nomenclature," *Human Genomics*, vol. 5, no. 2, pp. 90–98, 2011.

[12] P. P. Amaral, M. B. Clark, D. K. Gascoigne, M. E. Dinger, and J. S. Mattick, "LncRNAdb: a reference database for long noncoding RNAs," *Nucleic Acids Research*, vol. 39, no. 1, pp. D146–D151, 2011.

[13] T. Thomson and H. Lin, "The biogenesis and function of PIWI proteins and piRNAs: progress and prospect," *Annual Review of Cell and Developmental Biology*, vol. 25, pp. 355–376, 2009.

[14] J. S. Mattick and I. V. Makunin, "Small regulatory RNAs in mammals," *Human Molecular Genetics*, vol. 14, no. 1, pp. R121–R132, 2005.

[15] E. J. Adams and P. Parham, "Species-specific evolution of MHC class I genes in the higher primates," *Immunological Reviews*, vol. 183, pp. 41–64, 2001.

[16] T. A. M. Groothuis, A. C. Griekspoor, J. J. Neijssen, C. A. Herberts, and J. J. Neefjes, "MHC class I alleles and their exploration of the antigen-processing machinery," *Immunological Reviews*, vol. 207, pp. 60–76, 2005.

[17] P. Paul, F. Adrian Cabestre, E. C. Ibrahim et al., "Identification of HLA-G7 as a new splice variant of the HLA-G mRNA and expression of soluble HLA-G5, -G6, and -G7 transcripts in human transfected cells," *Human Immunology*, vol. 61, no. 11, pp. 1138–1149, 2000.

[18] A. N. Antoniou, S. J. Powis, and T. Elliott, "Assembly and export of MHC class I peptide ligands," *Current Opinion in Immunology*, vol. 15, no. 1, pp. 75–81, 2003.

[19] A. V. S. Hill, "Genetic susceptibility to malaria and other infectious diseases: from the MHC to the whole genome," *Parasitology*, vol. 112, supplement, pp. S75–S84, 1996.

[20] A. Neisig, C. J. M. Melief, and J. Neefjes, "Reduced cell surface expression of HLA-C molecules correlates with restricted peptide binding and stable TAP interaction," *Journal of Immunology*, vol. 160, no. 1, pp. 171–179, 1998.

[21] C. S. Falk and D. J. Schendel, "HLA-C revisited. Ten years of change," *Immunologic Research*, vol. 16, no. 2, pp. 203–214, 1997.

[22] M. Colonna, G. Borsellino, M. Falco, G. B. Ferrara, and J. L. Strominger, "HLA-C is the inhibitory ligand that determines dominant resistance to lysis by NK1- and NK2-specific natural killer cells," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 90, no. 24, pp. 12000–12004, 1993.

[23] S. I. Khakoo, C. L. Thio, M. P. Martin et al., "HLA and NK cell inhibitory receptor genes in resolving hepatitis C virus infection," *Science*, vol. 305, no. 5685, pp. 872–874, 2004.

[24] C. T. Tiemessen, M. Paximadis, G. Minevich et al., "Natural killer cell responses to HIV-1 peptides are associated with more activating KIR genes and HLA-C genes of the C1 allotype," *Journal of Acquired Immune Deficiency Syndromes*, vol. 57, no. 3, pp. 181–189, 2011.

[25] K. L. Collins, B. K. Chen, S. A. Kalams, B. D. Walker, and D. Baltimore, "HIV-1 Nef protein protects infected primary cells against killing by cytotoxic T lymphocytes," *Nature*, vol. 391, no. 6665, pp. 397–401, 1998.

[26] A. Alcami and U. H. Koszinowski, "Viral mechanisms of immune evasion," *Trends in Microbiology*, vol. 8, no. 9, pp. 410–418, 2000.

[27] R. Thomas, R. Apps, Y. Qi et al., "HLA-C cell surface expression and control of HIV/AIDS correlate with a variant upstream of HLA-C," *Nature Genetics*, vol. 41, no. 12, pp. 1290–1294, 2009.

[28] P. Moreau, F. Adrian-Cabestre, C. Menier et al., "IL-10 selectively induces HLA-G expression in human trophoblasts and monocytes," *International Immunology*, vol. 11, no. 5, pp. 803–811, 1999.

[29] V. Cirulli, J. Zalatan, M. McMaster et al., "The class I HLA repertoire of pancreatic islets comprises the nonclassical class Ib antigen HLA-G," *Diabetes*, vol. 55, no. 5, pp. 1214–1222, 2006.

[30] W.-H. Yan, "HLA-G expression in cancers: potential role in diagnosis, prognosis and therapy," *Endocrine, Metabolic and Immune Disorders*, vol. 11, no. 1, pp. 76–89, 2011.

[31] E. D. Carosella, P. Paul, P. Moreau, and N. Rouas-Freiss, "HLA-G and HLA-E: fundamental and pathophysiological aspects," *Immunology Today*, vol. 21, no. 11, pp. 532–534, 2000.

[32] N. Rouas-Freiss, R. E. Marchal, M. Kirszenbaum, J. Dausset, and E. D. Carosella, "The α1 domain of HLA-G1 and HLA-G2 inhibits cytotoxicity induced by natural killer cells: is HLA-G the public ligand for natural killer cell inhibitory receptors?" *Proceedings of the National Academy of Sciences of the United States of America*, vol. 94, no. 10, pp. 5249–5254, 1997.

[33] J. M. Lozano, R. González, J. M. Kindelán et al., "Monocytes and T lymphocytes in HIV-1-positive patients express HLA-G molecule," *AIDS*, vol. 16, no. 3, pp. 347–351, 2002.

[34] M. Onno, C. Pangault, G. Le Friec, V. Guilloux, P. André, and R. Fauchetz, "Modulation of HLA-G antigens expression by human cytomegalovirus: specific induction in activated macrophages harboring human cytomegalovirus infection," *Journal of Immunology*, vol. 164, no. 12, pp. 6426–6434, 2000.

[35] V. Piguet, L. Wan, C. Borel et al., "HIV-1 Nef protein binds to the cellular protein PACS-1 to downregulate class I major histocompatibility complexes," *Nature Cell Biology*, vol. 2, no. 3, pp. 163–167, 2000.

[36] M. Williams, J. F. Roeth, M. R. Kasper, R. I. Fleis, C. G. Przybycin, and K. L. Collins, "Direct binding of human immunodeficiency virus type 1 Nef to the major histocompatibility complex class I (MHC-I) cytoplasmic tail disrupts MHC-I trafficking," *Journal of Virology*, vol. 76, no. 23, pp. 12173–12184, 2002.

[37] O. Schwartz, V. Maréchal, S. Le Gall, F. Lemonnier, and J.-M. Heard, "Endocytosis of major histocompatibility complex class I molecules is induced by the HIV-1 Nef protein," *Nature Medicine*, vol. 2, no. 3, pp. 338–342, 1996.

[38] S. L. Gall, F. Buseyne, A. Trocha, B. D. Walker, J.-M. Heard, and O. Schwartz, "Distinct trafficking pathways mediate Nef-induced and clathrin-dependent major histocompatibility complex class I down-regulation," *Journal of Virology*, vol. 74, no. 19, pp. 9256–9266, 2000.

[39] M. E. Greenberg, A. J. Lafrate, and J. Skowronski, "The SH3 domain-binding surface and an acidic motif in HIV-1 Nef regulate trafficking of class I MHC complexes," *The EMBO Journal*, vol. 17, no. 10, pp. 2777–2789, 1998.

[40] M. R. Kasper and K. L. Collins, "Nef-mediated disruption of HLA-A2 transport to the cell surface in T cells," *Journal of Virology*, vol. 77, no. 5, pp. 3041–3049, 2003.

[41] G. B. Cohen, R. T. Gandhi, D. M. Davis et al., "The selective downregulation of class I major histocompatibility complex proteins by HIV-1 protects HIV-infected cells from NK cells," *Immunity*, vol. 10, no. 6, pp. 661–671, 1999.

[42] N. Pizzato, M. Derrien, and F. Lenfant, "The short cytoplasmic tail of HLA-G determines its resistance to HIV-1 Nef-mediated cell surface downregulation," *Human Immunology*, vol. 65, no. 11, pp. 1389–1396, 2004.

[43] J. Nattermann, H. D. Nischalke, V. Hofmeister et al., "HIV-1 infection leads to increased HLA-E expression resulting in impaired function of natural killer cells," *Antiviral Therapy*, vol. 10, no. 1, pp. 95–107, 2005.

[44] M. Barcova, L. Kacani, C. Speth, and M. P. Dierich, "gp41 envelope protein of human immunodeficiency virus induces interleukin (IL)-10 in monocytes, but not in B, T, or NK cells, leading to reduced IL-2 and interferon-γ production," *Journal of Infectious Diseases*, vol. 177, no. 4, pp. 905–913, 1998.

[45] C. Matte, J. Lajoie, J. Lacaille, L. S. Zijenah, B. J. Ward, and M. Roger, "Functionally active HLA-G polymorphisms are associated with the risk of heterosexual HIV-1 infection in African women," *AIDS*, vol. 18, no. 3, pp. 427–431, 2004.

[46] M. Derrien, N. Pizzato, G. Dolcini et al., "Human immunodeficiency virus 1 downregulates cell surface expression of the non-classical major histocompatibility class I molecule HLA-G1," *The Journal of General Virology*, vol. 85, no. 7, pp. 1945–1954, 2004.

[47] F. Lenfant, N. Pizzato, S. Liang, C. Davrinche, P. Le Bouteiller, and A. Horuzsko, "Induction of HLA-G-restricted human cytomegalovirus pp65 (UL83)-specific cytotoxic T lymphocytes in HLA-G transgenic mice," *The Journal of General Virology*, vol. 84, no. 2, pp. 307–317, 2003.

[48] J. M. Lozano, R. González, J. M. Kindelán et al., "Monocytes and T lymphocytes in HIV-1-positive patients express HLA-G molecule," *AIDS*, vol. 16, no. 3, pp. 347–351, 2002.

[49] L. Donaghy, F. Gros, L. Amiot et al., "Elevated levels of soluble non-classical major histocompatibility class I molecule human leucocyte antigen (HLA)-G in the blood of HIV-infected patients with or without visceral leishmaniasis," *Clinical and Experimental Immunology*, vol. 147, no. 2, pp. 236–240, 2007.

[50] G. Murdaca, P. Contini, M. Setti et al., "Behavior of non-classical soluble HLA class G antigens in human immunodeficiency virus 1-infected patients before and after HAART: comparison with classical soluble HLA-A, -B, -C antigens and potential role in immune-reconstitution," *Clinical Immunology*, vol. 133, no. 2, pp. 238–244, 2009.

[51] V. Rebmann, A. Busemann, M. Lindemann, and H. Grosse-Wilde, "Detection of HLA-G5 secreting cells," *Human Immunology*, vol. 64, no. 11, pp. 1017–1024, 2003.

[52] J. Lajoie, J. Fontaine, C. Tremblay, J.-P. Routy, J. Poudrier, and M. Roger, "Persistence of high levels of blood soluble human leukocyte antigen-G is associated with rapid progression of HIV infection," *AIDS*, vol. 23, no. 11, pp. 1437–1440, 2009.

[53] M. D. Hazenberg, S. A. Otto, B. H. B. Van Benthem et al., "Persistent immune activation in HIV-1 infection is associated with progression to AIDS," *AIDS*, vol. 17, no. 13, pp. 1881–1888, 2003.

[54] W. J. R. Turk, J. Kimani, T. Bielawny et al., "Associations of human leukocyte antigen-G with resistance and susceptibility to HIV-1 infection in the Pumwani sex worker cohort," *AIDS*, vol. 27, no. 1, pp. 7–15, 2013.

[55] M. Le Discorde, C. Le Danff, P. Moreau, N. Rouas-Freiss, and E. D. Carosella, "HLA-G$*$0105N null allele encodes functional HLA-G isoforms," *Biology of Reproduction*, vol. 73, no. 2, pp. 280–288, 2005.

[56] M. J. Castro, P. Morales, J. Martinez-Laso et al., "Lack of MHC-G4 and soluble (G5, G6) isoforms in the higher primates, Pongidae," *Human Immunology*, vol. 61, no. 11, pp. 1164–1168, 2000.

[57] E. C. Castelli, P. Moreau, A. O. E. Chiromatzo et al., "*In silico* analysis of microRNAS targeting the *HLA-G* $3'$ untranslated region alleles and haplotypes," *Human Immunology*, vol. 70, no. 12, pp. 1020–1025, 2009.

[58] X.-M. Zhu, T. Han, X.-H. Wang et al., "Overexpression of miR-152 leads to reduced expression of human leukocyte antigen-G and increased natural killer cell mediated cytolysis in JEG-3 cells," *American Journal of Obstetrics and Gynecology*, vol. 202, no. 6, pp. 592.e1–592.e7, 2010.

[59] I. Manaster, D. Goldman-Wohl, C. Greenfield et al., "MiRNA-mediated control of HLA-G expression and function," *PLoS ONE*, vol. 7, no. 3, Article ID e33395, 2012.

[60] D. Snary, C. J. Barnstable, W. F. Bodmer, and M. J. Crumpton, "Molecular structure of human histocompatibility antigens: the HLA-C series," *European Journal of Immunology*, vol. 7, no. 8, pp. 580–585, 1977.

[61] S. Kulkarni, R. Savan, Y. Qi et al., "Differential microRNA regulation of HLA-C expression and its association with HIV control," *Nature*, vol. 472, no. 7344, pp. 495–498, 2011.

[62] J. J. Neefjes and H. L. Ploegh, "Allele and locus-specific differences in cell surface expression and the association of HLA class I heavb chain with β2-microglobulin: differential effects of inhibition of glycosylation on class I subunit association," *European Journal of Immunology*, vol. 18, no. 5, pp. 801–810, 1988.

[63] A. Specht, A. Telenti, R. Martinez et al., "Counteraction of HLA-C-mediated immune control of HIV-1 by Nef," *Journal of Virology*, vol. 84, no. 14, pp. 7300–7311, 2010.

[64] J. Fellay, D. Ge, K. V. Shianna et al., "Common genetic variation and the control of HIV-1 in humans," *PLoS Genetics*, vol. 5, no. 12, Article ID 1000791, 2009.

[65] T. W. Corrah, N. Goonetilleke, J. Kopycinski et al., "Reappraisal of the relationship between the HIV-1-protective single-nucleotide polymorphism 35 kilobases upstream of the HLA-C gene and surface HLA-C expression," *Journal of Virology*, vol. 85, no. 7, pp. 3367–3374, 2011.

[66] A. Gatignol, S. Laine, and G. Clerzius, "Dual role of TRBP in HIV replication and RNA interference: viral diversion of a cellular pathway or evasion from antiviral immunity?" *Retrovirology*, vol. 2, article 65, 2005.

[67] S. Qian, X. Zhong, L. Yu, B. Ding, P. De Haan, and K. Boris-Lawrie, "HIV-1 Tat RNA silencing suppressor activity is conserved across kingdoms and counteracts translational repression of HIV-1," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 106, no. 2, pp. 605–610, 2009.

[68] S. Bivalkar-Mehla, J. Vakharia, R. Mehla et al., "Viral RNA silencing suppressors (RSS): novel strategy of viruses to ablate the host RNA interference (RNAi) defense system," *Virus Research*, vol. 155, no. 1, pp. 1–9, 2011.

[69] A. M. Hayes, S. Qian, L. Yu, and K. Boris-Lawrie, "Tat RNA silencing suppressor activity contributes to perturbation of lymphocyte miRNA by HIV-1," *Retrovirology*, vol. 8, no. 1, article 36, 2011.

[70] G. Sun, H. Li, X. Wu et al., "Interplay between HIV-1 infection and host microRNAs," *Nucleic Acids Research*, vol. 40, no. 5, pp. 2181–2196, 2012.

[71] D. C. Krakauer and M. Nowak, "T cell induced pathogenesis in HIV: bystander effects and latent infection," *Proceedings of the Royal Society B*, vol. 266, no. 1423, pp. 1069–1075, 1999.

[72] L. Houzet, M. L. Yeung, V. de Lame, D. Desai, S. M. Smith, and K.-T. Jeang, "MicroRNA profile changes in human immunodeficiency virus type 1 (HIV-1) seropositive individuals," *Retrovirology*, vol. 5, no. 1, article 118, 2008.

[73] K. W. Witwer, A. K. Watson, J. N. Blankson, and J. E. Clements, "Relationships of PBMC microRNA expression, plasma viral load, and CD4$^+$ T-cell count in HIV-1-infected elite suppressors and viremic patients," *Retrovirology*, vol. 9, no. 1, article 5, 2012.

[74] J. Huang, F. Wang, E. Argyris et al., "Cellular microRNAs contribute to HIV-1 latency in resting primary CD4$^+$ T lymphocytes," *Nature Medicine*, vol. 13, no. 10, pp. 1241–1247, 2007.

[75] J. K. Ahluwalia, S. Z. Khan, K. Soni et al., "Human cellular microRNA hsa-miR-29a interferes with viral nef protein expression and HIV-1 replication," *Retrovirology*, vol. 5, article 117, 2008.

[76] K. Chiang and A. P. Rice, "MicroRNA-mediated restriction of HIV-1 in resting CD4$^+$ T cells and monocytes," *Viruses*, vol. 4, no. 9, pp. 1390–1409, 2012.

[77] K. Chiang, H. Liu, and A. P. Rice, "miR-132 enhances HIV-1 replication," *Virology*, vol. 438, no. 1, pp. 1–4, 2013.

[78] S. T. Chang, M. J. Thomas, P. Sova, R. R. Green, R. E. Palermo, and M. G. Katze, "Next-generation sequencing of small RNAs from HIV-infected cells identifies phased microRNA expression patterns and candidate novel microRNAs differentially expressed upon infection," *MBio*, vol. 4, no. 1, 2013.

[79] T.-L. Sung and A. P. Rice, "miR-198 inhibits HIV-1 gene expression and replication in monocytes and its mechanism of action appears to involve repression of cyclin T1," *PLoS Pathogens*, vol. 5, no. 1, Article ID e1000263, 2009.

[80] J. M. Sisk, J. E. Clements, and K. W. Witwer, "miRNA profiles of monocyte-lineage cells are consistent with complicated roles in HIV-1 restriction," *Viruses*, vol. 4, no. 12, pp. 1844–1864, 2012.

[81] X. Wang, L. Ye, W. Hou et al., "Cellular microRNA expression correlates with susceptibility of monocytes/macrophages to HIV-1 infection," *Blood*, vol. 113, no. 3, pp. 671–674, 2009.

[82] S. Swaminathan, J. Zaunders, J. Wilkinson, K. Suzuki, and A. D. Kelleher, "Does the presence of anti-HIV miRNAs in monocytes explain their resistance to HIV-1 infection?" *Blood*, vol. 113, no. 20, pp. 5029–5030, 2009.

[83] Y. Bennasser, S.-Y. Le, M. L. Yeung, and K.-T. Jeang, "HIV-1 encoded candidate micro-RNAs and their cellular targets," *Retrovirology*, vol. 1, article 43, 2004.

[84] Y. Bennasser, S.-Y. Le, M. Benkirane, and K.-T. Jeang, "Evidence that HIV-1 encodes an siRNA and a suppressor of RNA silencing," *Immunity*, vol. 22, no. 5, pp. 607–619, 2005.

[85] S. Omoto, M. Ito, Y. Tsutsumi et al., "HIV-1 nef suppression by virally encoded microRNA," *Retrovirology*, vol. 1, article 44, 2004.

[86] S. Omoto and Y. R. Fujii, "Regulation of human immunodeficiency virus 1 transcription by nef microRNA," *Journal of General Virology*, vol. 86, no. 3, pp. 751–755, 2005.

[87] Z. Klase, R. Winograd, J. Davis et al., "HIV-1 TAR miRNA protects against apoptosis by altering cellular gene expression," *Retrovirology*, vol. 6, article 18, 2009.

[88] J. Lin and B. R. Cullen, "Analysis of the interaction of primate retroviruses with the human RNA interference machinery," *Journal of Virology*, vol. 81, no. 22, pp. 12218–12226, 2007.

[89] M. L. Yeung, Y. Bennasser, K. Watashi, S.-Y. Le, L. Houzet, and K.-T. Jeang, "Pyrosequencing of small non-coding RNAs in HIV-1 infected cells: evidence for the processing of a viral-cellular double-stranded RNA hybrid," *Nucleic Acids Research*, vol. 37, no. 19, pp. 6575–6586, 2009.

[90] A. W. Whisnant, H. P. Bogerd, O. Flores et al., "In-depth analysis of the interaction of HIV-1 with cellular microRNA biogenesis and effector mechanisms," *MBio*, vol. 4, no. 2, 2013.

[91] N. Alkhouly, I. Shehata, M. B. Ahmed, H. Shehata, S. Hassan, and T. Ibrahim, "HLA-G expression in acute lymphoblastic leukemia: a significant prognostic tumor biomarker," *Medical Oncology*, vol. 30, no. 1, 2013.

Hindawi Publishing Corporation
BioMed Research International
Volume 2013, Article ID 892106, 10 pages
http://dx.doi.org/10.1155/2013/892106

Review Article

Translational Potential into Health Care of Basic Genomic and Genetic Findings for Human Immunodeficiency Virus, *Chlamydia trachomatis*, and Human Papilloma Virus

Jelena Malogajski,[1] Ivan Brankovic,[1] Stephan P. Verweij,[2] Elena Ambrosino,[1] Michiel A. van Agtmael,[3] Angela Brand,[1] Sander Ouburg,[2] and Servaas A. Morré[1,2]

[1] *Institute for Public Health Genomics (IPHG), Department of Genetics and Cell Biology, Research School GROW School for Oncology & Developmental Biology, Faculty of Health, Medicine & Life Sciences, University of Maastricht, 6229 ER Maastricht, The Netherlands*
[2] *Laboratory of Immunogenetics, Department of Medical Microbiology and Infection Control, VU University Medical Center, 1081 BT Amsterdam, The Netherlands*
[3] *Department of Internal Medicine, VU University Medical Centre, 1081 HV Amsterdam, The Netherlands*

Correspondence should be addressed to Servaas A. Morré; samorretravel@yahoo.co.uk

Received 28 February 2013; Revised 1 May 2013; Accepted 7 May 2013

Academic Editor: Enrique Medina-Acosta

Individual variations in susceptibility to an infection as well as in the clinical course of the infection can be explained by pathogen related factors, environmental factors, and host genetic differences. In this paper we review the state-of-the-art basic host genomic and genetic findings' translational potential of human immunodeficiency virus (HIV), *Chlamydia trachomatis* (CT), and Human Papilloma Virus (HPV) into applications in public health, especially in diagnosis, treatment, and prevention of complications of these infectious diseases. There is a significant amount of knowledge about genetic variants having a positive or negative influence on the course and outcome of HIV infection. In the field of *Chlamydia trachomatis*, genomic advances hold the promise of a more accurate subfertility prediction test based on single nucleotide polymorphisms (SNPs). In HPV research, recent developments in early diagnosis of infection-induced cervical cancer are based on methylation tests. Indeed, triage based on methylation markers might be a step forward in a more effective stratification of women at risk for cervical cancer. Our review found an imbalance between the number of host genetic variants with a role in modulating the immune response and the number of practical genomic applications developed thanks to this knowledge.

1. Introduction

Infectious diseases representa major health threat worldwide and a significant part of the burden of disease in developing countries [1]. Public health policy has traditionally had an important role in tackling such threat through established measures of prevention, mostly by controlling social and environmental determinants of health and through vaccination. With the recent advances in public health genomics, public health moved its focus from a "one size fits all" approach in health promotion and prevention activities to targeting populations and subpopulations with defined genetic risks and developed its unique role, translation of genome-based knowledge and technologies into public health policy and practice, and its integration across disciplines [2].

Scientific developments in basic research and the development of public health genomics have changed many paradigms regarding infectious diseases. Indeed, the recent evidence of genetic factors in the pathogenesis of infectious diseases transformed the view of such diseases from strictly pathogen-centric to the one incorporating host genetic determinants that modulate immune response. Though research in the field of genetic susceptibility to infectious diseases started in 1954, recent progress in genomics led to the characterization of molecular biomarkers and pathways as

targets for diagnosis or intervention [3]. Furthermore, this understanding of infectious diseases explains the individual variation in susceptibility to an infection as well as the clinical course of the infection by pathogen related factors, environmental factors, and genetic differences. The field identifies genes responsible for influencing susceptibility to infections as well as their severity and response to treatment. This is predominantly achieved by studying candidate genes, genome wide associations, and twin studies [4].

A great amount of effort and resources have been directed to obtaining knowledge about host genetic components of infectious diseases and to confirm associations in order to develop genomic applications in everyday clinical practice and prevention.

Nonetheless, although the amount of genetic data in relation to disease is increasing exponentially [5, 6], there is a clear lack of translation of such findings to healthcare applications. Indeed, the amount of information about basic genome-based scientific findings present in the scientific journals is disproportionate to the number of patents and marketed products used in hospitals [7].

In this paper, priority was given to three sexually transmitted diseases of significant public health relevance: HIV, HPV, and *Chlamydia trachomatis* (CT) genital tract infections.

The aim of this review is to provide a state-of-the-art overview on the translational potential of basic genomic and genetic findings related to HIV, CT, and HPV infections, into applications in public health focusing on their diagnostics and treatment.

2. Methods

Based on our field of expertise in sexually transmitted diseases (STDs) we selected the most prevalent bacterial STD *Chlamydia trachomatis* (CT) and the 2 most prevalent viral STDs *Human Papilloma Virus* (HPV) and the *Human Immunodeficiency Virus* (HIV) knowing that from these infectious diseases human genetic and genomic markers are described.

We used the HuGE Navigator (Version 2.0: an integrated, searchable knowledge base of genetic associations and human genome epidemiology (http://hugenavigator.net/)) [8] to identify papers with a description of potential translation on the basic findings of genetic and genomic markers into diagnostic applications and ultimately into public health. Identified papers and authors were expanded using PubMed searches. For each infectious disease a general introduction will be given, the key genetic and genomic markers will be described, and the translational potential outlined. Finally, a general discussion and conclusions will be provided.

3. Results

3.1. HIV. Despite the decrease in incidence of HIV infection (in 2009 the number of newly infected individuals dropped by almost 20% compared to the previous year), the prevalence of HIV is still very high. At the end of 2009, it was estimated that there were 33.3 million people living with HIV. The growing prevalence and the reduction in the AIDS-related mortality are mainly attributed to the success of antiviral therapy [9]. Nonetheless, the public health relevance of the disease remains indisputable, as tackling HIV requires large financial expenditures, and it is still among the sexually transmitted diseases causing the highest morbidity and mortality and it is highly preventable [10].

As mentioned earlier, research in the field of infectious diseases has established that the susceptibility of an individual is also modulated by host genomic factors. In this context, recent genomic and genetic discoveries using candidate gene and genome wide association studies (GWAS) increased our knowledge of the association among genetic loci from the so-called "major susceptibility genes." HIV infection is the most studied infection by the aforementioned approaches. The research of a genetic role for the individual differences in the course of infection, besides offering new strategies for developing a treatment or a vaccine, also provides basic insights in the immunopathology of the infection. Moreover, this newly collected evidence could provide an opportunity of identifying persons at higher risk of getting or progression of the infection. On the other hand, this could detect patients having genes that make them long-term nonprogressors, thus with delayed or no progression to AIDS.

3.1.1. Review of the Host Genetic Variants Found to Influence HIV Infection. The review of papers written by the experts in the field of host genomic determinants of infection, disease progression, and disease outcome reveals the growing body of host genomic "suspects" by the year. However, few associations were positively confirmed. Among these, only 15–20% of observed genetic variants have been identified as influencing HIV infection [11].

Many studies and reviews place genetic variants of chemokine receptor and chemokine ligand genes, HLA and related genes on top of the list of influential genetic factors identified in HIV infection [11–16].

Chemokine receptors have an important role in modulating HIV-1 early infection. Particular attention has been given to *CCR5* and *CCR2* genes, encoding coreceptors on the surface of the CD4+ lymphocytes, crucial for HIV cell entry. In the initial stages of the infection, the HIV virus uses CCR5 as a preferred coreceptor [15]. As a result, a mutation in the chemokine receptor genes resulting in the absence or significant reduction of CCR5 molecules on the cell surface would have a protective effect. Indeed, the expression level of this coreceptor influences the HIV infection outcome, and mutation of this molecule is associated with the ability of the virus to enter the cells *in vitro*, the *in vivo* viral load, the CD4+ levels during highly active anti-retroviral therapy (HAART: combination of three or more antiviral drugs), and the progression of the diseases to AIDS.

In 1996, it was discovered that the deletion of 32 base pairs of *CCR5* (*CCR5*Δ32) results in shortened and inactive proteins. So far, *CCR5*Δ32 remains the only discovered mutation that completely protects homozygotes from HIV infection and in heterozygotes slows down the progression

of the disease [11]. Moreover, the discovery of *CCR5*Δ32 genetic variant opened the door for the development of a new type of anti-HIV medications. Data obtained from *CCR5* gene candidate studies have been rather timely applied in the pharmaceutical industry, leading to the development of novel therapies, as further discussed in the next section.

In addition, the association between the +190 A>G mutation of *CCR2* chemokine receptor and the delayed onset of AIDS was discovered in 1997. The resulting substitution of the amino acid valine, at the position 64 of CCR2, to isoleucine influences HIV progression, but not the risk of HIV infection. HIV positive patients carrying this mutation showed delayed progression to AIDS by 2–4 years [17].

3.1.2. Application of Research Based on Chemokine Receptors. As stressed earlier, the major goal of the research on host immunogenetics of HIV is to acquire knowledge of how differences in genetic variants are influencing individual susceptibility to infection and developing new drugs based on that. The research provided insights into the effects of CCR5 coreceptor blockade and downregulation on HIV infection [18]. As a result drugs with a new mechanism of action, the blockage of CCR5 receptors, were developed. These drugs are also known as entry inhibitors. So far there are only two approved such drugs in clinical use, Maraviroc (Pfizer) and Enfuvirtide (Roche) [19, 20]. Of the two, *Enfuvirtide* was the first to be FDA approved. The success of this drug, despite its proven antiviral efficacy in patients' treatment, was constrained by the difficulties related to its subcutaneous administration, causing skin abscesses. The first orally administered HIV entry inhibitor was *Maraviroc*, approved by the FDA for patients with R5 virus types in 2006. The drug binds to the CCR5 chemokine receptor causing a conformational change that blocks the gp41-mediated fusion of viral and cellular membranes [19]. The next most promising HIV entry inhibitor is *Vicriviroc* (Schering-Plough), a medicine with the same action mechanism as Maraviroc, but expected to be more effective. Vicriviroc has still not been approved by FDA, but phase III clinical trials have been recently completed [21].

A recent extensive review of HIV-1 entry inhibitors patented from 2004–2010, [20], revealed 35 small CCR5 antagonist molecules patented by 5 different pharmaceutical companies (Astra Zeneca, ViroChem Pharma, Anormed, Inc./Genzyme Corp., Euroscreen, and Ono Pharmaceuticals). In the same review, it was found that the number of patents for CXCR4 (coreceptors for X4 HIV strains) antagonists and dual CCR5/CXCR4 antagonists is significantly lower. Further, clinical developments of CXR4 antagonists have been delayed in preclinical and clinical studies due to serious side effects (cardiac abnormalities and liver toxicity) or lack of drug efficacy.

Human Leukocytes Antigen (HLA) genes encode proteins that present antigens to T and B lymphocytes. There are two classes of HLA genes: class I (loci A, B, and C) and class II genes. A strong association has been observed between HLA I alleles and protection/susceptibility to HIV [22]. The effect of HLA A, B, and C homozygosis in general is accelerated AIDS. Other confirmed associations include HLA alleles B*27 and B*57 and delayed progression to AIDS [15, 16, 22, 23]. On the other hand, the B*35 allele is associated with increased susceptibility and more rapid progression of the disease. The median time in which homozygous carriers of the B*35 allele develop AIDS is half the time of noncarriers of such alleles [24].

The association between genetic variants of HLA class I loci and CCR5 and the pathogenesis of HIV infection has been confirmed in recent years by many GWAS studies. However, GWAS did not identify further major susceptibility loci [25].

Association studies between HLA class II alleles and the susceptibility to the HIV infection has been less consistent.

HLA genes have also been shown to have a role in the Mother to Child Transmission (MTCT) of HIV infection. Indeed, HLA class I concordance between mother and child is associated with higher risk of transmission, *vice versa* HLA discordance is associated with a lower risk [16].

3.1.3. Application of Research on HLA Genes. Although none of the mentioned HLA genes have yet been identified as a target for new drugs, the information gathered on the disease progression modulated by different genotypes has provided valuable information for clinical trials [22]. Research on HLA alleles led to important pharmacogenetic applications. HLA B*5701 positive patients, who are at risk for hypersensitivity to *Abacavir* (a nucleoside reverse transcriptase inhibitor), cannot be treated with this drug. This serious, and possibly fatal, adverse drug reaction is present in 5% of patients [26]. Genetic testing of all the individuals before prescribing the drug prevents serious side effects, building a very strong case for a stratified medicine approach, tailored to individual genetic characteristics. The idea behind it is that our personal genetic differences create a need for accordingly different treatment approaches. In the case of Abacavir recognizing interpersonal variation in reaction to drug is an excellent example of stratifying HIV treatment based on genetic research.

In summary, HIV immunogenetic research provided some basic insights into the immunopathology of the infection and gave foundations to the development of new drugs for the therapy of the infection. Ideally this will be just the first step in advancing therapies. Information on individual susceptibility, higher or lower individual risks, and delayed or accelerated AIDS progression associated with certain gene variants will make a more individually tailored treatment possible in the future.

3.1.4. Chlamydia trachomatis. *Chlamydia trachomatis* is a leading cause for a variety of diseases including ocular, respiratory, and sexually transmitted diseases. This section of the review will only focus on the latter, since sexually transmitted *Chlamydia* infections are the most common worldwide, whereas, for instance, ocular infections are mostly seen in third world countries. Host genetic twin studies of *Chlamydia* have shown that 40% of the responses to *Chlamydia* are based on host genetics [27].

According to the WHO, "more cases of STD are caused by *Chlamydia trachomatis* than by any other bacterial pathogen" [28]. The persisting high incidence of 90–100 million cases per year worldwide makes *Chlamydia trachomatis* infection an enormous health problem throughout the world. The bacteria can be easily eliminated by antibiotic treatment; however, as a result of often being asymptomatic, the infection is frequently diagnosed too late or not at all. Infertility, premature delivery, PID, and ectopic pregnancy are some serious sequelae of the untreated infection [29].

Evaluation of the casual link between *Chlamydia* lower genital tract infection and tubal infertility is very challenging due to the fact that this is a "silent" complication, usually diagnosed years after the infection [30]. Infected women can either clear the bacteria without any damage to their reproductive functions or develop severe late complications, such as tubal occlusion and periadnexal adhesions, leading to infertility as the most severe of complications. The differences in disease outcome are often determined by genetic variations, such as single nucleotide polymorphisms (SNPs) in genes responsible for, amongst others, bacterial sensing receptors (and the pathways to which they belong) on cells such as macrophages as well as local vaginal and tubal epithelial cells. The higher the number of genes affected by SNPs, the more abnormal the immune response, leading to a higher chance of severe complications [31]. Inadequate recognition of the pathogen and consequent inadequate immune response lead to a higher risk of subfertility [32]. In a research performed on Gambian twins [27], it was estimated that 40% of variation in *Chlamydia* infection characteristics could be explained by differences in host genetic factors.

3.1.5. Review of the Host Genetic Variants Found to Influence Chlamydia Lower Genital Tract Infection

TLR Receptors. Toll-like receptors (TLRs), with their role in identifying pathogens and initiating innate immune response, have been recognized as the most important factors in influencing differences in susceptibility to course and outcome of *Chlamydia* infection [33, 34]. Indeed, much of immunogenetic research in this field is focused on TLR genes and genes involved in their pathways, not only by mRNA- and protein-based studies but also by studying the association between SNPs in TLR genes leading to the loss of function of the receptors and the potential higher risk of late complications such as tubal infertility. The application of such research could be in the area of early diagnosis of tubal infertility or subfertility. Based on this evidence, the time now being lost as a result of late or misdiagnosis of tubal infertility could be directed to IVF attempts.

So far, there are 10 TLRs identified in humans, recognizing different bacterial and viral components. TLRs activate signaling pathways of immune response against different pathogens by activating different inflammatory cytokines [35]. TLR2, TLR4, and TLR9 recognize pathogen-associated molecular patterns (PAMPs) of *Chlamydia trachomatis*. Genes for TLR receptors 2 and 4 are considered particularly important in modulating innate immune response to *Chlamydia trachomatis* [36].

Several studies showed that SNPs in *TLR4* have a role in making women more prone to subfertility as a late complication of *Chlamydia* infection. Nonetheless, the exact role of TLR4 in subfertility has not been yet clearly understood [33, 34]. Subfertile women who have IgG antibodies for *Chlamydia trachomatis* have a two times higher likelihood to be carriers of the *TLR4* +896 A allele, compared to women without tubal pathology [34]. Although this observation was not statistically significant, reported trends suggest that it could be worthwhile to further explore it in a larger cohort. Further, murine studies showed that TLR4 functional mice are more protected against reinfection compared with mice with dysfunctional or absent TLR4 [36]. In their study of genetic variants involved in the immune response regulation in genetic tract infections, Laisk et al. found that the *TLR4* +896 A>G and +1196 C>T polymorphisms protect against multiple infections with *C. trachomatis*, *N. gonorrhoeae*, *M. hominis*, *M. genitalium*, *U. parvum*, and *U. urealyticum*. Depending on the patient definition (i.e., including or excluding *C. trachomatis* serology), they found that specific *MBL2* high producing haplotypes can have a protection of a risk effect in tubal factor infertility. Low-producing *MBL2* haplotypes are associated with *C. trachomatis* serology positive tubal factor infertility patients [36].

In their study on the role of *TLR2* and *TLR4* in the development of tubal pathology on knock out (KO) mouse models, Darville et al. [33] showed that the amount of cytokines produced by macrophages depends on TLR2 but not on TLR4 receptors. Indeed, the deficiency of TLR2 receptors is associated with a decreased production of cytokines *in vitro*. *In vivo*, the deficiency or absence of TLR2 causes lower levels of inflammatory mediators, but the course of infection does not differ compared with naïve animals. Microscopic examination of the tubal tissue showed that mice with intact TLR2 are, however, more prone to the development of late inflammatory sequelae. Finally, their study concluded that TLR4 does not modulate innate immune response to *Chlamydia*, whereas *in vivo* experiments on TLR2 indicated its important role in protection against late inflammatory sequelae following *Chlamydia* genital tract infection [33].

In a study aiming at understanding the role of two *TLR2* SNPs in the susceptibility to infection and contribution to the development of the tubal pathology in Dutch women, Karimi et al. [37] revealed a statistically significant association between certain *TLR2* haplotypes and protection from tubal pathology and development of the late inflammatory complications (the absence of TLR2 is associated with an increase in the severity of the *Chlamydia* infection).

TLR9—as already mentioned, most of the studies assessing host genetic determinants of *Chlamydia* infections are focusing on the extracellular TLR2's and TLR4's contribution to the differences in the susceptibility and severity of the infection. However, there is also an interest in the relevance of the intracellular TLR9. So far, human cohort data have not shown significant differences between carriers of mutant alleles and controls in the susceptibility to infection, course

of the infection, or frequency of later tubal pathology. On the other hand, experiments in mice models found that *TLR9*-deficient mice had a higher level of protection against reinfection [38].

HLA Alleles. In addition to the research directed at *TLR* genes, there are also indications of association between tubal infertility caused by *Chlamydia trachomatis* and HLA alleles. Cohen et al. [39, 40] found that alleles of the HLA-DQ, DR1, and DRB5 loci modulate the severity of *Chlamydia* infections. Kinnunen et al. also found that specific HLA-DQ alleles are more frequently present in women with tubal infertility [41].

Besides the TLR and HLA alleles, in 2009, Morré et al. published an extensive overview of the then known genetic variants influencing susceptibility and severity of *Chlamydia* infections including SNPs in cytokines and other pathogen recognition receptors like NODs [42].

3.2. Application of Research. Immunogenetics research on *Chlamydia trachomatis* indicates that a proof of principle for the successful application of genetic and genomic markers for the prediction of late complications after the infection could have a strong public health impact.

Subfertility poses an enormous burden on healthcare and society throughout the world. Worldwide, 15% of couples trying to conceive suffer from subfertility [44, 45]. One of the major causes of female subfertility is tubal pathology (TP) [44], and CT is the single most common cause for infertility. If left untreated, CT may lead to ectopic pregnancy, tubal pathology, and ultimately infertility. The cost associated with subfertility is high, as it requires tubal surgery and *in vitro* fertilisation (IVF).

Currently, CT IgG serology is used to assess the risk of CT-associated TP in subfertile women (20%) (Figure 1) [46]. CT serology has limited sensitivity and specificity and the predictive value is poor thus, many women undergo additional diagnostic procedures while not needed (40–45%) or do not get intervention while needed (19%). Laparoscopy is widely used to assess the risk of TP in women positive for CT IgG. This procedure is invasive and expensive (on average 3000 Euros including additional costs) and requires general anaesthesia. Furthermore, it holds a 1.5% risk of surgical complications (e.g., bleeding, infection, or worse).

Therefore it is crucial to develop a companion diagnostic to improve the assessment of risk of TP in CT-positive and negative women. By doing so, one is able to prevent invasive procedures in patients without TP and reduce both the cost and the psychological burden associated with laparoscopy. This companion diagnostic should merge serology, taking into account serological positivity and titres and considering new serological responses (e.g., pgp3) [47] and add the predictive value of host genetic markers involved, for example, related to the innate immune response to pathogens. The genetic trait should consist of a series of markers with a so-called SNP load or gene load linked to decision making for performing laparoscopy or not. Future studies should be directed at performing studies in larger cohorts to access the true clinical potential of this approach.

3.3. HPV. Roughly 20% of cancers are linked to various infectious agents [48]. Human papilloma virus (HPV) is one of these agents, and the role of different HPV subtypes in the etiology of cervical cancer has been well established [49]. HPV infections are in most cases cleared by the actions of the immune system within one year and often remain asymptomatic throughout that period. However, a small percentage of the infections eventually lead to some form of cancer.

HPV-induced cancers account for approximately one-third of all cancers caused by infectious agents [50], and HPV is considered to be the most common sexually transmitted infectious agent [51]. However, studies have shown the existence of nonsexual modes of HPV transmission (including transplacental and transmission via fingers and objects [52–54]), and therefore, HPV cannot be referred strictly to as an STI [52].

The HPV virus infects skin or mucosal tissues in the anogenital area or the region of the head and neck. So far more than 100 types have been reported [50]. It has however been proven that approximately 15 out of these 100 types cause virtually all cases of cervical cancer [55]. Moreover, HPV types 16 and 18 account for around 70% of cervical cancer cases, and they—particularly type 16—have also been identified in anal, as well as some head and neck cancers [56].

The strong association between HPV infection and cervical carcinogenesis makes cervical cancer preventable, thus fulfilling an important criterion for public health relevancy. With the introduction of HPV vaccines, a major breakthrough in prevention has been made. Vaccines proved to be safe and efficacious [57] and vaccination programmes for girls and young women have been implemented in many countries.

3.3.1. Review of the Host Genetic Variants Found to Influence HPV Infection. Of all the women who are infected with HPV, only a small percentage develops cervical cancer. This observation suggests a role of host genetic factors influencing persistent HPV infections and progression into cervical cancer.

The Role of HLA. Alleles have been reported to be associated with the development of HPV-related cervical cancer. In their review of evaluating this association, Hildesheim and Wang [58] found several alleles of HLA class II to be associated with an higher risk of developing cervical cancer (DQB1*03 alleles and DRB1*1501, DQB1*0602). As for HLA genetic variants' protective effect, several studies consistently reported that DRB1*13 and DOB1*0603 are associated with it [58]. Associations between HLA and HPV infection and progression to cancer are reported to be population- and HPV type-dependent. Indeed, HLA DQB1*0301 allele carries an increased risk of cervical cancer in the British population in case of infection with all HPV subtypes [59], while researchers in Bolivia found a statistically significant association of HLA DRB1*1602 with susceptibility toinfection [60].

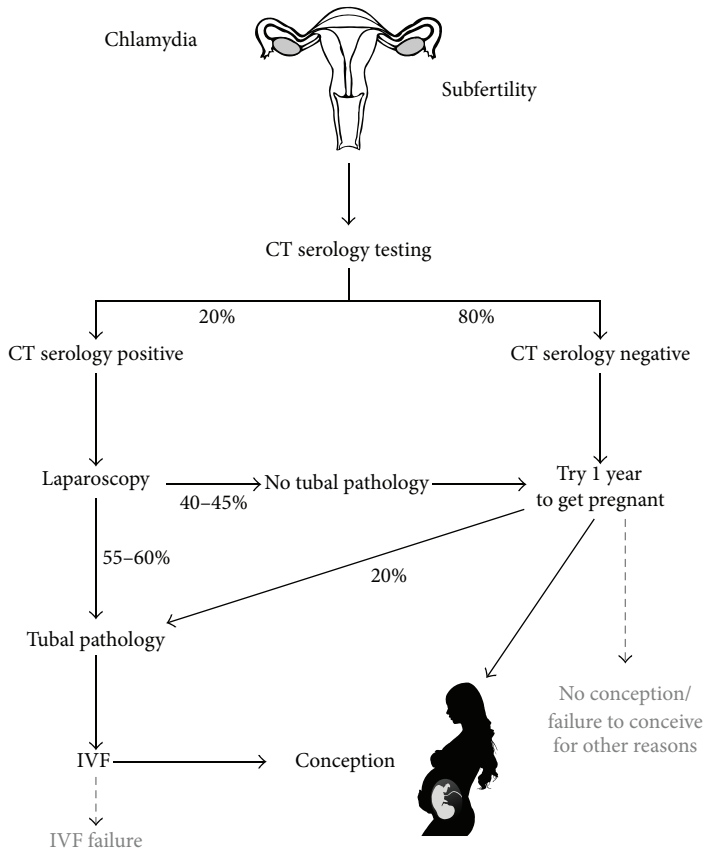


Figure 1: Current serology protocol for subfertility resulting from CT infection. Women with a negative CT serology are advised to try to conceive for one year; however, 20% of those women actually have tubal pathology and are thus misdiagnosed. Of the women with a positive CT serological test, 40–45% do not have tubal pathology after laparoscopic examination and are thus misdiagnosed. Figure adapted from Lal et al. [43].

In their recently published review of the genetic susceptibility to cervical cancer, Chen et al. [61] presented the most important genetic polymorphisms associated with the development of this disease. Their literature search identified, in addition to HLA genetic variants, genes encoding interleukin-1β, tumor necrosis factor α, interleukin-12 A and B, interferon-γ, interleukin-10, cytotoxic t-Lymphocyte antigen-4, p53, BRCA1, and LAMB3 as genes associated with persistent HPV infection and progression to cervical cancer [61]. In addition, certain genes encoding killer immunoglobulin-like receptors (KIR) also seem to be associated with cervical cancer [62].

So far, no genetic or genomic applications have been developed based on these findings. When it comes to applying genetic knowledge and discoveries into the field of HPV infection and cervical findings diagnosis and prevention, the strategy known as methylation takes the lead.

3.3.2. The Role of Methylation. Methylation is a common mechanism through which the silencing of genes, and among these tumor-suppressor genes, can be achieved [64]. It represents a chemical alteration in regions of DNA referred to as "CpG islands," commonly found in many promoter regions. The alteration leads to the inhibition of the transcription of genes controlled by such methylated promoters [65]. Methylation markers are easily detected in cervical scrapes, with, for example, methylation-specific PCR (MSP). Hence, positive MSP results in these samples are indicators of methylation of relevant genes in the tissue [65]. At the moment, the strategy for early detection of cervical neoplasia in screening programmes is cervical scraping cytomorphologic assessment (PAP test), which has a considerably low sensitivity. Data on sensitivity and specificity of the PAP test are highly heterogeneous. Depending on the study done and combination of tests and reference standard thresholds applied, they range from 18% to 98% for sensitivity and from 17% to 99% for specificity [66]. Furthermore, the National Cancer Institute assessed the sensitivity of the PAP smear to be 55–80% for high grade lesions and around 68% for low grade lesions [67]. Taking this into consideration, there is a need for the development of novel approaches, and additional tools based on methylation markers might be a step forward.

3.3.3. Application of Methylation in Triage of Cervical Carcinomas. In the study by Henken et al. [65], 29 tumor-suppressor

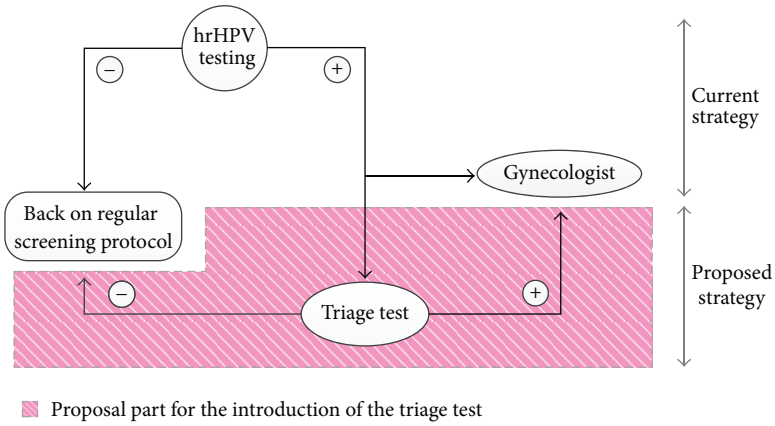


Figure 2: Introducing methylation as an addition to the primary hrHPV test would lower the number of unnecessary referrals to gynaecologists. Figure based on Yang et al. [63].

genes were analyzed as potential methylation targets, and 12 of them were found to have methylated gene promoters in cervical cancer tissue. Eight of those were also associated with consecutive stages in HPV-mediated transformation *in vitro*. The promoter that was most commonly methylated (in 92% of the examined carcinoma samples) was MGMT.

Methylation of the promoters CCNA1 and C13ORF18 in cervical scrapings is found to be strongly associated ($P < 0.0005$) with CIN2 (moderate cervical intraepithelial dysplasia) and higher grade stages of cervical dysplasia, as was determined in the study by Yang et al. [63]. Hence, these would be suitable markers for a triage test, referring a patient to a gynecologist upon a methylation-positive result. The more severe the lesion in the sample, the more methylation was present in these two gene promoters. Analysis of high methylation of these two markers has a high specificity (96% and 100%, resp.), as well as high positive predictive value. Further, Yang et al. [63] suggest that their methylation test should be used as a triage test in primary hrHPV testing (high risk HPV test identifies types of HPV which are linked to cervical cancer). hrHPV testing is more effective in preventing invasive cervical cancer; however, it is considered to be less sensitive than cytology in detecting CINs. Introducing methylation as a part of a triage test to the primary hrHPV test would lower the number of unnecessary referrals to gynecologists; especially in younger women who tend to be over diagnosed [68] (see Figure 2).

In another study evaluating the potential value of the methylation markers CADM1 and MAL as a triage tool for hrHPV+ women, it was found that there is a solid reasoning for combining markers which relate to different stages in cervical carcinogenesis [69]. They examined and confirmed the advantage of combining methylation patterns in the promoter region of more than one suppressor gene with the aim to increase the sensitivity for high grade CINs. A methylation-based test focuses on later phases of the carcinogenesis, given that these promoter alterations increase in these late stages. However, methylation-driven silencing of MAL promoter takes place at a very early point, before HPV-positive keratinocytes undergo tumor transformation. Whereas, silencing of CADM1 promoter by methylation correlates more with late stages. Overmeer et al. demonstrated that this marker combination is optimal for detection of CIN3 lesions [69].

In the process of progression into late stages, there are genes other than oncogenes and tumor suppressors also relevant. MicroRNAs (miRNAs) are short noncoding RNA molecules, which act in regulating expression of protein-coding genes, by pairing with sequences within such genes. hsa-miR-124 is an miRNA known to be silenced by methylation in many cancers, and Wilting et al. (2010) proved that this mode of silencing frequently occurs in cervical lesions as well [70]. No methylation was found in normal tissues, while almost 60% was detected in CIN3 lesions, and more than 93% methylation of hsa-miR-124 was present in cervical carcinomas. The methylation of this gene is not directly related to the presence of hrHPV. High positivity is however observed in CIN3 and cervical carcinomas, which altogether makes it a potentially very useful triage marker for hrHPV-positive women. This applies however not for setting where HPV genotyping is not implemented yet including under development countries.

Triage could serve as an additional step that would more aptly bridge screening and diagnosis in order for a better stratification of women at risk to be achieved [71]. It would be used on those with positive primary screening results to determine the further risk of the progression into later stages.

The effects of constructing this type of triage test based on methylation would be expected to land a formidable impact on policies that currently regulate screening intervals.

4. Discussion and Conclusion

To our knowledge, this is the first review on the translational potential of basic genomic and genetic findings for HIV, CT, and HPV into applications in public health and in diagnostics, treatment, and prevention of late complications of these infectious diseases. We found scarce examples of the

current application of genomic/genetic findings, in pharmacogenomics, and we found examples of genomic information with a promise of translation in the near future.

In our review, we did not focus on analytic validity, clinical validity, and clinical utility and other criteria generally considered to be the most important factors in evaluation of the genetic/genomic applications [72]. Since there are still no market-ready applications, so the aforementioned criteria could not be considered; we focused on an earlier step of this process. We focused on the promising examples of translation of the discovery into a possible application.

Based on the review of the relevant literature some examples can be considered promising.

The genes responsible for susceptibility to HIV infection can be basically divided in two groups, chemokine receptors genes and HLA genes. So far, the discovery of the *CCR5*Δ32 genetic variant opened the door for the development of new anti-HIV drugs. Although undoubtedly a very important step forward, CCR5 targeted therapy and the research behind it are just one of the possible applications of immunogenetic information. Indeed, there is a significant amount of knowledge of certain genetic variants having a positive or negative influence on the course and outcome of HIV infection. Possible future use of the knowledge about the expected course of the infection would be advancing the standard of care and therapy after routine genetic testing.

In the field of *Chlamydia trachomatis* caused subfertility there is a promise for a more accurate subfertility diagnosis based on SNPs. Research showed that SNPs in *TLR4* possibly increase the risk of tubal pathology. Specific *TLR2* haplotypes are associated with protection from tubal pathology and development of the late inflammatory complications.

These findings, together with the one carrying multiple SNPs in multiple pattern recognition receptors' (PRRs) encoding genes (*TLR9*, *TLR4*, *CD14*, and *CARD 15*/*NOD2*) doubles the risk of tubal pathology in *Chlamydia trachomatis* IgG-positive women compared to IgG-positive women carrying less than two SNPs, offer a proof of concept for the development of a genomic application in diagnosis of subfertility.

A genetic test as a part of routine subfertility diagnosis should be able to save time and money by decreasing the number of unnecessary laparoscopies and the time patients unsuccessfully spend trying to get pregnant.

In the field of HPV, there are some promising advancements in the early diagnosis of cervical cancer based on methylation tests. The methylation markers CADM1 and MAL were found to be an optimal combination for the detection of CIN3 lesions [69]. Moreover, the methylation of CCNA1 and C13ORF18 in cervical scrapings is found to be strongly associated with CIN2 and higher grade stages [63]. A triage test based on such methylation markers might be an important step towards a more effective stratification of patients at risk for cervical cancer.

The knowledge about the gene-disease associations should lead to growing numbers of genetic tests, which will in the future have an increasingly important role, in tailored clinical and drug treatment. However, in order for this translation process to succeed, the wide consensus among scientists, clinicians, policy makers, and the industry on necessity of going in this direction needs to be achieved [73].

Based on what we have shown here, there are many host genetic variants found to have a role in modulating the immune response to HIV, HPV, and *Chlamydia* infections. However, we found an imbalance between the number of host genetic variants with a role in modulating the immune response and the number of practical genomic applications. Thus, such new knowledge and technologies from basic research are not yet integrated in health in a timely, effective, and efficient manner [7].

This imbalance, the lack of translation from bench to bedside, is in favor of basic research that seems to be somewhat hermetic in quality, revealing confirmed positive association with a certain genetic variant and not exploring the future implications of these findings, should not represent a norm in the field.

The next step is needed in which gene-disease association leads to the development of the genetic/genomic application. Starting with interdisciplinary collaboration is very important in the process of evaluation of role of genetic variants in the etiology of human diseases [74].

There are some clear and well-supported genetic associations with particular infectious diseases; these should be driving forces of the successful translation process.

References

[1] A. J. Frodsham and A. V. S. Hill, "Genetics of infectious diseases," *Human Molecular Genetics*, vol. 13, no. 2, pp. R187–R194, 2004.

[2] W. Burke, M. J. Khoury, A. Stewart, and R. L. Zimmern, "The path from genome-based research to population health: development of an international public health genomics network," *Genetics in Medicine*, vol. 8, no. 7, pp. 451–458, 2006.

[3] A. V. S. Hill, "Aspects of genetic susceptibility to human infectious diseases," *Annual Review of Genetics*, vol. 40, pp. 469–486, 2006.

[4] A. V. S. Hill, "Immunogenetics and genomics," *Lancet*, vol. 357, no. 9273, pp. 2037–2041, 2001.

[5] J. L. Rowell, N. F. Dowling, W. Yu, A. Yesupriya, L. Zhang, and M. Gwinn, "Trends in population-based studiesof human genetics in infectious diseases," *PLoS One*, vol. 7, no. 2, Article ID e25431, 2012.

[6] M. J. Khoury, M. Gwinn, M. Clyne, and W. Yu, "Genetic epidemiology with a capital E, ten years after," *Genetic Epidemiology*, vol. 35, no. 8, pp. 845–852, 2011.

[7] J. A. . Lal, T. Schulte In den Baumen, S. A. Morre, and A. Brand, "Public health and valorization of genome-based technologies: a new model," *Journal of Translational Medicine*, vol. 9, article 207, 2011.

[8] W. Yu, M. Gwinn, M. Clyne, A. Yesupriya, and M. J. Khoury, "A navigator for human genome epidemiology," *Nature Genetics*, vol. 40, no. 2, pp. 124–125, 2008.

[9] UNAIDS, "UNAIDS report on the global AIDS epidemic 2010," Global Report, 2010.

[10] CDC, "Updated guidelines for evaluating public health surveillance systems: recommendations from the Guidelines Working Group," *Morbidity and Mortality Weekly Report*, vol. 50, no. RR-13, pp. 1–35, 2001.

[11] P. An and C. A. Winkler, "Host genes associated with HIV/AIDS: advances in gene discovery," *Trends in Genetics*, vol. 26, no. 3, pp. 119–131, 2010.

[12] J. Tang and R. A. Kaslow, "The impact of host genetics on HIV infection and disease progression in the era of highly active antiretroviral therapy," *AIDS*, vol. 17, supplement 4, pp. S51–S60, 2003.

[13] W. He, J. Castiblanco, E. A. Walter, J. F. Okulicz, and S. K. Ahuja, "Mendelian randomization: potential use of genetics to enable causal inferences regarding HIV-associated biomarkers and outcomes," *Current Opinion in HIV and AIDS*, vol. 5, no. 6, pp. 545–559, 2010.

[14] S. J. O'Brien, G. W. Nelson, C. A. Winkler, and M. W. Smith, "Polygenic and multifactorial disease gene association in man: lessons from AIDS," *Annual Review of Genetics*, vol. 34, pp. 563–591, 2000.

[15] R. A. Kaslow, T. Dorak, and J. Tang, "Influence of host genetic variation on susceptibility to HIV type 1 infection," *Journal of Infectious Diseases*, vol. 191, supplement 1, pp. S68–S77, 2005.

[16] K. K. Singh and S. A. Spector, "Host genetic determinants of human immunodeficiency virus infection and disease progression in children," *Pediatric Research*, vol. 65, no. 5, part 2, pp. 55R–63R, 2009.

[17] M. W. Smith, M. Dean, M. Carrington et al., "Contrasting genetic influence of CCR2 and CCR5 variants on HIV-1 infection and disease progression. Hemophilia Growth and Development Study (HGDS), Multicenter AIDS Cohort Study (MACS), Multicenter Hemophilia Cohort Study (MHCS), San Francisco City Cohort (SFCC), ALIVE Study," *Science*, vol. 277, no. 5328, pp. 959–965, 1997.

[18] G. Hütter and S. Ganepola, "The ccr5-delta32 polymorphism as a model to study host adaptation against infectious diseases and to develop new treatment strategies," *Experimental Biology and Medicine*, vol. 236, no. 8, pp. 938–943, 2011.

[19] J. A. McKinnell and M. S. Saag, "Novel drug classes: entry inhibitors [enfuvirtide, chemokine (C-C motif) receptor 5 antagonists]," *Current Opinion in HIV and AIDS*, vol. 4, no. 6, pp. 513–517, 2009.

[20] I. P. Singh and S. K. Chauthe, "Small molecule HIV entry inhibitors. Part I: chemokine receptor antagonists: 2004–2010," *Expert Opinion on Therapeutic Patents*, vol. 21, no. 2, pp. 227–269, 2011.

[21] B. L. Gilliam, D. J. Riedel, and R. R. Redfield, "Clinical use of CCR5 inhibitors in HIV and beyond," *Journal of Translational Medicine*, vol. 9, supplement 1, article S9, 2011.

[22] M. Carrington and S. J. O'Brien, "The Influence of HLA Genotype on AIDS," *Annual Review of Medicine*, vol. 54, pp. 535–551, 2003.

[23] D. den Uyl, I. E. van der Horst-Bruinsma, and M. van Agtmael, "Progression of HIV to AIDS: a protective role for HLA-B27?" *AIDS Reviews*, vol. 6, no. 2, pp. 89–96, 2004.

[24] S. J. O'Brien and G. W. Nelson, "Human genes that limit AIDS," *Nature Genetics*, vol. 36, no. 6, pp. 565–574, 2004.

[25] S. J. Chapman and A. V. Hill, "Human genetic susceptibility to infectious disease," *Nature Reviews Genetics*, vol. 13, no. 3, pp. 175–188, 2012.

[26] S. Mallal, D. Nolan, C. Witt et al., "Association between presence of HLA-B*5701, HLA-DR7, and HLA-DQ3 and hypersensitivity to HIV-1 reverse-transcriptase inhibitor abacavir," *Lancet*, vol. 359, no. 9308, pp. 727–732, 2002.

[27] R. L. Bailey, A. Natividad-Sancho, A. Fowler et al., "Host genetic contribution to the cellular immune response to Chlamydia trachomatis: heritability estimate from a Gambian twin study," *Drugs of Today*, vol. 45, pp. 45–50, 2009.

[28] WHO, *Sexually Transmitted Diseases. Disease Burden*, 2012.

[29] M. N. Starnbach and N. R. Roan, "Conquering sexually transmitted diseases," *Nature Reviews Immunology*, vol. 8, no. 4, pp. 313–317, 2008.

[30] J. A. Land, J. E. A. M. Van Bergen, S. A. Morré, and M. J. Postma, "Epidemiology of Chlamydia trachomatis infection in women and the cost-effectiveness of screening," *Human Reproduction Update*, vol. 16, no. 2, Article ID dmp035, pp. 189–204, 2009.

[31] J. E. den Hartog, S. Ouburg, J. A. Land et al., "Do host genetic traits in the bacterial sensing system play a role in the development of Chlamydia trachomatis-associated tubal pathology in subfertile women?" *BMC Infectious Diseases*, vol. 6, article 122, 2006.

[32] J. E. den Hartog, S. A. Morré, and J. A. Land, "Chlamydia trachomatis-associated tubal factor subfertility: immunogenetic aspects and serological screening," *Human Reproduction Update*, vol. 12, no. 6, pp. 719–730, 2006.

[33] T. Darville, J. M. O'Neill, C. W. Andrews, U. M. Nagarajan, L. Stahl, and D. M. Ojcius, "Toll-like receptor-2, but not toll-like receptor-4, is essential for development of oviduct pathology in chlamydial genital tract infection," *Journal of Immunology*, vol. 171, no. 11, pp. 6187–6197, 2003.

[34] J. E. Den Hartog, J. M. Lyons, S. Ouburg et al., "TLR4 in chlamydia trachomatis infections: knockout mice, STD patients and women with tubal factor subfertility," *Drugs of Today B*, vol. 45, pp. 75–82, 2009.

[35] T. Kawai and S. Akira, "TLR signaling," *Cell Death and Differentiation*, vol. 13, no. 5, pp. 816–825, 2006.

[36] T. Laisk, M. Peters, M. Saare, K. Haller-Kikkatalo, H. Karro, and A. Salumets, "Association of CCR5, TLR2, TLR4 and MBL genetic variations with genital tract infections and tubal factor infertility," *Journal of Reproductive Immunology*, vol. 87, no. 1-2, pp. 74–81, 2010.

[37] O. Karimi, S. Ouburg, H. J. C. De Vries et al., "TLR2 haplotypes in the susceptibility to and severity of Chlamydia trachomatis infections in Dutch women," *Drugs of Today B*, vol. 45, pp. 67–74, 2009.

[38] S. Ouburg, J. M. Lyons, J. A. Land et al., "TLR9 KO mice, haplotypes and CPG indices in Chlamydia trachomatis infection," *Drugs of Today B*, vol. 45, pp. 83–93, 2009.

[39] C. R. Cohen, S. S. Sinei, E. A. Bukusi, J. J. Bwayo, K. K. Holmes, and R. C. Brunham, "Human leukocyte antigen class II DQ alleles associated with Chlamydia trachomatis tubal infertility," *Obstetrics & Gynecology*, vol. 95, no. 1, pp. 72–77, 2000.

[40] C. R. Cohen, J. Gichui, R. Rukaria, S. S. Sinei, L. K. Gaur, and R. C. Brunham, "Immunogenetic correlates for Chlamydia trachomatis-associated tubal infertility," *Obstetrics & Gynecology*, vol. 101, no. 3, pp. 438–444, 2003.

[41] A. H. Kinnunen, H. M. Surcel, M. Lehtinen et al., "HLA DQ alleles and interleukin-10 polymorphism associated with Chlamydia trachomatis-related tubal factor infertility: a case-control study," *Human Reproduction*, vol. 17, no. 8, pp. 2073–2078, 2002.

[42] S. A. Morré, O. Karimi, and S. Ouburg, "Chlamydia trachomatis: identification of susceptibility markers for ocular and sexually transmitted infection by immunogenetics," *FEMS Immunology and Medical Microbiology*, vol. 55, no. 2, pp. 140–153, 2009.

[43] J. A. Lal, J. Malogajski, S. P. Verweij et al., "Chlamydia trachomatis infections and subfertility: opportunities to translate host pathogen genomic data into public health," *Public Health Genomics*, vol. 16, no. 1-2, pp. 50–61, 2013.

[44] J. L. H. Evers, "Female subfertility," *Lancet*, vol. 360, no. 9327, pp. 151–159, 2002.

[45] K. A. Broeze, B. C. Opmeer, F. van der Veen, P. M. Bossuyt, S. Bhattacharya, and B. W. J. Mol, "Individual patient data meta-analysis: a promising approach for evidence synthesis in reproductive medicine," *Human Reproduction Update*, vol. 16, no. 6, pp. 561–567, 2010.

[46] K. A. Broeze, B. C. Opmeer, S. F. P. J. Coppus et al., "Chlamydia antibody testing and diagnosing tubal pathology in subfertile women: an individual patient data meta-analysis," *Human Reproduction Update*, vol. 17, no. 3, pp. 301–310, 2011.

[47] G. S. Wills, P. J. Horner, R. Reynolds et al., "Pgp3 antibody enzyme-linked immunosorbent assay, a sensitive and specific assay for seroepidemiological analysis of Chlamydia trachomatis infection," *Clinical and Vaccine Immunology*, vol. 16, no. 6, pp. 835–843, 2009.

[48] E. Roman, J. Simpson, P. Ansell et al., "Childhood acute lymphoblastic leukemia and infections in the first year of life: a report from the United Kingdom Childhood Cancer Study," *American Journal of Epidemiology*, vol. 165, no. 5, pp. 496–504, 2007.

[49] H. J. An, K. R. Kim, I. S. Kim et al., "Prevalence of human papillomavirus DNA in various histological subtypes of cervical adenocarcinoma: a population-based study," *Modern Pathology*, vol. 18, no. 4, pp. 528–534, 2005.

[50] M. Lehoux, C. M. D'Abramo, and J. Archambault, "Molecular mechanisms of human papillomavirus-induced carcinogenesis," *Public Health Genomics*, vol. 12, no. 5-6, pp. 268–280, 2009.

[51] J. G. Baseman and L. A. Koutsky, "The epidemiology of human papillomavirus infections," *Journal of Clinical Virology*, vol. 32, supplement 1, pp. S16–S24, 2005.

[52] C. C. Pao, P. L. Tsai, Y. L. Chang, T. T. Hsieh, and J. Y. Jin, "Non-sexual papillomavirus transmission routes," *Lancet*, vol. 339, no. 8807, pp. 1479–1480, 1992.

[53] A. N. Burchell, R. L. Winer, S. de Sanjose, and E. L. Franco, "Chapter 6: epidemiology and transmission dynamics of genital HPV infection," *Vaccine*, vol. 24, supplement 3, pp. 52–61, 2006.

[54] S. K. Tay, "Genital oncogenic human papillomavirus infection: a short review on the mode of transmission," *Annals, Academy of Medicine, Singapore*, vol. 24, no. 4, pp. 598–601, 1995.

[55] M. Schiffman and P. E. Castle, "Human papillomavirus: epidemiology and public health," *Archives of Pathology and Laboratory Medicine*, vol. 127, no. 8, pp. 930–934, 2003.

[56] K. Syrjänen, "Mechanisms and predictors of high-risk human papillomavirus (HPV) clearance in the uterine cervix," *European Journal of Gynaecological Oncology*, vol. 28, no. 5, pp. 337–351, 2007.

[57] D. M. Harper, "Prevention of human papillomavirus infections and associated diseases by vaccination: a new hope for global public health," *Public Health Genomics*, vol. 12, no. 5-6, pp. 319–330, 2009.

[58] A. Hildesheim and S. S. Wang, "Host and viral genetics and risk of cervical cancer: a review," *Virus Research*, vol. 89, no. 2, pp. 229–240, 2002.

[59] J. Cuzick, G. Terry, L. Ho et al., "Association between high-risk HPV types, HLA DRB1* and DQB1* alleles and cervical cancer in British women," *British Journal of Cancer*, vol. 82, no. 7, pp. 1348–1352, 2000.

[60] J. Cervantes, C. Lema, L. V. Hurtado et al., "HLA-DRB1*1602 allele is positively associated with HPV cervical infection in Bolivian Andean women," *Human Immunology*, vol. 64, no. 9, pp. 890–895, 2003.

[61] X. Chen, J. Jiang, H. Shen, and Z. Hu, "Genetic susceptibility of cervical cancer," *Journal of Biomedical Research*, vol. 25, no. 3, pp. 155–164, 2011.

[62] L. Arnheim, J. Dillner, and C. B. Sanjeevi, "A population-based cohort study of KIR genes and genotypes in relation to cervical intraepithelial neoplasia," *Tissue Antigens*, vol. 65, no. 3, pp. 252–259, 2005.

[63] N. Yang, J. J. H. Eijsink, A. Lendvai et al., "Methylation markers for CCNA1 and C13ORF18 are strongly associated with high-grade cervical intraepithelial neoplasia and cervical cancer in cervical scrapings," *Cancer Epidemiology Biomarkers and Prevention*, vol. 18, no. 11, pp. 3000–3007, 2009.

[64] M. Esteller and J. G. Herman, "Cancer as an epigenetic disease: DNA methylation and chromatin alterations in human tumours," *Journal of Pathology*, vol. 196, no. 1, pp. 1–7, 2002.

[65] F. E. Henken, S. M. Wilting, R. M. Overmeer et al., "Sequential gene promoter methylation during HPV-induced cervical carcinogenesis," *British Journal of Cancer*, vol. 97, no. 10, pp. 1457–1464, 2007.

[66] K. Nanda, D. C. McCrory, E. R. Myers et al., "Accuracy of the papanicolaou test in screening for and follow-up of cervical cytologic abnormalities: a systematic review," *Annals of Internal Medicine*, vol. 132, no. 10, pp. 810–819, 2000.

[67] NIH, "Cervical Cancer Screening," 2012.

[68] G. Ronco, P. Giorgi-Rossi, F. Carozzi et al., "Efficacy of human papillomavirus testing for the detection of invasive cervical cancers and cervical intraepithelial neoplasia: a randomised controlled trial," *The Lancet Oncology*, vol. 11, no. 3, pp. 249–257, 2010.

[69] R. M. Overmeer, J. A. Louwers, C. J. Meijer et al., "Combined CADM1 and MAL promoter methylation analysis to detect (pre-)malignant cervical lesions in high-risk HPV-positive women," *International Journal of Cancer*, vol. 129, no. 9, pp. 2218–2225, 2011.

[70] S. M. Wilting, R. A. A. van Boerdonk, F. E. Henken et al., "Methylation-mediated silencing and tumour suppressive function of hsa-miR-124 in cervical cancer," *Molecular Cancer*, vol. 9, article 167, 2010.

[71] D. Solomon, "Chapter 14: role of triage testing in cervical cancer screening," *Journal of the National Cancer Institute Monographs*, no. 31, pp. 97–101, 2003.

[72] S. M. Teutsch, L. A. Bradley, G. E. Palomaki et al., "The evaluation of genomic applications in practice and prevention (EGAPP) initiative: methods of the EGAPP working group," *Genetics in Medicine*, vol. 11, no. 1, pp. 3–14, 2009.

[73] W. Burke, D. Atkins, M. Gwinn et al., "Genetic test evaluation: information needs of clinicians, policy makers, and the public," *American Journal of Epidemiology*, vol. 156, no. 4, pp. 311–318, 2002.

[74] J. Little, L. Bradley, M. S. Bray et al., "Reporting, appraising, and integrating data on genotype prevalence and gene-disease associations," *American Journal of Epidemiology*, vol. 156, no. 4, pp. 300–310, 2002.

Hindawi Publishing Corporation
BioMed Research International
Volume 2013, Article ID 297501, 11 pages
http://dx.doi.org/10.1155/2013/297501

Review Article

Genetic and Functional Profiling of Crohn's Disease: Autophagy Mechanism and Susceptibility to Infectious Diseases

Annalisa Marcuzzi,[1] Anna Monica Bianco,[1] Martina Girardelli,[1] Alberto Tommasini,[1] Stefano Martelossi,[1] Lorenzo Monasta,[1] and Sergio Crovella[1,2]

[1] *Institute for Maternal and Child Health—IRCCS "Burlo Garofolo" of Trieste, Via dell'Istria 65/1, 34137 Trieste, Italy*
[2] *University of Trieste, 34127 Trieste, Italy*

Correspondence should be addressed to Annalisa Marcuzzi; marcuzzi@burlo.trieste.it

Received 27 February 2013; Accepted 20 March 2013

Academic Editor: Enrique Medina-Acosta

Crohn's disease is a complex disease in which genome, microbiome, and environment interact to produce the immunological background of the disease. Disease in childhood is more extensive and characterized by a rapid progression, leading to severe repercussions in the course of the disorder. Several genetic variations have been associated with an increased risk of developing the disease and most of these are also implicated in other autoimmune disorders. The gut has many tiers of defense against incursion by luminal microbes, including the epithelial barrier and the innate and adaptive immune responses. Moreover, recent evidence shows that bacterial and viral infections, as well as inflammasome genes and genes involved in the autophagy process, are implicated in Crohn's disease pathogenesis. The aim of this review is to establish how much the diagnostic system can improve, thus increasing the success of Crohn's disease diagnosis. The major expectation for the near future is to be able to anticipate the possible consequences of the disease already in childhood, thus preventing associated complications, and to choose the best treatment for each patient.

1. Introduction

Crohn's disease (CD) is a chronic form of inflammatory bowel disease (IBD) that can affect any part of the gastrointestinal tract, from the mouth to the anus. However, it most commonly affects the colon and terminal ileum [1] with up to 75% of patients having ileal disease with or without colonic involvement [2]. It is a debilitating disorder with an overall prevalence of 0.5%–1% of the general population [3, 4]. CD differs from other types of IBDs because in patients with CD the inflammation is often continuous and with involvement of the mucosa [5].

Complications are common but not a constant: disease progression is marked by severe colitis, strictures and perianal fistulas, typically requiring surgery [6, 7].

Beaugerie et al. [8] recently reported three factors that at the time of the diagnosis increase the chance of developing a disabling disease in the following five years: (A) age <40 years, (B) presence of perianal lesions, and (C) the requirement of steroids to control the first flare [8]. However, as the median age at diagnosis is 27 years, patients may live with CD for more than 50 years in the Western world, where life expectancy of patients exceeds 70 years.

The age of onset is frequently in the second decade of life, and most patients progress to a relapsing disease characterized by abdominal pain, bloody diarrhea, vomit, and weight loss.

Although CD normally manifests in adulthood, it can be present in childhood before the age of 2 years [9]. The early onset Crohn's disease (EOCD) is typically more extensive (beyond the colon and/or oral or perianal disease) and characterized by rapid progression, leading to severe repercussions in disease development [10]. Diagnosis is particularly challenging in children in which presenting symptoms may vary widely and may only consist of subtle extraintestinal manifestations [11]. This often leads to a typical delay in the diagnosis of pediatric IBD, ranging from 4 weeks in severe colitis to 6-7 months in milder disease. Reducing

this diagnostic delay is important, since a long period of unmanaged symptoms can significantly impact on growth and early treatment is essential to preserve long-term quality of life [12, 13]. Thus, a sensitive yet noninvasive tool for the identification of patients at high risk of IBD, and therefore warranting endoscopic evaluation, would be a valuable diagnostic aid. There are specific clinical, therapeutic, and psychosocial issues specific to children with IBD that must be considered to ensure prompt diagnosis and appropriate medical management.

The etiopathogenesis of CD is unclear. It remains to be determined whether this disease represents an abnormal response to normal antigenic stimuli or an appropriate response to persistently abnormal stimuli [14, 15]. A better understanding of the origin of the disease and the mechanisms of action is necessary to improve the prognosis of CD.

CD is a complex disorder resulting from the interaction of genetic environmental and microbial factors. Given the difficult genotype-phenotype correlation and given the heterogeneous genetics, the different interactions among predisposing factors, and not only the number of genes involved, should be considered in order to understand the mechanisms of this disease. Many previous and ongoing studies have sought to identify genes and especially disease-causing variants such as risk factors for the disease. Identification and characterization of disease-causing variants represents one of the biggest challenges of genetics within the etiopathogenetic study of CD. Genomewide association studies already identified several distinct genetic polymorphisms associated with Crohn's disease. In European individuals, NOD2 gene polymorphisms confer by far the greatest risk for the disease. Other variations in ATG16L1, IRGM, and IL23R genes were reported to be highly associated with CD.

The genetic background has certainly a predisposing role, but several alternative explanations are possible, mostly related to lifestyle. The importance of environmental factors is shown by an increase in the incidence rate of diseases in ethnic groups previously less affected, as Hispanic, Asians, and immigrants that had moved from regions of low incidence into areas where the incidence of the disease is higher [16].

Observation of Crohn's patients and animal models suggests a role of bacteria in the disorder [17]. Among the most important bacteria that can adhere and invade the mucosa is *Escherichia coli* [18]. The onset of the disease is quite common after gastrointestinal infections and people suffering from this disorder have, generally, higher concentrations of mucosal bacteria if compared to healthy subjects [19].

A detailed picture of how genes work together and interact with environmental and microbial factors may better explain individual differences in CD susceptibility.

2. Etiology of CD

The complex pathophysiology of CD has, for a long time, been an enigma [20].

Although the precise etiology of CD remains elusive, epidemiological data conclusively indicate a deregulation of the immune response against the luminal flora in a genetically susceptible host [21] (Figure 1).

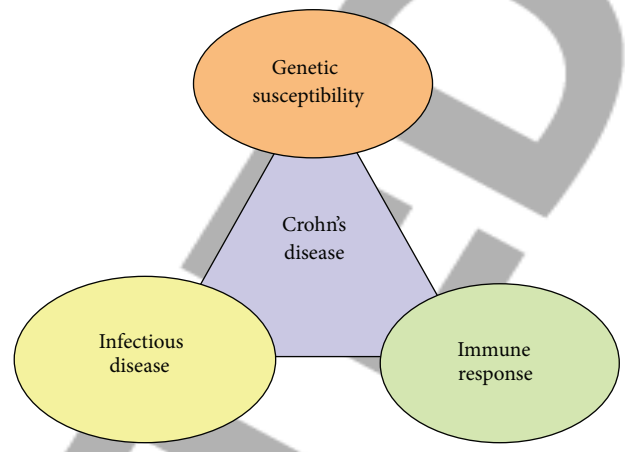


Figure 1: Crohn's disease is a heterogeneous disorder of multifactorial etiology in which genetic, environmental, and microbial factors, together with the immunological response, interact to produce the disease.

It is commonly assumed that CD is a heterogeneous disorder of multifactorial etiology in which genome, microbiome (hereditability), and environment interact to produce the immunological background of the disease. It is probable that patients have a genetic predisposition for the development of the disease coupled with immunoregulation disturbances [22].

2.1. Genetic Susceptibility: Greater Weighing Factor in Early Onset Crohn Disease. The epidemiologic evidence of the role of genetic factors in the pathogenesis of the disease came from studies demonstrating higher rates of CD among individuals of Caucasian and Jewish ethnicity, familial aggregation of CD, and higher concordance rates of both twins developing CD in monozygotic compared to dizygotic twins. Due to the complexity of the disease, the search for specific CD susceptibility genes has been very difficult so far. Despite the large number of genomewide associations (GWAS) established to date, most complex diseases (not monogenic) have only managed to explain some additional percentage of the hereditability estimates. The source of this *missing hereditability* is the subject of much debate with various explanations: overestimates of original heritability statistics, underpowered GWAS studies to detect common variants, poorly investigated epistasis and gene-environment interactions, and rare genetic variants [23]. In the attempt to explain some of this *missing hereditability*, researchers have adopted several complementary strategies. Combined genotypes, "private genes," and epigenetic markers may account for this *missing hereditability*: monogenic immunity disorders are increasingly diagnosed in patients with EOCD [24]. Advances in bioinformatics have now made it possible to perform GWAS using copy number variation probes. By several GWAS and meta-analysis studies many genes have been associated with CD: more than 90 distinct genomic loci have been found to be associated with an increased risk of

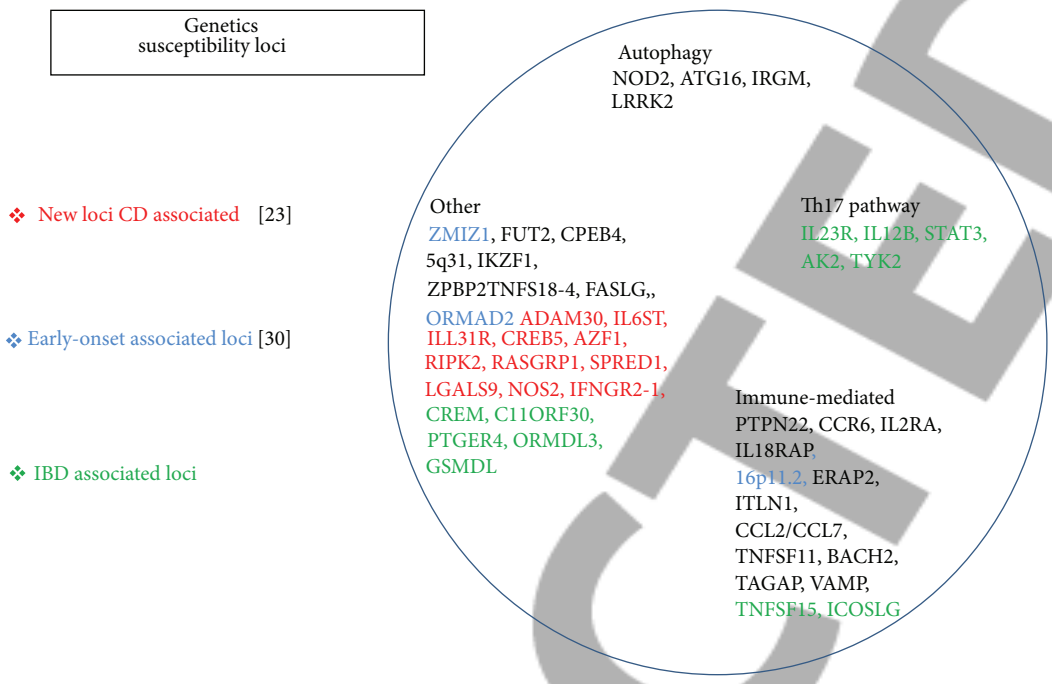


FIGURE 2: More than 90 distinct genomic susceptibility loci have been found to be associated with an increased risk of developing CD. The genes variants relate largely to the innate immunity genes, in particular to the disruption of the innate and adaptative arms of the immune systems, to the process of autophagy, to the epithelial barrier function, and to the activation of the endoplasmic reticulum stress response.

developing CD. Genes with replicated evidence for strong association suggest that these variants relate largely to the innate immunity genes, in particular to the disruption of the innate and adaptive arms of the immune system, to the process of autophagy, to the epithelial barrier function, and to the activation of the endoplasmic reticulum stress response [25–27] (Figure 2).

Most loci are in relation with the CD phenotype and many loci are implicated in other immune-mediated disorders, most notably with ankylosing spondylitis, erythema nodosum, and psoriasis [28]. Several genes are involved in primary immunodeficiencies, characterized by a dysfunctional immune system resulting in severe infections [26, 29, 30]. In the last years defective processing of intracellular bacteria has become a central theme. A considerable overlap has also been observed between susceptibility loci for CD and susceptibility for infectious diseases [27].

Genetic susceptibility is thought to play a more important role in the etiology of early rather than late onset CD [31]. This is supported by a higher rate of positive family history of CD in patients with a younger age at diagnosis with respect to patients with older age at diagnosis, suggesting that an earlier presentation may be due to a higher burden of disease-causing mutations in the genomes of these affected children compared to those in whom disease manifests later in life [32]. EOCD presents a more aggressive phenotype; in fact earlier age at diagnosis is associated with a greater need for surgery [33]. EOCD candidate susceptibility genes have been identified using linkage analysis and gene sequencing in two unrelated consanguineous families [34]. Some gene/loci may be specific to pediatric-onset CD and that is documented by recent GWAS focused on a pediatric cohort highlighting the implication of novel pathways and interaction between the two different onsets [35, 36].

Moreover, environmental factors such as smoking are less likely to be exerting an influence on the disease in pediatric cohorts [37].

2.2. Gastrointestinal Microbiota: Host Genome-Microbe Interactions in Crohn's Pathogenesis. Considering epidemiological, genetic and immunological data, it is probable that patients have a genetic predisposition for the development of the disease coupled with disturbances in both immunoregulation, and intestinal microbiota. The disease can be triggered by any of a number of different factors and sustained by an abnormal immune response to these factors. Rather, the intensive interaction between intestinal epithelial cells and immune competent cells is critical to maintain and perpetuate the chronic inflammatory process characteristic of CD [22]. The genetic and pathological complexity of CD is particularly well suited for testing whether interactively redefining disease diagnoses can enhance the value of genetic and pathogenetic studies. Precision in the characterization of the disease would make defining the impact of host-gene-microbial interactions on the disease process more robust.

The human body is inhabited by a vast number of bacteria, archaea, viruses, and unicellular eukaryotes. The microbiota represents a collection of microorganisms that live in peaceful coexistence with their hosts [38]. By far, the most heavily colonized organ is the gastrointestinal tract (GIT) and the colon alone is estimated to contain over 70% of all the microbes [39] and represents a major surface

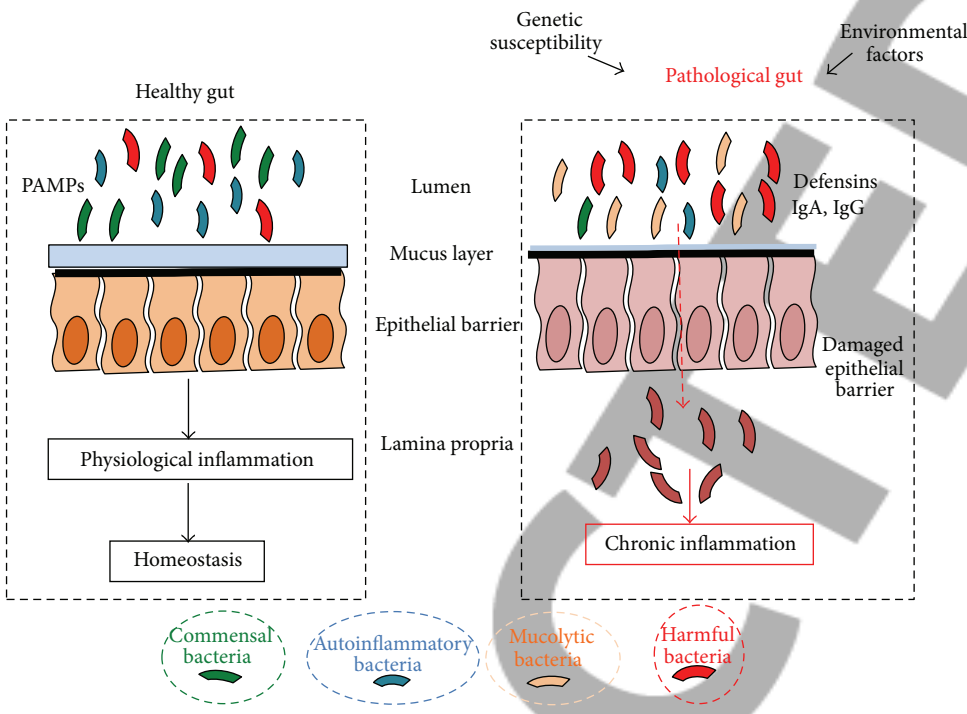


FIGURE 3: The role of microbiota in Crohn's disease pathogenesis. The interplay between the host microbiota and the environmental factors in a genetic susceptible host results in a progressive inflammatory damage to the host intestinal mucosa.

for microbial colonization. It is estimated that the human microbiota, not homogeneous in the GIT, contains as many as 1014 bacterial cells [40, 41] and the number of bacterial species present in the human gut is estimated to be 500 to 1000 [42]. Nevertheless, a recent analysis involving multiple subjects has suggested that the collective human gut microbiota is composed of over 35000 bacterial species [43].

Colonization of the human gut with microbes begins immediately at birth; in fact infants are exposed to a complex microbial population upon the passage through the birth canal [44] and it is known that infants delivered through cesarean section have different microbial compositions compared to vaginally delivered infants [45]. It has been shown that the microbiota of adult monozygotic and dizygotic twins was equally similar to that of their siblings, suggesting that the colonization by the microbiota from a shared mother was more decisive in determining their adult microbiota than their genetic makeup [46] (Figure 3).

Several studies have shown that host genetics can impact the microbial composition of the gut [47, 48]. The central role of gut microbiota in the development of mucosal immunity is not surprising considering that the intestinal mucosa represents the largest surface area in contact with the antigens of the external environment called PAMPs (pathogen-associated molecular patterns). Additionally, the dense carpet of the gut microbiota overlying the mucosa normally accounts for the largest proportion of the antigens presented to the resident immune cells and those stimulating the pattern recognition receptors such as the NOD-like receptors (NLRs) of the intestinal epithelial cells [49]. The GI (gastrointestinal gut) microbiome of healthy humans is dominated by four major bacterial phyla: *Firmicutes*, *Bacteroidetes*, and to a lesser degree *Proteobacteria* and *Actinobacteria* [50]. Many studies have observed imbalances or dysbioses in the GI microbiomes of CD patients [51, 52]. In CD patients biodiversity is decreased, with a lower proportion of *Firmicutes* and an increase in Gammaproteobacteria [53]. In CD, proportions of the Clostridia are altered: the *Roseburia* and *Faecalibacterium* genera of the *Lachnospiraceae* and *Ruminococcaceae* families are decreased, whereas *Ruminococcus gnavus* increases [54]. An important concept in the pathogenesis of CD is that bacterial and viral interactions occur in a host gene-specific manner [55, 56]. Surgical diversion of the fecal stream ameliorates inflammation. In addition, the evaluation of the microbial populations in surgically resected tissue samples of small bowel and colon from CD patients and non-CD controls, by rRNA sequence analysis, showed that specific flora was not enriched in small bowel or colon from CD patients. However, a subset of CD samples showed alterations in the representations of the *Bacteroides* and *Firmicutes* [43, 57]. Moreover, several studies have shown that the gut microbiota is altered in IBD patients. For example, biopsy samples from CD patients were used to prepare bacterial DNA which was amplified using universal bacterial 16S rRNA primers [58], and a significant increase in *Proteobacteria* and *Bacteroidetes* was found in CD patients compared to controls, with a decrease in *Clostridia*. Metagenomic approaches were used to analyze fecal samples from Crohn's patients and healthy donors and revealed reduced complexity of the *Firmicutes* in affected individuals [59]. Finally, the evidence that intestinal bacteria play an important role in CD patients is that antibiotics help some patients and can ameliorate

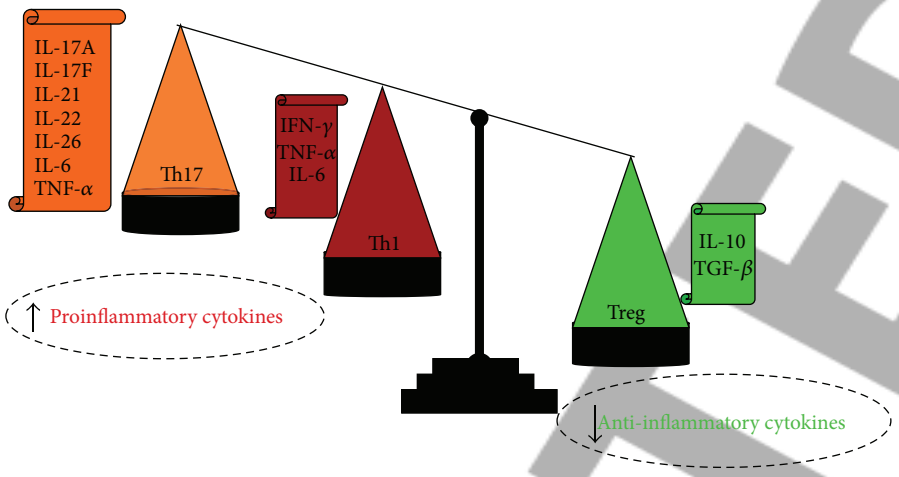


Figure 4: Up- and Downregulation of the proinflammatory cytokines evidenced in the immune system dysregulation of CD patients.

disease activity. Moreover metronidazole is an important therapeutic agent for certain complications of CD such as fistulising disease. Viral infection is required to generate the Paneth cell defect found in ATG16L1 mice [60], suggesting that in addition to human bacterial microbiota, viral or fungal commensals may play a role in CD pathogenesis.

2.3. Immunological Response in CD

2.3.1. Th1 and Th17 Implicated in CD Pathogenesis. Although the exact CD etiology is still not completely understood, several studies indicate that its pathogenesis is characterized by an exaggerated immune response in genetically susceptible individuals.

CD patients suffer from marked immune system deregulation. The inflammation seen in these patients is characterized by pronounced Th1 and Th17 responses [61] involving upregulation of proinflammatory cytokines IL-1, IL-6, IL-12, TNF-α, IFN-γ, IL-23, and IL-17 and downregulation of IL- 10, but it is not clear whether this is a cause or a consequence of the disease [62] (Figure 4).

Th1 cells are commonly assumed to be associated with CD development and produce IFN- γ, and their primary role is the protection against intracellular microbes. IFN-γ secreting lamina propria lymphocytes are abundant in the mucosa of CD patients: this condition is marked at CD onset (mucosal T cells appear to mount a typical Th1 response that resembles an acute infectious process) and disappears in late CD.

Recently, several studies showed the pivotal role of the imbalance of regulatory T cells (Treg) and Th17 in CD. Treg cells are important for the control of the immune response to self-antigens preventing autoimmunity and maintaining self-tolerance [63]. In contrast, IL-17 producing Th17 cells were recognized as a novel group of T cells which play a major role in autoimmunity. The gastrointestinal immune system has to maintain both a state of tolerance toward intestinal antigens and the ability to combat pathogens. In CD this balance is lost and the effects of proinflammatory T cells outnumber the tolerizing, anti-inflammatory effects of Treg cells. The discovery that Th17 cells, which express the IL-23 receptor (IL-23R), play a role in CD pathogenesis was supported by recent GWAS studies demonstrating that IL-23R and other genes involved in the differentiation of Th17 cells are susceptibility genes.

To confirm the link between immune response and genetic susceptibility in the pathogenesis of CD there are several recent lines of evidence that the key role is played by autophagy that includes the antigen presentation and the production of proinflammatory cytokines. The relationship between autophagy and microbes, indeed, has remained ill-defined until a recent convergence of studies showing that autophagy is an innate immune defense against bacteria, protozoa, and viral pathogens [64]. It is commonly assumed that the role of autophagy in addition to eliminating intracellular pathogens [65] contributes to MHC II restricted endogenous antigen presentation. It is an effector of Th1/Th2 polarization, affects B and T cell homeostasis and repertoire selection, delivers cytosolic PAMP or danger associated molecular patterns to endosomal toll-like receptors (TLR), and acts as an innate immunity effector downstream of TLR [66]. Polymorphisms in autophagy genes result in deregulation of these processes and affect gut homeostasis: genetic variants of autophagy genes have been linked to CD.

2.3.2. The Role of the NLRP3 Inflammasome in the Pathogenesis of CD. Inflammasomes are cytoplasmic multiprotein complexes that function as sensors of endogenous or exogenous PAMPs. They are composed of one of several nucleotide-binding oligomerization-domain protein-like receptors (NLRs), including NLRP1, NLRP3, NLRP6, and NLRPC4. Upon sensing the relevant signal, they assemble, typically together with an adaptor protein, an apoptosis-associated speck-like protein (ASC) or a caspase activating and recruitment domain 8 (CARD8), into a multiprotein complex that governs caspase-1 activation and subsequent cleavage of effector proinflammatory cytokines including pro-IL-1β and pro-IL-18.

Recently several studies highlighted with particular emphasis the relevance and the role of NLRP3 (previously known as CIAS1 and NLRP3) in the pathogenesis of CD [67, 68].

There is evidence suggesting that NLRP3 is able to respond to a variety of signals: adenosine triphosphate (ATP), nigericin, maitotoxin, *Staphylococcus aureus* and *Listeria monocytogenes* [69], and RNA and uric acid crystals (monosodium urate and calcium pyrophosphate dehydrate) released from dying cells [70, 71].

Literature data showed that the proinflammatory compound muramyl-dipeptide, the minimal bioactive peptidoglycan motif common to all bacteria, was an activator of the NLRP3 inflammasome, which suggested a very interesting connection between NOD2 and NALP3 [72].

On the other hand, Kanneganti et al. suggested that bacterial RNA and small antiviral compounds are the specific ligands of NLRP3 rather than MDP [70].

Anyway, NLRP3 plays a pivotal role in the inflammation regulating the activation of the caspase-1 and processing of IL-1β, two key mediators involved in the pathogenesis of the more common inflammatory disorders [73].

More recently, in an increasingly complicated picture, Elinav et al. described a novel regulatory sensing system in the colon, dependent on the NLRP6 inflammasome [74], and von Kampen et al. showed that CARD8 negatively regulates NOD-2 mediated signaling [75]. These current data further underline the link between the different components of the CD etiopathogenesis that are strongly correlated.

3. Autophagy in CD

Genomewide association studies and genetic analyses have emphasized the involvement of autophagy processes in the pathogenesis of inflammatory bowel diseases implicating three component genes in CD pathogenesis: ATG16L1 [76], IRGM [77], and NOD2 [78, 79]. These genes encode proteins critical for autophagy, a process that mediates degradation of intracellular proteins via vesicle-mediated delivery to the lysosome [80, 81]. Autophagy is involved in intracellular homeostasis, contributing to the degradation and recycling of cytosolic contents and organelles, as well as to resistance against infection and the removal of intracellular microbes. It is a major degradative pathway of the cell with several critical functions in innate and adaptive immunity [82] (Figure 5).

The ATG16L1 deficiency mouse showed Paneth cell dysfunction with aberrant exocytosis, as well as an altered transcriptional profile, characterized by increased expression of pro-inflammatory cytokines and lipid metabolism genes like Paneth cell phenotype of CD patients [83]. Nevertheless an important observation derived from the ATG16L1 mouse model was that the murine norovirus infection, as well as the presence of the commensal bacteria, was required for the generation of these specific Paneth cell abnormalities [84]. ATG16L1 is essential for all forms of autophagy, and the coding mutation T300A is associated with increased risk of CD. Despite its ubiquitous expression, the defects associated with ATG16L1 polymorphisms have so far been described only within the gut, probably owing to the high microbial load in this tissue.

Nucleotide-binding-oligomerization-domain- (NOD-) like receptors (NLRs) represent ancient sentinels of the host innate immune system, and genetic variants in NLR genes are associated with complex chronic inflammatory barrier diseases [85]. The NOD2 gene is an intracellular sensor for the bacterial cell wall component muramyl-dipeptide, and loss-of-function variants in the human NOD2 gene have been associated with an increased susceptibility for CD [86, 87] in Caucasian populations of European ancestry [88], and particularly for ileal disease [89], and were found to be an important regulator of the commensal gut microbiota in mice [90]. NOD2 recognizes components of the bacterial cell wall and elicits an NF-κB response and mediates the release of defensins, which are antimicrobial peptides. Evidence from MDP stimulation of NOD2-activated autophagy shows a link between genetic risk loci and highlights the importance of defining disease associated pathways and the potential of new roles for known genes [78]. Epithelial cells and dendritic cells containing Crohn's-disease-associated ATG16L1 and NOD2 variants show defects in antibacterial autophagy [79, 91]. In dendritic cells, these defects are associated with an impaired ability to present exogenous antigens to CD4+T cells [78]. A discussed model of Crohn's disease is the one in which individuals are genetically susceptible to a pathogen that triggers a compensatory and harmful immune response. Antibacterial autophagy, through ATG16L1, NOD2, and potentially other genes (IRGM), is consistent with this model. However, one of the most important experimental supports for this model comes from an unrelated study using *Citrobacter rodentium* to induce intestinal inflammation in NOD2 –/– mice [92]. These results illustrate a close relationship between NOD2, ATG16L1, and autophagy, affecting intracellular processing and communication with the adaptive immune system suggesting that genetic polymorphisms may affect both pathways concomitantly.

IRGM belongs to the p47 Immunity-Related GTPase (IRG) family and is linked to CD by GWAS as a protein that is implicated in the autophagy mechanism [93]. The analysis of the interactions between 44 autophagy-associated human proteins and 83 viral proteins belonging to different RNA virus families revealed that IRGM was the autophagy-associated protein most targeted by these viruses. IRGM can interact with 12 viral proteins belonging to different viruses, such as HCV and HIV-1 [94, 95]. A recent study suggests that a polymorphism in IRGM could affect the binding and the consequent misregulation by a specific miRNA (miR-196) that is highly expressed in the intestinal tissue of patients with Crohn's disease. The consequence is that the xenophagy flux is not well regulated leading to the accumulation of bacteria in the lysosomal compartment. This study showed that greater IRGM expression leads to both colocalization of adherent invasive *E. coli* (AIEC) with the autophagy machinery and increased intracellular survival of the bacteria [96]. This and other strains of *E. coli* are more abundant in the mucosa of CD patients [90]. The ability of IRGM to induce autophagy and limit the replication of intracellular bacteria has been demonstrated with mycobacteria by inducing mitochondrial

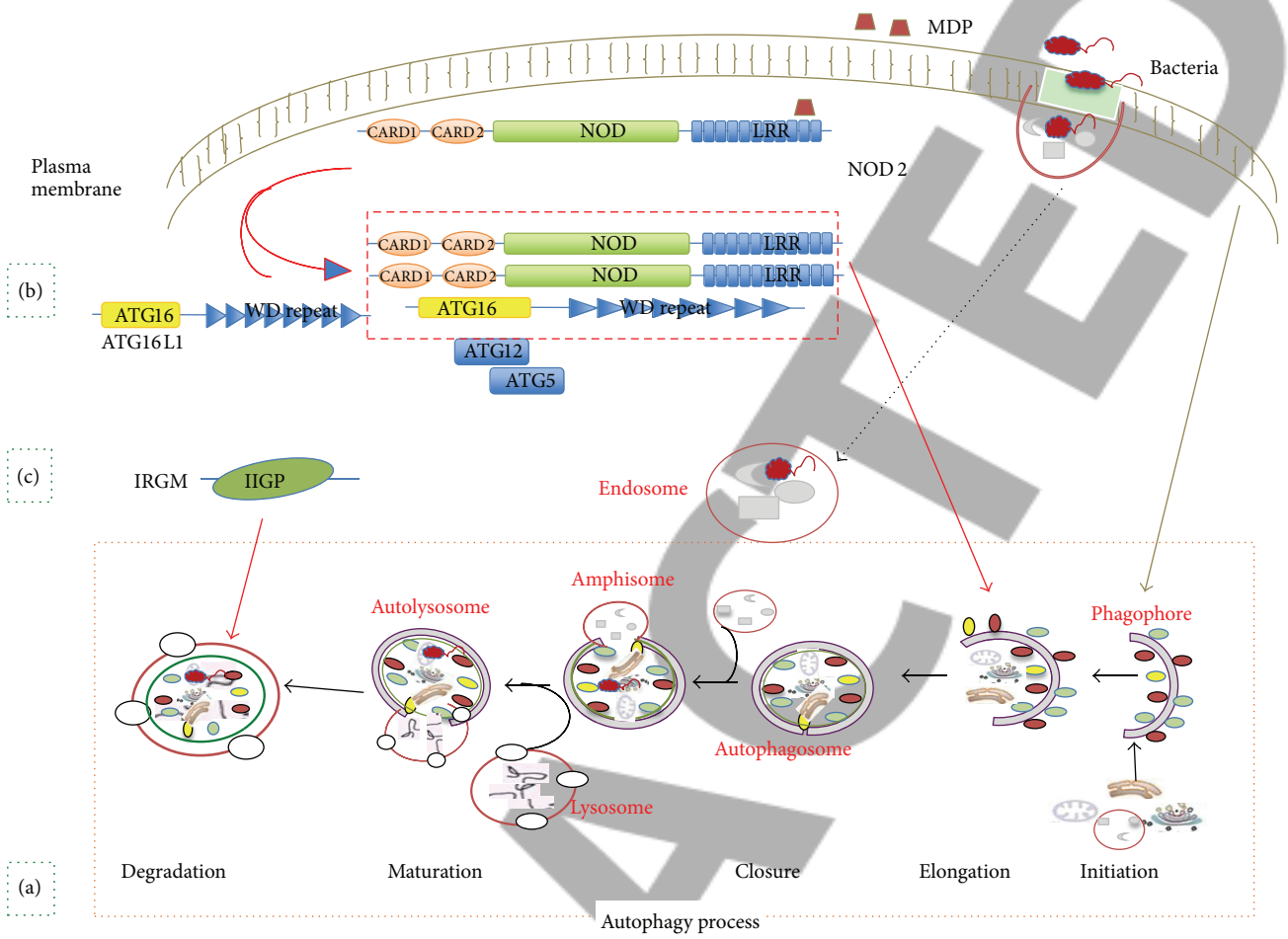


Figure 5: CD pathogenesis and autophagy: susceptible CD genes ATG16L1, NOD2, and IRGM are proteins critical for the autophagy process. (a) The process of mammalian autophagy is divided into the following principal steps: initiation, elongation, closure, maturation, and degradation. (b) At the bacterial entry site NOD2 activated by MDP recruit ATG16L1 to the plasma membrane. Follow the assembling of the ATG5-ATG12 complex, stabilized by ATG16L1, that facilitates the formation of an autophagosome around the invading bacterium. (c) IRGM, another autophagy-related gene, could be involved in the final steps of the degradation step.

depolarization and can increase ROS production and cell death [97]. Finally IRGM could regulate inflammation by either regulating intracellular pathogens or cellular homeostasis much like ATG16L1.

These data provide further information and support for the hypothesis that microbial/viral interactions with the intestinal mucosa are required for disease generation and suggest that combinatorial models for CD pathogenesis are most relevant for the study of human disease pathogenesis.

4. Concluding Remarks

The diagnosis of CD is reached through the results of clinical, laboratory, radiographic, endoscopic, and histologic analyses.

Radiological and endoscopic techniques are essential for the diagnosis of CD since its onset and are useful in assessing the inflammatory status of the intestinal mucosa. However, endoscopy is an invasive procedure. In children it can be traumatic and could have critical implications due to the more severe clinical manifestation and complication of the pediatric disease, that make the intestinal mucosal extremely thin and at risk of perforation.

Noninvasive tests for CD already exist, including antibodies, imaging-based screens, and fecal biomarkers [98]. The specificity of existing methods ranges from 89% to 95% for CD and other inflammatory bowel diseases. However, these methods are limited to active disease and poorly sensitive (~55%). Their outcome can be confounded by other diseases, further limiting their clinical utility. Recently, high expectations are placed in diagnostic studies of the gastrointestinal microbiota, but further validations will be necessary before this tool is accepted in clinical practice [32, 99].

Research is moving forward in order to identify new and valid biomarkers for the diagnosis of the disease with the aim of replacing the use of invasive techniques.

Currently, only the measurement of fecal calprotectin levels has achieved a place in clinical routine practice and is used as a marker for noninvasive determination of intestinal

inflammation [99, 100]. This protein is an ideal marker because it is not degraded by the human microbiota. Levels of fecal calprotectin significantly increase in patients with CD, ulcerative colitis, infectious colitis, and, to a lesser extent, in tumors of the colon rectum, but not in patients with functional disorders, as in the case of the irritable bowel syndrome. In CD, the calprotectin assay reflects the activity of the disease, monitoring its progression, and can contribute to the decision about the correct medication strategy.

The application of this test in the pediatric population is a good result. Although the test is not yet able to replace diagnostic colonoscopy, it can be a good indicator for the decision to use or delay the use of invasive investigations [99].

Recently, Vitali et al. suggested the use of high-mobility group box1 (HMGB1) as a novel marker of intestinal mucosal inflammation. HMGB1 is today regarded as a pleiotropic cytokine, that is passively released by necrotic cells, but not from apoptotic cells. Moreover HMGB1 could be actively secreted from some types of immune cells in response to lipopolysaccharide (LPS), IFN-γ, and TNF-α. There is evidence that HMGB1 is secreted in the stools of these patients and not detectable in controls [101].

The relationship between the genetic susceptibility and the microbiome could be considered in the disease diagnosis. There are several international human microbiome projects that have focused initially on the bacterial component of the microbiome. The evidence that bacteria play an important role in CD includes the observation that surgical diversion of the fecal stream ameliorates the inflammation and that antibiotics help some patients. Moreover other evidence is showed in mouse models of colitis where virus, bacteria, or both acting together can contribute to the pathology via signaling through innate immune sensors and regulation of pro- and anti-inflammatory cytokines [82]. The microbiome varies from person to person and such variation could provide environmental inputs that contribute to the incidence of CD, within the genetic foundation revealed by GWAS [102]. The concept of dysbiosis as a contributor to CD is correlated with intestinal bacteria communities, so the changes in the bacterial microbiota could have a potential role in the disease. However, this hypothesis needs to be expanded to include specific interactions between individual bacteria and host genes.

Moreover, another ambitious goal is the identification of a genetic pattern able to associate specific phenotypic characteristics to CD patients or to anticipate the possible consequences of the disease already in childhood and thus prevent complications associated with the disease and to choose the best treatment for each patient.

A rapid diagnosis is fundamental to avoid a growth delay or complications of the disease typical of the pediatric disease leading to surgery. In some cases, genetic studies have provided useful information for the identification of specific mutations that predict risk of stenosis and surgery and/or disease localization in pediatric-onset CD [103, 104].

The difficulty of finding a common genetic pattern of association is caused by the multifactorial feature of the disease that shows different characterizations by world region and race.

In conclusion, it would certainly be useful to be able to create a biological algorithm that helps clinicians in the identification and classification of the disease and to determine the pharmacological care. This algorithm could include not only the known and principal factors predisposing to the disease, but also the gene-microbiome interaction and could help identify novel markers in patients with familiar history of EOCD. This could represent a major advance for early-onset diagnosis as specific tests might be developed to improve counselling, while direct identification of modifier genes might assist in the recognition of new genetic, environmental, and microbial causes of CD.

Conflict of Interests

The authors declare they have no conflict of interests related to the issues discussed in this paper.

Authors' Contribution

Annalisa Marcuzzi and Anna Monica Bianco contributed equally to this study.

References

[1] D. C. Baumgart and W. J. Sandborn, "Crohn's disease," *The Lancet*, vol. 380, no. 9853, pp. 1590–1605, 2012.

[2] G. Radford-Smith and N. Pandeya, "Associations between *NOD2/CARD15* genotype and phenotype in Crohn's disease—are we there yet?" *World Journal of Gastroenterology*, vol. 12, no. 44, pp. 7097–7103, 2006.

[3] D. K. Bonen and J. H. Cho, "The genetics of inflammatory bowel disease," *Gastroenterology*, vol. 124, no. 2, pp. 521–536, 2003.

[4] S. K. Yang, S. Yun, J. H. Kim et al., "Epidemiology of inflammatory bowel disease in the Songpa-Kangdong district, Seoul, Korea, 1986–2005: a KASID study," *Inflammatory Bowel Diseases*, vol. 14, no. 4, pp. 542–549, 2008.

[5] R. J. Xavier and D. K. Podolsky, "Unravelling the pathogenesis of inflammatory bowel disease," *Nature*, vol. 448, no. 7152, pp. 427–434, 2007.

[6] J. Cosnes, S. Cattan, A. Blain et al., "Long-term evolution of disease behavior of Crohn's disease," *Inflammatory Bowel Diseases*, vol. 8, no. 4, pp. 244–250, 2002.

[7] E. Louis, A. Collard, A. F. Oger, E. Degroote, F. A. Aboul Nasr El Yafi, and J. Belaiche, "Behaviour of Crohn's disease according to the Vienna classification: changing pattern over the course of the disease," *Gut*, vol. 49, no. 6, pp. 777–782, 2001.

[8] L. Beaugerie, P. Seksik, I. Nion-Larmurier, J. P. Gendre, and J. Cosnes, "Predictors of Crohn's disease," *Gastroenterology*, vol. 130, no. 3, pp. 650–656, 2006.

[9] M. B. Heyman, B. S. Kirschner, B. D. Gold et al., "Children with early-onset inflammatory bowel disease (IBD): analysis of a pediatric IBD consortium registry," *Journal of Pediatrics*, vol. 146, no. 1, pp. 35–40, 2005.

[10] Z. Cannioto, I. Berti, S. Martelossi et al., "IBD and IBD mimicking enterocolitis in children younger than 2 years of age," *European Journal of Pediatrics*, vol. 168, no. 2, pp. 149–155, 2009.

[11] IBD Working Group of the European Society for Paediatric Gastroenterology, Hepatology and Nutrition, "Inflammatory

bowel disease in children and adolescents: recommendations for diagnosis—the Porto criteria," *Journal of Pediatric Gastroenterology and Nutrition*, vol. 41, no. 1, pp. 1–7, 2005.

[12] C. Spray, G. D. Debelle, and M. S. Murphy, "Current diagnosis, management and morbidity in paediatric inflammatory bowel disease," *Acta Paediatrica*, vol. 90, no. 4, pp. 400–405, 2001.

[13] J. D. Lewis, O. Abramson, M. Pascua et al., "Timing of myelosuppression during thiopurine therapy for inflammatory bowel disease: implications for monitoring recommendations," *Clinical Gastroenterology and Hepatology*, vol. 7, no. 11, pp. 1195–1201, 2009.

[14] K. Mitsuyama, N. Tomiyasu, K. Takaki et al., "Interleukin-10 in the pathophysiology of inflammatory bowel disease: increased serum concentrations during the recovery phase," *Mediators of Inflammation*, vol. 2006, Article ID 26875, 7 pages, 2006.

[15] S. J. Bickston, L. W. Comerford, and F. Cominelli, "Future therapies for inflammatory bowel disease," *Current Gastroenterology Reports*, vol. 5, no. 6, pp. 518–523, 2003.

[16] J. K. Hou, H. El-Serag, and S. Thirumurthi, "Distribution and manifestations of inflammatory bowel disease in Asians, Hispanics, and African Americans: a systematic review," *American Journal of Gastroenterology*, vol. 104, no. 8, pp. 2100–2109, 2009.

[17] C. Abraham and J. H. Cho, "Inflammatory bowel disease," *The New England Journal of Medicine*, vol. 361, no. 21, pp. 2066–2078, 2009.

[18] N. Barnich, F. A. Carvalho, A. L. Glasser et al., "CEACAM6 acts as a receptor for adherent-invasive *E. coli*, supporting ileal mucosa colonization in Crohn disease," *The Journal of Clinical Investigation*, vol. 117, no. 6, pp. 1566–1574, 2007.

[19] D. C. Baumgart and W. J. Sandborn, "Inflammatory bowel disease: clinical aspects and established and evolving therapies," *The Lancet*, vol. 369, no. 9573, pp. 1641–1657, 2007.

[20] D. K. Podolsky, "The current future understanding of inflammatory bowel disease," *Best Practice & Research Clinical Gastroenterology*, vol. 16, no. 6, pp. 933–943, 2002.

[21] A. Gutierrez, M. Scharl, L. Sempere et al., "Genetic susceptibility to increased bacterial translocation influences the response to biological therapy in patients with Crohn's disease," *Gut*, 2013.

[22] J. P. Achkar and R. Duerr, "The expanding universe of inflammatory bowel disease genetics," *Current Opinion in Gastroenterology*, vol. 24, no. 4, pp. 429–434, 2008.

[23] W. Bodmer and I. Tomlinson, "Rare genetic variants and the risk of cancer," *Current Opinion in Genetics & Development*, vol. 20, no. 3, pp. 262–267, 2010.

[24] D. A. van Heel, S. Ghosh, M. Butler et al., "Muramyl dipeptide and toll-like receptor sensitivity in *NOD2*-associated Crohn's disease," *The Lancet*, vol. 365, no. 9473, pp. 1794–1796, 2005.

[25] J. van Limbergen, D. C. Wilson, and J. Satsangi, "The genetics of Crohn's disease," *Annual Review of Genomics and Human Genetics*, vol. 10, pp. 89–116, 2009.

[26] C. W. Lees, J. C. Barrett, M. Parkes, and J. Satsangi, "New IBD genetics: common pathways with other diseases," *Gut*, vol. 60, no. 12, pp. 1739–1753, 2011.

[27] S. Haglund, S. Almer, C. Peterson, and J. Söderman, "Gene expression and thiopurine metabolite profiling in inflammatory bowel disease—novel clues to drug targets and disease mechanisms?" *PLoS ONE*, vol. 8, no. 2, Article ID e56989, 2013.

[28] L. A. Hindorff, P. Sethupathy, H. A. Junkins et al., "Potential etiologic and functional implications of genome-wide association loci for human diseases and traits," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 106, no. 23, pp. 9362–9367, 2009.

[29] L. D. Notarangelo, "Primary immunodeficiencies," *The Journal of Allergy and Clinical Immunology*, vol. 125, no. 2, supplement 2, pp. S182–S194, 2010.

[30] A. Tommasini, A. Pirrone, G. Palla et al., "The universe of immune deficiencies in Crohn's disease: a new viewpoint for an old disease?" *Scandinavian Journal of Gastroenterology*, vol. 45, no. 10, pp. 1141–1149, 2010.

[31] L. de Ridder, R. K. Weersma, G. Dijkstra et al., "Genetic susceptibility has a more important role in pediatric-onset Crohn's disease than in adult-onset Crohn's disease," *Inflammatory Bowel Diseases*, vol. 13, no. 9, pp. 1083–1092, 2007.

[32] V. Biank, U. Broeckel, and S. Kugathasan, "Pediatric inflammatory bowel disease: clinical and molecular genetics," *Inflammatory Bowel Diseases*, vol. 13, no. 11, pp. 1430–1438, 2007.

[33] M. Lacher, R. Kappler, S. Berkholz, H. Baurecht, D. von Schweinitz, and S. Koletzko, "Association of a CXCL9 polymorphism with pediatric Crohn's disease," *Biochemical and Biophysical Research Communications*, vol. 363, no. 3, pp. 701–707, 2007.

[34] E. O. Glocker, D. Kotlarz, K. Boztug et al., "Inflammatory bowel disease and mutations affecting the interleukin-10 receptor," *The New England Journal of Medicine*, vol. 361, no. 21, pp. 2033–2045, 2009.

[35] M. Imielinski, R. N. Baldassano, A. Griffiths et al., "Common variants at five new loci associated with early-onset inflammatory bowel disease," *Nature Genetics*, vol. 41, no. 12, pp. 1335–1340, 2009.

[36] D. K. Amre, D. R. Mack, K. Morgan et al., "Association between genome-wide association studies reported SNPs and pediatric-onset Crohn's disease in Canadian children," *Human Genetics*, vol. 128, no. 2, pp. 131–135, 2010.

[37] K. L. Helbig, M. Nothnagel, J. Hampe et al., "A case-only study of gene-environment interaction between genetic susceptibility variants in *NOD2* and cigarette smoking in Crohn's disease aetiology," *BMC Medical Genetics*, vol. 13, article 14, 2012.

[38] C. Kunz, S. Kuntz, and S. Rudloff, "Intestinal flora," *Advances in Experimental Medicine and Biology*, vol. 639, pp. 67–79, 2009.

[39] W. B. Whitman, D. C. Coleman, and W. J. Wiebe, "Prokaryotes: the unseen majority," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 95, no. 12, pp. 6578–6583, 1998.

[40] R. E. Ley, D. A. Peterson, and J. I. Gordon, "Ecological and evolutionary forces shaping microbial diversity in the human intestine," *Cell*, vol. 124, no. 4, pp. 837–848, 2006.

[41] H. Verstraelen, "Cutting edge: the vaginal microflora and bacterial vaginosis," *Verhandelingen—Koninklijke Academie voor Geneeskunde van België*, vol. 70, no. 3, pp. 147–174, 2008.

[42] J. I. Gordon, "Honor thy gut symbionts redux," *Science*, vol. 336, no. 6086, pp. 1251–1253, 2012.

[43] D. N. Frank, A. L. St Amand, R. A. Feldman, E. C. Boedeker, N. Harpaz, and N. R. Pace, "Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, no. 34, pp. 13780–13785, 2007.

[44] V. Redondo-Lopez, R. L. Cook, and J. D. Sobel, "Emerging role of lactobacilli in the control and maintenance of the vaginal bacterial microflora," *Reviews of Infectious Diseases*, vol. 12, no. 5, pp. 856–872, 1990.

[45] A. Huurre, M. Kalliomäki, S. Rautava, M. Rinne, S. Salminen, and E. Isolauri, "Mode of delivery—effects on gut microbiota

and humoral immunity," *Neonatology*, vol. 93, no. 4, pp. 236–240, 2008.

[46] E. G. Zoetendal, A. von Wright, T. Vilpponen-Salmela, K. Ben-Amor, A. D. L. Akkermans, and W. M. de Vos, "Mucosa-associated bacteria in the human gastrointestinal tract are uniformly distributed along the colon and differ from the community recovered from feces," *Applied and Environmental Microbiology*, vol. 68, no. 7, pp. 3401–3407, 2002.

[47] R. E. Ley, F. Bäckhed, P. Turnbaugh, C. A. Lozupone, R. D. Knight, and J. I. Gordon, "Obesity alters gut microbial ecology," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 31, pp. 11070–11075, 2005.

[48] C. Zhang, M. Zhang, S. Wang et al., "Interactions between gut microbiota, host genetics and diet relevant to development of metabolic syndromes in mice," *The ISME Journal*, vol. 4, no. 2, pp. 232–241, 2010.

[49] S. Rakoff-Nahoum and R. Medzhitov, "Innate immune recognition of the indigenous microbial flora," *Mucosal Immunology*, vol. 1, supplement 1, pp. S10–S14, 2008.

[50] P. B. Eckburg, E. M. Bik, C. N. Bernstein et al., "Diversity of the human intestinal microbial flora," *Science*, vol. 308, no. 5728, pp. 1635–1638, 2005.

[51] M. Baumgart, B. Dogan, M. Rishniw et al., "Culture independent analysis of ileal mucosa reveals a selective increase in invasive *Escherichia coli* of novel phylogeny relative to depletion of Clostridiales in Crohn's disease involving the ileum," *The ISME Journal*, vol. 1, no. 5, pp. 403–418, 2007.

[52] E. Li, C. M. Hamm, A. S. Gulati et al., "Inflammatory bowel diseases phenotype, *C. difficile* and *NOD2* genotype are associated with shifts in human ileum associated microbial composition," *PLoS ONE*, vol. 7, no. 6, Article ID e26284, 2012.

[53] H. Sokol and P. Seksik, "The intestinal microbiota in inflammatory bowel diseases: time to connect with the host," *Current Opinion in Gastroenterology*, vol. 26, no. 4, pp. 327–331, 2010.

[54] M. Joossens, G. Huys, M. Cnockaert et al., "Dysbiosis of the faecal microbiota in patients with Crohn's disease and their unaffected relatives," *Gut*, vol. 60, no. 5, pp. 631–637, 2011.

[55] H. W. Virgin, E. J. Wherry, and R. Ahmed, "Redefining chronic viral infection," *Cell*, vol. 138, no. 1, pp. 30–50, 2009.

[56] E. Elinav, J. Henao-Mejia, and R. A. Flavell, "Integrative inflammasome activity in the regulation of intestinal mucosal immune responses," *Mucosal Immunology*, vol. 6, no. 1, pp. 4–13, 2013.

[57] D. N. Frank, C. E. Robertson, C. M. Hamm et al., "Disease phenotype and genotype are associated with shifts in intestinal-associated microbiota in inflammatory bowel diseases," *Inflammatory Bowel Diseases*, vol. 17, no. 1, pp. 179–184, 2011.

[58] U. Gophna, K. Sommerfeld, S. Gophna, W. F. Doolittle, and S. J. O. Veldhuyzen van Zanten, "Differences between tissue-associated intestinal microfloras of patients with Crohn's disease and ulcerative colitis," *Journal of Clinical Microbiology*, vol. 44, no. 11, pp. 4136–4141, 2006.

[59] C. Manichanh, L. Rigottier-Gois, E. Bonnaud et al., "Reduced diversity of faecal microbiota in Crohn's disease revealed by a metagenomic approach," *Gut*, vol. 55, no. 2, pp. 205–211, 2006.

[60] K. Cadwell, "Crohn's disease susceptibility gene interactions, a NOD to the newcomer *ATG16L1*," *Gastroenterology*, vol. 139, no. 5, pp. 1448–1450, 2010.

[61] D. M. Glubb, R. B. Gearry, M. L. Barclay et al., "*NOD2* and *ATG16L1* polymorphisms affect monocyte responses in Crohn's disease," *World Journal of Gastroenterology*, vol. 17, no. 23, pp. 2829–2837, 2011.

[62] J. P. Y. Ting, D. L. Kastner, and H. M. Hoffman, "CATERPILLERs, pyrin and hereditary immunological disorders," *Nature Reviews Immunology*, vol. 6, no. 3, pp. 183–195, 2006.

[63] S. Brand, "Crohn's disease: Th1, Th17 or both? The change of a paradigm: new immunological and genetic insights implicate Th17 cells in the pathogenesis of Crohn's disease," *Gut*, vol. 58, no. 8, pp. 1152–1167, 2009.

[64] T. S. Plantinga, L. A. Joosten, J. W. van der Meer, and M. G. Netea, "Modulation of inflammation by autophagy: consequences for Crohn's disease," *Current Opinion in Pharmacology*, vol. 12, no. 4, pp. 497–502, 2012.

[65] B. Levine and V. Deretic, "Unveiling the roles of autophagy in innate and adaptive immunity," *Nature Reviews Immunology*, vol. 7, no. 10, pp. 767–777, 2007.

[66] V. Deretic, "Autophagy as an innate immunity paradigm: expanding the scope and repertoire of pattern recognition receptors," *Current Opinion in Immunology*, vol. 24, no. 1, pp. 21–31, 2012.

[67] A. C. Villani, M. Lemire, G. Fortin et al., "Common variants in the *NLRP3* region contribute to Crohn's disease susceptibility," *Nature Genetics*, vol. 41, no. 1, pp. 71–76, 2009.

[68] L. Ferrero-Miliani, O. H. Nielsen, P. S. Andersen, and S. E. Girardin, "Chronic inflammation: importance of *NOD2* and *NALP3* in interleukin-1β generation," *Clinical and Experimental Immunology*, vol. 147, no. 2, pp. 227–235, 2007.

[69] A. Gombault, L. Baron, and I. Couillin, "ATP release and purinergic signaling in *NLRP3* inflammasome activation," *Frontiers in Immunology*, vol. 3, article 414, 2012.

[70] T. D. Kanneganti, N. Özören, M. Body-Malapel et al., "Bacterial RNA and small antiviral compounds activate caspase-1 through cryopyrin/*NALP3*," *Nature*, vol. 440, no. 7081, pp. 233–236, 2006.

[71] S. R. Kingsbury, P. G. Conaghan, and M. F. McDermott, "The role of the *NLRP3* inflammasome in gout," *Journal of Inflammation Research*, vol. 4, no. 1, pp. 39–49, 2011.

[72] F. Martinon, L. Agostini, E. Meylan, and J. Tschopp, "Identification of bacterial muramyl dipeptide as activator of the *NALP3*/cryopyrin inflammasome," *Current Biology*, vol. 14, no. 21, pp. 1929–1934, 2004.

[73] S. Mariathasan and D. M. Monack, "Inflammasome adaptors and sensors: intracellular regulators of infection and inflammation," *Nature Reviews Immunology*, vol. 7, no. 1, pp. 31–40, 2007.

[74] E. Elinav, T. Strowig, A. L. Kau et al., "NLRP6 inflammasome regulates colonic microbial ecology and risk for colitis," *Cell*, vol. 145, no. 5, pp. 745–757, 2011.

[75] O. von Kampen, S. Lipinski, A. Till et al., "Caspase recruitment domain-containing protein 8 (CARD8) negatively regulates *NOD2*-mediated signaling," *The Journal of Biological Chemistry*, vol. 285, no. 26, pp. 19921–19926, 2010.

[76] J. Hampe, A. Franke, P. Rosenstiel et al., "A genome-wide association scan of nonsynonymous SNPs identifies a susceptibility variant for Crohn disease in *ATG16L1*," *Nature Genetics*, vol. 39, no. 2, pp. 207–211, 2007.

[77] S. A. McCarroll, A. Huett, P. Kuballa et al., "Deletion polymorphism upstream of IRGM associated with altered IRGM expression and Crohn's disease," *Nature Genetics*, vol. 40, no. 9, pp. 1107–1112, 2008.

[78] R. Cooney, J. Baker, O. Brain et al., "*NOD2* stimulation induces autophagy in dendritic cells influencing bacterial handling and antigen presentation," *Nature Medicine*, vol. 16, no. 1, pp. 90–97, 2010.

[79] L. H. Travassos, L. A. M. Carneiro, M. Ramjeet et al., "Nod1 and *NOD2* direct autophagy by recruiting *ATG16L1* to the plasma membrane at the site of bacterial entry," *Nature Immunology*, vol. 11, no. 1, pp. 55–62, 2010.

[80] D. Glick, S. Barth, and K. F. Macleod, "Autophagy: cellular and molecular mechanisms," *The Journal of Pathology*, vol. 221, no. 1, pp. 3–12, 2010.

[81] A. Huett and R. J. Xavier, "Autophagy at the gut interface: mucosal responses to stress and the consequences for inflammatory bowel diseases," *Inflammatory Bowel Diseases*, vol. 16, no. 1, pp. 152–174, 2010.

[82] J. M. Yuk, T. Yoshimori, and E. K. Jo, "Autophagy and bacterial infectious diseases," *Experimental & Molecular Medicine*, vol. 44, no. 2, pp. 99–108, 2012.

[83] C. R. Homer, A. L. Richmond, N. A. Rebert, J. Achkar, and C. McDonald, "*ATG16L1* and *NOD2* interact in an autophagy-dependent antibacterial pathway implicated in Crohn's disease pathogenesis," *Gastroenterology*, vol. 139, no. 5, pp. 1630.e2–1641.e2, 2010.

[84] B. C. Miller, Z. Zhao, L. M. Stephenson et al., "The autophagy gene ATG5 plays an essential role in B lymphocyte development," *Autophagy*, vol. 4, no. 3, pp. 309–314, 2008.

[85] P. Rosenstiel, G. Jacobs, A. Till, and S. Schreiber, "NOD-like receptors: ancient sentinels of the innate immune system," *Cellular and Molecular Life Sciences*, vol. 65, no. 9, pp. 1361–1377, 2008.

[86] S. J. Ott, M. Musfeldt, D. F. Wenderoth et al., "Reduction in diversity of the colonic mucosa associated bacterial microflora in patients with active inflammatory bowel disease," *Gut*, vol. 53, no. 5, pp. 685–693, 2004.

[87] J. Qin, R. Li, J. Raes et al., "A human gut microbial gene catalogue established by metagenomic sequencing," *Nature*, vol. 464, no. 7285, pp. 59–65, 2010.

[88] Z. M. Kanaan, M. R. Eichenberger, S. Ahmad et al., "Clinical predictors of inflammatory bowel disease in a genetically well-defined Caucasian population," *Journal of Negative Results in Biomedicine*, vol. 11, article 7, 2012.

[89] S. Lesage, H. Zouali, J. P. Cezard et al., "*CARD15*/*NOD2* mutational analysis and genotype-phenotype correlation in 612 patients with inflammatory bowel disease," *The American Journal of Human Genetics*, vol. 70, no. 4, pp. 845–857, 2002.

[90] T. Petnicki-Ocwieja, T. Hrncir, Y. J. Liu et al., "*NOD2* is required for the regulation of commensal microbiota in the intestine," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 106, no. 37, pp. 15813–15818, 2009.

[91] P. Kuballa, A. Huett, J. D. Rioux, M. J. Daly, and R. J. Xavier, "Impaired autophagy of an intracellular pathogen induced by a Crohn's disease associated *ATG16L1* variant," *PLoS ONE*, vol. 3, no. 10, Article ID e3391, 2008.

[92] Y. G. Kim, N. Kamada, M. H. Shaw et al., "The *NOD2* sensor promotes intestinal pathogen eradication via the chemokine CCL2-dependent recruitment of inflammatory monocytes," *Immunity*, vol. 34, no. 5, pp. 769–780, 2011.

[93] M. Parkes, J. C. Barrett, N. J. Prescott et al., "Sequence variants in the autophagy gene IRGM and multiple other replicating loci contribute to Crohn's disease susceptibility," *Nature Genetics*, vol. 39, no. 7, pp. 830–832, 2007.

[94] P. Brest, P. Lapaquette, M. Souidi et al., "A synonymous variant in IRGM alters a binding site for miR-196 and causes deregulation of IRGM-dependent xenophagy in Crohn's disease," *Nature Genetics*, vol. 43, no. 3, pp. 242–245, 2011.

[95] A. Darfeuille-Michaud, C. Neut, N. Barnich et al., "Presence of adherent *Escherichia coli* strains in ileal mucosa of patients with Crohn's disease," *Gastroenterology*, vol. 115, no. 6, pp. 1405–1413, 1998.

[96] S. B. Singh, W. Ornatowski, I. Vergne et al., "Human IRGM regulates autophagy and cell-autonomous immunity functions through mitochondria," *Nature Cell Biology*, vol. 12, no. 12, pp. 1154–1165, 2010.

[97] I. P. Gregoire, C. Richetta, L. Meyniel-Schicklin et al., "IRGM is a common target of RNA viruses that subvert the autophagy network," *PLoS Pathogens*, vol. 7, no. 12, Article ID e1002422, 2011.

[98] E. Papa, M. Docktor, C. Smillie et al., "Non-invasive mapping of the gastrointestinal microbiota identifies children with inflammatory bowel disease," *PLoS ONE*, vol. 7, no. 6, Article ID e39242,, 2012.

[99] R. M. Ayling, "New faecal tests in gastroenterology," *Annals of Clinical Biochemistry*, vol. 49, part 1, pp. 44–54, 2012.

[100] P. F. van Rheenen, E. van de Vijver, and V. Fidler, "Faecal calprotectin for screening of patients with suspected inflammatory bowel disease: diagnostic meta-analysis," *British Medical Journal*, vol. 341, p. c3369, 2010.

[101] R. Vitali, L. Stronati, A. Negroni et al., "Fecal HMGB1 is a novel marker of intestinal mucosal inflammation in pediatric inflammatory bowel disease," *The American Journal of Gastroenterology*, vol. 106, no. 11, pp. 2029–2040, 2011.

[102] S. Ehlers, S. H. E. Kaufmann, and Participants of the 99th Dahlem Conference, "Infection, inflammation, and chronic diseases: consequences of a modern lifestyle," *Trends in Immunology*, vol. 31, no. 5, pp. 184–190, 2010.

[103] A. Marcuzzi, M. Girardelli, A. M. Bianco et al., "Inflammation profile of four early onset Crohn patients," *Gene*, vol. 493, no. 2, pp. 282–285, 2012.

[104] A. M. Bianco, V. Zanin, M. Girardelli et al., "A common genetic background could explain early-onset Crohn's disease," *Medical Hyphoteses*, vol. 78, no. 4, pp. 520–522, 2012.

Hindawi Publishing Corporation
BioMed Research International
Volume 2013, Article ID 419256, 9 pages
http://dx.doi.org/10.1155/2013/419256

Research Article

Expression Pattern of Genes of RLR-Mediated Antiviral Pathway in Different-Breed Chicken Response to Marek's Disease Virus Infection

Ze-Qing Feng,[1] Ting Lian,[1] Yong Huang,[2] Qing Zhu,[1] and Yi-Ping Liu[1]

[1] *College of Animal Science and Technology, Sichuan Agriculture University, Ya'an, Sichuan 625014, China*
[2] *College of Veterinary Medicine, Sichuan Agriculture University, Ya'an, Sichuan 625014, China*

Correspondence should be addressed to Yi-Ping Liu; liuyp578@yahoo.com

Received 19 December 2012; Accepted 3 March 2013

Academic Editor: Enrique Medina-Acosta

It has been known that the chicken's resistance to disease was affected by chicken's genetic background. And RLR-mediated antiviral pathway plays an important role in detection of viral RNA. However, little is known about the interaction of genetic background with RLR-mediated antiviral pathway in chicken against MDV infection. In this study, we adopted economic line-AA broilers and native Erlang mountainous chickens for being infected with MDV. Upon infection with MDV, the expression of *MDA-5* was upregulated in two-breed chickens at 4, 7, and 21 d.p.i. It is indicated that MDA-5 might be involved in detecting MDV in chicken. Interestingly, the expression of *IRF-3* and *IFN-β* genes was decreased in spleen and thymus of broilers at 21 d.p.i, but it was upregulated in immune tissues of Erlang mountainous chickens. And the genome load of MDV in spleen of broiler is significantly higher than that in Erlang mountainous chickens. Meanwhile, we observed that the death of broiler mainly also occurred in this phase. Collectively, these present results demonstrated that the expression patters of *IRF-3* and *IFN-β* genes in chicken against MDV infection might be affected by the genetic background which sequently influence the resistance of chicken response to MDV.

1. Introduction

Innate immune system serves as the first line for detecting and defending against invading pathogens [1]. It detects pathogen associated molecular patterns (PAMP) by employing Pattern-Recognition Receptors (PRRs) and triggers the production of type I interferon for preventing viral replication and diffusion [2, 3]. PRRs are composed of toll-like receptors (TLRs), retinoic-acid-inducible-gene-I- (RIG-I-) like receptors (RLRs), NOD-like receptors (NLRs), and C-type lectin receptors (CLRs). RLRs, located in cytoplasm, consists of retinoic acid-induced gene-I (RIG-I) [4], melanoma differentiation associated gene-5 (MDA-5) [5], laboratory of genetics and physiology-2 (LGP-2) [6]. RIG-I and MDA-5 recognize different length of viral double-stranded RNA (dsRNA) by their RNA helicase domain [6, 7]. Additionally, *RIG-I* is capable of recognizing single-stranded RNA (ssRNA) containing 5′-triphosphate by its C-terminal regulator domain which inhibits the activation of RIG-I in the steady state [8–11]. Once RIG-I and MDA-5 bind with ssRNA or dsRNA derived from virus, it can activate downstream transcription factors such as NF-κB, IRF-3, and IRF-7, then these transcription factors translocate from cytoplasm into nucleus and efficiently induce expression of genes encoding type I interferon [12–14].

It has been established that RLR-mediated innate immune plays a crucial role in human and mouse response to viral infection. Previous study indicated that the absence of *RIG-I* in chicken results in more susceptibility of chickens to influenza viruses than ducks [15]. Recently, *MDA-5* and *LGP-2* have been identified in chicken, and MDA-5 has been shown to be involved in sensing dsRNA and influenza A virus in chicken cell [16, 17]. However, the exact role of MDA-5 *in vivo* of chicken against virus infection has not been clarified in detail, and little study has been devoted to investigate the role of RLR-mediated antiviral pathways in chicken response to DNA virus infection.

Marek's disease (MD), which is caused by Marek's disease virus (MDV), is lymphoproliferative tumour disease in chickens, which clinically shows the immune suppression,

polyneuritis, and formation of T-cell lymphoma in the visceral [18]. MDV belongs to α-herpesvirus subfamily owing to its molecular structure and genomic organization close to herpes simplex virus (HSV) [19–21]. Previous studies showed that expression of many proinflammatory cytokine genes, including *IFN-α, IFN-γ, iNOS, IL-1β, IL-6* and *IL-18*, have been enhanced in chicken following infection with MDV [22–25]. Additionally, the changes of these cytokines expression *in vivo* were influenced by genetic background of chicken and virulence of MDV [26–28]. Meanwhile, the expression of *TLR-3* and *TLR-7* genes was induced in the lungs of chicken response to MDV infection [23]. These results impel us to determine whether RLR-mediated innate immune pathways participate in chickens immune against MDV. Meanwhile we also want to know whether the expression of gene of RLR-mediated innate immune pathway is affected by genetic background.

To address these objectives, two-breed chickens including economic line-AA broilers and native Erlang mountainous chickens were chose for infection with MDV. Then the expression of *MDA-5, IRF-3, IFN-α* and *β* gene in the immune organ at 4, 7, and 21 d.p.i were measured by real-time PCR. These results will make us to understand the roles of genetic background and RLR-mediated immune pathway in chicken response to MDV infection.

2. Materials and Methods

2.1. Experimental Animals and Virus. Fertilized eggs of Erlang mountainous chickens and AA broilers were obtained from Long-Sheng Company and Zheng-Da Company of China, respectively. All eggs were hatched at incubation room of Long-Sheng Company; chickens hatched were unvaccinated and housed in the isolation laboratory of veterinary hospital of Sichuan agricultural University. All chickens used in the study were approved by the Sichuan Agricultural University Animal Care and Use Committee.

The virulent MDV J-1 strain used in the study was purchased from institute of animal and veterinary in Beijing. The virus was always kept in the liquid nitrogen until used.

2.2. Experimental Design and Samples Collection. One hundred and 3 days posthatched Erlang mountainous chickens and AA broilers were randomly divided into uninfected group and infected group. Every group has fifty chickens. Each chicken in the infected group was infected intraperitoneally with 1500 PFU of virulent MDV J-1 strain. The control group was mock infected with viral diluents. The MDV-infected group was kept under identical condition as the uninfected age-matched control.

At 4, 7, and 21 d.p.i, six broilers and eight Erlang mountainous chickens of each group were euthanized, and lymphoid tissues including spleen, thymus, and bursa of Fabricius were collected from euthanized chickens. Collected samples were snap frozen in liquid nitrogen and then stored at −80°C. Meanwhile, the rest of chickens in infected group were monitored for death until 21 d.p.i.

2.3. DNA and RNA Extraction and cDNA Synthesis. Total RNA was isolated from spleen, thymus, and bursa of Fabricius of infected and uninfected chicken by using TRIZOL reagent (Invitrogen Co., Ltd, Beijing, China) according to the manufacturers' instructions. Extracted RNA was dissolved into 40 μL RNase-free water and stored at −80°C until used.

DNA was extracted from spleen of MDV-infected chickens by TRIZOL reagent (Invetrogen Co., Ltd, Beijing, China) according to manufacturer's protocol and was dissolved in TE buffer, as well as stored at −20°C until used.

Reverse transcription of total RNA was carried out using PrimeScript RT reagent Kit (TAKARA, Dalian, China) according to the manufacturers' instructions. The reaction was performed in a volume of 20 μL containing 4 μL of 5 × PrimeScript Buffer, 1 μL of PrimeScript RT Enzyme Mix I, 1 μL of Oligo dT Primer, 1 μL of Random 6 mers, 11 μL of RNase-free water, and 2 μL of total RNA. The reaction was done at 37°C for 15 min and 85°C for 5 sec. The synthesized cDNAs preparation was stored at −20°C until used in the real-time PCR.

2.4. Primer Design. The absolute MDV genome load in the MDV-infected chicken's spleen was quantified using primers specific for MDV-*meq* gene. The primers specific for *meq MDA-5, IRF-3, IFN-α* and *IFN-β*, as well as *GAPDH* genes were designed by Primer 5.0 and used for relative quantification of gene expression in collected tissues. The specificity of the primers was confirmed by using BLAST program in NCBI. The sequence and parameters of primers were shown in Table 1.

2.5. Construct for Standard Curve. The real-time PCR for relative quantification of the target genes expression was performed using the standard curve. The fragment of target gene was PCR amplified using the specific primers. The condition of amplification included an initial heat denaturing at 94°C for 4 min, 30 cycles of 94°C for 30 s, 55°C for 30 s, 72°C for 2 min. PCR products were tested in the 1.5% agarose gel and cloned into the p-vector (TAKARA, Dalian, China). The plasmid DNA of target and reference genes was 10-fold serial diluted (10^{-1} to 10^{-9}) and was used to generate standard curves on the CFX96 real-time PCR according to the following PCR condition.

2.6. Real-Time PCR. The expression levels of target gene were detected by using the SsoFast-Evagreen assay on the CFX96 real-time PCR thermal cycle instrument (Bio-Rad). Dilution of the standards was used as calibrator in each real-time PCR assay. PCR reaction mixture of 20 μL contained 10 μL of SsoFast Evagreen (Bio-Rad), 1 μL of each specific primer, 6 μL of ddH_20, and 2 μL of cDNA. All Real-time PCR reaction was carried out in the triplicate for each sample. The thermal cycling conditions consisted of an initial heat denaturing at 98°C for 2 min, 39 cycles of 98°C for 2 s, and optimal annealing temperature of each primer pair for 15 s. Melting-curve analyses were applied in each amplification to test the specificity of amplification.

Table 1: Genes and primer pairs used in this study.

Genes		Primer pairs sequences (5′–3′)	Annealing temperatures (°C)	Amplicons (bp)	Accession numbers
Meq	Forward Reverse	ACGCAGGGAGCAGACGTACTAT CCATAGGGCAAACTGGCTCAT	63°C	155	YP_001033993
MDA-5	Forward Reverse	GTTGCTGTAGGAGATGCAAGTG ATCTGGCTCAGGTGAAGCTCT	60°C	114	NM_001193638
IRF-3	Forward Reverse	TACACTGAGGACTTGCTGGAGGT AAGATGGTGGTCTCCTGATCC	62°C	170	NM_205372
IFN-α	Forward Reverse	CAGGATGCCACCTTCTCTCAC AGGATGGTGTCGTTGAAGGAG	60°C	113	NM_205427
IFN-β	Forward Reverse	CCTCAACCAGATCCAGCATTAC CCCAGGTACAAGCACTGTAGTT	59°C	167	NM_001024836
GAPDH	Forward Reverse	AGGACCAGGTTGTCTCCTGT CCATCAAGTCCACAACACGG	62°C	153	NM_204305

2.7. Statistical Analysis. The efficiency of real-time PCR (E) was calculated by $10^{(-1/\text{slope of the standard curve})}$, and the level of mRNA expression of target gene was calculated relative to *GAPDH* gene expression and was expressed as ratios. The formula used to quantify the relative amount of gene expression was $2^{-\Delta CT}$. The absolute numbers of MDV genome per 100 ng of spleen DNA were calculated based on standard curve. The MDV genome load data and target gene expression data were subjected to t-test. T-test and comparisons were considered significant at $P < 0.05$.

3. Results

3.1. Generation of Standard Curves. Standard curves for relative quantification of *MDA-5, IRF-3, IFN-α* and *IFN-β*, and *GAPDH* gene were generated, and *GAPDH* was used as reference gene. The amplification efficiency of *MDA-5, IRF-3, IFN-α*, *IFN-β*, and *GAPDH* was 101.9%, 96%, 96.7%, 100.2%, and 99.4%, respectively.

3.2. The Mortality of Two-Breed Chickens after Being Infected with MDV. After being infected with MDV, the death of two-breed chickens was monitored and the data are shown in Figure 1. We found that the mortality of broilers was higher than Erlang mountainous chickens at the same condition upon infection with MDV, and the death rate of broilers had a gradually increasing trend from 9 d.p.i to 21 d.p.i. But the death of Erlang mountainous chickens had not presented in the phase. These results indicate that the Erlang mountainous chicken have more resistance to MDV than broiler.

3.3. MDV Genome Load in the Spleen of MDV-Infected Broilers and ErLang Mountainous Chickens. Spleen DNA extracted from MDV-infected chickens was analyzed by real-time PCR and the result is shown in Figure 2. MDV genome could be detected in all infected chickens, whereas uninfected-control chickens did not show any amplification of *Meq* gene. After infection with MDV, the MDV genome load in the spleens of broilers and Erlang mountainous chickens had a gradually increasing trend from 4 d.p.i to 21 d.p.i. The MDV genome load in spleens of broilers and Erlang mountainous chickens was significantly higher at 7 d.p.i when compared to that in spleens of the same line at 4 d.p.i ($P = 0.0076$ and $P = 0.0082$), respectively. In broilers, it was also significantly higher at 21 d.p.i than that at 7 d.p.i ($P = 0.0494$). Meanwhile, the MDV genome load in spleens of broilers was significantly higher than that in Erlang mountainous chickens at 4 d.p.i ($P = 0.003$) and 21 d.p.i ($P = 0.038$). These results suggest that Erlang mountainous chicken might have more capability of controlling MDV replication *in vivo*.

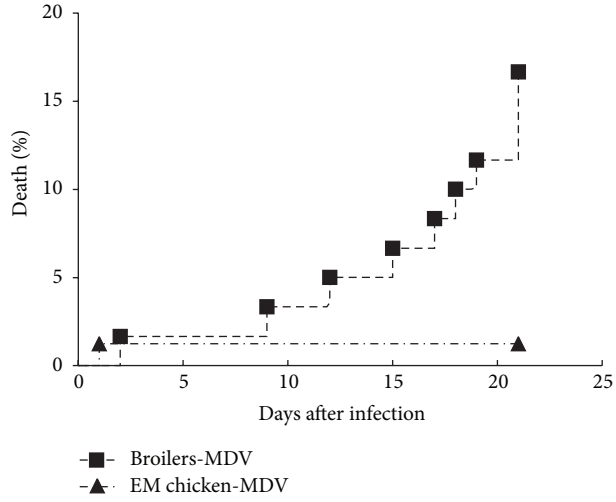


Figure 1: The death rate of two-breed chickens following infection with MDV. The groups were as follows: Broiler-MDV= chickens from broilers group infected with MDV, EM chicken-MDV = chickens from Erlang mountainous chickens group infected with MDV.

3.4. Detection of MDA-5, IRF-3, IFN-α, and IFN-β Genes in Spleens of MDV-Infected and MDV-Uninfected Chickens. The expression of *MDA-5* gene in spleens is shown in Figure 3(a). The expression of *MDA-5* gene had an increasing trend

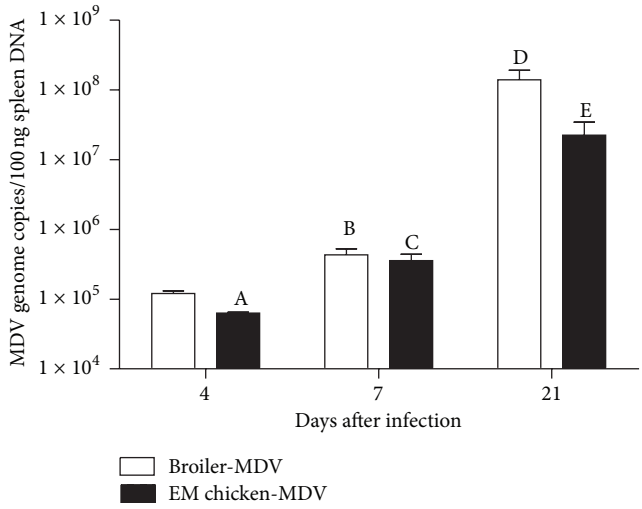


Figure 2: MDV genome load in spleen of broilers and Erlang mountainous chickens following infection with MDV. The groups were as follows: Broiler-MDV = chickens from the broilers infected with MDV, EM chicken-MDV=chickens from Erlang mountainous chickens infected with MDV. MDV infected group was infected with virulent strain of MDV. At 4, 7, and 21 d.p.i, six broilers and eight Erlang mountainous chickens of infected group were killed. The MDV genome loads in spleen of killed chickens were analyzed by real-time PCR. A: significant compared to broiler-MDV observed at 4 d.p.i. B: and C: significant compared to broiler-MDV and EM chicken-MDV observed at 4 d.p.i, respectively. D: significant compared to broiler-MDV observed at 7 d.p.i. E: significant compared to broiler-MDV observed at 21 d.p.i. Error bars represent standard error of the mean.

in spleens of both two-breed chickens infected with MDV compared to uninfected chickens. At 7 and 21 d.p.i, the MDV-infected broilers have significantly higher *MDA-5* mRNA expression in spleens compared to the uninfected-control same line ($P = 0.0117$ and $P = 0.0343$). Meanwhile, the expression of this gene in Erlang mountainous chickens had a dramatic rise compared to the uninfected-control same line at 4 and 7 d.p.i ($P = 0.0207$ and $P = 0.0027$).

The expression of *IRF-3* gene was observed in spleens of broilers and Erlang mountainous chickens (Figure 3(b)). It had a slightly increasing trend in spleens of two-breed chickens at 4 d.p.i, while the trend was not significant. However, at 21 d.p.i, the expression of this gene in the spleens of MDV-infected broilers was significantly lower than the uninfected ones ($P = 0.0375$). By contrast, the expression of the gene was significantly higher in the spleens of MDV-infected Erlang mountainous chickens than the uninfected ones ($P = 0.0212$). And the expression of *MDA-5* gene in spleens of MDV-infected broilers at 21 d.p.i was significantly lower when compared to that in spleens of MDV-infected Erlang mountainous chickens ($P = 0.0006$).

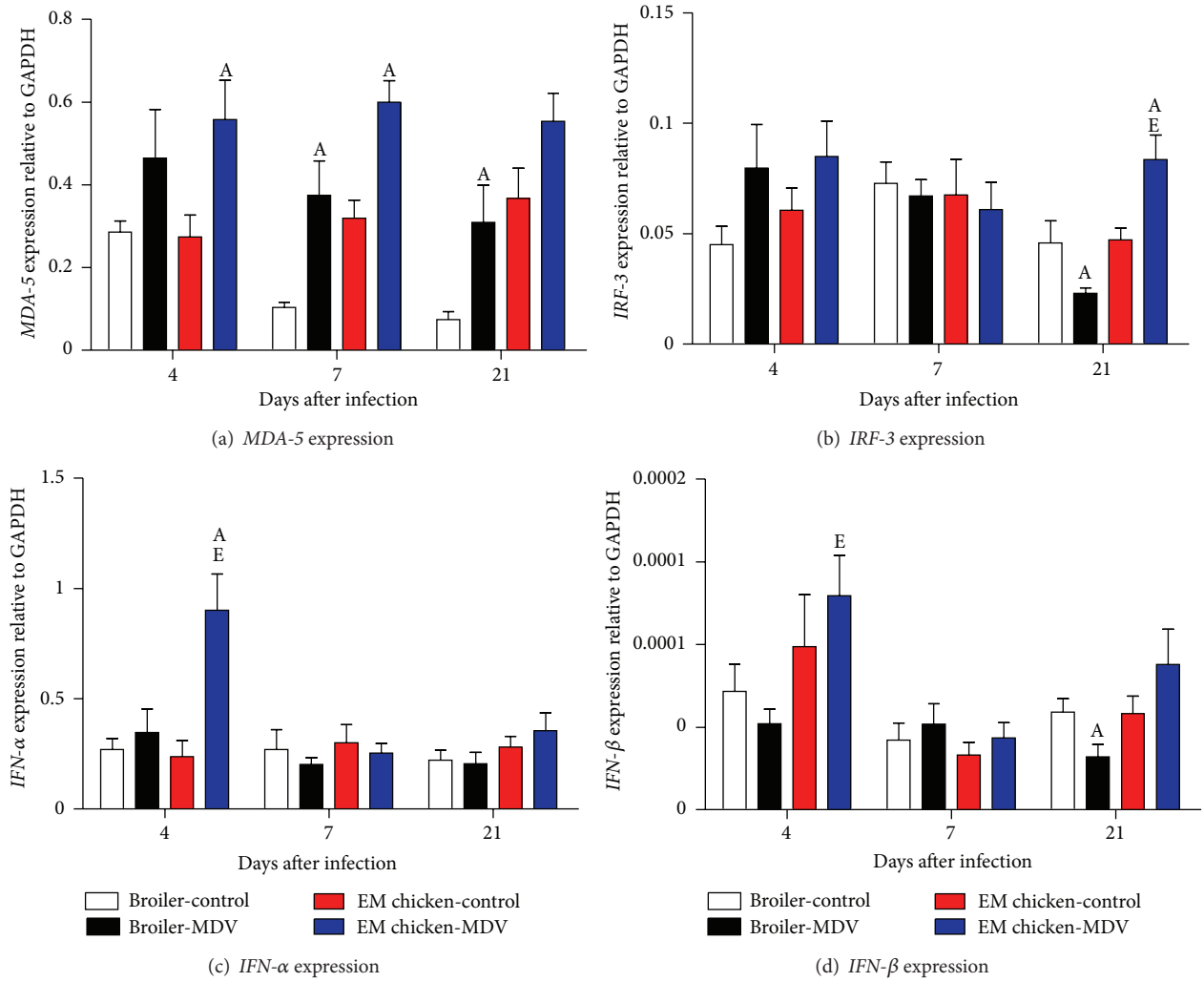
The expression of *IFN-α* and *IFN-β* in spleen was shown in Figures 3(c) and 3(d), respectively. The expression of *IFN-α* in spleen of MDV-infected Erlang mountainous chickens was significantly higher when compared to that in spleen of the uninfected-control same line and MDV-infected broilers at 4 d.p.i ($P = 0.0075$ and $P = 0.0179$). Even though the expression of *IFN-β* gene increased moderately in spleen of Erlang mountainous chickens during infection with MDV, the difference was not significant, and expression of this gene in spleen Erlang mountainous chickens was significantly higher than that in spleen of MDV-infected broilers at 4 d.p.i ($P = 0.011$). Interestingly, the expression of *IFN-β* gene had a substantial decrease in spleen of MDV-infected boiler chickens at 21 d.p.i ($P = 0.0428$).

3.5. The Expression of MDA-5, IRF-3, IFN-α and IFN-β in Thymus of MDV-Infected and MDV-Uninfected Chickens. The expression of *MDA-5* in thymus was shown in Figure 4(a). MDV infection caused upregulation of expression of *MDA-5* gene in thymus of broilers and Erlang mountainous chickens. At 7 and 21 d.p.i, MDV-infected broilers had significantly higher expression of *MDA-5* gene in thymus than the uninfected-control same line ($P = 0.0068$ and $P = 0.0102$). Furthermore, the expression of *MDA-5* gene in the thymus of MDV-infected Erlang mountainous chickens was also significantly higher than the uninfected-control same line at 4 and 21 d.p.i ($P = 0.0344$ and $P = 0.0242$).

The expression of *IRF-3* in thymus was shown in Figure 4(b). After infection with MDV, the expression of *IRF-3* gene was significantly higher in the thymus of the MDV-infected broilers when compared to that in the thymus of the control-uninfected broilers at 4 d.p.i ($P = 0.0112$) and 7 d.p.i ($P = 0.0344$). However, the expression of *IRF-3* gene was significantly higher in the thymus of Erlang mountainous chickens when compared to uninfected-control same line at 4 d.p.i ($P = 0.0138$), 7 d.p.i ($P = 0.0029$), and 21 d.p.i ($P = 0.0021$), respectively.

The expression data for *IFN-α* and *IFN-β* in spleen were shown in Figures 4(c) and 4(d), respectively. The expression of *IFN-α* in thymus of Erlang mountainous chickens has an increased tendency at 4 and 21 d.p.i, and the increased tendency reached significantly only at 21 d.p.i ($P = 0.0085$). Meanwhile the expression of *IFN-α* in thymus of MDV-infected Erlang mountainous chickens was significant higher when compared to that in the thymus of MDV-infected broilers ($P = 0.0314$). In addition, MDV infection caused the increase of expression of *IFN-β* in the Erlang mountainous chickens, and the increased trend reached significant at 21 d.p.i ($P = 0.0001$). By contrast, the expression of *IFN-β* in the thymus of MDV-infected broilers decreased significantly when compared to uninfected broilers at 21 d.p.i ($P = 0.0251$).

3.6. The Expression of MDA-5, IRF-3, IFN-α and IFN-β Genes in Bursa of Fabricius of MDV-Infected and Uninfected Chickens. The expression of *MDA-5* in bursa of Fabricius was shown in Figure 5(a). The expression of *MDA-5* gene in bursa of Fabricius of both two breeds had a rising trend following infection with MDV, which approached significant in broilers at 7 and 21 d.p.i ($P = 0.0077$ and $P = 0.0185$) and in Erlang mountainous chickens at 4 and 7 d.p.i ($P = 0.0042$ and $P = 0.0059$).



(a) *MDA-5* expression

(b) *IRF-3* expression

(c) *IFN-α* expression

(d) *IFN-β* expression

Figure 3: Expression of *MDA-5* (a), *IRF-3* (b), *IFN-α* (c), and *IFN-β* (d) genes in spleen of chicken infected with virulent of MDV or uninfected control chickens. The groups were as follows: Broiler-control = uninfected broilers, Broiler-MDV = MDV-infected chickens of broilers, EM chicken-control = uninfected chickens of Erlang mountainous chicken, and EM chicken-MDV = MDV-infected chickens of Erlang mountainous chicken. At 4, 7, 21 d.p.i, six broilers and eight Erlang mountainous chickens of each group were killed. The expression of genes in spleen of every killed chicken was analyzed. Error bars represent standard error of the mean. A: significant difference comparing MDV-infected chickens with uninfected chickens of the same line at the same point. E: significant difference comparing MDV-infected Erlang mountainous chicken with MDV-infected broilers at same point.

The expression of *IRF-3* in bursa of Fabricius was shown in Figure 5(b). The Erlang mountainous chickens infecting with MDV showed significant increase in expression of *IRF-3* in bursa of Fabricius tissues when compared to that in control-uninfected chickens at 4 d.p.i ($P = 0.0438$), 7 d.p.i ($P = 0.0345$), and 21 d.p.i ($P = 0.0009$). However, the significant increase in the expression of this gene of MDV-infected broilers occurred only at 4 d.p.i ($P = 0.0011$).

The expression data for *IFN-α* and *IFN-β* in bursa of Fabricius were shown in Figures 5(c) and 5(d), respectively. The expression of *IFN-β* gene in bursa of Fabricius of two breeds both was significantly higher than that in the uninfected same line at 4 d.p.i ($P = 0.0231$ and $P = 0.0013$), respectively. Although the expression of *IFN-β* revealed a sharp rise in the bursa of Fabricius of Erlang mountainous chickens infecting with MDV, it did not approach significant ($P = 0.0892$). Moreover, it was obtained that the expression of this gene was significantly higher in bursa of Fabricius of Erlang mountainous chickens than that in broilers at 21 d.p.i. ($P = 0.0398$).

4. Discussion

It has been proved that the resistance of chicken to MDV is influenced by different genetic backgrounds [29]. And the chicken's different haplotypes of major histocompatibility complex (MHC) affect the resistance of chicken to disease. It have been demonstrated that the B21 and B19 haplotypes are associated with resistance and susceptibility MDV, respectively [30]. Meanwhile, several quantitative trait

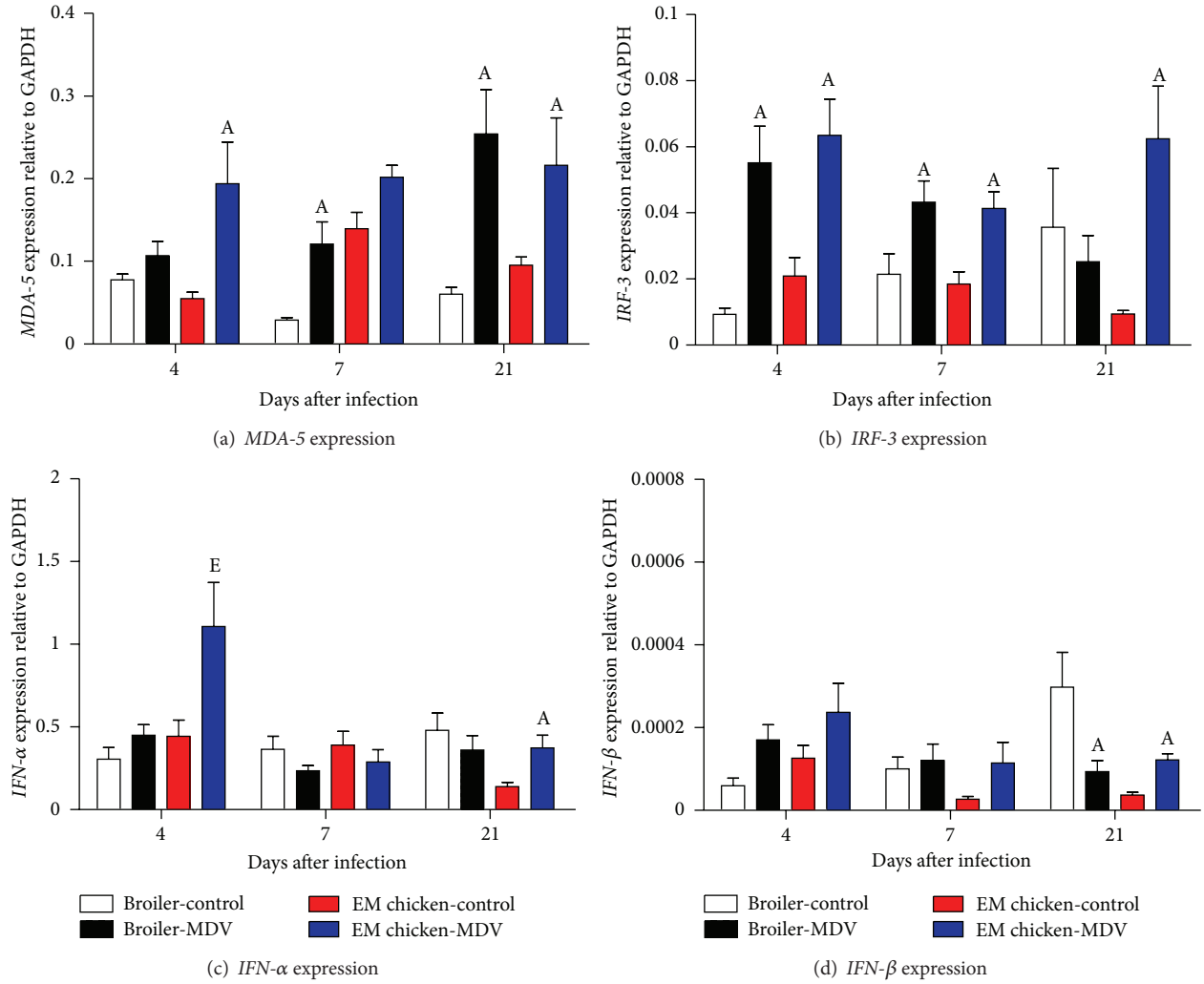


Figure 4: Expression of *MDA-5* (a), *IRF-3* (b), *IFN-α* (c), and *IFN-β* (d) genes in thymus of chicken infected with virulent of MDV or uninfected control chickens. The groups were as follows: Broiler-control = uninfected broilers, Broiler-MDV = MDV-infected chickens of broilers, EM chicken-control = uninfected chickens of Erlang mountainous chicken, and EM chicken-MDV = MDV-infected chickens of Erlang mountainous chicken. At 4, 7, and 21 d.p.i, six broilers and eight Erlang mountainous chickens of each group were killed. The expression of genes in thymus of every killed chicken was analyzed. Error bars represent standard error of the mean. A: significant difference comparing MDV-infected chickens with uninfected chickens of the same line at the same point. E: significant difference comparing MDV-infected Erlang mountainous chicken with MDV-infected broilers at the same point.

loci (QTL) against to MDV within the chicken's genome had been identified using genetic markers [31–33]. However, the underlying mechanism how genetic background influences the resistance of chicken to MDV remains unknown. In this study, two breeds, economic line-broilers and native line-Erlang mountainous chickens, were adopted for being infected with MDV. Broilers used in our experiment is special breed for meat production through a long-time high-intensity selection, and it has a higher growth speed in muscle tissue. On the contrary, Erlang mountainous chicken is a native breed, which have not been selected for a long time for any economic trait. After infection with MDV, Erlang mountainous chickens showed more resistance to MDV infection than broilers. It is indicated that overselection for economic trait indeed influence the resistance of chicken response to MDV infection. Previous study showed that the second cytolytic infection induced by MDV occurred in the susceptible chickens from approximately 18 d.p.i onward [29]. In our experiment, the death of broiler mainly occurred from 16 d.p.i to 21 d.p.i, and we speculated that the death of broilers might be the consequent of MDV-mediated second cytolytic infection during this phase.

Although both genetically susceptible and resistant chickens can be infected with MDV, genetically resistant chickens are capable of controlling the MDV genome load in spleens and feather [26, 34]. In agreement with this, in the current study, the MDV genome load appeared in spleens of MDV-infected two-breed chickens, and the MDV genome load in spleen of broilers was significantly higher when compared to Erlang mountainous chickens at 4 and 21 d.p.i. These results

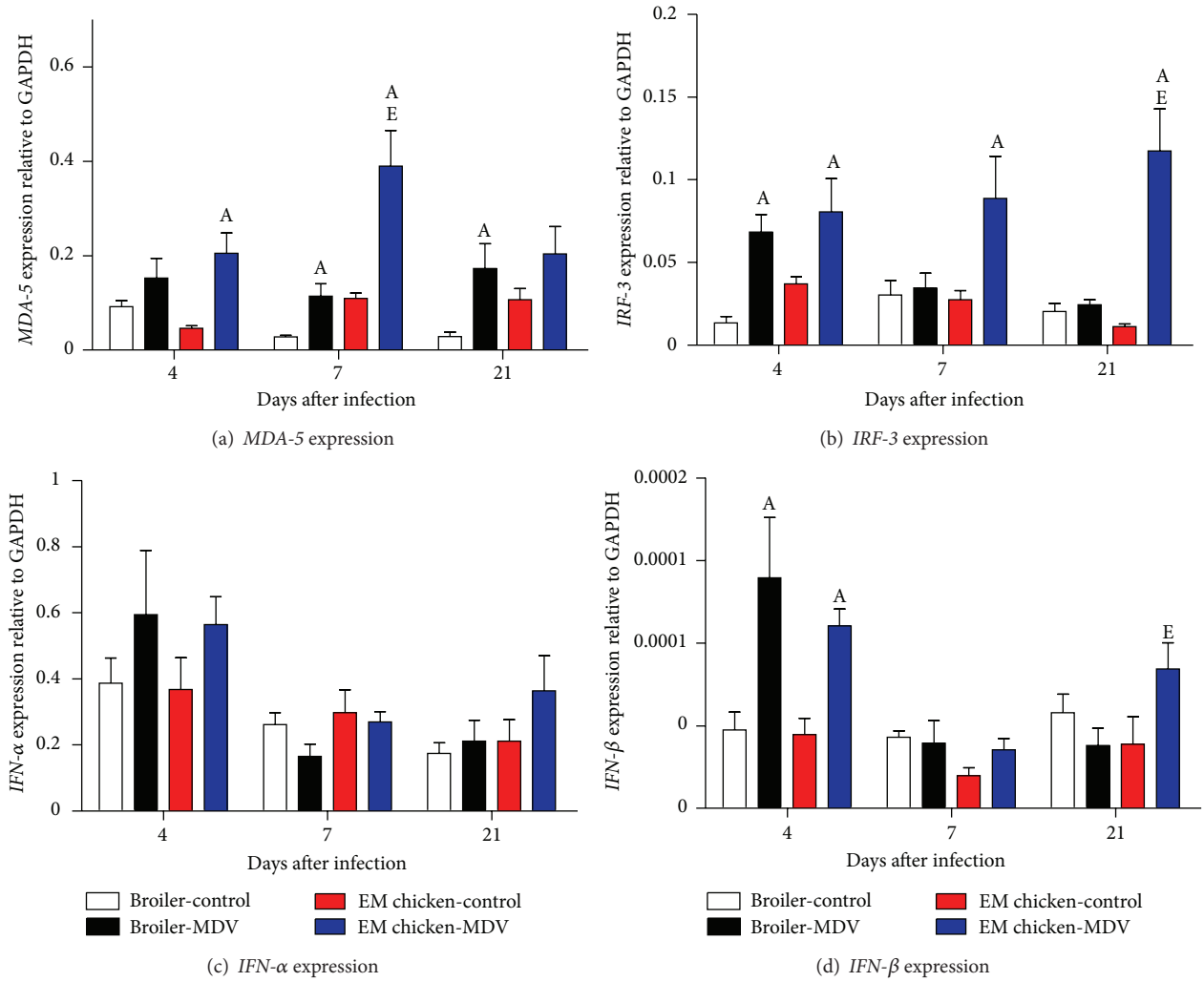


Figure 5: Expression of *MDA-5* (a), *IRF-3* (b), *IFN-α* (c), and *IFN-β* (d) genes in bursa of Fabricius of chicken infected with virulent of MDV or uninfected control chickens. The groups were as follows: Broiler-control = uninfected broilers, Broiler-MDV = MDV-infected chickens of broilers, EM chicken-control = uninfected chickens of Erlang mountainous chicken, and EM chicken-MDV = MDV-infected chickens of Erlang mountainous chicken. At 4, 7, and 21 d.p.i, six broilers and eight Erlang mountainous chickens of each group were killed. The expression of genes in bursa of Fabricius of every killed chicken was analyzed. Error bars represent standard error of the mean. A: significant difference comparing MDV-infected chickens with uninfected chickens of the same line at the same point. E: significant difference comparing MDV-infected Erlang mountainous chicken with MDV-infected broilers at the same point.

further indicate that genetic background function as crucial element for affecting MDV genome load in chicken.

It has been proved that RLR-mediated immune pathway mainly is involved in detection and response to RNA virus [35]. However, little is known about the exact role of RLR-mediated innate immune *in vivo* response to DNA virus. Due to the deficiency of RIG-I in chicken, chicken serve as a good animal modern for studying the role of MDA-5 *in vivo* response to DNA virus.

In our study, the expression of *MDA-5* gene was induced in three immune tissues of two-breed chickens at 4, 7, and 21 d.p.i. It is suggested that MDA-5 might be involved in detection and response against MDV. Because MDV belongs to DNA virus, how does chicken utilize MDA-5 to detect MDV? The study in human primary macrophages found that MDA-5 is responsible for recognition of HSV-1, and the process is dependent on viral replication [36]. Owing to dsRNA generated by positive-strand RNA viruses and DNA viruses during viral replication [37], we deduce that dsRNA produced by MDV during replication might serve as resources which are detected byMDA-5 and trigger RLR-mediated immune pathway. Meanwhile, some studies revealed that RNA polymerase III was involved in detection of cytosolic DNA and triggering production of type I in human cell, and inhibition of RNA polymerase III also blocked production of interferon induced by DNA virus, such as Herpes simplex virus-1 (HSV-1) and Epstein-Barr virus (EBV) [38–40]. However, the involvement of polymerase III in DNA virus is dependent on RIG-I-mediated immune pathway, independent on MDA-5. Owing to the absence of *RIG-I* in chicken, further study is needed to investigate whether chicken polymerase III and MDA-5 coordinately

detect MDV and promote the expression of interferon at cell level.

Chicken IRF-3 was firstly identified as the first example of a nonmammalian interferon regulatory factor [41], but it was thought as the homology of human IRF-7 due to its higher DNA sequence homology with human IRF-7, rather than human *IRF-3* [42]. Mammalian IRF-3 is mainly responsible for induction of *IFN-β* gene but not the *IFN-α*, yet *IRF-7* efficiently activated both *IFN-α* and *IFN-β* [43, 44]. In our experiment, we found that expression level of *IRF-3* was associated with the expression of *IFN-α* and *IFN-β*. It is suggested that chicken IRF-3, like human IRF-7, is also responsible for the expression *IFN-α* and *IFN-β* in chicken.

Previous study indicated that vaccinating with MDV vaccine could enhance the expression of the *IRF-3* gene in chicken during latent period of MDV infection [45]. And the role of interferon chicken response to MDV infection had been proved [24, 46]. In the present study, we discovered that the expression of both *IRF-3* and *IFN-β* genes had been downregulated in spleen and thymus of broiler at 21 d.p.i, but it showed an upregulation in Erlang mountainous chickens. Owing to the death of broilers observed in this phase, these results further highlight the role of interferon in chicken response against MDV infection. Meanwhile, these results further support the previous conclusion that expression pattern of interferon and cytokine was correlated with genetic background of chicken during MDV infection [26, 28, 34]. Besides, giving that the MDV-mediated secondly cytolytic replication might be occurred in chicken during this phase, we speculate that the change of these genes expression in broiler is the result of MDV-mediated secondly cytolytic replication which causes immunosuppression in broilers for inhibition of interferon expression. These results further suggest that the downregulation of expression of *IRF-3* and interferon gene also might be associated with MDV reactivation. If we could explore deeply the mechanism that MDV infection causes immunosuppression in susceptible chicken, it will make us better understand the interaction between viruses and host.

5. Conclusions

In summary, our study found that the expression of *MDA-5* gene was induced in chicken following infection with MDV, which suggested that MDA-5 might be involved in recognition of MDV in chicken. Importantly, we observed the different expression pattern of *IRF-3* and *IFN-β* genes in broilers and Erlang mountainous chickens at 21 d.p.i. We conclude that it might be affected by genetic background which serve as the main reason leading to the different resistance of two-breed response against MDV infection. Further study is required to elucidate the underlying mechanism between host innate immune and different genetic backgrounds.

Acknowledgments

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper. This study was supported by Open Fund of Sichuan Provincial Key Laboratory of Animal Breeding and Genetics, Sichuan Animal Science Academy, and by Sichuan Province (2011NZ0099-6, 11TD007, and 2011JTD0032), Yunnan Province (2009CI119), and the Ministry of Agriculture of China (2009ZX08009-159B).

References

[1] R. B. Seth, L. Sun, and Z. J. Chen, "Antiviral innate immunity pathways," *Cell Research*, vol. 16, no. 2, pp. 141–147, 2006.

[2] A. Pichlmair and C. Reis e Sousa, "Innate recognition of viruses," *Immunity*, vol. 27, no. 3, pp. 370–383, 2007.

[3] T. Kawai and S. Akira, "Innate immune recognition of viral infection," *Nature Immunology*, vol. 7, no. 2, pp. 131–137, 2006.

[4] M. Yoneyama, M. Kikuchi, T. Natsukawa et al., "The RNA helicase RIG-I has an essential function in double-stranded RNA-induced innate antiviral responses," *Nature Immunology*, vol. 5, no. 7, pp. 730–737, 2004.

[5] D. C. Kang, R. V. Gopalkrishnan, Q. Wu, E. Jankowsky, A. M. Pyle, and P. B. Fisher, "mda-5: an interferon-inducible putative RNA helicase with double-stranded RNA-dependent ATPase activity and melanoma growth-suppressive properties," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 99, no. 2, pp. 637–642, 2002.

[6] M. Yoneyama, M. Kikuchi, K. Matsumoto et al., "Shared and unique functions of the DExD/H-box helicases RIG-I, MDA5, and LGP2 in antiviral innate immunity," *Journal of Immunology*, vol. 175, no. 5, pp. 2851–2858, 2005.

[7] H. Kato, O. Takeuchi, E. Mikamo-Satoh et al., "Length-dependent recognition of double-stranded ribonucleic acids by retinoic acid-inducible gene-I and melanoma differentiation-associated gene 5," *Journal of Experimental Medicine*, vol. 205, no. 7, pp. 1601–1610, 2008.

[8] S. Cui, K. Eisenächer, A. Kirchhofer et al., "The C-terminal regulatory domain is the RNA 5′-triphosphate sensor of RIG-I," *Molecular Cell*, vol. 29, no. 2, pp. 169–179, 2008.

[9] M. Yoneyama and T. Fujita, "Function of RIG-I-like receptors in antiviral innate immunity," *Journal of Biological Chemistry*, vol. 282, no. 21, pp. 15315–15318, 2007.

[10] V. Hornung, J. Ellegast, S. Kim et al., "5′-Triphosphate RNA is the ligand for RIG-I," *Science*, vol. 314, no. 5801, pp. 994–997, 2006.

[11] A. Pichlmair, O. Schulz, C. P. Tan et al., "RIG-I-mediated antiviral responses to single-stranded RNA bearing 5′-phosphates," *Science*, vol. 314, no. 5801, pp. 997–1001, 2006.

[12] R. B. Seth, L. Sun, C. K. Ea, and Z. J. Chen, "Identification and characterization of MAVS, a mitochondrial antiviral signaling protein that activates NF-κB and IRF3," *Cell*, vol. 122, no. 5, pp. 669–682, 2005.

[13] L. G. Xu, Y. Y. Wang, K. J. Han, L. Y. Li, Z. Zhai, and H. B. Shu, "VISA is an adapter protein required for virus-triggered IFN-β signaling," *Molecular Cell*, vol. 19, no. 6, pp. 727–740, 2005.

[14] E. Meylan, J. Curran, K. Hofmann et al., "Cardif is an adaptor protein in the RIG-I antiviral pathway and is targeted by hepatitis C virus," *Nature*, vol. 437, no. 7062, pp. 1167–1172, 2005.

[15] M. R. W. Barber, J. R. Aldridge, R. G. Webster, and K. E. Magor, "Association of RIG-I with innate immunity of ducks to influenza," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 107, no. 13, pp. 5913–5918, 2010.

[16] M. Liniger, A. Summerfield, G. Zimmer, K. C. McCullough, and N. Ruggli, "Chicken cells sense influenza A virus infection through MDA5 and CARDIF-signaling involving LGP2," *Journal of Virology*, vol. 86, no. 2, pp. 705–717, 2012.

[17] A. J. Karpala, C. Stewart, J. McKay, J. W. Lowenthal, and A. G. D. Bean, "Characterization of chicken Mda5 activity: regulation of IFN-β in the absence of RIG-I functionality," *Journal of Immunology*, vol. 186, no. 9, pp. 5397–5405, 2011.

[18] B. W. Calnek, "Pathogenesis of Marek's disease virus infection," *Current Topics in Microbiology and Immunology*, vol. 255, pp. 25–55, 2000.

[19] J. Burnside and R. W. Morgan, "Genomics and Marek's disease virus," *Cytogenetic and Genome Research*, vol. 117, no. 1–4, pp. 376–387, 2007.

[20] N. Osterrieder, J. P. Kamil, D. Schumacher, B. K. Tischer, and S. Trapp, "Marek's disease virus: from miasma to model," *Nature Reviews Microbiology*, vol. 4, no. 4, pp. 283–294, 2006.

[21] S. J. Baigent, L. P. Smith, V. K. Nair, and R. J. W. Currie, "Vaccinal control of Marek's disease: current challenges, and future strategies to maximize protection," *Veterinary Immunology and Immunopathology*, vol. 112, no. 1-2, pp. 78–86, 2006.

[22] Z. Xing and K. A. Schat, "Expression of cytokine genes in Marek's disease virus-infected chickens and chicken embryo fibroblast cultures," *Immunology*, vol. 100, no. 1, pp. 70–76, 2000.

[23] M. F. Abdul-Careem, K. Haq, S. Shanmuganathan et al., "Induction of innate host responses in the lungs of chickens following infection with a very virulent strain of Marek's disease virus," *Virology*, vol. 393, no. 2, pp. 250–257, 2009.

[24] M. F. Abdul-Careem, B. D. Hunter, L. F. Lee et al., "Host responses in the bursa of Fabricius of chickens infected with virulent Marek's disease virus," *Virology*, vol. 379, no. 2, pp. 256–265, 2008.

[25] M. F. Abdul-Careem, B. D. Hunter, A. J. Sarson, A. Mayameei, H. Zhou, and S. Sharif, "Marek's disease virus-induced transient paralysis is associated with cytokine gene expression in the nervous system," *Viral Immunology*, vol. 19, no. 2, pp. 167–176, 2006.

[26] P. Kaiser, G. Underwood, and F. Davison, "Differential cytokine responses following Marek's disease virus infection of chickens differing in resistance to Marek's disease," *Journal of Virology*, vol. 77, no. 1, pp. 762–768, 2003.

[27] K. W. Jarosinski, B. L. Njaa, P. H. O'Connell, and K. A. Schat, "Pro-inflammatory responses in chicken spleen and brain tissues after infection with very virulent plus Marek's disease virus," *Viral Immunology*, vol. 18, no. 1, pp. 148–161, 2005.

[28] P. Quéré, C. Rivas, K. Ester, R. Novak, and W. L. Ragland, "Abundance of IFN-α and IFN-γ mRNA in blood of resistant and susceptible chickens infected with Marek's disease virus (MDV) or vaccinated with turkey herpesvirus; and MDV inhibition of subsequent induction of IFN gene transcription," *Archives of Virology*, vol. 150, no. 3, pp. 507–519, 2005.

[29] L. D. Bacon, H. D. Hunt, and H. H. Cheng, "Genetic resistance to Marek's disease," *Current Topics in Microbiology and Immunology*, vol. 255, pp. 121–141, 2001.

[30] L. D. Bacon, H. D. Hunt, and H. H. Cheng, "A review of the development of chicken lines to resolve genes determining resistance to diseases," *Poultry Science*, vol. 79, no. 8, pp. 1082–1093, 2000.

[31] R. L. Vallejo, L. D. Bacon, H. C. Liu et al., "Genetic mapping of quantitative trait loci affecting susceptibility to Marek's disease virus induced tumors in F2 intercross chickens," *Genetics*, vol. 148, no. 1, pp. 349–360, 1998.

[32] J. P. McElroy, J. C. M. Dekkers, J. E. Fulton et al., "Microsatellite markers associated with resistance to Marek's disease in commercial layer chickens," *Poultry Science*, vol. 84, no. 11, pp. 1678–1688, 2005.

[33] H. Cheng, M. Niikura, T. Kim et al., "Using integrative genomics to elucidate genetic resistance to Marek's disease in chickens," *Developments in Biologicals*, vol. 132, pp. 365–372, 2008.

[34] M. F. Abdul-Careem, L. R. Read, P. Parvizi, N. Thanthrige-Don, and S. Sharif, "Marek's disease virus-induced expression of cytokine genes in feathers of genetically defined chickens," *Developmental and Comparative Immunology*, vol. 33, no. 4, pp. 618–623, 2009.

[35] M. Schlee, E. Hartmann, C. Coch et al., "Approaching the RNA ligand for RIG-I?" *Immunological Reviews*, vol. 227, no. 1, pp. 66–74, 2009.

[36] J. Melchjorsen, J. Rintahaka, S. Søby et al., "Early innate recognition of herpes simplex virus in human primary macrophages is mediated via the MDA5/MAVS-dependent and MDA5/MAVS/RNA polymerase III-independent pathways," *Journal of Virology*, vol. 84, no. 21, pp. 11350–11358, 2010.

[37] F. Weber, V. Wagner, S. B. Rasmussen, R. Hartmann, and S. R. Paludan, "Double-stranded RNA is produced by positive-strand RNA viruses and DNA viruses but not in detectable amounts by negative-strand RNA viruses," *Journal of Virology*, vol. 80, no. 10, pp. 5059–5064, 2006.

[38] Y. H. Chiu, J. B. MacMillan, and Z. J. Chen, "RNA polymerase III detects cytosolic DNA and induces type I interferons through the RIG-I pathway," *Cell*, vol. 138, no. 3, pp. 576–591, 2009.

[39] M. K. Choi, Z. Wang, T. Ban et al., "A selective contribution of the RIG-I-like receptor pathway to type I interferon responses activated by cytosolic DNA," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 106, no. 42, pp. 17870–17875, 2009.

[40] A. Ablasser, F. Bauernfeind, G. Hartmann, E. Latz, K. A. Fitzgerald, and V. Hornung, "RIG-I-dependent sensing of poly(dA:dT) through the induction of an RNA polymerase III-transcribed RNA intermediate," *Nature Immunology*, vol. 10, no. 10, pp. 1065–1072, 2009.

[41] C. E. Grant, M. Z. Vasa, and R. G. Deeley, "cIRF-3, a now member of the interferon regulatory factor (IRF) family that is rapidly and transiently induced by dsRNA," *Nucleic Acids Research*, vol. 23, no. 12, pp. 2137–2146, 1995.

[42] L. Zhang and J. S. Pagano, "IRF-7, a new interferon regulatory factor associated with Epstein-Barr virus latency," *Molecular and Cellular Biology*, vol. 17, no. 10, pp. 5748–5757, 1997.

[43] K. Honda, H. Yanai, H. Negishi et al., "IRF-7 is the master regulator of type-I interferon-dependent immune responses," *Nature*, vol. 434, no. 7034, pp. 772–777, 2005.

[44] K. Honda and T. Taniguchi, "IRFs: master regulators of signalling by Toll-like receptors and cytosolic pattern-recognition receptors," *Nature Reviews Immunology*, vol. 6, no. 9, pp. 644–658, 2006.

[45] R. Kano, S. Konnai, M. Onuma, and K. Ohashi, "Cytokine profiles in chickens infected with virulent and avirulent Marek's disease viruses: interferon-gamma is a key factor in the protection of Marek's disease by vaccination," *Microbiology and Immunology*, vol. 53, no. 4, pp. 224–232, 2009.

[46] K. W. Jarosinski, W. Jia, M. J. Sekellick, P. I. Marcus, and K. A. Schat, "Cellular responses in chickens treated with IFN-α orally or inoculated with recombinant Marek's disease virus expressing IFN-α," *Journal of Interferon and Cytokine Research*, vol. 21, no. 5, pp. 287–296, 2001.