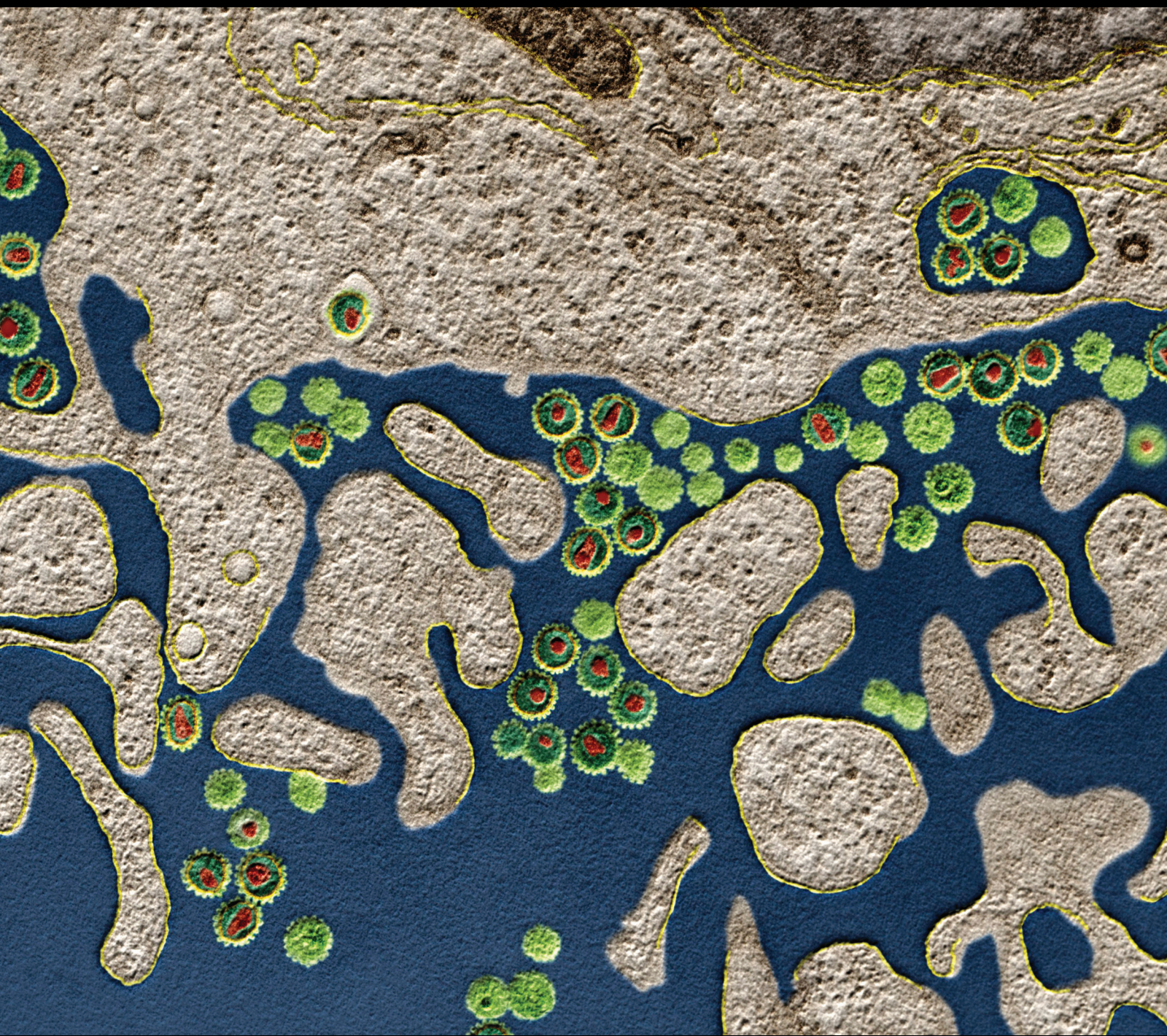
Journal of Immunology Research

Immune Responses to RNA viruses

Lead Guest Editor: Elias A. Said
Guest Editors: Ali A. Al-Jabri, Daniel Lamarre, Felipe Diaz-Griffero, and Dorine Bonte



Immune Responses to RNA viruses

Journal of Immunology Research

Immune Responses to RNA viruses

Lead Guest Editor: Elias A. Said
Guest Editors: Ali A. Al-Jabri, Daniel Lamarre, Felipe Diaz-Griffero, and Dorine Bonte

Copyright © 2018 Hindawi. All rights reserved.

This is a special issue published in "Journal of Immunology Research." All articles are open access articles distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Editorial Board

B. D. Akanmori, Congo
Jagadeesh Bayry, France
Kurt Blaser, Switzerland
Eduardo F. Borba, Brazil
Federico Bussolino, Italy
Nitya G. Chakraborty, USA
Cinzia Ciccacci, Italy
Robert B. Clark, USA
Mario Clerici, Italy
Nathalie Cools, Belgium
M. Victoria Delpino, Argentina
Nejat K. Egilmez, USA
Eyad Elkord, UK
Steven E. Finkelstein, USA
Maria Cristina Gagliardi, Italy
Luca Gattinoni, USA
Alvaro González, Spain
Theresa Hautz, Austria
Martin Holland, UK
Douglas C. Hooper, USA
Eung-Jun Im, USA
Hidetoshi Inoko, Japan
Juraj Ivanyi, UK
Ravirajsinh N. Jadeja, USA
Peirong Jiao, China
Taro Kawai, Japan
Alexandre Keller, Brazil
Hiroshi Kiyono, Japan
Bogdan Kolarz, Poland
Herbert K. Lyerly, USA
Mahboobeh Mahdavinia, USA
Giulia Marchetti, Italy
Eiji Matsuura, Japan
Chikao Morimoto, Japan
Hiroshi Nakajima, Japan
Paola Nistico, Italy
Enrique Ortega, Mexico
Patrice Petit, France
Isabella Quinti, Italy
Eirini Rigopoulou, Greece
Ilaria Roato, Italy
Luigina Romani, Italy
Aurelia Rughetti, Italy
Francesca Santilli, Italy
Takami Sato, USA
Senthamil R. Selvan, USA
Naohiro Seo, Japan
Trina J. Stewart, Australia
Benoit Stijlemans, Belgium
Jacek Tabarkiewicz, Poland
Mizue Terai, USA
Ban-Hock Toh, Australia
Joseph F. Urban, USA
Paulina Wlasiuk, Poland
Baohui Xu, USA
Xiao-Feng Yang, USA
Maria Zervou, Greece
Qiang Zhang, USA

Contents

Hindawi
Journal of Immunology Research
Volume 2018, Article ID 5473678, 2 pages
https://doi.org/10.1155/2018/5473678

Editorial

Immune Responses to RNA Viruses

Elias A. Said,[1] **Felipe Diaz-Griffero**,[2] **Dorine Bonte**,[3,4] **Daniel Lamarre**,[5] **and Ali A. Al-Jabri**[1]

[1]*Department of Microbiology and Immunology, College of Medicine and Health Sciences, Sultan Qaboos University, P.O. Box: 35, 123 Muscat, Oman*
[2]*Department of Microbiology and Immunology, Albert Einstein College of Medicine, New York City, NY, USA*
[3]*CNRS UMR8200, Gustave Roussy Cancer Institute, Paris, France*
[4]*College of Pharmacy, Paris-Sud University, Paris, France*
[5]*Centre de Recherche du CHUM (CRCHUM) et Faculté de Médecine, Université de Montréal, Montréal, QC, Canada*

Correspondence should be addressed to Elias A. Said; elias.said@gmail.com, Daniel Lamarre; daniel.lamarre@umontreal.ca, and Ali A. Al-Jabri; aaljabri@squ.edu.om

Received 9 May 2018; Accepted 9 May 2018; Published 12 June 2018

Copyright © 2018 Elias A. Said et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

RNA viruses constitute an important threat to human health around the globe. Several RNA viruses are pandemic and infect hundreds of millions around the world leading to the death of millions of people every year. These viruses include the human immunodeficiency virus (HIV), hepatitis C virus (HCV), Ebola virus, Zika virus, respiratory syncytial virus (RSV), influenza viruses, yellow fever virus, dengue virus, rhinoviruses (common cold), human T-lymphotropic virus type 1 (HTLV-I), poliovirus, and measles virus. Currently, no vaccine or specific treatment is available for many of these viruses and some of the available vaccines and treatments are not highly effective. Because infection with RNA viruses is a global issue, the Journal of Immunology Research arranged for the publication of a special issue dedicated to the topic of immune responses to RNA viruses. The special issue contains 3 reviews and 2 research articles submitted by researchers from 6 countries in Europe, Asia, America, and Africa. These articles emphasized on the importance of the relationship between the components of the immune system and the treatments and vaccines directed against RNA viruses. They also highlighted the role of the immune system in the pathogenesis of the infections with these viruses.

HIV infection is a good example of the interactions between an RNA virus and the immune system and how these interactions modulate the pathophysiology of the disease caused by the virus. In this special issue, 3 articles focused on HIV infection. I. E. Akase et al. showed in "Immune Dysfunction in HIV: A Possible Role for Pro- and Anti-Inflammatory Cytokines in HIV Staging" that the levels of proinflammatory cytokines IL-6 and IL-10 are elevated in the absence of anti-HIV treatment. These cytokines, particularly IL-6, were associated with the WHO clinical staging of the disease. A trend of association was also found between these cytokines and the CD4 T cell count in HIV-infected patients. Moreover, L. Herráiz-Nicuesa et al. reported in their article entitled "Impact of the Polymorphism *rs9264942* near the HLA-C Gene on HIV-1 DNA Reservoirs in Asymptomatic Chronically Infected Patients Initiating Antiviral Therapy" that the −35 C allele is associated with low viremia and low levels of HIV reservoir. However, these associations did not reach statistical significance after thirty-six months of therapy. In addition, G. V. Gonzalez-Enriquez et al. reviewed in "SERINC as a Restriction Factor to Inhibit Viral Infectivity and the Interaction with HIV" the capacity of some HIV proteins, that is, Nef, Env, and glycosylated Gag (glycoGag), to interfere with the activity of serine incorporator 5 (SERINC5) restriction factor that prevents fusion and hence inhibits viral infectivity. The authors proposed that SERINC5 can be considered as an element in "promising scenarios" aiming to develop anti-HIV treatments and to predict the prognosis of the

disease. Additionally, in their review "Viruses Seen by Our Cells: The Role of Viral RNA Sensors," E. A. Said et al. discussed the implication of Toll-like receptors 3, 7, and 8 (TLRs 3, 7, and 8) in mounting proper anti-HIV responses and they reviewed the possibilities of targeting these molecules in future anti-HIV therapies and vaccine preparations.

The innate immune system plays a key role in sensing RNA viruses. This has a major influence on the antiviral responses and the pathogenesis of diseases caused by these RNA viruses. In their article, E. A. Said et al. also reviewed the role of pathogen recognition receptors (PRRs) in detecting nucleic acids as pathogen-associated molecular patterns (PAMPs) present in RNA viruses and how they balance the need for innate defenses against pathogens and actively restrict involuntary pathway activation. Moreover, they described the nature of the ligands and the pathways related to classic RNA helicases of antiviral innate immunity (RIG-I, MDA5) and of sentinel sensors (LGP2, SNRNP200, and DDX60) as well as of TLRs 3, 7, and 8 upon infection. They also reported on the implication of these molecules in the pathogenesis of the diseases caused by RNA viruses and the potential therapies and vaccines that involve these PRRs. In addition, in the review "Pulmonary Susceptibility of Neonates to Respiratory Syncytial Virus Infection: A Problem of Innate Immunity?," C. Drajac et al. questioned the role of the innate immune system in the susceptibility of neonates to the infection with RSV. They discussed the knowledge about the immune environment in the lung during the early-life periods at steady state and following RSV infection. They also reviewed the different experimental strategies that aimed to modulate the neonatal susceptibility to RSV.

Altogether, the authors of these articles highlighted the danger that infections with RNA viruses represent to global health. They provided clues about how understanding and interfering with the immune responses during these infections can be a major asset in the attempt to eradicate these viral infections. In summary, this special issue is providing information about different aspects of the immune responses during infections with RNA viruses. It is reporting on the current challenges in the field and providing new perspectives for the development of efficient treatments and vaccines directed against RNA viruses.

Elias A. Said
Felipe Diaz-Griffero
Dorine Bonte
Daniel Lamarre
Ali A. Al-Jabri

Hindawi
Journal of Immunology Research
Volume 2018, Article ID 9480497, 14 pages
https://doi.org/10.1155/2018/9480497

Review Article

Viruses Seen by Our Cells: The Role of Viral RNA Sensors

Elias A. Said,[1] **Nicolas Tremblay**,[2] **Mohammed S. Al-Balushi**,[1] **Ali A. Al-Jabri**,[1] **and Daniel Lamarre**[2]

[1]*Department of Microbiology and Immunology, College of Medicine and Health Sciences, Sultan Qaboos University, P.O. Box 35, 123 Muscat, Oman*
[2]*Centre de Recherche du CHUM (CRCHUM) et Faculté de Médecine, Université de Montréal, Montréal, QC, Canada*

Correspondence should be addressed to Elias A. Said; elias.said@gmail.com, Ali A. Al-Jabri; aaljabri@squ.edu.om, and Daniel Lamarre; daniel.lamarre@umontreal.ca

Received 23 November 2017; Revised 20 February 2018; Accepted 13 March 2018; Published 30 April 2018

Academic Editor: Mario Clerici

Copyright © 2018 Elias A. Said et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

The role of the innate immune response in detecting RNA viruses is crucial for the establishment of proper inflammatory and antiviral responses. Different receptors, known as pattern recognition receptors (PRRs), are present in the cytoplasm, endosomes, and on the cellular surface. These receptors have the capacity to sense the presence of viral nucleic acids as pathogen-associated molecular patterns (PAMPs). This recognition leads to the induction of type 1 interferons (IFNs) as well as inflammatory cytokines and chemokines. In this review, we provide an overview of the significant involvement of cellular RNA helicases and Toll-like receptors (TLRs) 3, 7, and 8 in antiviral immune defenses.

1. Introduction

If a living organism wants to engage, control, and eliminate a pathogenic entity, it must first be able to detect it. At first, this simple yet elegant paradigm might seem easy enough to crack experimentally, but in retrospect, it has been a central research question for more than 50 years now.

From the pioneering studies to identify interferon- (IFN-) inducing compounds, to the discovery of Toll-like receptors (TLR), RIG-I-like receptors (RLR), and the cGAS-STING pathway, the quest to understand how pattern recognition receptors (PRR) recognized pathogen-associated molecular patterns (PAMP) has shed light on a complex network of signaling pathways that are spatially compartmentalized, mostly pathogen specific and highly/tightly regulated.

In this review, we will focus on the significant contribution of cellular RNA helicases and TLRs 3, 7, and 8 to antiviral immune defenses.

2. The Classical RNA Helicases of Antiviral Innate Immune Responses

In the wake of the discovery of TLRs, it was historically postulated that antiviral immunity was mediated via TLR3 because this membrane-anchored receptor was essential to trigger the production of type 1 IFNs and the activation of IFN stimulated genes (ISGs) when challenged with extracellular double-stranded RNA (dsRNA) poly(I:C), as a viral surrogate [1]. However, further investigation revealed that mouse TLR3−/− dendritic cells (BMDCs) can produce high levels of IFNα when stimulated with intracellular dsRNA suggesting the existence of another type of RNA sensor, beside the TLRs, that would survey the cytoplasmic space for pathogenic nucleic acids [2]. Further studies would identify RIG-I, MDA5, and LGP2; all RNA sensors of what is now known as the RLR signaling pathway.

The retinoic acid-inducible gene I (RIG-I) was first identified as a cytoplasmic sensor that recognizes viral

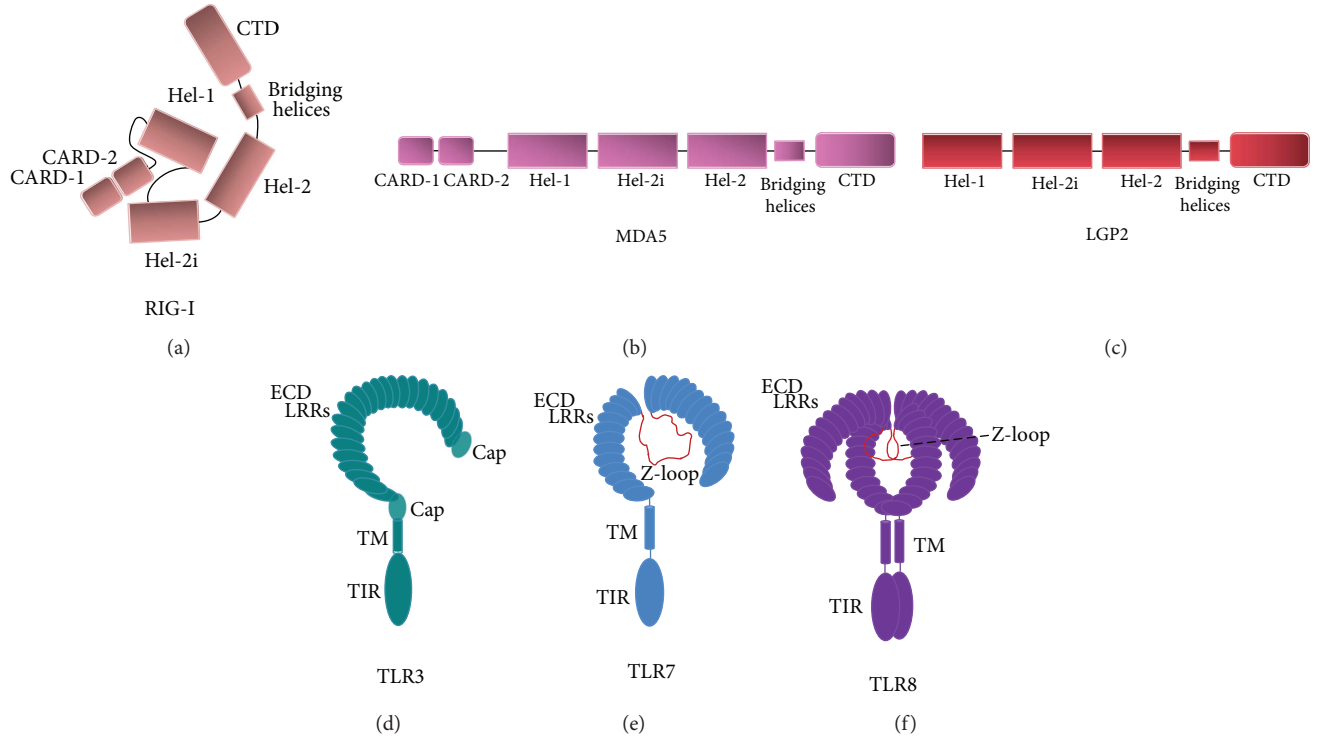


Figure 1: The structure of PRR implicated in detecting vRNA. RLRs are composed of a C-terminal domain (CTD), helicase domains (Hel), and two caspase-activation and recruitment domains (CARD-1, CARD-2) for (a) RIG-I and (b) MDA5, and only a CTD and helicase domain for (c) LGP2. TLRs 3, 7, and 8 are composed of an extracellular domain (ECD), a transmembrane (TM) domain, and a toll-interleukin 1 receptor (TIR) domain. The ECD contains 23 leucine-rich repeats (LRRs) for (d) TLR3 and 26 LRRs for (e) TLR7 and (f) TLR8. TLRs 7 and 8 have a Z-loop in the ECD. TLR8 exists as a dimer in the resting state.

nucleic acids and triggers a signal to induce innate immune responses during viral infection [3]. The protein comprises two caspase-activation and recruitment domains (2CARDs) at the N-terminal region, an RNA helicase domain, and a C-terminal domain (CTD) (Figure 1(a)). In resting cells, the CTD suppresses the N-terminal 2CARDs that are responsible for the association with mitochondrial antiviral-signaling (MAVS) (also called IPS-1, CARDIFF, and VISA) and required for triggering downstream signaling (Figure 2) [4]. After recognition of intracellular virus-derived RNA (vRNA), the binding of the CTD to vRNA induces the conformational change of the RIG-I protein, resulting in the release of the 2CARDs and allowing the proteins to assemble along the vRNA and to form a nucleoprotein filament. The released 2CARDs form a tetramer structure [5] that functions as a core for CARD-containing MAVS aggregation on the outer membrane of the mitochondria. RIG-I activation is tightly regulated by posttranslational modifications (PTMs) such as phosphorylation and ubiquitination [6, 7]. In resting cells, CK2, PKCα, and PKCβ protein kinases phosphorylate RIG-I, which keeps them in an inactive closed state to limit its activation (Figure 2) [8]. Upon viral infection, these PTMs are rapidly removed via two phosphatases (PP1α and PP1β) to shift RIG-I conformation into an active open state, which exposes its CARD domains and makes them available for subsequent ubiquitination [9]. Exposed CARDs are then targeted by TRIM25, Riplet, TRIM4, or MEX3C for K63 ubiquitin linkage, which is essential to allow its interaction with downstream adaptor protein MAVS and for the production of type 1 IFN (Figure 2) [10–18]. To prevent its overactivation, RIG-I is actively targeted by many cellular factors that inhibit K63 ubiquitination (CYLD, USP1, and USP3) or tag it for proteasome degradation via K48 ubiquitin linkage (RNF122, RNF125) [19–23]. Other PTMs, such as acetylation (HDAC6) and SUMOylation (P1AS2β, TRIM38, and SENP2), or direct association of cellular proteins with RIG-I to disrupt its interaction with MAVS (NLRC5, NLRX1), have also been shown to contribute to RIG-I activation or repression. However, their overall contribution to the canonical phosphorylation-ubiquitination system remains to be elucidated [24–28]. Once activated, RIG-I and MAVS interact via their CARD domains to form prion-like aggregates that become the immune platform for the phosphorylation of IRF3/NF-κB. This signaling relies on the recruitment of many regulatory subunits (TRAF2, TRAF5, TRAF6, and NEMO), which allows the phosphorylation of immune transcription factors via IKBKE, TBK1, and IKK protein kinases, leading to their nuclear translocation and the production of type 1 IFN with subsequent expression of ISGs (Figure 2) [29–34]. Based on sequence homology analysis, MDA5 and CARD-less LGP2 were identified as putative vRNA sensors (Figures 1(b) and 1(c)). The three proteins are collectively referred to as RLRs. Notably, these proteins have a similar helicase superfamily II (SF2) ATPase

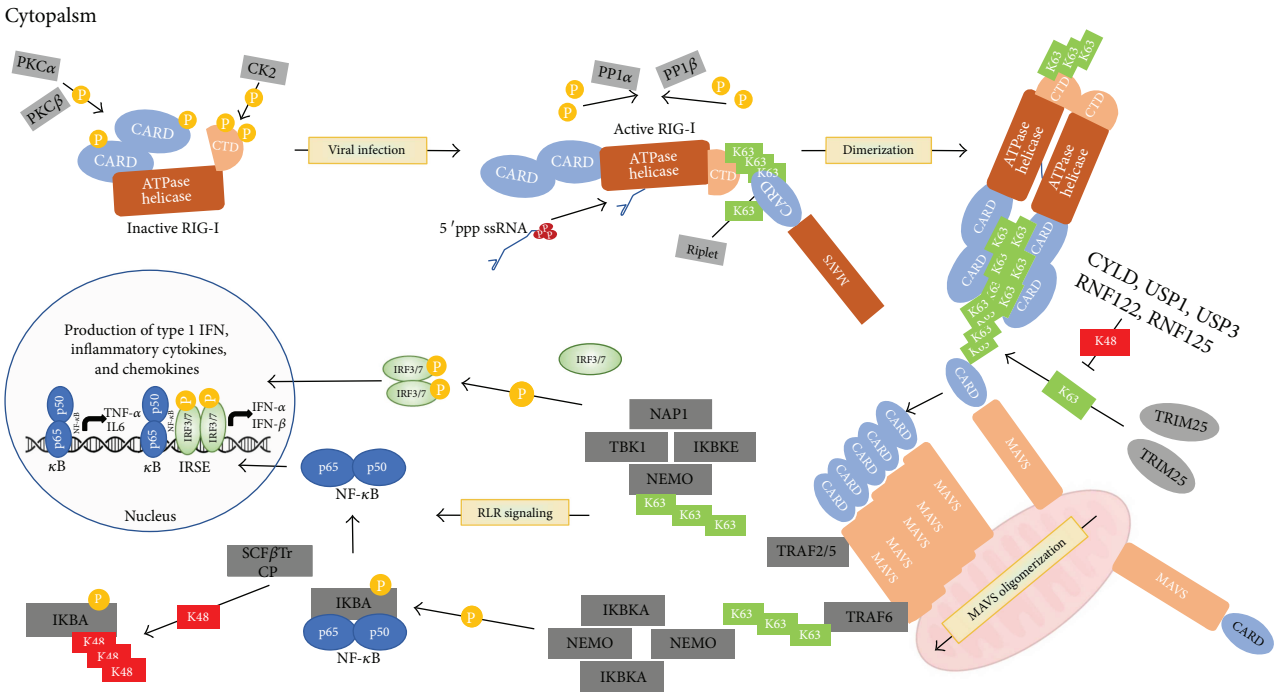


FIGURE 2: The pathways induced by RIG-I. Activation of RIG-I is regulated by many posttranslational modifications such as phosphorylation and ubiquitination. In resting cells, inactive RIG-I is kept in a close conformation by PKCα. PKCβ and CK2 phosphorylate both CARDs and CTD. Upon viral infection, PP1α and PP1β dephosphorylate RIG-I to allow the binding of viral RNA within its ATPase-helicase domain which shifts RIG-I to an open conformation and allows the CTD to be ubiquitinated by Riplet. Once activated, TRIM25 allows for the recruitment of K63-polyubiquitin chains via TRIM25 which allow RIG-I dimerization and recruitment to the adaptor protein MAVS. To balance immune activation, CYLD, UPS1, UPS3, RNF122, and RNF125 actively antagonize RIG-I activation by the degradation of K63-polyubiquitin chains and a switch to K48-polyubiquitin chains that tag RIG-I for proteasome degradation. This interaction allows for the oligomerization of MAVS and the recruitment of regulatory subunits TRAF2, TRAF5, TRAF6, and NEMO. This signaling culminates with the phosphorylation of immune transcription factors via IKBKE, TBK1, and IKK protein kinases, leading to their nuclear translocation and production of type 1 IFN with subsequent expression of ISGs.

domain and CTD that will prove to be essential for their nucleic acid sensing function and distinguishing between different RNAs.

3. RLR Distinction of RNA Ligands

RIG-I and MDA5 are RNA helicases that survey the cytoplasm in search of PAMP (Figure 2). They have distinct but overlapping pathogenic RNA preferences, which enable differentiation of cytosolic self and nonself RNA. Initial studies in mouse embryonic fibroblasts deficient for MDA5 (MDA5−/−) showed that they can initiate an antiviral response when challenged with intracellular nonself RNA molecules containing a triphosphate moiety at the 5′ region (5′ppp) while RIG-I−/− cells cannot [35]. Moreover, when the 5′ region is capped or is treated with calf intestinal alkaline phosphatase to remove the phosphates, no stimulations are observed [36]. These findings gave the first evidence that RIG-I can recognize uncapped and phosphorylated 5′ RNAs while MDA5 could not. Subsequent studies showed that RIG-I is more likely to recognize short double-stranded RNA (dsRNA) molecules while MDA5 is activated by long dsRNA [12, 37, 38]. More recently, influenza U/A-rich 3′ regions of viral RNA segments were shown to activate RIG-I in a 5′ppp-independent manner via an unknown mechanism (Table 1) [39]. This recognition might be mediated by RIG-I's helicase domain instead of the paradigmatic CTD. Additional studies, such as examination of the crystal structures of the full RIG-I/MDA5 proteins bound to vRNA, are required to understand the fine molecular mechanisms related to the vRNA and the sensor structural properties, in order to have one unifying and comprehensive theory. Nevertheless, the physical characteristics of RLR ligands correlate exceptionally well with the type of viruses that are recognized by RIG-I, such as Sendai virus (SeV), vesicular stomatitis (VSV), influenza A (FLUA), and hepatitis C virus (HCV), and by MDA5, such as encephalomyocarditis virus (EMCV), norovirus, or murine hepatitis virus (MHV) (see Table 1) [39–41]. In short, RIG-I can recognize viruses that produce short and phosphorylated replication intermediates through its CTD, whereas MDA5 tends to recognize long vRNA molecules. Altogether, these data support the concept that cytoplasmic RNA helicases are sensors of nonself RNA and work together to ensure an optimal coverage of the full spectrum of viral nucleic acids, including replication intermediates and copy-back defective interfering (DI) genomes. Furthermore, these observations emphasize the

Table 1: RNA viruses and ligands recognized by RLR and TLR.

RNA sensor	RNA preference	Representative viruses
Helicases		
RIG-I	Uncapped 5′ and phosphorylated ssRNA, short dsRNA, and U/A-rich 3′ regions of viral RNA	Adenovirus, DENV, EBOV, FLUA/B, HCV, HSV, JEV, LACV, LASV, MV, NDV, NV, PIV5, Reoviridae, RSV, RV, RVFV, SeV, VSV, and WNV
MDA5	Long dsRNA	Adenovirus, DENV, EBV, ECMV, enteroviruses, HCV, HSV, JEV, MV, NDV, norovirus, NV, PIV5, Reoviridae, RSV, SeV, SAFV3, TMEV, and WNV
LGP2*	dsRNA	ECMV and HCV*
DDX60	dsRNA	HCV, RSV, and VSV
SNRNP200	dsRNA	FLUA, HCV, and SeV
TLRs		
TLR3	dsRNA	CVB3, HSV-1, poliovirus, Reoviridae family (rotavirus), RSV, and WNV
TLR7	GU- and U-rich ssRNA	DENV, EBOV, FLUA, HCV, HIV, HTLV-I, MV, poliovirus, rhinoviruses, and YFV
TLR8	GU- and U-rich ssRNA	FLUA, HCV, HIV, rhinoviruses, and YFV

CVB3: Coxsackie B virus; DENV: dengue virus; EBOV: Ebola virus; EBV: Epstein-Barr virus; ECMV: encephalomyocarditis virus; FLUA: influenza A virus; FLUB: influenza B virus; HCV: hepatitis C virus; HIV: human immunodeficiency virus; HSV: herpes simplex virus; JEV: Japanese encephalitis virus; LACV: La Crosse virus; LASV: Lassa virus; MV: measles virus; NV: Nipah virus; PIV5: parainfluenzas virus 5; RSV: respiratory syncytial virus; RV: rabis virus; RVFV: Rift Valley fever virus; SAFV3: Saffold virus 3; TMEV: Theiler's virus; SeV: Sendai virus; VSV: vesicular stomatitis virus; WNV: West Nile virus; YFV: yellow fever virus. *More studies are required to clarify the capacity of LGP2 to detect viruses including ECMV and HCV.

importance of the 5′-triphosphate and dsRNA as molecular patterns that enable RIG-I/MDA5 to distinguish pathogenic from self RNA.

Now, how does the functioning of these RNA helicases differentiate self RNA from pathogenic RNA leading to the initiation of the RLR/MAVS antiviral signaling pathway? As discussed before, in resting cells, RIG-I and MDA5 are kept in a closed conformation (signal off) by their CTD. Upon contact with vRNA molecules, it is proposed that an ATP-dependent translocation along the dsRNA leads to the high-affinity binding with the CTD to expose the 2CARDs and to the promotion of the formation of stable RIG-I dimers [42–44]. Importantly, the ATP-dependent translocation was recently shown to contribute to the self versus nonself RNA recognition, as the ATPase/translocase activity removes RIG-I from abundant self RNA while locking it into the nonself RNA motifs following translocation, and binding it to the viral determinant such as 5′ppp, reducing background signaling and increasing sensitivity of vRNA detection [42, 44]. Following the recognition of the proper RNA ligand, RIG-I signaling is activated and cells enter an antiviral state characterized by the production of antiviral type 1 IFN and ISGs (Figure 2) [29–32, 34, 45]. If RIG-I binds to nonpathogenic RNA, it will be displaced by ATP hydrolysis to prevent the recognition of endogenous RNA and avoid unintentional signaling due to prolonged RNA binding. The lack of proper ATP hydrolysis by RNA sensors, such as RIG-I and MDA5, was recently linked to many genetic disorders whose pathogenesis is caused by an upregulated type 1 IFN signaling that leads to many autoimmune disorders such as Aicardi-Goutières syndrome (AGS), Singleton-Merten syndrome (SMS), systemic lupus erythematosus (SLE), and type 1 diabetes [46]. These disorders are caused by nonsynonymous point mutations located within the helicase/ATPase domain of MDA5 and RIG-I that confer a constitutive activation and implicate the aberrant sensing of nucleic acids for the inappropriate production of type 1 IFNs [47–50]. These studies emphasize the importance of a functional SF2 helicase domain for the discrimination of self and nonself RNA and the elicitation of an adequate and controlled immune response.

4. RNA Helicases as Sentinels for Cytoplasmic RNA and Antiviral Immune Responses

LGP2 is an RNA helicase, homologous in structure to RIG-I and MDA5, except that it lacks the 2CARDs that are required to initiate antiviral signaling via the MAVS adaptor protein (Figure 1(c)). Thus, LGP2 is not able to propagate the signal to produce type 1 IFN and must have a role that is different from RIG-I and MDA5 in the RLR pathway (Figure 2). Initially, LGP2 was proposed as a negative feedback regulator of the RLR pathway that would act by sequestering vRNA from RIG-I [51] or by displacing IKBKE from MAVS in order to terminate IRF3-dependent antiviral signaling [52]. Subsequent studies showed that CTDs of LGP2 and RIG-I are analogous and provided *in vitro* evidence that LGP2 CTD can interact with RIG-I to abolish its ability to initiate antiviral signaling [4, 53]. In addition, the latest study is reminiscent of the novel negative regulator of innate immunity KHSRP that associates with the CTD of RIG-I to maintain the receptor in an inactive state and attenuate its sensing of vRNA [54]. Upon viral infection, KHSRP competes with PAMP for the RNA recognition site located within RIG-I's CTD. This competition between KHSRP and vRNA is thought to be essential to maintain a proper activation threshold of RIG-I signaling and prevent unnecessary or disproportionate activation of the RLR pathway. Despite

some initial controversies about its function in antiviral signaling, LGP2 is emerging as a sentinel sensor that cooperates with RIG-I and MDA5 to enhance their recognition of the vRNA substrate and to initiate type 1 IFN response against viruses such as ECMV and HCV (Table 1) [55–57]. According to this model, LGP2 can leverage upon its ATP-dependent/RNA helicase activity to assist and increase interactions of a larger subset of nucleic acid-derived PAMP with RIG-I or MDA5 and finally potentiate antiviral signaling. Additionally, it was recently shown that LGP2 inhibits a DICER-mediated processing of vRNA [58]. In contrast to the elaborated protein-based system found in mammals, plants and invertebrates rely on their RNA interference (RNAi) machinery to degrade vRNA and subvert viral replication [59]. This recent report provides evidence that LGP2 antagonizes the degradation of vRNA by DICER to keep the cytosolic PAMPs intact and allow their detection by RNA sensors. Further studies should provide key insights about the relationship between the antiviral RNAi system, LGP2, and the RLR pathway in mammalian cells. Interestingly, LGP2 sentinel function seems to be shared by many other DExD/H box RNA helicases such as DDX3, DHX9, DHX29, and DDX41, which bind directly to nucleic acids and interact with either RIG-I or MAVS to activate the pathway (see [60, 61]). Furthermore, RNA helicases from the Ski-2-like family have been described to act as sentinels for RIG-I activation and viral RNA degradation, as well as negative regulators of the RLR pathway. Indeed, SKIV2L teams up with exosomes to degrade RNA and limits activation of the RLR pathway upon activation of the unfolded protein response (UPR), and humans with a deficiency in SKIV2L have a type 1 interferon signature in their peripheral blood [62]. In this review article, we will concentrate on DDX60 and SNRNP200 to show the prototypical characteristics of a Ski-2-like helicase as a sentinel for cytoplasmic antiviral response. The DDX60 RNA helicase also acts as a cofactor of the exosome complex, which is involved in the degradation of various types of RNA molecules to maintain the quality of host RNA. However, upon viral infection, DDX60 acts as an ISG that helps cells to suppress viral replication by increasing interactions between vRNA and RIG-I/MDA5 to enhance antiviral signaling and type 1 IFN production [63]. DDX60 is also able to promote exosome-mediated degradation of HCV RNA (Table 1) that reduces cell stress from viral replication as a first line of defense, but in turn produces degraded vRNA agonists that are likely to be recognized by RIG-I/MDA5 and other sentinels in a feed-forward mechanism that enhances type 1 IFN production [64]. Overall, while additional studies are required to assess the role of DDX60 against many viruses and across different cell lines, the first insight into its mechanism of action highlights two important features of Ski-2-like RNA helicases as sentinel for cytoplasmic RNA: (1) they are able to detect vRNA and bring them to RNA sensors (RIG-I) to augment antiviral signaling by allowing for a more efficient detection of a cytoplasmic PAMP and (2) they are able to target vRNA to the RNA exosome, which turns them into immune-stimulatory molecules by revealing a molecular signature (e.g., short 5′ppp dsRNA) that can be recognized by RIG-I. More recently, we identified a novel sentinel, SNRNP200, a member of the Ski-2 RNA helicase family that is critical in the RIG-I/MAVS signaling pathway by promoting vRNA sensing and IRF3 activation via a direct interaction with TBK1 [65]. SNRNP200 is an essential member of the spliceosome complex along with several other RNA helicases that are responsible for removing introns from the pre-mRNA and give rise to coding mRNA [66–70]. Upon viral infection, SNRNP200 binds vRNA through its amino-terminal Sec 63 (Sec63-1) domain, relocates to the perinuclear region, and acts as an adaptor protein to potentiate IRF3 signaling. Much like other DExD/H box RNA helicases, SNRNP200 requires a functional ATPase/helicase activity in addition to a competent Sec63-1 domain of unknown function to promote IRF3-dependent IFN induction upon virus infection. Directed mutagenesis experiments further showed that a defective SNRNP200 C502A variant within the ATP-binding motif leads to constitutive type 1 IFN production *in vitro* [71], reminiscent of a phenotype of type 1 interferonopathies [46–50]. Thus, the immunoregulatory function of SNRNP200 recapitulates properties of RIG-I/MDA5 and sentinels; they all leverage upon their ATPase/helicase domain to unwind vRNA and detect and bind to a specific RNA motif as they translocate along the RNA strand, serving as scaffolding proteins to initiate antiviral signaling. This mode of action limits recognition of nonpathogenic RNA and the unnecessary activation of RLR signaling (as reviewed in [72]). In this perspective, it is reasonable to propose that antiviral RNA helicases are involved in the larger picture of RNA responsiveness, where they balance the need for innate defenses against pathogens and actively restrict involuntary RLR pathway activation.

5. Toll-Like Receptors (TLRs)

TLRs have an important role in recognizing molecular patterns associated with different pathogens. 11 TLR genes are present in the human genome, with TLR11 being a nonfunctional pseudogene. The majority of the TLRs are found on the plasma membrane, while TLRs 3, 7, 8, and 9 are present in the endosomal compartment [73]. Whereas those expressed on the cell surface predominantly recognize molecules of the microbial membrane, for example, proteins, lipids, and lipoproteins, endosomal TLRs detect viral, bacterial, or self nucleic acids. In this review we will focus on TLRs 3, 7, and 8 for their role in detecting extracellular RNA and viral particles [73].

6. TLR3 Expression and Ligands

TLR3 is expressed in the endosomes of immune cells, that is, monocytes, macrophages, dendritic cells (DCs) (other than plasmacytoid DCs), natural killer (NK) cells, T and B lymphocytes, mast cells, eosinophils, and basophils. Nonimmune cells, such as epithelial and endothelial cells, keratinocytes, fibroblasts, hepatocytes, astrocytes, and microglia, also express TLR3 [74, 75]. TLR3 recognizes dsRNA, the synthetic polyinosinic-polycytidylic acid (poly I:C), and polyadenylic-polyuridylic acid (poly A:U) (Table 1) [74, 75].

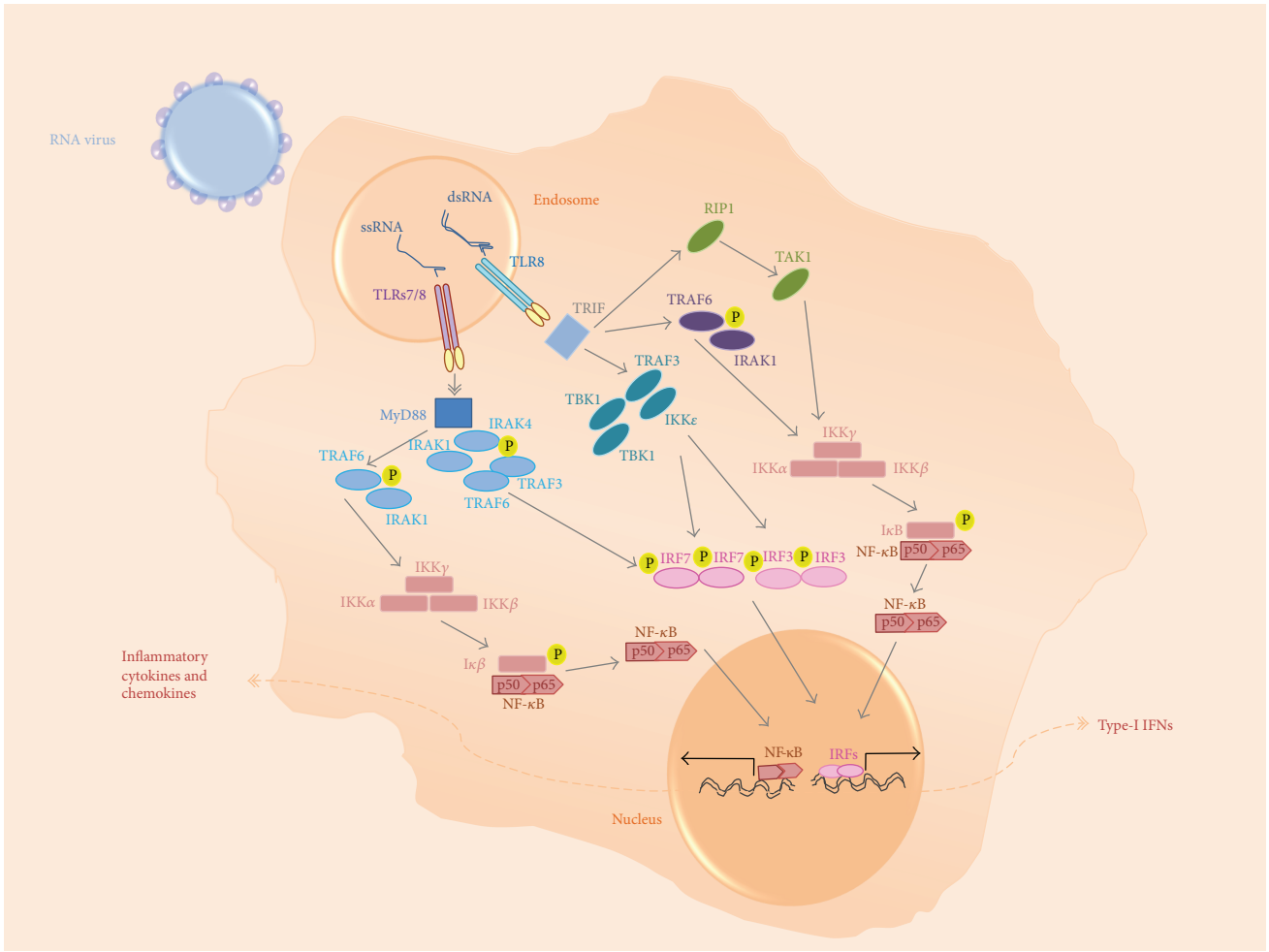


Figure 3: The pathways induced by TLRs 3, 7 and, 8. TLR3, TLR7, and TLR8 are expressed in the endosomes. The triggering through these molecules leads to the activation of NF-κB and IRF signaling pathways, which results in the production of inflammatory cytokines and chemokines as well as type 1 IFNs.

Moreover, TLR3 may be triggered by single-stranded RNA (ssRNA) with stable stem structures as described based on poliovirus RNA sequences [76]. However, further studies may be required to elucidate the exact mechanisms of such triggering.

TLR3 plays a significant role in the modulation of RNA and DNA virus-mediated innate immune responses. TLR3 senses dsRNA viruses such as members of the Reoviridae family including the rotavirus by sensing their genomic RNA; this recognition leads to the induction of inflammatory cytokines and type 1 IFNs [74, 77]. Moreover, TLR3 recognizes intermediate RNAs that are produced during the replication of other viruses such as the herpes simplex virus-1 (HSV-1), respiratory syncytial virus (RSV), West Nile virus (WNV), coxsackievirus B3 (CVB3), poliovirus, and influenza A virus (FLUA). The viral dsRNAs can reach the TLR3 in the endosomes upon phagocytosis of dying infected cells or by direct uptake from the medium by antigen presenting cells (Table 1) [74, 77]. The possibility of the presence of intermediate viral ssRNAs with stable stem structures as a reason for the detection of these viruses by TLR3, as observed in the case of poliovirus, remains to be investigated [76].

7. TLR3 Structure and Signaling Pathways

TLR3 has a C-terminal cytoplasmic toll-interleukin 1 receptor (TIR) domain used for signaling, an N-terminal extracellular domain (ECD), and a single transmembrane alpha helix. The ECD has 23 leucine-rich repeats (LRRs); it is responsible for the binding of dsRNA (Figure 1(d)). The dimerization of ECDs initiates the signaling [74, 78]. The TIR domain-containing adaptor protein-inducing IFN-β (TRIF) is then recruited and undergoes slight conformational changes [79] to form a signaling complex together with TNF receptor-associated factor 6 (TRAF6), TRAF3, TBK1, IKKε, and IKK (Figure 3). This leads to the activation of IRF3/IRF7 and NF-κB, which results in the production of type 1 IFNs and inflammatory cytokines, respectively [74, 78].

In order to control the levels of inflammation induced by the triggering of TLR3, its signaling pathway is regulated by different molecules. Some act as positive regulators such as serine/threonine kinase receptor-associated protein (STRAP) that interacts with TBK1 and IRF3 [80], munc18-1-interacting protein 3 (Mint3) that stimulates the K63-linked polyubiquitination of TRAF3 [81], Src-associated substrate

in mitosis of 68 kDa (Sam68) that may balance NF-κB p65 and c-Rel activation [82], and finally S100A9 that acts during the early stages of TLR3 activation by easing the maturation of TLR3-containing early endosomes into late endosomes [83]. Other molecules act as negative regulators, such as Rho proteins that decrease the production of proinflammatory cytokines upon TLR3 triggering [84], SUMO-specific protease 6 (SENP6) that inhibits the NF-κB-mediated expression of the proinflammatory genes [85], and miR-155 that controls TLR3 signaling by repressing molecules such as TAB2, IKK-ε, and RIP [86]. Interestingly, some oncogenic herpes viruses such as Kaposi's-sarcoma-associated herpes virus (KSHV) and Epstein-Barr virus (EBV) induce cellular miR-155 expression or encode the functional ortholog of miR-155, which might constitute a strategy to escape the immune responses induced upon TLR3 triggering [86]. In addition, several proteins in the TLR3 pathway are targeted by different PTMs, which also participate in the regulation of responses initiated by TLR3 triggering [6].

8. TLR3 and the Pathogenesis of Viral Infections

TLR3 has an important impact on the pathogenesis and outcome of several RNA virus infections. In fact, the level of expression of TLR3 is associated with the severity and outcome of HCV infection [87]. Moreover, single-nucleotide polymorphisms (SNPs) in the *TLR3* gene are associated with HCV-mediated liver disease progression and the development of hepatic fibrosis [88]. As mentioned above, TLR3 also plays an important role in establishing immune responses against HSV-1. Different studies showed that mutations in the *TLR3* gene are associated with the predisposition to HSV-1 encephalitis (HSE) in children [89–92] and adults [93, 94]. These mutations in TLR3 were shown to result in a lack of response to poly I:C and HSV-1 as observed in fibroblasts and induced pluripotent stem cell- (iPSC-) differentiated neural stem cells (NSCs), neurons, astrocytes, and oligodendrocytes [89, 90]. This impairment was characterized by the absence of production of IFN-β and IFN-λ in these cells [89, 90]. The association of mutations in the *TLR3* gene with varicella-zoster virus encephalitis was also shown [93]. Other studies have shown that TLR3 may influence the pathogenesis of RSV, CB3, and enterovirus 71 (EV71), severe fever with thrombocytopenia syndrome (SFTS), and HBV infections [95–99]. This highlights the important role played by TLR3 in the innate immune responses to viruses, although the exact mechanisms of recognition and how it is involved often remain elusive.

9. Targeting TLR3 in Antiviral Therapies and Vaccines

The potential use of TLR3 ligands in antiviral therapies and vaccines is suggested by different studies. For example, recently TLR3 ligands were shown to be efficient in reversing the latency of the human immunodeficiency virus (HIV) by the reactivation of HIV transcription in microglial cells [100]. Another study reported TLR3 ligands as candidates for anti-HIV immunotherapeutic strategies because these ligands increased the ability of HIV-infected DC to activate HIV-specific cytotoxic T lymphocytes [101]. TLR3 ligands were also shown to be potent adjuvants for vaccine preparations targeting influenza virus, HIV, and HSV-2 [102–104]. Interestingly, poly I:C derivatives (known as Ampligen) are potential adjuvants tested in vaccine preparations targeting influenza virus, HIV, and HPV [102].

10. TLRs 7 and 8: Expression and Ligands

TLRs 7 and 8 are expressed in the endosomes of a wide variety of cells including immune cells such as monocytes, macrophages, DC, and NK cells [105]. The expression of TLR7 is also reported in T and B cells [105, 106]. TLR8 is also expressed in mast cells and regulatory T cells [107, 108]. The expression of TLRs 7 and 8 is not restricted to immune cells, as they are also expressed in endothelial and epithelial cells, astrocytes, microglia, and hepatocytes, as well as tumor cells [109–111].

TLRs 7 and 8 share a lot of similarities, and recent findings suggest a potential compensatory role played by TLR8 in the absence of TLR7 [112]. TLRs 7 and 8 recognize guanosine and uridine- (GU-) rich or U-rich ssRNA sequences [113, 114]. However, we have shown that the presence of GU-rich sequences in ssRNA might not be sufficient, although necessary, to stimulate these TLRs [115]. In this study, several GU-rich sequences in the HCV genome were described; however, not all these sequences were able to trigger TLRs 7 and 8. In fact, the capacity of these sequences to trigger TLRs 7 and 8 was not influenced by their length or the number of GU repeats that they contain [115]. Interestingly, some cellular defense mechanisms that target vRNA may influence its detection by TLRs 7 and 8. In fact, the detection of phagocytosed vRNA by TLRs 7 and 8 is facilitated by the adenosine-to-inosine (A-to-I) editing, which is an important arm of the antiviral response [116]. Furthermore, $2'$-O-methylation within an RNA sequence shapes differential activation of TLRs 7 and 8 [117, 118]. This modification leads to the triggering of TLR8 but not TLR7 by an RNA that was initially able to trigger both TLRs. The hypothesis that this might be due to a stronger binding by TLR7 than TLR8 will require further investigation. This change in the triggering leads to a different secretion of proinflammatory cytokines as it impairs IFN-α production but not IL-6 [118].

Because of the capacity to sense ssRNA, TLRs 7 and 8 have an important role in detecting RNA viruses and inducing antiviral immune responses. They can be triggered by viral GU- and U-rich ssRNA sequences, such as those in highly conserved untranslated terminal regions (UTR) of viral genomes that have a crucial role in viral protein translation and RNA replication [119]. The implication of TLR7 or TLR8 in detecting RNA viruses is different depending on the virus and the cell in which these TLRs are expressed. Viruses, such as yellow fever virus (YFV), rhinoviruses, and HIV, can be detected by both TLR7 and TLR8 [113, 120, 121]. However, the expression of TLRs 7 and 8 in a cell does not always guarantee their triggering by an RNA virus, even though the latter has RNA sequences that can be detected by these TLRs.

This was shown in the case of the HCV genome, which has sequences that stimulate both TLRs 7 and 8 [115]. Nevertheless, the complete HCV particles do not induce responses through these TLRs in myeloid and plasmacytoid DC subsets and monocytes, whereas such stimulation takes place in macrophages without stimulating antiviral responses [115]. Differences in the ability of cells to detect an RNA virus via TLRs 7 and 8 were also described for Zika virus (ZIKV) infection, as no TLR7 activation was detected in primary human fibroblasts [122], while genes implicated in TLR7 and TLR8 pathways were found to be upregulated in the human neural progenitor cells (hNPCs) infected with this virus [123]. Moreover, some vRNAs are recognized by TLR7 but not by TLR8. This may suggest the presence of differences in the conditions that lead to the detection of ssRNA sequences by TLR7 and TLR8. For example, the measles virus (MV), Ebola virus (EV), dengue virus (DV), human T-lymphotropic virus type 1 (HTLV-I), and poliovirus are able to trigger TLR7 only, while the role of TLR8 in such recognition remains unclear (Table 1) [74, 124]. Nevertheless, SNPs in *TLR7* and *TLR8* genes were associated with immune responses to MV suggesting a role for both TLRs during MV infection [125].

11. TLRs 7 and 8: Structures and Signaling Pathways

TLRs 7 and 8 are single-pass transmembrane receptors composed of a pathogen-recognition LRR-containing ectodomain and a TIR domain [126]. TLRs 7 and 8 have 26 LRR motifs in their extracellular domain, which contain multiple insertions such as the Z-loop or undefined region situated between LRRs 14 and 15 (Figures 1(e) and 1(f)) [127]. Both TLRs are proteolytically cleaved in the endosomes at the level of the Z-loop by arginine endopeptidase and cathepsins, and the cleaved fragments are linked together [128]. This is essential for the dimerization and activation of these TLRs [129]. TLR7 and TLR8 dimers have a binding site for small chemical stimuli or degradation products of ssRNA and a second binding site that recognizes ssRNA oligonucleotides. Both these sites are required for ssRNA-induced activation [130, 131]. The TIR domains multimerize following the interaction of TLRs 7 and 8 with their agonists, which is important for the recruitment of myeloid differentiation primary response gene 88 (MyD88) [132]. MyD88 forms a complex with interleukin 1 receptor-associated kinase (IRAK) molecules. The pathway will eventually lead to the activation of transcription factors including IRF7 and NF-κB, which will cause the production of type 1 IFNs and inflammatory cytokines, respectively (Figure 3) [132].

A number of molecules regulate TLR7 and TLR 8 signaling pathways and control the immune responses that are triggered upon stimulation of these TLRs. Some of these molecules are positive regulators such as UNC93B1, which physically associates with TLRs 7 and 8 and delivers them to endolysosomes [133]; hepatocyte growth factor regulated tyrosine kinase substrate (HRS) that is required for proper TLR7 trafficking to endolysosomal networks [134]; CCAA T/enhancer-binding protein beta (C/EBPδ) that enhances the transcription of TLR8 [135]; triggering receptor expressed on myeloid cells like 4 (TREML4) that enhances TLR7 signaling [136]; and pyruvate dehydrogenase kinase isozyme 2 (PDK2) that physically interacts with TRAF6 [134]. Spleen tyrosine kinase (Syk) was also shown as a positive regulator of the TLR7 pathway in the plasmacytoid DC (pDC) subsets. However, Syk may also negatively regulate the TLR7 pathway upon the stimulation of the regulatory immunoreceptors CD303 and CD85g in pDC, which suggests the presence of a dual role for Syk in the regulation of the TLR7 pathway [137]. Other molecules are also considered as negative regulators for the TLR7 pathway such as tripartite motif 35 (TRIM35) that stimulates the K48-linked ubiquitination of IRF7 [138] and SENP6 described above in the TLR3 section [85]. More studies are required to identify molecules that negatively regulate TLR8 signaling. Furthermore, different proteins implicated in the TLR7/8 pathway are subject to PTMs, which have a direct impact on the regulation of TLR7- and TLR8-induced responses [6].

12. TLRs 7 and 8 and the Pathogenesis of Viral Infections

TLRs 7 and 8 influence the pathogenesis and outcome of several RNA virus infections such as HCV. In fact, the spontaneous resolution of the HCV infection has been shown to be associated with a sustained hyperresponsiveness of pDCs and mDCs to TLR7/8 stimulation [139], and the clearance and progression of the HCV infection is modulated by variations in the TLR7 and TLR8 genes [140]. Moreover, the potential capacity of the vRNA of different influenza strains to stimulate TLRs 7 and 8 was found to be correlated to the virulence of the strains [141]. In addition, SNPs in the *TLR7* and *TLR8* genes were associated with the CD4 T cell count during an HIV infection [142] as well as the levels of type 1 IFN and proinflammatory cytokines and the progression to hepatocellular carcinoma during an HCV infection [143, 144]. Also, the low copy numbers of the *TLR7* gene is associated with the establishment of chronic HBV infection [145].

The triggering of TLRs 7 and 8 by viruses is not always an advantage for the immune system. HIV infection provides several examples for this phenomenon. In fact, TLR7 stimulation by the HIV ssRNA in CD4 T cells induces the anergy of these cells [146]. HIV requires the stimulation of NF-κB upon the triggering of TLR8 to replicate in DCs [147]. In addition, HIV takes advantage of the cellular protein snapin that inhibits its detection by TLR8 in DCs to transinfect other cells [148]. In fact, inhibiting snapin expression leads to an increased localization of HIV-1 within the early endosomes that contain TLR8, the establishment of a proinflammatory response, and the inhibition of CD4 T cell transinfection [148].

13. Targeting TLRs 7 and 8 in Antiviral Therapies and Vaccines

TLR7 and TLR8 ligands are potential candidates for antiviral therapeutic and vaccine strategies. Hence, the capacity of

TLR7 and TLR8 ligands to inhibit HIV replication and to activate the HIV reservoir is being investigated [149, 150]. Moreover, TLR7 and TLR8 ligands were proposed to be used as adjuvants in FLU vaccine preparations [151]. Furthermore, the TLR7 agonist Imiquimod (R-837 or trade name Aldara) and TLR7/8 dual agonist Resiquimod (R-848) are topical treatments for HPV-induced warts [102]. Although systemic administration of Imiquimod may be highly toxic, Resiquimod showed promising results as an adjuvant in an anti-HSV trial [102].

14. Conclusion and Perspectives

Up to this point, we have established the key players and mechanisms of the antiviral innate immunity protecting the host from RNA viruses. We have shown that RNA helicases and TLRs 3, 7, and 8 are essential nucleic acid sensors that survey the cytoplasmic and endosomal spaces for extracellular threats and, upon engagement, elicit type 1 IFN responses to restrict viral replication. Recent findings showing the involvement of unconventional PTMs, such as SUMOylation and acetylation, to the regulation of these PRRs have cleared the way to a better understanding of antiviral signaling, host-factor interactions, and the etiology of various autoimmune diseases. Further studies using a system-based approach, similar to the one used to identify SNRNP200 and KHSRP, together with the understanding of the nature of ligands and inhibitors of PRRs should provide additional knowledge to identify novel approaches for treatments and vaccine preparations directed against RNA viruses and beyond, in autoimmune diseases and cancers [102, 152]. Moreover, the potential ability of RNA viruses to interfere with the mechanisms regulating the signaling of these PRRs in order to escape detection necessitates more investigations. Additionally, with the description of a myriad of novel host factors involved in RLR signaling, one might wonder which components (RNA sensors, sentinels, positive, and negative regulators) are required for the minimum or optimal antiviral response, and what are the differences in this hierarchy according to cell type or pathogen. There is a coordination between TLRs and RLRs, as seen in some autoimmune diseases and viral infections [153–156]. The mechanisms that control this cooperation in detecting RNA viruses, and the consequences of such collaboration, deserve to be investigated in more depth. Lastly, PRR-targeting therapies have gained great momentum in the field of cancer immunotherapy. Recent reports have shown that RIG-I activation can induce tumor cell death directly via the production of IFN, or indirectly via the activation of cytotoxic CD8 T cells and NK cells, and via DC-mediated antigen cross-presentation of tumor-associated antigens to CD8 T cells [68]. In addition, the modulation of TLR3 and 7 can be leveraged as anticancer therapies since their signaling can increase cytotoxic T cell activity and directly induce cancer cell death via apoptosis, pyroptosis, and autophagy. Thus, the recent advances in our understanding of innate antiviral immunity have clearly given a new momentum towards the development of therapeutic agents targeting PRR for infectious diseases and cancers. These strategies are in the preclinical or early clinical phase such that it is still unknown if these PPR-targeting agents will translate into effective, safe, and tolerable anticancer therapeutics.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Authors' Contributions

Elias A. Said and Nicolas Tremblay contributed equally.

Acknowledgments

The authors thank Dr. Mary Lane-Kelso and Mr. Alan Kelso for help in manuscript revision.

References

[1] L. Alexopoulou, A. C. Holt, R. Medzhitov, and R. A. Flavell, "Recognition of double-stranded RNA and activation of NF-κB by Toll-like receptor 3," *Nature*, vol. 413, no. 6857, pp. 732–738, 2001.

[2] S. S. Diebold, M. Montoya, H. Unger et al., "Viral infection switches non-plasmacytoid dendritic cells into high interferon producers," *Nature*, vol. 424, no. 6946, pp. 324–328, 2003.

[3] 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.

[4] T. Saito, R. Hirai, Y. M. Loo et al., "Regulation of innate antiviral defenses through a shared repressor domain in RIG-I and LGP2," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, no. 2, pp. 582–587, 2007.

[5] B. Wu, A. Peisley, C. Richards et al., "Structural basis for dsRNA recognition, filament formation, and antiviral signal activation by MDA5," *Cell*, vol. 152, no. 1-2, pp. 276–289, 2013.

[6] J. Liu, C. Qian, and X. Cao, "Post-translational modification control of innate immunity," *Immunity*, vol. 45, no. 1, pp. 15–30, 2016.

[7] Y. Zhou, C. He, L. Wang, and B. Ge, "Post-translational regulation of antiviral innate signaling," *European Journal of Immunology*, vol. 47, no. 9, pp. 1414–1426, 2017.

[8] N. P. Maharaj, E. Wies, A. Stoll, and M. U. Gack, "Conventional protein kinase C-α (PKC-α) and PKC-β negatively regulate RIG-I antiviral signal transduction," *Journal of Virology*, vol. 86, no. 3, pp. 1358–1371, 2012.

[9] E. Wies, M. K. Wang, N. P. Maharaj et al., "Dephosphorylation of the RNA sensors RIG-I and MDA5 by the phosphatase PP1 is essential for innate immune signaling," *Immunity*, vol. 38, no. 3, pp. 437–449, 2013.

[10] M. U. Gack, Y. C. Shin, C. H. Joo et al., "TRIM25 RING-finger E3 ubiquitin ligase is essential for RIG-I-mediated antiviral activity," *Nature*, vol. 446, no. 7138, pp. 916–920, 2007.

[11] M. U. Gack, A. Kirchhofer, Y. C. Shin et al., "Roles of RIG-I N-terminal tandem CARD and splice variant in TRIM25-

mediated antiviral signal transduction," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 105, no. 43, pp. 16743–16748, 2008.

[12] R. Y. Sanchez David, C. Combredet, O. Sismeiro et al., "Comparative analysis of viral RNA signatures on different RIG-I-like receptors," *eLife*, vol. 5, 2016.

[13] H. Oshiumi, M. Miyashita, N. Inoue, M. Okabe, M. Matsumoto, and T. Seya, "The ubiquitin ligase Riplet is essential for RIG-I-dependent innate immune responses to RNA virus infection," *Cell Host & Microbe*, vol. 8, no. 6, pp. 496–509, 2010.

[14] H. Oshiumi, M. Matsumoto, S. Hatakeyama, and T. Seya, "Riplet/RNF135, a RING finger protein, ubiquitinates RIG-I to promote interferon-β induction during the early phase of viral infection," *The Journal of Biological Chemistry*, vol. 284, no. 2, pp. 807–817, 2009.

[15] J. Yan, Q. Li, A. P. Mao, M. M. Hu, and H. B. Shu, "TRIM4 modulates type I interferon induction and cellular antiviral response by targeting RIG-I for K63-linked ubiquitination," *Journal of Molecular Cell Biology*, vol. 6, no. 2, pp. 154–163, 2014.

[16] K. Kuniyoshi, O. Takeuchi, S. Pandey et al., "Pivotal role of RNA-binding E3 ubiquitin ligase MEX3C in RIG-I-mediated antiviral innate immunity," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 111, no. 15, pp. 5646–5651, 2014.

[17] W. Zeng, L. Sun, X. Jiang et al., "Reconstitution of the RIG-I pathway reveals a signaling role of unanchored polyubiquitin chains in innate immunity," *Cell*, vol. 141, no. 2, pp. 315–330, 2010.

[18] X. Jiang, L. N. Kinch, C. A. Brautigam et al., "Ubiquitin-induced oligomerization of the RNA sensors RIG-I and MDA5 activates antiviral innate immune response," *Immunity*, vol. 36, no. 6, pp. 959–973, 2012.

[19] C. S. Friedman, M. A. O'Donnell, D. Legarda-Addison et al., "The tumour suppressor CYLD is a negative regulator of RIG-I-mediated antiviral response," *EMBO Reports*, vol. 9, no. 9, pp. 930–936, 2008.

[20] Y. Fan, R. Mao, Y. Yu et al., "USP21 negatively regulates antiviral response by acting as a RIG-I deubiquitinase," *The Journal of Experimental Medicine*, vol. 211, no. 2, pp. 313–328, 2014.

[21] J. Cui, Y. Song, Y. Li et al., "USP3 inhibits type I interferon signaling by deubiquitinating RIG-I-like receptors," *Cell Research*, vol. 24, no. 4, pp. 400–416, 2014.

[22] K. Arimoto, H. Takahashi, T. Hishiki, H. Konishi, T. Fujita, and K. Shimotohno, "Negative regulation of the RIG-I signaling by the ubiquitin ligase RNF125," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, no. 18, pp. 7500–7505, 2007.

[23] W. Wang, M. Jiang, S. Liu et al., "RNF122 suppresses antiviral type I interferon production by targeting RIG-I CARDs to mediate RIG-I degradation," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 113, no. 34, pp. 9581–9586, 2016.

[24] J. Cui, L. Zhu, X. Xia et al., "NLRC5 negatively regulates the NF-κB and type I interferon signaling pathways," *Cell*, vol. 141, no. 3, pp. 483–496, 2010.

[25] C. B. Moore, D. T. Bergstralh, J. A. Duncan et al., "NLRX1 is a regulator of mitochondrial antiviral immunity," *Nature*, vol. 451, no. 7178, pp. 573–577, 2008.

[26] S. J. Choi, H. C. Lee, J. H. Kim et al., "HDAC6 regulates cellular viral RNA sensing by deacetylation of RIG-I," *The EMBO Journal*, vol. 35, no. 4, pp. 429–442, 2016.

[27] M. M. Hu, C. Y. Liao, Q. Yang, X. Q. Xie, and H. B. Shu, "Innate immunity to RNA virus is regulated by temporal and reversible sumoylation of RIG-I and MDA5," *The Journal of Experimental Medicine*, vol. 214, no. 4, pp. 973–989, 2017.

[28] Z. Mi, J. Fu, Y. Xiong, and H. Tang, "SUMOylation of RIG-I positively regulates the type I interferon signaling," *Protein & Cell*, vol. 1, no. 3, pp. 275–283, 2010.

[29] F. Hou, L. Sun, H. Zheng, B. Skaug, Q. X. Jiang, and Z. J. Chen, "MAVS forms functional prion-like aggregates to activate and propagate antiviral innate immune response," *Cell*, vol. 146, no. 3, pp. 448–461, 2011.

[30] T. Kawai, K. Takahashi, S. Sato et al., "IPS-1, an adaptor triggering RIG-I- and Mda5-mediated type I interferon induction," *Nature Immunology*, vol. 6, no. 10, pp. 981–988, 2005.

[31] 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.

[32] 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.

[33] H. Xu, X. He, H. Zheng et al., "Structural basis for the prion-like MAVS filaments in antiviral innate immunity," *Biophysical Journal*, vol. 106, no. 2, article 684a, 2014.

[34] 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.

[35] 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.

[36] 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.

[37] A. Baum, R. Sachidanandam, and A. Garcia-Sastre, "Preference of RIG-I for short viral RNA molecules in infected cells revealed by next-generation sequencing," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 107, no. 37, pp. 16303–16308, 2010.

[38] 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," *The Journal of Experimental Medicine*, vol. 205, no. 7, pp. 1601–1610, 2008.

[39] W. G. Davis, J. B. Bowzard, S. D. Sharma et al., "The 3′ untranslated regions of influenza genomic sequences are 5′PPP-independent ligands for RIG-I," *PLoS One*, vol. 7, no. 3, article e32661, 2012.

[40] M. Schlee, "Master sensors of pathogenic RNA—RIG-I like receptors," *Immunobiology*, vol. 218, no. 11, pp. 1322–1335, 2013.

[41] L. Zinzula and E. Tramontano, "Strategies of highly pathogenic RNA viruses to block dsRNA detection by RIG-I-like receptors: hide, mask, hit," *Antiviral Research*, vol. 100, no. 3, pp. 615–635, 2013.

[42] C. Lässig, S. Matheisl, K. M. J. Sparrer et al., "Correction: ATP hydrolysis by the viral RNA sensor RIG-I prevents unintentional recognition of self-RNA," *eLife*, vol. 5, 2016.

[43] D. Luo, A. Kohlway, A. Vela, and A. M. Pyle, "Visualizing the determinants of viral RNA recognition by innate immune sensor RIG-I," *Structure*, vol. 20, no. 11, pp. 1983–1988, 2012.

[44] D. C. Rawling, M. E. Fitzgerald, and A. M. Pyle, "Establishing the role of ATP for the function of the RIG-I innate immune sensor," *eLife*, vol. 4, 2015.

[45] M. C. Boelens, T. J. Wu, B. Y. Nabet et al., "Exosome transfer from stromal to breast cancer cells regulates therapy resistance pathways," *Cell*, vol. 159, no. 3, pp. 499–513, 2014.

[46] C. Lassig and K. P. Hopfner, "Discrimination of cytosolic self and non-self RNA by RIG-I-like receptors," *The Journal of Biological Chemistry*, vol. 292, no. 22, pp. 9000–9009, 2017.

[47] M. A. Jang, E. K. Kim, H. Now et al., "Mutations in *DDX58*, which encodes RIG-I, cause atypical Singleton-Merten syndrome," *The American Journal of Human Genetics*, vol. 96, no. 2, pp. 266–274, 2015.

[48] H. Oda, K. Nakagawa, J. Abe et al., "Aicardi-Goutières syndrome is caused by *IFIH1* mutations," *The American Journal of Human Genetics*, vol. 95, no. 1, pp. 121–125, 2014.

[49] G. I. Rice, Y. Del Toro Duany, E. M. Jenkinson et al., "Gain-of-function mutations in *IFIH1* cause a spectrum of human disease phenotypes associated with upregulated type I interferon signaling," *Nature Genetics*, vol. 46, no. 5, pp. 503–509, 2014.

[50] F. Rutsch, M. MacDougall, C. Lu et al., "A specific *IFIH1* gain-of-function mutation causes Singleton-Merten syndrome," *The American Journal of Human Genetics*, vol. 96, no. 2, pp. 275–282, 2015.

[51] S. Rothenfusser, N. Goutagny, G. DiPerna et al., "The RNA helicase Lgp2 inhibits TLR-independent sensing of viral replication by retinoic acid-inducible gene-I," *The Journal of Immunology*, vol. 175, no. 8, pp. 5260–5268, 2005.

[52] A. Komuro and C. M. Horvath, "RNA- and virus-independent inhibition of antiviral signaling by RNA helicase LGP2," *Journal of Virology*, vol. 80, no. 24, pp. 12332–12342, 2006.

[53] A. Murali, X. Li, C. T. Ranjith-Kumar et al., "Structure and function of LGP2, a DE*X*(D/H) helicase that regulates the innate immunity response," *Journal of Biological Chemistry*, vol. 283, no. 23, pp. 15825–15833, 2008.

[54] S. Soonthornvacharin, A. Rodriguez-Frandsen, Y. Zhou et al., "Systems-based analysis of RIG-I-dependent signalling identifies KHSRP as an inhibitor of RIG-I receptor activation," *Nature Microbiology*, vol. 2, article 17022, 2017.

[55] A. M. Bruns, G. P. Leser, R. A. Lamb, and C. M. Horvath, "The innate immune sensor LGP2 activates antiviral signaling by regulating MDA5-RNA interaction and filament assembly," *Molecular Cell*, vol. 55, no. 5, pp. 771–781, 2014.

[56] A. M. Bruns, D. Pollpeter, N. Hadizadeh, S. Myong, J. F. Marko, and C. M. Horvath, "ATP hydrolysis enhances RNA recognition and antiviral signal transduction by the innate immune sensor, Laboratory of Genetics and Physiology 2 (LGP2)," *Journal of Biological Chemistry*, vol. 288, no. 2, pp. 938–946, 2013.

[57] M. Si-Tahar, F. Blanc, L. Furio et al., "Protective role of LGP2 in influenza virus pathogenesis," *The Journal of Infectious Diseases*, vol. 210, no. 2, pp. 214–223, 2014.

[58] A. G. van der Veen, P. V. Maillard, J. M. Schmidt et al., "The RIG-I-like receptor LGP2 inhibits Dicer-dependent processing of long double-stranded RNA and blocks RNA interference in mammalian cells," *The EMBO Journal*, vol. 37, no. 4, article e97479, 2018.

[59] B. R. tenOever, "Questioning antiviral RNAi in mammals," *Nature Microbiology*, vol. 2, no. 5, article 17052, 2017.

[60] A. Fullam and M. Schröder, "DExD/H-box RNA helicases as mediators of anti-viral innate immunity and essential host factors for viral replication," *Biochimica et Biophysica Acta (BBA) - Gene Regulatory Mechanisms*, vol. 1829, no. 8, pp. 854–865, 2013.

[61] H. Oshiumi, T. Kouwaki, and T. Seya, "Accessory factors of cytoplasmic viral RNA sensors required for antiviral innate immune response," *Frontiers in Immunology*, vol. 7, 2016.

[62] S. C. Eckard, G. I. Rice, A. Fabre et al., "The SKIV2L RNA exosome limits activation of the RIG-I-like receptors," *Nature Immunology*, vol. 15, no. 9, pp. 839–845, 2014.

[63] M. Miyashita, H. Oshiumi, M. Matsumoto, and T. Seya, "DDX60, a DEXD/H box helicase, is a novel antiviral factor promoting RIG-I-like receptor-mediated signaling," *Molecular and Cellular Biology*, vol. 31, no. 18, pp. 3802–3819, 2011.

[64] H. Oshiumi, M. Miyashita, M. Okamoto et al., "DDX60 is involved in RIG-I-dependent and independent antiviral responses, and its function is attenuated by virus-induced EGFR activation," *Cell Reports*, vol. 11, no. 8, pp. 1193–1207, 2015.

[65] N. Tremblay, M. Baril, L. Chatel-Chaix et al., "Spliceosome SNRNP200 promotes viral RNA sensing and IRF3 activation of antiviral response," *PLoS Pathogens*, vol. 12, no. 7, article e1005772, 2016.

[66] R. Bordonne, J. Banroques, J. Abelson, and C. Guthrie, "Domains of yeast U4 spliceosomal RNA required for PRP4 protein binding, snRNP-snRNP interactions, and pre-mRNA splicing in vivo," *Genes & Development*, vol. 4, no. 7, pp. 1185–1196, 1990.

[67] J. Lin and J. J. Rossi, "Identification and characterization of yeast mutants that overcome an experimentally introduced block to splicing at the 3′ splice site," *RNA*, vol. 2, no. 8, pp. 835–848, 1996.

[68] D. Xu, S. Nouraini, D. Field, S.-J. Tang, and J. D. Friesen, "An RNA-dependent ATPase associated with U2/U6 snRNAs in pre-mRNA splicing," *Nature*, vol. 381, no. 6584, pp. 709–713, 1996.

[69] P. Fabrizio, B. Laggerbauer, J. Lauber, W. S. Lane, and R. Lührmann, "An evolutionarily conserved U5 snRNP-specific protein is a GTP-binding factor closely related to the ribosomal translocase EF-2," *The EMBO Journal*, vol. 16, no. 13, pp. 4092–4106, 1997.

[70] J. P. Staley and C. Guthrie, "Mechanical devices of the spliceosome: motors, clocks, springs, and things," *Cell*, vol. 92, no. 3, pp. 315–326, 1998.

[71] N. Tremblay, M. Baril, L. Chatel-Chaix et al., "Correction: spliceosome SNRNP200 promotes viral RNA sensing and IRF3 activation of antiviral response," *PLoS Pathogens*, vol. 13, no. 1, article e1006174, 2017.

[72] P. Linder and E. Jankowsky, "From unwinding to clamping — the DEAD box RNA helicase family," *Nature Reviews Molecular Cell Biology*, vol. 12, no. 8, pp. 505–516, 2011.

[73] T. Kawasaki and T. Kawai, "Toll-like receptor signaling pathways," *Frontiers in Immunology*, vol. 5, 2014.

[74] S. Jensen and A. R. Thomsen, "Sensing of RNA viruses: a review of innate immune receptors involved in recognizing RNA virus invasion," *Journal of Virology*, vol. 86, no. 6, pp. 2900–2910, 2012.

[75] K. A. Zarember and P. J. Godowski, "Tissue expression of human toll-like receptors and differential regulation of toll-like receptor mRNAs in leukocytes in response to microbes, their products, and cytokines," *Journal of Immunology*, vol. 168, no. 2, pp. 554–561, 2002.

[76] M. Tatematsu, F. Nishikawa, T. Seya, and M. Matsumoto, "Toll-like receptor 3 recognizes incomplete stem structures in single-stranded viral RNA," *Nature Communications*, vol. 4, p. 1833, 2013.

[77] N. H. Chen, P. P. Xia, S. J. Li, T. J. Zhang, T. T. Wang, and J. Z. Zhu, "RNA sensors of the innate immune system and their detection of pathogens," *IUBMB Life*, vol. 69, no. 5, pp. 297–304, 2017.

[78] Y. Wang, L. Liu, D. R. Davies, and D. M. Segal, "Dimerization of Toll-like receptor 3 (TLR3) is required for ligand binding," *Journal of Biological Chemistry*, vol. 285, no. 47, pp. 36836–36841, 2010.

[79] J. Mahita and R. Sowdhamini, "Integrative modelling of TIR domain-containing adaptor molecule inducing interferon-β (TRIF) provides insights into its autoinhibited state," *Biology Direct*, vol. 12, no. 1, p. 9, 2017.

[80] H. D. Huh, E. Lee, J. Shin, B. Park, and S. Lee, "STRAP positively regulates TLR3-triggered signaling pathway," *Cellular Immunology*, vol. 318, pp. 55–60, 2017.

[81] W. W. Huai, H. Song, Z. X. Yu et al., "Mint3 potentiates TLR3/4- and RIG-I-induced IFN-β expression and antiviral immune responses," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 113, no. 42, pp. 11925–11930, 2016.

[82] J. A. Tomalka, T. J. de Jesus, and P. Ramakrishnan, "Sam68 is a regulator of Toll-like receptor signaling," *Cellular & Molecular Immunology*, vol. 14, no. 1, pp. 107–117, 2017.

[83] S. Y. Tsai, J. A. Segovia, T. H. Chang et al., "Regulation of TLR3 activation by S100A9," *Journal of Immunology*, vol. 195, no. 9, pp. 4426–4437, 2015.

[84] E. Borysiewicz, D. Fil, and G. W. Konat, "Rho proteins are negative regulators of TLR2, TLR3, and TLR4 signaling in astrocytes," *Journal of Neuroscience Research*, vol. 87, no. 7, pp. 1565–1572, 2009.

[85] X. Liu, W. Chen, Q. Wang, L. Li, and C. Wang, "Negative regulation of TLR inflammatory signaling by the SUMO-deconjugating enzyme SENP6," *PLoS Pathogens*, vol. 9, no. 6, article e1003480, 2013.

[86] X. M. Hu, J. Q. Ye, A. J. Qin, H. T. Zou, H. X. Shao, and K. Qian, "Both microRNA-155 and virus-encoded MiR-155 ortholog regulate TLR3 expression," *PLoS One*, vol. 10, no. 5, article e0126012, 2015.

[87] P. Kar, D. Kumar, P. K. Gumma, S. J. Chowdhury, and V. K. Karra, "Down regulation of TRIF, TLR3, and MAVS in HCV infected liver correlates with the outcome of infection," *Journal of Medical Virology*, vol. 89, no. 12, pp. 2165–2172, 2017.

[88] M. R. Al-Anazi, S. Matou-Nasri, A. A. Abdo et al., "Association of Toll-like receptor 3 single-nucleotide polymorphisms and hepatitis C virus infection," *Journal of Immunology Research*, vol. 2017, Article ID 1590653, 11 pages, 2017.

[89] F. G. Lafaille, I. M. Pessach, S. Y. Zhang et al., "Impaired intrinsic immunity to HSV-1 in human iPSC-derived TLR3-deficient CNS cells," *Nature*, vol. 491, no. 7426, pp. 769–773, 2012.

[90] Y. Q. Guo, M. Audry, M. Ciancanelli et al., "Herpes simplex virus encephalitis in a patient with complete TLR3 deficiency: TLR3 is otherwise redundant in protective immunity," *Journal of Experimental Medicine*, vol. 208, no. 10, pp. 2083–2098, 2011.

[91] L. Abel, S. Plancoulaine, E. Jouanguy et al., "Age-dependent Mendelian predisposition to herpes simplex virus type 1 encephalitis in childhood," *The Journal of Pediatrics*, vol. 157, no. 4, pp. 623–629.e1, 2010.

[92] S. Y. Zhang, E. Jouanguy, S. Ugolini et al., "TLR3 deficiency in patients with herpes simplex encephalitis," *Science*, vol. 317, no. 5844, pp. 1522–1527, 2007.

[93] M. Sironi, A. M. Peri, R. Cagliani et al., "*TLR3* mutations in adult patients with herpes simplex virus and varicella-zoster virus encephalitis," *The Journal of Infectious Diseases*, vol. 215, no. 9, pp. 1430–1434, 2017.

[94] N. Mork, E. Kofod-Olsen, K. B. Sorensen et al., "Mutations in the TLR3 signaling pathway and beyond in adult patients with herpes simplex encephalitis," *Genes & Immunity*, vol. 16, no. 8, pp. 552–566, 2015.

[95] P. X. Song, N. Zheng, L. Zhang et al., "Downregulation of interferon-β and Inhibition of TLR3 expression are associated with fatal outcome of severe fever with thrombocytopenia syndrome," *Scientific Reports*, vol. 7, no. 1, p. 6532, 2017.

[96] P. L. Geng, L. X. Song, H. J. An, J. Y. Huang, S. Li, and X. T. Zeng, "Toll-like receptor 3 is associated with the risk of HCV infection and HBV-related diseases," *Medicine*, vol. 95, no. 21, article e2302, 2016.

[97] D. Liu, Q. Chen, H. Zhu et al., "Association of respiratory syncytial virus toll-like receptor 3-mediated immune response with COPD exacerbation frequency," *Inflammation*, vol. 41, no. 2, 2017.

[98] R. Sesti-Costa, M. C. S. Francozo, G. K. Silva, J. L. Proenca-Modena, and J. S. Silva, "TLR3 is required for survival following *Coxsackievirus* B3 infection by driving T lymphocyte activation and polarization: the role of dendritic cells," *PLoS One*, vol. 12, no. 10, article e0185819, 2017.

[99] H. F. He, S. H. Liu, P. P. Liu et al., "Association of Toll-like receptor 3 gene polymorphism with the severity of enterovirus 71 infection in Chinese children," *Archives of Virology*, vol. 162, no. 6, pp. 1717–1723, 2017.

[100] D. Alvarez-Carbonell, Y. Garcia-Mesa, S. Milne et al., "Toll-like receptor 3 activation selectively reverses HIV latency in microglial cells," *Retrovirology*, vol. 14, no. 1, p. 9, 2017.

[101] S. Cardinaud, A. Urrutia, A. Rouers et al., "Triggering of TLR-3, -4, NOD2, and DC-SIGN reduces viral replication and increases T-cell activation capacity of HIV-infected human dendritic cells," *European Journal of Immunology*, vol. 47, no. 5, pp. 818–829, 2017.

[102] J. K. Dowling and A. Mansell, "Toll-like receptors: the Swiss army knife of immunity and vaccine development," *Clinical & Translational Immunology*, vol. 5, no. 5, article e85, 2016.

[103] E. Bardel, R. Doucet-Ladeveze, C. Mathieu, A. M. Harandi, B. Dubois, and D. Kaiserlian, "Intradermal immunisation using the TLR3-ligand poly (I:C) as adjuvant induces

mucosal antibody responses and protects against genital HSV-2 infection," *Npj Vaccines*, vol. 1, no. 1, article 16010, 2016.

[104] E. Poteet, P. Lewis, C. Y. Chen et al., "Toll-like receptor 3 adjuvant in combination with virus-like particles elicit a humoral response against HIV," *Vaccine*, vol. 34, no. 48, pp. 5886–5894, 2016.

[105] J. L. Cervantes, B. Weinerman, C. Basole, and J. C. Salazar, "TLR8: the forgotten relative revindicated," *Cellular & Molecular Immunology*, vol. 9, no. 6, pp. 434–438, 2012.

[106] M. Dominguez-Villar, A. S. Gautron, M. de Marcken, M. J. Keller, and D. A. Hafler, "TLR7 induces anergy in human $CD4^+$ T cells," *Nature Immunology*, vol. 16, no. 1, pp. 118–128, 2015.

[107] J. Dai, B. Liu, and Z. H. Li, "Regulatory T cells and Toll-like receptors: what is the missing link?," *International Immunopharmacology*, vol. 9, no. 5, pp. 528–533, 2009.

[108] H. Sandig and S. Bulfone-Paus, "TLR signaling in mast cells: common and unique features," *Frontiers in Immunology*, vol. 3, 2012.

[109] N. Fitzner, S. Clauberg, F. Essmann, J. Liebmann, and V. Kolb-Bachofen, "Human skin endothelial cells can express all 10 *TLR* genes and respond to respective ligands," *Clinical and Vaccine Immunology*, vol. 15, no. 1, pp. 138–146, 2008.

[110] K. Rosenberger, K. Derkow, P. Dembny, C. Kruger, E. Schott, and S. Lehnardt, "The impact of single and pairwise Toll-like receptor activation on neuroinflammation and neurodegeneration," *Journal of Neuroinflammation*, vol. 11, no. 1, p. 166, 2014.

[111] M. T. Abreu, "Toll-like receptor signalling in the intestinal epithelium: how bacterial recognition shapes intestinal function," *Nature Reviews Immunology*, vol. 10, no. 2, pp. 131–144, 2010.

[112] M. Awais, K. Wang, X. W. Lin et al., "TLR7 deficiency leads to TLR8 compensative regulation of immune response against JEV in mice," *Frontiers in Immunology*, vol. 8, 2017.

[113] F. Heil, H. Hemmi, H. Hochrein et al., "Species-specific recognition of single-stranded RNA via toll-like receptor 7 and 8," *Science*, vol. 303, no. 5663, pp. 1526–1529, 2004.

[114] S. S. Diebold, T. Kaisho, H. Hemmi, S. Akira, and C. R. Sousa, "Innate antiviral responses by means of TLR7-mediated recognition of single-stranded RNA," *Science*, vol. 303, no. 5663, pp. 1529–1531, 2004.

[115] Y. W. Zhang, M. El-Far, F. P. Dupuy et al., "HCV RNA activates APCs via TLR7/TLR8 while virus selectively stimulates macrophages without inducing antiviral responses," *Scientific Reports*, vol. 6, no. 1, 2016.

[116] S. T. Sarvestani, M. D. Tate, J. M. Moffat et al., "Inosine-mediated modulation of RNA sensing by Toll-like receptor 7 (TLR7) and TLR8," *Journal of Virology*, vol. 88, no. 2, pp. 799–810, 2013.

[117] F. C. F. Schmitt, I. Freund, M. A. Weigand, M. Helm, A. H. Dalpke, and T. Eigenbrod, "Identification of an optimized $2'$-O-methylated trinucleotide RNA motif inhibiting Toll-like receptors 7 and 8," *RNA*, vol. 23, no. 9, pp. 1344–1351, 2017.

[118] S. Jung, T. von Thulen, V. Laukemper et al., "A single naturally occurring $2'$-O-methylation converts a TLR7- and TLR8-activating RNA into a TLR8-specific ligand," *PLoS One*, vol. 10, no. 3, article e0120498, 2015.

[119] A. Forsbach, J. G. Nemorin, K. Volp et al., "Characterization of conserved viral leader RNA sequences that stimulate innate immunity through TLRs," *Oligonucleotides*, vol. 17, no. 4, pp. 405–418, 2007.

[120] T. Querec, S. Bennouna, S. K. Alkan et al., "Yellow fever vaccine YF-17D activates multiple dendritic cell subsets via TLR2, 7, 8, and 9 to stimulate polyvalent immunity," *Journal of Experimental Medicine*, vol. 203, no. 2, pp. 413–424, 2006.

[121] K. Triantafilou, E. Vakakis, E. A. J. Richer, G. L. Evans, J. P. Villiers, and M. Triantafilou, "Human rhinovirus recognition in non-immune cells is mediated by *Toll*-like receptors and MDA-5, which trigger a synergetic pro-inflammatory immune response," *Virulence*, vol. 2, no. 1, pp. 22–29, 2014.

[122] R. Hamel, O. Dejarnac, S. Wichit et al., "Biology of Zika virus infection in human skin cells," *Journal of Virology*, vol. 89, no. 17, pp. 8880–8896, 2015.

[123] A. J. Rolfe, D. B. Bosco, J. Y. Wang, R. S. Nowakowski, J. Q. Fan, and Y. Ren, "Bioinformatic analysis reveals the expression of unique transcriptomic signatures in Zika virus infected human neural stem cells," *Cell & Bioscience*, vol. 6, no. 1, p. 42, 2016.

[124] R. Colisson, L. Barblu, C. Gras et al., "Free HTLV-1 induces TLR7-dependent innate immune response and TRAIL relocalization in killer plasmacytoid dendritic cells," *Blood*, vol. 115, no. 11, pp. 2177–2185, 2010.

[125] H. D. Clifford, S. T. Yerkovich, S. K. Khoo et al., "Toll-like receptor 7 and 8 polymorphisms: associations with functional effects and cellular and antibody responses to measles virus and vaccine," *Immunogenetics*, vol. 64, no. 3, pp. 219–228, 2012.

[126] L. A. J. O'Neill and A. G. Bowie, "The family of five: TIR-domain-containing adaptors in Toll-like receptor signalling," *Nature Reviews Immunology*, vol. 7, no. 5, pp. 353–364, 2007.

[127] D. Werling, O. C. Jann, V. Offord, E. J. Glass, and T. J. Coffey, "Variation matters: TLR structure and species-specific pathogen recognition," *Trends in Immunology*, vol. 30, no. 3, pp. 124–130, 2009.

[128] U. Ohto, "Conservation and divergence of ligand recognition and signal transduction mechanisms in Toll-like receptors," *Chemical and Pharmaceutical Bulletin*, vol. 65, no. 8, pp. 697–705, 2017.

[129] U. Ohto, H. Tanji, and T. Shimizu, "Structure and function of toll-like receptor 8," *Microbes and Infection*, vol. 16, no. 4, pp. 273–282, 2014.

[130] Z. K. Zhang, U. Ohto, T. Shibata et al., "Structural analysis reveals that Toll-like receptor 7 is a dual receptor for guanosine and single-stranded RNA," *Immunity*, vol. 45, no. 4, pp. 737–748, 2016.

[131] H. Tanji, U. Ohto, T. Shibata et al., "Toll-like receptor 8 senses degradation products of single-stranded RNA," *Nature Structural & Molecular Biology*, vol. 22, no. 2, pp. 109–115, 2015.

[132] J. Q. Wang, Y. S. Jeelall, L. L. Ferguson, and K. Horikawa, "Toll-like receptors and cancer: MYD88 mutation and inflammation," *Frontiers in Immunology*, vol. 5, 2014.

[133] H. Itoh, M. Tatematsu, A. Watanabe et al., "UNC93B1 physically associates with human TLR8 and regulates TLR8-mediated signaling," *PLoS One*, vol. 6, no. 12, article e28500, 2011.

[134] C. Y. Chiang, A. Engel, A. M. Opaluch et al., "Cofactors required for TLR7-and TLR9-dependent innate immune

responses," *Cell Host & Microbe*, vol. 11, no. 3, pp. 306–318, 2012.

[135] C. Zannetti, F. Bonnay, F. Takeshita et al., "C/EBPδ and STAT-1 are required for *TLR8* transcriptional activity," *Journal of Biological Chemistry*, vol. 285, no. 45, pp. 34773–34780, 2010.

[136] Z. G. Ramirez-Ortiz, A. Prasad, J. W. Griffith et al., "The receptor TREML4 amplifies TLR7-mediated signaling during antiviral responses and autoimmunity," *Nature Immunology*, vol. 16, no. 5, pp. 495–504, 2015.

[137] B. Aouar, D. Kovarova, S. Letard et al., "Dual role of the tyrosine kinase Syk in regulation of Toll-like receptor signaling in plasmacytoid dendritic cells," *PLoS One*, vol. 11, no. 6, article e0156063, 2016.

[138] Y. M. Wang, S. S. Yan, B. Yang et al., "TRIM35 negatively regulates TLR7- and TLR9-mediated type I interferon production by targeting IRF7," *FEBS Letters*, vol. 589, no. 12, pp. 1322–1330, 2015.

[139] S. Pelletier, N. Bedard, E. Said, P. Ancuta, J. Bruneau, and N. H. Shoukry, "Sustained hyperresponsiveness of dendritic cells is associated with spontaneous resolution of acute hepatitis C," *Journal of Virology*, vol. 87, no. 12, pp. 6769–6781, 2013.

[140] F. Z. Fakhir, M. Lkhider, W. Badre et al., "Genetic variations in toll-like receptors 7 and 8 modulate natural hepatitis C outcomes and liver disease progression," *Liver International*, vol. 38, no. 3, pp. 432–442, 2018.

[141] C. W. Yang and S. M. Chen, "A comparative study of human TLR 7/8 stimulatory trimer compositions in influenza A viral genomes," *PLoS One*, vol. 7, no. 2, article e30751, 2012.

[142] E. A. Said, F. Al-Yafei, F. Zadjali et al., "Association of single-nucleotide polymorphisms in TLR7 (Gln11Leu) and TLR9 (1635A/G) with a higher CD4T cell count during HIV infection," *Immunology Letters*, vol. 160, no. 1, pp. 58–64, 2014.

[143] T. A. Abdel-Raouf, A. Ahmed, W. K. Zaki, H. M. Abdella, and M. A. Zid, "Study of toll-like receptor 7 expression and interferon α in Egyptian patients with chronic hepatitis C infection and hepatocellular carcinoma," *Egyptian Journal of Medical Human Genetics*, vol. 15, no. 4, pp. 387–392, 2014.

[144] C. H. Wang, H. L. Eng, K. H. Lin et al., "*TLR7* and *TLR8* gene variations and susceptibility to hepatitis C virus infection," *PLoS One*, vol. 6, no. 10, article e26235, 2011.

[145] F. Li, X. Li, G. Z. Zou, Y. F. Gao, and J. Ye, "Association between *TLR7* copy number variations and hepatitis B virus infection outcome in Chinese," *World Journal of Gastroenterology*, vol. 23, no. 9, pp. 1602–1607, 2017.

[146] L. Trautmann, E. A. Said, R. Halwani et al., "Programmed death 1: a critical regulator of T-cell function and a strong target for immunotherapies for chronic viral infections," *Current Opinion in HIV and AIDS*, vol. 2, no. 3, pp. 219–227, 2007.

[147] S. I. Gringhuis, M. van der Vlist, L. M. van den Berg, J. den Dunnen, M. Litjens, and T. B. H. Geijtenbeek, "HIV-1 exploits innate signaling by TLR8 and DC-SIGN for productive infection of dendritic cells," *Nature Immunology*, vol. 11, no. 5, pp. 419–426, 2010.

[148] E. Khatamzas, M. M. Hipp, D. Gaughan et al., "Snapin promotes HIV-1 transmission from dendritic cells by dampening TLR8 signaling," *EMBO Journal*, vol. 36, no. 20, pp. 2998–3011, 2017.

[149] R. A. Bam, D. Hansen, A. Irrinki et al., "TLR7 agonist GS-9620 is a potent inhibitor of acute HIV-1 infection in human peripheral blood mononuclear cells," *Antimicrobial Agents and Chemotherapy*, vol. 61, no. 1, pp. e01369–e01316, 2016.

[150] E. Schlaepfer and R. F. Speck, "TLR8 activates HIV from latently infected cells of myeloid-monocytic origin directly via the MAPK pathway and from latently infected $CD4^+$ T cells indirectly via TNF-α," *The Journal of Immunology*, vol. 186, no. 7, pp. 4314–4324, 2011.

[151] N. Van Hoeven, C. B. Fox, B. Granger et al., "A formulated TLR7/8 agonist is a flexible, highly potent and effective adjuvant for pandemic influenza vaccines," *Scientific Reports*, vol. 7, article 46426, 2017.

[152] S. T. Zhang, Z. Hu, H. Tanji et al., "Small-molecule inhibition of TLR8 through stabilization of its resting state," *Nature Chemical Biology*, vol. 14, no. 1, pp. 58–64, 2018.

[153] N. I. Maria, E. C. Steenwijk, A. S. IJpma et al., "Contrasting expression pattern of RNA-sensing receptors TLR7, RIG-I and MDA5 in interferon-positive and interferon-negative patients with primary Sjögren's syndrome," *Annals of the Rheumatic Diseases*, vol. 76, no. 4, pp. 721–730, 2017.

[154] W. X. Wu, W. Zhang, E. S. Duggan, J. L. Booth, M. H. Zou, and J. P. Metcalf, "RIG-I and TLR3 are both required for maximum interferon induction by influenza virus in human lung alveolar epithelial cells," *Virology*, vol. 482, pp. 181–188, 2015.

[155] N. I. Maria, C. G. van Helden-Meeuwsen, E. C. Steenwijk et al., "Nucleic acid sensing receptors TLR7, RIG-I and MDA5 collaborate in driving the systemic IFN signature and amplify the pathogenic loop: potential new targets for therapy in primary Sjogrens syndrome," *Arthritis & Rhematology*, vol. 66, pp. S1302–S1302, 2014.

[156] L. Slater, N. W. Bartlett, J. J. Haas et al., "Co-ordinated role of TLR3, RIG-I and MDA5 in the innate response to rhinovirus in bronchial epithelium," *PLoS Pathogens*, vol. 6, no. 11, article e1001178, 2010.

Hindawi
Journal of Immunology Research
Volume 2017, Article ID 8689313, 7 pages
https://doi.org/10.1155/2017/8689313

Research Article

Impact of the Polymorphism *rs9264942* near the *HLA-C* Gene on HIV-1 DNA Reservoirs in Asymptomatic Chronically Infected Patients Initiating Antiviral Therapy

Laura Herráiz-Nicuesa,[1,2] Diana Carolina Hernández-Flórez,[1,2] Lara Valor,[1,2] Sonia García-Consuegra,[1,2] Juan Paulo Navarro-Valdivieso,[1,2] Eduardo Fernández-Cruz,[1,2] and Carmen Rodríguez-Sainz[1,2]

[1]*Servicio de Inmunología Clínica, Hospital General Universitario Gregorio Marañón, Madrid, Spain*
[2]*Instituto de Investigación Sanitaria Gregorio Marañón, Madrid, Spain*

Correspondence should be addressed to Carmen Rodríguez-Sainz; crodriguezs@salud.madrid.org

Received 28 July 2017; Accepted 17 October 2017; Published 28 December 2017

Academic Editor: Daniel Lamarre

Copyright © 2017 Laura Herráiz-Nicuesa et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Several genome-wide association studies have identified a polymorphism located 35 kb upstream of the coding region of *HLA-C* gene (*rs9264942*; termed −35 C/T) as a host factor significantly associated with the control of HIV-1 viremia in untreated patients. The potential association of this host genetic polymorphism with the viral reservoirs has never been investigated, nor the association with the viral control in response to the treatment. In this study, we assess the influence of the polymorphism −35 C/T on the outcome of virus burden in 183 antiretroviral-naïve HIV-1-infected individuals who initiated antiviral treatment (study STIR-2102), analyzing HIV-1 RNA viremia and HIV-1 DNA reservoirs. The *rs9264942* genotyping was investigated retrospectively, and plasma levels of HIV-1 RNA and peripheral blood mononuclear cell- (PBMC-) associated HIV-1 DNA were compared between carriers and noncarriers of the protective allele −35 C before antiretroviral therapy (ART), one month after ART and at the end of the study (36 months). HIV-1 RNA and HIV-1 DNA levels were both variables significantly different between carriers and noncarriers of the allele −35 C before ART. HIV-1 DNA levels remained also significantly different one month posttherapy. However, this protective effect of the −35 C allele was not maintained after long-term ART.

1. Introduction

The clinical outcome of HIV-1 infection is highly variable and determined by complex interactions between virus, host, and environment. Part of this epidemiological heterogeneity could be attributed to host genetic factors, which have been extensively studied using whole genome approaches (reviewed in [1, 2]). Several genome-wide association studies (GWAS) have identified a polymorphism located 35 kb upstream of the coding region of the gene *HLA-C* (−35 C/T; *rs9264942*) as a host factor significantly associated with the control of HIV-1 viremia. Fellay et al. reported the first AIDS GWAS in 2007 [3]. Their study tracked the viral set point (mean plasma RNA level over several months once the immune system has settled to a steady-state level after primo-infection) in 486 European AIDS patients (Cohort EURO-CHAVI) that were genotyped. The variant *rs9264942* was the second most significant independent hit in the EURO-CHAVI study and resulted the top hit in the International HIV Controller Study involving 974 controllers, as reported three years later [4]. Another HIV-1 viral set point GWAS with 2554 Caucasian participants provided overwhelming confirmation of the SNP *rs9264942* [5]. This SNP also associates strongly with differences in *HLA-C* expression levels. The protective allele (*−35 C*) leads to a lower viral load and is associated with higher expression of the *HLA-C* gene [3].

Recently, more work has focused on elucidating the functional significance of the *−35 C/T* SNP, and several groups now have demonstrated HLA-C surface expression to be a

Table 1: Baseline characteristics for the cohort of patients (study STIR-2102).

	ART + HIV-immunogen	ART + placebo	Overall
Number of subjects	86	97	183
Age, mean (S.D.) (years)	35 (8)	33 (7)	34 (7)
Gender, *N* (%)			
Male	61 (70.9)	65 (67.0)	126
Female	25 (30.0)	32 (33.0)	57
$CD4^+$ T cells, mean (S.D.), cells $\times 10^{-6}$ L^{-1}	405.1 (72.1)	401.0 (74.9)	402.9 (73.4)
HIV-1 RNA, median (range), copies per mL	14,150 (459,801)	14,650 (243,801)	14,525 (459,801)

S.D.: standard deviation.

key element in the control of HIV viral load [6]. HLA-C surface expression has been correlated with the presence of microRNA binding sites that affect *HLA-C* expression and control of HIV disease. It has been identified a binding site for the microinterference RNA, miR-148a, that is present in the sequence of some *HLA-C* alleles, but missing in others due to polymorphism in the region. This polymorphism includes an insertion/deletion variant at the position 263 of the 3′UTR of the *HLA-C* gene, which is in very strong linkage disequilibrium with *rs9264942* in Caucasians [7]. The linkage disequilibrium of the −35 SNP with the 3′UTR miRNA-148a binding site polymorphisms offers a functional explanation for the observed differences in HLA-C surface expression among individuals and the associated control of HIV disease. Globally, all these data support a role for *HLA-C* in early and/or chronic infection [8, 9].

HLA-C is the most recently evolved of the classical *MHC-I* alleles and is restricted to humans and great apes [10, 11]; and fewer alleles of *HLA-C* have been identified. HLA-C plays a dual role in that it can present antigens to CTLs and it can inhibit natural killer (NK) cell (and possibly also CTL) lysis via its interaction with inhibitory receptors (killer immunoglobulin-like receptors, KIR). For reasons that are poorly understood, HLA-C is normally expressed on the cell surface at levels approximately 10-fold less than most *HLA-A* and *HLA-B* allotypes [12–14]. In HIV-infected individuals, HLA-A and HLA-B molecules expressed on the surface of the infected cells are preferentially downmodulated by the viral accessory protein Nef, but not HLA-C molecules [15], and so HLA-C may have a unique role in presenting antigens to CTLs in HIV disease and modulating cytotoxicity.

Since the first GWAS study of Fellay, involving the variant *rs9264942* near the HLA-C as a genetic determinant in HIV-1 infection, most of the GWAS performed to date in cohorts involving various outcomes of HIV infection are focus on the viremia or the control of circulating virus and all of them have been designed in the absence of antiviral therapy. Viral burden has been extensively investigated in the HLA-C background analyzing plasmatic HIV-1 RNA, but HIV-1 reservoirs, particularly HIV-1 DNA levels are lesser known. The potential association of the variant *rs9264942* with the viral control under antiviral treatment is also unknown.

The objective of this study was to assess the influence of the polymorphism *rs9264942* on the outcome of virus burden in 183 antiretroviral-naïve individuals who initiated antiviral treatment (study STIR-2102), analyzing HIV-1 RNA viremia and HIV-1 DNA reservoirs associated to PBMC. We quantified HIV-1 RNA from plasma and HIV-1 DNA obtained from PBMCs before antiretroviral therapy (ART) and during the treatment with a follow-up of 36 months.

2. Materials and Methods

2.1. Patients. One hundred eighty-three patients with asymptomatic HIV-1 chronic infection who had participated in the clinical trial STIR-2102 [16, 17] were retrospectively genotyped for the polymorphism *rs9264942*. The study STIR-2102 was a multicenter, randomized, double-blinded, placebo-controlled phase II clinical trial of antiretroviral therapy (ART) in combination with an HIV-1 immunogen (Remune®) in antiretroviral-naïve HIV-1-infected subjects with $CD4^+$ T lymphocytes between 300 and 700 cells/μL. ART consisted of zidovudine and didanosine. Patients started therapy one month prior randomization to receive ART plus the immunogen ($N = 86$) or ART plus placebo ($N = 97$). Treatment arms showed no differences in baseline factors, such as age, gender, risk group, viral load, and $CD4^+$ T cells. The baseline characteristics for the study STIR-2102 are shown in Table 1. The immunovorological response to the therapy was similar at the end of the study with no significant differences between both arms. Prior to the commencement of the study STIR-2102, conducted between 1997 and 2001, Institutional Review Board approval by each participant hospital was obtained. Furthermore, informed consents from all participants, jointly with the review and approval of the protocol by the Spanish Agency of Medicament and Sanitary Products, were also obtained prior to the initiation of the trial.

2.2. Plasma HIV-1 RNA Viremia and $CD4^+$ T Cell Subset. Laboratory test including $CD4^+$ T cell counts and plasma HIV-1 RNA levels were carried out every three months throughout the study (36 months). Plasma levels of HIV-1 RNA were assessed using the Amplicor assay (Hoffman La Roche, Nutley, NJ) with a lower limit of quantification of 200 copies/mL (2.30 $\log_{10}$ copies/mL) [16, 17].

2.3. Polymorphism rs9264942 Genotyping Assessment. Blood samples were collected from patients during the trial, and DNA was isolated from the peripheral blood mononuclear cells by standard protocols. PBMC were separated by Ficoll gradient (Pharmacia, Uppsala, Sweden) and stored as

Table 2: Frequencies of the genotypes for the SNP rs9264942 in the *HLA-C* 5′ region, where T is the major allele and C is the minor allele. HIV-1 RNA and HIV-1 DNA levels are shown in the patients naïve for antiretroviral therapy (pre-ART), one month (1 m) post-ART, and at the end of the study (follow-up: 36 months).

rs9264942 genotypes		Frequency *N* (%)	HIV-1 RNA, median (min–max)			HIV-1 DNA, median (min–max)		
			Pre-ART	1 m post-ART	End of study	Pre-ART	1 m post-ART	End of study
Total $N = 183$	TT	54 (29.5%)	24,976 (200–460,000)	200 (200–60,000)	5400 (200–56,543)	927.5 (250–32,560)	1080 (250–10,645)	250 (250–2570)
	CT	95 (51.9%)	11,450 (200–274,500)	200 (200–25,000)	1125 (200–46,200)	520.0 (250–12,985)	440 (250–18,855)	250 (250–10,120)
	CC	34 (18.6%)	9250 (200–206,500)	200 (200–16,100)	5252 (200–41,200)	448.5 (250–17,425)	420 (250–7475)	250 (250–930)
			N.S. $p = 0.068$	N.S.	N.S.	$\boldsymbol{p = 0.001}$	$\boldsymbol{p = 0.020}$	N.S.

Median and minimum–maximum values of viremia and cellular-associated viral loads are indicated for the respective genotypes, HIV-1 RNA in copies/mL, and HIV-1 DNA in copies/10^6 PBMCs. The p values are for median comparisons; N.S.: not significant.

dry pellets at −80°C. DNA was purified from each PBMC pellet by Wizard Genomic DNA Purification Kit (Promega Corporation, Madison, WI, USA), and DNA content was determined by spectrophotometric analysis.

The *rs9264942* genotyping was retrospectively performed by real-time PCR amplification with the LightSNiP *rs9264942 HLA-C* (TIB MOLBIOL GmbH, Berlin, Germany; under license from Roche Diagnostics GmbH) using LightCycler® FastStart DNA Master HybProbe (Roche Diagnostics) and a LightCycler 1.5 Instrument, according to the manufacturer's protocol. The genotype was verified by sequencing.

2.4. HIV-1 DNA Level. HIV-1 DNA was quantified with a real-time quantitative PCR method using SYBR Green and primers of the *pol* gene as previously described [18]. Fluorescence was monitored specifically in a single point of each amplification cycle, allowing the determination of initial DNA copies through comparison with a standard curve constructed with DNA from the T-lymphoblastoid cell lines Jurkat and 8E5LAV that do not contain HIV-1 LAV or only in the amount of one single copy of HIV-1 LAV per cell, respectively. The detection limit of our assay was 250 copies/10^6 PBMC. Results of the PBMC-associated HIV-1 DNA in this cohort of patients were reported partially in a previous study [18].

2.5. Endpoints and Statistical Analysis. In STIR-2102 trial, the virologic endpoint was defined as time to the first increase of viral load above 5000 copies per milliliter and the immunological endpoint as time to the first decrease of $CD4^+$ T cell count below 250 cells/μL [16]. Subjects who at the end of the study at month 36 had not developed a primary endpoint were censored at the last available visit. Kaplan-Meier analysis was used to construct event-free survival curves, which were compared using the log-rank test. Adjusted hazard ratios (HRs) and the 95% confidence intervals (CIs) were calculated using multivariate Cox proportional-hazard models. Proportional hazard assumptions were assessed as previously described [19]. Virologic data did not show a normal distribution and were expressed as median. Mann–Whitney test was used to compare groups. We used the Statistical Package for the Social Sciences (SPSS) and Stata for statistical analysis.

3. Results

3.1. Distribution of the rs9264942 Genotypes in the Study Cohort. Among the 183 chronically infected study subjects, thirty-four (18.6%) displayed the genotype homozygous for the protective allele (*−35 CC*), whereas ninety-five (51.9%) were heterozygous (*−35 CT*) and fifty-four were homozygous for the allele *−35* T (29.5%) (Table 2). The allelic frequency of the minor allele (*−35 C*) was 44.5%. The study population was in Hardy-Weinberg equilibrium.

3.2. Association of the Allele −35 C with HIV-1 Viral Load. To evaluate the impact of the protective allele *−35 C* on the steady-state plasma HIV-1 RNA levels in the study cohort, we compared viral load levels between carriers and noncarriers of the allele, at the baseline when individuals were ART-naïve. Patients included in this study were asymptomatic and maintained a relatively stable viral load off therapy. The analysis revealed decreased set points of the plasmatic viremia in *−35 C* carriers as compared to those with the genotype *−35 TT* reaching statistical significance ($p = 0.035$; median comparison for independent samples). HIV-1 DNA levels were also significantly different between patients with and without the allele *−35 C* ($p < 0.001$; median comparison for independent samples, Table 3). This allele was associated with lesser levels of HIV-1 DNA in PBMCs.

HIV-1 DNA levels in PBMCs remained also significantly different one month posttherapy [median = 425 cop/10^{-6} PBMCs (max = 18,855, min = 250 cop/10^{-6} PBMCs) in *−35 C* carriers versus median = 1080 cop/10^{-6} PBMCs (max = 10,645, min = 250 cop/10^{-6} PBMCs) in the individuals with the genotype −35 TT; $p = 0.010$, median comparison for independent samples].

3.3. Association of the Allele −35 C with HIV-1 Disease Progression in Response to the Therapy. We did not find any protective effect of the allele *−35 C* in Kaplan-Meier survival analysis of the 183 therapy-naïve chronically infected individuals during the thirty-six months of the study STIR-2102 after the initiation of the antiretroviral therapy. Carriers

Table 3: Frequencies of the individuals carrying the protective allele rs9264942–35 C (genotypes CC + CT) versus noncarriers of this allele (genotype TT). HIV-1 RNA and HIV-1 DNA levels are shown in the patients naïve for antiretroviral therapy (pre-ART), one month (1 m) post-ART, and at the end of the study (follow-up: 36 months).

rs9264942 (T > C) −35 C allele (genotypes)		Frequency (%)	HIV-1 RNA median (min–max)			HIV-1 DNA median (min–max)		
			Pre-ART	1 m post-ART	End of study	Pre-ART	1 m post-ART	End of study
Total $N = 183$	Noncarriers (TT)	54 (29.5%)	24,976 (200–460,000)	200 (200–60,000)	3600 (200–56,543)	927,5 (250–32,560)	1080 (250–10,645)	250 (250–2570)
	Carriers (CT + CC)	129 (70.5%)	10,100 (200–274,500)	200 (200–25,000)	1730 (200–132,000)	500 (250–17,425)	425 (250–18,855)	250 (250–15,920)
			$\boldsymbol{p = 0.035}$	N.S.	N.S.	$\boldsymbol{p < 0.001}$	$\boldsymbol{p = 0.010}$	N.S.

Median and minimum–maximum values of viremia and cellular-associated viral loads are indicated for the respective genotypes, HIV-1 RNA in copies/mL, and HIV-1 DNA in copies/10^6 PBMCs. The p values are for median comparisons; N.S.: not significant.

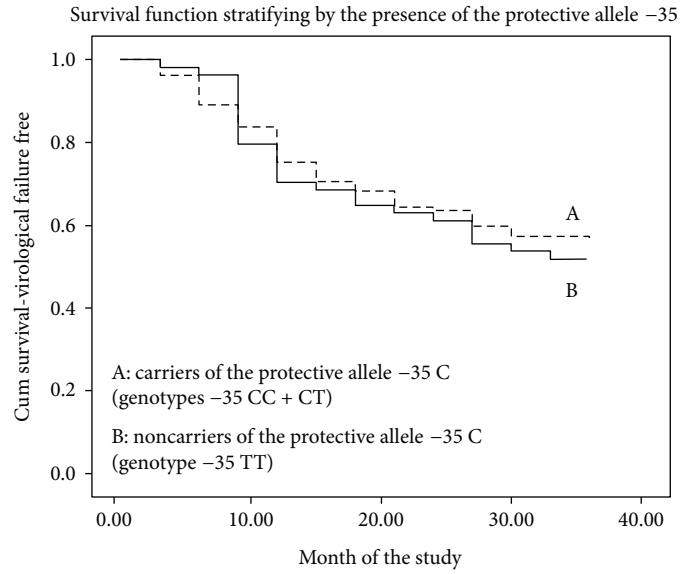


Figure 1: Kaplan-Meier estimates of virological failure-free survival during antiviral therapy according to the stratification by the presence of the protective allele −35 C (SNP *rs9264942*).

Table 4: Cox regression model for the virological failure during antiviral therapy.

Variable	HR (95% CI)	Endpoint p (%)	p
HIV-1 RNA ($\log_{10}$ copies/mL)[a]	1.86 (1.27–2.72)	65.5	0.001
HIV-1 DNA ($\log_{10}$ copies/10^6 PBMC)[a]	1.74 (1.10–2.77)	63.0	0.018
Protective allele −35 C[b]	0.90 (0.54–1.50)	47.3	0.698

Cox regression model for the virological failure according to the presence of the protective allele −35 C for the SNP rs9264942 adjusting for HIV-1 RNA levels and HIV-1 DNA levels. Hazard ratio (HR), 95% confidence intervals (CIs), endpoint p (probability to reach an endpoint, as defined by HR/(1 + HR)), and p values are shown. [a]HRs calculated for a 1-$\log_{10}$ increased. [b]HR calculated for the allele −35 C as a categorical variable (presence of the allele −35 C for the SNP rs9264942[T > C]).

and noncarriers of the allele *−35 C* reached the study endpoint (VL > 5000 copies/mL or CD4 T cells < 250 cells/mL) without showing differences statistically significant [mean time to the endpoint = 30.0 months (CI, 27.4–32.5) versus mean time to the endpoint = 29.0 months (CI, 25.1–32.9), respectively; log-rank test $p = 0.616$; Figure 1].

A multivariate Cox model analysis was performed by introducing as variable the presence of the protective allele *−35 C* and covariating with the variables HIV-1 RNA level and HIV-1 DNA level at the baseline pre-ART (Table 4). HIV-1 DNA and HIV-1 RNA were both independently associated with virological failure. The adjusted HR was significant for each 1-$\log_{10}$ increase in the baseline HIV-1 RNA level (adjusted HR, 1.86 [95% IC, 1.27–2.72, $p = 0.001$]) and for each 1-$\log_{10}$ increase in the baseline HIV-1 DNA (HR 1.74 [95% CI, 1.10–2.77, $p = 0.018$]). The protective allele *−35 C* was not significantly associated with the virological failure in this model ($p = 0.69$). Adjustment

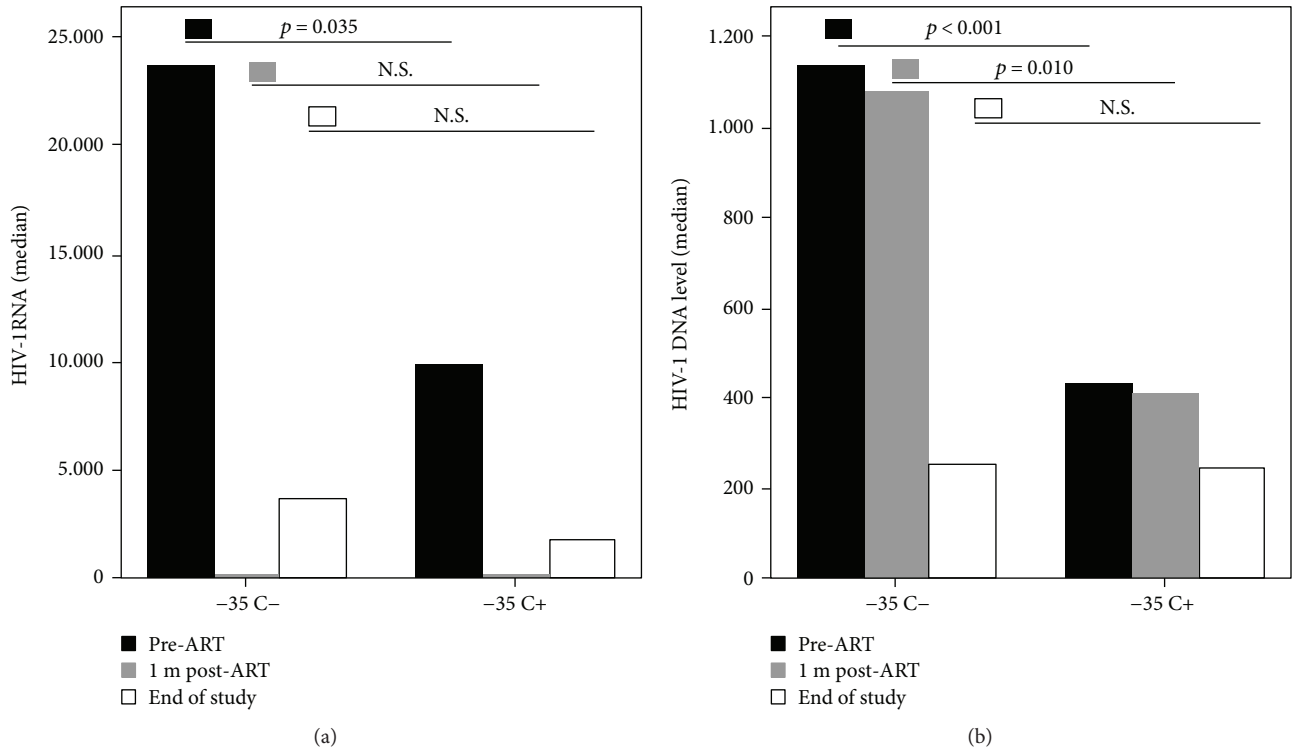


Figure 2: Graph showing the median of HIV-RNA viral load (a) and the median of HIV-1 DNA reservoirs (b) over time (pre-ART, one month post-ART, and at the end of the study) in the patients carrying the protective allele −35 C+ (genotypes CC + CT) and patients noncarrying −35 C (genotype TT); p values are indicated.

for treatment arms (IMN and IFA), age of patients, and $CCR5^+$, $HLA\text{-}B^*27^+/HLA\text{-}B^*57^+$ genotypes affected on the Cox model analysis marginally.

The immunovirological response to the antiretroviral therapy was not significantly different between carriers and noncarriers of the allele *−35 C* in each measure of the surrogate markers of disease progression ($CD4^+$ T lymphocyte counts and RNA HIV-1 levels measured every three months during 36 months of follow-up). The SNP did not predict $CD4^+$ T cell recovery after 36 months on ART.

Finally, although HIV-1 DNA levels, measured retrospectively at the baseline pre-ART and one month post-ART were significantly different between patients with and without the protective allele, the measurements of HIV-1 DNA levels at the end of the study were not significantly different ($p = 0.458$).

4. Discussion

HIV-1 infection exhibits a considerably phenotypic heterogeneity that may be attributed to a complex interplay between viral, environmental, and host genetic factors. Host genes have been extensively explored by several genome-wide association studies, which compiled common human genetic variations across diverse continental populations. More than 16 GWAS targeting various HIV-linked phenotypes have been published since 2007 [1–5, 20]. Surprisingly, only the two HIV-1 chemokine coreceptors and *HLA loci* have exhibited consistent and reproducible statistically significant genetic associations. GWAS focusing on viral load control allowed to identify the association between plasma RNA viral load and the polymorphism *rs9264942* located 35 kb upstream *HLA-C*. Potential association of this SNP with HIV-1 reservoirs, particularly HIV-1 DNA levels had not been investigated. HIV-RNA level from plasma and HIV-1 DNA level from PBMCs are closely related variables but each one provides slightly different information about the replicative history and spread of the virus [21–23]. Both viral markers do not necessarily show the same trend.

Here, we have retrospectively genotyped the *−35 C/T* polymorphism *rs9264942* in 183 HIV-1 chronically infected asymptomatic subjects and have confirmed the association of the *−35 C/T* variant with the HIV-1 viral control. In our untreated patients, the protective allele *−35 C* is associated with lower viremias as previously demonstrated and also with lower levels of cellular reservoirs of HIV-1 DNA. Even one month posttherapy, when the majority (78.5%) of the 183 patients in our cohort showed complete suppression of HIV-1 RNA viremia, they maintained significantly different amounts of HIV DNA load between carriers and noncarriers of the protective allele *−35 C*. However, the trend for diminished HIV-1 DNA levels did not reach statistically significance at the end of the study STIR-2102, in response to antiviral therapy after thirty-six months on therapy. Carriers and noncarriers of the allele *−35 C* reached the study endpoint without showing statistically significant differences. Probably the allele *−35 C* has a moderate impact on the viral control only in the natural history of the infection, disappearing when treated with antiretrovirals (Figure 2). The mechanisms that regulate HLA-C expression and the link between

this molecule and HIV infection are still not fully understood. Higher levels of HLA-C associated to the protective allele *−35 C* may be important for the viral control in natural history of infection, involving both lower viremias and lower cellular HIV-1 reservoirs, possibly promoting effective CTL recognition and lysis of HIV-infected cells and modulating NK cell activity through the interaction with KIR receptors [24]. Whether HLA-C expression levels are directly responsible for the protective effect of −35 SNP (or 263 insertion/deletion polymorphism) or the strong linking disequilibrium between the protective variant *−35 C* and other protective genes in the *HLA* locus exerts viral control is still unclear. This is extremely difficult to unravel; therefore, we cannot exclude the possibility that some protective *HLA*-alleles have an effect on the viral control independently or in conjunction with −35 SNP [20, 25]. Certain *HLA* variants have been previously associated with viral control such as the protective alleles *HLA-B*57* and *HLA-B*27* [1–4]. In our study, we have also investigated these alleles (data not shown): eight patients carried the allele *HLA-B*57* and other 8 patients carried the allele *HLA-B*27*. We did not find any protective effect of these alleles on HIV-1 RNA or HIV-1 DNA viral load before or after the antiretroviral treatment. Probably, the statistical power of our study was limited taking into account the low frequency of both *HLA-B*57* and *HLA-B*27* in our population and the sample size. Therefore, our data cannot demonstrate an additive and independent protective effect of the alleles *HLA-B*57*, *HLA-B*27*, and *−35 HLA-C*.

Finally, synergistic interactions between loci may affect outcome after infection, as suggested by associations of specific, functionally relevant HLA and KIR variants with HIV disease outcomes and these require further consideration as well. Several association studies have shown that certain HLA/KIR pairs of genotypes (*HLA-Bw4*/*KIR3DL* and *HLA-Bw4*/*KIR3DS1*) are associated with lower rates of disease progression [26–28]. As HLA-C is the natural ligand for the inhibitory receptors KIR2DL1 and KIR2DL2/3, it would be worthy to study HLA-C group haplotypes (C1/C2) in relation with the corresponding polymorphic receptors KIR, in addition to the polymorphism *rs9264942*. Inhibitory signals derived from the KIR/HLA interactions play a pivotal role in discriminating normal from pathologic tissue and NK cell responses resulting in target infected-cell lysis [29]. To date, it is not clear the relevance of HLA-C levels and haplotypes in combination with different KIR in the HIV viral control.

The design of future studies might consider that the host and viral genetic variation are operating in an interacting system and probably each factor is not in itself sufficient to confer perdurable or complete protection for the disease outcome [30]. Considering the dynamic nature of host-pathogen interactions can shape optimized approaches to long-term HIV-1 management.

5. Conclusions

HIV-1 RNA viral load and HIV-1 DNA are both, in our study, variables significantly different between carriers and noncarriers of the protective allele −35 C before ART. Higher levels of HLA-C associated to the protective allele −35 C may be important for the viral control in natural history of infection, involving both lower viremias and lower cellular HIV-1 reservoirs, possibly promoting effective CTL recognition and lysis of HIV-infected cells and modulating NK cell activity through the interaction with KIR receptors. This protective effect of the −35 C allele on viral control was not maintained after the antiretroviral treatment. Probably, this allele has a moderate impact on the viral control only in the natural history of the infection, disappearing when treated with antiretrovirals (Figure 2).

Additional Points

Highlights. (1) The polymorphism *rs9264942* (C/T) impacts on HIV-1 DNA reservoirs. (2) The *rs9264942* allele C is associated with lower levels of HIV-1 DNA. (3) This protective effect is observed in untreated patients. (4) The SNP *rs9264942* is not associated with the viral control after 36 months on ART. (5) The SNP *rs9264942* do not influence T cell recovery in response to ART

Disclosure

An earlier version of this work was presented as an abstract at Congreso de la Sociedad Española de Inmunología 2016.

Conflicts of Interest

The authors declare no conflict of interest.

Authors' Contributions

Eduardo Fernández-Cruz and Carmen Rodríguez-Sainz conceived and designed the experiments. Laura Herráiz-Nicuesa, Diana Carolina Hernández-Flórez, Lara Valor, and Carmen Rodríguez-Sainz performed the experiments. Eduardo Fernández-Cruz and Carmen Rodríguez-Sainz analyzed the data. Eduardo Fernández-Cruz and Carmen Rodríguez-Sainz wrote the paper.

Acknowledgments

The authors thank the patients and investigators for contributing the samples studied in the STIR-2102 Study. This work was supported by Fondo de Investigación Sanitaria (FIS), Grants PI 06/1255 and PI 09/2558.

References

[1] M. P. Martin and M. Carrington, "Immunogenetics of HIV disease," *Immunological Reviews*, vol. 254, no. 1, pp. 245–264, 2013.

[2] S. Limou and J.-F. Zagury, "Immunogenetics: genome-wide association of non-progressive HIV and viral load control: *HLA* genes and beyond," *Frontiers in Immunology*, vol. 4, article 118, 2013.

[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] The International HIVCS, "The major genetic determinants of HIV-1 control affect HLA class I peptide presentation," *Science*, vol. 330, no. 6010, pp. 1551–1557, 2010.

[5] 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.

[6] 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.

[7] S. Kulkarni, R. Savan, Y. Qi et al., "Differential micro RNA regulation of HLA-C expresión and its association with HIV control," *Nature*, vol. 472, no. 7344, pp. 495–498, 2011.

[8] T. W. Corrah, N. Goonetilleke, J. Kopycinski et al., "A reappraisal of the relationship between the HIV-1-protective single nucleotide polymorphism 35 kb upstream of the HLA-C gene and surface HLA-C expression," *Journal of Virology*, vol. 85, no. 7, pp. 3367–3374, 2011.

[9] 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.

[10] E. J. Adams and P. Parham, "Species-specific evolution of MHC class I genes in the higher primates," *Immunological Reviews*, vol. 183, no. 1, pp. 41–64, 2001.

[11] A. M. Older Aguilar, L. A. Guethlein, E. J. Adams, L. Abi-Rached, A. K. Moesta, and P. Parham, "Coevolution of killer cell Ig-like receptors with HLA-C to become the major variable regulators of human NK cells," *Journal of Immunology*, vol. 185, no. 7, pp. 4238–4251, 2010.

[12] 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.

[13] J. A. McCutcheon, J. Gumperz, K. D. Smith, C. T. Lutz, and P. Parham, "Low HLA-C expression at cell surfaces correlates with increased turnover of heavy chain mRNA," *The Journal of Experimental Medicine*, vol. 181, no. 6, pp. 2085–2095, 1995.

[14] 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, pp. 171–179, 1998.

[15] S. Le Gall, L. Erdtmann, S. Benichou et al., "Nef interacts with the l subunit of clathrin adaptor complexes and reveals a cryptic sorting signal in MHC I molecules," *Immunity*, vol. 8, no. 4, pp. 483–495, 1998.

[16] E. Fernandez-Cruz, S. Moreno, J. Navarro et al., "Therapeutic immunization with an inactivated HIV-1 Immunogen plus antiretrovirals versus antiretrovirals therapy alone in asymptomatic HIV-infected subjects," *Vaccine*, vol. 22, no. 23.24, pp. 2966–2973, 2004.

[17] L. Valor, J. Navarro, J. Carbone et al., "Immunization with an HIV-1 immunogen induces CD4+ and CD8+ HIV-1-specific polyfunctional responses in patients with chronic HIV-1 infection receiving antiretroviral therapy," *Vaccine*, vol. 26, no. 22, pp. 2738–2745, 2008.

[18] C. Rodríguez-Sáinz, R. Ramos, L. Valor et al., "Prognostic value of peripheral blood mononuclear cell-associated HIV-1 DNA for virological outcome in asymptomatic HIV-1 chronic infection," *Journal of Clinical Virology*, vol. 48, no. 3, pp. 168–172, 2010.

[19] P. Grambsch and T. Therneau, "Proportional hazards tests and diagnostics based on weighted residual," *Biometrika*, vol. 81, no. 3, pp. 515–526, 1994.

[20] P. J. McLaren, C. Coulonges, I. Bartha et al., "Polymorphisms of large effect explain the majority of the host genetic contribution to variation of HIV-1virus load," *PNAS*, vol. 112, no. 47, pp. 14658–14663, 2015.

[21] V. Avettand-Fèncël, L. Hocqueloux, J. Ghosn et al., "Total HIV-1 DNA, a marker of viral reservoir dynamics with clinical implications," *Clinical Microbiology Reviews*, vol. 29, no. 4, pp. 859–880, 2016.

[22] R. Banga, F. A. Procopio, and M. Perreau, "Current approaches to assess HIV-1 persistance," *Current Opinion in HIV and AIDS*, vol. 11, no. 4, pp. 424–431, 2016.

[23] M. J. Churchill, S. G. Deeks, D. M. Margolis, R. F. Siliciano, and R. H. I. V. Swanstrom, "Reservoirs: what, where and how to target them," *Nature Reviews. Microbiology*, vol. 14, no. 1, pp. 55–60, 2016.

[24] M. Sips, Q. Liu, M. Draghi et al., "HLA-C levels impact natural killer cell subset distribution and function," *Human Immunology*, vol. 77, no. 12, pp. 1147–1153, 2016.

[25] M. Carrington, A. A. Bashirova, and P. J. McLaren, "On stand by: host genetics of HIV control," *AIDS*, vol. 27, no. 18, pp. 2831–2839, 2013.

[26] 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.

[27] K. Pelak, A. C. Need, J. Fellay et al., "Copy number variation of KIR genes influences HIV-1 control," *PLoS Biology*, vol. 9, no. 11, article e1001208, 2011.

[28] M. P. Martin, Y. Qi, X. Gao et al., "Innate partnership of HLA-B and KIR3DL1 subtypes against HIV-1," *Nature Genetics*, vol. 39, no. 6, pp. 733–740, 2007.

[29] J. Hens, W. Jennes, and L. Kestens, "The role of NK cells in HIV-1 protection: autologous, allogeneic or both?," *AIDS Research and Therapy*, vol. 13, no. 1, article 15, 2016.

[30] P. J. McLaren and J. Fellay, "Human genetic variation in HIV disease: beyond genome-wide association studies," *Current Opinion in HIV and AIDS*, vol. 10, no. 2, pp. 110–115, 2015.

Hindawi
Journal of Immunology Research
Volume 2017, Article ID 1548905, 9 pages
https://doi.org/10.1155/2017/1548905

Review Article

SERINC as a Restriction Factor to Inhibit Viral Infectivity and the Interaction with HIV

Gracia Viviana Gonzalez-Enriquez,[1] Martha Escoto-Delgadillo,[2,3] Eduardo Vazquez-Valls,[2] and Blanca Miriam Torres-Mendoza[1,2]

[1]*Departamento de Disciplinas Filosóficas, Metodológicas e Instrumentales, Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara, Guadalajara, JAL, Mexico*
[2]*Laboratorio de Inmunodeficiencias y Retrovirus Humanos, Centro de Investigación Biomédica de Occidente, Instituto Mexicano del Seguro Social, Guadalajara, JAL, Mexico*
[3]*Centro Universitario de Ciencias Biológicas y Agropecuarias, Universidad de Guadalajara, Guadalajara, JAL, Mexico*

Correspondence should be addressed to Blanca Miriam Torres-Mendoza; bltorres1@hotmail.com

Received 24 July 2017; Revised 22 October 2017; Accepted 2 November 2017; Published 22 November 2017

Academic Editor: Elias Said

Copyright © 2017 Gracia Viviana Gonzalez-Enriquez et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

The serine incorporator 5 (SERINC5) is a recently discovered restriction factor that inhibits viral infectivity by preventing fusion. Retroviruses have developed strategies to counteract the action of SERINC5, such as the expression of proteins like negative regulatory factor (Nef), S2, and glycosylated Gag (glycoGag). These accessory proteins downregulate SERINC5 from the plasma membrane for subsequent degradation in the lysosomes. The observed variability in the action of SERINC5 suggests the participation of other elements like the envelope glycoprotein (Env) that modulates susceptibility of the virus towards SERINC5. The exact mechanism by which SERINC5 inhibits viral fusion has not yet been determined, although it has been proposed that it increases the sensitivity of the Env by exposing regions which are recognized by neutralizing antibodies. More studies are needed to understand the role of SERINC5 and to assess its utility as a therapeutic strategy.

1. Introduction

The promising and within-reach goal of eradicating acquired immunodeficiency syndrome (AIDS) is not being accomplished. Despite the fact that the number of infected people has stabilized to around 35 million people living with HIV/AIDS, it has not been possible to decrease the numbers of infections since 2010 [1].

The current challenge includes finding a robust vaccine and, on the other hand, controlling infectivity in viral sanctuaries. Through various strategies, there are attempts to avoid viral replication using epigenetics and starting new antiretroviral regimens earlier [2].

Among others, there are proposals that include the endogenous proteins, especially the family of serine-incorporating proteins called SERINC [3]. Within the members of this family, the SERINC5 participates in the defense of the host and has the potential for the development of innovative, antiviral treatments [4].

2. Retrovirus and Host Defense

Retroviruses have developed strategies to evade and counteract the host's immune response and achieve successful infection that allows them to spread [5, 6]. Within these capabilities is the blocking of the host proteins that interfere at different stages of the viral cycle [7, 8]. These proteins are called "viral restriction factors" and they seek to counteract the action of viruses by providing a cellular barrier, being the first line of defense against viral infection [9, 10].

The restriction factors are highly conserved and are multitaskers, one of their function is regulated cross-species infections. Consequently, they are less effective against viruses in their natural hosts [10, 11].

In this sense, the human immunodeficiency virus (HIV) is not the exception among retroviruses. The HIV has developed mechanisms to overcome these types of cellular barriers that hinder replication (the restriction factors) through viral proteins that interfere with, or nullify, the activity of the host's defense factors [12, 13]. HIV uses its accessory proteins such as the Vif that counteracts the apolipoprotein B mRNA-editing enzyme 3G (APOBEC3G), Vpr for SLX4 endonuclease complex, Vpu for bone marrow stromal antigen 2 (BST-2) or tetherin, and Vpx for SAM-domain HD-domain containing protein 1 (SAMHD1) [14–19].

Antagonists have been found for all accessory viral proteins except for the negative regulatory factor (Nef). Recently, through Nef-deficient cell cultures, the family of proteins called SERINC was discovered [4, 20]. The SERINC have a high antiviral activity against retroviruses such as lentivirus (HIV, simian immunodeficiency virus (SIV), and equine infectious anemia virus (EIAV)) and gammaretrovirus (murine leukemia virus (MLV)) [4, 21, 22].

3. Nef Activity on HIV Virulence

The HIV-1 evades the host's immune response through manipulation of the cell machinery [23]. This process involves the use of vesicular traffic to efficiently direct cell markers such as CD4 and the major histocompatibility complex class 1 (MHC-1) from the plasma membrane to the endosomes, to final degradation in the lysosome [24–27] (Figure 1(a)). To accomplish this activity, the virus uses its Nef accessory protein, which is expressed during the early stages of viral infection [28]. The importance of Nef participation in viral pathogenesis was evidenced by the fact that in SIV, the lack of a functional Nef protein showed a decrease in clinical disease progression and a lower viral load [24, 29].

Nef has a weight of 27–35 kDa and presents myristoylation at the N-terminal end that facilitates association with the cytosolic side of lipids and cell membrane proteins [30]. It has a proline-rich structure that allows it to interact with host tyrosine kinase proteins and AP-2 domain [31–33]. This helps in the recognition of proteins involved in vesicular traffic, such as the family of heterotetrameric adapter protein (AP) complexes, the AP-1, AP-2, phosphofurin acidic cluster sorting protein 1 (PACS1), and the PACS2 [34–39]. The Nef favors the intrinsic infectivity of HIV virions necessary for the full deployment of virus infectivity. The mechanism by which it performs this action is not well defined, yet it is known to cause downregulation of CD4 lymphocytes [31]. The goal is to prevent reinfection and prevent antibody-mediated cytotoxicity; and, it also reduces the MHC-1 to protect infected cells from death by cytotoxic T lymphocytes [40, 41]. Furthermore, it induces the release of virions and contributes to maintaining high viral load in patients [42]. Some patients with deletion of the Nef gene also have downregulation levels of CD4 T lymphocytes, which suggest that it is not the only protein involved in infectivity, although its role is of great importance. In a cohort of 8 individuals infected by transfusion from the same donor who had a deletion of the *nef* gene, they progressed very slowly or did not progress at all, which makes the Nef protein a pathogenic factor [43, 44]. Nef and Vpu contribute to HIV-infected cells evading recognition by the immune system and its consequential cell lysis of cytotoxic T lymphocytes and natural killer cells [45, 46].

4. SERINC Family

The SERINC proteins are part of a family of transmembrane proteins present in all eukaryotic cells. These proteins are highly conserved and unique and are not homologous with other eukaryotic proteins, which possibly makes their function in the cell membrane indispensable [3, 47]. The SERINC family is comprised of five members, from SERINC1 to SERINC5, structurally characterized as having 10 to 11 transmembrane domains. SERINC proteins participate in the transport of the serine amino acid through the lipid bilayer and in the biosynthesis of sphingolipids and phosphatidylserine by incorporating serine into membrane lipids [3]. However, the exact physiological function of the SERINC family is still unknown [7, 48].

Of all the members in the SERINC family, only the SERINC3 and SERINC5 have the ability to inhibit viral infection at an early stage of the viral cycle, inhibiting viral fusion and acting as restriction factors [4, 20].

The SERINC5 protein has five isoforms generated by alternative splicing (Figure 2). These isoforms have similar topology but differ at the terminal carbon end and in the number of transmembrane domains. Only the SERINC5-001 isoform has 10 transmembrane domains and consequently presents the longest sequence with 461 amino acids, of which 12 are located at their C-terminal end. The remaining isoforms lack the transmembrane domain 10 and have different numbers of amino acids at their C-terminus, located after domain nine: the SERINC5-004, -005, -008a, -008b, and -201 isoforms have 47, 11, 8, 5, and 5 amino acids, respectively. SERINC3 does not have isoforms [49].

The SERINC5-001 isoform is expressed in greater quantities compared to other isoforms. It is the only one that is in the plasma membrane and involved in the inhibition of HIV infectivity. Therefore, the transmembrane domain 10 is key to the SERINC5 activity as a viral restriction factor. The SERINC5-005 and SERINC5-008 isoforms are in the cytoplasm but have a short half-life because they are rapidly degraded. This demonstrates that the 10-domain and the carbon-terminal end are necessary to stabilize and increase the expression of SERINC5 [49, 50].

5. Nef Mechanisms to Counter SERINC3 and SERINC5

The antiviral activity of SERINC5 is counteracted by Nef (by leading to a decrease in its incorporation in the virions) because Nef removes it from the plasma membrane and sequesters it in the endosomes for its subsequent degradation

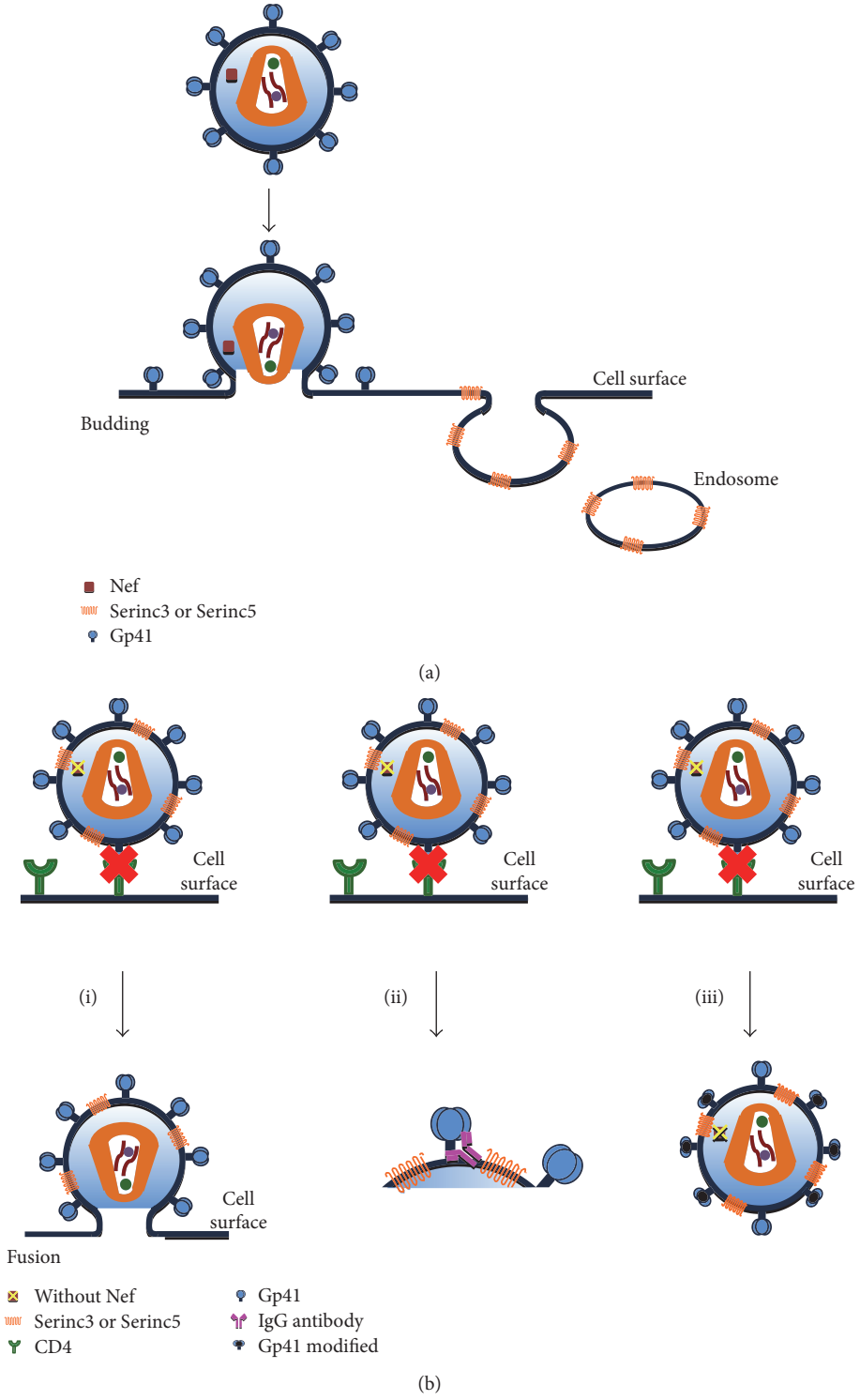


Figure 1: SERINC and Nef interaction in HIV infection. (a) HIV-1 evades the host immune response through manipulation of cell machinery. This process involves the use of vesicular traffic from the plasma membrane to the endosomes and finally be degraded in the lysosome. The HIV uses Nef protein to carry out this activity. In cells infected with viruses expressing the Nef protein, it is observed that SERINC5 was sequestered in the endosomes. (b) HIV-1 with Nef deletion. SERINC5 blocks the activities involved in viral infectivity and does not participate in other Nef-mediated processes. This interaction could have three possible actions and could have as a consequence block or slow the fusion of virus. (i) SERINC5 alters the enlargement of the fusion pore decreasing the ability of the virions to fuse with the target cells; (ii) by slowing the fusion, it would be promoted that gp41 to adopt an open conformation, which would remain exposed for a long time making it susceptible to the neutralizing antibodies; and (iii) SERINC5 could promote structural changes in envelope glycoproteins leading to preventing the entry of the virus prior to small pore formation.

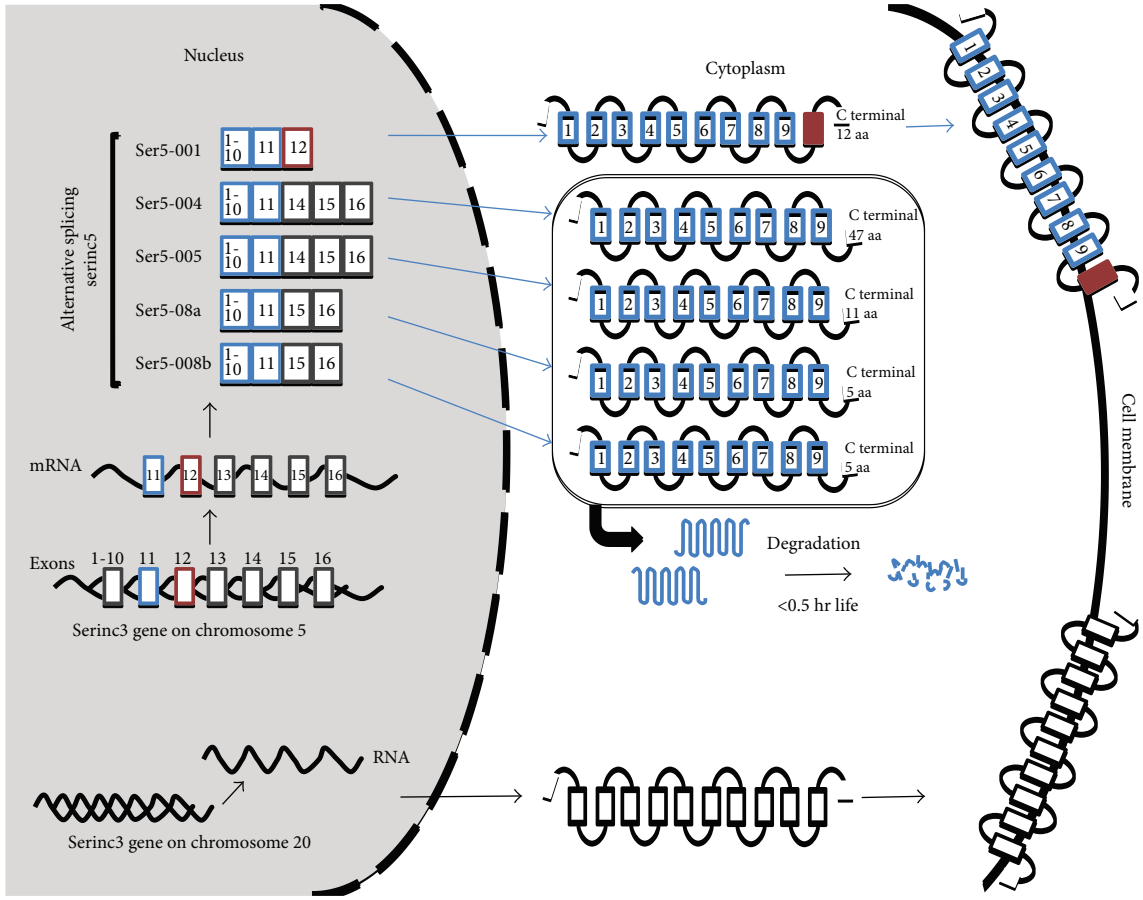


Figure 2: SERINC3 and SERINC5 isoforms. SERINC3 does not have isoforms and is transported directly to the cell membrane. SERINC5 protein has five isoforms generated by alternative splicing. These differ at the terminal carbon end and in the transmembrane domains. The SERINC5-001 isoform has 10 transmembrane domains, only these are in the cell membrane and are involved in the inhibition of HIV infectivity.

[4, 20] (Figure 1(a)). To accomplish this action, Nef requires certain structural characteristics as defined below.

5.1. Transport Pathways. Nef induces downregulation of SERINC3 and SERINC5 from the cell membrane by using the cellular transport machinery, mainly using the endolysosomal system and the trans-Golgi network, in a mechanism similar to that used for CD4 downregulation [4, 51–53].

Clathrin-covered vesicles are the main carriers of the endocytic and late secretory pathways, regulating the transport of proteins from the plasma membrane and endosomes to compartments such as other endosomes or lysosomes [54]. Nef sequesters the vesicular traffic of the cells through the modulation of some adapter and accessory proteins involved in the formation of clathrin-coated vesicles [55, 56]. Within these adaptive proteins are the AP complexes and dynamin [57–59]. Nef has conserved sequences such as dileucine motifs (ExxxLL) and carboxy terminal diacid residues (EDAA) that allow it to interact with endocytic machinery, particularly AP1 and AP2 proteins [37, 60, 61]. These motifs are indispensable for the downregulation of MHC-1 and CD4 [51, 57]. Also, Nef requires both regions to antagonize SERINC5 and send it to the endosomes. Therefore, Nef-mediated SERINC5 removal requires the cell to produce AP2 and dynamin [20, 51, 52].

5.2. Myristoylation. Myristoylation is a posttranslational change that requires Nef to anchor in the membrane and execute sequestration of the proteins [30, 62, 63]. This modification is carried out in the glycine residue of Nef and when, in experiments, this was substituted by alanine, there was a decrease in the internalization of SERINC5 towards the endosomes for its subsequent degradation [4, 52].

The ability of SERINC3 and SERINC5 to inhibit viral infectivity in cell cultures that had HIV-1 with Nef deletion was demonstrated with genomic and proteomic tools. However, SERINC5 is more potent in its capacity for viral inhibition than SERINC3. SERINC5 reduces wild-type (WT) HIV infectivity in a range between 50% and 90%, whereas SERINC3 only showed a 20% decrease in infectivity [4, 49]. Even when SERINC5 was expressed ectopically, it was observed that the infection was inhibited up to 40 times. SERINC5 inhibition of Nef-defective HIV-1 occurs in a dose-dependent relationship. In contrast, in cells infected with viruses expressing the Nef protein they observed that SERINC5 was sequestered in the endosomes, which

prevented their incorporation into viral particles and increased viral infectivity up to 20 to 30 times that observed without Nef-expression cells [4, 20].

When Nef is present, it interacts with SERINC5 to restrict its activity. However, in certain cellular models, the SERINC5 overexpression is able to suppress the capacity of Nef. Thus, it is assumed that Nef activity can be saturated with overexpression of this viral restriction factor [4, 49]. Also, it was found that in few virion-associated SERINC5, the enlargement of the fusion pore is altered, which implies a higher energy spending for pore formation [20] (Figure 1(b)).

The expression of SERINC5 decreases the ability of the virions to fuse with the target cells [4, 20]. SERINC5 only blocks the activities involved in viral infectivity and does not participate in other Nef-mediated processes such as decreased CD4 or MHC-1 [52].

The exact mechanism by which SERINC5 acts to inhibit viral infectivity is still unknown, and there are other elements in addition to Nef that mediate the SERINC5 restriction activity [52]. The type of envelope glycoprotein (Env) that the virus carries is the other determinant able to counteract and resist the SERINC5 action, and this could account why in some cells infected with HIV-1, SERINC5 does not block the fusion [64–66]. Despite *in vitro* experiments not detecting any physical interaction between Env and SERINC5, the presence of virion-associated SERINC5 did not interfere with the incorporation or distribution of Env [65]. So, Nef and Env act by distinct mechanisms to counteract SERINC5 [64].

One possible explanation of the mechanism is that SERINC5 acts to form large oligomers, restricting lipid diffusion and/or inducing hardening of the membranes, leading to decreased mobility of viral particles and interrupting their fusion [65]. The membrane stiffness would slow the folding of the envelope for fusion and promote that the Env would adopt an open conformation, which would remain exposed for a long time and make it susceptible to the neutralizing antibodies [65, 67]. In fact, it was found that the incorporation of SERINC5 increased sensitivity of the antibody 4E10 that targets the membrane-proximal region (MPER) of the gp41, so SERINC5 sensitizes HIV to neutralizing antibodies and inhibitory peptides that recognize conserved gp41 domains [64, 65] (Figure 1(b)).

There are HIV-Env isolates that are resistant to SERINC5. The regions of envelope glycoproteins that contribute to this resistance are located in loops V1, V2, and V3 [66], and this resistance may be impaired when there are inhibitory pressures sensitizing Env to SERINC5 activity [64, 68, 69]. Like when the antiretroviral maraviroc was present, the virions carrying SERINC5 were more sensitive to the maraviroc and neutralizing antibodies [64]. Maraviroc decreases the level of CCR5 receptors on the cell surface that could bind to the Env [70, 71]. This action is potentiated in SERINC5-associated virions by promoting a change in Env conformation that delays the entry of the virus through preventing or slowing the formation of the fusion pore [64]. *In vitro*, SERINC5 causes a conformational change in Env and exposes conserved regions that were identified by the use of neutralizing antibodies [64]. Thus, *in vivo*, subjects infected with certain HIV variants might be more susceptible to maraviroc, for the action of SERINC5, than other subjects with variants resistant to this restriction factor. The greatest susceptibility could be the combination of the conformational changes that generate SERINC5 on Env that delay viral fusion and the recognition of regions conserved through neutralizing antibodies circulating in the subject.

The hypothesis raised by Rosa et al. [4], on the antiviral activity of SERINC5 and its participation in the lipid composition of host cell membranes and HIV-1, was not confirmed by Trautz et al. [4, 48] who found no alterations in the lipid composition of membranes between SERINC5-associated virions with Nef-defective and WT [48]. Although the HIV-1 particles have a higher concentration of saturated lipid species than the cell membranes, the absence of Nef and the presence of SERINC5 did not change this condition [48, 72]. However, a subpopulation of Nef-associated to lipids rafts can alter the lipid composition of this microdomain of the cell host and facilitate signal transduction activities of Nef [73, 74]. In MT-4 T lymphocytes, Nef enhanced sphingomyelin uptake and exclusion of polyunsaturated phosphatidylcholine from the virions, thereby increasing the lipid raft character [73]. In contrast, HIV-1 particles produced from 293 T cells did not show Nef-mediated sphingomyelin enrichment [48]. The diversity reported in the action of Nef may be a consequence of the different cellular models used, similar to the observed variability of SERINC5 to counteract the infectivity of HIV-1 [4]. Another factor is the methodology used, for example, transfecting proviral DNA allowed the Nef-mediated recruitment of Gag into microdomains of 293 T cells; on the contrary, in MT-4 and Jurkat T lymphocytes cells that were not transfected, Gag was not found in lipid rafts [75, 76]. *In vitro* studies do not always correlate with the physiological phenomenon and can yield controversial results [77], so the action of Nef and SERINC5 on the lipid composition should continue to be investigated in different cell models.

6. Other Proteins Equivalent to Nef

SERINC5 is fundamental to the restriction of virus infectivity, so those retroviruses that lack the Nef protein count on proteins with a similar activity to block SERINC3 and SERINC5, as is the case with glycosylated Gag (glycoGag) of the gammaretrovirus, MLV, and S2 of the lentivirus EIAV. For this reason, it is considered a potent viral restriction factor [21, 22, 67].

Nef, glycoGag, and S2 do not present structural homology between them, but they have characteristic patterns that allow for inhibiting SERINC3 and SERINC5, since they all have regions of myristoylation and have conserved the domain of dileucine to obtain the union with AP2 [21, 22, 78].

6.1. GlycoGag. The glycoGag protein is identical to the Gag protein except for the presence of 88 additional residues at the amino-terminal end. This amino acid sequence acts as a signal to be transported through vesicular traffic to the cell surface to the N-terminal end towards the cytosolic face,

and the C-terminal is cut by a protease to be free in the extracellular space [78, 79]. The function of glycoGag has not been completely clarified but it increases the infectivity of MLV particles [80]. The MLV envelope glycoproteins are highly polymorphic and glycoGag has been shown to have a greater contribution to infectivity when certain types of Env that are more sensitive to their action are present [21, 66]. SERINC5 reduces the infectivity of MLV in the absence of glycoGag, and, as with HIV-1, the inhibition is dose-dependent: the greater the amount of SERINC5, the greater the inhibition of MLV [4, 21].

6.2. S2. The EIAV expresses the auxiliary protein S2 that has a molecular weight of 7 kDa and is not homologous with other proteins. Its function remained unknown until it was discovered that S2 antagonizes SERINC3 and SERINC5 [22, 81, 82].

The HIV-1 with S2 expression was six times more infectious than the Nef-defective HIV-1. The S2 activity complements but does not add to restoration of the Nef and glycoGag infectivity function. Furthermore, like Nef and glycoGag, S2 has a similar dependence on cell type and envelope glycoprotein to exert its action [21, 22]. The S2 relocalizes SERINC to the endosomes and significantly reduces its incorporation into virions; thus, the S2 executes HIV-1 infectivity by counteracting SERINC3 and SERINC5. Despite the lack of homology that S2 has with others, the Nef, glycoGag, and S2 share two similar sequences, one of which is the site of myristoylation that is located in the glycine of position seven, and the other is the dileucine motif, and both, as mentioned above, counteract the activity of SERINC5 [22, 81]. This points out that the power of SERINC5 to inhibit infectivity is twice that in cells infected with HIV-1 than in cells infected with EIAV envelope glycoprotein [22]. Therefore, the Env type plays a role in the susceptibility of retroviruses to SERINC and participates in the variability of the activity that occurs with different strains of HIV-1, vesicular stomatitis virus (VSV), and the Ebola virus [4, 83].

7. Evolution of Nef and SERINC

The fitness to inhibit SERINC5 arose independently in lentiviruses and gammaretroviruses as each developed its proteins to escape the action of this cellular restriction factor [22, 80].

The activity of Nef to counteract SERINC5 is essential for guaranteeing viral infectivity, which is highly conserved among primate lentiviruses, and correlates with the prevalence of these species. Nef mutations that guaranteed highly effective activity against SERINC5 were selected during the adaptation of chimpanzees; in fact, they appear to have gained greater anti-SERINC5 activity after transmission between species of monkeys to apes and from apes to humans [84]. It is also observed that although Nef is one of the most variable proteins among primate lentiviruses, the dileucine motif is highly conserved among species because it is a region that is required to abolish the activity of SERINC5 [52, 84].

SERINC5 has a different evolutionary history than other restriction factors, as it does not present a high frequency of nonsynonymous substitutions in its coding sequence as it occurs with APOBEC3G and SAMHD1. The other functions that SERINC5 performs within the cell may be those that determined its evolution and avoided the arms race or limited it to the introns [85].

8. SERINC5: The Promise in HIV

SERINC5 presents new and promising scenarios for both the generation of treatments for HIV infection and the prognosis of the disease. SERINC5 would be as an adjunctive treatment with current antiretrovirals, primarily maraviroc, based on results found in cell cultures where the expression of SERINC5 made the cells more sensitive to the action of three antiretrovirals [64]. To achieve this, SERINC5 would be synthesized by genetic engineering (a fragment that includes the transmembrane domain 10) and incorporated into nanosomes loaded with maraviroc-like signal for delivery to the plasma membrane of the CD4 T cells and counteract the Nef-mediated infectivity. *In vitro* studies have to develop synthetic cell surface receptors that are inserted into the cell surface for the execution of their biologic functions and have the potential of biological drugs [86]. As proteins anchored by glycosylphosphatidylinositol are incorporated in the plasma membrane, they retain native protein function [87]. The use of synthetic cell surface receptors is a better strategy than the gene transfer for the manipulation of the components of the plasmatic membrane [86, 87]. With these strategies, the immunological mechanisms of the host are potentiated by combining the action of restriction factors such as SERINC5 with neutralizing antibodies directed against the MPER of gp41 that guarantee a more successful treatment for the cure and/or eradication of HIV-1 [64, 65].

Levels of SERINC5 expression in serum should be investigated as a possible diagnostic tool to predict response to treatment when using maraviroc. Finally, the results found by Trautz et al. [48] and the evolutionary history of SERINC5 [48, 85] present new questions to elucidate on the SERINC3 and SERINC5 functions within cells and in identifying the biochemical mechanism through which they inhibit viral fusion and the infectivity of retroviruses.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

Acknowledgments

The authors wish to thank the Centro Universitario de Ciencias de la Salud de la Universidad de Guadalajara, for their support of this research.

References

[1] UNAIDS, "AIDS Update 2016. Joint United Programme on HIV/AIDS," 2017, July-2017 http://www.unaids.org/sites/default/files/media_asset/global-AIDS-update-2016_en.pdf.

[2] A. S. Fauci and H. D. Marston, "Ending AIDS — is an HIV vaccine necessary?," *The New England Journal of Medicine*, vol. 370, no. 6, pp. 495–498, 2014.

[3] M. Inuzuka, M. Hayakawa, and T. Ingi, "Serinc, an activity-regulated protein family, incorporates serine into membrane lipid synthesis," *The Journal of Biological Chemistry*, vol. 280, no. 42, pp. 35776–35783, 2005.

[4] A. Rosa, A. Chande, S. Ziglio et al., "HIV-1 Nef promotes infection by excluding SERINC5 from virion incorporation," *Nature*, vol. 526, no. 7572, pp. 212–217, 2015.

[5] F. Kirchhoff, "Immune evasion and counteraction of restriction factors by HIV-1 and other primate lentiviruses," *Cell Host and Microbe*, vol. 8, no. 1, pp. 55–67, 2010.

[6] R. E. Randall and D. E. Griffin, "Within host RNA virus persistence: mechanisms and consequences," *Current Opinion in Virology*, vol. 23, pp. 35–42, 2017.

[7] O. T. Fackler, "Spotlight on HIV-1 Nef: SERINC3 and SERINC5 identified as restriction factors antagonized by the pathogenesis factor," *Virus*, vol. 7, no. 12, pp. 6730–6738, 2015.

[8] C. Goffinet, "Cellular antiviral factors that target particle infectivity of HIV-1," *Current HIV Research*, vol. 14, no. 3, pp. 211–216, 2016.

[9] F. Bin Hamid, J. Kim, and C. Shin, "Cellular and viral determinants of retroviral nuclear entry," *Canadian Journal of Microbiology*, vol. 62, no. 1, pp. 1–15, 2015.

[10] S. F. Kluge, D. Sauter, and F. Kirchhoff, "SnapShot: antiviral restriction factors," *Cell*, vol. 163, no. 3, pp. 774–774.e1, 2015.

[11] R. A. Lever and A. M. L. Lever, "Intracellular defenses against HIV, viral evasion and novel therapeutic approaches," *Journal of the Formosan Medical Association*, vol. 110, no. 6, pp. 350–362, 2011.

[12] M. Imran, S. Manzoor, M. Saalim et al., "HIV-1 and hijacking of the host immune system: the current scenario," *Acta Pathologica, Microbiologica et Immunologica Scandinavica*, vol. 124, no. 10, pp. 817–831, 2016.

[13] K. Strebel, "HIV accessory proteins versus host restriction factors," *Current Opinion in Virology*, vol. 3, no. 6, pp. 1–13, 2014.

[14] N. Laguette, C. Brégnard, P. Hue et al., "Premature activation of the SLX4 complex by Vpr promotes G2/M arrest and escape from innate immune sensing," *Cell*, vol. 156, no. 1-2, pp. 134–145, 2014.

[15] V. Simon, N. Bloch, and N. R. Landau, "Intrinsic host restrictions to HIV-1 and mechanisms of viral escape," *Nature Immunology*, vol. 16, no. 6, pp. 546–553, 2015.

[16] M. W. McNatt, T. Zang, and P. D. Bieniasz, "Vpu binds directly to tetherin and displaces it from nascent virions," *PLoS Pathogens*, vol. 9, no. 4, pp. 40–44, 2013.

[17] A. Ara, R. P. Love, T. B. Follack, K. A. Ahmed, M. B. Adolph, and L. Chelico, "Mechanism of enhanced HIV restriction by virion coencapsidated cytidine deaminases APOBEC3F and APOBEC3G," *Journal of Virology*, vol. 91, no. 3, pp. e02230–e02216, 2016.

[18] K. Hrecka, C. Hao, M. Gierszewska et al., "Vpx relieves inhibition of HIV-1 infection of macrophages mediated by the SAMHD1 protein," *Nature*, vol. 474, no. 7353, pp. 658–661, 2011.

[19] N. Laguette, B. Sobhian, N. Casartelli et al., "SAMHD1 is the dendritic- and myeloid-cell-specific HIV-1 restriction factor counteracted by Vpx," *Nature*, vol. 474, no. 7353, pp. 654–657, 2011.

[20] Y. Usami, Y. Wu, and H. G. Göttlinger, "SERINC3 and SERINC5 restrict HIV-1 infectivity and are counteracted by Nef," *Nature*, vol. 526, no. 7572, pp. 218–223, 2015.

[21] Y. S. Ahi, S. Zhang, Y. Thappeta et al., "Functional interplay between murine leukemia virus glycogag, serinc5, and surface glycoprotein governs virus entry, with opposite effects on gammaretroviral and ebolavirus glycoproteins," *MBio*, vol. 7, no. 6, pp. 1–14, 2016.

[22] A. Chande, E. C. Cuccurullo, A. Rosa, S. Ziglio, S. Carpenter, and M. Pizzato, "S2 from equine infectious anemia virus is an infectivity factor which counteracts the retroviral inhibitors SERINC5 and SERINC3," *Proceedings of the National Academy of Science of the United States of America*, vol. 113, no. 46, pp. 13197–13202, 2016.

[23] A. Landi, V. Iannucci, A. Van Nuffel, P. Meuwissen, and B. Verhasselt, "One protein to rule them all: modulation of cell surface receptors and molecules by HIV Nef," *Current HIV Research*, vol. 9, no. 7, pp. 496–504, 2011.

[24] B. R. E. Benson, A. Sanfridson, J. S. Ottinger, C. Doyle, P. Bryan, and N. Carolina, "Downregulation of cell-surface CD4 expression by simian immunodeficiency virus Nef prevents viral super infection," *Journal of Experimental Medicine*, vol. 177, no. 6, pp. 1561–1566, 1993.

[25] N. Michel, I. Allespach, S. Venzke, O. T. Fackler, and O. T. Keppler, "The Nef protein of human immunodeficiency virus establishes superinfection immunity by a dual strategy to downregulate cell-surface CCR5 and CD4," *Current Biology*, vol. 15, no. 8, pp. 714–723, 2005.

[26] J. V. Garcia and A. D. Miller, "Serine phosphorylation-independent downregulation of cell-surface CD4 by *Nef*," *Nature*, vol. 350, no. 6318, pp. 508–511, 1991.

[27] 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.

[28] O. Schwartz, V. Marechal, O. Danos, and J. M. Heard, "Human immunodeficiency virus type 1 Nef increases the efficiency of reverse transcription in the infected cell," *Journal of Virology*, vol. 69, no. 7, pp. 4053–4059, 1995.

[29] F. Kirchhoff, T. Greenough, D. Brettler, J. Sullivan, and R. Desrosiers, "Brief report: absence of intact Nef sequences in a long-term survivor with nonprogressive HIV-1 infection," *The New England Journal of Medicine*, vol. 332, no. 4, pp. 228–232, 1995.

[30] O. T. Fackler, N. Kienzle, E. Kremmer et al., "Association of human immunodeficiency virus Nef protein with actin is myristoylation dependent and influences its subcellular localization," *European Journal of Biochemistry*, vol. 247, no. 3, pp. 843–851, 1997.

[31] X. Ren, S. Y. Park, J. S. Bonifacino, and J. H. Hurley, "How HIV-1 Nef hijacks the AP-2 clathrin adaptor to downregulate CD4," *eLife*, vol. 3, article e01754, 2014.

[32] R. Chaudhuri, O. W. Lindwasser, W. J. Smith, J. H. Hurley, and J. S. Bonifacino, "Downregulation of CD4 by human immunodeficiency virus type 1 Nef is dependent on clathrin and involves direct interaction of Nef with the AP2 clathrin adaptor," *Journal of Virology*, vol. 81, no. 8, pp. 3877–3890, 2007.

[33] K. Saksela, G. Cheng, and D. Baltimore, "Proline-rich (PxxP) motifs in HIV-1 Nef bind to SH3 domains of a subset of Src kinases and are required for the enhanced growth of Nef+ viruses but not for down-regulation of CD4," *The EMBO Journal*, vol. 14, no. 3, pp. 484–491, 1995.

[34] S. Basmaciogullari and M. Pizzato, "The activity of Nef on HIV-1 infectivity," *Frontiers in Microbiology*, vol. 5, p. 232, 2014.

[35] S. M. Sugden, M. G. Bego, T. N. Pham, and É. A. Cohen, "Remodeling of the host cell plasma membrane by fitness and persistence," *Virus*, vol. 8, no. 67, pp. 1–30, 2016.

[36] L. Erdtmann, K. Janvier, G. Raposo et al., "Two domains of HIV Nef are required for interaction with the endocytic machinery through binding to the μ1 chain of AP1 complex," *Traffic*, vol. 1, no. 11, pp. 871–883, 2000.

[37] H. M. Craig, T. R. Reddy, N. L. Riggs, P. P. Dao, and J. C. Guatelli, "Interactions of HIV-1 Nef with the mu subunits of adaptor protein complexes 1, 2, and 3: role of the dileucine-based sorting motif," *Virology*, vol. 271, no. 1, pp. 9–17, 2000.

[38] J. D. Dikeakos, L. Thomas, G. Kwon, J. Elferich, U. Shinde, and G. Thomas, "An interdomain binding site on HIV-1 Nef interacts with PACS-1 and PACS-2 on endosomes to down-regulate MHC-I," *Molecular Biology of the Cell*, vol. 23, no. 11, pp. 2184–2197, 2012.

[39] 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.

[40] 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.

[41] S. A. Swann, M. Williams, C. M. Story, K. R. Bobbitt, R. Fleis, and K. L. Collins, "HIV-1 Nef blocks transport of MHC class I molecules to the cell surface via a PI 3-kinase-dependent pathway," *Virology*, vol. 282, no. 2, pp. 267–277, 2001.

[42] H. W. Kestler 3rd, D. J. Ringler, K. Mori et al., "Importance of the Nef gene for maintenance of high virus loads and for development of AIDS," *Cell*, vol. 65, no. 4, pp. 651–662, 1991.

[43] J. C. Learmont, A. F. Geczy, J. Mills et al., "Immunologic and virologic status after 14 to 18 years of infection with an attenuated strain of HIV-1 — a report from the Sydney Blood Bank Cohort," *The New England Journal of Medicine*, vol. 340, no. 22, pp. 1715–1722, 1999.

[44] J. Learmont, B. Tindall, J. Kaldor et al., "Long-term symptomless HIV-1 infection in recipients of blood products from a single donor," *The Lancet*, vol. 340, no. 8824, pp. 863–867, 1992.

[45] B. K. Chen and R. T. Gandhi, "CD4 down-modulation during infection of human T cells with human immunodeficiency virus type 1 involves independent activities of Vpu, Env, and Nef," *Journal of Virological Methods*, vol. 70, no. 9, pp. 6044–6053, 1996.

[46] S. Wildum, M. Schindler, J. Münch, and F. Kirchhoff, "Contribution of Vpu, Env, and Nef to CD4 down-modulation and resistance of human immunodeficiency virus type 1-infected T cells to superinfection," *Journal of Virology*, vol. 80, no. 16, pp. 8047–8059, 2006.

[47] M. Bossolasco, F. Veillette, R. Bertrand, and A.-M. Mes-Masson, "Human TDE1, a TDE1/TMS family member, inhibits apoptosis in vitro and stimulates *in vivo* tumorigenesis," *Oncogene*, vol. 25, no. 33, pp. 4549–4558, 2006.

[48] B. Trautz, H. Wiedemann, C. Lüchtenborg et al., "The host-cell restriction factor SERINC5 restricts HIV-1 infectivity without altering the lipid composition and organization of viral particles," *Journal of Biological Chemistry*, vol. 114, no. 35, pp. 9379–9384, 2017.

[49] X. Zhang, T. Zhou, J. Yang et al., "Identification of SERINC5-001 as the predominant spliced isoform for HIV-1 restriction," *Journal of Virology*, vol. 91, no. 10, pp. e00137–e00117, 2017.

[50] T. Kurosaki and L. E. Maquat, "Nonsense-mediated mRNA decay in humans at a glance," *Journal of Cell Science*, vol. 129, no. 3, pp. 461–467, 2016.

[51] E. A. Pereira and L. L. P. daSilva, "HIV-1 Nef: taking control of protein trafficking," *Traffic*, vol. 17, no. 9, pp. 976–996, 2016.

[52] B. Trautz, V. Pierini, R. Wombacher et al., "The antagonism of HIV-1 Nef to SERINC5 particle infectivity restriction involves the counteraction of virion-associated pools of the restriction factor," *Journal of Virology*, vol. 90, no. 23, pp. 10915–10927, 2016.

[53] C. Aiken, J. Konner, N. R. Landau, M. E. Lenburg, and D. Trono, "Nef induces CD4 endocytosis: requirement for a critical dileucine motif in the membrane-proximal CD4 cytoplasmic domain," *Cell*, vol. 76, no. 5, pp. 853–864, 1994.

[54] D. E. Wakeham, J. A. Ybe, F. M. Brodsky, and P. K. Hwang, "Molecular structures of proteins involved in vesicle coat formation," *Traffic*, vol. 1, no. 5, pp. 393–398, 2000.

[55] A. Burtey, J. Z. Rappoport, J. Bouchet et al., "Dynamic interaction of HIV-1 Nef with the clathrin-mediated endocytic pathway at the plasma membrane," *Traffic*, vol. 8, no. 1, pp. 61–76, 2007.

[56] G. H. Renkema and K. Saksela, "Interactions of HIV-1 NEF with cellular signal transducing proteins," *Frontiers in Bioscience*, vol. 5, no. 1, pp. D268–D283, 2000.

[57] S. Le Gall, L. Erdtmann, S. Benichou et al., "Nef interacts with the μ subunit of clathrin adaptor complexes and reveals a cryptic sorting signal in MHC I molecules," *Immunity*, vol. 8, no. 4, pp. 483–495, 1998.

[58] L. Johannes, V. Pezo, F. Mallard et al., "Effects of HIV-1 Nef on retrograde transport from the plasma membrane to the endoplasmic reticulum," *Traffic*, vol. 4, no. 5, pp. 323–332, 2003.

[59] M. Pizzato, A. Helander, E. Popova et al., "Dynamin 2 is required for the enhancement of HIV-1 infectivity by Nef," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, no. 16, pp. 6812–6817, 2007.

[60] M. Greenberg, L. DeTulleo, I. Rapoport, J. Skowronski, and T. Kirchhausen, "A dileucine motif in HIV-1 Nef is essential for sorting into clathrin-coated pits and for downregulation of CD4," *Current Biology*, vol. 8, no. 22, pp. 1239–12S3, 1998.

[61] P. A. Bresnahan, W. Yonemoto, S. Ferrell, D. Williams-Herman, R. Geleziunas, and W. C. Greene, "A dileucine motif in HIV-1 Nef acts as an internalization signal for CD4 downregulation and binds the AP-1 clathrin adaptor," *Current Biology*, vol. 8, no. 22, pp. 1235–12S1, 1998.

[62] K. E. Seaton and C. D. Smith, "N-Myristoyltransferase isozymes exhibit differential specificity for human immunodeficiency virus type 1 Gag and Nef," *The Journal of General Virology*, vol. 89, no. 1, pp. 288–296, 2008.

[63] M. Bentham, S. Mazaleyrat, and M. Harris, "Role of myristoylation and N-terminal basic residues in membrane association of the human immunodeficiency virus type 1 Nef protein," *Journal of General Virology*, vol. 87, no. 3, pp. 563–571, 2006.

[64] S. Beitari, S. Ding, Q. Pan, A. Finzi, and C. Liang, "The effect of HIV-1 Env on SERINC5 antagonism," *Journal of Virology*, vol. 91, no. 4, pp. e02214–e02216, 2016.

[65] C. Sood, M. Marin, A. Chande, M. Pizzato, and G. B. Melikyan, "SERINC5 protein inhibits HIV-1 fusion pore formation by promoting functional inactivation of envelope glycoproteins," *Journal of Biological Chemistry*, vol. 292, no. 14, pp. 6014–6026, 2017.

[66] Y. Usami and H. Göttlinger, "HIV-1 Nef responsiveness is determined by Env variable regions involved in trimer association and correlates with neutralization sensitivity," *Cell Reports*, vol. 5, no. 3, pp. 802–812, 2013.

[67] P. R. Tedbury and S. G. Sarafianos, "Exposing HIV's weaknesses," *Journal of Biological Chemistry*, vol. 292, no. 14, pp. 6027-6028, 2017.

[68] M. Veillette, M. Coutu, J. Richard et al., "The HIV-1 gp120 CD4-bound conformation is preferentially targeted by antibody-dependent cellular cytotoxicity-mediating antibodies in sera from HIV-1-infected individuals," *Journal of Virology*, vol. 89, no. 1, pp. 545–551, 2015.

[69] R. P. Lai, J. Yan, J. Heeney et al., "Nef decreases HIV-1 sensitivity to neutralizing antibodies that target the membrane-proximal external region of TMGP41," *PLoS Pathogens*, vol. 7, no. 12, pp. 1–16, 2011.

[70] S. M. Woollard and G. D. Kanmogne, "Maraviroc: a review of its use in HIV infection and beyond," *Drug Design, Development and Therapy*, vol. 9, pp. 5447–5468, 2015.

[71] H. Arberas, A. C. Guardo, M. E. Bargallo et al., "*In vitro* effects of the CCR5 inhibitor maraviroc on human T cell function," *Journal of Antimicrobial Chemotherapy*, vol. 68, no. 3, pp. 577–586, 2013.

[72] B. Brügger, B. Glass, P. Haberkant, I. Leibrecht, F. T. Wieland, and H.-G. G. Kräusslich, "The HIV lipidome: a raft with an unusual composition," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 103, no. 8, pp. 2641–2646, 2006.

[73] B. Brügger, B. Brügger, E. Krautkrämer et al., "Human immunodeficiency virus type 1 Nef protein modulates the lipid composition of virions and host cell membrane microdomains," *Retrovirology*, vol. 4, no. 70, pp. 1–12, 2007.

[74] S. I. Giese, I. Woerz, S. Homann, N. Tibroni, M. Geyer, and O. T. Fackler, "Specific and distinct determinants mediate membrane binding and lipid raft incorporation of HIV-1SF2 Nef," *Virology*, vol. 355, no. 2, pp. 175–191, 2006.

[75] Y. H. Zheng, A. Plemenitas, T. Linnemann, O. T. Fackler, and B. M. Peterlin, "Nef increases infectivity of HIV via lipid rafts," *Current Biology*, vol. 11, no. 11, pp. 875–879, 2001.

[76] N. Sol-Foulon, C. Esnault, Y. Percherancier et al., "The effects of HIV-1 Nef on CD4 surface expression and viral infectivity in lymphoid cells are independent of rafts," *Journal of Biological Chemistry*, vol. 279, no. 30, pp. 31398–31408, 2004.

[77] J.-M. Cardot and B. M. Davit, "*In vitro–in vivo correlations*: tricks and traps," *The AAPS Journal*, vol. 14, no. 3, pp. 491–499, 2012.

[78] Y. Usami, S. Popov, and H. G. Gottlinger, "The Nef-like effect of murine leukemia virus glycosylated Gag on HIV-1 infectivity is mediated by its cytoplasmic domain and depends on the AP-2 adaptor complex," *Journal of Virology*, vol. 88, no. 6, pp. 3443–3454, 2014.

[79] A. Corbin, A. C. Prats, J. L. Darlix, and M. Sitbon, "A non-structural gag-encoded glycoprotein precursor is necessary for efficient spreading and pathogenesis of murine leukemia viruses," *Journal of Virology*, vol. 68, no. 6, pp. 3857–3867, 1994.

[80] M. Pizzato, "MLV glycosylated-Gag is an infectivity factor that rescues Nef-deficient HIV-1," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 107, no. 20, pp. 9364–9369, 2010.

[81] F. Li, C. Leroux, J. K. Craigo, S. J. Cook, C. J. Issel, and R. C. Montelaro, "The S2 gene of equine infectious anemia virus is a highly conserved determinant of viral replication and virulence properties in experimentally infected ponies," *Journal of Virology*, vol. 74, no. 1, pp. 573–579, 2000.

[82] A. J. Fagerness, M. T. Flaherty, S. T. Perry, B. Jia, S. L. Payne, and F. J. Fuller, "The S2 accessory gene of equine infectious anemia virus is essential for expression of disease in ponies," *Virology*, vol. 349, no. 1, pp. 22–30, 2006.

[83] B. A. Sponseller, W. O. Sparks, Y. Wannemuehler et al., "Immune selection of equine infectious anemia virus *Env* variants during the long-term inapparent stage of disease," *Virology*, vol. 363, no. 1, pp. 156–165, 2007.

[84] A. Heigele, D. Kmiec, K. Regensburger et al., "The potency of Nef-mediated SERINC5 antagonism correlates with the prevalence of primate lentiviruses in the wild," *Cell Host and Microbe*, vol. 20, no. 3, pp. 381–391, 2016.

[85] B. Murrell, T. Vollbrecht, J. Guatelli, and J. O. Wertheim, "The evolutionary histories of antiretroviral proteins SERINC3 and SERINC5 do not support an evolutionary arms race in primates," *Journal of Virology*, vol. 90, no. 18, pp. 8085–8089, 2016.

[86] D. Hymel and B. R. Peterson, "Synthetic cell surface receptors for delivery of therapeutics and probes," *Advanced Drug Delivery Reviews*, vol. 64, no. 9, pp. 797–810, 2012.

[87] M. Notohamiprodjo, R. Djafarzadeh, A. Mojaat, I. Von Lüttichau, H. J. Gröne, and P. J. Nelson, "Generation of GPI-linked CCL5 based chemokine receptor antagonists for the suppression of acute vascular damage during allograft transplantation," *Protein Engineering, Design and Selection*, vol. 19, no. 1, pp. 27–35, 2006.

Hindawi
Journal of Immunology Research
Volume 2017, Article ID 8734504, 13 pages
https://doi.org/10.1155/2017/8734504

Review Article

Pulmonary Susceptibility of Neonates to Respiratory Syncytial Virus Infection: A Problem of Innate Immunity?

Carole Drajac, Daphné Laubreton, Sabine Riffault, and Delphyne Descamps

VIM, INRA, Université Paris-Saclay, Jouy-en-Josas, France

Correspondence should be addressed to Delphyne Descamps; delphyne.descamps@inra.fr

Received 28 July 2017; Accepted 9 October 2017; Published 9 November 2017

Academic Editor: Elias Said

Copyright © 2017 Carole Drajac et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Human respiratory syncytial virus (RSV) is a common and highly contagious viral agent responsible for acute lower respiratory infection in infants. This pathology characterized by mucus hypersecretion and a disturbed T cell immune response is one of the major causes of infant hospitalization for severe bronchiolitis. Although different risk factors are associated with acute RSV bronchiolitis, the immunological factors contributing to the susceptibility of RSV infection in infants are not clearly elucidated. Epidemiological studies have established that the age at initial infection plays a central role in the severity of the disease. Thus, neonatal susceptibility is intrinsically linked to the immunological characteristics of the young pulmonary mucosa. Early life is a critical period for the lung development with the first expositions to external environmental stimuli and microbiota colonization. Furthermore, neonates display a lung immune system that profoundly differs to those from adults, with the predominance of type 2 immune cells. In this review, we discuss the latest information about the lung immune environment in the early period of life at a steady state and upon RSV infection and how we can modulate neonatal susceptibility to RSV infection.

1. Introduction

Human respiratory syncytial virus (RSV) was isolated for the first time in chimpanzees and identified in 1957 in children with severe lower respiratory illness [1–3]. RSV is an enveloped negative-sense single-stranded RNA (ssRNA) virus of the Pneumoviridae family [4]. RSV consists of a single serotype and two antigenic subtypes, A or B. The RSV genome is about 15 kb nucleotides and encodes nine structural proteins and two nonstructural proteins (NS1 and NS2). The envelope of RSV contains three transmembrane surface proteins, which are the fusion glycoprotein (F protein), the G glycoprotein (G protein), and the SH protein. F and G are the only RSV proteins that induce neutralizing antibodies [5].

RSV is the commonest viral agent causing acute lower respiratory infection (ALRI) in infants, a disease usually named bronchiolitis [6]. Bronchiolitis is characterized by mucus hypersecretion and inflammatory cell infiltration into the airspaces leading to airway lumen narrowing [7]. RSV infection is the main cause of hospitalization for severe bronchiolitis and is responsible for important infant mortality in developing countries [8]. RSV is highly contagious, and it is estimated that 95% of children have experienced at least one RSV infection before the age of two. Thus, the development of new treatment strategies is the World Health Organization's priority. A growing concern is that severe RSV infection may adversely affect pulmonary development and may lead to long-term respiratory disorders. Indeed, infants exposed to severe bronchiolitis or even to mild RSV disease are at much higher risk to develop recurrent wheeze up to teenage years [9].

In the sixties, the administration of formaldehyde-inactivated RSV vaccine (FIRSV) to a cohort of infants resulted in 80% of hospitalization due to an enhanced form of RSV-mediated disease (RSV disease). Two infants died and eighteen developed bronchiolitis and/or pneumonia, characterized by an excess of monocytes and eosinophils in the lungs [10]. High levels of anti-inflammatory type 2 T helper cell (T_H2) cytokines, such as interleukin-4 (IL-4), IL-13, and IL-15, have also been detected in mice vaccinated with FIRSV [11, 12]. This dramatic episode highlights the need to improve our knowledge of infant immune responses to viral infection as well as of RSV pathogenesis in newborn airways. Although different risk factors (preterm birth,

polymorphisms in host immune genes) are associated with acute RSV bronchiolitis [13, 14], the immunological factors contributing to the susceptibility of RSV infections in infants are not clearly elucidated. Different human epidemiological studies have established that the age at initial infection plays a key role in the susceptibility of RSV disease and the development of an asthma-like phenotype [15]. At birth, neonates, which rely on maternally derived antibodies (MDA) and innate responses, have a limited ability to defend themselves against pathogens. Indeed, the critical period of susceptibility to RSV arises between 2 and 6 months of age when MDA decrease beyond protective levels and before host neutralizing antibodies reach sufficient titers [16, 17]. Protection of preterm infants with higher occurrence of severe bronchiolitis (measured as the duration of wheezing) can be achieved through prophylactic treatment with a neutralizing humanized antibody called palivizumab [18]. Innate immune components that are the first available line of defense in neonates will also contribute to covering the "hole" in acquired immunity, educating the adaptive immune system, and strengthening it. Yet little is known about innate immunity in the lungs of neonates and how it will imprint further acquired immunity to RSV.

To better understand the immune pathways mobilized by RSV infection in infants and their long-term effects on the lungs, a mouse model of neonatal infection has been developed in BALB/c mice [19]. Mice infected under age 7 days (neonatal mice) develop an asthma-like pathology upon adult reinfection, characterized by weight loss, airway hyperresponsiveness, mucus hypersecretion, type 2 immune responses (neutrophil and eosinophil recruitment and IL-13 and IL-4 secretion), and airway remodelling [19, 20]. Thereby, as in human infants, the age of neonatal mice at initial RSV infection determines the clinical outcome upon RSV reexposure at adult age. These data suggest that RSV infection during the neonatal period is responsible for an immunopathological imprinting in the lungs that could influence the development and the severity of disease and finally long-term respiratory disorders. Thus, neonatal mice are an experimental model of interest to study the causes of this age-specific susceptibility.

The infant susceptibility to RSV infection is intrinsically linked to the immunological characteristics of the pulmonary mucosa. To date, neonatal innate immune responses and their effects on RSV disease progression remain poorly described. In this review, we resume the latest information about the immune environment in the young lung (mice and human). Then, the advanced researches on the mechanisms of the innate response to RSV infection in neonates are described. Finally, we discuss different approaches to modulate the young susceptibility to RSV infection by targeting the neonatal window of intervention.

2. Lung Tissue in Neonatal Life: A Moving Landscape

In mice, lung development begins at embryonic day 9 (ED9) and can be divided in three main periods, referred to as embryonic, fetal, and postnatal periods [21]. Before birth, lung development proceeds to pseudoglandular (ED12–16.5), canalicular (ED16.5–17.5), and saccular (ED18–PND4) stages forming the branching tree and future air space [21]. After birth, the formation of the secondary septa occurs resulting in the formation of the alveoli. This alveolarization phase takes place from postnatal day (PND) 4 to approximately PND21, with the development of the first mature alveoli and microvascular system around PND14 [21, 22]. During this period, the young lungs are exposed to a specific pattern of chemokines and cytokines, physical stress, and/or external environmental stimuli that will influence the immune system development [23, 24].

Immune cells start to colonize the lungs during the pseudoglandular phase (ED12) with the major population consisting of $CD45^+$ yolk sac-derived macrophages, followed by fetal liver monocytes that enter the lungs at the beginning of the saccular phase (ED18) [25]. Following birth, lung epithelial cells produce elevated levels of GM-CSF (granulocyte-macrophage colony-stimulating factor or Csf-2). GM-CSF is a hematopoietic growth factor that promotes alveolar macrophage (AM) development in the lungs from fetal monocytes [25, 26]. AMs appear after PND1 in lung tissue and reach their maximum abundance in the lungs at PND3 where they fully colonize the alveolar space, coinciding with the start of the alveolar phase [25] (Figure 1). At PND14, monocytes, macrophages, and granulocytes reach adult-like cell frequencies [25, 27]. An influx of granulocytes ($CD11b^+$ $CD11c^{int}$ $Ly6G/SiglecF^+$ cells probably corresponding to neutrophils) is quickly observed at PND1 [27]. Similar observations have been reported for circulating neutrophils. In human, following the first 24 hours after birth, the circulating neutrophil count abruptly raises and gradually stabilizes by 48 to 72 hours of life [28]. In neonatal mice, there is also a marked increase in circulating neutrophil numbers from PND1 to PND3 reaching an adult number from PND14 [29].

A recruitment of type 2 innate cells (type 2 innate lymphoid cells or ILC2s, mast cells, eosinophils, and basophils) occurs in the lungs at the start of the alveolarization period. Their frequency reaches a maximum at PND14 and then declines until weaning [27, 30]. A recent study showed that following the first breathes, the lung epithelium also produces high amount of IL-33 [30]. IL-33 is an alarmin that belongs to the IL-1 family and is mainly secreted by stromal cells such as epithelial and endothelial cells [31]. IL-33 signals through its receptor ST2 present in particular at the membrane of macrophages, dendritic cells (DCs), mast cells, and ILC2 [32]. IL-33 contributes to the promotion of T_H2 immunity [31], particularly in the lungs of newborn mice [27, 30]. Indeed, IL-33 released by lung epithelial cells has been recently associated with the accumulation of ILC2 during the alveolar period [27, 30]. ILC2 cells belong to the family of innate lymphoid cells (ILCs). This family comprises three cellular groups that have been divided according to their cytokine production profile. Group 1 comprises both ILC1 and NK cells and is defined by the production of the signature cytokine IFNγ, while ILC2 has been defined by their ability to produce T_H2-type cytokines such as IL-4 and IL-5 and ILC3 through their IL-17 production [33]. Thus, IL-33 secretion stimulates steady-state IL-5 and IL-13 production

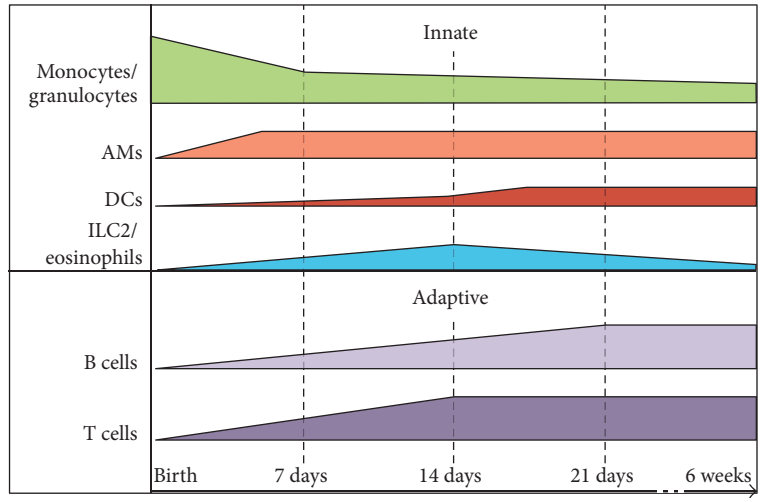


Figure 1: Immune cell colonization of the lungs during the postnatal period (schematization of cellular frequencies in $CD45^+$ lung cells). Adapted from [25, 27, 30, 38] and personal unpublished data.

by ILC2 that in turn promotes a neonatal AM or DC phenotype switch towards type 2 immune response [27, 30]. To our knowledge, neither ILC1 nor ILC3 has been found in murine lungs [34, 35]. It is well known that NK cells represent up to 10% of resident lymphocytes in the lungs of adult mice [36], but their proportion in neonates has not been described yet.

GM-CSF also controls DC development in the lungs [37]. DCs are extremely rare in the respiratory tract after birth, but their frequency gradually increases over time. From E20 until PND2, $CD11b^+CD64^+$ monocyte-derived DCs (moDCs) form the majority of DCs. Our group showed that 6-day-old BALB/c neonatal lungs display less conventional (cDCs) and plasmacytoid DCs (pDCs), with a lower $CD103^+$ to $CD11b^+$ cDC ratio, as compared to adult lungs [38]. However, a study using a gating strategy that separates cDCs from moDCs in C57Bl/6J newborn mice showed that lung $CD11b^+$ cDCs developed more slowly, leading to a predominance of $CD103^+$ cDCs until PND7 [27]. During the alveolarization phase, neonatal DCs and pDCs display increased levels of OX40L (CD134) [27, 39] and this is related to their preferential ability to promote T_H2 responses. Both IL-33 [27] and TSLP [39] production by lung epithelial cells seem to influence OX40-L expression in neonates.

As for DCs, T and B cells progressively accumulate in the lungs from birth to weaning [27]. Lung tissue of 6-day-old mice contain fourfold less $CD3^+$ lymphocytes than adult tissue [38]. Neonatal T cell population is enriched in $CD4^-CD8^-GATA3^+$ T cells while $CD4^+$ and $CD8^+$ T cells are less represented than those in in adults [38]. No difference in both NKT and $\gamma\delta$ pulmonary cells are observed between adults and neonates. Little is known about B cells in neonatal lungs. At PND6, the neonatal lung tissues contain fivefold less $CD19^+$ B cells than adult tissue [27]. Our group observed that the neonatal B cell population is enriched in both immature B cells and innate-like $CD5^+$ B1a cells (Laubreton D. and Descamps D., unpublished data). A previous study has demonstrated that the $CD5^+$ B cell population is more abundant in the spleen of 6-day-old C57Bl/6J neonates than in the adult spleen [40]. An equivalent subset named neonatal regulatory B cells (nBreg) has recently been identified in human cord blood [41].

First breaths not only provide signals that will shape lung maturation but also carry microbes that will form the microbiota. In neonatal mice, bacteria start to colonize the lungs around PND3, with their number and diversity progressively increasing until weaning [42, 43]. Interestingly, microbiota installation is closely related to the alveolarization phase. Indeed, Yun et al. suggest that bacteria influence lung development and barrier functions [44]. Bacteria can also influence the lung immune environment. In germ-free (GF) mice, invariant NKT accumulates in the lungs [45], and CD40 and programmed death-ligand 1 (PD-L1) expression by neonatal DCs is affected [42].

In conclusion, neonates display a lung immune system that is profoundly different from that of adults especially in regard to the presence of innate immune cells able to induce T_H2 immunity. Moreover, the early life is a critical period for the lung development with the first expositions to external environmental stimuli and microbiota colonization. All these events affect the maturation of the pulmonary immune capacity and thus the lung susceptibility to respiratory pathogens [23, 24, 46].

3. Innate Sensing of RSV in Neonatal Lungs

At a steady state, the age-specific cellular composition of the neonatal lungs naturally promotes the initial development of T_H2 immune responses [27, 30]. This ability can be influenced by innate responses of resident airway cells that produce different mediators following RSV sensing. Innate immune responses to RSV are important to control the early phase of viral infection but also to influence the polarization of anti-RSV immune responses and thus the outcome of RSV infection. The difficulty in studying lung cells in infants with bronchiolitis and in healthy controls leads to an incomplete knowledge of innate pulmonary immunity and

factors influencing it in neonates. However, using animal models, numerous studies have identified that innate responses to RSV are decisive immunological events in neonatal RSV susceptibility [14, 47].

3.1. RSV Detection by Pattern Recognition Receptors (PRRs). Several Toll-like receptors (TLRs), RIG-I-like receptors (RLRs), or nucleotide-binding domain and leucine-rich repeat-containing proteins (NLRs) are particularly involved in antiviral defenses and cytokine production upon RSV infection [14, 47]. Recognition of the virus by these PRRs is well defined in human and adult mouse but is still poorly described in neonates [48–50].

3.1.1. Membrane TLRs Involved in RSV Recognition. The F fusion protein is recognized by TLR4, which is also known to detect lipopolysaccharide (LPS) of Gram-negative bacteria [51]. TLR4 stimulation leads to the production of proinflammatory cytokines and type I interferons (IFN-I), involving the signalling pathways dependent on two adaptive molecules which are the myeloid differentiation primary response 88 protein (MyD88) or the TIR domain-containing adapter inducing interferon β (TRIF). In TLR4-deficient C57BL10/ScCr mice, RSV clearance as well as activation and recruitment of NK cells is impaired [51, 52]. However, RSV infection is not affected in another TLR4-deficient BALB/c mice [53]. Human and murine pulmonary epithelial cells and macrophages express a broad range of TLRs including TLR4 [50, 54–56]. Interestingly, TLR4 signalling and IL-4Rα/STAT6 and IFN-β pathway engagement in murine AMs promote a type 2 immune response in the course of RSV infection [57].

TLR2/TLR6 complex is also involved in the detection of RSV, but the mechanisms of sensing remain unknown. Activation of these receptors promotes the production of IFN-I and proinflammatory cytokines through the MyD88-dependent pathway [48–50]. In the lungs of TLR2- and TLR6-deficient adult mice, viral load is increased and neutrophil recruitment is impaired following RSV infection. Moreover, isolated AMs from these mice produce decreased levels of IFN-I and inflammatory cytokines [58].

The PRR expression by other mucosal innate immune cells like ILCs is better documented for the digestive tract than for the lungs [59]. Nevertheless, a recent work showed that TLR2 and TLR4 are expressed in pulmonary ILC2 and drive together a type 2 immune response by inducing IL-13 production [60].

3.1.2. Endosomal TLRs Involved in RSV Recognition. TLR3 detects the double-stranded RNA form of the RSV genome, which is generated during the virus replication cycle [61]. TLR3 exclusively signals through the TRIF pathway. TRIF recruitment leads to the activation of the transcription factor interferon regulatory factor 3 (IRF-3), which generates IFN-I production by the cell. TLR3 is constitutively expressed in numerous cell types including nasal and pulmonary epithelial cells, AMs, and DCs [62, 63]. Rudd et al. showed that RSV promotes a type 2 immunity in TLR3-deficient adult mice with eosinophilic infiltration, mucus overproduction, and T_H2-type cytokine secretion (IL-5, IL-8, and IL-13) while viral load remains unchanged [61].

TLR7 recognizes the single-stranded RNA genome of RSV and triggers subsequent expression of genes encoding IFN-I and proinflammatory cytokines via IRF-7 and NF-κB activation through the MyD88-dependent pathway [48–50]. Lung epithelial cells, DCs, and eosinophils are able to sense RSV via TLR7 activation [56, 64–66]. RSV infection in TRL7-deficient mice induces a significant increase in inflammation and mucus production in the lungs [65]. Interestingly, Schlender et al. showed that RSV prevents IFN-I production in human pDCs in a TLR7-dependant manner, but they cannot explain the mechanism involved in such inhibition [67].

3.1.3. Cytosolic Location of RLRs and NLRs. Retinoic acid-inducible gene I (RIG-I) and melanoma differentiation-associated gene 5 (MDA5) are both RNA helicases that can bind to the double-stranded RNA form of the RSV genome and 5′-triphosphorylated uncapped viral RNA in the cytosol [68, 69]. RIG-I signalling is particularly involved in IFN-I responses in lung epithelial cells, DCs, and AMs [56, 64, 68, 70]. RIG-I and MDA5 contain two N-terminal caspase activation and recruitment domains (CARDs), which, upon virus sensing in the cytosol, interact with the mitochondrial antiviral signalling (MAVS) protein to trigger the NF-κB and IRF-3 pathways [48–50].

Nucleotide-binding oligomerization domain 2 (Nod2), a member of the NLR family, can also detect single-stranded viral RNA and triggers innate immune activation by binding with MAVS [71]. Upon RSV infection, MAVS-deficient mice displayed higher viral load in the lungs and profound defects in antiviral defenses in comparison with control WT mice, although RSV clearance is still effective in the absence of RIG-I, MDA5, and Nod signallings [64, 72]. Johansson's group showed that AMs are the main IFN-I producers through the MAVS-dependent pathway in adult lungs of RSV-infected mice [70, 73].

3.2. PRR Expression or Functionality, a Factor of Neonatal Susceptibility to RSV Infection. In infants, several genetic polymorphisms in innate immune genes have been associated with the susceptibility to develop RSV-mediated bronchiolitis [13, 74–76]. Thus, single-nucleotide polymorphisms (SNPs) in genes coding for PRRs have been considered attractive targets for clinical decision-making [77]. However, contradictory studies with other cohorts have failed to correlate SNPs in RIG-I or TLR4 genes with the severity of RSV disease [78]. Adult mouse models similarly show a variable role of the TLR4 pathway in the development of RSV disease [51–53]. Consequently, the severity of RSV disease appears likely dependent on both genetic and environmental factors (microbiota and coinfections) during the neonatal period [47]. Accordingly, a combination of the TLR4 genotype and environmental exposure to LPS during early life is involved in the occurrence of RSV bronchiolitis [79]. Most studies using cord blood cells suggest that TLR expression is not a modulator of the degree of cytokine responsiveness during the perinatal period [69, 80]. Furthermore, Marr

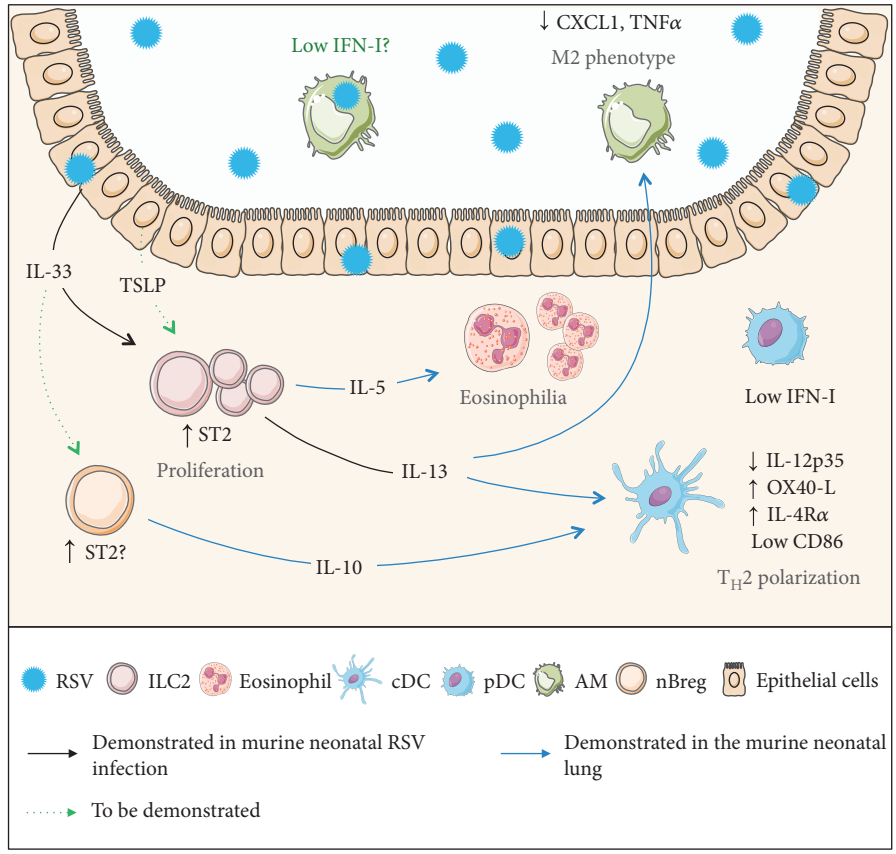


Figure 2: Immediate immune responses of pulmonary resident cells to RSV infection in neonates. Servier Medical Art has provided images. Neonatal RSV exposure leads to an early IL-33 secretion by respiratory epithelial cells [96]. IL-33 signals through its receptor ST2 localized at the membrane of ILC2. This alarmin supports the increase in the ILC2 number and IL-13 production in the lungs of RSV-infected neonatal mice [74]. ILC2 can promote a switch towards a type 2 phenotype for AMs or lung DCs at a steady state or in a house dust mite-induced asthma model [27, 30]. Concerning the IFN-I pathway, neonatal pDCs display a poor pulmonary mobilization and a weak activation of the IFN-I pathway following RSV infection [29]. AMs are the main source of IFN-I in RSV-infected adult lungs, but the question remains open during the neonatal period [54]. Therefore, it is strongly suspected that ILC2 cells are indirectly responsible for the inability of neonatal mice to mount an effective IFN-I response to counteract RSV infection. In addition, IL-10-secreting nBregs may constitute another cellular subset contributing to the type 2 immunity induced by RSV infection in neonates [40, 99].

et al. showed that RIG-I expression is similar between neonatal and adult pDCs [69]. They propose that IFN-I responses following RSV infection are decreased in neonatal pDCs compared to adult pDCs because of different signalling events downstream of MAVS or posttranslational modifications affecting either RIG-I or MAVS pathways [69].

Nevertheless, a correlation between pulmonary PRR acquisition after birth and RSV susceptibility cannot be excluded. Several studies in the mouse model showed that TLR4 expression is very low in the fetal lungs and increases throughout development [81, 82]. Harju et al. proposed an association between reduced pulmonary TLR4 expression at baseline and neonatal hyporesponsiveness to LPS [82]. Currently, there are virtually no studies on the maturation of TLR3 and 7 and RIG-I signalling in the neonatal lungs at a basal state and upon RSV infection.

3.3. Immediate Innate Responses of Pulmonary Resident Immune Cells to RSV Infection. RSV infection in neonatal mice promotes a type 2 immunity characterized by a strong proliferation of an IL-4Rα^+-CD4$^+$ T_H2 subset together with a defect in CD8$^+$ T cell activation and IFNγ production [83, 84]. In this part, we describe the first innate responses of pulmonary resident cells to RSV infection that contribute to the development and/or maintenance of anti-RSV T_H2 immunity (Figure 2).

Adaptive immune responses are initiated by DCs that traffic from the infected lungs to the draining respiratory lymph nodes in order to prime T cell responses. Our group and others have described major deficiencies in the functionality of DCs in neonatal lungs following RSV infection [85–87]. As compared to adults, cDCs are poorly represented in the lungs and in the lymph nodes of RSV-infected neonates, with an increased proportion of a CD103$^+$ DC subset [85]. These neonatal DCs also have lower expression of the costimulatory molecule CD86 and thus are less effective in antigen presentation [85]. Moreover, a poor pulmonary mobilization of pDCs, potent producers of IFNα/β, and a weak activation of the IFN-I pathway are described in RSV-infected neonatal mice [86, 87]. IFN-I production is important not only to

induce antiviral responses but also to amplify proinflammatory responses in the lungs of adult mice [88]. It has been demonstrated that IFNα treatment or an increased lung DC number (by adoptive transfer of adult pDCs or administration of a hematopoietic cell proliferation factor, the Flt3 ligand (Flt3-L)) reboots the IFN-I pathway upon RSV neonatal infection and decreases T_H2-biased immunopathology upon adult reinfection [86, 87]. Thus, IFN-I production clearly appears as a key factor in neonatal susceptibility to RSV infection [86, 87, 89].

The role of AMs in primary RSV infection only begins to be appreciated. Thus, the depletion of AMs in the early period of life has been associated with a reduction in RSV clearance and a delay in weight gain [90]. In adult mice, AMs have also been described to play an essential role in early inflammatory molecule production (TNFα, IL-6, CCL3, and IFNα) and activation/recruitment of NK cells [91, 92]. Recently, it has been reported that adult AMs are the main source of IFN-I following RSV infection [70]. To date, these observations have not been checked in neonatal mice. AMs are known to be a flexible cellular subset that adapts to the microenvironment of the airway lumen [93]. It is not known yet whether neonatal AMs have the same reactivity to RSV infection than adult AMs. Addressing this issue seems critical to understand the causes of inability of a neonate to generate IFN-I response following RSV infection. Similar to the classification of T cells in a T_H1/T_H2 phenotype, macrophages have been also categorized into classically activated macrophages (CAMs or M1) or alternatively activated macrophages (AAMs or M2), based on activating cytokines (IFNγ and IL-4, resp.) and functional activities (inflammation and airway remodelling, resp.) [94]. Interestingly, Empey et al. have demonstrated that neonatal AMs present a delay of their differentiation toward a CAM phenotype following RSV infection, likely due to undetectable IFNγ production [95]. In adult mice, RSV infection induces AAMs that are important to reduce lung pathology [57]. Thus, pulmonary AM polarization seems to depend on age. Altogether, in the particular context of the neonatal airway environment, it becomes important to understand the mechanism that triggers the polarization of neonatal AMs following RSV infection and to evaluate its relationship with exacerbated airway responses upon adult reinfection.

The contribution of ILC2 and IL-33 to the neonatal RSV susceptibility has been recently investigated. Neonatal RSV exposure leads to an early IL-33 secretion by respiratory epithelial cells; this is not observed in adult mice. IL-33 plays a major function in the immunopathogenesis of RSV infection by supporting an increase in the ILC2 number and IL-13 production in the lungs of neonatal mice that will impact on disease severity in reinfected mice [96]. Additionally, it has been reported that hospitalized infants with viral bronchiolitis have detectable levels of nasal IL-13, IL-33, and thymic stromal lymphopoietin (TSLP) [96, 97]. Importantly, TSLP-deficient adult mice are unable to mount ILC2 proliferation and activation upon RSV infection [98]. The link between TSLP from respiratory epithelium and ILC2 proliferation/activation is not yet reported in RSV-infected neonatal mice. Nevertheless, the release of TSLP is identified as an important event for pulmonary DC polarization during RSV infection in the neonatal period and for RSV-mediated long-term respiratory disorders [39]. ILC2 is known to secrete T_H2-type cytokines, such as IL-5 and IL-13. In the neonatal lungs, these cells can promote an AM or DC phenotype switch towards type 2-polarizing abilities at a steady state or in a house dust mite-induced asthma model [27, 30]. Therefore, it is strongly suspected that ILC2 cells are indirectly responsible for the inability of neonatal mice to mount an effective IFN-I response following RSV infection.

Finally, nBregs (or $CD5^+$ B1a subset) may constitute another cellular subset contributing to the type 2 immunity induced by RSV infection in neonates. In the neonatal spleen, these nBregs have been previously described for their ability to produce IL-10 and to control the DC activation *in vivo* for T_H1/T_H2 polarization [40]. Moreover, IL-10-producing nBregs in the lungs could be induced by IL-33, as previously described in the intestines [99]. An equivalent subset of nBregs has been recently characterized in the blood of human neonates, and its frequency is identified as a predictive factor for the severity of RSV-mediated bronchiolitis in infants [41].

All these results point to the existence of T_H2-like innate immune responses that are early induced by RSV infection in the neonatal lungs. These specific immunological properties must be considered in order to develop relevant therapeutic approaches against RSV infection.

4. Experimental Strategies to Modulate the Neonatal Susceptibility to RSV

An increasing set of data supports the concept of a "neonatal window of opportunity". The early life is the critical period for the development of immunity and therefore for the newborn sensitivity to the development of pulmonary pathologies. Immunomodulatory interventions targeting this period of life are likely to have profound effects on immune system homeostasis and hence on an individual's susceptibility to pathogens [23]. Different studies using neonatal or adult mice have shown the possibility to take advantage of immunomodulation strategies on innate defenses to modify the neonatal pulmonary susceptibility to RSV infection and to fight RSV disease (Table 1).

4.1. Counteracting the Ineffective IFN-I Secretion in the Lungs

4.1.1. Increase and/or Activation of IFN-I-Producing Cell Population. IFN-I production in neonatal RSV infection is decisive for the severity of RSV pathology [86, 87, 89]. Thus, with recombinant IFN-I intranasal instillation prior to mouse infection, Cormier et al. suggested that boosting the antiviral response of pDCs during the neonatal period limits RSV pathology [86]. Additionally, the treatment of neonatal mice with the Flt3-L, a growth factor for hematopoietic cells, before RSV infection increases pDCs in the lungs, partially restores the IFN-I pathway, and reduces the long-term pathological pulmonary consequences of RSV infection [87]. However, AMs have been identified as predominant producers of IFN-I in RSV-infected adult mice [70]. Therefore, the role of these cells in neonates and in the development

Table 1: Biological therapeutic interventions to modulate neonatal innate immunity following RSV infection.

Strategy	Target	Design	Biological product	Category	Administration	Models	Ref.
Counteracting the ineffective IFN-I secretion	IFN-I-producing cells	Activation & recruitment	IFN-I and Flt3-L	rIFN-α and growth factor	Inhalation & injection	Mouse (N)	[86, 87]
	IFN-I signalling	Activation	CpG and SB 9200	TLR or RLR agonist	Oral	Mouse (A & N)	[100, 102]
Modulating the pulmonary T_H2 bias	Th2-polarizing cytokines	Blocking	Anti-IL-13, anti-TSLP, anti-IL-33	Antibodies	Injection	Human (A) & mouse (A & N)	[39, 96, 98, 103–105]
	Signalling pathways	Blocking	Anti-IL-4Rα and anti-STAT6	Antisense oligonucleotide and inhibitory peptide	Inhalation	Mouse (N)	[107, 108]
	Alveolar macrophages	Activation	IFNγ	rIFNγ	Inhalation	Mouse (N)	[90, 95, 110]
Modulating the mucosal microbiota	Respiratory & intestinal mucosa	Maturation	*Lactobacillus rhamnosus* CRL1505	Live or heat-killed bacteria	Oral or inhalation	Human (C) & mouse (N)	[112–115]
	Respiratory mucosa	Maturation	Primocolonizing lung bacteria strains	Live bacteria	Inhalation	Mouse (N)	[43]

A: adult; C: children; N: neonate.

of pulmonary anti-RSV immunity in the long term should not be ignored.

4.1.2. Promoting IFN-I Signalling via TLR/RLR Agonists. By targeting PRRs, several groups propose the use of TLR or RLR agonists to boost antiviral responses. Thus, the preexposure of neonates to CpG (TLR9 ligand) prior to the first RSV infection reduces RSV pathology observed in the second RSV exposure at adult age. TLR9 stimulation induces the alteration of neonatal T_H2 skewing, probably by accelerating maturation of neonatal antigen-presenting cells as well as NK cell recruitment in the lungs [100]. In an adult mouse model, others propose to target TLR3 with the synthetic dsRNA agonist poly IC stabilized with poly-L-lysine carboxymethyl cellulose (poly ICLC). However, the administration of poly ICLC fails to induce an appropriate innate immune response following RSV infection in the cotton rat model, which is not the case in BALB/c mice [101]. These results highlight the importance of the choice of an animal model used for therapeutic evaluation in the context of RSV infection. Recently, SB 9200, a dinucleotide prodrug targeting RIG-I and NOD2 activation, has been presented as a novel prophylactic and therapeutic anti-RSV immunomodulatory agent by Spring Bank Pharmaceuticals. In the mouse model, it has been observed that SB 9200 reduces viral load and lung inflammation while increasing IRF3-dependent IFN-I production [102]. To our knowledge, it is the first immunomodulation strategy targeting RLRs that could be considered in RSV treatment. However, because of the known dissimilarities in PRRs and cell immune responses according to age, it would be necessary to test SB 9200 in a neonatal model for assessing innate immune response following RSV infection.

4.2. Modulating the Activation of Cells Promoting the Pulmonary T_H2 Environment in the Neonatal Period

4.2.1. Modulating by Blocking T_H2-Polarizing Cytokines. Several studies point out the major role of IL-13 in airway hyperresponsiveness of adult mice during RSV infection [20, 103]. Thus, IL-13 targeting could represent a good strategy to modulate neonatal responses to RSV infection. In adult mice, anti-IL-13 treatment prior to RSV infection reduces both viral load and mucus hypersecretion and increases IL-12 production in the lungs [103]. It would be interesting to study the effect of anti-IL-13 treatment on neonatal mice because it has been shown that IL-13 is highly secreted in the lungs upon neonatal RSV infection [96].

A recent study suggests that TSLP might also represent a therapeutic target for IL-13-driven immunopathology to RSV. Indeed, Stier et al. showed in adult mice that TSLP signalling is required for IL-13 production by ILC2. RSV-infected adult mice receiving an anti-TSLP neutralizing antibody presented a reduction in IL-13 production as well as a decrease in viral load and airway mucus secretion [98]. Furthermore, administration of anti-TSLP before neonatal RSV infection has been shown to reduce OX40-L expression on DCs thereby reducing their capacity to promote T_H2 polarization and to decrease eosinophil numbers in the bronchoalveolar lavage fluids [39]. Both strategies (anti-IL-13 and anti-TSLP) are currently under clinical trial evaluation for adult patients with asthma [104, 105]. In the same way, the administration of an IL-33-neutralizing antibody during primary RSV infection in neonatal mice reduces IL-13 production and ILC2 numbers in the lungs and consequently decreases disease severity after reinfection at adult age [96].

4.2.2. Modulating by Blocking Signalling Pathways Involved in the Type 2 Immunity Induction. Several groups propose to interfere with the T_H2-biased immunopathogenesis of neonatal RSV infection by targeting receptors of T_H2-type cytokines or proteins involved in their downstream signalling. Recently, Shrestha et al. have revealed that cDCs and pDCs downregulate their IL-4 receptor α (IL-4Rα) with age. Interestingly, the elevated IL-4Rα expression on $CD11b^+$ cDCs is related to the immunopathology upon RSV reinfection [106]. Accordingly, the downregulation of pulmonary IL-4Rα expression with antisense oligonucleotides (ASO) enhances the presence of maturation markers (CD80 and CD86) at the membrane of $CD11b^+$ cDCs and leads to a shift of T cell responses toward T_H1 cells producing IFNγ. Besides, IL-4Rα ASO-treated neonates display higher level of T_H1-like IgG2a antibodies in response to RSV exposure than nontreated mice, while viral load is unchanged. Subsequently, long-term respiratory disorders associated with RSV reinfection are reduced by the neonatal administration of IL-4Rα ASO [107]. In accordance with these results, the inhibition of STAT6 activity, an essential transcription factor in IL-4Rα signalling, by a specific inhibitory peptide during neonatal RSV infection, decreases IL-4 secretion and AAM number in the lungs and prevents from pulmonary eosinophil recruitment and airway hyperresponsiveness upon adult RSV reinfection [108].

4.2.3. Modulating by Activation of Neonatal AMs. Several studies have reported that neonatal AMs present an immature phenotype upon RSV infection [90, 95] and this has been associated with T_H2-biased airway immunopathology upon adult reinfection [108]. AM differentiation is controlled by local IL-4 or IFNγ secretion [94]. However, in neonatal mice, IFNγ production is absent following RSV infection [95]. Furthermore, it has been demonstrated that IFNγ production during neonatal infection influences the outcome of RSV pathology upon adult reinfection [109]. Indeed, it has been shown that intranasal injection of recombinant IFNγ in neonatal RSV-infected mice induces a better AM activation characterized by the expression of CAM markers ($CD86^+$, MHC II^+ and $CCR7^+$, and mannose $receptor^-$) on neonatal AMs and reduced viral load in the lungs [90, 95, 110].

4.3. Promoting the Maturation of the Pulmonary Immune System by Modulating the Microbiota.

Several groups focused their research on the capacity of probiotic microorganisms to stimulate the lung immune system and to prevent RSV infection during the first years of life [111]. Studies were carried out with *Lactobacillus rhamnosus* isolated from goat milk in order to control RSV infection via TLR3 modulation [112–114]. Oral treatment of 3-week-old BALB/c mice with *L. rhamnosus* CRL1505 significantly reduces viral load and

pulmonary tissue damage due to inflammation following RSV infection with respect to the control group [114]. Hence, *L. rhamnosus* CRL1505 administrated orally is able to beneficially modulate the respiratory mucosal immunity to RSV infection. The nasal administration of heat-killed probiotic *L. rhamnosus* CRL1505 is also able to increase the resistance of adult mice to the challenge with RSV [115]. Moreover, oral administration of this probiotic has reduced the frequency and severity of respiratory infections in a randomized clinical trial involving 298 children aged from two to five years in Argentina [112].

In parallel with the gut microbiota, a lung microbiota gradually colonizes the airways during the neonatal period [42, 43]. Its composition and diversity can affect host physiology and the immune capacity of the airway tissue [23, 111]. Thus, it has been observed that the nasopharyngeal microbiota of young children can influence the spread of RSV infection to the lower respiratory tract and can modulate the host immune response to virus [116, 117]. Thomas's group has recently isolated different primocolonizing bacterial strains of the mouse neonatal lungs in order to propose a new approach to modulate the immune response to respiratory pathologies. Thus, Remot et al. showed in a neonatal mouse model of asthma that the repeated administration of one strain of these primocolonizing lung bacteria positively or negatively impacts the outcome of pathology [43]. Therefore, it will be interesting to evaluate this strategy on RSV disease in neonates.

All these results indicate that the control of type 2 immune responses during primary RSV infection in the neonatal period can prevent RSV-mediated long-term respiratory problems. Altogether, the existence of the pulmonary neonatal environment displaying specific immunological properties must be considered in order to develop relevant therapeutic approaches against RSV infection.

5. Conclusion

In conclusion, the early response to RSV infection is closely associated with specific immunological characteristics of the developing lungs. That is why the RSV researches of new preventive or curative treatments against RSV must take advantage of experimental models in young animals. A better understanding of anti-RSV innate immune responses in neonates, and their relative contributions to long-term pulmonary immunopathology, is required to develop new immunomodulation—but also vaccination—strategies specific to this early period of life.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

Acknowledgments

The authors thank Dr. Isabelle Schwartz-Cornil (INRA, Jouy-en-Josas) for the helpful discussion and critically reading the manuscript. Carole Drajac was the recipient of a PhD fellowship of Région Ile-de-France (DIM Malinf). Daphné Laubreton was supported by a grant from the Agence Nationale de la Recherche (ANR-13-BSV3-0016 SyncBreg).

References

[1] R. E. Blount Jr., J. A. Morris, and R. E. Savage, "Recovery of cytopathogenic agent from chimpanzees with goryza," *Proceedings of the Society for Experimental Biology and Medicine*, vol. 92, no. 3, pp. 544–549, 1956.

[2] R. Chanock and L. Finberg, "Recovery from infants with respiratory illness of a virus related to chimpanzee coryza agent (CCA). Epidemiologic aspects of infection in infants and young children," *American Journal of Epidemiology*, vol. 66, no. 3, pp. 291–300, 1957.

[3] R. Chanock, B. Roizman, and R. Myers, "Recovery from infants with respiratory illness of a virus related to chimpanzee coryza agent (CCA). Isolation, properties and characterization," *American Journal of Epidemiology*, vol. 66, no. 3, pp. 281–290, 1957.

[4] C. L. Afonso, G. K. Amarasinghe, K. Bányai et al., "Taxonomy of the order Mononegavirales: update 2016," *Archives of Virology*, vol. 161, no. 8, pp. 2351–2360, 2016.

[5] M. Connors, P. L. Collins, C. Y. Firestone, and B. R. Murphy, "Respiratory syncytial virus (RSV) F, G, M2 (22K), and N proteins each induce resistance to RSV challenge, but resistance induced by M2 and N proteins is relatively short-lived," *Journal of Virology*, vol. 65, no. 3, pp. 1634–1637, 1991.

[6] S. Jain, W. H. Self, R. G. Wunderink, and CDC EPIC Study Team, "Community-acquired pneumonia requiring hospitalization," *New England Journal of Medicine*, vol. 373, no. 24, p. 2382, 2015.

[7] A. Jha, H. Jarvis, C. Fraser, and P. J. M. Openshaw, "Respiratory syncytial virus," in *SARS, MERS and Other Viral Lung Infections*, D. S. Hui, G. A. Rossi and S. L. Johnston, Eds., vol. 72, pp. 84–109, European Respiratory Society, Sheffield UK, 2016.

[8] T. Shi, D. A. McAllister, K. L. O'Brien et al., "Global, regional, and national disease burden estimates of acute lower respiratory infections due to respiratory syncytial virus in young children in 2015: a systematic review and modelling study," *The Lancet*, vol. 390, no. 10098, pp. 946–958, 2017.

[9] N. Sigurs, F. Aljassim, B. Kjellman et al., "Asthma and allergy patterns over 18 years after severe RSV bronchiolitis in the first year of life," *Thorax*, vol. 65, no. 12, pp. 1045–1052, 2010.

[10] H. W. Kim, J. G. Canchola, C. D. Brandt et al., "Respiratory syncytial virus disease in infants despite prior administration of antigenic inactivated vaccine," *American Journal of Epidemiology*, vol. 89, no. 4, pp. 422–434, 1969.

[11] M. Connors, N. A. Giese, A. B. Kulkarni, C. Y. Firestone, H. C. Morse 3rd, and B. R. Murphy, "Enhanced pulmonary histopathology induced by respiratory syncytial virus (RSV) challenge of formalin-inactivated RSV-immunized BALB/c mice is abrogated by depletion of interleukin-4 (IL-4) and IL-10," *Journal of Virology*, vol. 68, no. 8, pp. 5321–5325, 1994.

[12] M. E. Waris, C. Tsou, D. D. Erdman, S. R. Zaki, and L. J. Anderson, "Respiratory synctial virus infection in BALB/c mice previously immunized with formalin-inactivated virus induces enhanced pulmonary inflammatory response with a predominant Th2-like cytokine pattern," *Journal of Virology*, vol. 70, no. 5, pp. 2852–2860, 1996.

[13] R. Janssen, L. Bont, C. L. Siezen et al., "Genetic susceptibility to respiratory syncytial virus bronchiolitis is predominantly associated with innate immune genes," *The Journal of Infectious Diseases*, vol. 196, no. 6, pp. 826–834, 2007.

[14] L. Lambert, A. M. Sagfors, P. J. Openshaw, and F. J. Culley, "Immunity to RSV in early-life," *Frontiers in Immunology*, vol. 5, p. 466, 2014.

[15] S. A. Cormier, D. You, and S. Honnegowda, "The use of a neonatal mouse model to study respiratory syncytial virus infections," *Expert Review of Anti-Infective Therapy*, vol. 8, no. 12, pp. 1371–1380, 2010.

[16] R. Ochola, C. Sande, G. Fegan et al., "The level and duration of RSV-specific maternal IgG in infants in Kilifi Kenya," *PLoS One*, vol. 4, no. 12, article e8088, 2009.

[17] C. J. Sande, P. A. Cane, and D. J. Nokes, "The association between age and the development of respiratory syncytial virus neutralising antibody responses following natural infection in infants," *Vaccine*, vol. 32, no. 37, pp. 4726–4729, 2014.

[18] M. O. Blanken, M. M. Rovers, J. M. Molenaar et al., "Respiratory syncytial virus and recurrent wheeze in healthy preterm infants," *New England Journal of Medicine*, vol. 368, no. 19, pp. 1791–1799, 2013.

[19] F. J. Culley, J. Pollott, and P. J. Openshaw, "Age at first viral infection determines the pattern of T cell-mediated disease during reinfection in adulthood," *The Journal of Experimental Medicine*, vol. 196, no. 10, pp. 1381–1386, 2002.

[20] A. Dakhama, J. W. Park, C. Taube et al., "The enhancement or prevention of airway hyperresponsiveness during reinfection with respiratory syncytial virus is critically dependent on the age at first infection and IL-13 production," *Journal of Immunology*, vol. 175, no. 3, pp. 1876–1883, 2005.

[21] J. C. Schittny, "Development of the lung," *Cell and Tissue Research*, vol. 367, no. 3, pp. 427–444, 2017.

[22] S. I. Mund, M. Stampanoni, and J. C. Schittny, "Developmental alveolarization of the mouse lung," *Developmental Dynamics*, vol. 237, no. 8, pp. 2108–2116, 2008.

[23] E. S. Gollwitzer and B. J. Marsland, "Impact of early-life exposures on immune maturation and susceptibility to disease," *Trends in Immunology*, vol. 36, no. 11, pp. 684–696, 2015.

[24] C. M. Lloyd and B. J. Marsland, "Lung homeostasis: influence of age, microbes, and the immune system," *Immunity*, vol. 46, no. 4, pp. 549–561, 2017.

[25] M. Guilliams, I. De Kleer, S. Henri et al., "Alveolar macrophages develop from fetal monocytes that differentiate into long-lived cells in the first week of life via GM-CSF," *The Journal of Experimental Medicine*, vol. 210, no. 10, pp. 1977–1992, 2013.

[26] C. Schneider, S. P. Nobs, M. Kurrer, H. Rehrauer, C. Thiele, and M. Kopf, "Induction of the nuclear receptor PPAR-γ by the cytokine GM-CSF is critical for the differentiation of fetal monocytes into alveolar macrophages," *Nature Immunology*, vol. 15, no. 11, pp. 1026–1037, 2014.

[27] I. M. de Kleer, M. Kool, M. J. de Bruijn et al., "Perinatal activation of the interleukin-33 pathway promotes type 2 immunity in the developing lung," *Immunity*, vol. 45, no. 6, pp. 1285–1298, 2016.

[28] B. L. Manroe, A. G. Weinberg, C. R. Rosenfeld, and R. Browne, "The neonatal blood count in health and disease. I. Reference values for neutrophilic cells," *The Journal of Pediatrics*, vol. 95, no. 1, pp. 89–98, 1979.

[29] H. S. Deshmukh, Y. Liu, O. R. Menkiti et al., "The microbiota regulates neutrophil homeostasis and host resistance to *Escherichia coli* K1 sepsis in neonatal mice," *Nature Medicine*, vol. 20, no. 5, pp. 524–530, 2014.

[30] S. Saluzzo, A. D. Gorki, B. M. J. Rana et al., "First-breath-induced type 2 pathways shape the lung immune environment," *Cell Reports*, vol. 18, no. 8, pp. 1893–1905, 2017.

[31] A. M. Miller, "Role of IL-33 in inflammation and disease," *Journal of Inflammation*, vol. 8, no. 1, p. 22, 2011.

[32] J. Lu, J. Kang, C. Zhang, and X. Zhang, "The role of IL-33/ST2L signals in the immune cells," *Immunology Letters*, vol. 164, no. 1, pp. 11–17, 2015.

[33] H. Spits, D. Artis, M. Colonna et al., "Innate lymphoid cells — a proposal for uniform nomenclature," *Nature Reviews Immunology*, vol. 13, no. 2, pp. 145–149, 2013.

[34] G. Gasteiger, X. Fan, S. Dikiy, S. Y. Lee, and A. Y. Rudensky, "Tissue residency of innate lymphoid cells in lymphoid and nonlymphoid organs," *Science*, vol. 350, no. 6263, pp. 981–985, 2015.

[35] C. H. Kim, S. Hashimoto-Hill, and M. Kim, "Migration and tissue tropism of innate lymphoid cells," *Trends in Immunology*, vol. 37, no. 1, pp. 68–79, 2016.

[36] C. Gregoire, L. Chasson, C. Luci et al., "The trafficking of natural killer cells," *Immunological Reviews*, vol. 220, pp. 169–182, 2007.

[37] M. Greter, J. Helft, A. Chow et al., "GM-CSF controls nonlymphoid tissue dendritic cell homeostasis but is dispensable for the differentiation of inflammatory dendritic cells," *Immunity*, vol. 36, no. 6, pp. 1031–1046, 2012.

[38] X. Roux, A. Remot, A. Petit-Camurdan et al., "Neonatal lung immune responses show a shift of cytokines and transcription factors toward Th2 and a deficit in conventional and plasmacytoid dendritic cells," *European Journal of Immunology*, vol. 41, no. 10, pp. 2852–2861, 2011.

[39] J. Han, A. Dakhama, Y. Jia et al., "Responsiveness to respiratory syncytial virus in neonates is mediated through thymic stromal lymphopoietin and OX40 ligand," *The Journal of Allergy and Clinical Immunology*, vol. 130, no. 5, pp. 1175–1186.e9, 2012.

[40] C. M. Sun, E. Deriaud, C. Leclerc, and R. Lo-Man, "Upon TLR9 signaling, $CD5^+$ B cells control the IL-12-dependent Th1-priming capacity of neonatal DCs," *Immunity*, vol. 22, no. 4, pp. 467–477, 2005.

[41] D. Zhivaki, S. Lemoine, A. Lim et al., "Respiratory syncytial virus infects regulatory B cells in human neonates via chemokine receptor CX3CR1 and promotes lung disease severity," *Immunity*, vol. 46, no. 2, pp. 301–314, 2017.

[42] E. S. Gollwitzer, S. Saglani, A. Trompette et al., "Lung microbiota promotes tolerance to allergens in neonates via PD-L1," *Nature Medicine*, vol. 20, no. 6, pp. 642–647, 2014.

[43] A. Remot, D. Descamps, M. L. Noordine et al., "Bacteria isolated from lung modulate asthma susceptibility in mice," *The ISME Journal*, vol. 11, no. 5, pp. 1061–1074, 2017.

[44] Y. Yun, G. Srinivas, S. Kuenzel et al., "Environmentally determined differences in the murine lung microbiota and their relation to alveolar architecture," *PLoS One*, vol. 9, no. 12, article e113466, 2014.

[45] T. Olszak, D. An, S. Zeissig et al., "Microbial exposure during early life has persistent effects on natural killer T cell function," *Science*, vol. 336, no. 6080, pp. 489–493, 2012.

[46] T. Gensollen, S. S. Iyer, D. L. Kasper, and R. S. Blumberg, "How colonization by microbiota in early life shapes the immune system," *Science*, vol. 352, no. 6285, pp. 539–544, 2016.

[47] C. Johansson, "Respiratory syncytial virus infection: an innate perspective," *F1000Research*, vol. 5, p. 2898, 2016.

[48] T. H. Kim and H. K. Lee, "Innate immune recognition of respiratory syncytial virus infection," *BMB Reports*, vol. 47, no. 4, pp. 184–191, 2014.

[49] N. Marr, S. E. Turvey, and N. Grandvaux, "Pathogen recognition receptor crosstalk in respiratory syncytial virus sensing: a host and cell type perspective," *Trends in Microbiology*, vol. 21, no. 11, pp. 568–574, 2013.

[50] R. Zeng, Y. Cui, Y. Hai, and Y. Liu, "Pattern recognition receptors for respiratory syncytial virus infection and design of vaccines," *Virus Research*, vol. 167, no. 2, pp. 138–145, 2012.

[51] E. A. Kurt-Jones, L. Popova, L. Kwinn et al., "Pattern recognition receptors TLR4 and CD14 mediate response to respiratory syncytial virus," *Nature Immunology*, vol. 1, no. 5, pp. 398–401, 2000.

[52] L. M. Haynes, D. D. Moore, E. A. Kurt-Jones, R. W. Finberg, L. J. Anderson, and R. A. Tripp, "Involvement of toll-like receptor 4 in innate immunity to respiratory syncytial virus," *Journal of Virology*, vol. 75, no. 22, pp. 10730–10737, 2001.

[53] S. Ehl, R. Bischoff, T. Ostler et al., "The role of Toll-like receptor 4 versus interleukin-12 in immunity to respiratory syncytial virus," *European Journal of Immunology*, vol. 34, no. 4, pp. 1146–1153, 2004.

[54] L. Armstrong, A. R. Medford, K. M. Uppington et al., "Expression of functional toll-like receptor-2 and -4 on alveolar epithelial cells," *American Journal of Respiratory Cell and Molecular Biology*, vol. 31, no. 2, pp. 241–245, 2004.

[55] K. Suzuki, T. Suda, T. Naito, K. Ide, K. Chida, and H. Nakamura, "Impaired toll-like receptor 9 expression in alveolar macrophages with no sensitivity to CpG DNA," *American Journal of Respiratory and Critical Care Medicine*, vol. 171, no. 7, pp. 707–713, 2005.

[56] M. Vareille, E. Kieninger, M. R. Edwards, and N. Regamey, "The airway epithelium: soldier in the fight against respiratory viruses," *Clinical Microbiology Reviews*, vol. 24, no. 1, pp. 210–229, 2011.

[57] K. A. Shirey, L. M. Pletneva, A. C. Puche et al., "Control of RSV-induced lung injury by alternatively activated macrophages is IL-4Rα-, TLR4-, and IFN-β-dependent," *Mucosal Immunology*, vol. 3, no. 3, pp. 291–300, 2010.

[58] M. R. Murawski, G. N. Bowen, A. M. Cerny et al., "Respiratory syncytial virus activates innate immunity through Toll-like receptor 2," *Journal of Virology*, vol. 83, no. 3, pp. 1492–1500, 2009.

[59] G. F. Sonnenberg and D. Artis, "Innate lymphoid cells in the initiation, regulation and resolution of inflammation," *Nature Medicine*, vol. 21, no. 7, pp. 698–708, 2015.

[60] T. Ishii, K. Hosoki, Y. Niikura, T. Nagase, and N. Yamashita, "The activation of pulmonary type2 innate lymphoid cells through toll-like receptors," *American Journal of Respiratory and Critical Care Medicine*, vol. 195, article AS284, 2017.

[61] B. D. Rudd, E. Burstein, C. S. Duckett, X. Li, and N. W. Lukacs, "Differential role for TLR3 in respiratory syncytial virus-induced chemokine expression," *Journal of Virology*, vol. 79, no. 6, pp. 3350–3357, 2005.

[62] L. Guillot, R. Le Goffic, S. Bloch et al., "Involvement of toll-like receptor 3 in the immune response of lung epithelial cells to double-stranded RNA and influenza A virus," *The Journal of Biological Chemistry*, vol. 280, no. 7, pp. 5571–5580, 2005.

[63] F. Qi, D. Wang, J. Liu et al., "Respiratory macrophages and dendritic cells mediate respiratory syncytial virus-induced IL-33 production in TLR3- or TLR7-dependent manner," *International Immunopharmacology*, vol. 29, no. 2, pp. 408–415, 2015.

[64] T. Demoor, B. C. Petersen, S. Morris et al., "IPS-1 signaling has a nonredundant role in mediating antiviral responses and the clearance of respiratory syncytial virus," *Journal of Immunology*, vol. 189, no. 12, pp. 5942–5953, 2012.

[65] N. W. Lukacs, J. J. Smit, S. Mukherjee, S. B. Morris, G. Nunez, and D. M. Lindell, "Respiratory virus-induced TLR7 activation controls IL-17-associated increased mucus via IL-23 regulation," *Journal of Immunology*, vol. 185, no. 4, pp. 2231–2239, 2010.

[66] S. Phipps, C. E. Lam, S. Mahalingam et al., "Eosinophils contribute to innate antiviral immunity and promote clearance of respiratory syncytial virus," *Blood*, vol. 110, no. 5, pp. 1578–1586, 2007.

[67] J. Schlender, V. Hornung, S. Finke et al., "Inhibition of toll-like receptor 7- and 9-mediated alpha/beta interferon production in human plasmacytoid dendritic cells by respiratory syncytial virus and measles virus," *Journal of Virology*, vol. 79, no. 9, pp. 5507–5515, 2005.

[68] Y. M. Loo, J. Fornek, N. Crochet et al., "Distinct RIG-I and MDA5 signaling by RNA viruses in innate immunity," *Journal of Virology*, vol. 82, no. 1, pp. 335–345, 2008.

[69] N. Marr, T. I. Wang, S. H. Kam et al., "Attenuation of respiratory syncytial virus-induced and RIG-I-dependent type I IFN responses in human neonates and very young children," *Journal of Immunology*, vol. 192, no. 3, pp. 948–957, 2014.

[70] M. Goritzka, S. Makris, F. Kausar et al., "Alveolar macrophage-derived type I interferons orchestrate innate immunity to RSV through recruitment of antiviral monocytes," *The Journal of Experimental Medicine*, vol. 212, no. 5, pp. 699–714, 2015.

[71] A. Sabbah, T. H. Chang, R. Harnack et al., "Activation of innate immune antiviral responses by Nod2," *Nature Immunology*, vol. 10, no. 10, pp. 1073–1080, 2009.

[72] V. G. Bhoj, Q. Sun, E. J. Bhoj et al., "MAVS and MyD88 are essential for innate immunity but not cytotoxic T lymphocyte response against respiratory syncytial virus," *Proceedings of the National Academy of Sciences*, vol. 105, no. 37, pp. 14046–14051, 2008.

[73] S. Makris, M. Bajorek, F. J. Culley, M. Goritzka, and C. Johansson, "Alveolar macrophages can control respiratory syncytial virus infection in the absence of type I interferons," *Journal of Innate Immunity*, vol. 8, no. 5, pp. 452–463, 2016.

[74] A. A. Awomoyi, P. Rallabhandi, T. I. Pollin et al., "Association of TLR4 polymorphisms with symptomatic respiratory syncytial virus infection in high-risk infants and young children," *Journal of Immunology*, vol. 179, no. 5, pp. 3171–3177, 2007.

[75] G. Tal, A. Mandelberg, I. Dalal et al., "Association between common Toll-like receptor 4 mutations and severe respiratory syncytial virus disease," *The Journal of Infectious Diseases*, vol. 189, no. 11, pp. 2057–2063, 2004.

[76] M. K. Tulic, R. J. Hurrelbrink, C. M. Prêle et al., "TLR4 polymorphisms mediate impaired responses to respiratory syncytial virus and lipopolysaccharide," *Journal of Immunology*, vol. 179, no. 1, pp. 132–140, 2007.

[77] M. Ramet, M. Korppi, and M. Hallman, "Pattern recognition receptors and genetic risk for rsv infection: value for clinical decision-making?," *Pediatric Pulmonology*, vol. 46, no. 2, pp. 101–110, 2011.

[78] N. Marr, A. F. Hirschfeld, A. Lam, S. Wang, P. M. Lavoie, and S. E. Turvey, "Assessment of genetic associations between common single nucleotide polymorphisms in RIG-I-like receptor and IL-4 signaling genes and severe respiratory syncytial virus infection in children: a candidate gene case-control study," *PLoS One*, vol. 9, no. 6, article e100269, 2014.

[79] M. T. Caballero, M. E. Serra, P. L. Acosta et al., "TLR4 genotype and environmental LPS mediate RSV bronchiolitis through Th2 polarization," *The Journal of Clinical Investigation*, vol. 125, no. 2, pp. 571–582, 2015.

[80] T. R. Kollmann, O. Levy, R. R. Montgomery, and S. Goriely, "Innate immune function by Toll-like receptors: distinct responses in newborns and the elderly," *Immunity*, vol. 37, no. 5, pp. 771–783, 2012.

[81] K. Gabehart, K. A. Correll, J. E. Loader, C. W. White, and A. Dakhama, "The lung response to ozone is determined by age and is partially dependent on toll-like receptor 4," *Respiratory Research*, vol. 16, p. 117, 2015.

[82] K. Harju, M. Ojaniemi, S. Rounioja et al., "Expression of toll-like receptor 4 and endotoxin responsiveness in mice during perinatal period," *Pediatric Research*, vol. 57, no. 5, Part 1, pp. 644–648, 2005.

[83] J. S. Tregoning, Y. Yamaguchi, J. Harker, B. Wang, and P. J. Openshaw, "The role of T cells in the enhancement of respiratory syncytial virus infection severity during adult reinfection of neonatally sensitized mice," *Journal of Virology*, vol. 82, no. 8, pp. 4115–4124, 2008.

[84] A. F. Christiaansen, C. J. Knudson, K. A. Weiss, and S. M. Varga, "The CD4 T cell response to respiratory syncytial virus infection," *Immunologic Research*, vol. 59, no. 1–3, pp. 109–117, 2014.

[85] T. J. Ruckwardt, A. M. Malloy, K. M. Morabito, and B. S. Graham, "Quantitative and qualitative deficits in neonatal lung-migratory dendritic cells impact the generation of the CD8+ T cell response," *PLoS Pathogens*, vol. 10, no. 2, article e1003934, 2014.

[86] S. A. Cormier, B. Shrestha, J. Saravia et al., "Limited type I interferons and plasmacytoid dendritic cells during neonatal respiratory syncytial virus infection permit immunopathogenesis upon reinfection," *Journal of Virology*, vol. 88, no. 16, pp. 9350–9360, 2014.

[87] A. Remot, D. Descamps, L. Jouneau et al., "Flt3 ligand improves the innate response to respiratory syncytial virus and limits lung disease upon RSV reexposure in neonate mice," *European Journal of Immunology*, vol. 46, no. 4, pp. 874–884, 2016.

[88] M. Goritzka, L. R. Durant, C. Pereira, S. Salek-Ardakani, P. J. Openshaw, and C. Johansson, "Alpha/beta interferon receptor signaling amplifies early proinflammatory cytokine production in the lung during respiratory syncytial virus infection," *Journal of Virology*, vol. 88, no. 11, pp. 6128–6136, 2014.

[89] T. R. Johnson, S. E. Mertz, N. Gitiban et al., "Role for innate IFNs in determining respiratory syncytial virus immunopathology," *Journal of Immunology*, vol. 174, no. 11, pp. 7234–7241, 2005.

[90] K. M. Eichinger, L. Egaña, J. G. Orend et al., "Alveolar macrophages support interferon gamma-mediated viral clearance in RSV-infected neonatal mice," *Respiratory Research*, vol. 16, p. 122, 2015.

[91] P. K. Pribul, J. Harker, B. Wang et al., "Alveolar macrophages are a major determinant of early responses to viral lung infection but do not influence subsequent disease development," *Journal of Virology*, vol. 82, no. 9, pp. 4441–4448, 2008.

[92] D. Kolli, M. R. Gupta, E. Sbrana et al., "Alveolar macrophages contribute to the pathogenesis of human metapneumovirus infection while protecting against respiratory syncytial virus infection," *American Journal of Respiratory Cell and Molecular Biology*, vol. 51, no. 4, pp. 502–515, 2014.

[93] T. Hussell and T. J. Bell, "Alveolar macrophages: plasticity in a tissue-specific context," *Nature Reviews Immunology*, vol. 14, no. 2, pp. 81–93, 2014.

[94] D. M. Mosser and J. P. Edwards, "Exploring the full spectrum of macrophage activation," *Nature Reviews Immunology*, vol. 8, no. 12, pp. 958–969, 2008.

[95] K. M. Empey, J. G. Orend, R. S. Peebles Jr. et al., "Stimulation of immature lung macrophages with intranasal interferon gamma in a novel neonatal mouse model of respiratory syncytial virus infection," *PLoS One*, vol. 7, no. 7, article e40499, 2012.

[96] J. Saravia, D. You, B. Shrestha et al., "Respiratory syncytial virus disease is mediated by age-variable IL-33," *PLoS Pathogens*, vol. 11, no. 10, article e1005217, 2015.

[97] M. L. Garcia-Garcia, C. Calvo, A. Moreira et al., "Thymic stromal lymphopoietin, IL-33, and periostin in hospitalized infants with viral bronchiolitis," *Medicine*, vol. 96, no. 18, article e6787, 2017.

[98] M. T. Stier, M. H. Bloodworth, S. Toki et al., "Respiratory syncytial virus infection activates IL-13-producing group 2 innate lymphoid cells through thymic stromal lymphopoietin," *The Journal of Allergy and Clinical Immunology*, vol. 138, no. 3, pp. 814–824.e11, 2016.

[99] S. Sattler, G. S. Ling, D. Xu et al., "IL-10-producing regulatory B cells induced by IL-33 ($Breg^{IL\text{-}33}$) effectively attenuate mucosal inflammatory responses in the gut," *Journal of Autoimmunity*, vol. 50, pp. 107–122, 2014.

[100] Y. Yamaguchi, J. A. Harker, B. Wang, P. J. Openshaw, J. S. Tregoning, and F. J. Culley, "Preexposure to CpG protects against the delayed effects of neonatal respiratory syncytial virus infection," *Journal of Virology*, vol. 86, no. 19, pp. 10456–10461, 2012.

[101] M. S. Boukhvalova, T. B. Sotomayor, R. C. Point, L. M. Pletneva, G. A. Prince, and J. C. Blanco, "Activation of interferon response through toll-like receptor 3 impacts viral pathogenesis and pulmonary toll-like receptor expression during respiratory syncytial virus and influenza infections in the cotton rat *Sigmodon hispidus* model," *Journal of Interferon & Cytokine Research*, vol. 30, no. 4, pp. 229–242, 2010.

[102] S. Challa, A. Sheri, S. Padmanabhan et al., "Prophylactic and therapeutic anti-RSV activity of SB 9200 – a novel agent that activates RIG-I and NOD2," in *Spring Bank Pharmaceuticals Presents Data on Immunomodulatory Agent SB 9200 at the 29th International Conference on Antiviral Research*, La Jolla, CA, 2016.

[103] K. K. Tekkanat, H. F. Maassab, D. S. Cho et al., "IL-13-induced airway hyperreactivity during respiratory syncytial virus infection is STAT6 dependent," *Journal of Immunology*, vol. 166, no. 5, pp. 3542–3548, 2001.

[104] G. M. Gauvreau, P. M. O'Byrne, L. P. Boulet et al., "Effects of an anti-TSLP antibody on allergen-induced asthmatic responses," *New England Journal of Medicine*, vol. 370, no. 22, pp. 2102–2110, 2014.

[105] E. H. De Boever, C. Ashman, A. P. Cahn et al., "Efficacy and safety of an anti-IL-13 mAb in patients with severe asthma: a randomized trial," *Journal of Allergy and Clinical Immunology*, vol. 133, no. 4, pp. 989–996, 2014.

[106] B. Shrestha, D. You, J. Saravia et al., "IL-4Rα on dendritic cells in neonates and Th2 immunopathology in respiratory syncytial virus infection," *Journal of Leukocyte Biology*, vol. 102, no. 1, pp. 153–161, 2017.

[107] M. J. Ripple, D. You, S. Honnegowda et al., "Immunomodulation with IL-4Rα antisense oligonucleotide prevents respiratory syncytial virus-mediated pulmonary disease," *Journal of Immunology*, vol. 185, no. 8, pp. 4804–4811, 2010.

[108] B. T. Srinivasa, K. H. Restori, J. Shan et al., "STAT6 inhibitory peptide given during RSV infection of neonatal mice reduces exacerbated airway responses upon adult reinfection," *Journal of Leukocyte Biology*, vol. 101, no. 2, pp. 519–529, 2017.

[109] Y. M. Lee, N. Miyahara, K. Takeda et al., "IFN-γ production during initial infection determines the outcome of reinfection with respiratory syncytial virus," *American Journal of Respiratory and Critical Care Medicine*, vol. 177, no. 2, pp. 208–218, 2008.

[110] K. M. Eichinger, E. Resetar, J. Orend, K. Anderson, and K. M. Empey, "Age predicts cytokine kinetics and innate immune cell activation following intranasal delivery of IFNγ and GM-CSF in a mouse model of RSV infection," *Cytokine*, vol. 97, pp. 25–37, 2017.

[111] J. P. Lynch, M. A. Sikder, B. F. Curren et al., "The influence of the microbiome on early-life severe viral lower respiratory infections and asthma-food for thought?," *Frontiers in Immunology*, vol. 8, p. 156, 2017.

[112] J. Villena, S. Salva, M. Núñez et al., "Probiotics for everyone! The novel immunobiotic *Lactobacillus rhamnosus* CRL1505 and the beginning of social probiotic programs in Argentina," *International Journal of Biotechnology for Wellness Industries*, vol. 1, no. 3, pp. 189–198, 2012.

[113] J. Villena, E. Chiba, Y. Tomosada et al., "Orally administered *Lactobacillus rhamnosus* modulates the respiratory immune response triggered by the viral pathogen-associated molecular pattern poly(I:C)," *BMC Immunology*, vol. 13, p. 53, 2012.

[114] E. Chiba, Y. Tomosada, M. G. Vizoso-Pinto et al., "Immunobiotic *Lactobacillus rhamnosus* improves resistance of infant mice against respiratory syncytial virus infection," *International Immunopharmacology*, vol. 17, no. 2, pp. 373–382, 2013.

[115] Y. Tomosada, E. Chiba, H. Zelaya et al., "Nasally administered *Lactobacillus rhamnosus* strains differentially modulate respiratory antiviral immune responses and induce protection against respiratory syncytial virus infection," *BMC Immunology*, vol. 14, p. 40, 2013.

[116] W. A. de Steenhuijsen Piters, S. Heinonen, R. Hasrat et al., "Nasopharyngeal microbiota, host transcriptome, and disease severity in children with respiratory syncytial virus infection," *American Journal of Respiratory and Critical Care Medicine*, vol. 194, no. 9, pp. 1104–1115, 2016.

[117] S. M. Teo, D. Mok, K. Pham et al., "The infant nasopharyngeal microbiome impacts severity of lower respiratory infection and risk of asthma development," *Cell Host & Microbe*, vol. 17, no. 5, pp. 704–715, 2015.

Hindawi
Journal of Immunology Research
Volume 2017, Article ID 4128398, 5 pages
https://doi.org/10.1155/2017/4128398

Research Article

Immune Dysfunction in HIV: A Possible Role for Pro- and Anti-Inflammatory Cytokines in HIV Staging

Iorhen Ephraim Akase,[1] Bolanle O. P. Musa,[1,2] Reginald Onyedumarakwe Obiako,[1,3] Abdurrahman Ahmad Elfulatiy,[2] and Abdullahi Asara Mohammed[1]

[1]*Department of Medicine, Ahmadu Bello University Teaching Hospital, Shika-Zaria, Nigeria*
[2]*Immunology Unit, Ahmadu Bello University, Zaria, Nigeria*
[3]*Neurology Unit, Ahmadu Bello University, Zaria, Nigeria*

Correspondence should be addressed to Iorhen Ephraim Akase; akasephraim@yahoo.com

Received 16 June 2017; Revised 3 October 2017; Accepted 10 October 2017; Published 2 November 2017

Academic Editor: Elias Said

Copyright © 2017 Iorhen Ephraim Akase et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

HIV infection is a chronic infection that almost inevitably progresses to AIDS. The infection is characterized by the deterioration in the immune function leading to opportunistic infections and malignancies. Additionally, there is an associated immune dysfunction characterized by a persistent inflammatory state and unhealthy elaboration of both pro- and anti-inflammatory cytokines. The $CD4^+$ T cell count has been used as a surrogate for the level of immune dysfunction that exists in patients with HIV infection. Eighty-eight (88) patients with HIV infection, forty-four (44) of whom were treatment naïve patients and forty-four (44) who were treatment-experienced patients, were recruited. The serum concentrations of cytokines IL-6 and IL-10 were carried out using R&D human *Quantikine* ELISA kits, while patients' $CD4^+$ T cell counts were evaluated using the *Partec easy count kit*. The serum IL-6 and IL-10 concentrations were significantly higher among the AR-naïve participants compared to the ART-experienced group. Additionally, the IL-6 and IL-10 concentrations were higher in patients with lower $CD4^+$ T cell count compared to those with higher cell counts though this was not statistically significant. Also, both IL-6 and IL-10 concentrations were higher in patients with higher WHO clinical staging of disease, significantly so for IL-6.

1. Introduction

The human immunodeficiency virus type-1 (HIV-1) infection and its sequelae, the acquired immune deficiency syndrome (AIDS), are the major causes of morbidity and mortality worldwide, accounting for over 35 million deaths since the first reported cases in 1983 [1]. AIDS is characterized by various abnormalities of immune function which manifests as opportunistic diseases that eventually lead to increased morbidity and mortality in involved individuals [2]. An estimated 36.7 million people are infected with HIV worldwide with 69.5% of this number living in sub-Saharan Africa [1]. In Nigeria, about 3.1% of the population are said to be infected with HIV [1].

Cytokines are a group of low molecular weight proteins that mediate communication between immune system cells. They contribute to chemical signaling pathways that regulate cell development, tissue repair, haematopoiesis, inflammation, and immune responses. They act in a complex network where one cytokine can influence the production of, and response to, many other cytokines [3]. Cytokines play a crucial role in entry of HIV into host tissues, progression of disease, and in the occurrence of opportunistic infections [4]. HIV infection is characterized by immune activation, with the production of both pro- and anti-inflammatory cytokines. An immune deficiency state results when HIV infection produces a significant decrease in number of $CD4^+$ T lymphocytes, defective function of both the T lymphocytes and macrophages, and dysregulation of cytokine production [5].

The CD4 T lymphocytes, also called T helper cells, are important in the coordination of the immune system by directing immune responses against pathogens either towards a predominantly humoral or a cell-mediated

response depending on the nature of the antigen and the immune receptor that has been activated by the antigen. Additionally, subsets of the T helper cells are crucial in the regulation of immune responses in immunocompetent individuals. By selectively targeting the $CD4^+$ T cell, infection with HIV not only renders an immunodeficient individual but also produces a state of immune disarray which manifests as a clinical predisposition to infections and a persistent inflammatory state which has been demonstrated even in individuals who are on cART and who have had virological suppression with viral suppression below detected serum levels.

Improvement of patients with HIV is documented clinically by the improvements in clinical conditions, virologically by the reductions in HIV RNA viral loads, and immunologically by the $CD4^+$ T cell count. For long, clinical decisions in HIV-infected individuals have been based on the $CD4^+$ T cell counts of such individuals, but questions remain as to how well the peripheral $CD4^+$ T cell count mirrors the true state of the immune dysfunction seen in patients who have been infected with HIV.

The aim of this study was therefore to assess the relationship between the $CD4^+$ T cell count and the serum levels of IL-6 and IL-10 in patients infected with HIV whether on treatment or not, who were attending the ART clinic of the Ahmadu Bello University Teaching Hospital, Shika-Zaria.

2. Methods

This study was carried out in the outpatient clinic of the Ahmadu Bello University Teaching Hospital (ABUTH) HIV program. The HIV program is situated in the NASARA complex and is part of the 700-bed referral hospital located in Shika-Zaria, Kaduna State. Eighty-eight (88) patients were selected consecutively. They included forty-four (44) newly diagnosed ART- (antiretroviral treatment-) naïve patients to comprise the test group and another forty-four (44) ART-experienced patients to serve as the comparison group. The comparison group included patients who had been receiving ARTs for at least 6 months. All eighty-eight samples (88) had IL-6 and IL-10 assays carried out on them, as well as $CD4^+$ T cell enumeration.

The study was a comparative cross-sectional study. Ethical approval for the study was obtained from the ABUTH Ethical and Scientific committee. Adequate counseling and informed consent were applied confidentially throughout the course of the research. Data was collected using a researcher-administered questionnaire, which captured the biodata/sociodemographic characteristics, medical history, and the physical examination of the participants.

The levels of both serum IL-6 and IL-10 were assayed using the *Quantikine*® ELISA human IL-6 and IL-10 immunoassay kits by R&D Systems Inc., USA. The $CD4^+$ T cell estimation was done with the *Partec Cyflow* flow cytometer using the CD4 *easy count kit* (Sysmex Partec GmbH, Germany).

All data was analyzed using Statistical Package for the Social Sciences (SPSS), version 20.0, Chicago, IL, USA. Qualitative variables were reported as percentages. Normally distributed quantitative variables (e.g., age) were presented as mean and standard deviation, while those with uneven distribution (e.g., CD4 count and cytokine levels) were presented as median and range. Statistical test of significance was set at 5% alpha level using the chi-square and Pearson's correlation for qualitative and quantitative variables, respectively. The Student *t*-test was used to compare the mean age while the Mann–Whitney *U* test was used to compare the medians of CD4 cells, interleukin-6, and interleukin-10. Linear regression model was used to measure the relationship between the interleukin levels and the WHO clinical stage, antiretroviral (ARV) regimens, and ARV treatment categories.

3. Results

3.1. Sociodemographic Characteristics. Males constituted 54.5% (48) of the study participants while 45.5% (40) were female. The mean age in the study was 36.6 ± 8.8 years, and the majority of the respondents were of the Hausa/Fulani extraction, constituting 51% (45) of the participants. Participants of Ibo and Yoruba extraction each constituted 4.5% (4) of the respondents.

3.2. Immunological Parameters. The median serum interleukin-6 concentration was lower among the female participants compared to the male counterparts, with median values of 3.74 pg/mL (0.00–171.00 pg/mL) and 2.36 pg/mL (0.00–45.31 pg/mL) among the male and female participants, respectively ($p = 0.88$, Mann–Whitney *U* test). Similarly, the median serum concentration of interleukin-10 was lower among the female participants compared to the male participants, with median values of 6.76 pg/mL (0.00–134.20 pg/mL) and 7.22 pg/mL (0.38–47.37 pg/mL) among the female and the male participants, respectively ($p = 0.68$, Mann–Whitney *U* test).

The median $CD4^+$ T cell count in the ART-naïve group was 166 cells/μL with a range of 6–809 cells/μL, while among the ART-experienced participants, the median was 463 cells/μL with a range of 37–1519 cells/μL ($p < 0.001$, Mann–Whitney *U* test). The median value of the serum concentration of IL-6 among the ART naïve group was 6.8 pg/mL with a range of 1.2–316.0 pg/mL. Conversely, the median serum IL-6 concentration among the ART-experienced subjects was 1.40 pg/mL with a range of 1.0–27.5 pg/mL ($p < 0.001$, Mann–Whitney *U* test). Similarly, the median IL-10 concentration among the ART-naïve subjects was 10.1 pg/mL with a range of 2.1–870 pg/mL, while a median value of 6.0 pg/mL and a range of 3–39.9 pg/mL ($p = 0.14$, Mann–Whitney *U* test) was observed among the ART-experienced subjects (see Table 1).

The association between the patient categories (i.e., ART naïve versus ART experienced) and serum interleukin-6 levels was not affected after adjustment for the gender of the participants, with the regression coefficient for patient category reducing by 0.13% ($b = 15.76$ versus $b = 15.74$). Similarly, the effect of the participant gender on the difference in the serum interleukin-10 among the study participants was not significant, with the regression coefficient only

Table 1: Immunological parameters of participants.

Parameter	ART naïve	ART experienced	p value*
Median† CD4 concentration	166 cells/μL (6–609)	463 cells/μL (37–1519)	*<0.001*
Median IL-6 concentration	6.8 pg/mL (1.2–316)	1.4 pg/mL (1.0–27.5)	*<0.001*
Median IL-10 concentration	10.1 pg/mL (2.1–870)	6.0 pg/mL (3.0–39.9)	*0.14*

*$\alpha < 0.05$, Mann–Whitney U test. †Median (range).

Table 2: Median concentration of interleukins according to the WHO clinical stage of HIV.

WHO clinical stage	IL-6 concentration (pg/mL)	IL-10 concentration (pg/mL)
1	2.22 (0.00–37.97)*	10.15 (0.00–47.37)
2	2.22 (0.00–45.31)	26.32 (5.64–43.23)
3	7.76 (1.39–65.13)	30.83 (12.40–48.50)
4	171.00 (4.70–175.84)	134.20 (14.66–800.00)

*Median (range).

Table 3: Antiretroviral stage of treatment based on the sex of the participants.

ARV category	Male	Female	Total
ART naïve	26	18	44
1st line regimen	18	16	34
2nd line regimen	4	6	10

reducing by 3.33% after adjustment for gender ($b = -1.80$ versus $b = -0.06$).

Both the serum IL-6 ($r = -0.43$, $p = 0.01$) and IL-10 ($r = -0.27$, $p = 0.12$) concentrations showed an inverse correlation with the $CD4^+$ T cell count of the participants in the study participants. The serum concentrations of both interleukin-6 and interleukin-10 demonstrated a positive relationship with the WHO clinical stage of participants (see Table 2).

There was no significant increase of interleukin-6 levels with the WHO clinical stage II of HIV compared to stage I ($b = 3.98$, $t(83) = 0.51$, and $p = 0.62$); however, the increase in serum interleukin-6 levels was significant at stage III ($b = 20.27$, $t(83) = 2.31$, and $p = 0.02$) and stage IV ($b = 141.64$, $t(83) = 9.66$, and $p < 0.001$) compared to levels at WHO clinical stage I, with the regression model predicting 53.5% ($R^2 = 0.535$) of the variance ($F_{(3,52)} = 32.24$, $p < 0.001$). There was also a nonsignificant increase in the serum interleukin-10 levels at WHO clinical stage II ($b = 15.56$, $t(52) = 0.55$, and $p = 0.56$) and stage III ($b = 23.06$, $t(52) = 0.77$, and $p = 0.44$) compared to stage I; however, the increase in interleukin-10 was significant in stage IV compared to stage I ($b = 301.34$, $t(52) = 6.06$, and $p < 0.001$), with the regression model predicting 41.7% ($r^2 = 0.417$) of the variance ($F_{(3,84)} = 12.40$, $p < 0.001$).

3.3. Interleukin Levels and HAART. As shown above, there was a significant difference in the median serum concentrations of IL-6 among the study categories of patients, with levels significantly higher among the treatment-naïve subjects compared to the ART-experienced subjects ($p < 0.001$, Mann–Whitney U test).

The IL-6 levels were affected by the ARV regimen the patient was currently taking, with the highest levels found in participants who were not on any medications and the lowest levels among those who were taking Tenofovir/Lamivudine/Efavirenz combination (1.45 pg/mL, $b = -22.00$, $t(82) = -1.78$, and $p = 0.08$), while participants who were on Zidovudine/Lamivudine/Nevirapine combination had higher levels among those who were on the other regimens (2.29 pg/mL, $b = -14.53$, $t(82) = -1.88$, and $p = 0.06$) compared to those who were yet to commence treatment, with the ARV regimen explaining only 8% of the variance in the interleukin-6 levels ($r^2 = 0.08$, $F_{(5,81)} = 1.41$, and $p = 0.23$). Participants who were on Tenofovir/Emtricitabine/Efavirenz combination had the lowest median serum interleukin-10 concentrations (9.71 pg/dL, $b = -45.58$, $t(50) = -1.19$, and $p = 0.24$) with the highest serum concentrations of interleukin-10 in the ART-experienced group observed among patients who were on Tenofovir/Lamivudine/Lopinavir-ritonavir regimen (15.8 pg/mL, $b = -43.04$, $t(50) = -0.67$, and $p = 0.51$) compared to the ART-naïve group, with the ARV regimen explaining 4.1% of the variance ($r^2 = 0.041$, $F_{(5,50)} = 0.43$, and $p = 0.83$).

The levels of IL-6 were affected by the stage of treatment, with the highest levels detected among those yet to commence medications, followed by those on the 2nd line drugs (2.22 pg/mL, $b = -19.15$, $t(84) = -1.83$, and $p = 0.07$) while the lowest levels were detected among those on the 1st line medications (1.80 pg/mL, $b = -16.03$, $t(84) = -2.34$, and $p = 0.02$). The stage of ARV treatment explained 7.6% of the variation ($r^2 = 0.076$, $F_{(2,\ 84)} = 3.46$, and $p = 0.04$). The IL-10 levels were also noticed to be higher among the untreated participants but lower among the participants who were on the 1st (11.65 pg/mL, $b = -45.38$, $t(53) = -1.50$, and $p = 0.14$) and 2nd (12.59 pg/mL, $b = -47.41$, $t(53) = -1.02$, and $p = 0.31$), with the regression model predicting 47% of the variation ($r^2 = 0.47$, $F_{(2,53)} = 1.31$, and $p = 0.28$). The distribution of the participants according to the stage of treatment is shown in Table 3.

There was a significant negative correlation between the duration from diagnosis to time of study and the levels of interleukin-6 ($r = -0.25$, $p = 0.03$). The duration from diagnosis of HIV infection to time of study demonstrated

a nonsignificant negative correlation with the serum interleukin-10 ($r = -0.19$, $p = 0.18$).

4. Discussion

This study showed higher interleukin levels among patients with lower $CD4^+$ T cell counts, which is consistent with more pronounced inflammation in subjects with more advanced disease. These findings are also consistent with similar studies done elsewhere [6, 7]. Similarly, the higher serum concentrations of both IL-6 and IL-10 in patients with advanced WHO clinical stage of disease mirrored the pattern observed with low $CD4^+$ T cell counts and corresponding high interleukin levels. These findings add credence to the usefulness of the WHO clinical staging in decision-making, especially in resource poor settings that do not have access to routine $CD4^+$ T cell measurements. The higher serum levels of both IL-6 and IL-10 in ART naïve compared to the ART-experienced HIV-positive participant categories are also consistent with studies that show higher degrees of inflammation with deteriorating immune function [6–9], in line with the thinking that the HIV virus drives the inflammation that is persistent in HIV infections [2]. Similarly, the improvement that is associated with the use of ARTs as evidenced by immunological improvement in the patients has been observed by other researchers [10, 11]. It is also worthy of note that the ART-experienced patients also have persistently elevated levels of inflammatory markers albeit in lower concentrations than in the ART-naïve participants. This is consistent with the findings by other researchers that, despite successful suppression of viral replication to undetected levels, a degree of inflammation persists, which is vital to the overall pathogenesis of HIV, which may be of consequence in the long-term outcomes of these patients [2, 12].

The findings in this study suggest that participants on Tenofovir/Emtricitabine/Efavirenz and Tenofovir/Lamivudine/Efavirenz combinations had lower levels of both interleukin-6 and interleukin-10, with higher levels observed among participants who were on Zidovudine/Lamivudine/Nevirapine combination and the protease inhibitor-containing regimens. This is in contrast to findings by workers elsewhere that suggest the suppression of interleukin levels was without respect to the ART regimen used [11, 13]. The methodology of this study however is insufficient to adequately address this issue, and a well-designed randomized clinical trial will be better suited to evaluate this.

Patients who had a longer duration between diagnosis and the conduct of this study were observed to have lower interleukin levels. Whether this observation is due to a more sustained viral suppression is uncertain as we did not assay for HIV viral load in our participants.

5. Conclusion

There are higher concentrations of IL-6 and IL-10 levels among HIV patients that are not on HAART, and the levels of IL-6 and IL-10 are reduced by HAART. There is a higher level of IL-6 and IL-10 among HIV patients with low $CD4^+$ T cell counts and advanced WHO clinical stage, which was not affected by the gender of the participants. This relationship between interleukin-6 and interleukin-10 suggests that there may be a role for the use of cytokine measurements in the staging of HIV infections and this may be very useful in the setting of poor immune reconstitution with poor CD4 T lymphocyte response which is not uncommon in resource poor settings, partly on account of a severely damaged lymphoid system due to late commencement of ARVs.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Acknowledgments

The authors wish to thank the management of Ahmadu Bello University for the logistic and laboratory support in the course of this study.

References

[1] World Health Organization (WHO), "Global Health Observatory on HIV/AIDS," June 2017, http://www.who.int/gho/hiv/en/.

[2] A. S. Fauci and C. H. Lane, "Human immunodeficiency virus disease: AIDS and related disorders," in *Harrison's Infectious Diseases*, D. L. Kasper and A. S. Faici, Eds., pp. 792–885, McGraw Hill Inc, New York, NY, USA, 2010.

[3] M. S. Reitz and R. C. Gallo, "Human immunodeficiency viruses," in *Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases*, G. L. Mandell, J. E. Bennett and R. Dolin, Eds., pp. 2323–2333, Churchill Livingstone Elsevier, Philadelphia, PA, USA, 2010.

[4] S. S. Ramaseri, S. R. Hanumanth, R. T. Nagaraju et al., "IL-10 high producing genotype predisposes HIV infected individuals to TB infection," *Human Immunology*, vol. 73, no. 6, pp. 605–611, 2012.

[5] K. Kedzierska, J. Mak, A. Mijch et al., "Granulocyte-macrophage colony-stimulating factor augments phagocytosis of Mycobacterium avium complex by human immunodeficiency virus type 1- infected monocytes/macrophages in vitro and in vivo," *The Journal of Infectious Diseases*, vol. 181, no. 1, pp. 390–394, 2000.

[6] J. Neuhaus, D. R. Jacobs, J. V. Baker et al., "Markers of inflammation, coagulation, and renal function are elevated in adults with HIV infection," *The Journal of Infectious Diseases*, vol. 201, no. 12, pp. 1788–1795, 2010.

[7] J. P. Bastard, C. Soulie, S. Fellahi et al., "Circulating IL-6 levels correlate with residual HIV viremia and markers of immune dysfunction in treatment controlled HIV-infected patients," *Antiviral Therapy*, vol. 17, no. 5, pp. 915–919, 2012.

[8] P. J. Norris, B. L. Pappalardo, B. Custer, G. Spotts, F. M. Hetch, and P. M. Busch, "Elevations in IL-10, TNF-α, and IFN-γ from the earliest point of HIV-1 infection," *AIDS Research and Human Retroviruses*, vol. 22, no. 8, pp. 757–762, 2006.

[9] C. E. Osakwe, C. Bleotu, M. C. Chifiriuc et al., "TH1/TH2 cytokine levels as an indicator for disease progression in human immunodeficiency virus type 1 infection and response

to antiretroviral therapy," *Roumanian Archives of Microbiology and Immunology*, vol. 69, no. 1, pp. 24–34, 2010.

[10] M. A. Brockman, D. S. Kwon, D. P. Tighe et al., "IL-10 is up-regulated in multiple cell types during viremic HIV infection and reversibly inhibits virus-specific T cells," *Blood*, vol. 114, no. 2, pp. 346–356, 2009.

[11] S. M. Keating, E. T. Golub, M. Nowicki et al., "The effect of HIV infection and HAART on inflammatory biomarkers in a population-based cohort of women," *AIDS*, vol. 25, no. 15, pp. 1823–1832, 2011.

[12] E. Stylianou, P. Aukrust, D. Kvale, F. Müller, and S. S. Frøland, "IL-10 in HIV infection: increasing serum IL-10 levels with disease progression—down-regulatory effect of potent anti-retroviral therapy," *Clinical and Experimental Immunology*, vol. 116, no. 1, pp. 115–120, 1999.

[13] N. Amirayan-Chevillard, H. Tissot-Dupont, C. Capo et al., "Impact of highly active anti-retroviral therapy (HAART) on cytokine production and monocyte subsets in HIV-infected patients," *Clinical and Experimental Immunology*, vol. 120, no. 1, pp. 107–112, 2001.