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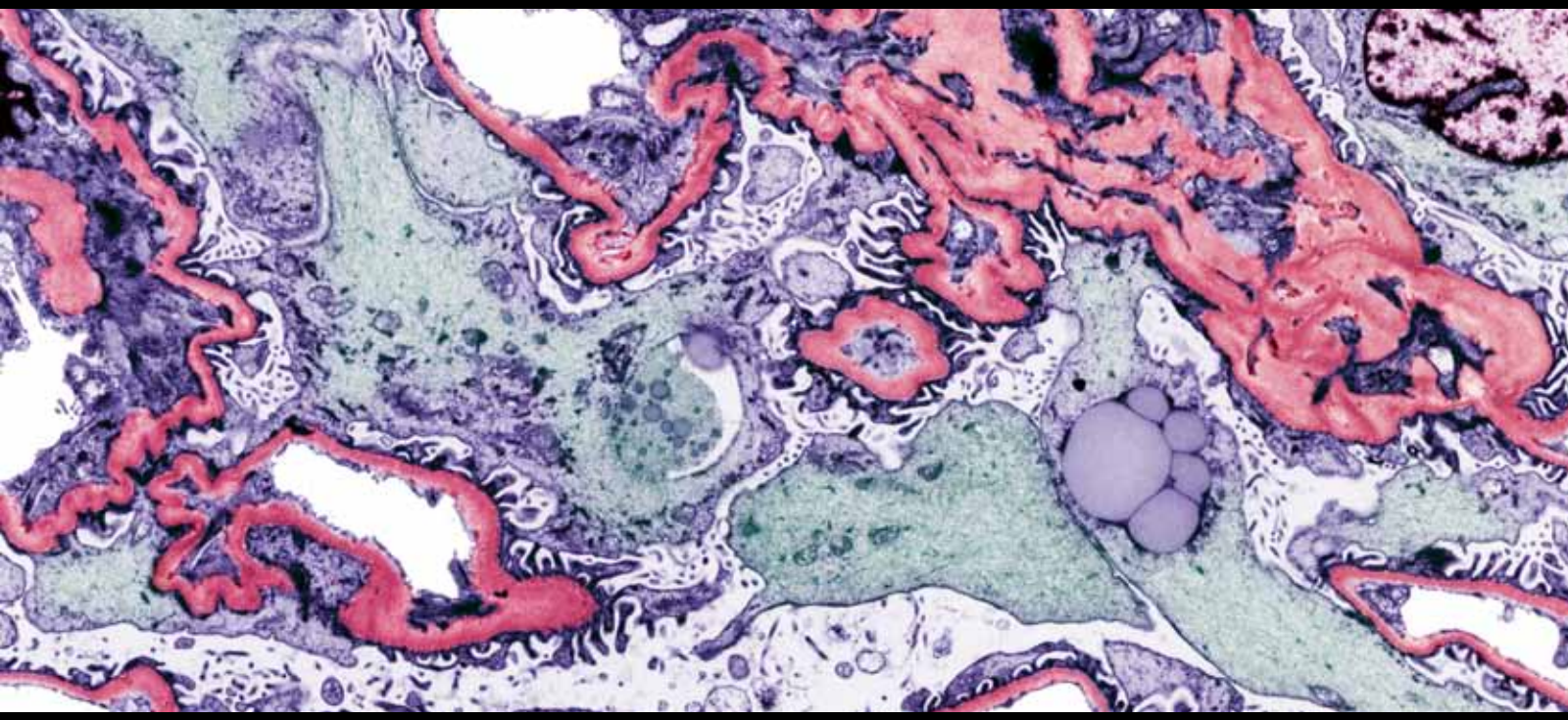
Guest Editors: Juan-Manuel Anaya, Adriana Rojas-Villarraga, and Mario García-Carrasco



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Editorial Board

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Editorial

The Autoimmune Tautology: From Polyautoimmunity and Familial Autoimmunity to the Autoimmune Genes

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Autoimmune diseases (ADs) are chronic conditions initiated by the loss of immunological tolerance to self-antigens and represent a heterogeneous group of disorders that afflict specific target organs or multiple organ systems [1]. The chronic nature of these diseases places a significant burden on the utilization of medical care, direct and indirect economic costs, and quality of life. The fact that ADs share several clinical signs and symptoms (i.e., subphenotypes), physiopathological mechanisms, and genetic factors has been called autoimmune tautology and indicates that they have common mechanisms [2–8].

In clinical practice, there are two conditions supporting this theory: polyautoimmunity and familial autoimmunity. Polyautoimmunity is the presence of two or more ADs in a single patient while familial autoimmunity occurs when different relatives from a nuclear family present with diverse ADs [4]. These conditions indicate that similar genetic, epigenetic, and environmental factors influence ADs [8].

In rhetoric, tautology (from Greek tauto, "the same" and logos, "word/idea") is an obvious statement. In medical practice, Sjögren's syndrome could be considered the "autoimmune diabetes" or the "celiac disease" of the salivary and lachrymal glands. In logic, tautology is a formula, which is true in every possible interpretation. Thus, autoimmune tautology means that an AD is similar to a second one, to a third one, and so on. Its formula is $\mathrm{Vpq} = \mathrm{AD}_1 \simeq \mathrm{AD}_2 \simeq \mathrm{AD}_3$, where Vpq represents the symbol of tautology. ADs cannot be all identical because the target cell and the affected organ may differ from one AD to another one. In addition, trigger factors as well as the age at onset may vary among them and from one patient to another. Yet, autoimmune mechanisms of injury may be common including predisposing and protective factors. One step forward to the demonstration of this logically valid formula will be a new taxonomy of ADs based on common and specific subphenotypes that will allow us to predict and prevent them, tailor individual medical decisions, and provide personalized healthcare while facilitating patient's participation in their treatment and eventual cure of their disease.

In Table 1, ten shared characteristics supporting the autoimmune tautology are summarized. In this special issue of *Autoimmune Diseases,* a dozen of papers are devoted to these characteristics. Evolution and genetics of ADs, including the biological significance of evolution in autoimmune phenomena, a meta-analysis of HLA class II in six ADs in Latin America, genetic factors of autoimmune thyroid diseases in Japanese, and an *in silico* approach of the autoimmune tautology are included. An updated review on epigenetics and ADs is also presented.

Environmental factors play an important role in the induction of ADs. This special issue also contains a very interesting hypothesis about local cartilage trauma as a pathogenic trigger factor of autoimmunity, a review about the induction of autoimmunity by microbial infections, and another one on the effect of selenium on HLA-DR expression of thyrocytes. How does age at onset influence the outcome

Table 1: Shared characteristics among autoimmune diseases (ADs) supporting the autoimmune tautology*.

Characteristic	Comment
Female predominance	The more frequent the AD and the later it appears, the more women are affected.
Similar pathophysiology	Damage induced by T or B cells, or both, plays a major pathogenic role in ADs. Although the autoimmune phenotype varies depending on the target cell and the affected organ, the local mechanisms for tissue injury are similar.
Shared subphenotypes	Mathematical approaches for precisely defining subphenotypes based on accurate clinical and immunological databases, combined with strengthening molecular genetics analyses, have significant promise for a better understanding of ADs.
Age at onset influences severity	Early age at onset is a poor prognostic factor for some ADs.
Similar environmental factors	Although a latitudinal gradient of infectious agents exists, Epstein-Barr virus and cytomegalovirus are notorious as they are consistently associated with multiple ADs. Some infections could be protective against ADs development. Smoking has also been consistently associated with several ADs.
Ancestry influences clinical presentation	Amerindian ancestry influences the risk of acquiring ADs as well as its severity.
Common genetic factors	The genetic risk factors for ADs consist of two forms: those common to many ADs and those specific to a given disorder. Combinations of common and disease-specific alleles at HLA and non-HLA genes in interaction with epigenetic and environmental factors over time will determine the final phenotype.
Polyautoimmunity	Factors significantly associated with polyautoimmunity are female gender and familial autoimmunity.
Familial autoimmunity	Unlike familial AD, which corresponds to the presence of one specific AD in various members of a nuclear family, familial autoimmunity uses the term "autoimmune disease" as a trait that encompasses all accepted pathologies for which evidence suggests an autoimmune origin.
Similar treatment	Similar biological and nonbiological therapies are used to treat diverse ADs.

* Adapted from references [2–8].

of ADs is also reviewed, and an update on lupus nephritis is offered.

Last but not least, a careful analysis of concomitant ADs in patients with systemic lupus erythematosus, rheumatoid arthritis (RA), multiple sclerosis (MS), and systemic sclerosis is reported. Polyautoimmunity is the term proposed for this association of disorders, which encompasses the concept of a common origin for these diseases. A lack of association between spondyloarthropathies (SpAs) and ADs is described, highlighting the fact that SpAs correspond more to autoinflammatory diseases rather than to ADs.

We hope that readers of *Autoimmune Diseases* will find in this special issue not only accurate data and updated reviews on the common mechanisms of ADs, but also important questions to be resolved such as their missing heritability, the antagonisms of some disorders (i.e., RA and MS), their prevention, the effect of ethnicity, socioeconomic status and health care system on their outcome, and the role of the autoimmunologist, among others.

Juan-Manuel Anaya
Adriana Rojas-Villarraga
Mario García-Carrasco

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Review Article

Shared HLA Class II in Six Autoimmune Diseases in Latin America: A Meta-Analysis

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The prevalence and genetic susceptibility of autoimmune diseases (ADs) may vary depending on latitudinal gradient and ethnicity. The aims of this study were to identify common human leukocyte antigen (HLA) class II alleles that contribute to susceptibility to six ADs in Latin Americans through a meta-analysis and to review additional clinical, immunological, and genetic characteristics of those ADs sharing HLA alleles. DRB1*03:01 (OR: 4.04; 95%CI: 1.41–11.53) was found to be a risk factor for systemic lupus erythematosus (SLE), Sjögren's syndrome (SS), and type 1 diabetes mellitus (T1D). DRB1*04:05 (OR: 4.64; 95%CI: 2.14–10.05) influences autoimmune hepatitis (AIH), rheumatoid arthritis (RA), and T1D; DRB1*04:01 (OR: 3.86; 95%CI: 2.32–6.42) is a susceptibility factor for RA and T1D. Opposite associations were found between multiple sclerosis (MS) and T1D. DQB1*06:02 and DRB1*15 alleles were risk factors for MS but protective factors for T1D. Likewise, DQB1*06:03 allele was a risk factor for AIH but a protective one for T1D. Several common autoantibodies and clinical associations as well as additional shared genes have been reported in these ADs, which are reviewed herein. These results indicate that in Latin Americans ADs share major loci and immune characteristics.

1. Introduction

Autoimmune diseases (ADs) are chronic conditions initiated by the loss of immunological tolerance to self-antigens. They are a heterogeneous group of disorders that affect specific target organs or multiple organ systems [1]. Almost all ADs disproportionately affect middle-aged women and are among the leading causes of death for this group of patients [2]. The etiology of ADs is unknown, but these complex diseases are known to feature genetic and environmental factors in their development [1, 3]. Although they exhibit contrasting epidemiological features and clinical manifestations, there is evidence that ADs share similar immunogenetic mechanisms [4].

Three related lines of evidence sustain the common origin for ADs. First, clinical evidence highlights the co-occurrence of distinct ADs within an individual (i.e., polyautoimmunity) and within members of a nuclear family (i.e., familial autoimmunity). Second, physiopathologic evidence indicates that the pathologic mechanisms may be similar among ADs. Third, genetic evidence shows that autoimmune phenotypes might represent pleiotropic outcomes of the interaction of nonspecific disease genes [5].

The study of HLA, which carries the major genetic influence on susceptibility to ADs, will allow us to understand its common or specific influence on these diseases and to identify genetic prediction markers. The large and diverse population of Latin America (LA) is a powerful resource for elucidating the genetic basis of complex traits due to its admixture [6]. Modern day LA resulted from the encounter of Europeans with the indigenous people of the Americas in 1492, followed by waves of migration from Europe and Africa. As a result, the genomic structure of present day Latin Americans is determined by both the genetic structure of the founding populations and the numbers of migrants from

these different populations [7]. Analysis of multiple Latino populations in gene association studies could also strengthen the potential associations as well as provide opportunities for examining gene-environment and gene-gene interactions [8].

The purpose of this paper was to estimate the common effect size of HLA class II on ADs across LA populations through a meta-analysis and to evaluate the additional characteristics (i.e., other genes, autoantibodies, and clinical characteristics) of genetically associated ADs in Latin America.

2. Materials and Methods

2.1. Study Selection. Five meta-analyses of HLA class II polymorphisms in LA patients with ADs published from 2007 to 2010 by our group were included [9–13]. The ADs included were rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), autoimmune hepatitis (AIH), multiple sclerosis (MS), and type 1 diabetes (T1D). In addition, the results from the only study of Sjögren's syndrome (SS) reported on the LA population were included [14]. Briefly, the strategies to search for, select, and analyze the studies used for each meta-analysis are mentioned hereinafter.

In all of the cases, a systematic review of the electronic databases (MEDLINE, PubMed, SciELO, BIREME, EMBASE, Cochrane, and LILACS) was done independently by two experts. The searches only included publications on HLA-Class II alleles and susceptibility to ADs in LA published in any of these three languages: Spanish, English, or Portuguese. All of the search strategies included MeSH terms: "HLA DR/DQ antigens" and "Major Histocompatibily Complex". However, other major topics were used depending on the specific AD: "Arthritis, Rheumatoid", "Lupus Erythematosus, Systemic", "Hepatitis, Autoimmune", "Hepatitis, Chronic", "Multiple Sclerosis", "Type 1 Diabetes", or "Autoimmune Diabetes".

The inclusion criteria were the following: (1) AD diagnosis established using international validated criteria for RA [15], for SLE [16], for AIH [17, 18], for MS [19, 20], and for T1D [21, 22]; (2) case-control design of the study; (3) publication of sufficient information to calculate odds ratios (ORs); (4) a focus on a well-defined LA population; (5) use of molecular techniques to determine HLA polymorphisms (i.e., allele-specific oligonucleotides—ASO, polymerase chain reaction with sequence-specific primers—PCR/SSP, restriction fragment length polymorphism—RFLP, specific oligonucleotide probes—SOP, or sequence-specific oligonucleotide probes—SSOP); and (6) manuscript's publication in a peer-reviewed journal as a full paper. Summaries or abstracts were not accepted.

Data were analyzed using the Comprehensive Meta-Analysis version 2 program (Biostat, Englewood, NJ, 2004). For each polymorphism group, the effect summary odds ratio (OR) and 95% confidence interval (CI) were obtained by the random effect model. The systematic review and meta-analysis were done following the PRISMA guidelines and the respective checklist completion [23].

2.2. Meta-Analysis. Calculations were carried out for each HLA-DR and HLA-DQ allele using low or high resolution based on information available in each meta-analysis. The final pooled OR was done by weighing individual OR by the inverse of their variance. For each allele, the final effect OR and 95%CI were obtained by means of a random model. This model was used because of the assumption that there is a distribution of true effect sizes rather than one true effect, assigning a more balanced weight to each study. It was also used because all the studies were considered to be functionally unequal. Values less than 1.0 suggest a protective effect while values greater than 1.0 suggest a risk for each AD. Heterogeneity was calculated by means of Cochran's (Q) and Higgins's (I^2) tests. The I^2 test measures the degree of inconsistency in the studies by calculating the percentage of total variation across studies due to heterogeneity rather than chance and was expressed as a ratio with a range of 0% to 100%. A qualitative classification of low, moderate, and high were assigned to I^2 values of 25%, 50%, and 75%, respectively. A significant Q-statistic ($P < 0.10$) indicated heterogeneity across studies. Publication bias was determined using Funnel plots, Egger's regression asymmetry tests, and sensitivity analysis. Data were analyzed by using Comprehensive Meta-Analysis version 2 program.

2.3. Literature Review. An updated systematic literature review was done following the PRISMA guidelines [23] for the prevalence of autoantibodies in RA, SLE, AIH, T1D, SS, and MS (Figure 1). Publications were identified through a systematic search done in Pubmed. The inclusion criteria were the following: (1) studies in humans, (2) restricted by title, (3) articles published in the last 20 years, (4) the sample size must be higher than 100 patients for SLE and RA studies and higher than 50 patients for SS, AIH, T1D, and MS studies, and (5) enough data available to calculate the prevalence of the antibodies in each AD. All of the search strategies included MeSH terms: "diabetes mellitus, type 1", "lupus erythematosus, systemic", "arthritis, rheumatoid", "Sjögren's syndrome", "hepatitis autoimmune", and "multiple sclerosis". In addition, key words for searching 20 antibodies were used including ANAs: antinuclear antibodies (ANAs), antidouble stranded DNA antibodies (Anti-dsDNA), antiribonucleoprotein antibodies (Anti-RNP), anti-Smith antibodies (Anti-Sm), Anti-Ro, Anti-La, lupic anticoagulant (LAC), IgG anticardiolipins, IgM anticardiolipins, anti-beta-2-glicoprotein I (Anti-β2GPI), rheumatoid factor (RF), anti-cyclic citrullinated peptide (Anti-CCP) antibodies, antiglutamic acid decarboxylase (Anti-GAD) antibodies, anti-islet cell antibodies (ICAs), anti-insulin antibodies (IAAs), antimitochondrial antibodies (AMAs), antismooth muscle antibodies (ASMAs), antithyroglobulin (Anti-TG) antibodies, and antithyroid peroxidase (Anti-TPO) antibodies. The complete search is described in detail in Table 1 in Supplementary Material available online at doi:10.1155/2012/569728.

3. Results

3.1. Meta-Analysis for Association between HLA-II Alleles and ADs. A total of five meta-analysis of HLA class II

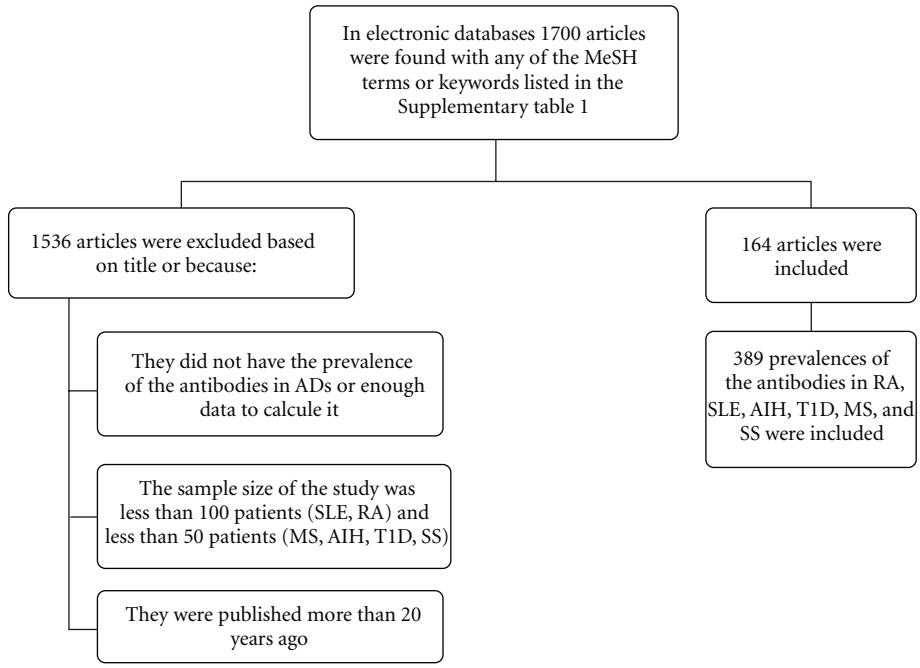

Figure 1: Flow chart of the systematic literature review.

polymorphisms in LA patients with ADs (RA, SLE, AIH, MS, and T1D) and the unique report for SS in LA were evaluated (Figure 2).

A total of 3727 cases and 8465 controls were analyzed, and different types of association between alleles and ADs were found (Table 1). These included three risk alleles for two or more ADs, four opposite associations (the same allele is a risk factor for one AD, but a protective factor for other AD), thirteen risk alleles for a particular AD, and eight protective alleles that are disease-specific. The associations were grouped through network in Figure 3.

There are two risk alleles associated with three ADs. The first is DRB1*03:01 that was found to be a risk for SLE, SS, and T1D while the second is DRB1*04:05 that was associated with AIH, T1D, and RA. Similarly, there is one risk allele associated with two ADs. It is DRB1*04:01 which was found to impart risk for RA and T1D.

Interestingly, two opposite associations were found between MS and T1D. DQB1*06:02 and DRB1*15 alleles were risk factors for MS but protective factors for T1D. Likewise, an opposite association was found between AIH and T1D in that DQB1*06:03 was a risk factor for AIH but protective factor for T1D.

In addition, thirteen risk disease-specific alleles were found. Those are seven for T1D, three for MS, two for RA, and one for AIH while, conversely, eight protective alleles for a particular AD were reported. Those are five for T1D, two for AIH, and one for SLE (Table 1).

3.2. Study Quality. Significant heterogeneity was not seen for the DRB1*04:01 allele ($I^2 = 0\%$; $Q = 0$; $P = 0.98$). Moderate heterogeneity for the DRB1*04:05 allele was observed ($I^2 = 57\%$; $Q = 4.65$; $P = 0.098$). High heterogeneity was found by meta-analysis for the DRB1*03:01 allele ($I^2 = 87.93\%$; $Q = 16.57$; $P < 0.001$). There was no evidence of publication bias in the current meta-analysis according to the Funnel plot and Egger's regression test (data not shown).

3.3. Sharing of Autoantibodies in ADs. Findings are summarized in Supplementary Table 2. Presence of ANAs was found in all of the analyzed ADs. These autoantibodies, as expected, were more prevalent in SLE (even over 75%) than other ADs. However, prevalence of ANAs over 60% was observed in SS, RA, and AIH. Anti-dsDNA, Anti-RNP and Anti-Sm antibodies were observed in SLE, RA, and SS. Anti-Ro and Anti-La antibodies were presented mainly in SS over 50%. Also, these two antibodies were presented in SLE, RA and AIH. In our revision, LAC was only present in SLE patients, but not in other ADs. IgG anticardiolipins were found in all ADs with different prevalences, SLE being the most frequent one. Otherwise, IgM anticardiolipins were presented in all ADs, but they were less prevalent than IgG subtype. Anti-β2GPI antibodies (IgG and IgM subtypes) were observed mainly in SLE, but they were present in all diseases, except in SS. RF was present in other ADs different to RA, such as SLE, SS, MS, and AIH. Likewise Anti-CCP antibodies were found in all ADs except in MS, although the prevalence was lower than 28%. Shared autoantibodies in ADs also were Anti-TPO and Anti-TG (present in all ADs except in AIH). Conversely, Anti-GAD, ICA, and IAA were observed only in T1D and AIH.

The prevalence of autoantibodies varied widely due to laboratory techniques, population, type of study, and activity of AD.

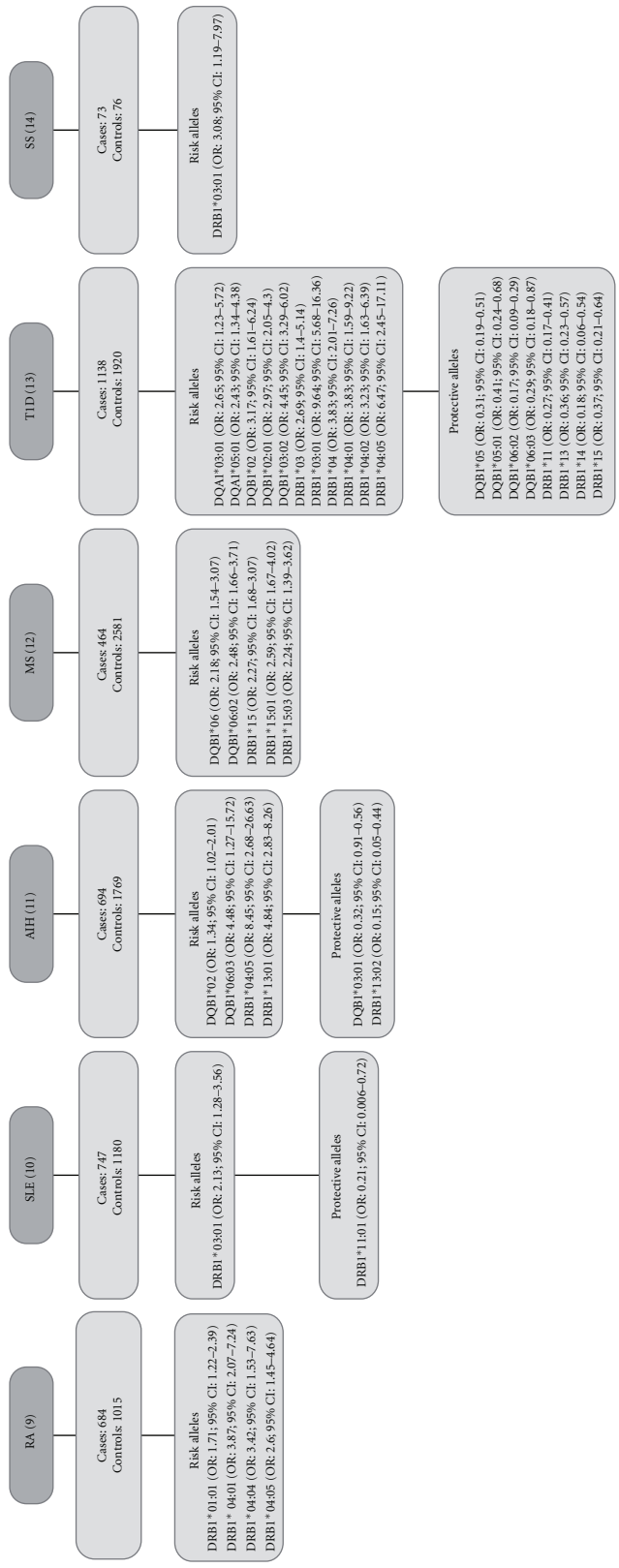

Figure 2: Previous results obtained from five meta-analyses and one original article.

Table 1: Associations between HLA class II and six ADs: SLE, RA, T1D, AIH, SS, and MS.

Association	Allele	AD	OR	Lower limit	Upper limit	P value[a]
Risk (for only one AD)	DQA1*03:01	T1D	2.65	1.23	5.72	0.013
	DQA1*05:01	T1D	2.43	1.34	4.38	0.003
	DQB1*02:01	T1D	2.97	2.05	4.30	<0.001
	DQB1*03:02	T1D	4.45	3.29	6.02	<0.001
	DRB1*03	T1D	2.69	1.41	5.15	0.003
	DRB1*04	T1D	3.83	2.02	7.27	<0.001
	DRB1*04:02	T1D	3.23	1.63	6.39	0.001
	DQB1*06	MS	2.18	1.55	3.08	<0.001
	DRB1*15:01	MS	2.59	1.68	4.02	<0.001
	DRB1*15:03	MS	2.24	1.39	3.62	0.001
	DRB1*01:01	RA	1.71	1.23	2.39	0.002
	DRB1*04:04	RA	3.42	1.54	7.63	0.003
	DRB1*13:01	AIH	4.84	2.83	8.26	<0.001
Risk (for more than one AD)	DRB1*04:01	T1D and RA	3.86	2.32	6.42	<0.001
	DRB1*03:01	SLE, SS and T1D	3.56	1.42	11.54	0.009
	DRB1*04:05	AIH, T1D and RA	4.64	2.14	10.05	<0.001
Protection (for only one AD)	DQB1*05	T1D	0.31	0.19	0.51	<0.001
	DQB1*05:01	T1D	0.41	0.24	0.68	<0.001
	DRB1*11	T1D	0.27	0.17	0.42	<0.001
	DRB1*13	T1D	0.37	0.24	0.58	<0.001
	DRB1*14	T1D	0.18	0.06	0.55	0.002
	DQB1*03:01	AIH	0.33	0.19	0.56	<0.001
	DRB1*13:02	AIH	0.16	0.05	0.45	0.001
	DRB1∗11:01	SLE	0.21	0.006	0.72	<0.001
Opposite associations	DQB1*06:02	MS risk	2.49	1.67	3.71	<0.001
		T1D protection	0.17	0.09	0.29	<0.001
	DQB1*06:03	AIH risk	4.48	1.28	15.73	<0.001
		T1D protection	0.29	0.18	0.87	<0.001
	DRB1*15	MS risk	2.28	1.69	3.07	<0.001
		T1D protection	0.38	0.22	0.65	<0.001

[a] $\alpha = 0.05$.

Each OR and its CI show the effect size and precision for individual studies and for the combined effect calculated by the random model.

AD: autoimmune disease; RA: rheumatoid arthritis; SLE: systemic lupus erythematosus; AIH: autoimmune hepatitis; T1D: type 1 diabetes; SS: Sjögren's syndrome; MS: multiple sclerosis; OR: odds ratio.

4. Discussion

In this meta-analysis, the genetic commonality in ADs was analyzed by examining the contributions from HLA-II alleles which confer associated risk or protection to six ADs: RA, SLE, AIH, MS, SS, and T1D in the LA population [9–14]. Two types of genetic risk factors were found: those common to many diseases and those specific to a given disorder. In addition, opposite associations between two different ADs and the same allele were found.

The LA population is a mixed group with ancestries that include blacks, Caucasians, and Amerindians, which reflects a notable racial, genetic, and cultural diversity [8]. However, our results showed that the effect of HLA-class II alleles on ADs in LA is similar to the reported effect on other populations regardless of latitudinal gradient and admixture. For instance, DRB1*03:01, DRB1*04:05, DRB1*04:01, and DQB1*02:01 risk alleles for T1D in LA also confer susceptibility in Caucasians and Asians [13]. DRB1*03:01 allele, which has been described in the Colombian population to be a risk factor for SS, was also associated with the disease at the worldwide level [54]. Furthermore, some non-HLA genes that influence the risk of developing ADs in Caucasians also have the same effect in Latin Americans (i.e., *C8orf13-BLK* and *CD226* genes) [55]. In contrast, some non-HLA genes influencing the developing ADs in a particular population are not replicated in another one (i.e., *PADI4* and *SLC22A4* genes) [56].

Several studies have indicated that the major histocompatibility complex (MHC) is one of the central loci

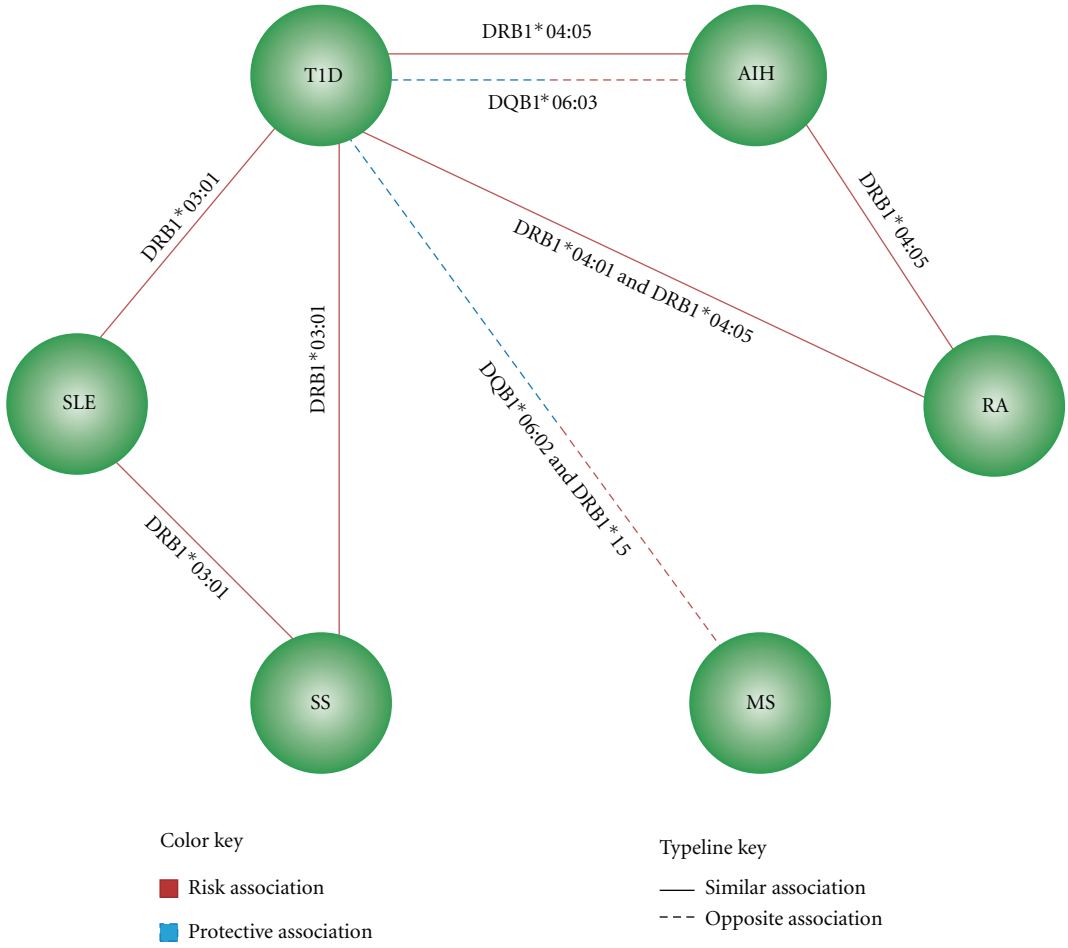

Figure 3: The complex interplay of HLA in six autoimmune diseases in Latin Americans.

contributing to the development of ADs [25, 57]. Our results show that three alleles identified in previous analyses [9–14] of a particular disease were found to influence the risk of at least two diseases. The DRB1*03:01 allele was found to be a risk for SLE, SS, and T1D while DRB1*04:05 allele was associated with AIH, T1D, and RA. In addition, DRB1*04:01 allele confers susceptibility to T1D and RA. Analyses of other polymorphic genes related to autoimmune response and inflammation have been carried out. Results indicated that *PTPN22* 1858T/C [25] and *TNF-α*-308G/A [31, 58, 59] alleles are associated with SLE, SS, and T1D. Likewise, the *CTLA4* gene has been reported as a risk factor for AIH, T1D, and RA [37, 60, 61]. Other non-HLA genes that impart risk to develop two or more ADs in LA population have been also identified. For instance, *ITGAM* and its variant (rs1143679, Arg77His) are associated with SLE and systemic sclerosis (SSc) [62]. Another example is the association of rs6822844 in the IL2-IL21 region with SLE, T1D, and SS in non-European populations [24].

Our results demonstrated that there are both common susceptibility and protective alleles for ADs and single alleles involved in the development of ADs (Table 1). The DRB1*04:04 allele, which specifically influences susceptibility to acquire RA, was identified. It has a conserved motif (L-LE-[Q/R]-[R/K]-R-A-A) comprising residues 67–74 in the third hypervariable region of the DRβ1 chain, known as the shared epitope (SE). These residues constitute an α-helical domain which forms one side of the antigen binding site, a site likely to affect antigen presentation [63]. Thus, the SE might selectively bind an arthritogenic peptide which could favor an autoimmune response. LA individuals carrying SE alleles have 3.5-fold higher risk of developing RA than noncarriers [9].

Although we identified common HLA class II alleles that contribute to susceptibility to different ADs, there is evidence indicating that two clinically distinct ADs with different susceptibility HLA-II alleles share other common genetics variants. Using a very large sample set, Zhernakova et al. compared the genetic basis of RA and celiac disease (CD). They found 14 loci that contribute to the risk of both diseases including *CD247*, *UBE2L3*, *DDX6*, *UBASH3A*, *SH2B3*, *8q24.2*, *STAT4*, and *TRAF1-C5*. However, it is known that RA and CD have different HLA risk alleles (HLA-DQ*A1 and DQ*B1 alleles in CD and HLA-DRB1

Table 2: Relationship between genetic and clinical features with HLA-ADs associations.

Associations	Genetic associations (ref)	Clinical association (ref)
SLE, SS, T1D	Shared risk genes (i) *IL2–IL21* (rs6822844) [24] (ii) *PTPN22* (1858T/C) [25, 26] (iii) 8.1 Ancestral Haplotype [30] (iv) *TNF-α* (-308G/A) [31–33]	Common clinical characteristics (i) Human endogenous retroviruses (HERVs) are associated with multiple ADs including SLE, SS, and T1D [27] (ii) Coexistence of SLE and SS has been reported [28, 29]
AIH, RA, T1D	Shared risk genes (i) DRB1*04:05 [34–36] (ii) *CTLA4* [37–39]	(iii) Hepatitis C virus has been related to ADs such as RA, AIH, T1D, SLE, SS, and others [40] (iv) AIH was found in 0% to 1.7% of patients with SS. However, the prevalence of abnormal liver function test in SS patients is close to 47% [41] (v) High prevalence of ADs in siblings of probands affected by AITD, MS, RA, T1D, SLE, and others ADs [42]
MS, T1D	Shared risk genes (i) *CD226* (rs763361), *CLEC16A* (rs12708716), *SH2B3* (rs3184504) [43, 44] (ii) *ZSCAN23* (rs11752919) [45] (iii) *KIF5A* (rs1678542), *SH2B3* (rs3184504), *CD226* (rs763361) [46] Shared protective genes: (i) HLA-DRB1*01, HLA-DRB1*10, HLA-DRB1*11, and HLA-DRB1*14 [43] Opposite gene associations: (i) Risk for T1D but protection for MS [45]: *TAP2* (rs10484565), *VARS2* (rs1264303), *CDSN* (rs1265048), *NOTCH4* (rs2071286), *BTNL2* (rs2076530), *TRIM40* (RS757262) (ii) Risk for MS but protection for T1D [45, 49]: *CDSN* (rs3130981), *HLA-DMB* (rs151719) *IL2RA* (rs35285258), *IL2RA* (rs7090530)	Common clinical characteristics (i) A latitudinal gradient characterizes both diseases. MS and T1D each become increasingly common as distance from the Equator increases [43] (ii) Protective effect of vitamin D levels [43] (iii) Association to Epstein-Barr virus infection [43] (iv) Both MS and T1D are characterized by T cell-mediated autoimmunity. The targets of T cells are pancreatic islet and central nervous system antigens in both diseases [43] (v) Familial aggregation [47, 48]
AIH, T1D	Shared protective alleles (i) DQB1*03:01 [11, 50] Controversial genetic and clinical characteristics: (ii) In children with AIH, the frequency of high-risk HLA DQB1*03:02 or DQB1*02 alleles was low and similar to control frequencies, indicating low risk for DM despite the presence of DM-related autoimmunity markers [51]	Controversial characteristics (i) One case report with Grave's disease, AIH and T1D [52] (ii) One cohort of 278 patients with AIH presented only two cases of T1D [53] (iii) One study reported that the prevalence of ICA and IAA antibodies in children with AIH was 60.7 and 18.5% respectively. However, only one patient developed T1D [51]

"SE" alleles in RA). According to the authors' hypothesis, the HLA-II molecules in these two ADs confer risk by preferentially presenting disease-specific antigens (gluten in CD, most likely citrullinated antigens in RA) to autoreactive T cells. Therefore, the disease specificity is determined in large part by the inheritance of specific HLA alleles and exposure to disease-specific antigens. The specific genes could be influencing downstream signaling events common to both diseases that may lead to altered T-cell activation and differentiation [64].

With regard to opposite associations DQB1*06:02 and DRB1*15 alleles were found to be risk factors for MS but protective factors for T1D. Our results are similar to those from other studies reporting that other MHC genes such as *CDSN* and *HLA-DMB* (rs3130981-A and rs151719-G, resp.) are risk factors for MS, but protective ones for T1D [45]. However, there is also evidence of the inverse relation. For instance, *TAP2* (rs10484565-T), *VARS2* (rs1264303-G), *NOTCH4* (rs2071286-A), *BTNL2* (rs2076530-G), and *TRIM40* (rs757262-T) were found to be risk factors for T1D but protective factors for MS [45]. Despite the presence of these genetically opposite associations, it is important to mention that clinical evidence supporting the coexistence of MS and T1D has been reported [65, 66]. Thus, these pleiotropic effects can be explained by the combined action of different alleles of several genes and environmental factors that change the biological context of the SNPs in different individuals and populations (Table 2).

Shared autoantibodies in ADs are described also. ANAs were presented in multiple ADs such as SLE, SS, RA, T1D,

AIH, and MS. These autoantibodies are not specific for one AD. Furthermore, no autoantibody that was exclusive to a single AD was found. The theory that ADs have a common origin and similar pathogenic mechanisms receives support from these findings (Supplementary Table 2). These serological results reinforce the genetic findings of the present meta-analysis. In addition, there are pathophysiological mechanisms and clinical features supporting our findings (Table 2). There is evidence that an AD can be induced or triggered by infectious agents (i.e., viruses or bacteria) via different mechanisms, such as an alteration of expression of some genes involved in immune regulation, the induction of foreign proteins that could trigger the production of autoantibodies in B cells, and molecular mimicry [27]. Several epidemiologic studies have demonstrated that human endogenous retroviruses (HERVs), hepatitis C virus (HCV) [40], and Epstein-Barr virus (EBV) [43] are associated with different ADs (Table 2). Furthermore, elevated prevalence of HCV has been reported in ADs and suggests that it plays a pathogenic role triggering the production of ANAs, RF, anticardiolipin, and Anti-TG antibodies [40].

Another consideration concerning genetic findings is the familial aggregation. Relatives of patients with ADs have a higher risk for developing the same or other ADs than general population. These findings have been reported in AITD, RA, MS, SLE, and T1D [42, 47, 48].

Regarding the opposite association between AIH and T1D, there is one study with more than 250 AIH patients in which only two cases of T1D were presented [53]. Also, there is a report of one patient with AIH, T1D, and Grave´s disease (i.e., multiple autoimmune syndrome) [52]. Finally, one study in which children with AIH were evaluated for T1D-related autoantibodies and susceptibility alleles has been reported. da Silva et al. found a high prevalence of autoantibodies but despite these findings, the prevalence of risk alleles for T1D was similar to controls and only one patient developed T1D after 3 years [51].

In summary, our results validate the common origin of ADs paradigm. The finding of significant risk and protective alleles in LA and the fact that they are shared with other populations around the world highlights the primary role of some HLA regions in the genetic susceptibility to ADs regardless of latitudinal gradient and ethnicity.

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Review Article

Lupus Nephritis: An Overview of Recent Findings

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Lupus nephritis (LN) is one of the most serious complications of systemic lupus erythematosus (SLE) since it is the major predictor of poor prognosis. In susceptible individuals suffering of SLE, *in situ* formation and deposit of immune complexes (ICs) from apoptotic bodies occur in the kidneys as a result of an amplified epitope immunological response. IC glomerular deposits generate release of proinflammatory cytokines and cell adhesion molecules causing inflammation. This leads to monocytes and polymorphonuclear cells chemotaxis. Subsequent release of proteases generates endothelial injury and mesangial proliferation. Presence of ICs promotes adaptive immune response and causes dendritic cells to release type I interferon. This induces maturation and activation of infiltrating T cells, and amplification of Th2, Th1 and Th17 lymphocytes. Each of them, amplify B cells and activates macrophages to release more proinflammatory molecules, generating effector cells that cannot be modulated promoting kidney epithelial proliferation and fibrosis. Herein immunopathological findings of LN are reviewed.

1. Introduction

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease in which diverse immunological events can lead to a similar clinical picture, characterized by a wide range of clinical manifestations and target organs (phenotypes) with unpredictable flares and remissions that eventually lead to permanent injury. Sociodemographic factors such as sex, race, and ethnicity play an important role in the incidence of the disease, frequency of its manifestations, and therapeutic response.

The overall prevalence and incidence of SLE ranges from 1.4 to 21.9% and from 7.4 to 159.4 cases per 100,000 people, respectively [1]. SLE can affect several organs and systems, including the joints, skin, brain, heart, lungs, blood vessels, and kidneys.

Lupus nephritis (LN) is one of the most serious SLE complications since it is the major predictor of poor prognosis. The incidence and prevalence of LN varies depending on the studied population. The LN cumulative incidence is higher in people of Asian (55%), African (51%), and Hispanic (43%) ancestry compared with Caucasians (14%) (1). Up to 25% of these patients still develop end-stage renal disease (ESRD) 10 years after onset of renal compromise [2]. In terms of outcome, the 5- and 10-year renal survival rates of LN in the 1990s ranged between 83–93% and 74–84%, respectively [2]. In addition, LN develops early in the course of SLE thus becoming a major predictor of poor prognosis [3]. However, in about 5% of the cases, LN may appear several years after the onset of SLE (i.e., delayed LN) [4]. The group with delayed LN is positively associated with Sjögren syndrome (SS), lung involvement, and antiphospholipid syndrome as compared with early LN (i.e., those SLE patients who develop LN during the first 5 years of the disease) [4].

LN has been looked upon as a classic example of immune complex-induced microvascular injury which results from circulating double-stranded DNA polynucleotide antigens/anti-DNA antibody complexes and other mechanisms including *in situ* reactivity for free antibodies with fixed antigens and the presence of sensitized T cells which are an important part of the picture [5]. Early deposits of immune complexes (ICs) include nucleosomes, DNA-extractable nuclear antigen antibodies (ENAS), and antibodies against C1q complex of the complement system as byproducts of

inefficient phagocytosis of apoptotic bodies. This results in an autoimmune response through epitope expansion. These ICs have predominance over immunoglobulin G (IgG) 2 and 3. Deposits of ICs are initially located at the glomerular mesangium and interstitial tissue within the proximal tubular epithelial cells (PTECs) [5]. These deposited ICs initiate the release of proinflammatory cytokines and chemokines such as monocyte chemoattractant protein-1 (MCP-1) and cell adhesion molecules (CAMs) thus establishing a chronic inflammatory process. The resulting overload of the mesangial phagocytic system leads to deposits of subendothelial ICs becoming an easy target for monocyte migration and infiltration [5]. This migration and infiltration is due to a general response of the innate immune system that releases inflammatory proteases thus causing endothelial injury and proliferation. In turn, the innate immune system response promotes the activation of adaptive immune system secondary to the presence of ICs and dendritic cells (DCs), which subsequently trigger release of type 1 interferon and induce maturation and activation of infiltrating T cells. This activation leads to sequential amplification of T helper 2 lymphocytes, (Th2) T helper 1 (Th1), and T helper 17 (Th17). Each of these amplifies lymphocyte B cell response, and activates macrophages. This generates a second general response, which increases recruitment of effector cells that can no longer be modulated by regulatory T cells and, in the end, results in epithelial glomerular proliferation and fibrosis [5] (Figure 1).

2. Factors Influencing LN: Role of Ethnicity

So far, it has been difficult to predict the course of LN. Renal compromise in SLE has been markedly heterogeneous in terms of clinical presentation and course. One of the most important factors influencing LN is ethnicity. Prevalence in populations varies depending on ethnicity. In a recent case control study, Sisó et al. found an overall prevalence of 31% of LN in a large cohort of white Spanish biopsy-proven patients. One third of these patients developed end-stage renal disease (ESRD) [6]. Most studies have reported rates of up to 31% ESRD in Africans and 18% in Hispanics compared to 10% ESRD in Caucasians [7]. However, more than a decade ago, Molina et al. described African and Latin American patients with LN in a study with cohorts of 222 and 300 patients, respectively, which showed a higher prevalence of LN 46% for both populations [8].

SLE patients from 9 different Latin American countries were evaluated in the GLADEL Multinational Latin American Prospective Inception Cohort of 1,214 Patients in 2004. Amongst the statistical significant results; Afro Latin Americans (ALA) mestizos had more severe disease than did whites, as evidenced by a higher frequency of renal disease, pericarditis, polyadenopathy, and discoid lesions in ALA. In addition, both ALA and mestizos had higher maximum disease activity indices than whites, but this was lost when controlled by country. However, damage scores tended to be lower in ALA than in both mestizos and whites, a surprising finding that might be explained by shorter disease duration or by the more recent incorporation of Brazilian and Cuban groups into the study. A peculiar observation was that of a significantly lower frequency of both xerophthalmia and sicca syndrome [8].

3. Murine Models

3.1. Spontaneous Murine Models. There has been a renewed interest in the use of animal models in the study of IC mediated LN, which has focused on immune and inflammatory mechanisms involved in the disease process. The majority of the murine models have been created to mimic LN [9]. This research has led to a better understanding of the disease by learning about the role of new cells and molecules that have been involved in the pathogenesis of LN. There are many known lupus murine models, which include spontaneous mice with inherited susceptibility, transgenic, and deletion knockout mouse models [9].

Specifically, three spontaneous lupus (inherited susceptibility) mouse models have been extensively studied: New Zealand Black (NZB), New Zealand White F1 mice (NZWF1), inbred strains of mice (BXSB), and mice homozygous for the apoptosis-defective Faslpr mutation (MRL-Faslpr). These models share some similarities with human SLE including the presence of antinuclear antibodies (ANAs), ICs, activation of T and B cells, and kidney disease. Nevertheless, there are sharp differences in the genetic origin and target organ involvement in murine models. The MRL mice are the result of a mutation of Fas with diminished apoptosis in lymphocytes, which generates hyper proliferation and secondary organomegaly [9, 10].

3.2. Transgenic Mice Models. Transgenic as well as deficient (knockout) models have clarified the function of many molecules as well as their potential role in autoimmunity. This, however, does not necessarily mean that these genes are relevant to human SLE. For instance, deletion of the Fc receptor in immunoglobulins (FcR) in NZB mice prevents injury despite the deposit of ICs [11, 12]. The above result is consistent with the fact that anti-DNA antibodies can modulate gene expression in mesangial cells through Fc-gamma-receptor- (FcγR-) dependent and independent pathways, which can induce proliferation, extracellular matrix synthesis, and production of proinflammatory cytokines [13, 14].

Transgenic models with deleted genes (knockout models) have altered tolerance to B cells or T cells. These gene deletions include FcγR, Bim, CD22, Lyn, (src-tyrosine kinase involved in B-cell activation) CD72, and co-stimulatory receptor (PD-1). In the MRL model, the removal of interactions of the programmed death 1/programmed death ligand 1 (PD-1/PD-L1) pathway provided a negative regulatory checkpoint in mediating tolerance and autoimmune disease. PD-L1 caused early death by autoimmune myocarditis and pneumonitis [15]. In addition, Lyn gene deletion in transgenic models affects the ability of B cell receptors (BCR) to edit. A T cell role has been demonstrated to be implicated in LN through the deletion of CD4+ T cells in transgenic models. The CD28 molecule, in turn, appears to be essential to initiate the activation of lymphocyte CD4 + T cells and also to induce costimulatory proteins (ICOS), which are

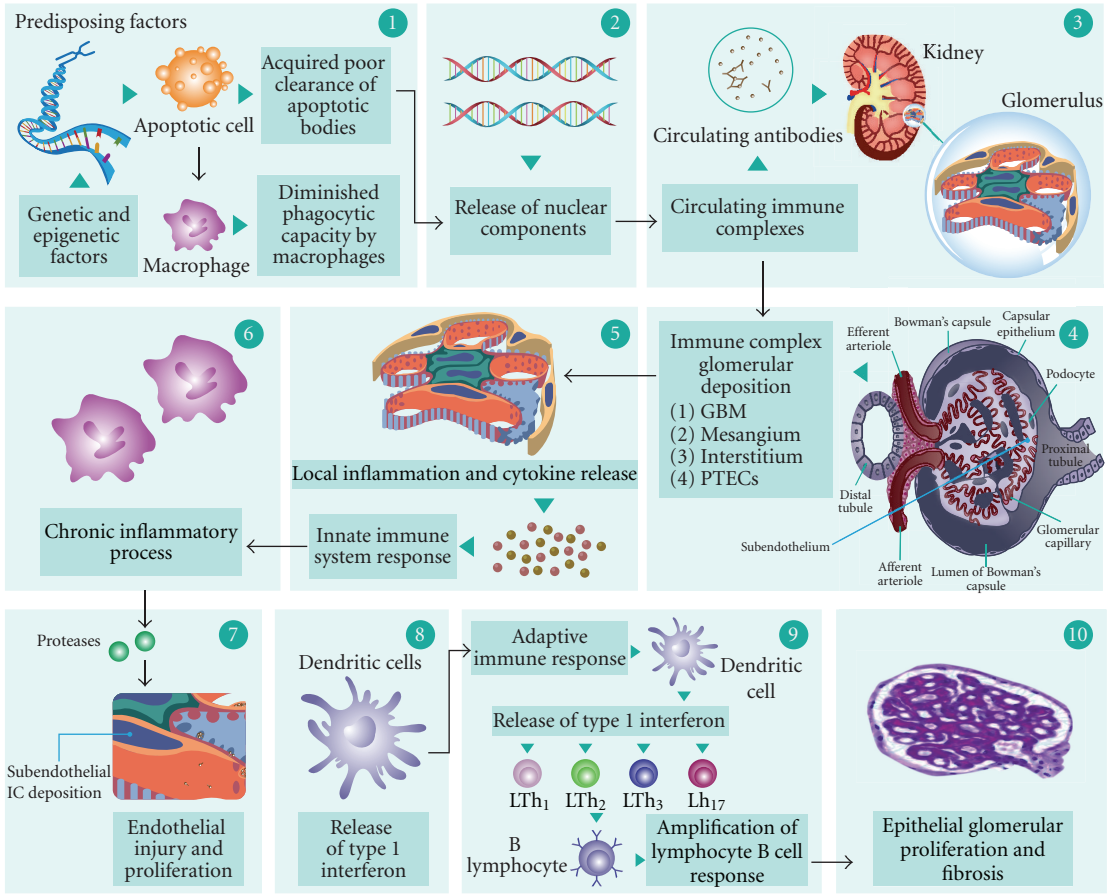

Figure 1: Lupus nephritis: an imbalance between cytokine homeostasis and immune complex deposition. In predisposing susceptible individuals who develop systemic lupus erythematosus (SLE), there is an acquired poor clearance of apoptotic bodies and a diminished phagocytic capacity by macrophages (1). Early formation of immune complexes (ICs) include antinucleosomes, anti-double-stranded DNA (anti-dsDNA), DNA extractable nuclear antigen antibodies (ENAS), antibodies against C1q complex of the complement system, free DNA, antiribonucleoproteins (anti-RNP), and histones as byproducts of inefficient phagocytosis of apoptotic bodies (2). Circulating ICs are deposited initially at the glomerular base membrane (GBM), mesangium, and interstitial tissue within the proximal tubular epithelial cells (PTECs) (3) and (4). The deposited ICs initiate the release of proinflammatory cytokines and chemokines such as monocyte chemoattractant protein 1 (MCP-1), interleukins 1 and 6 (IL-1, IL-6) and adhesion molecules (CAMs) thus establishing a chronic inflammatory process (5). The resulting overload of the mesangial phagocytic system (innate immune system) leads to deposits of subendothelial ICs becoming an easy target for monocyte migration and infiltration and generating endothelial injury and proliferation (6) and (7). In turn, the adaptive immune system is activated secondary to the presence of ICs and dendritic cells (DCs) (8), which subsequently trigger release of type 1 interferon and induce maturation and activation of infiltrating T cells. This activation leads to sequential amplification of T helper 2 lymphocytes (Th2), T helper 1 (Th1), and T helper 17 (Th17) (9). Each of these amplifies lymphocyte B cell response and further activates macrophages, generating a second general response, which increases recruitment of effector cells that can no longer be modulated by regulatory T cells and resulting in the end in epithelial glomerular proliferation and fibrosis (10).

more important in the activation of previously differentiated effector T cells. An induced deficiency of ICOS reduces autoantibody titers of IgG and *in situ* survival of T cells but does not affect the condition [16].

Natural inhibitors of the CD28/B7 pathway include the cytotoxic T-lymphocyte antigen 4 (CTLA-4) receptor in T cells and PD-1. Both of these recruit inhibitor protein tyrosine phosphatase (SHP-2). PD-1 chronically inhibits activated T cells and makes them respond in peripheral tissues but not in lymphoid organs. This is essential in maintaining T cell tolerance. The fine control between T regulator cells and PD-1 pathway may depend on the completion of an uncontrolled reactive autoimmune response [17]. The PD-1 pathway has the ability to simultaneously remove self-reactive T cells and promote the development of LT regulator cells.

4. Genetic Susceptibility of SLE

Patients with SLE have defects in all branches of the immune system including innate immunity, antigen presentation, apoptosis, impaired tolerance in T and B cells, and defective release of regulatory cytokines and chemokines. SLE should be considered a failure of immune tolerance in one or more of the central or peripheral checkpoints with summation effects of multiple genes related to the immune response [18].

The tendency to self-reactivity is a natural phenomenon as it is estimated that 75% of recently formed B cells in the bone marrow in adults and 40% of the B cells located in germinal centers are autoreactive [19, 20]. In murine models, defects have been detected in both central and peripheral tolerance in B and T cells by introducing self-reactive receptors [21]. However, in humans a natural selection mechanism is currently believed to be the major one for reducing reactive immature B cells by as much as 75% in the bone marrow [22]. An altered edition of this mechanism has been reported in some patients with SLE. B cells that get through this defective mechanism will be subjected to control in the periphery by induced deletion, anergy, or apoptosis. Both biological processes require strong BCR signals that activate an inhibitory pathway via the CD22-tyrosine phosphatase SHP-1 thus avoiding clonal amplification through the inhibition of the interaction between B and Tfh cells [22].

For years now, human susceptibility to systemic autoimmunity has been related to several genes with polymorphisms or mutations that encode defective proteins involved in the immune system. HLA and non-HLA genes contribute to the polygenic susceptibility of the disease, and about 30 genes have been consistently replicated and confirmed to influence the predisposition of SLE. For instance, a genome-wide association study (GWAS) evaluating 317,501 single nucleotide polymorphisms (SNPs) in 720 women of European ancestry with SLE and 2,337 controls disclosed four loci associated with the disease harboring the following genes: *ITGAM*, *KIAA1542*, *PXK,* and the SNP rs10798269 in chromosome 1q25.1 [23]. In addition to the already established gene associations with SLE and other autoimmune diseases, *FCGR2A*, *PTPN22,* and *STAT4* were confirmed. These results are only an example to show that several genes, some with known immune-related functions, predispose to SLE [23].

One of the most interesting genes associated with SLE is *PTPN22*. This gene encodes for the protein tyrosine phosphatase Lyp, in which a missense mutation that changes residue 1858 from cytosine to thymidine (1858C/T) is associated with multiple autoimmune disorders including SLE, rheumatoid arthritis (RA), and type 1 diabetes (T1D) [24, 25]. The protein, encoded under normal circumstances, is involved in B cell signaling. However, with the presence of autoantibodies associated with the 1858T variant, B cell signal transduction is impaired thus contributing to autoimmunity.

A polymorphic variant of *IRF5* has been linked to SLE and high circulating levels of Type I interferon (IFN). The genetic alterations may lead to sustained overproduction of IFN $\alpha\beta$ in human SLE, which will result in increased bioavailability and activation of immature DCs that control peripheral tolerance by deleting autoreactive lymphocytes [26, 27]. IFN mature DCs activate and expand autoreactive T cells thus helping autoreactive B cells to differentiate. In addition to its indirect effect through DCs, IFN also directly allows the expansion and survival of CD4+ and CD8+ T cells as well as the differentiation of B cells into plasma cells. The increased frequency of autoreactive B cells depends on a second set of genetic alterations that target B cell tolerance checkpoints. These early events create a first level of autoimmune injury, which is clinically silent but might generate apoptotic cells and nucleic acid-containing immune complexes. The capture of these apoptotic cells by myeloid DCs and nucleic acid-containing ICs by peripheral DCs and autoreactive B cells broadens the autoimmune reaction thereby leading to disease manifestations [26, 27].

Many of the genes associated with more severe forms of SLE such as *HLA* genes have also been associated with LN. Certain alleles in the HLA-DR2 and the HLA-DQ haplotypes seem to be particularly associated with LN in specific ethnic groups [28, 29]. In addition, in a cohort of 2,366 patients with SLE and 2,931 controls with common European ancestry, a variant at exon-3 (rs1143679 A) of Integrin-α-M (*ITGAM)* was strongly associated ($P < 0.0003$) with renal criteria in these patients. Among non-HLA genes associated with LN, *ITGAM* has been consistently reported to influence this SLE manifestation [30].

In African Americans, a strong risk factor has been associated with the presence of a monocyte receptor polymorphism in FcγRII-H131 that interacts with IgG2, which reduces the hepatosplenic clearance of circulating ICs [31]. In the context of the pathogenesis of LN, this may be important because it will facilitate glomerular IC deposit (Table 1).

5. Pathogenesis and Antibodies

5.1. Glomerular Immune Complex Deposit and Anti-dsDNA. An appropriate understanding of the current model of glomerular immune complex deposit is based on several experimental models of LN that use double-stranded anti-DNA antibodies (anti-dsDNA) with different affinities and physicochemical properties and correlate them in the eluate of patients with LN.

Apparently, renal involvement begins with the glomerular ICs deposit. These ICs are predominantly antibodies against single-stranded (ss) and double-stranded (ds) DNA as well as some polyreactive reagents that include anti-Sm, anti-RNP, anti-histones, anti-Ro/SS-A, anti-La/SS-B, and anti-C1q antibodies [5]. The formation of ICs seems to be predominantly *in situ*. However, although anti-dsDNA ICs are present in LN most of the time, it has not yet been proven that these types of ICs are enough to induce LN [69].

Three mechanisms have been proposed to explain the ability of anti-dsDNA to settle in the kidney [13]. First, anti-dsDNA reactive antibodies can form ICs with DNA/nucleosome previously released from apoptotic cells. These ICs may be deposited in the kidney and initiate an inflammatory cascade. There is another postulated theory commonly known as the planted antigen theory. This theory proposes that anti-dsDNA reacts with DNA/ nucleosome trapped in the glomerular base membrane (GBM). In addition, the trapping of DNA/nucleosome has been associated with the negatively charged DNA and positively charged GBM. The third theory relates to the cross-reactivity between kidney antigens and anti-dsDNA. Nephritogenic anti-dsDNA antibodies have been shown to cross-react with alpha-actinin, laminin, and heparan sulfate (Figure 2).

The amount of deposited ICs, isotypes, and their affinity correlates with the severity of LN. The ICs located at

Table 1: Adapted from [32] and [33]. Susceptibility genes in SLE associated with LN.

Chromosome	Gene	SNPs	Population	OR[1] with LN	References
6p21	HLA region	DRB1*0301 and several other Alleles.	European, Several Asian, African American, mixed European-Amerindian, and Latin American.	2.4	[29, 34–37]
7q32	*IRF5*	5bp promoter indel, rs2004640, rs2070197, 10954213 rs10954213 rs 729302	European, several Asian, mixed European-Amerindian, African American, Latin american.	1.6	[23, 38–42]
2q32	*STAT4*	rs7574865, rs3821236, rs7582694	European, mixed European-Amerindian, several Asian, African-American	1.5	[23, 38, 40, 43, 44]
6q23	*TNFAIP3*	rs5029939 rs223096 rs223096	European, Asian, African American	2.0	[23, 38, 40, 45–48]
16p11	*ITGAM*	rs9888739, rs1143679, rs4548893	European, mixed European-Amerindian, Asian, African American, Latin Americans	1.6	[30, 38–40, 46, 49, 50]
4q24	*BANK1*	rs10516487 rs1726654 rs3733197 rs1051647 rs10516483	European, European-Amerindian, Asian, Caucasian	1.2	[38, 46, 51–53]
1p13	*PTPN22*	rs2476601	European, Latin Americans	1.4	[23, 54]
8p23	*BLK*	rs13277113, rs2736340 rs2248932	European, several Asian	1.3	[23, 38–40, 46, 55, 56]
2q37	*PDCD (CD279)*	PD1.3A	European, European-Amerindian, Chinese, Latin Americans	1.2	[57]
1q25	*TNFSF4*	Risk haplotype; rs3850641	European, Asian	1.4	[23, 38, 40, 46, 52, 58]
18q22.3	*CD226*	rs763361 rs727088	European, European-Amerindian, Asian	NA[2]	[59, 60]
1q21–23	*FCGR2A*	ARG131HIS	European, European-Amerindian, African American	2.2	[23, 39, 40]
19p13.2	TYK2	rs280519 rs2304256 rs12720270	European	1.2	[40, 42]
3p21.3	*TREX1*	rs72556554	European, Asian, Hispanic, African	25	[61, 62]
Xq28	*MECP2-IRAK1*	rs2269368 rs17435 rs3027933 rs1734791	European, Chinese, Korean, European-Amerindian (Mexican)	1.4	[40, 63–65]
3p14.3	*PXK*	rs6445975 rs2176082	European	1.2	[23, 40]

Table 1: Continued.

Chromosome	Gene	SNPs	Population	OR[1] with LN	References
2q24	*IFIH1*	rs1990760	European	NA[2]	[40]
11p15.5	*KIAA1542 (PHRF1)*	rs4963128	European	NA[2]	[23]
8p23.1	*XKR6*	rs6985109	European	NA[2]	[23]
6q21	*ATG5-PRMD1*	rs6568431, rs2245214 rs548234	European, Chinese	NA[2]	[23, 40, 66]
22q11.2	*UBE2L3*	s5754217	European, Chinese	1.2	[40, 45, 67]
5q33.3	*PTTG1*	rs2431099	European	1.2	[40, 45, 67]
6p21	*UHRF1BP1*	rs11755393	European	NA[2]	[40, 67]
5q32	*TNIP1*	rs7708392	European, Chinese, Thai, Japanese.	1.3	[38, 40, 67, 68]
7p15.2	*JAZF1*	rs849142	European	NA[2]	[40, 67]
7p21.3	*ICA1*	rs10156091	European	1.2	[23, 67]
1q24	*IL10*	rs3024505	European	NA[2]	[40, 67]
1q25.3	*NMNAT2*	rs2022013	European, Chinese	1.1	[23, 38]
11q23.3	*ETS1*	rs6590330	Chinese, Thai	NA[2]	[38, 46]
10q11.23	*WDFY4*	rs877819	Chinese, Thai	NA[2]	[38, 46]
7p12.2	*IKZF1*	rs4917014	Chinese	0.7	[38]
12q24.32	*SLC15A4*	rs10847697 rs1385374	Chinese	1.31	[38]
2p22.3	*RASGRP3*	rs13385731	Chinese	0.64	[38]

OR[1]: Approximate odds ratio.
NA[2]: Data not available.

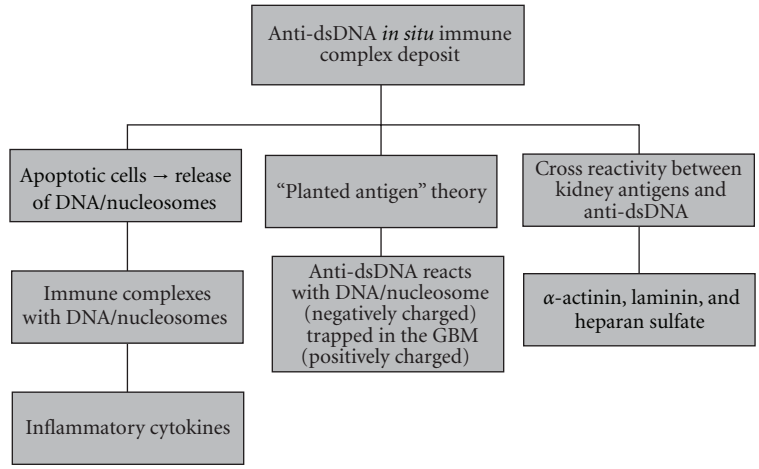

Figure 2: Proposed theories for anti-dsDNA *in situ* immune complex deposit [13]. GBM: glomerular base membrane.

the mesangium and subendothelium subsequently contribute to the recruitment of inflammatory cells. Although there is a predominant deposit of IgG and isotypes IgG2 and IgG3, there are also IgM and IgA deposits as well as C3, C4, and C1q molecules, which are part of the complement system [69].

The activation of the inflammatory cascade is achieved through Fc gamma receptors in macrophages, DCs, neutrophils, mesangial cells, and kidney cells [70]. It is also achieved by cross-reactivity with nephritogenic proteins expressed in renal parenchymal cells, PTEC, and mesangial cells thus generating the release of proinflammatory mediators and vascular adhesion molecules. Mesangial cells and PTEC are the most involved in releasing cytokines such as Interleukin-6 (IL-6), interleukin-1 (IL-1), tumoral necrosis factor (TNF), and chemokines such as MCP-1 [71]. It is worth highlighting that these nephritogenic compounds, as has already been mentioned, are related to the expression of laminin or collagen IV.

In addition, once ICs are deposited, they cannot be phagocytosed by mesangial cells and so will be deposited in

the subendothelium. This leads to the first migration and posterior infiltration of monocytic effector cells and polymorphonuclear cells (PMNs). This cell recruitment is mainly mediated by the action of proinflammatory cytokines and by the complement system thus causing tissue damage [12]. This, in turn, increases the release of more proinflammatory cytokines (IL-1, IL-6, and TNF-α) and chemokines such as MCP-1, secreted cytokine (RANTES), TNF-related weak inducer of apoptosis (TWEAK), and activation of CAMs (ICAM-1, VCAM-1), all of which enhance amplification of the innate immune response. Moreover, the dysregulation in the synthesis of cytokines could be responsible for mesangial proliferation, crescent formation, and progressive glomerulosclerosis. The cytokines involved are IL-4, IFN-γ, transforming growth factor (TGF), platelet-derived growth factor (PDGF), and MCP-1 [12].

To support the idea that Fcγ receptors are directly involved in the activation of the inflammatory cascade, LN has been attenuated in the knockout models [72].

The adaptive immune response is simultaneously promoted by the presence of ICs, which causes a reaction within the DCs, and this induces the release of type I IFN. As a result of the subsequent maturation of the DCs, antigens are presented and infiltrating T cells undergo further activation. This leads to amplification of Th2 responses, Th1, Th3, Th17 and B cells and further activates a new wave of effector cells such as monocytes and PMNs.

Based on murine models and neonatal studies in class V NL (i.e., membranous), there is also an *in situ* glomerular deposit of ICs. In this case, the antibody recognizes the receptor of phospholipase 2 expressed in podocytes. However, the target antigen in class V LN has not yet been identified. The subepithelial ICs then trigger a cascade of events that generate podocyte injury with flattening and sloughing through the activation of the complement membrane attack complex (MAC). Ultimately, this disruption is responsible for proteinuria. In contrast to the endothelium and mesangium, podocytes do not proliferate in response to injury but produce thickening of the GBM due to increased synthesis of extracellular matrix proteins [73].

Aside from anti-dsDNA being directly involved in *in situ* IC formation, high-affinity anti-DNA plays a central role in some of the manifestations of SLE, especially LN. They are relatively specific and are good markers of activity in some patients. This has been confirmed in a large cohort of 1,000 patients reported by Cervera et al. [74]. Not all anti-dsDNA antibodies are related to LN. As mentioned before, this depends mainly on their specificity, affinity, isotype and idiotype, cross-reactivity with glycosaminoglicanos, and ability to interact with nucleosomes or DNA-linked collagen. The lack of IgM anti-dsDNA secretion is associated with apparently more severe LN [75]. However, the disease can develop in the absence of anti-dsDNA [13, 76].

5.2. Role of Complement in LN. Low total complement hemolytic activity and decreased C3 and C4 levels are detected in 75% of the patients with class III and 90% of those with class IV LN. The settling of IgG isotypes, IgA, IgM, C1q, C4, C3, and C5b-9 is called a *full house, which* is almost exclusive to LN. Complement degradation products such as C3d and C5b-9 can also be detected in urine thus providing circumstantial evidence of the role of the complement system in LN. However, C3 deficiency does not reduce the risk of LN and its true role is unknown [70]. Some studies suggest a predominant mechanism via Fcγ receptors [12, 77].

5.3. Antinucleosomes. Chromatin is the complex of histone-native DNA in eukaryotic cells. It is the packaging unit of DNA and controls the expression of genetic information by regulating access to transcription factors. There has been increasing evidence that nucleosomes are the main targets of the IC deposits [78].

Apparently, antinucleosome complexes adhere to heparan sulfate and have been detected in the human glomerulus. The main source of nucleosome release is from lymphocyte apoptotic bodies. It seems that they are generated at a very early stage even before DNA is released [79].

Free DNA has very few antigenic properties. It becomes more immunogenic as DNA-protein complexes show tridimensional epitopes of chromatin. Several histone fractions are shown to be able to bind glycosaminoglican proteins. It seems likely that the immune response begins with antinucleosome and anti-DNA antibodies and is the result of epitope amplification response. When these complexes are given to murines, they cause a lupus-like syndrome (SLE-like) [80]. Histone DNA complexes have a higher affinity for glycosaminoglycans *in vitro* and serve as a histone anchor for a larger deposit of DNA. Kalaaji et al. demonstrated antichromatin deposits in human and murine lupus LN by electron microscopy [81, 82]. This chromatin appeared to originate from glomerular apoptotic cells.

5.4. Anti-C1q. In 2004, Trouw et al. demonstrated in a mouse model that antibodies against C1q of the complement system (anti-C1q) play a pathogenic role in LN in the presence of ICs [83]. Anti-C1q could participate in glomerular injury by reducing the clearance of circulating ICs.

5.5. Alterations in Apoptosis. In healthy individuals, dead cells, mainly T and B cells as well as PMNs, are rapidly removed by macrophages in a noninflammatory context. In SLE patients, poor clearance of apoptotic bodies leads to the release of self-antigens that are subsequently submitted to T cell presentation by follicular DCs and B cells in secondary lymphoid organs thus challenging peripheral self-tolerance [84].

In 1998, mice exposed to syngeneic apoptotic thymocytes intravenously induced development of ANAs, anticardiolipin, and anti-ssDNA antibodies as well as deposits of ICs in the kidney [85]. Some of the autoantibodies generated react with nuclear products as a result of degradation by granzymes present in membrane vesicles of apoptotic cells [86]. This leads to the release of DNA-histone complexes, free DNA, small RNA, SS-A SS-B, and overexpression of phospholipid molecules in the membrane. The clearance of apoptotic cells is finely regulated through the activation of multiple receptors in phagocytic cells (scavenger receptor, phosphatidylserine receptors) that detect apoptotic cells

[87]. A decreased ability on the part of macrophages to clear apoptotic bodies in a considerable number of patients has been previously described. This appears to be a defect since they have diminished phagocytic capacity at different stages of maturation [88, 89]. This defect alters the balance of peripheral tolerance and generates a first phase of autoimmune activation leading to a reaction of natural autoreactive B cells with subsequent epitope amplification [90].

A slight increase in apoptosis at the tubule-interstitial level that correlated with mononuclear infiltrates in 35 kidney biopsies of patients with LN was reported. In addition to these findings, the level of apoptosis of tubular cells had a positively significant statistical correlation with the activity index score for mononuclear cell infiltration but not with scores for other chronicity index components [91].

5.6. Dendritic Cells in LN. DCs are the most powerful antigen presenting cells (APCs) and are crucial in both innate and adaptive immune responses [92]. DCs are classified as type I or tolerogenic cells, which release interleukin 10 (IL-10), and type II or immunogenic cells, which release interleukin 12 (IL-12).

DCs are found in peripheral tissues where they capture antigens and then migrate to lymph nodes to exert their APC function on follicular helper T cells (fhT) by regulating the activation and differentiation of cell populations. They can also interact directly with B cells. DCs descend from two lines, myeloid and lymphoid. They differ in the expression of toll-like receptors (TLRs). Lymphoid DCs release cytokines such as IL-12 and IL-18. Myeloid DCs (mDCs) are the largest population and are differentiated from monocytes. Their synthesis rises in bacterial infections. Moreover, DCs can synthesize multiple cytokines and chemokines such as IL-1, IL-6, IL-8, IL-12, IL-18, granulocyte macrophage colony stimulating factor (GM-CSF), MCP-1, IL-10, and TGF. DCs are considered to be the largest producers of IL-18 and promote lymphocyte Th1 responses [92].

DCs are crucial for maintenance of immune tolerance. Circulating immature DCs capture antigens and migrate to lymph nodes, where they present self-peptides in the absence of costimulatory signals to T cells, which induce their anergy or deletion [93].

Human DCs instruct naïve CD4+ T cells to become IL-21-producing Fh T cells through the secretion of IL-12 [92]. IL-21 is a B cell growth factor required to induce differentiation and isotype switching and cooperates with IL-6 and the B lymphocyte stimulator (BlyS). In turn, IL-12 appears to induce both Th1 γ interferon production as well as IL-2 [92].

In SLE, DCs are activated by self-antigens through TLR 3, 7, 8, 9 or Fcγ receptors and are induced to release IFN-α, a crucial molecule in autoimmunity that also plays a key role in LN [94].

Several subtypes of DCs are detected in normal human kidneys, predominantly the myeloid cells. Only 25% of them are plasmacytoid DCs (BDCA2 +). In murine models of LN, DCs in GBM are increased in NZB mice. In proliferative forms of murine LN, a population increase has been shown in CD68+ myeloid/macrophage cells at the glomerular interstitium [85] as well as an increase in lymphoid DCs [95, 96]. Apparently, the extent of infiltration is higher in proliferative classes. Most of the DCs detected in LN are immature in contrast to SLE patients that show a marked reduction of mature DCs and lymphoid cells [95]. This could be the consequence of their migration to the kidneys and other tissues during the activity of the disease.

LN has been attributed to an imbalance between cytokine homeostasis and IC deposits. High synthesis of cytokines and chemokines by DCs may contribute to LN pathogenesis. Therefore, the increased migration of DCs, which has been recognized in the kidney may be due to the early release of IL-18, IL-1, and C-C chemokine receptor type 5 (CCR5) [97]. These, in turn, are central in regulating the secretion of more cytokines and chemokines and determining the prevalence of the response of Th1 and Th2 cells.

5.7. Role of T and B Cells in LN Pathogenesis

5.7.1. T Lymphocytes. T cells are divided into effectors and regulators. The former includes CD4+ Th1, Th2, Th3, cytotoxic CD8+, and Th17. Regulator T cells include (FOXP3+ CD25+) T cells and natural killer T cells (NKs) [97]. Together, these cells participate in initiation, amplification, and regulation of the immune response in LN as well as migration, destruction, fibrosis, resolution, and exacerbations of the disease [97]. Therefore, they have become one of the targets for therapeutic intervention [97].

Central defects detected in SLE patients not only include the substitution or replacement of the T cell receptor (TCR)/CD3δ by TCR/Fcγ [97] but also display constitutive changes in the grouping of lipid vesicles that carry transcription factors with the consequence of early aberrant TCR signaling [98] and decreased threshold of activation. The molecular mechanisms of the above mentioned are not clear but appear to be both transcriptional and posttranscriptional modifications. In SLE, the entry of calcium activates calcineurin, which, in turn, activates the nuclear factor of activated T cells (NFAT) thus increasing the expression of CD40L and stimulating B cell activation and the synthesis of immunoglobulins [98].

In LN, activated CD4 and some CD8 T cells as well as activated macrophages and DCs infiltrate the renal interstitium, thus worsening renal function [99]. The restricted use of Vβ chains on recruited T cells suggests their oligoclonality and are potentially specific antigens or autoreactive [100]. Crispín et al. reported infiltrates of double negative (DN) T cells and Th17, which were presumably derived from a population of blood-producing IL-17 DN T cells [101]. T memory CD45RO+ expressing cells were also detected in the urine of patients with LN [102].

It is worth mentioning that DN T cells constitute a small population (less than 5%) in healthy subjects and are significantly increased in patients with SLE [101]. Having a mixed profile of Th1, Th2, and Th17, they synthesize IL-4, IL-17, IL-1, and IFN-γ. DN T cells are also found in patients with LN. However, why this cellular differentiation happens is not yet clear.

In LN, T cells are able to interact with epitopes like nucleosome histone complexes. T cells also help autoreactive nephritogenic B cells, modulate the differentiation of T cell subpopulations, recruit macrophages and NK cells, and induce renal cell damage through the release of cytokines or direct cytotoxicity [103]. T cells also activate proximal tubular cells and promote parenchymal fibrosis [103].

CD40 and CD40L interaction between B and T cells induces clonal expansion, which makes differentiation into plasma cells (isotype switching) possible. Interestingly, the use of CD40L monoclonal antibodies (mAb) in NZB mice has been shown to delay the onset of the disease, reduce the number of B cells, suppress the isotype switching, and decrease the titles of anti-DNA antibodies [104].

At first, clarifying the role of Th1 and Th2 cells in murine LN was given great importance but their biological or genetic modulation showed some inconsistent results [105]. Both populations appear to contribute to a greater or lesser extent since giving both IL10 and IFN-γ to the NZ and WNZ (BWF1) hybrid mice accelerated nephritis, and antagonism of the two delays the disease as the antagonism of IL-4 or IFN-γ does in MRL mice [105]. However, there is evidence of a mayor predominance of the role played by Th1 cells in the pathogenesis of LN since the lymphocytic infiltration is abolished in the knockout model [106, 107]. The IFN-γ, in contrast, facilitates the interaction between T cells and parenchymal cells, especially PTEC, and increases the expression of HLA class II and accessory molecules [16].

Some of these contradictory results seem to depend on the confusing effect generated by the action of Th17 cell products, which are, in turn, promoted by the action of IL-6, IL-23, and TGF-β [108].

When IL-18 is administered to mice, LN is accelerated and the accumulation of DN T cells is fostered. As a result, IFN-γ is synthesized and DN T cells are differentiated into CD4+ cells and CD8+ cells. IL-18 antagonism reduces lymphoproliferation, production of IFN-γ, and progression of LN thus also implying a role for Th1 cells [109]. In addition, serum levels of IL-18 nearly double in patients with LN [110].

Microarray analysis suggests that production of nephritogenic autoantibodies in murine models depends on Th1 cells [111]. IFN-γ promotes the switch from IgG2 to IgG3, which is typical of LN unlike a predominance of IgG1 in the skin in SLE [112]. Therefore, IFN-γ seems to be crucial in modulating the activity of LN in murine models and promoting the synthesis of IgG2 in MRL-Faslpr and NZB mice [111].

Likewise, the expression of genes in infiltrating kidney T cells strongly suggests the presence of dominant Th1 though there is also some expression of Th2 GATA-3 cells (transcription factor) [113]. Chan et al. reported T bet (Th1 transcription factor) overexpression, IFN-γ, IL-2, IL-12, IL-18, MCP-1, and IL-10, which had a significant correlation with the histological activity index of LN [113]. Therefore, measurement of pro-Th1 in urine can be a promising biomarker for LN activity. This pro-Th1 response appears to be associated with proliferative LN class III and IV [114] and induces the switch towards Th1 response. This apparently worsens the disease and correlates with the histological activity index [115, 116]. In contrast, Th2 response appears to be predominant in Type V membranous LN models [117].

Nevertheless, the role of T cells in humans in the course of LN is less clear, and it cannot be resolved on the basis of murine models of gene deletion or costimulation. Other authors have confirmed the proliferative LN Th1 dominance in humans [114, 115]. However, in pediatric LN, a balance between Th1/Th2 on the basis of IgG subclasses has been detected [118, 119]. In proliferative LN, there is overexpression of TNF-related apoptosis-inducing ligand (TRAIL) in the glomerular tubules of patients [120]. These findings may play a protective role by enhancing PTEC survival while also exerting a proinflammatory effect that may contribute to local inflammation and injury by inducing expression of ICAM-1 and IL-8, which may also be caused by TNF-α and IFN-γ [120].

5.7.2. B Lymphocytes. B cells are also abnormal and hyperactive in SLE. The uncontrolled activation of B cells may be the result of aberrant editing, increased signaling, an increase in co-stimulatory receptors B7 and CD40, increased subpopulations of plasmablastic DCs and plasma cells in the blood, and alterations of cytokines (BAFF, IFN-α, IL-6, and IL-21). The B-cell-activating factor (BAFF) rescues autoreactive B cells from deletion and induces isotype switching to IgG [121–124].

There has recently been a resurgence of interest in the role played by effector B cells not only through the synthesis of autoantibodies but also as regulators. To support this, some autoimmune models that were thought to be primarily mediated by T cells have shown potential roles for B cells through gene deletion or administration of CD20 monoclonal antibody in mice (independent autoantibody effects) [125].

B cells can also modulate some cellular responses by direct interaction with memory T cells and regulation of DC development. Indirectly, B cells are involved in cytokine synthesis: IL-10, IL-4, IL-6, IFN-γ, IL-2, IL-12, IL-23, IL-27, and BAFF. Under inflammatory conditions, they can function as B regulatory cells by releasing IL-10 and TGF-β through TLR stimulation. The increase in plasmablastic cells and B lymphopenia has been correlated with SLE clinical activity [125].

In humans, B cells seem to have some degree of organization rather than being random. Formation of ectopic germinal centers with organized follicles and DCs that correlate with the severity of tubule interstitial disease and deposit of ICs has been demonstrated as well [126]. However, to some authors, there is a predominance of the APC phenotype rather than synthesis of immunoglobulins as well as an increased expression of receptors for chemokine-type CXCR5 BCA-1 [127].

One of the most recent findings regarding B cells relates to circulating levels of BAFF (BLyS) in SLE, RA, and Sjögren's syndrome (SS). BAFF seems to contribute to B cell survival in germinal centers in a high percentage of patients and in NZB and MRL models. In these, it correlates with the amount of proteinuria and anti-DNA levels. BAFF acts synergistically with IL-6 and IL-21 to foster survival and differentiation of B

cells in humans. It is synthesized by monocytes, neutrophils, DCs, and T cells [128, 129].

Interestingly, in transgenic murine models with BAFF over expression, there is an induced lupus-like syndrome with LN even in the absence of T cells [130, 131]. For some reason, it appears to mostly favor the maturation of autoreactive clones. Both plasmablastic and plasma cells express the receptor that is involved in the homeostasis of peripheral B cells [132].

Results from a GWAS pointed to B cell having an important role in the development of SLE through signaling and the involvement of TLR 7 and TLR 9 [67]. In SLE, the role of T cell regulators CD4+CD25 + Fox P3 has been demonstrated to suppress activity of B cells *in vitro* and *in vivo* [133].

5.7.3. Th17 Lymphocytes (LTh17). LTh17 are a subpopulation of CD4+ T cells and a subtype of high-producing IL-17 derived from Th1 cells [134]. LTh17 do not lend themselves to regulation by T regulator cells (Tregs) [135]. LTh17 do not lend themselves to regulation by T regulator cells (Tregs) [136]. The differentiation of naive cells into this proinflammatory Th17 subtype apparently occurs inversely to the development of Treg cells. Although both populations are induced by TGF-β, Th17 require the presence of inflammatory signals like IL-6, IL-21, and IL-23 as well in order to favor their differentiation and inhibit the Treg cells. In humans, it seems Th17 cells also synthesize IFN-γ.

When Th17 cells produce IL-17 in response to TGF-β, they activate kappa beta nuclear factor ($\kappa\beta$NF). Consequently, a MAP kinase cascade is generated and activates the ROR transcription factor [135]. This exerts a powerful proinflammatory effect and fosters increased recruitment of macrophages and neutrophils thus inducing the production of IL-8 and MCP-1. ROR transcription factor also induces CAM expression on T cells and the production of IL-6 and GM-CSF. This is how a second phase of inflammation is generated and becomes self-perpetuated. Th17 subpopulations do not appear to be antigen specific [135].

Zhao et al. evaluated IL-17 serum levels in fifty-seven patients with confirmed SLE and 30 healthy volunteers [137]. They found significantly elevated levels in patients with SLE. However, there was no positive association with activity of the disease measured by Systemic Lupus Erythematosus Disease Activity Index (SLEDAI), which indicates that there is still no concluding data regarding the role of Th17 and SLE and, therefore, LN [137, 138].

5.7.4. T Regulatory Cells. The concept that Treg play an important role in maintenance of autoimmune response is well accepted. A decreased number and function of Treg cells implicated in murine lupus have been shown. However, human studies, many of which appear to be the result of clinical activity or immunosuppressive therapy [139, 140], are inconclusive [141, 142].

5.7.5. T Cells and Adhesion Molecules. Cell adhesion molecules seem central and CD44 is greatly increased in patients with SLE [143]. Through alternative splicing and posttranslational mechanisms, this gene has several isoforms such as CD44v3 and CD44v6 that are high in SLE patients and correlate with the disease activity and the presence of LN. Infiltrating T cells express these isoforms. Patients with active NL have high urinary concentrations of VCAM-1. Their expression is regulated by IL-1 and TNF [144].

5.8. Effector Cells and Molecules. In proliferative LN, there is predominance of mononuclear infiltrates and, to a lesser extent, of neutrophils and platelets. Mononuclear activation depends on chemokines, complement system activation, and ICs and cause cytotoxicity in the target organ. When there is cytotoxicity, mononuclear cells become effector cells [97].

Also involved in LN immunopathogenesis, proteases that have been detected in urine of LN patients are presumably involved in the degradation of extracellular matrix proteins of the GBM and mesangium (serine proteases, elastases, cathepsin G, and collagenases) thus generating tissue necrosis [97].

In LN, many PMNs are located close to the crescents and their proteases and oxygen radicals and derivatives of nitrogen may contribute to tissue damage and necrosis. One of the proteases, collagenase B-associated lipocalin has recently been reported as a good biomarker of active LN [97].

In addition to releasing free radicals, nitric oxide, and proteases, macrophages also release proinflammatory cytokines such as IL-1, TNF-α, and IFN-α, PDGF, TGF-β, complement components, coagulation factors, and chemokines [97].

There is a prominent recruitment of type II activated macrophages mainly in the tubules, interstitium, and glomeruli both in murine models and in humans [145]. Their activation may also be enhanced during Th1 responses. The release of growth factors by macrophages may contribute to mesangial proliferation (PDGF and TGF β) and sclerosis in LN and MRL [146, 147].

5.9. Role of Intrinsic Renal Cells in LN. The main intrinsic kidney cells include mesangial, endothelial, and epithelial cells. Apparently, they are not innocent bystanders but may be signal amplifiers. This has been observed in murine models and appears to contribute on three levels: proinflammatory mediator release, fibrogenesis, and possibly APC.

Kidney mesenquimal cells (mesangial, tubular epithelial, and endothelial cells) synthesize and release significant amounts of MCP-1. They may also overexpress α actinin in the presence of IFN γ and IL-1. All of the above has been previously shown in murine models [148].

5.10. Cytokines, Chemokines, and LN. Although the picture is still unclear in terms of proinflammatory molecules, an upregulation of cytokines such as TNF, IL-1, IL-6, IL-18, IFN-γ, and IL-4, induce Th1 and Th2, cells, respectively. In contrast, a downregulation of TGF-β in an inflammatory context has been proven [149].

5.11. Interferon α. IFN type I or α is produced by all cell types but particularly by DCs in response to viral stimuli and in the presence of ICs [150]. Both pathways act by stimulating

the TLRs types 3, 7, 8, 9 and thus induce maturation in DCs through the increase in the expression of costimulatory molecules such as ICAM-1, CD86, HLA class I and class II molecules. IFN-α activates hundreds of genes including viral transcripts (OAX, MX1), the IFN regulatory factor *(IRF) 5* and *IRF7*, BLyS, chemokines (MCP-1 and IP-10) and enhances Th1 responses by inducing the synthesis of IFN-γ and expression of CXCR3 cells. IFN-α is a potent inducer of BlyS [151].

In SLE patients, a microarray analysis of thousands of genes has shown an over expression of IFN-α-inducible genes in about 40% of patients [152]. Specifically, three inducible genes showed a high IFN score and had a significantly higher prevalence of renal disease, increased SLE activity and presence of antibodies specific to Ro, U1 RNP, Sm, and ds-DNA but not phospholipids [94]. Steroid pulses dramatically decrease IFN-α expression by inducing significant depletion of pDC but their action is of short duration (one week) [153].

IFN-α seems to be a potent inducer of mesangial proliferation in the kidney, and several studies report that IL-6 is a critical mediator of the production of human nephritogenic antibodies [154].

5.12. IFN-γ in LN. It has been suggested that, in addition to inducing *in situ* synthesis of autoantibodies, IFN-γ increases the expression of CD40 molecules as well as that of MCP-1, ICAM-1, and VCAM-1. This seems fundamental in the pathogenesis of exacerbations of LN.

Recent recognition of the role of pro-Th1 cytokines such as IL-18, IL-12, and IL-27 makes this molecule central to the regulation of these responses [155]. According to multiple studies, higher circulating levels of IL-18 are found in patients with SLE and LN and may be crucial in the development of Th1 responses [156].

5.13. Interleukin 10. In patients with SLE, high levels of IL-10 are from three to twelve times higher than in controls, but there seem to be little correlation with the disease activity [157].

5.14. Transforming Growth Factor β. TGF-β ligands signal and activate intracellular effectors thus regulating transcription. TGF-β is a cytokine involved in both normal renal function and the development of glomerulosclerosis. It is produced by NKs, lymphocytes, monocytes, macrophages, and renal mesangial cells. It also has a stimulatory effect on T cells and a downregulatory effect on antibody production. In human SLE, several studies demonstrated the nephrotoxic effects of TGF-β within kidney cells. There is a strong relationship between expression of TGF-β and podocyte depletion and apoptosis. TGF-β also increases epithelial to mesenchymal cell transdifferentiation, induces peritubular capillary loss, and causes glomerular endothelial cell apoptosis. In contrast, the cytoprotective effects are mediated by the hepatocyte growth factor (HGF). Therefore, studies found that the balance between TGF-β and HGF seems to be an important prognostic factor in LN TGF-β and HGF [158, 159].

5.15. Interleukin-4. The IL-4 role in the synthesis of autoantibodies in murine models and in humans is controversial in LN [160, 161]. It also seems to promote the depositing of collagen type III in human mesangial cells and could contribute towards renal failure progression [162]. CD4+ cells, which produce IL-4 in patients with Class III and Class IV LN, were demonstrated by immunohistochemistry [163].

5.16. Chemokines. Ex vivo monocytes have shown increased synthesis of chemokines that correlate with SLE activity: interferon-gamma-induced protein 10 (IP-10), RANTES, monokine induced by IFN-gamma (MIG), MCP-1, and IL-18. They also seem to correlate with LN activity as does IL-8 [164].

Of the above molecules, the best studied is MCP-1 and more recently TWEAK. Chemokines not only play a preponderant role in inducing and regulating the selective chemoattraction but also participate in the regulation of cellular activation and exert angiogenic, fibrogenic, and hematopoietic effects.

There is increasing evidence that MCP-1 plays a role in the progression of renal failure based on different mouse models and in various proliferative LN [148, 165]. In murine knockout MRL, there is marked prolongation of survival and no monocytic or lymphocytic infiltrates. They seem to be the initiator of locally produced early tubule interstitial damage. As a result, MCP-1 is synthesized mainly by mesangial cells but also endothelial, mononuclear, and tubular epithelial cells and excreted in the urine. Therefore, MCP-1 in the urine of patients is a promising biomarker of LN activity [166]. In addition to its chemoattractant and releasing properties on mononuclear cells, MCP-1 appears to play a role *in situ* in inducing renal tubular and mesangial cells to synthesize proinflammatory cytokines such as IL-6, adhesion molecules, for instance, ICAM-1, and promote transcription of NF $\kappa\beta$ by PKC [165]. In addition to that, MCP-1 promotes mesangial and endocapillary proliferation.

Chemokine receptors on T cells participate in regulating their trafficking. Native T cells express primarily CCR4, which interacts with chemokine C-X-C motif ligand 12 (CXCL12). Its pathogenic role has gained much attention recently. There is over expression in kidneys from NZBW, MRL, BXSB models as well as in LN in humans [167]. The use of murine C-C chemokine receptor type 4 (CCR4) antagonists has reduced the number of phenotypes and the use of C-C chemokine receptor type 3 (CCR3) antagonists reduced the infiltration of LTh1 and LTh17 and, therefore, the production of IFN-γ [168].

High expression of CXCR3 in 60% of infiltrating cells in biopsy material at the tubule-interstitial level has been recently reported in human type IV LN [169]. The CXCR3 receptor is a great candidate to explain the influx of LTh1 cells in LN. There are three CXCR3 ligands (CXCL9, CXXL10, CXCL11) with CXCL10 (IP-10) being the most potent inducer of IFN-γ synthesis. It has been proven to be a major chemokine expressed early on or preceding inflammation in murine LN [170, 171]. It is produced by endothelial cells, fibroblasts and monocytes stimulated with IFN-γ. In human LN, it appears to identify class IV nephritis [134]. In SLE

patients, levels of IP-10 are very high and correlate significantly with the histological activity index. The expression of CCR5 may also play a role in Th1 chemotaxis [40]. CXCL16 is among other chemokines involved [172].

5.17. TNF Superfamily Cytokine (TWEAK). This is widely expressed in human kidneys, specifically in mesangial cells, podocytes, and tubular cells. It is a proximal inducer of chemokine release. It also induces proliferation of mesangial cells and podocytes [173]. TWEAK has been recently studied as a biomarker for LN, and results have shown promising and significant results [174].

In summary, it is, therefore, possible that the deposit of ICs triggers the release by mesangial and tubule interstitial cells of MCP-1, TWEAK, and proinflammatory cytokines, which will contribute to the chemotaxis and after that, activation of monocytes and macrophages. This activation, in turn, releases chemokines such as CXCL10, which favors type LTh1 chemotaxis. LTh1 chemotaxis releases IFN-γ, which amplifies a further increased production of proinflammatory cytokines by monocytes and promotes IgG2 and IgG3 subclass synthesis. These immunoglobulins are responsible for generating anti-DNA antibodies, which induce glomerular cell proliferation.

6. Pathology

Classification of LN is critical to the issue of patient care and helps the physician make therapeutic decisions, follow up on the patient, and compare outcome results. In May 2002, a consensus conference of nephrologists, pathologists, and rheumatologists was held in order to redefine the different LN classes, and the meaning of the pathology terminology in order to standardize the way biopsies are interpreted and reported by different centers worldwide [175] (Table 2). The detailed pathological characteristics and their descriptions are beyond the scope of this paper. Readers are invited to consult pertinent and recent references about this topic [176].

6.1. Tubulointerstitial Disease. Active glomerular lesions have abundant interstitial inflammatory infiltrates of CD4+ cells and some CD8+ T cells, abundant monocytes, and plasma cells [99], which correlate with glomerular filtration rate and creatinine levels [177]. Others have found correlation between interstitial IC and serological activity [162]. Typically, the tubular damage, fibrosis, and atrophy are related linearly with renal function and are less responsive to treatment. These lesions often coexist with Classes III and IV.

7. Recent Advances in LN Therapy

LN impacts the clinical outcome of SLE both directly, in the form of target organ damage, and indirectly, through adverse effects of therapy [178]. On the other hand, the histological patterns of LN provide the basis for therapeutic guidelines and decisions to prevent target organ damage, as they are predictive. Despite improvements in survival rates and ESRD as mentioned before, LN is a marker of a bad prognosis [179]. Recent advances in therapy include a number of randomized controlled trials (RCTs), in which the goal has been to achieve clinical efficacy by inducing a remission of LN while at the same time minimize severe side-effects of treatment. The concept of two phases of therapy, an induction phase and a maintenance phase, is still widely accepted [180].

Patients with Class II LN and I do not require directed immunosuppressive treatment, and usually maintenance of adequate blood pressure control and blockade of the rennin angiotensin aldosterone system is the cornerstone of treatment. Patients with LN treated with ACE inhibitors have a better rate of renal involvement-free survival at 10 years (88.1%) as compared to patients with ACE inhibitors with a rate of renal involvement-free survival at 10 years of 75.4% ($P < 0.01$) [181].

7.1. Induction Therapy for Proliferative LN. Most patients with active proliferative LN are initially treated with a pulse of an intravenous steroid followed by a high-dose oral steroid, or by this method in conjunction with other immunosuppressive agents. These include cyclophosphamide, mycophenolate mofetil, and azathioprine.

7.1.1. Cyclophosphamide (CY). RCTs held at the National Institutes of Health (NIH) have provided strong evidence for the efficacy of IVCY in the treatment of proliferative LN. An IVCY pulse (0.5–1 g/m^2) each month for six consecutive months followed by a follow-up pulse of low-dose corticosteroid every third month has been shown to be effective and prevent relapses better than a shorter regimen limited to the six monthly doses of IVCY alone [182].

The Euro-Lupus Nephritis Trial (ELNT) was a multicenter European RCT in which 90 patients with proliferative LN were randomized to either high-dose IVCY (0.5–1 g/m^2) in 6 monthly pulses followed by two additional quarterly doses or to low-dose IVCY (500 mg) every 2 weeks to a total of 6 doses followed by azathioprine (AZA) maintenance therapy (2 mg/kg daily). After a median follow-up period of 41 months, there was no difference between the two groups in the rate of achievement of renal remission or in the rate of renal relapse [183]. The results of the ELNT of the follow-up period (73 months) showed similar results [184].

7.1.2. Mycophenolate Mofetil (MMF). The active metabolite of MMF suppresses B- and T-cell proliferation due to the absence of the salvage pathway necessary for DNA synthesis. That is the reason why results of several recent controlled trials have led to MMF being recommended as one of the first-choice regimens for inducing a remission in active proliferative LN. Chan et al. [185] randomized 42 patients with diffuse proliferative LN to either 12 months of oral MMF (2 g daily for 6 months followed by 1 g daily for 6 months) or to 6 months of oral CYC (2.5 mg/kg daily) followed by oral AZA (1.5 mg/kg/day) for 6 months, and both groups also received oral prednisolone (0.8 mg/kg). After a median follow-up period of 12 months, there were no significant differences between the remission rates (81 versus 76%), partial remission rates (14 versus 14%), or relapse rates

Table 2: International Society of Nephrology/Renal Pathology Society (ISN/RPS) 2003 classification of LN [175].

Class I minimal mesangial lupus nephritis
Normal glomeruli by light microscopy, but mesangial immune deposits by immunofluorescence.
Class II mesangial proliferative lupus nephritis
Purely mesangial hypercellularity of any degree or mesangial matrix expansion by light microscopy, with mesangial immune deposits. May be a few isolated subepithelial or subendothelial deposits visible by immunofluorescence or electron microscopy, but not by light microscopy.
Class III focal lupus nephritis
Active or inactive focal, segmental, or global endo- or extracapillary glomerulonephritis involving <50% of all glomeruli, typically with focal subendothelial immune deposits, with or without mesangial alterations.
Class IV diffuse lupus nephritis
Active or inactive diffuse, segmental, or global endo- or extracapillary glomerulonephritis involving ≥50% of all glomeruli, typically with diffuse subendothelial immune deposits, with or without mesangial alterations. This class is divided into diffuse segmental (IV-S) lupus nephritis when ≥50% of the involved glomeruli have segmental lesions, and diffuse global (IV-G) lupus nephritis when ≥50% of the involved glomeruli have global lesions. Segmental is defined as a glomerular lesion that involves less than half of the glomerular tuft. This class includes cases with diffuse wire loop deposits but with little or no glomerular proliferation.
Class V membranous lupus nephritis
Global or segmental subepithelial immune deposits or their morphologic sequelae by light microscopy and by immunofluorescence or electron microscopy, with or without mesangial alterations. Class V lupus nephritis may occur in combination with class III or IV in which case both will be diagnosed Class V lupus nephritis show advanced sclerosis
Class VI advanced sclerosis lupus nephritis
≥90% of glomeruli globally sclerosed without residual activity.

(15 versus 11%) for both treatments; however, infections were less common in the MMF group.

The Aspreva Lupus Management Study (ALMS) reported by Appel et al. [186] was one of the largest RCTs of a treatment of proliferative LN ever reported involving 370 patients with WHO class III, IV, or V LN randomized to 24 weeks of treatment with either MMF (3 g daily) or IVCY (0.5–1 g/m^2). Both groups were also treated with prednisolone that started at 60 mg daily and was tapered. After 6 months of therapy, there was no significant difference between the two groups in the combined complete remission plus partial remission rates. Moreover, there was no difference in mortality between the two groups, and a total of 14 of the 370 patients died [186].

Overall, RCTs have shown no real difference in induction therapy for LN between CY and MMF in terms of complete and partial remission rates. However, infection rates as adverse events of immunosuppressants are lower with the use of MMF leaving to the physician's choice whether to start MMF or CY as induction therapy in order to achieve remission and prevent progression of renal disease.

7.1.3. Tacrolimus. Recent findings regarding treatment in LN involve tacrolimus, which is a macrolide calcineurin inhibitor that potently suppresses human T-cell proliferation by inhibiting the intranuclear translocation of cytoplasmic nuclear factors in activated T cells by binding to tacrolimus-binding proteins and inhibiting calcineurin. Miyasaka et al. [187] reported a RCT that was undertaken to evaluate the efficacy and safety of tacrolimus in patients with persistent LN patients treated with a glucocorticoid. This RCT showed significant decrease in LN disease activity index (LNDAI) with tacrolimus when compared to placebo. A case-control study conducted by Szeto et al. [188] compared tacrolimus with standard protocols of oral CYC or AZA for the treatment of class V LN. Complete remission rate and partial remission rate were 38.9% and 44%, respectively, in the tacrolimus group, and 36.8% and 57.9%, respectively. It is important to remark that no significant adverse effects occurred in the tacrolimus group.

Five open-label prospective studies of the treatment of LN have been conducted [189–193], with preliminary evidence regarding the use of tacrolimus as induction-phase therapy. However, there is a need to conduct RCT with more proliferative LN patients in order to evaluate results and establish tacrolimus as on-label frequent use for the treatment of LN.

7.2. Maintenance Therapy for Proliferative LN. Maintenance therapy for proliferative LN focuses on maintaining renal remission previously achieved in the induction therapy. By avoiding flares or relapses, progression of renal disease can be achieved and, therefore, ESRD. The MAINTAIN Nephritis Trial [194], conducted on 105 patients with proliferative LN, was randomized to maintenance-phase therapy with AZA (target dose 22 mg/kg daily) or MMF (target dose 2 g daily). The MAINTAIN Nephritis Trial was predominantly Caucasian, and the results may not be applicable on populations of different ethnicities. Some meta-analyses have unequivocally favored the additional benefit of treating with immunosuppressive agents during the maintenance phase of LN therapy [195–197]. The selection and dosage in order to reduce long-term toxicities especially in childbearing age women must be done along with the patient. In addition, it is important to highlight the role of corticosteroids as

a major component of treatment in the maintenance phase of LN therapy, despite the side effects of long-term steroid use.

7.3. New Agents for the Treatment of Lupus Nephritis

7.3.1. Rituximab. This biological agent is a chimeric half murine-half human monoclonal antibody directed against the B cell marker CD20. Label indications of this biologic agent include RA and more recent SLE. Catapano et al. used Rituximab to treat 31 patients with relapsing or refractory SLE, 2 of whom developed relapsing/refractory LN during treatment with Rituximab (375 mg/m^2/week for 4 weeks in one patient and 1000 mg $\times$ 2 doses in the other) [198]. After a 30-month follow-up period, peripheral B cells had been depleted in 97% of the patients, and a remission had been achieved in 87% of the patients (complete in 17 and partial in 10) [198]. A renal remission occurred in 10 of the 11 patients with active LN. Clinical improvement was manifested by reductions in disease activity, proteinuria, and daily prednisolone dose. A relapse occurred in 67% of the patients treated after a median interval of 11 months. In 50% of the patients who experienced a relapse, the relapse was associated with the return ossf circulating B cells. A second course of treatment with rituximab was effective. A recent systematic review, which covered the period from 2002 to 2007, demonstrated that 171 (91%) of the 188 patients with SLE treated with rituximab for severe, refractory disease had a significant improvement in at least one lupus manifestation, and 94 (91%) of the 103 patients with LN exhibited a therapeutic response [199].

There is more need for RCTs using biological agents such as rituximab and other agents that are in course of study like Belimumab and Abatacept. One may infer due to the important role of B cells and T cells in LN pathogenesis that directed target therapy against them could bring new insights for effective treatment in LN.

8. Conclusion

LN is considered to be the major complication or outcome in SLE. Its incidence varies widely between populations. Over the years, a better understanding of immunopathogenesis and natural history has developed, which ultimately results in effective therapeutic decisions for the benefit of the patient and prevent end-stage renal disease. In addition, this appropriate comprehension of NL gives hope to future therapy aimed directly towards specific cells, autoantibodies, cytokines, and chemokines in order to regulate inflammation and tissue injury.

LN results from a complex interaction between autoantibodies in association with anti-dsDNA, nucleosomes and histones that end up forming kidney ICs and permanently activated inflammatory cells that stimulate and induce proliferation in local cells, which, in turn, stimulate complement, cytokines and chemokines.

So far, therapy for LN has shown to be partially effective in terms of renal remission. Directed target therapy against B and T cells could bring new insights for real effective treatment in LN and thus achieving a better outcome in patients.

Abbreviations

SLE:	Systemic lupus Erythematosus
LN:	Lupus nephritis
ICs:	Immune complexes
ENAS:	Extractable nuclear antigen antibodies
IgG2:	Immunoglobulin G subclass 2
PTEC:	Proximal tubular epithelial cells
MPC-1:	Monocyte chemoattractant protein-1
CAMs:	Cell adhesion molecules
DCs:	Dendritic Cells
Th2, th1, th17:	Lymphocyte T helper 2, T helper1, and T helper 17
ESRD:	End-stage renal disease
NZB:	New Zealand Black mice
NZWF1:	New Zealand White F1 mice
BXSB:	In-bred strains of mice
MRL-Faslpr:	Mice homozygous for the apoptosis-defective Faslpr mutation
ANAs:	Antinuclear antibodies
FcR:	Fc receptor of immunoglobulins
FcγR:	Fc gamma receptor
BCR:	B cell receptors
ICOS:	Inducing costimulator
CTLA4:	Cytotoxic T-Lymphocyte Antigen 4
(PD-1):	Programmed death 1 costimulatory receptor
SHP-2:	Nonreceptor protein tyrosine phosphatase
GWAS:	Genome-wide association study
SNPs:	Single nucleotide polymorphisms
HLA:	Human leukocyte antigen complex
Anti dsDNA:	Double-stranded anti-DNA antibodies
Anti RNP:	Antiribonucleoprotein antibody
Anti Ro/SS-A, *Anti La/SS-B:*	Extractable nuclear antigens
GBM:	Glomerular base membrane
IL-6:	Interleukin-6
IL-1:	Interleukin-1
TNF:	Tumoral necrosis factor
PMNs:	Polymorphonuclear cells
RANTES:	Regulated upon activation, normal T-cell expressed, and secreted cytokine
TWEAK:	TNF-related weak inducer of apoptosis
TGF:	Transforming growth factor
PDGF:	Platelet-derived growth factor
(APC):	Antigen presenting cells

MAC:	Complement membrane attack complex
FhT cells:	Follicular helper T cells
TLR:	Toll-like receptors
GM-CSF:	Granulocyte macrophage colony stimulating factor
BlyS:	B lymphocyte stimulator
CCR5:	C-C chemokine receptor type 5
(NK):	Natural Killer T cells
TCR:	T cell receptors
NFAT:	Nuclear factor of activated T cells
DN T cells:	Double negative T cells
mABs:	Monoclonal antibodies
BWF:	1hybrid between NZ and WNZ
TRAIL:	TNF related apoptosis-inducing ligand
Tregs:	T regulatory cells
RCTs:	Randomized clinical trials.

Conflicts of Interests

The authors declare no conflict of interests.

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Review Article

Epigenetics and Autoimmune Diseases

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Epigenetics is defined as the study of all inheritable and potentially reversible changes in genome function that do not alter the nucleotide sequence within the DNA. Epigenetic mechanisms such as DNA methylation, histone modification, nucleosome positioning, and microRNAs (miRNAs) are essential to carry out key functions in the regulation of gene expression. Therefore, the epigenetic mechanisms are a window to understanding the possible mechanisms involved in the pathogenesis of complex diseases such as autoimmune diseases. It is noteworthy that autoimmune diseases do not have the same epidemiology, pathology, or symptoms but do have a common origin that can be explained by the sharing of immunogenetic mechanisms. Currently, epigenetic research is looking for disruption in one or more epigenetic mechanisms to provide new insights into autoimmune diseases. The identification of cell-specific targets of epigenetic deregulation will serve us as clinical markers for diagnosis, disease progression, and therapy approaches.

1. Introduction

Epigenetics was defined by Conrad Waddington in the early 1940s as *the branch of biology that studies the causal interactions between genes and their products which bring the phenotype into being* [1]. Currently, epigenetics is defined as the *study of changes in gene function that are inheritable and that do not entail a change in DNA sequence* [2]. As has been mentioned before, all these mechanisms are inheritable thus the epigenetic markers have the ability to persist during development and potentially be transmitted from offspring to offspring. These mechanisms play an essential role in regulation of gene and miRNA expression, DNA-protein interactions, cell differentiation, embryogenesis, X-chromosome inactivation, and genomic imprinting [3].

One of the main functions of epigenetics is gene regulation. Gene regulation plays an important role in determining individual gene function and activity, the sets of genes which are functional in each specific cell type, cell type development and differentiation, and metabolic plasticity of the cell that allows it to adapt itself to environmental changes. However, it is important to note that epigenetics is not the only determinant of gene function. There are intrinsic components that are stable over time and are the same in each cell type. These intrinsic components, which include polymorphism and mutations, are among the mechanisms that affect gene expression. Also, the environment (virus, hormones, nutrition, and chemicals) influences epigenetics and thus, the intrinsic component altering gene function [4].

The interaction between environment and epigenetics is only one of the mechanisms by which a large range of different phenotypes arise from the same genotype such as in the case of monozygotic twins [5, 6]. Monozygotic twins have an identical DNA sequence, but studies have found some phenotypic differences that may be consequences of different exposures to environmental stressors. These exposures produce alterations in the DNA methylation pattern and histone modification. This condition may be one of the causes of the differences found in the concordance rate of autoimmune diseases between monozygotic twins (Table 1) [7–22].

Another example of how epigenetics interact with the environment is in the study of pregnant *Agouti* rodents. In this study, researchers fed pregnant *Agouti* rodents with food rich in methyl donors such as folate, methionine,

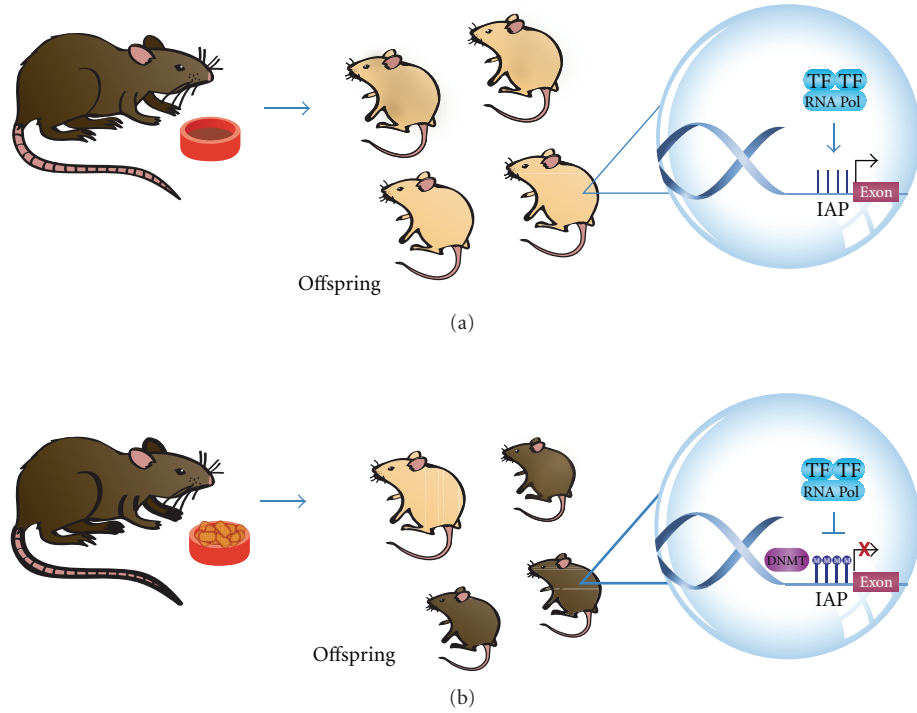

Figure 1: Epigenetic-environmental interaction. Offspring of pregnant *Agouti* rodents fed with food rich in methyl donors had a different color of coat (brown, (b)) due to an increased DNA methylation status in the viable yellow allele (A^{vy} allele), in comparison to offspring of pregnant rodents fed a normal diet (yellow or mottle, (a)). Intracisternal A Particle (IAP), Transcription Factor (TF), RNA Polymerase (RNA Pol), Methylated Cytosine (M).

Table 1: Concordance rate of autoimmune diseases between monozygotic twins.

Autoimmune disease	Concordance rate	References
Systemic lupus erythematosus	11–25%	[7] [8]
Type I diabetes mellitus	13–48%	[9] [10] [11] [12] [13]
Rheumatoid arthritis	12–22%	[14] [15] [16]
Grave's disease	22.2%	[17]
Multiple sclerosis	9–31%	[18] [19] [20] [21]
Celiac disease	75–83%	[22]

and choline. They found that, in comparison to offspring of pregnant rodents fed a normal diet (yellow or mottle, Figure 1(a)), the offspring of these rodents had a different color of coat (brown, Figure 1(b)) due to an increased DNA methylation status in the viable yellow allele (A^{vy} allele). These authors demonstrated that the percentage of phenotypes with a darker brown coat rises as increasing levels of methyl supplement are added to the diet. The lack of a methyl supplement has important implications because it indicates a pattern of future obesity and insulin resistance. In other words, mice with yellow or mottle coats have altered metabolism and obesity. It also results in increased cancer susceptibility, adult diabetes, and twice the mortality seen in normal mice [58].

Other researchers showed that Dutch who were exposed prenatally to famine during the Dutch Famine of 1944 in World War II had less DNA methylation of the imprinted Insulin-like growth factor 2 (IGF-2) gene 6 decades later compared to their unexposed, same-sex siblings. Because of the lack of nutrients that these individuals suffered during prenatal life, there was a deficiency of methyl donors such as the amino acid methionine that causes the hypomethylation of the differentially methylated region (DMR) in the maternally imprinted IGF-2 gene in comparison to same sex siblings who were not exposed. Even though the IGF-2 gene plays a key role in human growth and development, there was no evidence with respect to the relationship between hypomethylation of IGF-2 and birth weight. Thus,

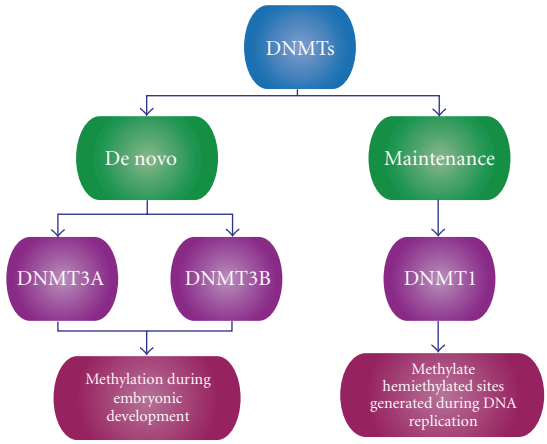

Figure 2: Classification of DNMTs. DNMTs can be classified into *de novo* and maintenance. *De novo* DNMTs are involved in methylation during embryonic development, and maintenance DNMTs are involved in methylation during DNA replication.

this finding supports the hypothesis that early mammalian development is important for establishing and maintaining epigenetic markers [59, 60].

Many studies had been done of the cohort from the Dutch Winter of Famine in World War II. One of them looked for differences in birth weight between offspring of mothers who were exposed to famine in either early or late gestation. The authors found that individuals who were exposed to famine in early gestation had epigenetic differences but a normal birth weight. In contrast, individuals exposed to famine in late gestation had a low birth weight but did not have any epigenetic changes [58]. At the same time, other studies have demonstrated that those individuals exposed to famine during the gestational period have a higher risk of developing schizophrenia and dyslipidemia. One of these studies demonstrated that there are sex-specific differences in the pattern of atherogenic lipids at the age of 58. Women showed elevated serum concentrations of total cholesterol, low-density lipoprotein (LDL), and triglycerides in comparison to unexposed women [61, 62]. It was also found that exposed women had a wide range of body mass indexes and thus have a higher risk of obesity and developing chronic diseases [63–65]. Other studies have shown that individuals exposed to famine in early gestation, both males and females, have an increased risk of schizophrenia while individuals who were exposed in later gestation have a higher risk of developing affective disorders [66–68].

2. Epigenetic Mechanisms

There are different epigenetic mechanisms that regulate gene expression whether this is to activate or repress it: DNA methylation, histone modification, nucleosome positioning, and RNA interference (RNAi) (miRNAs and small interfering RNA (siRNAs)) [4]. It is important to mention that all these epigenetic mechanisms act together at the same time and not separately to regulate gene expression.

2.1. DNA Methylation. There are evidence that DNA methylation occurs in different regions of the genome and it has great importance in embryogenesis, cellular differentiation, and tissue-specific development. It is noteworthy that DNA methylation varies among tissues and cell type because it is a dynamic process involving methylation and demethylation events [69–71] and plays a role in normal regulatory functions. Therefore, a dysfunction of normal state DNA methylation would lead to disease. Methylation is mediated by the DNA methyltransferase (DNMTs) family which is responsible for donating a methyl group to the DNA 5-cystosine. This family of enzymes has 5 members: DNMT1, DNMT2, DNMT3a, DNMT3b, and DNMT3L. At the same time, DNMTs can be classified into *de Novo* and maintenance DNMTs (Figure 2) [4]. *De Novo* DNMTs are DNMT3a, and DNMT3b and they are responsible for methylation during embryonic development. DNMT1 is the maintenance DNMT which is responsible for methylate hemimethylated sites that are generated during DNA replication. DNMT2 acts on transfer RNA, and DNMT3L acts on embryogenesis [72].

The other mechanism that counteracts DNA methylation is demethylation. Demethylation can be passive or active [4]. The first is induced by inhibition of DNMTs activities such as in the case of several drugs that are used as therapeutic compounds to eliminate aberrant hypermethylation. Active demethylation, in turn, occurs in cell differentiation and has been found in the activation of immune cells [73]. This process depends on the action of cytosine deaminase, which, when it is activated, induces cytidine deaminase (AICDA) that deaminates 5-methylcytosine [74].

It is important to understand that when there is a methylation state, transcription will be repressed; in contrast, when there is an unmethylated state, transcription will be permitted. Transcription inhibition is achieved because methyl groups interfere with the binding of transcription factors that activate transcription from a specific gene. Many of these transcription factors recognize mainly CpG sequences, but when these sequences are methylated, they are unable to bind DNA. An additional mechanism of transcriptional repression involves proteins that are attracted to methylated CpG sequences. These protein families are part of the methyl-CpG-binding domain (MBD), and they recognize methylated sequences thus providing a further signal to alter chromatin structures by formation of a corepressor complex [75].

There are four possible DNA methylation patterns. The first methylation pattern and the most widely studied is the methylation of CpG islands at promoter regions of genes. These CpG islands are regions of more than 200 bases with a G + C content of at least 50%. Many human gene promoters (60%) are associated with CpG islands and their basal state must be unmethylated to allow transcription (Figure 3(a)) [75, 76]. The second pattern is DNA methylation of CpG island shores, which are regions of lower CpG density in close

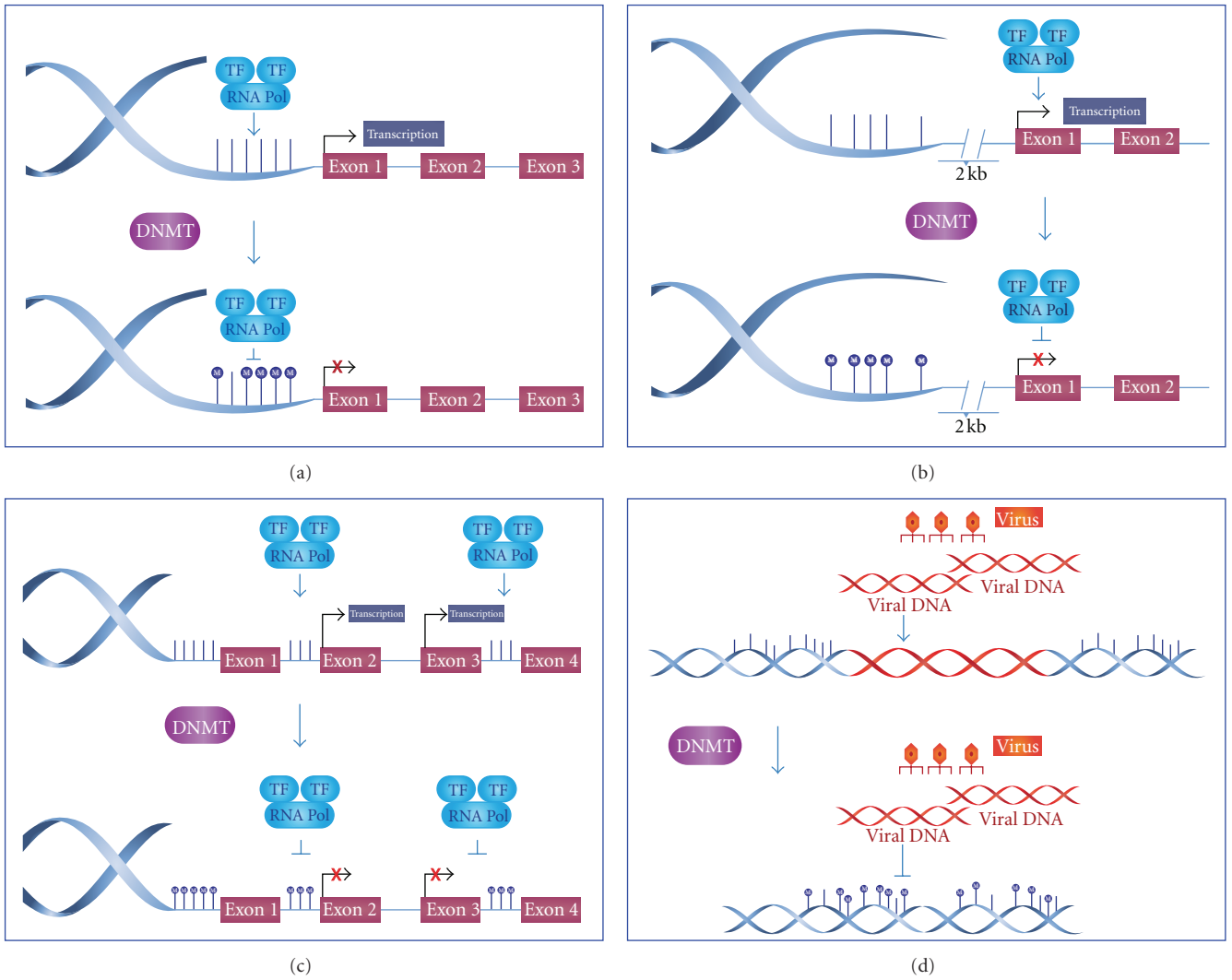

Figure 3: DNA methylation patterns. (a) In basal state, CpG Islands are unmethylated to allow the transcription, but when they are methylated at promoter regions of genes, the transcription will be inhibited. (b) At the same time, CpG island shores (located up to ~2 kb from CpG islands) have a methylation pattern that is similar to the CpG islands in that methylation is closely associated with transcriptional inactivation. (c) Gene bodies are methylated to prevent spurious transcription initiations. (d) Repetitive sequences which are hypermethylated to protect chromosomal integrity by preventing reactivation of endoparasitic sequences that cause chromosomal instability.

proximity (~2 kb) to CpG islands. This pattern is similar to the CpG island methylation pattern in which methylation is closely associated with transcriptional inactivation. It is important to note that most of the tissue-specific DNA methylation occurs in these regions (Figure 3(b)) [75, 77].

In contrast with both mechanisms mentioned above, the third pattern occurs in gene bodies which, in their basal state, are methylated to facilitate correct transcription thus preventing spurious transcription initiations (Figure 3(c)) [78]. In disease, gene bodies are demethylated to allow transcription to be initiated at incorrect sites. DNA methylation can also take place in CHG and CHH (H = A, C or T) sites in the human genome. This methylation has been found predominantly in stem cells and seems to be enriched in gene bodies directly correlated with gene expression. The last pattern to mention is hypermethylation of repetitive sequences that protect chromosomal integrity by preventing reactivation of endoparasitic sequences that cause chromosomal instability, translocations, and gene disruption (Figure 3(d)) [79].

2.2. Histone Modifications. Histones are conserved proteins that package and organize DNA. These proteins can be grouped in core histones (H2A, H2B, H3, and H4) and linker histones (H1 and H5). The linker histones bind to the DNA by sealing off the nucleosome at the location where DNA enters and leaves [80].

Histones suffer some posttranslational modifications such as lysine acetylation, and methylation, phosphorylation, ubiquitination, SUMOylation, and ADPribosylation. Histone modifications play an important role in transcriptional

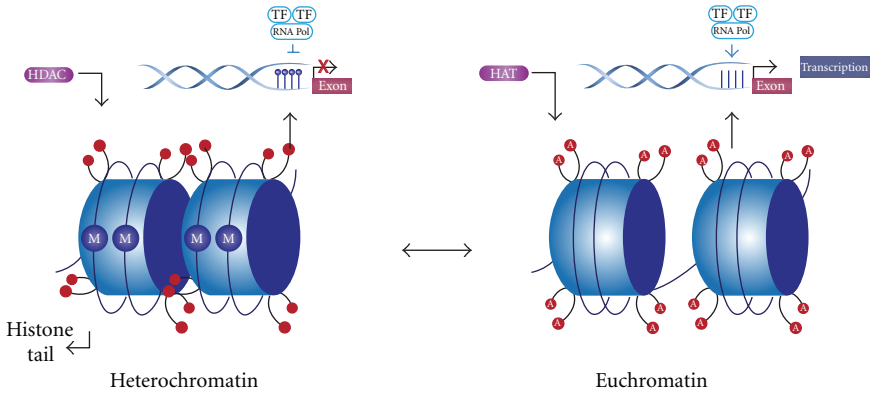

Figure 4: Histone modification. To form heterochromatin, histone deacetylation of histone tails caused by HDACs enzymes in association with DNA methylation (M) confers a dense configuration of DNA that prevents its transcription. In the euchromatin state, there is an acetylation of histone tails (A) by HATs enzymes in association with DNA demethylation to promote gene expression.

regulation, DNA repair, DNA replication and chromosome condensation [80, 81]. Of all these modifications, the one most widely studied is lysine acetylation. In this process, histones are acetylated and deacetylated on lysine residues in the N-terminal tail. These reactions are catalyzed by histone acetyltransferases (HATs), or histone deacetylases (HDACs) respectively [82, 83]. HATs promote gene expression by transferring an acetyl group to lysine and HDACs promote gene repression by removing an acetyl group from the lysine tail (Figure 4).

An example of how these modifications affect transcriptional regulation is the histone deacetylation with the association of 5′ methylcytosine in the DNA, which confers a heterochromatin configuration that makes DNA inaccessible to transcription factors. On the other hand, acetylation of histone tails such as lysine acetylation on histone 3 (H3K9) and DNA demethylation causes euchromatin configuration, which is accessible to transcription machinery [84]. It is important to mention that many posttranslational modifications can occur on the same histone tail and at same time produce the repression or the activation of gene expression [85]. For example, during cell cycle there is a regulatory relationship between methylation of H3K9 and phosphorylation of H3 serine 10 (H3S10). Phosphorylation of H3S10 is required for chromosomal condensation. During early prophase and anaphase, there are high quantities of H3S10 phosphorylation. In contrast, during late anaphase, dephosphorylation occurs and H3K9 methylation reemerges. Therefore, H3S10 phosphorylation blocked methylation of H3K9, which gave transcription factors access to DNA during mitosis. Also phosporylation preserves methylation patterns during cell division [86].

2.3. Nucleosome Positioning. Nucleosomes are a complex form of DNA packaged by histones. There are nucleosome positioning patterns that play an important role in transcriptional regulation. Depending on how close nucleosomes are to transcription start sites (TSSs), they may block the activators' and transcription factors' access to the DNA strand thus inhibiting elongation of the transcripts. Active gene promoters have a nucleosome-free region at the 5′ and 3′ untranslated region (UTR) to facilitate the assembly and disassembly of the transcription machinery [87]. For example, nucleosome displacements of as few as 30 bp at TSS have been implicated in changes in the activity of RNA polymerase II. When there is a loss of a nucleosome upstream from the TSS, transcription factors can bind to the TSSs and gene expression is achieved. In contrast, when there is an occlusion of the TSS by a nucleosome, transcription machinery does not bind to the TSSs and gene repression occurs. Interestingly, nucleosome positioning can influence DNA methylation because DNA methyltransferases preferentially target nucleosome-bound DNA [88].

2.4. microRNAs. miRNAs are RNAs that are 18–23 nucleotides in length and function as posttranscriptional regulators. They regulate mRNA translation by binding to complementary sequences that are cut or repressed. Many miRNAs are transcribed from intergenic regions or from introns of protein-coding genes and, sometimes, they are expressed at the same time that the protein gene is transcribed. Just a few miRNAs have been located in exons of protein-coding genes. Of all these miRNAs, the intergenic miRNAs are the only ones which have their own gene promoter and regulatory region [89].

The translational repression and target degradation of mRNAs is achieved by the level of complementarity between miRNA strands and the site in the 3′ UTR targets. If there is complete complementation, there will be cleavage of the mRNAs and this will produce degradation. On the other hand, if there is incomplete complementation, translation will be prevented by taking the transcripts into P bodies to keep them silenced using proteins that prevent translation or removal of the cap structure (Figure 5). Another mechanism by which miRNAs affect gene expression is by histone modification and DNA methylation of promoter sites. This mechanism occurs thanks to the RNA-induced transcriptional silencing (RITS) complex. This protein complex binds to miRNAs to perform posttranslational modification of histone tails such as methylation of H3K9 to form

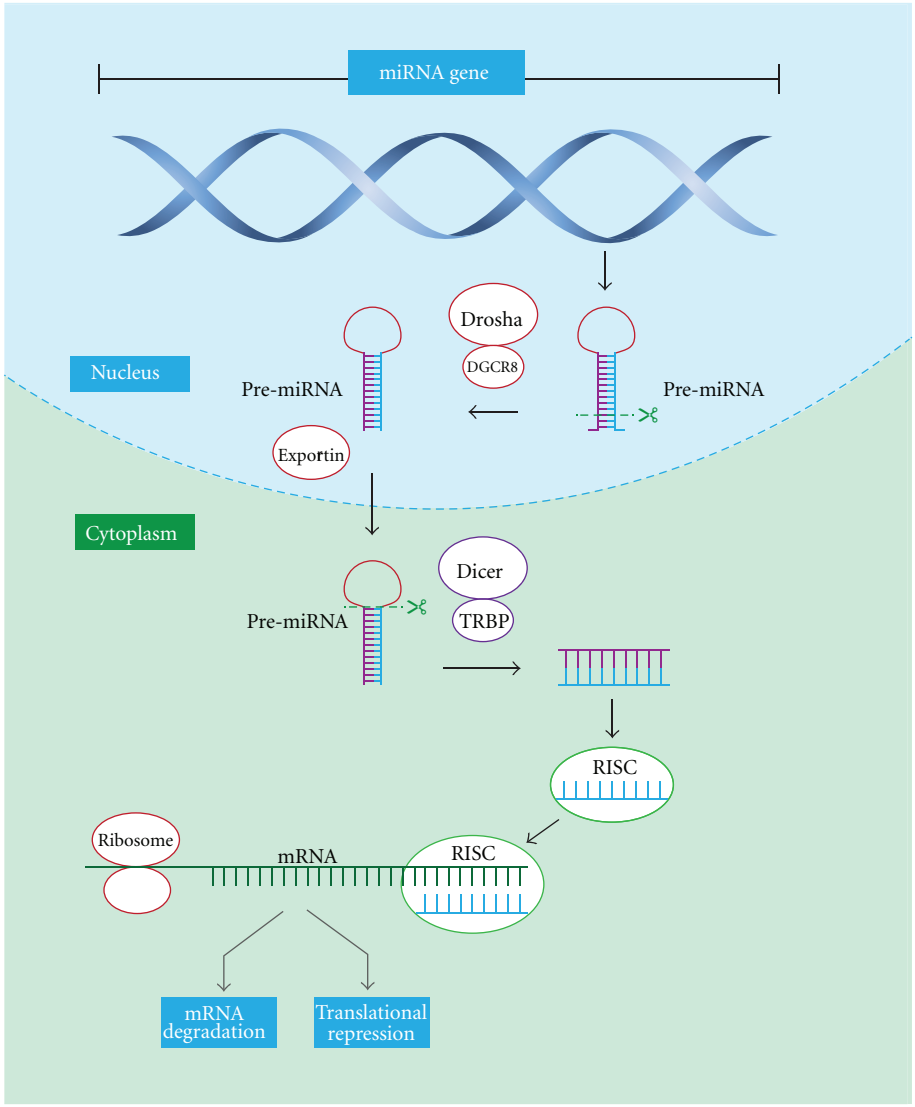

Figure 5: miRNA biogenesis. miRNA genes are transcribed by RNA Polymerase II in the nucleus to form a primary miRNA (pri-miRNA) that is, 100 to 1000 nucleotides in length. This pri-miRNA is recognized by nuclear enzymes Drosha, Pasha, or DGCR8 (in humans), which cleave about 11 nucleotides off of it to produce hairpin structures known as pre-miRNA, which are ~70 nucleotides in length. Once pre-miRNA hairpins are made, they are exported from the nucleus to the cytoplasm by the Exportin-5 enzyme. In the cytoplasm, the Dicer enzyme cleaves pre-miRNAs to form a duplex miRNA that is 18–23 nucleotides in length. Of these 2 strands, the one with lower stability in the 5′ end is the guide strand, and it will be associated with the RNA-induced silencing complex (RISC), where miRNAs interact with the mRNA targets. The RISC complex needs to interact with other proteins such as Argonaute (Ago) proteins and TRBP to function appropriately. The translational repression and target degradation of mRNAs can be achieved by the level of complementarity between miRNAs strand and the site in the 3′ UTR targets. If there is a complete complementation, there will be a cleavage of the mRNAs and it will produce the degradation. On the other hand, if there is an incomplete complementation, translation will be repressed by taking the transcripts into P bodies to keep them silenced.

heterochromatin and to cause transcriptional repression [89, 90].

3. Epigenetics and Autoimmunity

Autoimmune diseases are a complex group of diseases that do not have the same epidemiology, pathology, or symptoms but do have a common origin [91]. All autoimmune diseases share immunogenetic mechanisms mediated in part by several pleiotropic genes. Many studies over the years have shown that these diseases are caused by alterations in many loci and genes in the human genome [92]. However, until recent years, epigenetic studies have focused on autoimmune diseases. Therefore, it is important to underline the fact that autoimmune diseases may be generated by several alterations in the same epigenetic mechanism. Also, it is essential to understand that epigenetics is not the only mechanism

that may cause autoimmunity. In fact, there are intrinsic and extrinsic components (mutations, polymorphisms, and environmental factors) that predispose to autoimmunity.

3.1. DNA Methylation and Autoimmune Diseases. As was mentioned at the beginning of this paper, DNA methylation is the most widely studied mechanism in autoimmune diseases. Several studies done so far have found that some diseases such as systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) have global hypomethylation in the cells they target in promoter regions of DNA (Table 2). Studies of other autoimmune diseases in search of methylation patterns are just beginning.

3.1.1. Systemic Lupus Erythematosus. SLE is a systemic multiorgan autoimmune disease characterized by autoantibody response to nuclear and/or cytoplasmic antigens. Several studies have shown that there is a global hypomethylation of promoter regions, which contain the genes that are overexpressed in the disease such as *ITGAL, CD40LG*, *PRF1, CD70, IFGNR2, MMP14, LCN2,* and in the ribosomal RNA gene promoter (18S and 28S) [23–27]. The DNA hypomethylation may also affect the chromatin structure of T-cells thus enhancing the overexpression of these genes. This gene overexpression will cause cell hyperactivity, perpetuation of the immune response and consequently, and perpetuation of inflammatory response [93–95].

An example of how hypomethylation alters gene expression in SLE is the hypomethylation of the E1B promoter of CD5 in resting B cells. CD5 is a protein found in B cells that serves to mitigate activating signals from the B cell receptor (BCR) so that B cells are only activated by strong stimuli and not by normal tissue proteins. CD5 has two isoforms: E1A that is expressed on the membrane and E1B that is retained in the cytoplasm. The hypomethylation of E1B promoters may be the consequence of a reduced expression of DNMT1. Therefore, there is an increase in the expression of this CD5 isoform that will cause impairment of cell receptor signaling, which will then promote autoimmunity [28].

Another example is in the Lupus like disease caused by procainamide and hydralazine. These two drugs are DNA methylation inhibitors. As a result, they produce hypomethylation of DNA [96]. Procainamide is a competitive inhibitor of DNMT1 [97]. In contrast, hydralazine inhibits T and B cell signal-regulated kinase pathways [98]. The kinase signaling pathway plays an important role in the regulation of methylation [99]. These two mechanisms produce a reduction in DNMTs that will enhance the genetic expression of adhesion molecules on lupus-drug-induced lymphocytes [100–102].

3.1.2. Rheumatoid Arthritis. RA is a disease characterized by the progressive destruction of joints by invasive synovial fibroblasts. The RA synovial fibroblasts (RASFs) play a major role in the initiation and perpetuation of the disease [103]. They are the reason why several epigenetic studies of RA are focused on these synovial cells. Researchers have found a global hypomethylation of these cells, which could be responsible for the overexpression of inflammatory cytokines in synovial fluid [104, 105].

Some examples of hypomethylation in RA are in CpG islands upstream of an L1 open-reading frame and the Interleukin-6 (IL-6) promoter gene in monocytes. L1 is one of the major classes of repetitive elements that are spread throughout the genome. They are used as markers because they are methylated in normal synovial tissue. In synovial tissue from patients with RA, L1 is hypomethylated as a consequence of reduced expression of DNMTs. This reduction of methylation in inflammatory response promoter genes causes an overexpression of growth factors and receptors, adhesion molecules, and cytokines. In the end, they will cause irreversible phenotypic changes in synovial fibroblasts [29, 106].

The other example is the hypomethylation in CpG islands within the IL-6 promoter gene in monocytes. IL-6 is a proinflammatory cytokine that participates in B cell response. When this promoter is hypomethylated, there is an overexpression of IL-6 that will cause an overexpression of pro-inflammatory cytokines at the same time. This will be associated with a local hyperactivation of the inflammation circuit [30]. But there is evidence that we can also find a hypermethylation mechanism in monocytes such as in the case of the CpG islands within the promoter of death receptor 3 (DR-3). DR-3 is a protein that causes apoptosis and activation of transcription factor NF-kappa-B (NF-κB). However, when there is a downregulation of this protein because of the hypermethylation of its promoter, the RA synovial cell will be resistant to apoptosis [31, 107, 108].

3.1.3. Type 1 Diabetes (T1D). T1D is a T-cell-mediated autoimmune disease that develops in genetically susceptible individuals and affects their endocrine pancreas. There are some mechanisms by which epigenetics may play an important role in T1D by modulating lymphocyte maturation and cytokine gene expression and by differentiation of subtype T helper cells ruled by epigenetic controls. In this autoimmune disease, in contrast to SLE and RA, there is a global hypermethylation activity caused by altered metabolism of homocysteine [109].

Glucose and insulin levels are determinants of methylation [32]. They alter homocysteine metabolism by increasing cell homocysteine production through its inhibition of transsulfuration [110, 111]. When there is an increase in the levels of homocysteine, methionine in cells will be catalyzed by DNMTs in S-adenosylmethionine. This will enhance DNMT activity that will subsequently lead to increased global DNA methylation. Also, an increase in maternal homocysteine during pregnancy as a result of a low protein diet can produce an altered methionine metabolism that will cause a decrease in islet mass and vascularity in the fetus with a subsequent glucose intolerance in adult life [112, 113].

3.1.4. Multiple Sclerosis (MS). MS is a chronic inflammatory disease characterized by myelin destruction followed by a progressive degree of neurodegeneration. Recent studies have shown that the promoter region of peptidyl arginine

Table 2: Summary of epigenetic mechanisms involved in autoimmune diseases.

	DNA methylation	
Systemic lupus eErithematosus	Global Hypomethylation of promoter region of genes:	References
	ITGAL	[23]
	CD40LG	[24]
	PRF1	[25]
	CD70	[26]
	IFGNR2	[27]
	MMP14	[27]
	LCN2	[27]
	Ribosomal RNA gene promoter (18S and 28S)	[27]
	e1B promoter of CD5 in resting B cells	[28]
Rheumatoid arthritis	Hypomethylation:	
	CpG islands upstream of an L1 open-reading frame	[29]
	IL-6 promoter gene in monocytes	[30]
	Hypermethylation:	
	Promoter of death receptor 3 (DR-3)	[31]
Type 1 diabetes	Global hypermethylation by altered metabolism of homocysteine	[32]
Multiple sclerosis	Hypomethylation of promoter region of peptidyl arginine deiminase type II (PAD2)	[33]
Systemic sclerosis	Hypermethylation of CpG islands in Fli1 promoter	[34]
	Histone modification	
Systemic lupus erithematosus	Predisposition to apoptotic nucleosomes	
	H3K4me3	
	H4K8 triacetylation	
	H3K27me3	[35]
	H2BK12ac	
	Global acetylation of histone H3 and H4 in active CD4+ T cells	[36]
Rheumatoid arthritis	HDAC inhibitors:	
	Block induction of MMPs	[37]
	Repress of ADAMTs enzymes	
	Hyperacetylation of histones induces p16 and p21	[38]
Type 1 diabetes	Increase H3K9me2 in lymphocytes genes:	
	CLTA4	
	TGF-B	
	NF-κB	[39]
	p38	
	IL-6	
	Hyperglcemia causes H3K4 and H3K9 methylation	[40]
Multiple sclerosis	Hyperacetylation of H3 promoter region in white matter	[41]
	miRNAs	
Systemic lupus Erithematosus	Decreased expression:	
	miR-146a	[42]
	miR-125a	[43]
	Upregulation:	
	miR-21 and miR-148a	[44]
	miR-155	[45]

Table 2: Continued.

	DNA methylation	
Rheumatoid arthritis	Overexpression:	
	miR-155	[46]
	miR-203	[47]
	miR-146	[48]
	Decreased expression of miR-124	[49]
Multiple sclerosis	Upregulation:	
	miR-326	[50]
	miR-34a	[51]
	miR-155	[51]
	Expression in Treg cells: miR-17-5p, miR-497, miR-193 and miR-126	[52]
	Disease Relapse: miR-18b and miR-599 Disease Remission: miR-96	[53]
	Brain-specific: miR-124	[54]
Type 1 diabetes	Overexpression of miRNA-510	[55]
	Decreased expression of miRNA-342 and miRNA-191	[55]
	Beta cell failure: miR-21, miR-34a, and miR-146a	[56]
Sjögren's syndrome	Overexpression: miR-547-3p and miR-168-3p	[57]
	Upregulated: miR-150 and miR-149	[57]

deiminase type II (PAD2) is hypomethylated [33]. PAD2 plays a key role in the citrullination process of myelin basic protein (MBP). This citrullination process has important biologic effects. It promotes protein autocleavage, which increases the probability of creating new epitopes and also modulates the immune response. In MS, an increase has been found in demethylase enzyme activity, which will cause hypomethylation of the PAD2 promoter region [114]. Because of this hypomethylation, there will be an overexpression of PAD2 that will increase the MBP citrullination process with a subsequent increase in the production of immunodominant peptides. These peptides will increase the autocleavage of MBP thereby causing irreversible changes in its biological properties, which will produce proteolytic digestion, myelin instability, and a chronic inflammation response [115–117].

3.1.5. Systemic Sclerosis (SSc). SSc is a rare condition of unknown etiology that is characterized by excessive collagen deposits on skin and other tissues with a progressive vasculopathy. In SSc, there is a hypermethylation of CpG islands in the Fli1 promoter, which is a transcription factor that inhibits collagen production. The reduced expression of Fli1 increases collagen synthesis, that will not be balanced by metalloproteinase activity. This promotes collagen accumulation and, subsequently, the tissue fibrosis that is a characteristic of the disease [34, 118].

3.2. Histone Modifications and Autoimmune Diseases

3.2.1. Systemic Lupus Erythematosus. Histone modifications in SLE have been studied in murine models and in humans. These studies have found that, during apoptosis, histones can be modified to make them immunogenic. It is noteworthy that in the pathogenesis of SLE, antibodies are directed against components of the cell nucleus that are exposed at the cell surface during apoptosis [119, 120].

The nucleosomes, the primary inciting antigen in SLE, are released in patients with SLE as a result of a disturbed apoptosis or an insufficient clearance of apoptotic debris. During apoptosis, the nucleosome is modified, thereby creating more immunogenic epitopes. Subsequently, epitope spreading will lead to the formation of autoantibodies against unmodified chromatin components [35, 121]. Histone modifications such as histone 3 lysine 4 trimethylation (H3K4me3), histone 3 lysine 8 (H4K8) triacetylation, histone 3 lysine 27 trimethylation (H3K27me3), and histone 2B lysine 12 acetylation (H2BK12ac) will cause an increase in apoptotic nucleosomes (Table 2). These apoptotic nucleosomes will generate autoimmunogenicity that will cause activation of antigen-presenting cells and autoantibody production with a subsequent inflammatory response [36, 122].

There are other studies that have shown a global acetylation pattern of histone H3 and H4 in active SLE CD4+ T cells [123]. Also, monocytes, which are important in SLE renal disease, have been shown to have an altered acetylation pattern of histone H4 thus increasing the expression of interferon (IFN) genes that play a key role in SLE pathogenesis [124–126].

3.2.2. Rheumatoid Arthritis. RA synovial tissue is characterized by an imbalance between HAT and HDAC activity. Cartilage destruction is thought to be mediated by matrix metalloproteinases (MMPs) and enzymes from the ADAMTS

(a disintegrin and metalloproteinase domain with thrombospondin motifs) family. Many of these genes are regulated by modifications in the chromatin including acetylation of histones [127–129].

Many studies have shown that HDAC inhibitors inhibit cartilage degradation by blocking the induction of key MMPs by proinflammatory cytokines at both the mRNA and protein levels. Also, ADAMTs enzymes are inhibited at the mRNA level [37]. In fact, hyperacetylation of synovial cell histones induces p16 and p21 (cyclin-dependent kinase inhibitors that regulate cell cycle) expression with a subsequent decrease in Tumor Necrosis Factor-alpha (TNF-α) synthesis (Table 2). These mechanisms will inhibit joint swelling, synovial inflammation, and joint destruction in murine RA models [38, 129]. Also, the hyperacetylation of histones will downregulate HIF-1a (hypoxia inducible factor) and vascular endothelial growth factor (VEGF) to block angiogenesis in synovial cells [130].

It is noteworthy that HDAC inhibitors may, therefore, be new chondroprotective therapeutic agents in arthritis due to their ability to inhibit the expression of destructive metalloproteinases and ADAMTs in synovial tissue [131–133].

3.2.3. Type 1 Diabetes. There are just a few epigenetic studies associated with histone modifications and the pathogenesis of T1D. Patients with T1D show a subset of genes with an increase in histone 3 lysine 9 dimethylation (H3K9me2) in lymphocytes. This subset of genes includes the *CLTA4,* which is a type 1 diabetes susceptibility gene and has increased methylation of H3K9 in its promoter region. Other genes that have altered H3K9me2 are transforming growth factor-beta (TGF-B), NF-κB, p38 (mitogen-activated protein kinase), toll-like receptors (TLRs), and IL-6 (Table 2). The transcription factor NF-κB is also upregulated by H3K4 methyltransferase thus causing an increase in inflammatory gene expression in diabetic mice. All these genes are associated with autoimmune and inflammation-related pathways [39, 134, 135].

Histone modifications are also among the mechanisms that cause cardiovascular complications in T1D patients. Chemical modification of the H3K4 and H3K9 has recently been found to be related to the gene expression conferred by hyperglycemia. Transient hyperglycemia promotes gene-activating epigenetic changes and signaling events critical in the development and progression of vascular complications. These epigenetic changes are H3K4 and H3K9 methylation in genes associated with vascular inflammation [40, 136, 137].

3.2.4. Multiple Sclerosis. The oligodendrocyte identity is modulated by posttranslational modifications of histones. In rodents, histone deacetylation produces oligodendrocyte differentiation, whereas acetylation is associated with transcriptional inhibitors of differentiation. In patients with MS, there is a shift toward histone acetylation in the white matter. Thus, hyperacetylation of H3 in the promoter region of inhibitory genes will produce high levels of transcriptional inhibitors of oligodendrocyte differentiation such as *TCF7L2*, *ID2*, and *SOX2* (Table 2) [41].

3.3. Nucleosome Positioning and Autoimmune Diseases. Not many studies have been done on how nucleosome positioning causes autoimmune diseases. But in RA, histone variant macroH2A interferes with the binding of transcription factor NF-κB and impedes the action of some proteins that restructure nucleosomes [66]. Also, it has been reported that an SNP in the 17q12-q21 region, which is associated with a high risk of T1D, Crohn's disease, and Primary Biliary Cirrhosis, leads to allele-specific differences in nucleosome distribution [138].

3.4. microRNAs and Autoimmune Diseases

3.4.1. Systemic Lupus Erythematosus. Studies have shown that most lupus-related genes contain at least one miRNA target site for more than hundred miRNAs. In SLE, there is evidence of the key role some miRNAs play in its pathogenesis (Table 2). For example, miR-146a is a negative regulator of TLR signaling and its expression is decreased in patients with SLE. Also, this miRNA is a negative regulator of type I IFN pathway and carries out its function by targeting IFN regulatory factor 5 (IRF-5) and STAT-1 (Signal transduction and transcription protein). Therefore, decreased expression of miR-146a in peripheral blood mononuclear cells (PBMCs) may contribute to the enhanced type I IFN production in SLE [42]. Other studies have found that miR-125a was reduced in patients with lupus. This miRNA is expressed in T cells and is a critical transcription factor in the regulation of the chemokine RANTES (Regulated upon Activation, Normal T-cell Expressed, and Secreted). The decreased expression of miR-125a results in the upregulation and elevation of the inflammatory chemokine RANTES in lupus T cells [43].

Additional studies identified the upregulation of miR-21 and miR-148a in CD4+ T cells. One way these miRNAs may act in SLE is through the production of states of hypomethylation of some promoters by repressing DNMTs, which increases the expression of autoimmune-associated methylation-sensitive genes, CD70, and lymphocyte function-associated antigen [44]. Another way would be to inhibit DNMT1 translation via interaction with its 3′-UTR, as is the case with miR-126 [139]. There are other miRNAs that regulate B and T cell immunity such as miR-155. Therefore, the upregulation of miR-155 in lupus B and T lymphocytes may lead to abnormal B-cell activation and abnormal inflammatory T-cell development and cytokine production in patients with lupus [45, 46].

3.4.2. Rheumatoid Arthritis. miRNAs are also critical for RA pathogenesis (Table 2). For example, miR-155 and miR-146 are overexpressed in RASFs. miR-155 expression is enhanced by TNF-α and interleukin-1beta (IL-1B), and this enhancement produces an inhibitory effect on metalloproteinase expression in synovial fibroblasts [47]. In addition, miR-146 is an miRNA that is upregulated by proinflammatory

cytokines and its function is to downregulate the NF-κB pathway in monocytes. This miRNA has a strong correlation with the levels of TNF-α and interleukin-17 (IL-17) [48, 140, 141]. Another miRNA in RA is miR-203, which also causes repression of several metalloproteinase and inhibition of IL-6 [142].

Another miRNA implicated in RA is miR-124, which targets cyclin-dependent kinase 2 (CDK-2). In the basal state, CDK2 represses cell proliferation and arrests the cell cycle at the G1 phase, but in pathologic conditions such as RA, its level decreases. miR-124 also targets monocyte chemoattractant protein 1 (MCP-1), which is responsible for mononuclear phagocytes into the joint. Thus in RA, this miRNA increases cell proliferation and MCP-1 production [49, 143].

3.4.3. Multiple Sclerosis. Currently, many studies have been focusing on miRNAs involved in MS pathogenesis (Table 2). A recent study found that miR-326 plays a critical role in the pathogenesis of MS since it upregulates the Th-17 cell differentiation by targeting Ets-1, which is a negative regulator of Th-17 differentiation**.** This miRNA was significantly upregulated in patients with relapsing-remitting MS which produced an increase in Th-17 cell numbers and more severe symptoms [50]. Other miRNAs involved in MS are miR-34a and miR-155, which are upregulated in active MS lesions and contribute to MS pathogenesis by targeting CD47. CD47 is a "don't eat me" signal, and macrophages with low levels of this molecule are released from the inhibitory control signal, which causes increased phagocytosis of myelin. Also, miR-155 promotes development of inflammatory Th1 and Th17 cells [51].

In addition, differentially expressed miRNAs such as miR-17-5p, miR-497, miR-193, and miR-126 have been identified in different lymphocyte subsets including CD4+ T cells, CD8+ T cells, B cells, and CD4+ CD25+ Treg cells from patients with MS. Nevertheless, direct involvement and contribution of dysregulated miRNAs in MS has largely remained unknown and needs additional investigation [52]. It is noteworthy that all miRNAs are involved in the pathogenesis of the disease. There are miRNAs that can serve as prognostic markers. For example, the expression of miR-18b and miR-599 is related to relapse and miR-96 is involved in remission [53].

Other miRNAs such as miR-124, which is expressed in microglia but not in peripheral monocytes or macrophages, are brain specific. Their function may be to reduce activation of myelin-specific T cells with a marked suppression of the disease, which would make it a key regulator of microglia quiescence and a good prognostic factor for MS [54].

3.4.4. Type 1 Diabetes. There are few studies related to miRNAs and T1D pathogenesis. But there are some hypotheses that the function of regulatory T cells (Tregs) is influenced by changes in the expression of specific miRNAs (Table 2). In Tregs of diabetic patients, there is an increase in the expression of miRNA-510 and decreased expression of both miRNA-342 and miRNA-191. The exact function of these two is not yet known. There are other studies which demonstrate that miRNAs may be the cause of cytokine-mediated beta-cell cytotoxicity. This cytotoxicity is achieved when IL-1B and TNF-α induce the expression of miR-21, miR-34a, and miR-146a in pancreatic islets thus producing beta-cell failure by increasing proinflammatory cytokines [55, 56, 144].

3.4.5. Sjögren's Syndrome (SS). This syndrome is characterized by the inflammation and dysfunction of salivary and lacrimal glands, which cause dry mouth and eyes. Some miRNAs seem to play an important role in SS: miR-547-3p, miR-168-3p, miR-150, and miR-149 (Table 2). The first two are overexpressed in salivary glands, while the last two are upregulated in salivary glands and lymphocytes. The exact function of each one of these miRNAs has not yet been elucidated, but their overexpression may be the cause of the downregulation of some mRNAs that are important for correct immune function and for the downregulation of proinflammatory cytokines [57, 109].

4. Conclusions

Epigenetic research has grown and is now providing new insights into autoimmune diseases. This is possible thanks to advances in technological development, which are enabling epigenomic analysis on a large scale. This improvement in the genetic field has enabled us to find new causes that may explain the etiology of autoimmune diseases and, once again, has shown us that this group of diseases is not caused by a single altered component.

The candidate gene studies have identified a small set of genes that undergo aberrant DNA demethylation and overexpression in SLE and RA, which are the autoimmune diseases that have been the most widely studied in the last few years. This identification of cell-specific targets of epigenetic deregulation in autoimmune rheumatic disorders will provide clinical markers for diagnosis, disease progression, and response to therapies. However, to achieve this, high-throughput approaches are necessary for screening epigenetic alterations in autoimmune diseases related to specific tissue and cell types that are relevant to disease pathogenesis.

Once we have mapped all the altered epigenetic mechanisms that produce each one of the autoimmune diseases, even more research can be done on the therapeutic potential of compounds directed against those epigenetic mechanisms. But to do this, detailed human DNA methylomes, histone modification, and nucleosome positioning maps in healthy and diseased tissues are needed.

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Review Article

The Biological Significance of Evolution in Autoimmune Phenomena

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It is an inherent part of living to be in constant modification, which are due to answers resulting from environmental changes. The different systems make adaptations based on natural selection. With respect to the immune system of mammals, these changes have a lot to do with the interactions that occur continuously with other living species, especially microorganisms. The immune system is primarily designed to defend from germs and this response triggers inflammatory reactions which must be regulated in order not to generate damage to healthy tissue. The regulatory processes were added over time to prevent such damage. Through evolution the species have stored "an immunological experience," which provides information that is important for developing effective responses in the future. The human species, which is at a high level of evolutionary immunological accumulation, have multiple immune defense strategies which, in turn, are highly regulated. Imbalances in these can result in autoimmunity.

"*There is nothing permanent except change.*"
(Heraclitus)

1. Introduction

Life began on earth more than 3.5 billion years ago and evolution has allowed the development of myriads of species from very simple to highly complex ones. Initially, unicellular microorganisms without a nucleus (prokaryotes) similar to modern bacteria appeared and after that others with a nucleus similar to amoebas. These ancestral amoebas developed in groups and have been called "social amoebas." These feed on bacteria in the soil and they aggregate when there is a serious lack of food to form a migrating group. A type of amoeba in these groups differentiated and facilitated the process of detoxification through immunological mechanisms and became what is called a sentinel (S-cell) [1]. The S-cell engulfs bacteria and sequesters toxins. This may be the origin of the immune system. Subsequently, these unicellular eukaryotes differentiated into diverse functions and developed forms of signaling and adhesion molecules which allowed them to aggregate. This could have been the beginning of evolution of multicellular organisms (metazoans) which later migrated from the sea [2]. Around 600 million years ago, terrestrial metazoans began to develop in conjunction with an important increase in the oxygen concentration in the atmosphere. The vertebrate animals with their remarkable diversification appeared 500 million years ago in a relatively short time period termed the "evolutionary big bang." Since the beginning of life, the most important element of evolution has been the increasing ability of living things to accumulate information about these processes at different levels of memory.

Recently Dawkins [3], in his masterpiece, showed us that the different forms of memory were the most relevant foundation for evolution. Information required for handling the present so as to survive into the future is necessarily

obtained from the past. In fact, he proposed four levels of information gathering, which he called the "four memories," which would be the foundation of evolution. The "first memory" is DNA, the inherited database each species has, and which is the result of nonrandom evolution. It is the record of recurrent ancestral and cumulative details resulting from interaction with the environment which led to the primary characteristics of each species. The "second memory" is the adaptive immune system, which is cumulative information about dangerous microorganisms with which the individual interacts and through this interaction acquires the ability to defend itself from subsequent exposure with high efficiency. This process is present during the life of the individual. The "third memory" is the fact that we can think and it resides in the nervous system. By mechanisms that we do not yet fully understand, our brain records past experiences and works by a trial-and-error process that can be seen as yet another analogy to natural selection. In the human species, this level contains lists of faces, places, music, social customs, rules and words. The "fourth memory" is the collective memories inherited nongenetically from past generations and the culture. It also includes the information gathered through oral tradition and writing and, most recently, computer systems and Internet. This latter level of memory is necessary today for human survival.

Over time, the recorded information of repeated processes may be stored at a lower level of memory. Like wings or lungs that are necessary for survival, each adaptive change was developed as a secondary information-gathering system and later moved to the primary level. In fact, the mechanisms of evolution were also involved in the immune system development. The innate immunity which provides the early line of defense against microbes was developed by the perennial need for protection.

Based on these concepts, we are proposing a way to look at the autoimmune processes from the point of view of evolution with special attention to immune receptors which are crucial for sensing damage-causing agents and fine tuning the immune and inflammatory response that results. These receptors go through constant selection changes caused by the pressure of evolving pathogens. These pathogens stimulate the development of effective immune reactivity in order to maximize the destruction of the pathogens while avoiding an excessive immune and inflammatory response that could lead to consequences such as autoimmunity or septic shock [4].

In Figure 1, the evolutionary pathways of animal species are traced and the way the immune system becomes more complex in cumulative strategies is outlined.

It is important to note that the majority of species alive today are not from species that still exist but from previous ones. That is, man did not descend from monkeys, but shares a common ancestor. Through the study of species existing today, it is possible to describe with some degree of precision what their ancestors were like and how they evolved. Many species have disappeared and we only know about them through their fossils [5].

2. Influence of the First Form of Evolutionary Memory on the Autoimmune Phenomena

Innate immunity is a system that does not create a new form of memory and should be included in the first memory of evolution. The innate immunity is natural, nonspecific, nonanticipatory, and does not generate an accumulation of information. This system contains cells that resemble phagocytes which have generic receptors that recognize conserved patterns of pathogens and lectine-like soluble proteins, and they are essential in arthropods, nematodes, sipunculids, mollusks, annelids, platyhelminths, echinoderms, cephalochordates, and urochordates. Adaptive immunity, which has a highly diverse repertoire of lymphocytes, was added in agnathas (vertebrates without jaws) and gnathostomes (vertebrates with jaws). In fact, in the most complex species like mammals, the immune system consists of innate and adaptive immunities which include T and B lymphocytes and the production of cytokines and antibodies.

2.1. The Pattern Recognition Receptors (PRRs). Human innate immunity shares similar cells, cellular structures, and molecules with invertebrates. The PRRs are of special interest. These include members of nucleotide oligomerization domain proteins containing leucine-rich repeats (NLRs), retinoic acid inducing gene (RIG)-like helicases (RLHs), and toll-like receptors (TLRs) [6]. TLRs deserve special attention and are one of the largest and best-studied PRRs. They are expressed on macrophages, dendritic cells, epithelial cells, and endothelial cells, where they provide rapid responses including the induction of proinflammatory cytokine secretion that recruits and activates additional immune responses. Such receptors were highly conserved during evolution and were first identified in *Drosophila melanogaster* [7]. The TLRs are necessary for defense from various microorganisms. For example, it has been demonstrated that mutant *Drosophila*, which carries loss-of-function mutations in the toll receptor, resulted in high susceptibility to fungi infection. The defective induction of an antifungal peptide provided the first evidence that *Drosophila* expresses a specific receptor responsible for sensing a fungi infection [8]. TLR has a leucine-rich extracellular domain interrupted by cysteine motifs and an intracytoplasmic domain similar to the interleukin-1 (IL-1) receptor all of which contain ITAMs domains that make it possible to follow the cascade of signaling events through phosphorylation of tyrosine residues [9]. This similarity between the TRL and the IL-1 receptor domains has the same phylogenetic origin in invertebrates and is highly conserved among them. Nowadays, this domain has been named TIR (Toll/1L-R). Unlike other human TLRs that are typically present on the surface of cells and recognize bacterial danger signals, a group of TLRs including TLR3, TLR7, and TLR9 localize cell endosomes and recognize viral danger signals (dsRNA, ssRNA, and hypomethylated dsDNA, resp.). This group of endosomal TLRs has been particularly implicated in the pathogenesis of autoimmune diseases. Human-derived RNAs and DNAs that are targets of autoimmune responses in systemic lupus erythematosus (SLE) and related conditions have been found to induce activation of these receptors [10].

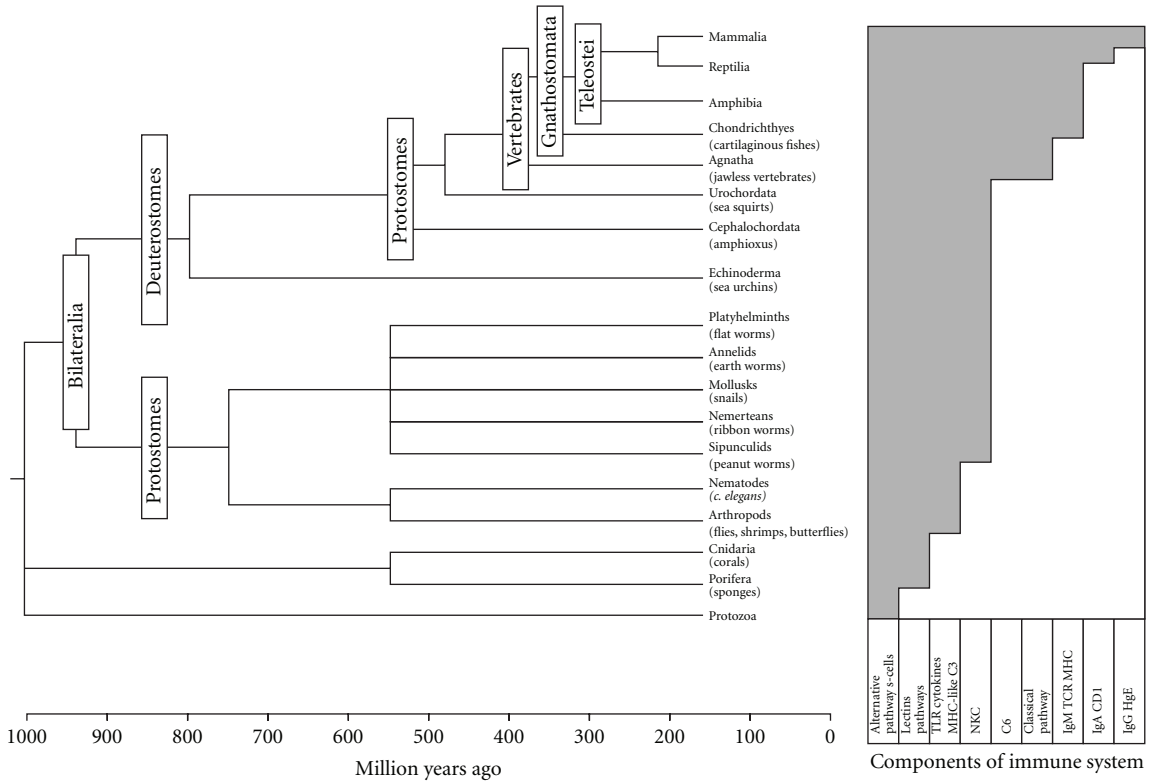

Figure 1: Phylogeny of animals and their immune system. Notice the form of accumulative evolution of the immune system from an innate to an adaptive system.

Altered expression and function of these receptors have been linked to clinical manifestations of lupus-like autoimmunity in animal models [11, 12]. In the case of rheumatoid arthritis (RA), it has been postulated that after the activation of TLR by exogenous stimuli, these receptors recognize endogenous proteins. Potential endogenous TLR ligands are head shock protein (HSP)-60, HSP-70, gp96, high mobility group box 1 protein (HMGB-1), serum amyloid A, and low molecular weight hyaluronic acid. Therefore, they are capable of inducing a self-perpetuating inflammatory process which plays an important role in the pathogenesis of RA [13]. This is a form of autoimmunity related to innate immunity and it occurs when regulatory systems are scarce and ancestral mechanisms such as RNA interference (RNAi) are involved [14]. In the case of primitive animals that possessed only an innate defense system, such animals might have suffered occasional disregulation which resulted in reactions against their own bodies and thus, parallel mechanisms to prevent self-injury had to be developed [15].

2.2. Cytokines. Proinflammatory cytokines and their receptors are present in early representatives of metazoans, such as cnidarians, and seem to be conserved in the entire animal kingdom. They are a family of secreted and regulatory molecules with a hormone-like activity and molecular mass ranging from 10 to 50 kDa. Cytokines are produced transiently and locally. Their mechanism of action is mainly paracrine or autocrine with the ability to induce a potent response in very small amounts. Cytokines interact with high-affinity cell surface receptors specific for each cytokine or cytokine group, which, when bound, leads to changes in the pattern of cellular RNA and protein synthesis [16]. They facilitate communication between cells, especially those of the haemopoietic and neuroendocrine systems. The evolution of the genes that encode the current spectrum of cytokines and receptor complexes involved multiple duplications from a smaller set of genes followed by the divergence of sequence and product function [17]. Recognition between distant cells is a phenomenon that is almost as old as metazoa itself dating back at least one billion years. A clear example of cell-to-cell recognition can be found in protozoa during sexual reproduction when recognition and signaling occurs between cells having a cell surface-associated set of "permissive" molecules which allow conjugation and exchange of genetic material between individual cells [18]. A marine ciliate protozoa (*Euplotes raikovi*) produces and releases specific pheromones into seawater which bind to receptors present on cells that are at the same point in the cell cycle and trigger molecular pathways which lead to a reciprocal search for permissive partners and to sexual reproduction [19, 20]. One of these pheromones was capable of binding to the α and β subunits of the IL-2 receptor on mammalian cells, and interleukin-2 (IL-2) was able to bind to its putative receptors on the ciliate protozoa cell surface. The implications of distant kinship suggested by these findings between IL-2 and pheromone families are

supported by similarities in the structures of these molecules which suggest a conservation of this cell signaling system during evolution [21]. A similar cross reaction that confirms an ancestral relationship between ligands and receptors is seen in a cytokine-like factor with proinflamatory functions found in the blood of the starfish *Asterias forbesi*. This factor stimulates monocyte chemotaxis and macrophage activation in mammals [22, 23]. The mussel *Mytilus edulis* has been the subject of studies to determine whether the relationships between the immune and neuroendocrine systems, observed in vertebrates, may also be present in invertebrates. The effects of rIL-1 and tumor necrosis factor α (TNF-α) were studied in *Mytilus* hemocytes previously shown to produce and react to opioid peptides. These cells responded to these cytokines both *in vitro* and *in vivo*, in a manner similar to that of human granulocytes. In addition, the presence of immunoreactive IL-1 and TNF in *Mytilus* hemolymph was demonstrated using polyclonal antibodies against mammalian cytokines [24].

2.2.1. IL-1. The family of the IL-1 is made up of IL-1α, IL-1β, IL-18, which are proinflamatory cytokines, and the IL-1 receptor antagonist (IL-1ra) with pivotal roles in the regulation of acute inflammation. The inactive IL-1 precursors of mammals must be cleaved intracellularly by the IL-1 converting enzyme (ICE) to release the biologically active form [25]. The nonmammalian vertebrate IL-1 lacks the sequence coding for the ICE cleavage site and requires another mechanism to active the cytokine [26]. IL-1 activity occurs as a consequence of binding to its receptor complex (IL-1R) on the cell surface of target cells. The binding of IL-1 to its receptor triggers complex intracellular pathways that result in the activation of new genes or modification of proteins. As already mentioned, the intracellular domain of the IL-1 receptor is a "TIR domain" and is similar to the intracellular domain of TLR. TIR domains participate in host defense and inflammation and are present in mammals, insects, and plants [27]. An IL-1-like cytokine, which induces increased vascular permeability in rabbit skin, has been reported in ascidians. This effect was neutralized by a polyclonal antihuman IL-1 antiserum [28]. In humans, a different form of polymorphisms, SNPs, are implicated in the severity of a number of autoimmune diseases such as RA in which an adequate balance between IL-1 and IL-1ra is also required [29].

2.2.2. TNF-α. TNFα, mainly produced by monocytes/macrophages, regulates inflammation and cellular immune responses [30]. One of its functions is the modulation of the expression of IL-1, IL-6, and chemokines [31]. TNF-α requires a converting enzyme (a metalloproteinase), which generates a 17 kDa soluble mature peptide. The active form of TNF-α is a homotrimer that binds to two distinct receptors on the cell surface, TNFR1 and TNFR2, which elicit different cellular responses including cellular differentiation, proliferation, and apoptosis. Several proteins that interact with the cytoplasmic domains of these receptors have been identified and include the signaling cascades that lead to activation of NF-kB, c-Jun N-terminal kinase, and the apoptotic pathway. Teleost fish has TNF-α and TNF-α receptors and the human recombinant TNF-α produces biological effects such as macrophage respiratory burst activity, neutrophil migration, and lymphocyte proliferation [32]. A similar cross-reaction observed with IL-1 confirms an ancestral relationship with other species. Infliximab, a chimeric antibody in which the Fab portion has a mouse origin effectively blocks the human TNFα molecule and provides a clinical benefit for patients with active RA [33]. TNF-α and their receptors have been implicated in the pathogenesis of diverse autoimmune diseases with special interest in their polymorphisms [34]. A regulatory mechanism for reducing the inflammatory response in infections such as tuberculosis (TB) is presumably the development of polymorphism by natural selection. The -308 and -238 single nucleotide polymorphisms (SNP) of TNF-α may influence the presence of autoimmune diseases and TB. In fact, TNF -308G was both associated with TB and protective for autoimmunity, TNF -238A allele was protective for autoimmunity but represented a susceptibility factor for TB, and the haplotype -308A -238G was a protective factor against TB while, at the same time, it carried susceptibility for RA, SLE, and Sjögren's syndrome (SS) [35]. These results support the hypothesis that autoimmune diseases are a consequence of natural selection for enhanced TB resistance. Likewise, it is important to know that evolutionary mechanisms have been developed for the production of TNF-α regulatory mechanisms, and their disruption can lead to an increase in action and be associated with autoimmunity. Tristetraproline (TTP) is one of them. The TTP family of CCCH tandem zinc-finger proteins consists of three known members in mammals with a fourth member recently identified in frogs and fish [36]. TTP is now known to bind to the so-called class II AU-rich elements within the mRNAs that encode TNF-α and the granulocyte/macrophage colony-stimulating factor (GM-CSF). In both cases, this binding results in destabilization of the mRNA and decreased secretion of the protein. Recent evidence suggests that TTP can accomplish this accelerated mRNA degradation by first promoting removal of the polyadenylated tail from the mRNA (deadenylation) [37]. A TTP deficient mouse develops a deep inflammatory syndrome with erosive arthritis, autoimmunity, and myeloid hyperplasia [38]. In patients with RA, a low TTP/TNF-α gene expression ratio could indicate failure to produce adequate amounts of TTP in response to increased TNF-α production [39].

2.3. Complement System. Serine proteinases appeared early in evolution. They have even been found in bacteria [40] and evolved to supply several physiological needs in the immune system and others. A serine proteinase cascade which shows similarities to the blood clotting system and the complement system of vertebrates is involved. There is even a functional link between immunity and haemostasis, so coagulation factors activate immunological processes and various components of the complement also activate coagulation factors [41, 42]. Substrates of these protease cascades show evolutionary relationships. The complement system has more than 30 components. About one-fourth of them

are serine proteases that are important for the activation or regulation of the system. The three branches of the complement in mammals are classical, lectin, and alternative pathways that converge in C3 protein and continue until the terminal phase with the C9 assembled to form the membrane attack complex (MAC). The complement system plays important roles not only in defense but also in normal tissue regeneration and development. The complement participates in the removal of immune complexes, aberrant and apoptotic cells, and cell debris and has important functions which, if they fail, are implicated in autoimmunity.

2.3.1. Initiating Enzymes. The classical pathway is triggered by antigen-antibody complexes and the proteases involved are C1r, C1s and C2. The lectin pathway is triggered by mannan-binding lectine and the proteases involved are mannan-binding-protein-associated serine proteases (MASP)-1, MASP-2, and MASP-3. The alternative pathway is triggered by pathogen and the proteases involved are factor D and factor B. The classical pathway is the newest phylogenetically and participates in the link between innate and adaptive immune systems. The deficiencies of C1q, C1r, C4, C2 are associated with SLE development by the failure to remove circulating immune complexes which may be deposited in blood vessel walls and tissues [43].

2.3.2. C3. The central component of the complement system is the C3 protein when the three pathways converge. It has been identified in jawless vertebrate and derives from a common ancestor, α-2-macroglobulin. This has been found in vertebrates, arthropods, and mollusks thus suggesting an early evolutionary origin and showing its importance as a defense molecule [44]. It contains an unstable internal thioether bond that, in nonactivated form, is buried in a hydrophobic pocket. When the protein is active, there is a cleavage of the thioether bond which allows the formation of a stable covalent bond with an adjacent substrate or water. C3 can be cleaved spontaneously in the alternative pathway or by other enzymes in the classical and lectin pathways. These processes must be regulated by proteases that promote proteolytic degradation of C3 (factor I and factor H in an alternative pathway), and their deficiency is associated with immune complex-mediated glomerulonephritis.

2.3.3. MAC. In the terminal complement component or lytic pathway, the C5, C6, C7, C8, and C9 proteins are present. The later configures the MAC which forms pores on the plasma membrane of the target cell, disturbs the membrane potential, and finally leads to cell lysis by a mechanism similar to perforin, which is the lytic protein of natural killer cells and cytotoxic lymphocytes [45]. Molecules homologous to mammalian C5 have been described in several species of teleost fish [46]. All these molecules share common structural motifs, that is, thrombospondin (TS), low-density lipoprotein receptor (LDL-R), and epidermal growth factor precursor (EGFP) domains. The cloning of a C6-like gene from the most primitive of present-day chordates, the amphioxus *Branchiostoma*, suggests an ancient origin of the C6/C7/C8/C9/perforin gene family. It seems reasonable for the duplication of an ancestral gene to have proceeded through these pathways. One pathway presumably led to the simple form of perforin while the second produced the ancestor of C6-C7 with its complex modular structure. Further duplication and loss of modules may have led to the creation of C8 and C9 molecules. The MAC present in teleost fish closely resembles the mammalian complex. CD59 is a regulatory factor of the terminal complement system. It blocks C9 binding and prevents the formation of MAC. Its deficiency is associated with hemolytic anemia.

2.4. Receptors for the Fc Region of IgG. (FcgR) provides a type of link between the humoral and cellular immune system. Inherited FcgR polymorphisms influence human phagocyte function. Single-aminoacid/SNP substitutions within the extracellular domains of FCgR alter the ability of the receptor to bind IgG and have been associated with the development of autoimmune and infectious diseases [47]. FCgRII (CD32) has two isoforms, FCgRIa and FCgRIIb, which are expressed on mononuclear phagocytes, neutrophils, and platelets. FCgRIIa has 2 codominantly expressed alleles, H131 and R131, which differ at aminoacid position 131 in the extracellular domain (histidine or arginine, resp.) and differ substantially in their ability to bind human IgG2 [48, 49]. H131 is the high-binding allele, R131 the low-binding allele and heterozygotes have an intermediate function [50]. FcgRIIa-H131 is essential for handling IgG2 immune complexes. These immune complexes are removed from circulation, primarily in the liver and spleen, by the mononuclear phagocyte system. Impaired removal of immune complexes is present in SLE which leads to an increase in the probability of tissue deposition of immune complexes, release of inflammatory mediators, influx of inflammatory cells, and damage to target-organs such as in the case of nephritis [51, 52]. FCgRIIb is the only FCgR inhibitor and regulates signalling which is crucial for the maintenance of B-cell tolerance and for the fine tuning of inflammatory and immune responses [53]. FCgRIIb-deficient mice are prone to inducible autoimmunity and, in some circumstances, develop spontaneous SLE [54]. In contrast, they are protected from both bacterial infection [55] and malaria [56]. In humans, several SNPs that modify the expressions or function of FCgRIIb have been described. A promoter polymorphism that may influence expression and be associated with SLE has been described [57]. An SNP in exon 5 of *FCGR2B* results in an isoleucine to threonine substitution within the transmembrane domain (1232T, rs1050501) which leads to the loss of the inhibitory function associated with the exclusion of FCgRIIb from lipid raft [58]. Homozygosis for this SNP is strongly associated with SLE [59, 60] and is found in 1% of Europeans, 5–7% of South-East Asian and Kenyans and in more than 10% of African-Americans. The high prevalence of this SNP in African and Asian populations may be due in part to the observation that it not only predisposes to SLE, but also protects children from malaria [61]. This is another example of how the effects of evolution based on natural selection can influence the genesis of autoimmune phenomena. FcγRIII

(CD16) has two isoforms, FcγRIIIa and FcγRIIIb. FcγRIIIb has two codominantly expressed alleles: NA1 and NA2 with changes in the aminoacid sequences that may also alter the affinity to immune complexes. The first has a low binding to the immune complex and is more associated with autoimmune processes such as antineutrophil cytoplasmic antibody (ANCA)-positive systemic vasculitis [62]. In SS, there is a similar correlation with the presence of FCgRII [63] and FCgRIII [64] polymorphisms.

Therefore, we postulate that primitive animals with an innate immune system may have been attacked by their own system at different times during evolution and, consequently, developed regulatory mechanisms such as RNAi, TTP, IL-1ra, regulator complement cascade proteins, and FCgRIIb presented above. Another regulatory mechanism which is implicated in autoimmune phenomena when it fails is the one associated with the functions of suppressor T cells [65].

2.5. Class III Major Histocompatibility Complex (Class III MHC). This loci contains several genes that encode secreted proteins that play innate immune functions: components of the complement system (such as C2, C4 and factor B) and inflammation-related molecules (cytokines such as TNF-α, LTA, LTB) or HSP. Class-III has a completely different function classes-I and II (described below in the text), but is between the other two in the short arm of human chromosome 6.

3. Influence of the Second Form of Evolutionary Memory in Autoimmune Phenomena

Adaptive immunity is a form of second evolutionary memory and stores molecular information in microbes in order to have a quicker and more effective defense against them in future exposures through cytokines and specific antibodies. The most important mechanism that nature has used to obtain and retain this type of information has been the immunoglobulin superfamily gene system which provides information to create multiple receptors. The adaptive immune system, as defined by rearranging antigen receptor genes in the immunoglobulin superfamily and by the major histocompatibility complex, has only been found in the jawed vertebrates (gnathostomes). The mechanism of recombination-activating gene (RAG)-mediated rearrangement exists in all jawed vertebrates, but the organization and structure of immunoglobulin (Ig) genes, as they differ among fish and fish species, reveal their capability for rapid evolution. Recombination among these loci created hybrid genes, the strangest of which encodes variable (V) regions that function as part of secreted molecules and, as the result of an ancient translocation, are also grafted onto the T-cell receptor [66]. Other groups of proteins that belong to the immunoglobulin superfamily with a common ancestral origin are FcγR and immunoglobulin-like receptor (KIR) which have an important function in the infection inflammatory response and the alteration of which is associated with autoimmune-type responses.

3.1. Autoantibodies, Autoantigens. The antibodies directed against their own structures (autoantibodies) have a primary role in autoimmunity being pathogenic in diseases caused by an attack on cell or tissue antigens (autoantigens), or in immune complex-mediated diseases. Several factors are implicated in this deviation from the primary role of the antibodies humeral immunity: (1) the formation of a repertoire of B cells that attack their own structures as they do not do adequate receptor editing or negative selection, (2) the recognition of autoantigens as foreign because they are similar to the structures of microorganisms (molecular mimicry), or (3) the possibility of not adequately removing immune complexes. The autoantigens may be from different sources.

3.1.1. Protein Structures That Are Present in the Organs and Which Are "Visible" to the Immune System. These molecules may have structural or functional roles and are usually common among mammals. Humans and mice share most of their genome. The passive immunization of mice with antibodies from human patients with autoimmune diseases can reproduce components of the diseases.

3.1.2. Proteins That Are Hidden in the Tissues and by Factors Such as Trauma Begin to Be Recognized by the Immune System as Foreign. The system starts processes to eliminate them with innate mechanisms initially and after that with acquired immunity mechanisms. An example of this condition is the sympathetic ophthalmia (SO) where breaching of systemic ocular barriers compromises the relative immune privilege of the eye and causes sensitization to previously sequestered uveoretinal antigens [67]. A similar mechanism is observed in relapsing polychondritis which begins with cartilage trauma and is triggered by an immune response to other cartilaginous or noncartilaginous tissues [68].

3.1.3. Even Though There Are Proteins That Are Not Present in the Human Structure, the Genetic Information Is Present. These genes are ancestrally repressed and hidden. As a result of different causes, they start the protein synthesis and this "foreign" protein is attacked by the immune system. An example is the endogenous retrovirus (ERV) which belongs to the large family of retrotransposable elements of human genoma [69]. ERV may have originated from an exogenous retrovirus that integrated ancestrally into the genoma and became trapped owing to mutations of essential genes and is transmitted genetically in the classical Mendelian form [70]. ERV is strand RNA viruses with a mode of replication in which the RNA genome is transcribed into DNA by reverse transcriptase. ERV may be activated by radiation, bacteria, chemicals, or recombination with an exogenous retrovirus [71, 72] and starts the "autoantigen" protein synthesis that is the source for autoimmune processes implicated in the pathogenesis of SLE [73]. Another mechanism is the biological effect of a viral product. For example, certain components derived from endogenous retroviruses pl5E present in several species, including murine, feline, and human, induce the immune abnormalities observed in SLE lymphocytes [74].

3.1.4. Ancestral Proteins Become Visible to the Immune System Through Diverse Stimuli Because They Are Trying to Perform Functions That Are no Longer Present in Our Species. This concept is part of an "atavist" hypothesis of pemphigus vulgaris based on the mechanism of shedding in reptiles [75, 76].

3.2. Classes I and II MHC. In vertebrates, allorecognition depends on proteins encoded by MHC genes. An MHC-like region is certainly very ancient and is believed to be present in the common ancestor of proto- and deuterostomes [77]. The function of MHC is to present antigens to T cell receptors. It has been proposed that the MHC region arose as a result of chromosomal duplications. In higher vertebrates, MHC is represented by two distinct classes, MHC I and MHC II. In the intracellular processes, the participation of chaperons and transporter molecules is necessary. The most likely hypothesis is that the ancestral MHC molecule had a class II-like structure which later gave rise to a class I molecule [78, 79].

3.2.1. Class I MHC. This is widely distributed and is expressed on most nucleated cells. As a general rule, antigens generated within the cell (endogens) are processed in the cytosolic pathway, transported with class I MHC molecules to the plasma membrane of most nucleated cells and presented to T-cells. Class I MHC requires proteasomes for antigen generation. These structures are phylogenetically ancient as they are found both in bacteria and eukaryotes. Peptides generated by the proteasome in *Drosophila* and yeast, which lack MHC, are rerouted towards a new biochemical pathway *via* the peptide transporters. Agnathans lack the ability to produce immunoproteasomes [80].

3.2.2. Class II MHC. Antigens taken up by phagocytosis (exogenous) are processed in the endocytic pathway, transported with class II MHC molecules, and presented in the membrane of macrophages, dendritic cells, and B cells. They are assembled within the rough endoplasmic reticulum where they associate with a glycoprotein called invariant chain (Ii, CD74) which prevents premature binding of any endogenously derived peptides. Afterwards, CD74 is digested by cathepsins S and L in order to free the binding fragment (CLIP). The removal of CLIP and peptide loading requires HLA-DM, an endosome-resident accessory molecule. In mammalian B cells, peptide loading is further modulated by another molecule, HLA-DO. The invariant chain glycoprotein, CD74, is found only in the gnathostome vertebrates. Several cathepsins seem to have been associated with class II MHC for peptide presentation several times during evolution at the level of exogenous peptide processing and processing of CD74 [81]. Class II MHC polymorphisms have been studied and their presence is a risk factor for various autoimmune diseases, for example, HLA DRB1*04 in RA, HLA DRB1*0301 in SLE o HLA DR1*0301-DQB1*0201 in SS [82]. Sometimes the risk is caused by the combination of polymorphisms (haplotypes) such as the 8.1 ancestral haplotype [83]. The presence of these molecules is associated with the common genesis of several autoimmune diseases (autoimmune tautology) [84]. In other mammals, similar associations have been shown [85].

4. Influence of the Third Form of Evolutionary Memory in the Autoimmune Phenomena

Memory related to neuronal function in relation to autoimmune phenomena is a crucial factor for storing diverse experiences during life.

4.1. Neuroimmunoendocrine Network. An emotion such as stress triggers endocrine responses which, in turn, affect the immune system and cause its activation and inappropriate response in the setting of autoimmune and infectious diseases. Many retrospective studies found that a high number of patients reported uncommon emotional stress before disease onset. Other studies suggest that stress is not only a participating factor but may also be a cause of disease exacerbations. Unfortunately, it is a vicious cycle because stress not only causes disease but the disease itself causes considerable stress in patients [86]. Neuroendocrine hormones triggered during stress may lead to immune dysregulation or altered or amplified cytokine production thus resulting in autoimmune diseases. Various types of transmitter substances in the neuroendocrine-immune network include epinephrine, norepinephrine, acetylcholine, substance P, vasoactive intestinal peptide, glucagon, insulin, cytokines, and growth factors. The stress response and induction of a disregulation in the cytokine balance can trigger the hypothalamic-pituitary-adrenal axis and sympathetic nervous system [87].

4.2. Neuron-Glial Relationship. Another type of information stored in the brain is related to the experience of pain. Somatic pain induces an immediate response that generates the reflex which moves the injured body part and prevents an increase in the damage. A second form of pain is the subacute or chronic somatic pain that is caused by the rest of the affected area during the repair process. Another form of pain is the neuropathic pain which is due to the persistent response that is generated after an injury to peripheral nerve structures [88]. In this condition the microglia, which are the resident macrophages of the brain and spinal cord, plays an important role. It seems that the nerve injury activates the receptor TLR4 which is only expressed in the central nervous system on microglia. Genetically altered mice lacking TLR4 showed markedly reduced microglial activation after nerve injury as well as reduced sensitivity to pain [89]. Another candidate trigger for microglial activation after nerve injury is fractalkine, a CX3C chemokine, expressed on the surface of neurons [90]. But, how does microglia excite sensory neurons? Many researchers have suggested that proinflammatory cytokines secreted by activated microglia increase neural excitability and sensitivity to pain when injected into the spinal cords of rats. It is possible that many brain functions allocated exclusively to neurons are dependent on a neuron-microglia interaction. Ontogenetically both microglia and

other glial cells are important in the process of migration and location of neurons in the central nervous system and are also closely related to their control electrolyte and nutrient supply [91]. Not only glial cells but also neurons respond to the presence of several cytokines [92]. The concept of integrated neuroendocrine-immune regulation has now been widely accepted although the physiological impact on normal development and homeostasis or conditions of mental stress or physical disease are still poorly understood [93]. The actions of cytokines via their receptors may also have physiopathological implications in other conditions such as multiple sclerosis, Alzheimer's disease, and AIDS dementia syndrome [94, 95].

4.3. Role of IL-1 and IL-1Rs. A well-known example is the role of IL-1 in the stress-axis, the hypothalamis-pituitary-adrenal (HPA) axis in mammals [96]. This axis is activated by inflammatory cytokines like IL-1 through the release of the corticotrophin releasing hormone (CRH) and proopiomelanocortin (POMC)-derived adrenocorticotropic hormone (ACTH) from the hypothalamus and anterior pituitary gland, respectively [97, 98]. Upon the release of ACTH, corticosteroid production, and release from the adrenal cortex will be stimulated which, in turn, causes redistribution of leukocytes, inhibition of antibody production and release of inflammatory cytokines. IL-1R family members are encoded on the X chromosome and they are present in multiple teleost orthologues as well as in chickens. In mammals, they are abundantly expressed in the hippocampal memory system of the brain and therefore might contribute to brain development or function [99]. Interestingly, mutations in this loci are responsible for a hereditary form of mental retardation [100, 101].

5. Influence of the Fourth Form of Evolutionary Memory in the Autoimmune Phenomena.

The fourth form of mankind's evolutionary memory is culture, and with this, there are many conditions that are related to the genesis of autoimmune phenomena. Humans who have changed to an urban environment have undergone changes in their exterior stimuli which their biological structure cannot be adequately adapted to given the limited period for that adaptation.

5.1. Changes in the Diet. Dietary modifications for humans that led to protein-calorie malnutrition or otherwise to weight excess have been assessed and taken into account when seeking nutritional factors associated with development of autoimmunity [102, 103]. The lack of exposure to various antigens in early childhood, including the newly born and those growing up in an "aseptic," environment results in a very low exposure to antigens required for the development of cell-mediated immunity (leading to better and safer protection) and, at later stages, requires an antibody-mediated protection which can lead to allergy and autoimmunity phenomena [104].

5.2. Changes in Exposure to Ultraviolet (UV) Radiation. It is well known that industrialization decreases exposure to sunlight with the consequent development of vitamin D deficiency, now a well-studied factor related to autoimmunity [105]. At the other extreme, the overexposure to sunlight due to working conditions or the cultural practice of changing the color of the skin (tanning) increases UV radiation. This is related to the development or exacerbation of cutaneous lupus and SLE by different mechanisms [106, 107].

5.3. Effects Related to Jobs and Habits. Some jobs are related to risks of SLE. For example, school teachers are exposed to many viruses that can be the basis for an abnormal immune response in a genetically predisposed individual [108]. Various habits such as smoking [109] or exposure to smog-related particles [110] are also associated with a similar outcome.

5.4. Effects of Exposure to Drugs. With the evolution of human culture, pharmacology also developed and diverse drugs could induce autoimmune phenomena through various factors including epigenetic ones (Example: procainamide, hydralazine, Chlorpromazine, isoniazid, phenytoin, and penicillamine) [111]. Women have a greater predisposition to developing autoimmunity in part as a result of the effect of estrogen, which, when used for therapeutic or contraceptive reasons, increases the risk [112].

5.5. Effects of Migrations and Social Aspects. It has been postulated that older factors such as migration and exposure for long periods of time to different forms of environment were determining factors for the development of different human races which have different risks for the development of autoimmune diseases [113–116]. More recently there has been a genetic mixing of races due to migration and risk conditions for developing diseases have changed for each race. Race can also be modified without requiring migration; for instance, by religious or political influences that encourage the choice of partner for reproduction in order to ensure a race with certain characteristics which are more related to beauty and purity [117]. Human society has the same dynamic processes as nature, which has a tendency to maintain a status of "entropy" within constant "chaos" in physicochemical terms, or constant "changes" in socioanthropological terms [118].

6. Conclusions

One way to understand autoimmunity is through knowledge of the biological significance of evolution. Since a specialized system for defense against microorganisms was set up, living things have theoretically been vulnerable to developing autoimmune phenomena. The existence of different regulatory mechanisms can only be explained as strategies that were developed to avoid these phenomena of self-destruction. The human species have a cumulative evolution of the most complex mechanisms of innate and acquired immunity which makes it very vulnerable to autoimmunity

especially when the regulatory mechanisms fail. Other risk factors are not inherited and are the product of the evolution of the brain and culture in the human species.

Conflict of Interests

The authors declare no conflict of interests.

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Research Article

The Autoimmune Tautology: An In Silico Approach

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There is genetic evidence of similarities and differences among autoimmune diseases (AIDs) that warrants looking at a general panorama of what has been published. Thus, our aim was to determine the main shared genes and to what extent they contribute to building clusters of AIDs. We combined a text-mining approach to build clusters of genetic concept profiles (GCPs) from the literature in MedLine with knowledge of protein-protein interactions to confirm if genes in GCP encode proteins that truly interact. We found three clusters in which the genes with the highest contribution encoded proteins that showed strong and specific interactions. After projecting the AIDs on a plane, two clusters could be discerned: Sjögren's syndrome—systemic lupus erythematosus, and autoimmune thyroid disease—type1 diabetes—rheumatoid arthritis. Our results support the common origin of AIDs and the role of genes involved in apoptosis such as *CTLA4*, *FASLG,* and *IL10*.

1. Introduction

There are clinical and genetic grounds for assuming similar immunogenetic mechanisms in autoimmune diseases (AIDs). Clinical evidence highlights the cooccurrence of distinct AIDs within members of a nuclear family and within an individual [1]. Individuals with a multiple autoimmune syndrome (MAS) have been grouped into three basic groups in which various AIDs cluster around one of three "main" AIDs, namely, systemic lupus erythematosus (SLE), autoimmune thyroid disease (AITD), and primary Sjögren's syndrome (SS). These three might be considered the "chaperones" of the other AID [2]. Along the same line of clinical evidence, there are therapies such as tumor necrosis factor inhibitors, rituximab, or a gluten-free diet that are already proving effective for more than one AID [3, 4]. With regards to genetic evidence, it has also been stated that around 44% of the single nucleotide polymorphisms (SNPs), which were found in genome-wide association studies (GWAS) on AIDs, are shared by two or more of the following diseases: celiac disease, Crohn's disease, psoriasis, multiple sclerosis (MS), rheumatoid arthritis (RA), type 1 diabetes (T1D), and SLE [5].

There are also genetic differences among AIDs. In spite of sharing several susceptibility genes, the differences among most AIDs, in particular systemic ones such as SLE and RA, seem to reside in the contribution of each gene to each disease [6]. Additionally, clusters of AIDs have been described where SNPs that make an individual susceptible to one class of AIDs also protect from another class of AIDs [7]. Furthermore, it is already known that different AIDs are associated with some different alleles from the human leukocyte antigen (HLA) [6].

As a consequence, it is important to obtain a general panorama of the problem in order to understand the origin of the AIDs. However, in biomedical research, the amount of experimental data and published scientific information is overwhelming. Therefore, literature-based discovery (LBD) tools emerge as useful to make the biomedical literature accessible for research purposes [8]. Thus, different LBD methods have been used to mine large amounts of literature and find the necessary information (Table 1) [8–11] with

Table 1: Examples of literature-based knowledge discovery tools.

Tool	Mined data	URL
ANNI	MedLine	http://www.biosemantics.org
Arrowsmith[1],	MedLine, OVID	http://wiki.uchicago.edu/
	UMLS concepts in	
Arrowsmith[2]	title words (MedLine)	http://arrowsmith.psych.uic.edu/
BITOLA	MeSH and LocusLink	http://www.mf.uni-lj.si/bitola/
LitLinker	UMLS	http://litlinker.ischool.washington.edu/
FACTA	MedLine	http://refine1-nactem.mc.man.ac.uk/facta/
FAUN	MedLine	https://grits.eecs.utk.edu/faun/

1 University of Chicago
2 University of Illinois at Chicago
For more information about biomedical text mining tools visit
http://arrowsmith.psych.uic.edu/arrowsmith_uic/tools.html.

two main approaches in the biomedical domain [12]. One approach focuses on the extraction of precise relationships between concepts, and the other relates biomedical concepts one to each other based on the statistical properties of their occurrence and cooccurrence in literature. A known LBD method based on concept occurrence is the concept profile (CP), in which a concept is characterized by a list of associated concepts, together with weights that indicate the strength of the association [13].

The output of the concept profiling method is a list of associations ordered by the strength of their relationship that needs verification. It is typically done with domain-relevant knowledge usually based on expert human judgments or even experimental validation [8, 14]. The latter approach is currently more feasible in the biomedical field given the increase in experimentally identified binary interactions between proteins that has made it possible to see how these components come together to form large functional regulatory networks [15]. There are several network approaches [16] that could be organized based on the type of biological or molecular interactions [17] and that analyze diverse databases (Table 2) [18–24]. Thus, the information related to protein-protein interactions helps us to study these associations from the perspective of biochemistry, signal transduction, and biomolecular networks [25]. Identification of functional roles of unknown pathogenic genes can also make it possible to understand pathogenic mechanisms. Proteins that are tightly connected in biological networks often work in similar processes [26].

This complex panorama shows that we are still distant from knowing everything, that is to know about genes, their interactions with other genes, and their impact on biological functions [6]. Therefore, the aim of this study was to obtain information from the literature and annotated databases to find main common genes in autoimmunity and determine to what extent they contribute to different clusters of AIDs.

2. Methods

Our analysis was made by using experimental knowledge of protein-protein interaction to evaluate the top ranked genes, which had been found through the CP approach to mine the biomedical literature (Figure 1).

2.1. Literature-Based Knowledge Discovery. The concepts selected as input for the LBD software were the three AIDs referred to as chaperones of autoimmunity (i.e., AITD, SS, and SLE). We also selected as input concepts the AIDs mentioned in literature as present in relatives of probands of these three diseases: MS, RA, T1D, vitiligo (VIT), and systemic sclerosis (SSc) [2].

To evaluate the genetic similarity of those AIDs, we chose the Anni software because it uses the CP methodology that has proven to be effective for finding information in the form of associations in the biological domain [27]. First, the mapping of those concepts in the thesaurus of the Anni software that uses the concept profile methodology was evaluated [28]. At this point, we eliminated the VIT concept because it showed ambiguity in mapping. Next, the CP for each one of the seven remaining AIDs was built. Those profiles corresponded to the weighted list made by all the genes mentioned in MedLine, so they were called genetic CPs (GCPs). To do this, we selected the 25.010 genes that belong to human beings from the thesaurus in Anni, and, then, we mined all the MedLine records that contained these genes in their text. Next, the associations between GCP were explored through hierarchical clustering. The clusters were generated by matching the GCP for each one of the mapped AIDs, as the CP can be described as vectors. Then, the similarities between the GCP in the found clusters were analyzed. For this purpose, we obtained a cohesion score by using as an inclusive filter for matching the described 25.010 genes. Briefly, the cohesion score is an average of the inner products of all possible pairs of profiles corresponding to the concepts in the group of interest. The contribution of each gene in the profile to the cohesion score was assessed in terms of percentage. To interpret the cohesion score we used a P value that gives the probability that the same score or higher would be found in a random group of the same size. This P-value was obtained by using the default parameter in Anni of 200 iterations. Finally, the distances between concepts that reflect the matching value between GCPs were projected in

Table 2: Examples of tools to analyze biological pathways.

Tool	Analyzed data	URL
Cytoscape	220 diverse databases.	http://www.cytoscape.org/
BIANA	uniprot, GenBank, IntAct, KEGG and PFAM.	http://sbi.imim.es/web/BIANA.php
Pathway studio	MedLine.	http://www.ariadnegenomics.com/products/pathway-studio/expression-analysis/algorithms
Patika	Reactome, UniProt, Entrez Gene, and GO.	http://www.patika.org/
Genes2networks	BIND, DIP, IntAct, MINT, pdzbase, SAVI, Stelzl, vidal, ncbi hprd, and KEGG mammalian	http://actin.pharm.mssm.edu/genes2networks/

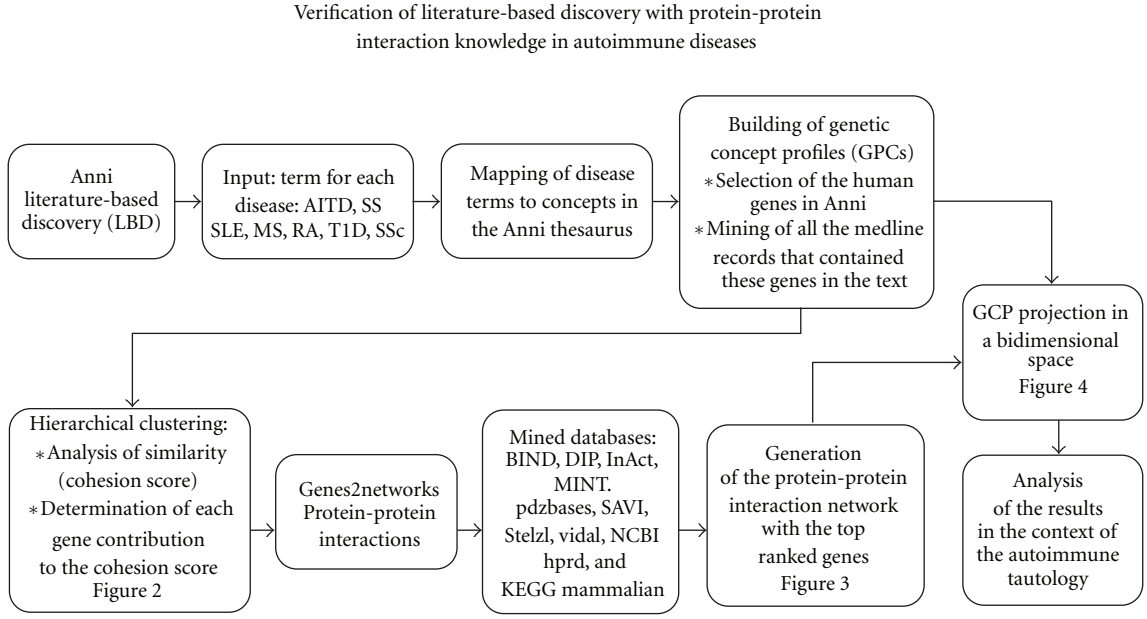

Figure 1: Flowchart of the methodology. AITD: autoimmune thyroid disease, SS: primary Sjögren's syndrome, SLE: namely systemic lupus erythematosus, MS: multiple sclerosis, RA: rheumatoid arthritis, T1D: type 1 diabetes, and SSc: systemic sclerosis.

a two-dimensional space, in order to understand the AID clustering.

2.2. Network Analysis. To analyze if the genes in the clusters previously found through LBD corresponded to proteins with a known interaction, a network analysis was done with the genes that contributed at least 0.1% to any of the clusters found by the method described in Section 2.1. For this purpose, the software, Genes2networks, was selected because it finds relationships between proteins by using ten high quality mammalian protein-protein interaction databases that take into account not only filtered high throughput but also low throughput experiments that have a lower probability of false positives [29]. Then, in order to find tightly connected proteins, the settings that were used in Genes2networks to build the network were (1) no filter for minimum number of references, (2) the maximum links per reference were four, (3) a maximum pathway length of two, and (4) a significant Zscore of 2.5 of the intermediate nodes, which was calculated through a binomial proportions test, as previously described [29].

2.3. Systematic Search. We did a classical systematic search, as previously done by our group [30], to understand the relevance of the genes found by our approach on AIDs. The genes selected were ones that contributed more than 1% to two or more clusters of AIDs and were close to each other in subnetworks where they were separated by a maximum of one node. To do this, we did a systematic search of the Catalog of Published Genome-Wide Association Studies at http://www.genome.gov/26525384 and on PubMed by using three terms: the gene name, the MeSH term "genome-wide association study" and the MeSH term for each AIDs that belonged to the found clusters. Consequently, the terms for the AIDs were chosen from the next MeSH terms: "arthritis, rheumatoid," "multiple sclerosis," "diabetes mellitus, type 1,"

Table 3: Genes with a contribution higher than 0.1% to the found clusters of the studied autoimmune diseases.

Cluster 1. SLE-SS		Cluster 2. T1D-AITD		Cluster 4. RA-MS	
Gene	%	Gene	%	Gene	%
TRIM21	27.91	*TPO*	32.4	*TNF*	39.5
TNFSF13B	27.46	*CTLA4*	28.6	*HLA-DRB1*	20.7
TROVE2	19.8	*TNFRSF25*	6.7	*IL10*	5.2
SSB	6.6	*HLA-DRB1*	6.7	*IL6*	2.2
FAS	2.7	*PTPN22*	6.4	*CCL2*	0.6
DLAT	2.6	*GAD1*	4.6	*CD4*	0.6
IRF5	1.0	*GAD2*	3.6	*MMP9*	0.6
IL10	0.9	*AIRE*	1.7	*IL1B*	0.5
FASLG	0.8	*PTPRN*	1.5	*IL4*	0.5
TNFRSF25	0.6	*HLA-DQB1*	0.5	*TNFSF13B*	0.5
CR1	0.5	*IDDM2*	0.5	*IL23A*	0.4
CALR	0.5	*SUMO4*	0.5	*CCR2*	0.4
SPTAN1	0.4	*ICA1*	0.4	*IL1RN*	0.4
RNPC3	0.4	*FOXP3*	0.3	*CCL5*	0.3
CR2	0.2	*FCRL3*	0.2	*ICAM1*	0.3
SNRNP70	0.2	*CD4*	0.2	*CXCR3*	0.3
SERPIND1	0.2	*FASLG*	0.2	*HLA-DQB1*	0.3
C1QA	0.2	*CXCL10*	0.2	*VCAM1*	0.2
IL18	0.2	*CD8A*	0.2	*CTLA4*	0.2
IL6	0.2	*IL1B*	0.2	*PADI4*	0.2
		TSHR	0.2	*IFNB1*	0.2
				CRP	0.2
				CCR5	0.2
				IL12B	0.2

SLE: systemic lupus erithematosus, SS: Sjögren's syndrome, T1D: type 1 diabetes, AITD: autoimmune thyroid disease, RA: rheumatoid arthritis, MS: multiple sclerosis, %: percentage of contribution to the cluster.

"lupus erythematosus, systemic," "scleroderma, systemic" and "Sjögren's syndrome." In the case of thyroid disease, the term "thyroid" was used. The information from PubMed was excluded when the retrieved information did not explicitly refer to the specific gene, for instance when CD4 referred to a type of cell (i.e., lymphocyte) but not to the gene.

3. Results

There were three paired clusters with a probability equal to or less than 3 percent that their cohesion score would be found in a random group of the same size: SLE with SS ($P = 0.02$), T1D with AITD ($P = 0.02$), and RA with MS ($P = 0.03$) (Figure 2). Regarding the genes that contributed to building the clusters, 55 of them had a contribution higher than 0.1% to the cohesion score of any of those clusters. Some of them were shared by more than one cluster: *HLA-DQB1, CD4, TNFSF25, FASLG, IL1B, IL6, IL10, TNFSF13B, CTLA4 and HLA-DRB1*. The later three had a contribution higher than 20% to any of the three specific clusters. The other genes contributed to only one cluster. It should be mentioned that there were also specific genes for one cluster that had a contribution of around 20% to their clusters, such as *TRIM21* and *TROVE2* in the cluster made up of SLE and SS, *TPO* in the cluster made up of T1D and AITD, and *TNF* in the cluster made up of RA and MS (Table 3).

Concerning to the network analysis, we used as input the previously mentioned 55 genes. 29 of these 55 entries were identified and described on the graph (Figure 3). Some genes such as *IL6* and *HLA-DRB1* did not appear in the network. This could have been because of the strict threshold, a maximum pathway length of two, established to avoid weak interactions or because they did not have protein-protein interactions already reported in the used database. For instance, some genes relating to antigen presentation such as *HLA-DRB1* may be absent in protein interaction networks.

The network had 20 intermediary nodes, 19 significant with a Z score above the cutoff of 2.5 (Table 4), thus indicating that they may be specific to interact with components from the inputted seed list of genes. In other words, those results indicated that the seed genes encode proteins that had strong and specific interactions. In the graph, it can also be seen that the genes common to more than one cluster belonged to the same connected network (Figure 3). There were two subnetworks of genes that had

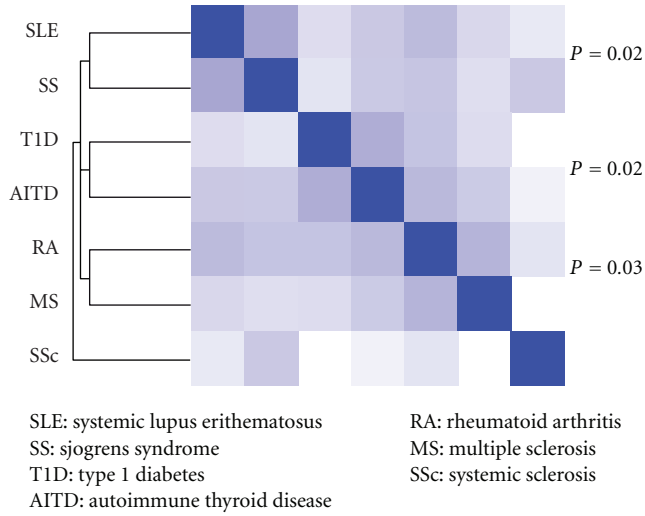

Figure 2: Clustering of seven autoimmune diseases. SLE: systemic lupus erithematosus, SS: Sjögren's syndrome, T1D: type 1 diabetes, AITD: autoimmune thyroid disease, RA: rheumatoid arthritis, MS: multiple sclerosis, SSc: systemic sclerosis.

Table 4: Significance of intermediates sorted by z-score.

Gene name	Link	Link in background	Links to seed	Links in subnetwork	z-score
HLA-DQA2	3	11429	2	60	15,852
DARC	4	11429	2	60	13,692
LCK	67	11429	6	60	9,548
PRTN3	9	11429	2	60	9,007
APCS	10	11429	2	60	8,522
FN1	62	11429	5	60	8,215
IGFBP7	11	11429	2	60	8,103
PTPN13	12	11429	2	60	7,737
CASP1	18	11429	2	60	6,215
A2M	24	11429	2	60	5,293
DCN	25	11429	2	60	5,171
NCL	30	11429	2	60	4,655
C3	31	11429	2	60	4,566
JAK2	116	11429	4	60	4,356
PTPRC	35	11429	2	60	4,248
THBS1	37	11429	2	60	4,108
ARRB1	44	11429	2	60	3,690
TRADD	63	11429	2	60	2,910
PIK3R1	133	11429	3	60	2,761
FYN	153	11429	3	60	2,457

a contribution higher than 0.1% and that were shared by more than one cluster. The first was made up of *HLA-DQB1*, *CD4*, *CTLA4* and *FASLG* that were genes connected through only one internode (*TNFRSF25* is also connected through three internodes with *FASLG*) and the second subnetwork was made up of *IL1B* and *IL10* that was connected to TNF, the gene with the highest contribution to the cluster made by RA and MS. There was also another subnetwork made with the directly connected *C1QA, CR1, and CR2* genes that belonged to the cluster made by SLE and SS (Figure 3).

We also observed that some of the genes with a contribution higher than 0.1% to only one cluster belonged to three little separate networks. The first little network had the genes *GAD1* and *GAD2* from the cluster of T1D-AITD, the second

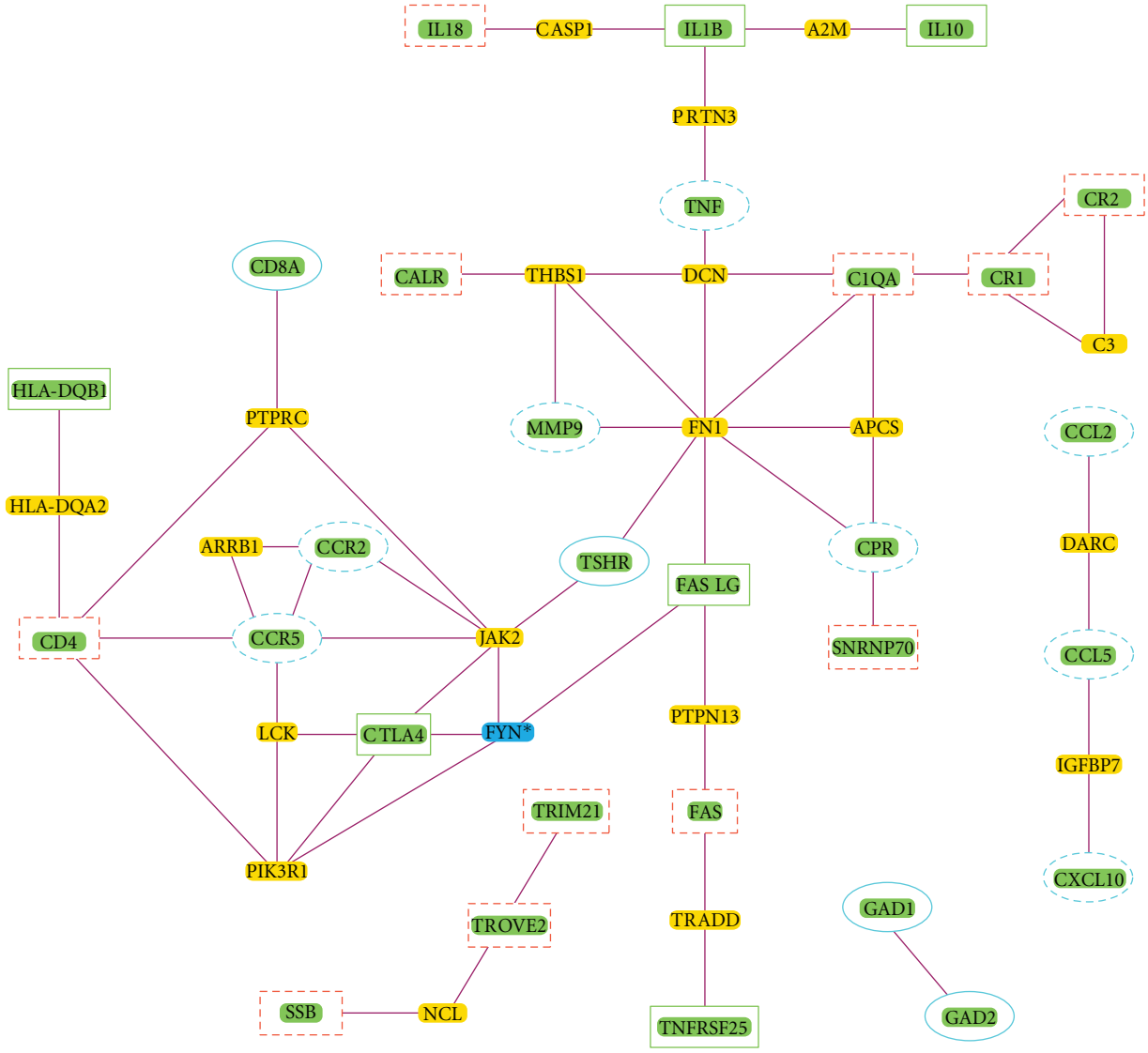

Figure 3: Network analysis of the genes that contribute to the clusters of autoimmune diseases. Solid squares: genes with a contribution higher than 0.1% that are shared by more than one cluster. Dotted squares: genes with a contribution higher than 0.1% from the SLE-SS cluster. Solid ovales: genes with a contribution higher than 0.1% from the T1D-AITD cluster. Dotted ovales: genes with a contribution higher than 0.1% from the RA-MS cluster. The other nodes correspond to significant intermediary ones (the asterisk indicates a nonsignificant intermediary node).

had the sgenes *TRIM21*, *TROVE2,* and *SSB* from the cluster of SLE-SS, and the third had the genes *CCL5* and *CCL2* from the cluster RA-MS (Figure 3).

Through the systematic search, we looked for GWAS information on six genes (Table 5). *HLA-DQB1* [31], *CTLA4* [32, 33], and *FASLG* and *IL10* [34] were related to AIDs in GWAS. In contrast, to date *CD4 and IL1B* have not been related by GWAS data to any of the above-mentioned AIDs.

Finally, according to the distances obtained through the LBD approach, the evaluated AIDs were projected into two main spaces that are near each other. The first included SS and SLE, and the second, AITD, T1D, and RA. Both were distant from SSc and a little closer to MS, especially in the case of the RA (Figure 4).

4. Discussion

Our *in silico* approach that combined LBD and network analysis of protein-protein interactions allowed us to confirm common genes involved in autoimmunity as well as to estimate their contribution into the clusters of AIDs. Some common genes made an important contribution to only one specific cluster such as *TRIM21*, *TROVE2,* or *SSB*, but others were present in more clusters of AIDs such as *HLA-DQB1*, *FASLG*, *CTLA4,* or *CD4*. However, our approach did not intend to find all the genes shared among AIDs. In fact, not all the genes could be validated through protein-protein interactions, and others did not make a significant contribution to the described clusters of AIDs.

Table 5: Relevance on autoimmunity GWAS of the genes with a contribution higher than 1% to two or more clusters of the studied autoimmune diseases.

Gene	Full name	Location	GWAS catalogue	Reference
HLA-DQB1	Major histocompatibility complex, class II, DQ beta 1	6p21.3	MS, PBC, RA, SSc, CD, UC, CrD	[31]
CD4	CD4 molecule	12pter-p12	—	—
CTLA4	Cytotoxic T-lymphocyte-associated protein 4	2q33	T1D, RA, MS, SLE, CD	[32, 33]
FASLG	Fas ligand (TNF superfamily, member 6)	1q23	CD, CrD	—
IL1B	Interleukin 1, beta	2q14	—	—
IL10	Interleukin 10	1q31-q32	T1D, SLE, UC, CrD	[34]

MS: multiple sclerosis, PBC: primary biliar cirrhosis, RA: rheumatoid arthitis, SSc: systemic sclerosis, CD: celiac disease, CrD: crohn disease, T1D: Type 1 diabetes, SLE: systemic lupus erithematosus, UC: ulcerative colitis, PSO: Psoriasis.

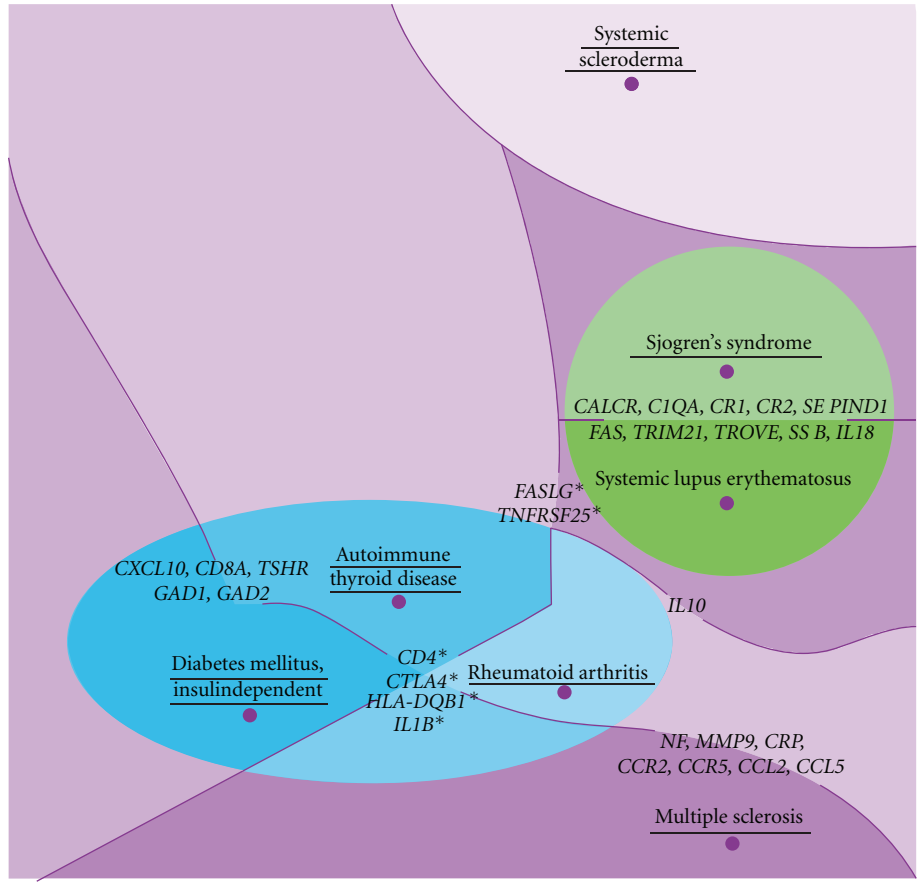

Figure 4: Projection of the seven studied autoimmune diseases on a plane. This figure shows the shared space of the genetic concept profiles from the studied AIDs (underlined), according to the matching value of their genetic concept profiles. We can see the genes with a contribution to clustering higher than 0.2%, the asterisk indicates the genes shared by two clusters.

With regards to genes shared by more than one cluster of AIDs, it can be seen that they were typically found to be significant in GWAS. However, there were exceptions. In the case of *CD4*, an association was not found with any AID by GWAS, but another approach that combines biological similarities found that *CD4* is a likely causal gene of RA [35], one that had been seen as high risk by recent studies [36, 37]. In contrast to GWAS, the genes that were found to be related to RA by the approach that combines biological similarities could be easily classified into related functional categories or biological processes [35], thus making these finding similar to our results.

In contrast, there were genes that contributed mainly to specific clusters of AIDs such as *TRIM21* (*Ro52*), *TROVE2* (*Ro60*) and *SSB* (*La*) that were found to be important for the SLE-SS cluster. In spite of the fact that they were not significant at the GWAS level, this observation agreed with the fact that anti-SS-A (Ro52/Ro60) autoantibodies have

been described as serological markers for both SS and SLE [38–40]. Ro52 works as an E3 ligase and mediates ubiquitination of several members of the interferon regulatory factor (IRF) family. Its deficiency has been associated with enhanced production of proinflammatory cytokines that are regulated by the IRF transcription factors including cytokines involved in the Th17 pathway [41]. Although Ro ribonucleoproteins such as Ro60 and La were discovered many years ago, their function is still poorly understood [42]. It has been suggested that *TROVE2* acts as a modulator in the Y RNA-derived miRNA biogenesis pathway. The hypothesis is that Ro RNPs are "latent" pre-miRNAs that can be converted into miRNAs under certain circumstances [42]. In addition, it was observed that narrow-band ultraviolet B irradiation provoked significant alterations of the keratinocyte morphology and led to the membrane expression of antigens recognized by anti-La and anti-Ro 60 kDa sera [43].

Another observation about genes that contributed mainly to specific clusters was that genes typically involved in one AID such as C1QA and CR1 in the case of SLE, or GAD1 and GAD 2 in the case of T1D, were found by our approach to be shared with SS or AITD, respectively. These findings agree with the observations that around 24% of patients with T1D expressed antithyroid autoantibodies and that 17% of them had AITD in comparison to 6% of age-matched controls [44].

The projection of the AIDs on a plane agreed with the similarity between genetic variation profiles of T1D and AITD found by another approach, which builds genetic variation profiles taking into account *P* values and odds-ratios of significant SNPs in GWAS, but does not totally agree with the claimed opposition between MS and RA [7]. It can be seen that RA has some similarity with MS in spite of being closer to AITD. This projection also agreed with the behavior of HLA, even in admixed Latin-American populations, as diseases that were closer in it shared risk alleles. This is the case for SLE, SS, and T1D that have the *DRB1*03:01* allele as a risk factor [30, 45, 46]. Furthermore, in diseases that are distant in our clustering analysis, such as MS and T1D, the same *DQB1*06:02* allele gives protection to the first but risk to the second disease [47].

From the biological perspective, our results showed the central role of *FASLG* as it is connected through one node to *CTLA4*, which is connected to *CD4* through one node and that, in turn, is connected to *HLA-DQB1* the same way (Figure 3). *FASLG* is also connected with *TNF* through two nodes, and this is connected, in turn, through one node to *IL1B*, which is also connected through one node to *IL10* and *IL18*. It is interesting that these two pathways are involved in similar processes since *CTLA4,* and *IL10* are implicated in peripheral immunologic tolerance [48]. *FASLG* is also connected to two other pathways. It is connected through one node to *C1QA,* which is directly connected to *CR1*. Lastly, it is also indirectly connected to the pathway of *TROVE2, TRIM21,* and *SSB* through a route that was not shown on the graph. This route involved *SUMO1*, a gene that has been associated with a blockage of the FAS pathway in RA, thus preventing apoptosis [49]. Taken together, our results highlight the autoimmunity role of genes involved in the process of apoptosis such as *CTLA4*, *FASLG,* and *IL10* that work together with genes involved in the inflammatory process such as *IL1B* [50].

Biomedical informatics involves a core set of methodologies that can provide a foundation for crossing the "translational barriers" associated with translational medicine [51]. Since the classical systematic review of literature could be incomplete because a significant amount of conceptual information present in literature is missing from the manually indexed terms [10], it seems to be advisable to combine the classical approach for searching literature with these new techniques.

In summary, the bioinformatics approach that combines text mining and network analysis of proteins allowed functional modules of interacting disease genes to be identified and can be used to predict additional disease gene candidates. Our approach also gave further evidence of the common origin of AIDs as the clustering of these diseases took into account thousands of genes that contribute to make the genetic concept profiles. Furthermore, this mining approach identified the specific contribution of a number of genes to causing some AIDs to cluster. These genes could be useful for further research.

Abbreviations

AIDs: Autoimmune diseases
AITD: Autoimmune thyroid disease
CP: Concept profile
GCP: Genetic concept profiles
GWAS: Genome-wide association studies
HLA: Human leukocyte antigen
IRF: Interferon regulatory factor
LBD: Literature-based discovery
MAS: Multiple autoimmune syndrome
MS: Multiple sclerosis
RA: Rheumatoid arthritis
SLE: Systemic lupus erythematosus
SNPs: Single nucleotide polymorphisms
SS: Primary Sjögren's syndrome
SSc: Systemic sclerosis
T1D: Type 1 diabetes
VIT: Vitiligo.

Conflict of Interests

The authors declare that they have no conflict of interests.

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Review Article

Introducing Polyautoimmunity: Secondary Autoimmune Diseases No Longer Exist

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Similar pathophysiological mechanisms within autoimmune diseases have stimulated searches for common genetic roots. Polyautoimmunity is defined as the presence of more than one autoimmune disease in a single patient. When three or more autoimmune diseases coexist, this condition is called multiple autoimmune syndrome (MAS). We analyzed the presence of polyautoimmunity in 1,083 patients belonging to four autoimmune disease cohorts. Polyautoimmunity was observed in 373 patients (34.4%). Autoimmune thyroid disease (AITD) and Sjögren's syndrome (SS) were the most frequent diseases encountered. Factors significantly associated with polyautoimmunity were female gender and familial autoimmunity. Through a systematic literature review, an updated search was done for all MAS cases (January 2006–September 2011). There were 142 articles retrieved corresponding to 226 cases. Next, we performed a clustering analysis in which AITD followed by systemic lupus erythematosus and SS were the most hierarchical diseases encountered. Our results indicate that coexistence of autoimmune diseases is not uncommon and follows a grouping pattern. Polyautoimmunity is the term proposed for this association of disorders, which encompasses the concept of a common origin for these diseases.

1. Introduction

Autoimmune diseases (ADs) have particular clinical characteristics and phenotypes depending on their nature (i.e., organ specific or systemic diseases). However, there is strong evidence that ADs share several clinical signs and symptoms, physiopathological mechanisms, and environmental and genetic factors, and this fact indicates that they have a common origin [1], which has been called the autoimmune tautology.

The clinical evidence of the autoimmune tautology highlights the cooccurrence of distinct ADs within an individual (i.e., polyautoimmunity) [1]. In an earlier paper, we described the foremost systematic literature review grouping all published cases of multiple autoimmune syndromes (MAS), defined by the presence of three or more well-defined ADs in a single patient, up until 2006. Initially, MAS was first mentioned by Pirofsky and Vaughn [2] and deeply described by Humbert and Dupond [3]. They provided a taxonomy for the cooccurrent phenotypes [4, 5]. MAS together with polyglandular autoimmune syndromes (PAS) II through IV, which are all MAS, represent the best example of polyautoimmunity [4]. Three basic, large clusters were found. Each of them had a predominant disease that was named the "chaperones" of autoimmunity, namely, autoimmune thyroid disease (AITD), Sjögren's syndrome (SS), and systemic lupus erythematosus (SLE). Study of the literature and clinical observation led to a similar clustering nomenclature which included the thyrogastric cluster and lupus-associated cluster [6, 7].

This coexistence of ADs in a single individual has lead researchers to consider different terms like autoimmune diathesis [8] or kaleidoscope of autoimmunity [9] both of which point to a common genetic background of ADs [10, 11]. The genetic basis of autoimmune clustering can depict part of the patterns of clustering across the spectrum of the implicated diseases [6].

Polyautoimmunity is also important for the current discussion because it may influence on the severity of ADs. In fact, some authors argue that there is a more severe presentation of a particular AD when polyautoimmunity is present [12–14], while others have found no influence or even a better prognosis in such cases [15–17].

In order to demonstrate one of the edges of autoimmune tautology, this study describes the presence of polyautoimmunity in four cohorts of ADs and analyses the main factors associated with its presence. In addition, an update of MAS cases is presented.

2. Material and Methods

2.1. Study Subjects. Four previously published series of ADs patients were evaluated. All of them had cross-sectional designs analyzing the presence of polyautoimmunity in patients with SLE [18], rheumatoid arthritis (RA) [19], multiple sclerosis (MS) [20], and systemic sclerosis (SSc) [21]. All the patients were recruited from a multicenter cohort of ADs patients followed at the Center for Autoimmune Diseases Research (CREA) at the Universidad del Rosario in Bogota, Colombia. Patients fulfilled the American College of Rheumatology (ACR) criteria for the classification of SLE, SSc, and RA [22–24] and McDonald's criteria for MS [25]. The institutional review board approved the study design.

Each patient was evaluated by a rheumatologist or a neurologist depending on the case. The information on patient demographics and cumulative clinical and laboratory data was obtained by physical examination, interview, and chart review. All data were collected in an electronic and secure database.

There were 23 ADs investigated in the cohorts based on international validated criteria including autoimmune adrenal insufficiency (AAI: Addison's disease), alopecia areata (AA), autoimmune hepatitis (AIH), AITD, antiphospholipid syndrome (APS), biliary inflamatory disease including primary sclerosing cholangitis and primary biliary cirrhosis (BID), celiac disease (CD), demyelinating autoimmune diseases (DAD) including transverse myelitis (TM) and MS, dermatomyositis, polymyositis (DM/PM), inflamatory bowel disease including ulcerative colitis and Crohn disease (IBD), myastenia gravis (MG), pernicious anemia (PA), pemphigus (PF), psoriasis (Pso), RA, relapsing polychondritis (RePo), sarcoidosis (Sar), SS, SSc, SLE, type 1 diabetes mellitus (T1D), vasculitis (Vas), and vitiligo (VIT).

The presence of familial autoimmunity, including the presence of the same group of ADs evaluated in the search for polyautoimmunity, was estimated by interviewing the patients and, in most of the cases, by clinical evaluation of the affected family members as previously reported [26]. First-degree relatives (FDR) were defined as parents and siblings.

2.2. Systematic Literature Review. An updated systematic literature review was done for all MAS cases reported from January 2006 to September 2011 (Figure 1). Publications were identified through a systematic search done by two independent experts in Pubmed. The only limits applied were Human. The [Majr] terms "multiple autoimmune diseases," "multiple autoimmune syndrome," "multiple autoimmune disease," "polyautoimunity," "co-occurrent," "co-occurrence," "coexistence," "overlap," "associated," "concurrent"; [Mesh] terms: diabetes mellitus, type 1; antiphospholipid syndrome; lupus erythematosus, systemic; arthritis, rheumatoid; arthritis, juvenile rheumatoid; arthritis, psoriatic; spondylitis, ankylosing; spondylarthropathies; Sjögren's syndrome; Churg-Strauss syndrome; giant cell arteritis; microscopic polyangiitis; cryoglobulinemia; polyarteritis nodosa; Wegener granulomatosis; scleroderma, localized; scleroderma, systemic; scleroderma, diffuse; scleroderma, limited; dermatomyositis; colitis, ulcerative; Crohn disease; inflammatory bowel diseases; anemia, pernicious; thyroiditis, autoimmune; Hashimoto disease; Graves disease; celiac disease; hepatitis, autoimmune; liver cirrhosis, biliary; cholangitis, sclerosing; myasthenia gravis; multiple sclerosis; myelitis, transverse; polychondritis, relapsing; Addison's disease; purpura, thrombocytopenic, idiopathic; psoriasis; sarcoidosis; autoimmune gastritis; alopecia areata; autoimmune pancreatitis; pemphigus vulgaris; pemphigus bulloso; pemphigus foliaceous; vitiligo; autoimmune anemia.

Finally, the systematic literature review up to 2006 was checked and updated. The previous and new cases were compiled into a new table (see Supplementary Material available online at doi:10.1155/2012/254319).

2.3. Statistical Analysis. The prevalence of coexisting ADs was figured out separately by individual. The difference in the proportion of the associated ADs between two index conditions was calculated by chi-square, multinomial test corrected by Yate's continuity depending on the case. The degrees of freedom and Cramer's V were calculated.

A multivariate analysis was done for each of the series (SLE, MS, SSc, and RA) to identify factors associated with polyautoimmunity using logistic regressions models adjusted for age, gender, and duration of disease. Adjusted odds ratios (AORs) were calculated with 95% confidence intervals (CIs). A P value of less than 0.05 was considered significant. The Hosmer and Lemeshow Goodness-of-Fit Test was applied [27].

A hierarchical cluster procedure analysis was done to identify relatively homogeneous subgroups of variables based on selected cases with MAS reported in the systematic review of the literature. The reported cases from the previous systematic review [4] were computed with the results of the updated search. The objective of this analysis was to find out which ADs agglomerate more frequently. The cluster method implemented [28] was Single Linkage Sneath and the measure of similarity was Matching Coefficient. SPSS (V17 for Windows, Chicago, IL) software was used for all the analysis.

3. Results

3.1. Polyautoimmunity Patients. The 1,083 individuals studied included 335 SLE, 304 RA, 154 MS, and 290 SSc patients. There were 373 patients with polyautoimmunity (34.4%).

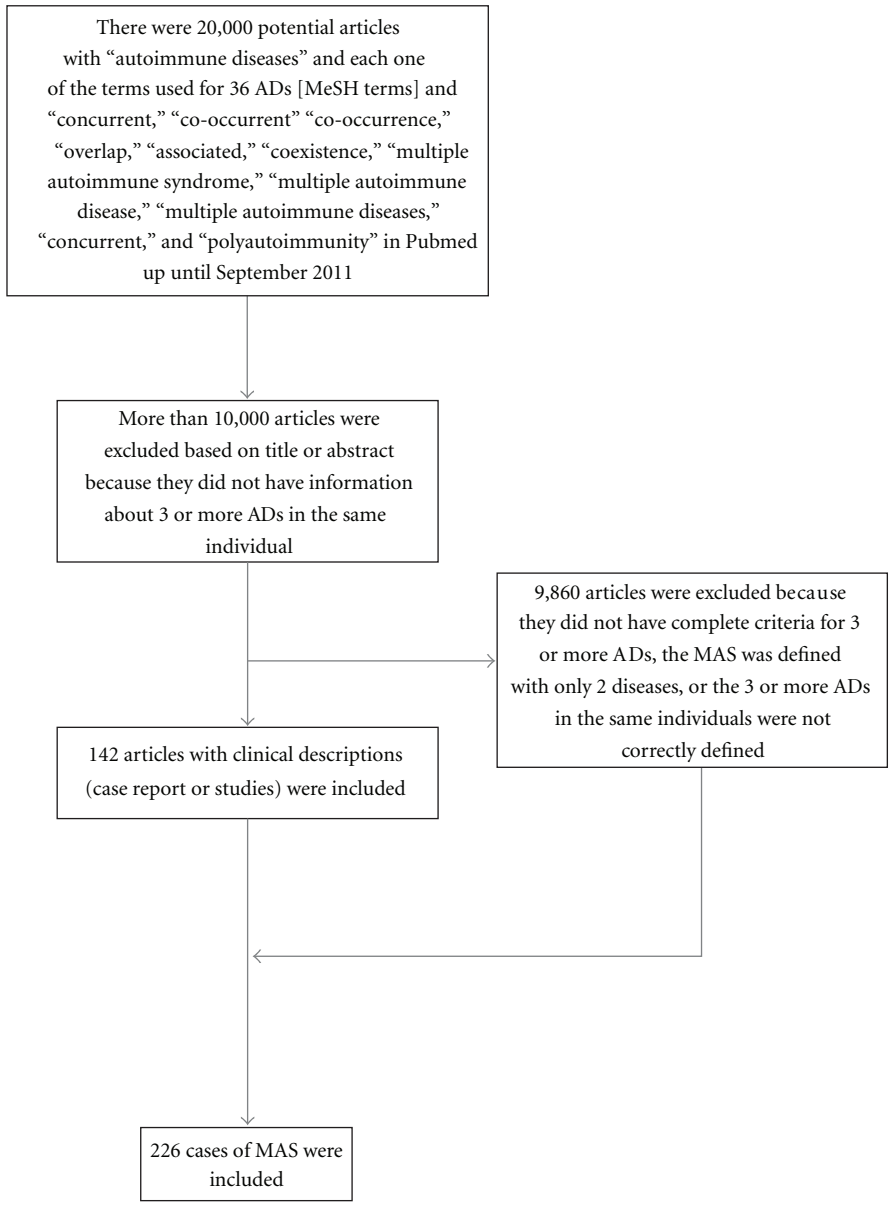

Figure 1: Flow chart of the systematic literature review. ADs: autoimmune diseases; MAS: multiple autoimmune syndrome.

The prevalence of polyautoimmunity was significantly different among the four ADs, being less frequent in MS (Table 1).

AITD was the most frequent coexisting AD and was associated with SSc in 23% ($N = 67$) of the cases, RA in 21% ($N = 64$), SLE in 17.9% ($N = 60$), and MS in 9.1% ($N = 14$). This was followed by SS which was associated with SSc in 14.8% ($N = 43$) of the cases, SLE in 14% ($N = 47$), RA in 11.8% ($N = 36$), and MS in 2.6% ($N = 4$). MAS was found in 11.6% ($N = 39$), 9.7% ($N = 28$), 5.3% ($N = 16$), and 1.9% ($N = 3$) of SLE, SSc, RA, and MS patients, respectively.

Factors significantly associated with polyautoimmunity are depicted in Table 2. Female gender was a shared factor that was significantly associated with polyautoimmunity in the four ADs. Familial autoimmunity was significantly associated with polyautoimmunity in SLE and SSc patients.

3.2. Systematic Literature Review. The flow chart for the systematic literature review and the articles included are shown in Figure 1. A total of 142 articles corresponding to 226 cases of MAS were included.

According to the dendogram (Figure 2), the most hierarchical AD in the MAS cases is represented by AITD followed by SLE and SS. Otherwise, the least representative diseases in the same context are juvenile chronic arthritis (JCA), ankylosing spondylitis (AS), and RePo. Although there were several articles about combined AIH and BID polyautoimmunity, the two were not close to each other

Table 1: Polyautoimmunity in 1,083 patients with four index autoimmune diseases.

	SLE		RA		MS		SSc		chi	df	*P*	Cramer's V
	N	%	*N*	%	*N*	%	*N*	%				
N	335	30.9	304	28.1	154	14.2	290	26.8				
Polyautoimmunity	136	40.6	98	32.2	21	13.6	118	40.7	40.81	3	0.001	0.19
AITD	60	17.9	64	21.1	14	9.1	67	23.1	14.63	3	0.0022	0.11
SS	47	14.0	36	11.8	4	2.6	43	14.8	16.4	3	0.0009	0.12
VIT					2	1.3						
APS	48	14.3	8	2.6					25.83	1	0.001*	0.2
Primary biliary cirrosis							15	5.2	—	—	—	—
MAS	39	11.6	16	5.3	3	1.9	28	9.7	17.99	3	0.0004	0.12

SLE: systemic lupus erythematosus; RA: rheumatoid arthritis; MS: multiple sclerosis; SSc: systemic sclerosis; AITD: autoimmune thyroid disease; SS: Sjögren's syndrome; VIT: vitiligo; APS: antiphospholipid syndrome; MAS: multiple autoimmune syndrome; chi: chi-square test; df: degree freedom; *P*: *P* value; *Yates chi-square.

Table 2: Significant factors associated with polyautoimmunity.

	SLE		RA		MS		SSc	
	AOR; CI95%	*P*	AOR; CI95%	*P*	AOR; CI95%	*P*	AOR; CI95%	*P*
Female gender	2.3; 1.03–5.15	0.043	1.8; 1.22–6.31	0.015	8.5; 1.02–70.8	0.048	9.08; 2.09–39.3	0.003
Familial autoimmunity	1.61; 1.14–2.28	0.007	NS		NS		2.62; 1.24–5.54	0.01
Articular Involvement	2.02; 1.26–3.23	0.003	NS		NE		NS	
Anti-Ro positivity	1.54; 1.10–2.16	0.013	NS		NE		NS	
Cardiovascular disease	NS		2.2; 1.17–3.94	0.014	NE		NE	
ANAs	NS		2.0; 1.08–3.84	0.027	NS			
SSEP	NE		NE		10.86; 1.31–89.6	0.027	NE	

SLE: systemic lupus erythematosus; RA: rheumatoid arthritis; MS: multiple sclerosis; SSc: systemic sclerosis; AOR: adjusted odd ratio; CI95%: confidence interval; ANAs: antinuclear antibodies; SSEP: somatosensory evoked potentials; NS: nonsignificant; NE: not evaluated.

on the dendogram nor did they show a suitable degree of agreement.

4. Discussion

Herein, we report one of the largest series of polyautoimmunity with an emphasis on its associated factors. Some authors had shown that so far the evidence suggesting that ADs tend to coexist within both individuals and families was anecdotal corresponding to the concept of autoimmune diathesis [29]. By grouping diverse ADs in the same patient (i.e., polyautoimmunity) including organ specific (i.e., MS) and systemic ADs, we have demonstrated that they are true associations as a part of the autoimmune tautology rather than the chance findings that were previous hypothesized [4].

Polyautoimmunity is a term that can group all the taxonomy terms referring to coexistence of well-defined ADs in a single individual because some of the terms previously used are confusing and exclude various associations. Polyautoimmunity was used by Sheenan and Stanton-King [30] for the first time while describing a patient with ITP, PA, AITD, SSc, pancreatic exocrine insufficiency, and CD before dying from vasculitic complications. The case they depicted corresponds to a typical MAS, which is already included in the term polyautoimmunity. Also, when patients fully develop two or more diseases simultaneously or sequentially, these diseases have frequently been classified as overlap syndromes; some of these were frequent enough to have been given names like rhupus and sclerodermatomyositis [31]. In another case, some authors have historically postulated that mixed connective tissue disease (MCTD) is a very homogeneous entity with shared clinical manifestations rather than shared diseases or autoantibodies [32, 33], while others have not. The existence of MCTD as a distinct disease entity has been a matter of controversy among researchers since it was first described [34, 35]. In fact, the coexistence of several sets of classification criteria for MCTD indicates how difficult it is to give a precise definition of the disease [33]. In addition, some patients will develop SLE, SSc, or RA, during the course of MCTD, and some will present with a longstanding MCTD [36]. In real-life conditions, searching for the specific phenotypes (antibodies and clinical) over the course of disease and constantly looking for associated ADs, including organ specific and systemic, are more useful for developing an exact description of polyautoimmunity than taxonomic discussions.

The fact that many ADs share a similar underlying pathology and have a tendency to cluster supports the involvement of shared susceptibility genes and similar molecular mechanisms. In fact, recent studies have identified several common genes associated with multiple ADs supporting the presence of autoimmunity genes as part of the autoimmune tautology [10, 37, 38].

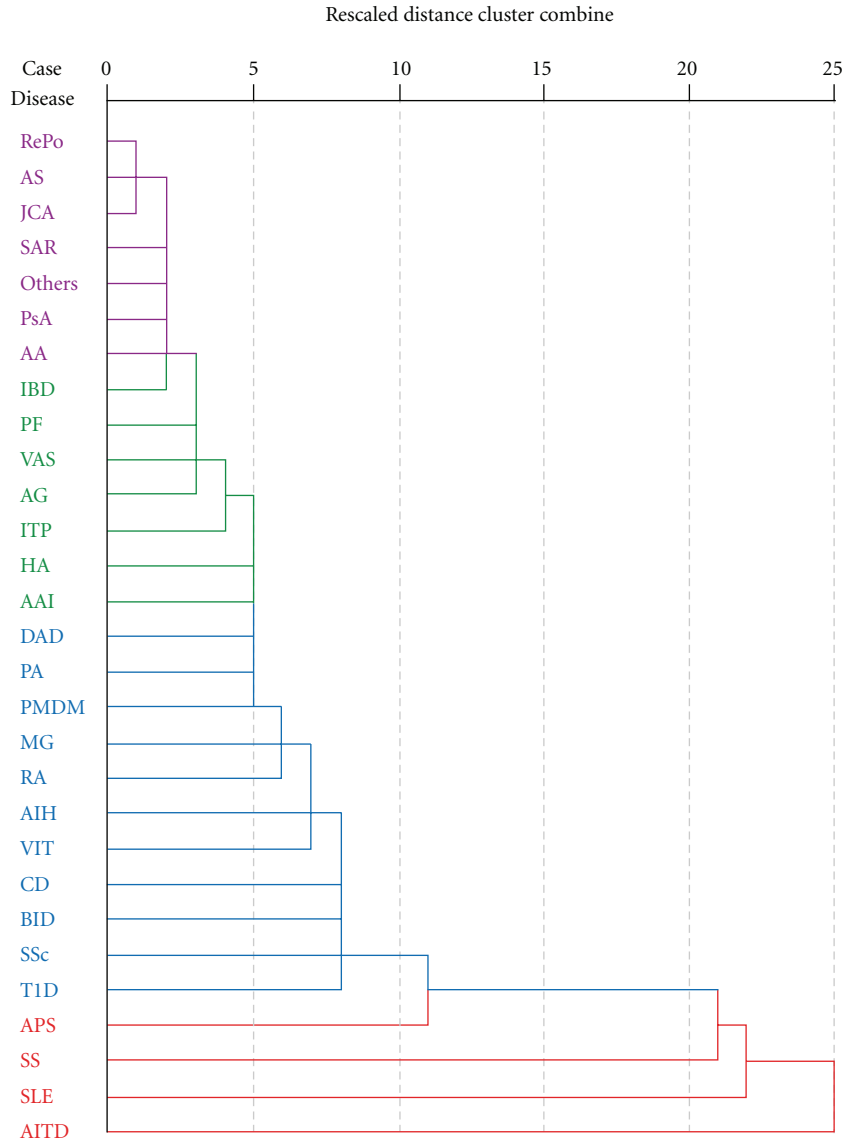

Figure 2: Cluster analysis dendogram. Each node represets a stage from the clustering process. There were four clusters. The most hierarchical was composed of four ADs. AITD: autoimmune thyroid disease (including thyroiditis, Hashimoto disease, Graves disease); SLE: systemic lupus erythematosus; SS: Sjögren's syndrome; APS: antiphospholipid syndrome; T1D: type 1 diabetes mellitus; SSc: scleroderma (including localized, systemic, diffuse, limited); BID: billiary inflammatory disease (including primary biliary cirrhosis, primary sclerosing cholangitis); CD: celiac disease; VIT: vitiligo; AIH: autoimmune hepatitis; RA: rheumatoid arthritis; MG: myasthenia gravis; PMDM: polymyositis/dermatomyositis; PA: pernicious anemia; DAD: demyelinating autoimmune diseases (including multiple sclerosis, transverse myelitis, optic neuromyelitis); AAI: autoimmune adrenal insufficiency (Addison disease); HA: autoimmune anemia; ITP: idiopathic thrombocytopenic purpura; AG: autoimmune gastritis; VAS: vasculitis (including Churg-Strauss syndrome, giant cell arteritis, microscopic polyangiitis, cryoglobulinemia, polyarteritis nodosa, Wegener granulomatosis); PF: pemphigus (including vulgaris, bulloso, foliaceous); IBD: inflammatory bowel disease (including ulcerative colitis, Crohn's disease); AA: alopecia areata; PsA: psoriasis (including psoriatic arthritis); SAR: sarcoidosis; JCA: juvenile chronic arthritis; AS: ankylosing spondylitis; RePo: relapsing polychondritis.

Familial autoimmunity and female gender were confirmed as risk factors for polyautoimmunity. Female gender was a shared factor associated with polyautoimmunity in the four index conditions here studied. This fact gives us a glimpse of one facet of the shared commonalities between ADs. The majority of ADs predominate in females [39] and constitute a leading cause of death among young and middle-aged women [40]. In searching for a reason behind female predominance, most attention has focused on hormonal changes while other factors have included genetic differences, both direct (i.e., influence of genes on sex chromosomes) and indirect such as microchimerism, as well as gender differences in lifestyle [39, 41]. Our results support previous studies including a meta-analytic approach demonstrating that most of the patients in 54 studies quantifying the coexistence of ADs among 4 selected ADs were female [29].

In addition, some authors have shown in specific ADs that women are distinguished from men by higher frequencies of concurrent immune diseases [42, 43].

AITD was the most frequent polyautoimmunity found in our series of 1,083 patients. This finding was supported by the analysis of the systemic literature review and depicted in the dendogram (Figure 2) where AITD was the main "chaperon" of autoimmunity. AITD has been described as the most prevalent AD as well as being associated with other organ-specific and non-organ-specific ADs [44].

Possible explanations for the relationship of these ADs include (a) immunomodulatory effects of antithyroid antibodies, (b) molecular mimicry between thyroid and disease-specific epitopes, and (c) a genetic link between antithyroid autoimmunity and the susceptibility to AD [45]. In population-based database studies, other authors have demonstrated that AITD is frequently associated with other ADs [46]. All of this information indicates that AITD is clinically important in the context of autoimmunity and it is mandatory for screening patients with hypothyroidism or hyperthyroidism symptoms for the autoimmune etiology when there is suspicion of the coexistence of AITD with another AD [44].

The prevalence of SS was demonstrated to be high and in fact the second most frequently associated AD in our series as well as in the MAS cases through the literature review. Many authors have recognized that it is quite difficult to categorize concomitant SS as primary or secondary, and there is disagreement about this issue in the literature [47]. Other authors believe that salivary changes in patients with an AD (i.e., SLE) might reflect a multisystem presentation of the disease [48]. Regarding the association of SS with other ADs, some authors have argued that the etiopathogenic mechanism for the simultaneous or sequential development of multiple ADs in one individual is not well understood [49]. The concept is more developed nowadays, and the idea that common genetic backgrounds and additional immunogenetic, environmental, or hormonal factors are responsible for the formation of subsets of AD clusters is becoming more established.

We previously evaluated the prevalence of SS in a large series of patients with SLE ($n = 969$) and the potential risk factors for this association [50]. SS patients fulfilled the American-European classification criteria (the presence of anti-Ro antibodies or a positive minor salivary gland biopsy was mandatory). There were 9.3% patients with SS, 42% had familial autoimmunity, of which 7% had familial SS as compared to 2% in the group of SLE without SS. The factors significantly associated with SS in SLE were familial SS, anti-La and anti-Ro antibodies, as well as pulmonary involvement. Anti-Sm antibodies and Colombian origin (i.e., ethnicity) were protective factors. Our results together with other series [51, 52] using similar strict classification criteria indicate that the prevalence of SS in SLE is close to 10%. SLE-SS appears to constitute a subgroup of patients with distinct clinical, serologic, pathologic, and immunogenetic features, in whom SS is expressed as an associated entity and is largely similar to what has been called primary SS [52]. Clinical and immunological factors observed in our study might serve as predictors for this association. Because variations in both additive and nonadditive genetic factors and the environmental variance are specific to the investigated population, family history of autoimmunity and patient origin are important characteristics to be considered.

While the currently most accepted classification criteria [53] designate these cases as "secondary" SS, the terms "overlapping" or "associated" SS are frequently used in the literature to describe them [49]. We consider these terms to be confusing and propose that SS always be taken into account and properly investigated in patients diagnosed with any AD because of the high possibility of the presence of the concomitant, well-defined phenotypes as demonstrated here in the context of polyautoimmunity or as demonstrated previously with a prevalence near to 10% in SLE patients when strict classification criteria are used.

It is of interest that the primary/secondary designation for classification of APS was introduced by rheumatologists who already used the primary/secondary terms to differentiate subgroups of patients with SS. In introducing the primary APS (PAPS) subgroup, it was unclear whether one would expect that the clinical features, disease course, or management of patients would be different based on their subclassification [54]. In fact, the international (Sydney) consensus statement on an update of the classification criteria for definite APS [55] advises against using the term "secondary" APS. They did not find differences in the clinical consequences of antiphospholipid antibodies among patients in these two categories (Evidence Level I). They state most patients with the so-called secondary APS have SLE. However, they discuss that it is unknown if APS and SLE are two diseases coinciding in an individual, if underlying SLE offers a setting for the development of APS, or if APS and SLE represent two elements of the same process [55]. Some cases with "secondary" APS are classified as lupus like disease (LLD). The Sydney consensus raised up that the interface between SLE, LLD, and APS merits further consideration. Finally, the consensus states that rather than distinguishing between patients with PAPS and secondary APS, documenting the coexistence of SLE (or other disease) is more advantageous for classification and that the disorder associated with APS, such as SLE, be listed; hence, one would report "APS associated with SLE" or "APS associated with rheumatoid arthritis" rather than "secondary APS" [54]. Studies showing no significant differences between PAPS and SAPS were cited. Patients with APS plus SLE and PAPS have similar clinical profiles, although heart valve disease, hemolytic anemia, low C4 levels, and neutropenia seem to be more common in patients with APS plus SLE [56].

Indeed evidence that there are any differences in presentation or course of PAPS versus SAPS is not persuasive [55, 56], and this has led to the suggestion that PAPS/SAPS designations be replaced by APS and "APS associated with the name of the autoimmune disease." It might suggest that such a distinction exists if there are differences in clinical complications, the timing of these complications, prognosis, or frequency of positive anticardiolipin, lupus anticoagulant, or other autoantibody tests. Studies that have addressed this question have found no difference in any of

these parameters [57, 58]. As an instance, some authors have compared intima-media thickness (IMT), arterial stiffness, and presence of plaques in APS patients and controls. A significant difference was found between IMT, arterial stiffness, and the presence of plaques in patients and controls, but no differences in these parameters were found between patients with primary APS and those with secondary APS [59]. Additional arguments have been raised for some authors [60, 61] who have explored the concept of an intermediate APS (having at least one but less than four of the 11 criteria for SLE) and did not found differences between PAPS, intermediate, and the so-called secondary APS when comparing the prevalence of the thrombotic or pregnancy manifestations.

The true prevalence of the development of PAPS in SLE will require decades of followup for this determination. The distinction between PAPS and SAPS can be difficult and at times seems a rather artificial convention [60]. It may be underestimated by some studies that have a follow-up period that is shorter than the interval between PAPS and SLE diagnoses noted in most case reports [60]. We agree on the proposition of the Sydney committee [55] against using the term "secondary" APS and encourage clinicians to follow adequately the patients and searching for specific phenotypic characteristic to classify patients as having polyautoimmunity.

Results with respect to the severity of the disease in patients with polyautoimmunity are not unanimous. In the case of associated SS, some authors have demonstrated as we did previously [17] that there is no influence on the course of the disease. Some have found that the appearance of SS in RA patients has no relationship with RA duration or activity [62]. Others demonstrated that the subset of patients with SLE and SS has a distinct clinical and laboratory phenotype with a lower frequency of renal disease and anti-dsDNA antibodies [63]. This has not been true for other examples of polyautoimmunity when there is a severe presentation of the diseases as is the case of associated ADs in MG patients with a severe presentation [12], a severe clinical onset of T1D and increased prevalence of other ADs in children with CD diagnosed before T1D [13], and a severe SLE compromise when associated with vasculitis [14].

In conclusion, we suggest searching for well-defined phenotypes by looking for clusters of ADs in the same individual. It is our contention that the term "secondary diseases" should not longer be used because it detracts from the reality that these patients have two or more well-established ADs sharing the same etiopathogenesis [64]. Our results indicate that coexistence of ADs is not uncommon and follows a grouping pattern. Polyautoimmunity is the term proposed for this association of disorders, which encompasses the concept of a common origin for these diseases.

Conflict of Interests

The authors declare no competing financial interests.

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Review Article

Autoimmunity in Rheumatic Diseases Is Induced by Microbial Infections via Crossreactivity or Molecular Mimicry

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A general consensus supports fundamental roles for both genetic and environmental, mainly microbial, factors in the development of autoimmune diseases. One form of autoimmune rheumatic diseases is confined to a group of nonpyogenic conditions which are usually preceded by or associated with either explicit or occult infections. A previous history of clinical pharyngitis, gastroenteritis/urethritis, or tick-borne skin manifestation can be obtained from patients with rheumatic fever, reactive arthritis, or Lyme disease, respectively, whilst, other rheumatic diseases like rheumatoid arthritis (RA), ankylosing spondylitis (AS), and Crohn's disease (CD) are usually lacking such an association with a noticeable microbial infection. A great amount of data supports the notion that RA is most likely caused by *Proteus* asymptomatic urinary tract infections, whilst AS and CD are caused by subclinical bowel infections with *Klebsiella* microbes. Molecular mimicry is the main pathogenetic mechanism that can explain these forms of microbe-disease associations, where the causative microbes can initiate the disease with consequent productions of antibacterial and crossreactive autoantibodies which have a great impact in the propagation and the development of these diseases.

1. Introduction

The exact triggering factor in most autoimmune diseases is unknown, yet an infectious cause has long been suggested to have an important role in the development of autoimmunity. Many epidemiologic and clinical reports show a prompt increase in the incidence of several immune-mediated disorders, such as rheumatoid arthritis (RA), inflammatory bowel disease (IBD), and primary biliary cirrhosis in the Western populations throughout the world. This rapid rise in the number of autoimmune diseases cannot be explained solely on the basis of genetic association, but also through the involvement of exogenous (environmental) factors predominantly in the form of microbial infections [1]. In this review, we are discussing the role of microbes in some immune-mediated rheumatologic disorders, such as rheumatic fever, Lyme disease, reactive arthritis (ReA), RA, ankylosing spondylitis (AS), and Crohn's disease (CD).

2. Interplay of Genetic and Environmental Factors in the Development of RA, AS, and CD

It is generally agreed that genetics form the main components of the aetiologic factors in the development of autoimmune diseases. For example, more than 95% of patients with AS possess HLA-B27, a class I major histocompatibility complex (MHC) gene, whilst its frequency among the general population is less than 10% [2, 3]. So far, this relationship is considered as the most powerful genetic-disease association holding true for many different populations [4]. Meanwhile, the frequency of HLA-B27 allelotypes in CD patients without associated arthritis is usually the same as in the normal population, but it is increased to up to 60% in those with involvement of the spinal joints [5].

In RA, however, class II MHC gene, HLA-DR4, is the most strongly linked genetic marker to this disease. The frequency of this allelotype has been found to be around 70% in RA patients but it is detected in less than 30% of the

general population [6]. A homologous molecular structure, consisting of a glutamic acid, glutamine, arginine, arginine, alanine, and alanine "EQRRAA" amino acid sequence (present in some subtypes of HLA-DRβ1 molecules, such as HLA-DRβ1*0401 and HLA-DRβ1*0404, but not in others such as HLA-DRβ1*0402) has been described in patients with RA and was given the name of "shared epitope" [7]. The frequency of the "shared epitope" was found to be increased to more than 90% in patients with RA [8].

The high associations between genetic haplotypes and these diseases cannot explain the considerably low incidence of these conditions among the relatives and even twins of patients with these diseases. For example, the concordance rate, the chance of the second twin of developing the disease, in monozygotic twins was found to be 40% in AS [9], 15% in RA [10], and less than 14% in CD patients [11], which indicates that other nongenetic environmental, probably microbial, factors are also involved in the aetiopathogenesis of these conditions.

3. Evidence for Associated or Preceding Bacterial Infections in Some Rheumatic Diseases

Extensive evidence supports the role for microbial infections in the development of various rheumatic diseases. The infection whether being associated with or preceding these conditions usually takes either an overt or occult form. Furthermore, these rheumatic disorders are usually associated with certain elements of autoimmune features in the form of elevated levels of autoantibodies to systematically distributed or organ-specific tissues. Certain examples of these microbe-triggered immune-mediated rheumatic disorders are discussed below.

3.1. Rheumatic Diseases Following Overt Bacterial Infections

3.1.1. Rheumatic Fever and Streptococcal Infections. Rheumatic fever is the prototype of postinfectious rheumatologic conditions following upper respiratory tract infections by group A beta hemolytic streptococcus pyogenes. This disease is considerably commoner in developing countries and its incidence may reach up to 50 per 100,000 [12]. Apart from involving the joints with a classical migratory polyarthritis, this condition is also characterized by other nonmusculoskeletal features which are induced by pathological lesions involving the heart (rheumatic carditis), and brain (Sydenham's chorea) [13]. Patients with rheumatic fever showing any of these clinical presentations are usually investigated for the evidence of previous infections by this microbe.

Apart from increased levels of anti-streptolysin O titres, serological analysis in these patients will also show elevated levels of auto-antibodies against the M protein and carbohydrate antigens which are crossreactive with the streptococcal antigens and expressed on the tissues of joints [14], heart [15], and basal ganglia in brain [16].

In a recent study, it has been found that passive immunization with anti-streptococcal exotoxin B monoclonal antibodies which also bind cardiac endothelial cells have caused IgG deposition, complement activation, and apoptotic cell death in the experimental mouse heart valve [17]. In the same study, it was shown that the binding ability of these monoclonal antibodies to the endothelial cells was blocked significantly by pretreatment with crossreactive amino acid peptide sequences taken from N-Acetyl-β-D-glucosamine, when conjugated with bovine serum albumin (BSA), but not with BSA alone.

3.1.2. Lyme Disease and Spirochetal Infections. Lyme disease or borreliosis, the most common vector-borne illness in the United States, is a multisystemic disorder caused by infections with spirochete microbes, *Borrelia burgdorferi*, and transmitted via Ixodes (deer) tick bites [18]. In this disease, different clinical stages with their probable explanations have been recognized [19]. Patients with Lyme disease are usually presented with or give a past history of localized skin manifestations in the form of well-characterized cutaneous rash and itching described as "erythema migrans" with or without a flu-like illness resulting from the tick bite. After a considerable period of time, patients with this illness show disseminated or wide-spread features due to the involvement of the cardiac, nervous, and musculo-skeletal systems as the result of infections by the causative microbes and/or their associated antimicrobial immune responses. The later phases of this illness, referred to as chronic Lyme disease and post-Lyme disease syndrome, are characterized by persistent arthritic and neurological features occurring as the result of the tissue damages induced by the effects of the cross-reacting antibodies to spirochetal and self-antigens [20, 21].

3.1.3. Reactive Arthritis and Its Association with Enteropathic and Uropathogenic Bacterial Infections. Reactive arthritis (ReA) is included as one component of a group of several inter-related but phenotypically different disorders that are collectively named as "spondylarthritis" (SpA) which comprises AS, psoriatic arthritis, undifferentiated SpA, and IBD consisting of two subsets; CD and ulcerative colitis [22].

It was in the early twentieth century when a link between infection and the occurrence of a triad of urethritis, conjunctivitis and arthritis (Reiter's syndrome) was discovered [23]. Reiter's syndrome was later recognized as a form of ReA which has commonly been associated with explicit preceding infections by enteropathic microbial agents including *Campylobacter*, *Salmonella*, *Shigella* and *Yersinia,* as well as the uropathogenic, *Chlamydia* bacteria [24].

In a most recent study from Finland, ReA was identified in 21 out of 45 referred patients suspected of having the disease after an extensive sewage contamination of the water supply system in the town of Nokia. Enteropathic microbial agents, including *Campylobacter*, *Yersinia,* and *Salmonella,* were isolated in 33% of these patients. These findings indicate that mere exposures to infections are not enough and the interplay of genetic and other susceptibility factors play a role in the disease pathogenesis [25].

The pathogenetic mechanism in this disease can be explained on the basis that secretory antibodies against these microbes which are produced in the gut are transferred into the joint spaces where they bind tissues expressing crossreactive self-antigens such as HLA-B27 molecules [26, 27].

3.2. Rheumatic Diseases Associated with Occult or Hidden Bacterial Infections

3.2.1. Evidence of Immunological, Molecular, and Microbiological Link between Proteus and RA. Since the mid 1980s, extensive efforts including many studies have emphasized a role of *Proteus mirabilis* microbes in the aetiopathogenesis of RA. Briefly, evidence for the role of *Proteus* in the initiation and development of RA can be summarized as follows.

(i) Rabbits injected with HLA-DR4-positive lymphocytes were found to produce antibodies which will only bind to *P. mirabilis* but not 18 other microbes [28].

(ii) Tissue-typing sera from pregnant women having anti-HLA-DR4 specificity were found to bind more significantly to *P. mirabilis* than to *E. coli* [29].

(iii) Molecular similarities were found between "ESRRAL" amino acid motifs present in the hemolysins enzyme products of *Proteus* microbes and "EQRRAA" molecular sequences present in HLA-DR4/1 haplotypes [30]. Furthermore, "IRRET" amino acid motifs expressed on surface antigens of *Proteus* urease enzymes were found to be homologous to "LRREI" molecular sequences present in type XI collagen fibres (Figure 1) [31].

(iv) Significant reciprocal bindings were detected between "EQRRAA" synthetic peptides and ESRRAL antisera raised in rabbits, and also between "ESRRAL" peptides with anti-EQRRAA antibodies from immunized rabbits. Furthermore, anti-ESRRAL peptide antibodies were found to bind preferably to mouse fibroblast transfectant cell line expressing HLA-DRβ1*0401, containing EQRRAA sequence, but not to HLA-DRβ0402, lacking EQRRAA sequence [32]. These results clearly indicate that antibodies to the "shared epitope" have tissue binding activity.

(v) IgG antibodies from patients with RA were found to have cytotoxic activities against HLA-DR4-peptide-bearing cells as shown by increased haemolysis for the sheep red blood cells coated with HLA-DRβ1*0404 peptides when compared to sera from AS and healthy control subjects [27].

(vi) Several independent groups have found that antibodies to *P. mirabilis* microbes were significantly elevated in patients with RA compared to those with other diseases or corresponding healthy subjects recruited from 15 different countries throughout the world (Table 1) [33, 34].

(vii) Evidence for the microbiological link between RA and *Proteus* microorganisms are mainly based on the findings of a group from Scotland, where the isolation rates of *P. mirabilis* bacteria from urine samples of patients with RA were found to be twice as high as that of *E. coli* [35]. A similar result was previously reported by our group from England, where *Proteus* microbes were isolated more significantly in female (63%) and male (50%) patients with RA than healthy women (32%) and men (11%) control subjects [36]. Moreover, urine samples from patients with RA were also shown to contain elevated levels of antibodies to *P. mirabilis* [35], and a positive correlation was found between the levels of these antibodies in sera and urine samples of RA patients [37]. Further evidence has come from results of previous studies, where patients with RA had increased incidence of urinary tract infections [38, 39].

These immunological, molecular, and microbiological findings support the notions that there is a crucial role for *Proteus* microorganisms in the initiation and perpetuation of RA. Furthermore, evidence exists which indicate that in RA *Proteus* infections usually occur in subclinical or asymptomatic forms [40].

3.2.2. Evidence of Immunological, Molecular, and Microbiological Link between Klebsiella and AS. The roles of *Klebsiella pneumonia* pathogens in the aetiopathogenesis of AS are mainly based on results of many studies which have been carried by several independent groups. These results can be summarized as follows.

(i) Sera from rabbits immunized with lymphocytes expressing HLA-B27 haplotypes were binding significantly to antigenic extracts of *Klebsiella* but not to those of other microbes [43]. Anti-HLA-B27 allogeneic human tissue typing sera were found to bind more preferably to *Klebsiella* microbes in comparison to other HLA-specific antisera [44].

(ii) HLA-B27 monoclonal antibodies were found to bind *Klebsiella*, *Shigella,* and *Yersinia* enterobacteria indicating the existence of some crossreactive antigens in these microbes [45]. Other anti-HLA-B27 monoclonal antibodies, however, were found to bind *Klebsiella* more preferably than *Shigella* and *Yersinia* microbial antigens [46].

(iii) Molecular similarities, comprising a hexameric amino acid sequence; glutamine, threonine, aspartic acid, arginine, glutamic acid, and aspartic acid, "QTDRED," have been found between *Klebsiella* nitrogenase reductase enzymes and HLA-B27 self-antigen molecules [47]. A quadrimeric homologous structure was also found to exist in both *Klebsiella* pullulanase pul-D secretion proteins, comprising; aspartic acid, arginine, aspartic acid, and glutamic acid, "DRDE" molecules and HLA-B27 haplotype, comprising aspartic acid, arginine, glutamic acid, and aspartic acid, "DRED" molecules, as well as

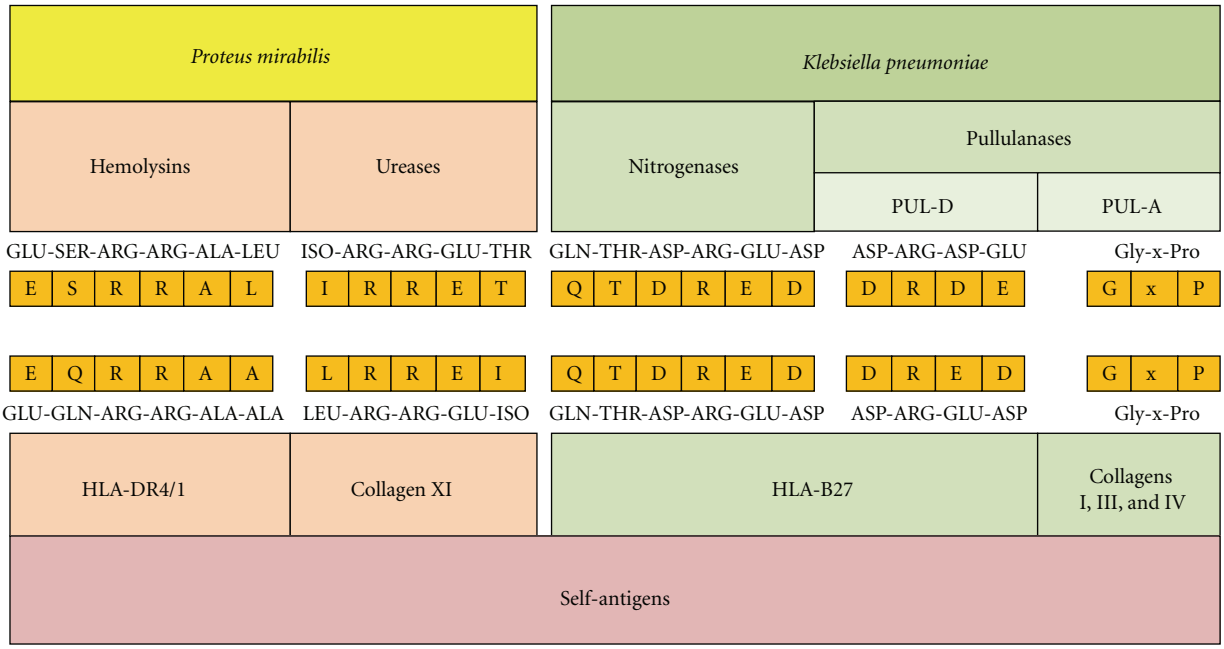

Figure 1: Schematic representation of the molecular similarities between bacterial, *Klebsiella* or *Proteus*, and self-antigens.

a similarity which involves repeated molecular motifs, consisting of glycine-x-proline (Gly-x-Pro) amino acid sequences present in both *Klebsiella* pullulanase Pul-A secretion proteins and collagen type I, III, and IV fibres (Figure 1) [48].

(iv) Antisera from immunized rabbits with *Klebsiella* were found to bind equally to B27-positive lymphocytes whether obtained from AS patients or healthy controls but not to lymphocytes taken from HLA-B27-negative individuals [49]. This indicates that there is no immunological discrepancy between surface antigens of both diseased and normal HLA-B27 molecules when treated with anti-*Klebsiella* antibodies.

(v) *Klebsiella* antibodies were significantly elevated in the serum compared to the synovial fluid of AS patients [50], indicating that these antibodies are produced in extra-articular regions such as the enteric mucosal lymphatic system before gaining entry into the joints.

(vi) Extensive immunological studies have been carried out during the last three decades by different independent groups throughout the world. The results of these studies indicate that antibodies against *K. pneumoniae* and/or crossreactive self-antigens but not against other microbial agents are significantly elevated among patients with AS when compared to patients with other diseases or to healthy individuals (Table 1) [41, 42].

(vii) IgG antibodies from AS patients were found to possess significant *in vitro* cytotoxic activities to HLA-B27 peptide-bearing cells when compared to RA patients or healthy subjects, when they showed increased percentage lysis of the sheep red blood cells coated with HLA-B27 peptides containing the crossreactive antigens [27].

(viii) Antibodies to *Klebsiella* nitrogenase "QTDRED-" containing peptides were found to bind the synovial tissues of AS patients more significantly when compared to those from patients with other rheumatic diseases [51].

(ix) Microbiological evidence for a link between *Klebsiella* microorganisms and AS are mainly based on the results of various studies, where increased isolation rates of *Klebsiella* from the bowel of AS patients have been reported to correlate with disease activity status [52–55]. Other groups, however, could not find such an association [56, 57]. These discrepancies in the results could be explained by the differences in the method of collection and culture of the faecal specimens and the disease activity status. Furthermore, in patients with AS elevated levels of IgA *Klebsiella* antibodies were found to be associated with a higher degree of gut inflammation [58] and the source of these bacterial antibodies were shown to be the jejunal region of the gut [59].

(x) HLA-B27 transgenic rats raised in a germ-free environment do not develop many features of SpAs, particularly the gut and arthritic lesions, which may indicate that the commensal gut flora plays an important role in the pathogenesis of B27-associated arthropathies [60].

The results of these studies together with those which have shown histological signs of inflammations [61, 62] and an increase in the gut permeability [63] in patients with

Table 1: Characteristics of bacterial immune responses in patients with rheumatoid arthritis and ankylosing spondylitis (see [33, 34, 41, 42]).

	Rheumatoid arthritis (RA)	Ankylosing spondylitis (AS)
Disease-triggering bacteria	*Proteus mirabilis*	*Klebsiella pneumoniae*
Bacterial antigens	Hemolysin; Urease	Nitrogenase; Pullulanase
Self-antigens*	HLA-DR4/1; collagen XI	HLA-B27; collagens I, III and IV
Antibody isotypes	IgG	IgA and IgG
Source of bacterial infections	Upper urinary tract	Large bowel
Bacterial isolations	*P. mirabilis* isolated more significantly from urine of active RA patients	*K. pneumonia* microbes are more abundant in the large bowel of active AS patients
Evidence for cytotoxic activities	*Proteus* antibodies are cytotoxic to cells coated with crossreactive self antigens	*Klebsiella* antibodies are cytotoxic to cells coated with crossreactive self antigens
Countries**	England; Ireland; Scotland; USA; Canada; France; Norway; Bermuda; Japan; Taiwan; India; Netherlands; Spain; Russia and Finland	England; Scotland; USA; Canada; Slovakia; China; Netherlands; Turkey; Japan; Finland; Sweden; Mexico; Germany; Taiwan; India; Russia; Spain
Microbial controls$^{\Delta}$	*Klebsiella, Escherichia, Yersinia, Salmonella, Chlamydia, Shigella, Pseudomonas, Campylobacters,* and viruses	*Proteus, Escherichia, Yersinia, Salmonella, Streptococci, Borrelia, Pseudomonas, Candida,* and *Campylobacters*
Disease controls$^{\Delta\Delta}$	AS, systemic lupus erythematosus, sarcoidosis, acute anterior uveitis, spondyloarthropathy	RA, psoriatic arthritis, osteoarthritis, reactive arthritis, systemic lupus erythematosus

* Self-antigens crossreactive with the corresponding bacterial antigens
** Countries with recruited cohort patients showing elevated levels of antibodies against *Proteus* (in RA), *Klebsiella* (in AS) as well as against the corresponding crossreactive self-antigens
$^{\Delta}$Microbial agents used as controls but showing no enhanced humoral immune responses in patients with RA or AS
$^{\Delta\Delta}$Disease controls showing normal immune responses to *Proteus* (in RA) and *Klebsiella* (in AS).

AS support the hypothesis that the main bacterial immune response involves mucosal immunity with signs of overt or more commonly occult or asymptomatic intestinal infections by *Klebsiella* microorganisms.

3.2.3. Evidence of Immunological and Microbiological Link between Klebsiella and CD. Evidence exists which supports direct and indirect roles for the environmental factors, mostly bacterial, and more specifically involves *Klebsiella* microbes in the pathogenesis of CD. These can be summarized as follows.

(i) Identical twins of patients with CD are less prone to develop the disease [11], indicating the role for an environmental factor in the development of this disease.

(ii) Increased incidences of CD have been reported among closely living friends of CD patients [64] as well as among small town populations in Southern Italy [65]. Furthermore, a slow increase in the incidence of CD was observed among migrants who are moving from a low to high risk area [66].

(iii) Pretreatment of mice with antibiotics has been shown to alleviate intestinal inflammation in experimental animal models [67, 68], which gives further support to the involvement of gut bacteria in CD.

(iv) Various independent groups have shown that antibodies to *Klebsiella* and/or to crossreactive collagen antigens were elevated more significantly in patients with CD when compared to the control groups [69].

(v) Evidence of the microbiological links of *Klebsiella* with CD has been mainly based on the isolation of these microbes from large bowel specimens in more than 25% of patients with CD [70]. Moreover, the disease relapses in patients with CD were found to be associated with *Klebsiella oxytoca* colitis [71].

It appears from these data results that unlike rheumatic fever, Lyme disease, and ReA, infections by *Proteus* and *Klebsiella* microbes in RA and AS/CD, respectively, usually occur in occult or subclinical forms, and that antibodies to the causative microbes and crossreactive self-antigens are detected frequently in active patients with these diseases when compared to control groups.

4. Exogenous (Microbial) and Endogenous Factors Are the Most Likely Causes of Autoantibody Productions in RA

It should be emphasized that apart from antibodies against the self-antigens, HLA-DR4/1, and collagen fibres, most if not all other autoantibodies such as rheumatoid factor (RF), anticyclical citrullinated protein/peptide antibodies (anti-CCP), and antineutrophil cytoplasmic antibodies (ANCA), which are commonly detected in patients with RA [72, 73], are most likely to be produced as the result of B cell stimulation by exogenous (microbial) agents and/or the effect of some endogenous enzymatic factors.

The interrelation between RF and RA could be explained on the basis of the following findings: firstly, RF can also be detected in increased levels in patients with various viral, bacterial, and parasitic infections [74]. Secondly, RFs can be

induced in the mice by polyclonal B cell stimulation with lipopolysaccharides [75]. Thirdly, RFs could be generated by immunization with collagen II antigen-antibody [76] and RF-like immune complexes [77] in experimental mice. Finally, RFs were found to disappear in patients with subacute bacterial endocarditis when the causative microbes, *Streptococcus*, were eradicated by antibiotic therapy [78].

Anti-CCP antibodies, which have been found in early cases of RA [79], can be linked to *Proteus* infections through the effects of peptidyl arginine deiminase (PAD) enzyme on the arginine-containing structures of *Proteus* hemolysin and urease antigens and the counterpart crossreactive HLA-DR4/1 and collagen XI self-antigens, with the production of citrulline-containing compounds which form the main antigenic components of citrullinated proteins [34, 80]. The association of RFs and anti-CCP antibodies with RA explains the existence of a positive correlation between increased levels of these antibodies and the presence of HLA-DR4/1 shared epitope [81] as well as the disease activity and severity in patients with RA [82]. Furthermore, anti-CCP antibodies have also been reported in patients with various microbial infections [83].

Unlike other autoantibodies, ANCAs were recognized in a lower proportion, usually in less than 25% [84, 85] of patients with RA. Apart from RA, however, these antibodies have also been reported in many other diseases, including microbial infections, especially when associated with systemic vasculitis [73]. Moreover, proteinase-3, which is considered as one of the predominant antigens that specifically binds to ANCA, is found to have similarities with some bacterial antigenic profiles [86].

5. Molecular Mimicry Hypothesis and Pathogenetic Mechanism in the Development of Microbe-Triggered Rheumatic Diseases

Although the avidity of the interactions between antigenic determinants and specific antibodies is considerably high, these antigen-binding sites can allow epitopes of similar shapes expressed on completely different microbial or animal cells to bind these antibodies, albeit with a lower binding avidity. These so-called crossreactive epitopes are made up of essentially the same amino acid and carbohydrate molecules, and such crossreactions are in fact common and may account for the undesirable production of antibodies against self-molecules which occurs in some autoimmune diseases.

Molecular mimicry or crossreactivity hypothesis proposes that an exogenous substance, mostly produced or possessed by infectious agents, may trigger an immune response against self-antigens. According to this theory susceptible individuals acquire an infection by a microbial agent that has antigenic similarity to self-antigens. As the result, these pathogen-specific antibodies bind to the host structures possessing crossreactive self-antigens and cause tissue damage and disease.

Molecular mimicry has been linked to the pathogenesis of several important diseases, such as rheumatic heart disease [87], multiple sclerosis [88], and type 1 diabetes mellitus [89]. In rheumatic fever carditis, for example, the basic pathogenetic process involves production of antibodies against *Streptococcus* which express high levels of M protein antigens, a molecule that shares structural similarities with those found in the heart valves and endocardial membrane. If antibodies to these bacterial proteins reach high levels, there may be sufficient binding to the host cells possessing these cross-reactive antigens with activation of the complement system and induction of the pathological damages at these sites.

The mechanism of molecular mimicry, however, can also be used in the explanation for the development of RA, AS, and CD after infections by the causative microbes. In AS, for example, after a preliminary gut mucosal activation by *Klebsiella* microbes and production of the secretory anti-*Klebsiella* IgA antibodies, recurrent bouts of subclinical *Klebsiella* infections in the large bowel of susceptible individuals carrying HLA-B27 will lead to production of increased levels of *Klebsiella* IgG antibodies. When the level of these antibodies reaches a certain limit, they will be able to activate the classical cascade of complement system and destroy tissues via the effect of end products of the complement components, mainly C8 and C9, "membrane attack complex." At the same time, certain activated complement components such as C3a and C5a help in the propagation of the inflammatory process through recruitment (chemoattraction) and activation of the neutrophils and phagocytes with the release of cytotoxic and destructive enzymes by these cells. Other chemoattractants, such as leukotriene B4, can be released by the autoantibody targeted cells. Inflammatory cells are further activated by binding to autoantibody Fc regions and fixed complement C3 fragments on the tissue cells, thus causing further tissue injury via effects of the products of activated inflammatory cells.

There is a requirement for the presence of high levels of anti-*Klebsiella* IgG antibodies in order that classical complement cascades will be activated and this will occur in patients with AS mainly during the active phases of the disease [90, 91]. The same pathogenetic process can also be applied to RA being caused by recurrent bouts of *Proteus* asymptomatic urinary tract infections.

6. Conclusions

The aetiopathogenetic mechanism which plays a major role in the causation and development of one group of autoimmune diseases involves interplay between the genetic and environmental factors. Microbes form an important part in the disease causations in most immune-mediated rheumatic diseases, such as rheumatic fever, ReA, Lyme disease, RA, AS, and CD. Molecular mimicry is considered as the basic mechanism which leads to the development of these diseases, in genetically susceptible individuals, where the causative microbe triggers formation of antimicrobial antibodies which could bind the crossreactive self-antigens and cause tissue damages via the effects of activated complement system and the cytotoxic products from recruited inflammatory cells.

Conflict of Interest Disclosure

The authors declare no competing financial interests.

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Clinical Study

Spondyloarthropathies in Autoimmune Diseases and Vice Versa

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Polyautoimmunity is one of the major clinical characteristics of autoimmune diseases (ADs). The aim of this study was to investigate the prevalence of ADs in spondyloarthropathies (SpAs) and vice versa. This was a two-phase cross-sectional study. First, we examined the presence of ADs in a cohort of patients with SpAs ($N = 148$). Second, we searched for the presence of SpAs in a well-defined group of patients with ADs ($N = 1077$) including rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and Sjögren's syndrome (SS). Among patients with SpAs, ankylosing spondylitis was observed in the majority of them (55.6%). There were two patients presenting with SS in the SpA group (1.4%) and 5 patients with autoimmune thyroiditis (3.5%). The global prevalence of ADs in SpAs was 4.86%. In the ADs group, there were 5 patients with SpAs (0.46%). Our results suggest a lack of association between SpAs and ADs. Accordingly, SpAs might correspond more to autoinflammatory diseases rather than to ADs.

1. Introduction

Spondyloarthropathies (SpAs) are a group of interrelated diseases with joint inflammatory involvement such as arthritis (axial and peripheral) and extraarticular involvement such as uveitis, enthesitis, psoriasis, and inflammatory bowel disease (IBD). This group of diseases is characterized by familial aggregation, absence of rheumatoid factor, and association with human leukocyte antigen (HLA)-B27 [1]. Classically, SpAs have been classified as ankylosing spondylitis (AS), Reiter syndrome (RS), reactive arthritis (ReA), psoriatic arthritis (PsA), IBD-associated SpA, and forms called undifferentiated SpA (uSpA) that do not meet the criteria for previous categories [2]. However, currently, there is a new classification for SpAs. This new classification includes two types of SpAs: axial and peripheral SpA depending on the predominant spinal or peripheral involvement [3, 4] and extraarticular involvement such as anterior uveitis or IBD, which are also considered part of the SpA group [5].

Autoimmune diseases (ADs), in turn, are a clinical syndrome caused by the loss of immune tolerance and characterized by T- or B-cell activation leading to tissue damage in the absence of any other evident cause [6]. Criteria for AD definition have been described and revisited [7]. These criteria, which include direct and indirect proof as well as circumstantial evidence [6], are described in Table 1. However, in many diseases labeled as autoimmune, there are several limitations to fulfill the concept of autoimmunity, which are mainly related to the lack of direct proof (autoantibodies and cell-mediated immunity). Conversely, autoinflammation, defined as self-directed tissue inflammation, is characterized by activation of the innate immune system determined by local factors at specific disease-prone sites [8]. Since polyautoimmunity (i.e., the presence of two or more well-defined ADs in a single patient) is one of the major clinical characteristics of ADs, our purpose was to look for the association between SpAs and ADs. To do so, a cross-sectional two-phase study was undertaken. First, the presence of ADs in a cohort of patients with SpAs was examined. Second, we searched for the presence of SpAs in a well-defined group of patients with ADs including rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and Sjögren's syndrome (SS).

Table 1: Classification criteria for autoimmune diseases in humans. Comparison between ADs and autoinflammatory diseases.

			Diseases					
			SLE	RA	SS	AS	PsA	IBD
Direct proof	Antibody-mediated	Circulating ABs which alter the function	+	+	+			
		Localized ABs	+	+	+			
		IC located at lesion	+	+	+	+		
		Passive transference	+		+			
	Cells-mediated	In vitro T-cell proliferation in respond to autoantigen	+					
		In vitro T-cell transference to immune-deficient mice	+					
		In vitro T-cell cytotoxicity against target organ cells	+	+	+			
Indirect proof		Disease reproduction by experimental immunization	+	+	+	+	+	+
		Disease reproduction by idiotypes	+	+				+
		Spontaneous animal models	+	+	+	+	+	+
		Animal models produced by immune system deregulation	+	+	+	+	+	+
Circumstantial evidence		Auto-ABs	+	+	+			
		Other AD association	+	+	+		+	+
		HLA association	+	+	+	+	+	+
		Lymphocytic infiltration in target organ	+	+	+	+	+	
		Good response to immune suppression	+	+	+	+	+	+

AS: ankylosing spondylitis, PsA: psoriatic arthritis, IBD: inflammatory bowel disease, SS: Sjögren's syndrome, RA: rheumatoid arthritis, SLE: systemic lupus erythematosus, AD: autoimmune disease, ABs: antibodies, and IC: immune complexes.

2. Materials and Methods

2.1. Study Population. A total population of 1,077 patients from our RA, SLE, and SS database was reviewed. This database consists of 671 patients with confirmed RA, 239 with confirmed SLE, and 167 with confirmed SS. All patients are followed at the Center for Autoimmune Diseases Research (CREA) in Bogota, Colombia. All patients met four or more of the American College of Rheumatology (ACR) criteria for classification of RA and SLE [9, 10]. All patients with SS satisfied 4 or more of the diagnostic criteria for primary SS proposed by the European Community Study Group [11]. All of them required objective salivary gland involvement (i.e., focus score >1).

A cohort of 148 patients with SpAs was consecutively evaluated and their clinical records reviewed. All SpAs patients were classified according to accepted international criteria for each disease. AS patients met modified New York criteria [12] and PsA patients satisfied CASPAR criteria [13]. For ulcerative colitis (UC) diagnosis was made on the basis of clinical suspicion supported by appropriate macroscopic findings on sigmoidoscopy or colonoscopy, typical histological findings on biopsy, and negative stool examinations for infectious agents. For Crohn's disease (CD) the diagnosis depended on demonstrating focal, asymmetric, and often granulomatous inflammation. However, the studies selected varied according to the presenting manifestations, physical findings, and complications [14]. IBD-associated SpA diagnosis required criteria for any type of SpA plus findings of UC or CD as was explained. For ReA, diagnosis was done based on the European Spondylarthropathy Study Group (ESSG) preliminary criteria for the classification of SpAs [15] and taking into account the fact that an antecedent of previous infection was required. Patients that did not meet the criteria for any SpA but satisfied the criteria for SpAs were classified as uSpA. Patients were also classified on the basis of the Assessment of SpondyloArthritis International Society (ASAS) criteria for axial and peripheral involvement [3, 4]. Patients with only extraarticular manifestations were classified as extraarticular SpA [5]. Patients with previous diagnostic of hypothyroidism were evaluated for autoimmune thyroiditis (AT) by searching of both antithyroglobulin (anti-Tg) and antithyroperoxidase antibodies (anti-TPO).

2.2. Clinical Variables. Information on patient demographics and cumulative clinical and laboratory manifestations over the course of the disease was obtained either by verification during discussion with the patient, an expert evaluation by a rheumatologist, or by chart review and were recorded in a standard and validated data-collection form for that purpose. A search was done for data on patients with any type of SpA and concomitant diagnoses of RA, SLE, and SS. Each patient's diagnosis was confirmed by review of clinical records using the criteria listed above (RA, SLE, SS, and SpAs).

The institutional review board at the School of Medicine and Health Sciences of the Universidad del Rosario approved the study design, and all the patients signed the informed consent form.

2.3. Laboratory Tests. HLA-B27 was determined by flow cytometry or DNA typing. Antinuclear antibodies were determined by indirect immunofluorescence on HEp-2 cells. Rheumatoid factor was measured by turbidimetry. The detection of the specific antibodies, such as anti citrullinated

cyclic peptide (anti-CCP), native anti-DNA, anti-RNP, anti-Sm, anti-Ro, anti-Tg, and anti-TPO antibodies were measured by Enzyme-Linked ImmunoSorbent Assay (ELISA) by using commercial kits (QUANTA Lite ELISAs, INOVA Diagnostics, Inc. San Diego, CA, USA) following the manufacturer's instructions.

2.4. Statistical Analysis. Univariate analysis was done on SpA patients. For the normal variables, mean and standard deviations (SD) are reported, and, for the nonnormal variables, interquartile ranges (IQR) are reported. Kolmogorov-Smirnov or Shapiro-Wilk tests were done to evaluate normality as appropriate. Differences between variables were analyzed by using T student, X^2, Fisher's exact test, ANOVA test, or Kruskall-Wallis as appropriate. Groups presenting $n \leq 5$ were excluded from analysis. Bonferroni test was done when significant differences were found in ANOVA. In all the cases, a P value < 0.05 was considered as significant. Data were managed using the Statistical Package for the Social Sciences software (SPSS v18 for Windows).

3. Results

3.1. General Characteristics of Two Groups of Study. A total of 148 patients with SpAs were included. AS was observed in 55.6%, PsA in 21.5%, uSpA in 16.7%, IBD-associated SpA in 4.2%, and ReA in 2.1% of the patients evaluated. Disease was predominant in males for all subtypes, except for uSpA, where a higher prevalence of females as compared with males was observed, 66.7% versus 33.3%, respectively. Table 2 summarizes the main clinical findings observed in the patients with SpAs at any time during the course of the disease, and Table 3 shows laboratory characteristics in patients with SpA and hypothyroidism.

Regarding the group of ADs, age at onset was significant lower in SLE patients than RA and SS patients. Otherwise, mean age of patients was higher in patients with RA than in patients with SS and SLE. As expected, all the ADs in this study were more frequent in women (Table 4).

3.2. ADs in Patients with SpAs. There were two patients presenting with SS in the SpA group (1.4%), one with AS, and other with PsA. There were no patients with RA or SLE observed in the SpA group; however, one patient with uSpA presented with HLA-B27, rheumatoid factor, and anti-CCP antibodies but did not fulfill the classification criteria for RA.

Hypothyroidism was present in 14 patients (9.5%). All of them were women. Of these patients, five (3.5%) met criteria for AT. Three were observed in the AS group, one in the PsA group and one in the uSpA group. Global prevalence of ADs in the SpA group was 4.86%. According to the new classification of SpAs, the presence of ADs was similar in both axial and peripheral SpA (Table 5).

3.3. SpAs in Patients with ADs. There was no patient with concomitant AS in the group of patients with RA or in that of patients with SLE (Table 6). Only one case of AS (0.59%) was found among 167 patients with SS. This patient did not belong to the same group of SpAs. Among all patients with ADs analyzed, the prevalence of AS was <1%. With respect to PsA, one case was found in the RA group (0.15%) and none in SLE or SS groups. The prevalence of PsA in all patients analyzed was also low (<1%) just as in the case of RA. IBD was presented in two patients (0.3%) in the RA group and in one patient (0.41%) in SLE group. Prevalence of IBD in the groups of ADs was extremely low (<1%). The overall prevalence of SpAs was also low (0.46%).

3.4. Comparison between SpAs and ADs Prevalences. Prevalence of ADs in the general population is considered to be 3.23% [16]. No significant differences between the prevalence of ADs in SpAs and the prevalence of ADs in the general population were found in our study ($P > 0.05$).

The prevalence of SpAs in the general population is about 0.4% [17]. Significant differences between the prevalence of SpAs in ADs in our patients as compared to the prevalence of SpAs in the general population were not found ($P > 0.05$).

4. Discussion

The prevalence of SpAs in ADs observed in our study (0.46%) was similar to the prevalence described in the general population (<1%) [17–19]. For example, Haglund et al. [18] found a prevalence of 0.45% for SpAs in southern Sweden. In North America, the prevalence of SpAs has been reported to be 0.4% [17]. Other studies on Caucasians have shown that the frequency of AS ranges between 0.15% and 1.8% and for PsA between 0.02% and 0.2% [20].

Sundquist et al. [21] analyzed the concordant and discordant associations between RA, SLE, and AS as well as the risk of siblings to develop these associations by using standardized incidence ratios (SIRs). They observed concordant association in siblings when AS was compared with AS (SIRs = 17.14). In contrast, AS was not associated with RA or SLE [21]. Information about the association of SpAs and RA is scarce, and few case reports have been published [22–24]. In 1981, one study including 184 patients with AS or RS showed that five of them had concomitant diagnostic of RA and two of these five patients presented also with Felty's syndrome [25]. In our study, no patient with coexisting RA and AS was observed. However, one patient with uSpA presented with anti-CCP antibodies, rheumatoid factor, and HLA-B27 but, at the time of the inclusion, the patient did not meet criteria for RA.

There are reports of IBD in RA [26] and SLE [27]. The present study reports a prevalence of 0.28% for IBD in all ADs, 0.3% in RA patients, and 0.41% in SLE. No patient with IBD was observed in SS. One study on North Americans reported elevated risk for RA in patients with IBD, showing an odds ratio (OR) of 2.7 with 95% confidence intervals (95% CI) between 2.4 to 3.0. However, the same study indicated a higher risk for AS (OR: 7.8; 95% CI: 5.6–10.8) than for RA [28]. Another study including 37 patients with IBD showed only one patient with peripheral arthritis and positive anti-CCP antibodies [29].

Concerning SLE, coexistence of AS is very rare, and this association has been suggested to occur in patients who

Table 2: General characteristics of patients with SpAs.

	Classical classification [2]						New classification [3, 4]		
	All ($n = 144$)[a]	AS ($n = 80$)	PsA ($n = 31$)	uSpA ($n = 24$)	IBD-associated SpA ($n = 6$)	P value	Axial ($n = 89$)	Peripheral ($n = 55$)	P value
Mean (SD)									
Age, years	43.78 (11.08)	42.23 (10.33)	50.81 (13.00)	41.28 (8.45)	38.96 (6.02)	$P < 0.05$[b,c]	41.80 (10.15)	46.54 (11.84)	$P = 0.018$
Age at onset, years	34.77 (11.39)	33.24 (10.71)	41.23 (11.20)	33.71 (11.52)	29.33 (6.77)	$P < 0.05$[b]	33.05 (10.42)	37.62 (12.35)	$P = 0.017$
Age at diagnostic, years	39.24 (10.98)	37.58 (10.27)	45.65 (12.43)	37.54 (9.31)	34.17 (7.83)	$P < 0.05$[b,c]	37.40 (10.03)	42.00 (11.95)	$P = 0.017$
Characteristic, n (%)									
Percentage	100	55.6	21.5	16.7	4.2	NA	60.13	37.16	NA
Male[d]	82 (56.9)	45 (56.3)	20 (64.5)	13 (54.2)	2 (33.3)	NS	51 (57.39)	31 (56.4)	NS
Female	62 (43.1)	35 (43.8)	11 (35.5)	11 (45.8)	4 (66.7)	NS	38 (42.7)	24 (43.6)	NS
Low back pain	111 (77.1)	80 (100)	10 (32.3)	15 (62.5)	4 (66.7)	$P < 0.001$[b,c,e]	89 (100)	50 (90.9)	$P < 0.001$
Peripheral arthritis	73 (50.7)	22 (27.5)	24 (77.4)	22 (91.7)	3 (50)	$P < 0.001$[b,c]	23 (25.8)	50 (90.9)	$P < 0.001$
Enthesitis (heel)	26 (18.1)	18 (22.5)	2 (6.5)	4 (16.7)	2 (33.3)	NS	20 (22.5)	6 (10.9)	NS
Enthesitis (other sites)	34 (23.6)	19 (23.8)	6 (16.1)	8 (33.3)	2 (33.3)	NS	22 (24.7)	12 (21.8)	NS
Dactylitis	28 (19.4)	10 (12.5)	9 (29)	8 (33.3)	1 (16.7)	NS	11 (12.4)	17 (30.9)	$P = 0.006$
Psoriasis	28 (19.4)	0	28 (90.3)	0	0	$P < 0.001$[b,f,g]	2 (2.2)	26 (47.3)	$P < 0.001$
IBD	6 (4.2)	0	0	0	6 (100)	$P < 0.001$[e,g,h]	2 (2.2)	4 (7.3)	NS
HLA-B27									
Positive	43/86 (50)	27/54 (50)	2/11 (18.2)	14/17 (82.35)	0	NA	29/59 (49.15)	14/27 (51.85)	NA
Negative	43/86 (50)	27/54 (50)	9/11 (81.8)	3/17 (17.65)	2/2 (100)	NA	30/59 (50.85)	13/27 (48.15)	NA
No data available	58/144 (40.3)	26/80 (32.5)	20/31 (64.5)	7/24 (29.16)	4/6 (66.66)	NA	30/89 (33.7)	28/55 (50.9)	NA

AS: ankylosing spondylitis, PsA: psoriatic arthritis, uSpA: undifferentiated spondyloarthropatty, IBD: inflammatory bowel disease, ReA: reactive arthritis, AD: autoimmune disease, HLA: human leukocyte antigen, NA: not applicable, and NS: non significant.

[a] Neither ReA ($n = 3$) nor exclusive extraarticular SpA were included.

[b] Significant differences between AS and PsA.

[c] Significant differences between AS and uSpA.

[d] Significant differences in gender for all categories.

[e] Significant differences between AS and IBD-associated SpA.

[f] Significant differences between PsA and uSpA.

[g] Significant differences between PsA and IBD-associated SpA.

[h] Significant differences between uSpA and IBD-associated SpA.

Table 3: Hypothyroidism in SpAs.

	Classical classification [2]					New classification [3, 4]		
	All ($n = 144$)[a]	AS ($n = 80$)	PsA ($n = 31$)	uSpA ($n = 24$)	P value	Axial ($n = 89$)	Peripheral ($n = 55$)	P value
Hypothyroidism (%)	14/148 (9.5)	8/80 (10)	4 (12.9)	2 (8.3)	NS	9 (10.1)	5 (9.1)	NS
Anti-TPO positive	5/14 (35.7)	3/8 (37.5)	1/4 (25)	1/2 (50)	NS	3/9 (33.33)	2/5 (40)	NS
Anti-Tg positive	1/14 (7.14)	1/8 (12.5)	0	0	NS	1/9 (11.11)	0	NS
Both anti-TPO and anti-Tg positives	1/14 (7.14)	1/8 (12.5)	0	0	NS	1/9 (11.11)	0	NS

AS: ankylosing spondylitis, PsA: psoriatic arthritis, uSpA: undifferentiated spondyloarthropatty, NS: nonsignificant, anti-TPO: anti-thyroperoxidase, and anti-Tg: antithyroglobulin.
[a]Neither ReA ($n = 3$) nor exclusive extraarticular SpA were included.

Table 4: Age and gender of patients with ADs and SpAs.

Characteristic	RA ($n = 671$)	SLE ($n = 239$)	SS ($n = 167$)	SpAs ($n = 148$)	P value
Age, mean (SD)	51.8 (12.34)	37.1 (14.63)	50.5 (13.91)	43.78 (11.08)	<0.001[†]
Age at onset, mean (SD)	38.7 (13.47)	29.03 (13.02)	44.2 (13.72)	34.77 (11.39)	<0.001[¶]
Male (%)	18.3	18.2	5.3	56.9	
Female (%)	81.7*	81.8*	94.7*	43.1	

SS: Sjögren's syndrome, RA: rheumatoid arthritis, SLE: systemic lupus erythematosus, SpAs: spondyloarthropathies, and SD: standard deviation.
[†]Significant differences were observed between the following groups: RA versus SLE, RA versus SpAs, SLE versus SS, SLE versus SpAs, and SS versus SpAs.
[¶]Significant differences were observed between the following groups: RA versus SLE, RA versus SS, RA versus SpAs, SLE versus SS, SLE versus SpAs, and SS versus SpAs.
*Females were more prevalent in each group ($P < 0.001$), but not in the SpAs group.

Table 5: ADs in patients with SpAs.

	Classical classification [2]				New classification [3, 4]		
	All ($n = 144$)[a]	AS ($n = 80$)	PsA ($n = 31$)	uSpA ($n = 24$)	Axial ($n = 89$)	Peripheral ($n = 55$)	P value
SS	2 (1.39)	1 (1.3)	1 (3.22)	0	1 (1.12)	1 (1.81)	NS
RA	0*	0	0	0*	0	0*	NA
SLE	0	0	0	0	0	0	NA
AT	5 (3.47)	3 (3.75)	1 (3.22)	1 (4.16)	3 (3.37)	2 (3.63)	NS
Total	7 (4.86)	4 (5)	2 (6.45)	1 (4.16)	4 (4.5)	3 (5.45)	NS

AS: ankylosing spondylitis, PsA: psoriatic arthritis, uSpA: undifferentiated spondyloarthropatty, SS: Sjögren's syndrome, RA: rheumatoid arthritis, SLE: systemic lupus erythematosus, AT: autoimmune thyroiditis, AD: autoimmune disease, SpAs: spondyloarthropathies, NA: not applicable, and NS: nonsignificant.
[a]Neither ReA ($n = 3$) nor exclusive extraarticular SpA were included.
*One patient presented with monoarthritis, HLA-B27, anti-CCP, and rheumatoid factor.

carry one or two susceptibility alleles for both diseases [30]. Furthermore, shared environmental factors that remain to be identified may also contribute to triggering both diseases [30]. The recently published cases of the coexistence of SLE and AS corresponded to drug-induced SLE or lupus-like syndrome associated with anti TNF treatment in SpA patients [31–33]. In our study, we did not observe patients with SpAs in the SLE group and vice versa.

There are few reports about the coexistence of SS and AS. Kobak et al. found SS in 10% of patients with AS in Turkey [34]. Brandt et al. reported a prevalence of 7.6% for SS in 105 patients with SpAs in Germany [35]. In our study, the prevalence of SS was 1.4% in all the patients with SpA. Different results could be related to diagnostic strategies (i.e., active search by performing autoantibodies and minor salivary gland biopsy systematically), ethnicity, and geography (i.e., environmental factors). We have no additional evidence of SpAs in other series stressing the scarce information in this regard.

Hypothyroidism (of any cause) was observed in 9.5% of SpAs, and all were women. This prevalence is significantly higher than the prevalence of hypothyroidism in the general population, which is considered to be 1%-2% [36] ($P < 0.001$). Although these results might indicate that SpAs patients have an increased risk of hypothyroidism, the design of this study was not intended to answer this question. Therefore, further research in this topic is required. Of the 14 patients presenting with hypothyroidism, 5 were diagnosed

Table 6: Prevalence of SpAs in patients with ADs.

n (%)	RA ($n = 671$)	SLE ($n = 239$)	SS ($n = 167$)	All ($n = 1077$)
AS	0	0	1 (0.59)	1 (0.092)
PsA	1 (0.15)	0	0	1 (0.092)
IBD	2 (0.3)	1 (0.41)	0	3 (0.28)
All SpAs	3 (0.44)	1 (0.41)	1 (0.59)	5 (0.46)

SLE: systemic lupus erythematosus, RA: rheumatoid arthritis, SS: Sjögren's syndrome, AS: ankylosing spondylitis, PsA: psoriatic arthritis, IBD: inflammatory bowel disease, and SpAs: spondyloarthropathies.

with AT. Thus, the prevalence of AT in SpAs in our study was 3.5%. According to the subtype of SpA, the prevalence of AT in AS, PsA, and uSpA was 3.8%, 3.2%, and 4.2%, respectively. One study carried out in Italian women with PsA showed a high prevalence of AT as compared with controls. In such study, 28% of PsA had anti-Tg and 14% anti-TPO antibodies [37]. In our study, we observed a lower prevalence of AT in PsA (Table 5), which could be due to differences in gender, sample size, ethnicity, geography, and searching strategies.

A case of AS and multiple sclerosis (MS) has been reported [38]; however, no patients with MS were observed in our cohort of SpAs.

Concerning the clinical findings of our group of patients with SpAs, the results differ from other local studies to some degree. Londoño et al. [39] found a higher prevalence of uSpA (35.3%) than we did and a lower prevalence of PsA (9.4%) among their group of patients with SpAs. They observed a familial history of SpA in 18% of patients and did not find patients with IBD-associated SpA in contrast with our findings. Likewise, they observed male : female rate of 3 : 1, but the study was made in a military hospital, where male patients are more frequent. Another study done by Marquez et al. [40] in the city of Medellin, included 71 patients and showed similar results to ours, although a high prevalence of enthesitis (67%) was observed in their patients. These differences could be due to the heterogeneity of the Colombian population and limited number of patients analyzed in both studies as well as to ascertainment bias. Our patients come predominantly from the city of Bogota where the population admixture is higher than that in Medellin. One genetic study performed on Colombians with AS did not find significant differences in HLA-B27 subtypes between patients from Bogota (mestizos) and Cartagena (mulattos). However, clinical characteristics were not evaluated [41].

5. Conclusion

The findings presented in this study suggest a lack of association between SpAs and ADs. As a corollary, SpAs seem to be diseases that are not autoimmune in the strict sense even though they involve immunological reactions such as the overproduction of particular cytokines. Therefore, we consider them autoinflammatory diseases instead of autoimmune ones (Table 1) [8]. However, in spite of having included a large number of patients, our data were underpowered. Thus, to accurately investigate the association between SpAs and ADs, further multicenter and collaborative research is required, involving about 2000 cases and the same number of controls to allow a statistical power of 80% with a 5% of error.

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Research Article

Effect of Selenium on HLA-DR Expression of Thyrocytes

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Autoimmune thyroid diseases (ATDs) represent the most frequent forms of the organ-specific autoimmune thyroid disorders that result from interaction between genetic and environmental factors. Selenium has been shown to exert a beneficial effect on the autoimmune thyroiditis. In spite of therapeutical effect of selenium on autoimmunity, the mechanism of its action has not been revealed. *Objective*. To determine whether selenium in vitro thyrocytes cultures are able to influence the HLA-DR molecule expression of human thyrocytes and production of free oxygen radicals. *Method*. Thyrocytes were prepared from human thyroid gland and cultured in vitro in the presence of interferon-γ and sodium selenite. The expression of HLA-DR molecules induced by interferon-γ in the presence of sodium selenite of various concentration was measured by fluorescence-activated cell sorter. *Results*. Selenium has a dose-dependent inhibitory effect on the expression of HLA-DR molecules of thyrocytes induced by interferon-γ. This effect of selenium was in the inverse correlation with antioxidative capacity. *Conclusion*. Beneficial effect of selenium on autoimmune mechanism is a complex mechanism in which the inhibitory effect on HLA-DR molecule expression and antioxidative capacity are involved into therapy of autoimmune thyroiditis.

1. Introduction

Recently published clinical studies on possible effects of selenium (Se) in autoimmune thyroiditis evoked exciting discussion. The conflicting data were published on effect of Se; the one part of investigators provided evidences that Se intakes may be beneficial with respect to autoimmune diseases [1–7], the others were not able to show the significant effect of Se on autoimmune thyroiditis [8, 9]. Furthermore, the authors who published the beneficial effect of Se on the levels of autoantibodies, advised to use Se therapy for patients with autoimmune thyroiditis (AIT) [1, 4, 10]. Recently, we published our prospective placebo-controlled prospective study including 132 patients with autoimmune thyroiditis [4]. L-thyroxine substitution therapy was made in both groups and the level of TSH remained in the normal range. Se therapy was continued by L-seleno-methionine (per os $2 \times 100\,\mu$g/day) for one year. The level of Se in the untreated patients' sera was significantly lower than in treated patients and controls, and after three-month therapy with it, Se was normalized. The titer of antithyroid antibodies (mostly the anti-TPO) significantly decreased at the end of study. An inverse correlation was found between antioxidant capacity and level of anti-TPO antibodies. This observation suggests that Se deficiency by itself might be responsible for the precipitation of inflammatory process. Although the precise mechanisms of action and the possible targets of Se have not been clarified yet, the beneficial influence of Se can be explained by different points of views. Growing evidence supports that the selenium-containing enzymes and their antioxidant capacity somehow modify the autoimmune mechanism [11–15]. Previously, it was published that unlike in thyroids from healthy individuals thyroid epithelial cells from patients with AITD were able to express HLA class II antigen molecules similar to normally expressed on antigen presenting cells (APCs) such as macrophages and dendritic cells [16–19]. The aberrant expression of HLA Class II molecules on thyroid cells may initiate and perpetuate thyroid autoimmunity via direct autoantigen presentation. We removed the repeated references from the highlighted part. Please check similar

Table 1: Investigation of HLA-DR expression on thyrocytes induced by IFN-γ in the absence and presence of selenium. Figures in the table represent mean percentage DR positive human thyroid cells ±SD. All experiments were made in triplicate. ns. = not significant.

Culture of human thyrocytes	Expression of HLA-DR on thyrocytes (%)			
Culture media ($n = 4$)	3.7 ± 2.4	$P < 0.001$	n.s	$P < 0.001$
IFN-γ (100 U/mL)	35.2 ± 5.2			
IFN-γ (100 U/mL) + sodium selenite (10 nM/mL) ($n = 3$)	33.2 ± 14.7		$P < 0.05$	
IFN-γ (100 U/mL) + sodium selenite (50 nM/mL) ($n = 3$)	26.4 ± 12.7			
IFN-γ (100 U/mL) + sodium selenite (100 nM/mL) ($n = 3$)	11.5 ± 5.2			

cases throughout the paper. Previously we provided evidence for the role of HLA-DR expression on thyrocytes induced by interferon-γ (IFN-γ) and its modification by methimazole which has a significant anti-oxidative capacity [20]. It was assumed that the Se, like methimazole, can modify the expression of HLA-DR molecules on thyrocytes culture in the presence of Se; therefore, we made in vitro experiments using human thyrocytes cultures to study this hypothesis.

2. Materials and Methods

We cultured human thyrocytes and analyzed HLA-DR antigen expressions induced by IFN-γ in the various concentrations of sodium selenite (Sigma) in culture media by previously published method [16]. Briefly, thyroid epithelial cells were separated from surgical specimens. $4–6 \times 10^6$ cells were obtained from 10 g of tissue with viability of >90% which was determined by trypan blue exclusion. 2×10^5 cells were placed in each well of a 24-well Costar culture plate and cultured in minimum essential medium containing 15% fetal calf serum (FCS) with 0.2% sodium bicarbonate either alone (control wells) or in the presence of IFN-γ (Hoffmann-La Roche), and to other wells 10.0, 50.0, and 100 nmol/mL of sodium selenite (Sigma) were added. In most of experiments, thyrocytes were cultured for 3 days and then detached by 0.2% trypsin. HLA-DR expression was investigated initially (0 day) and on day 3 and 7 of culture. Cells were recovered in Ca^{++} and Mg^{++} free EGTA solution with rubber policeman. The detached cells were resuspended in RPMI containing 10% FCS, 10 mM HEPES (Sigma). For indirect immunofluorescence, cells were resuspended in 200 μL RPMI (Sigma) containing 10% FCS, in 10 mM HEPES and were incubated for 30 min at 4°C with 5.0 μL monoclonal anti-DR framework antibody (DAKO). The cells were washed twice and the pellet incubated for 30 min at 4°C with 1 : 100 dilution of FITC-conjugated rabbit anti-mouse immunoglobulin (Cooper Biomedical, Inc., Malvern, PA). After two washes, the cells were analyzed in fluorescence-activated cell sorter (FACS III, Beckton-Dickinson Co., Sunnyvale, CA). All experiments were made in triplicate. The total antioxidant status (TAS) was determined by Randox kit (Randox Laboratories Ltd, Crumlin, UK) [4, 12, 14].

2.1. Statistical Methods. The computerized program "Stat View" (Version 4.5, SAS Institute Corp., North Carolina, USA) was used for evaluating data. Descriptive statistics, Pearson's chi-square test, Fisher's exact test, and ANOVA analysis were performed. Values of $P < 0.05$ were considered as significant.

3. Results

We found that IFN-γ (100 U/mL) was able to induce a significant stimulation of expression of HLA-DR molecules in thyrocytes (Table 1) (35.2 ± 15.2 versus 3.7 ± 2.4, $P < 0.001$). The peak of HLA-DR expression was at day three and then decreased abruptly. Therefore, we tested the expression of HLA-DR positive cells induced by IFN-γ at day three in absence and presence of Se of various concentrations. Se in two different concentrations (50 nM/mL and 100 nM/mL, resp.) significantly inhibited the expression of HLA-DR positive cells induced by IFN-γ (Table 1). If we added the Se to thyrocytes cultures after or before exposition of IFN-γ, there were not observed significant changes in HLA-DR expression. Time-dependent effect of sodium selenite (100 nM/mL) on IFN-γ-induced HLA-DR expression(100 U/mL) was on Figure 1. Then we studied the possible connection with HLA-DR expression on thyrocytes and antioxidant capacity of Se and found an inverse correlation between antioxidant status and expression of HLA-DR positive cells ($r = -0.72$, $P < 0.01$) (Figure 2).

4. Discussion

The trace element of Se plays an important role in the thyroid gland under physiological conditions and in diseases as well. Se supplementation decreased inflammatory activity in patients with autoimmune thyroiditis, and the reduction of titres of anti-TPO antibodies was correlated with serum levels of Se [2, 4, 6, 7]. Convincing observation was published for beneficial effect of Se in a patent with autoimmune thyroiditis when a marked decrease in thyroid 18FDG uptake after Se supplementation was found [21]. In spite of great efforts, the precise mechanism of Se has not yet been clarified. The role of antioxidant property of Se was published to be involved into beneficial effect in autoimmune thyroiditis [12–15]. Previously, we found that methimazole proved to have antioxidant capacity decreased the expression of HLA-DR molecules on the surface of thyrocytes [20]. Our experiments confirmed that the Se has a significant radical scavenging effect and the decrease the expression of HLA-DR molecules induced by IFN-γ was in an inverse correlation with

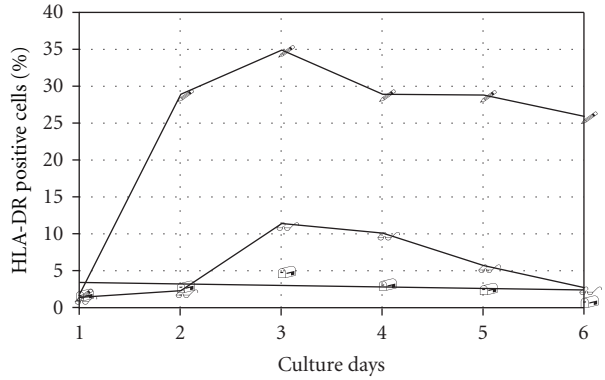

Figure 1: Time-dependent effect of sodium selenite (100 nM/mL) on IFN-γ (100 U/mL) induced HLA-DR expression. (i) Dots: HLA-DR expression without sodium selenite. (ii) Square: HLA-DR expression with sodium selenite (100 nM/mL). (iii) Filled square: HLA-DR expression with sodium selenite.

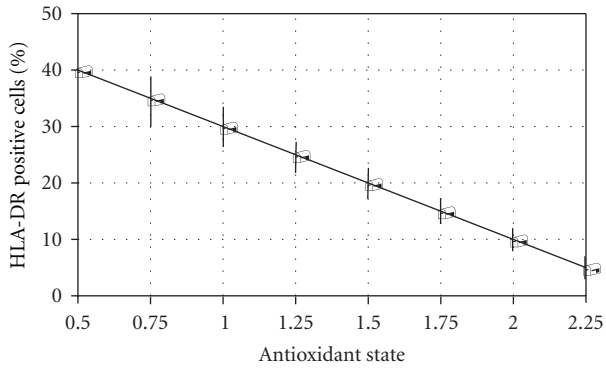

Figure 2: Study of connection between antioxidant status and expression of HLA-DR positive cells. Bars show the ±SD, $r = -0.72$.

antioxidative capacity of thyrocytes supernatant. The exogenous factors including iodine and oxidative stress have been published to be precipitating factors in genetically susceptible individuals [5, 14, 22–26]. The antigenicity of thyroid autoantigens (thyroglobulin and TPO) is increased after iodine exposition. The iodine is able to increase the amount of free radicals which are produced in the process of physiological hormonogenesis in the thyroid gland. In addition, there are accumulating data for antiviral capacity of Se. Both epidemiological and in vitro data demonstrated that Se deficiency might be important in viral infections as well [11]. Since the viruses have been published to induce IFN-γ, consequently HLA-DR expression, therefore, it is hypothesized that the trigger in autoimmune thyroiditis might be a virus infection [27–29]. Nowadays, the suggestion of viral origin of autoimmunity appears to be a speculation; however, the "selenium story" might open a new window not only for better understanding of beneficial effect of Se in autoimmune thyroiditis but also in the research of origin of autoimmunity [9, 11–13, 15, 22]. A new perspective has been opened by the investigations of Se on the role of T regulatory cells (Treg) with CD4CD25 FoxP3 markers [24, 25, 30, 31]. Accumulating data demonstrated that deficiency of $CD4^+CD25^+$ Treg cells was closely correlated with development of ATD [24, 25, 30, 31]. Recently, it was published in animal experiments that the CD4CD25 FoxP3 T cells displayed preventive effect on development of ATD [26]. Surprisingly, Se upregulated $CD4^+CD25^+$ regulatory T cells in iodine-induced autoimmune thyroiditis model of NOD.H-2^{h4} mice [24]. Our observation and experiments provide evidence that Se has a complex effect on immune system including decreased expression on HLA-DR molecules and by this way can prevent the induction and perpetuation of autoimmune thyroid processes.

5. Conclusions

Se has a dose-dependent inhibitory effect on the expression of HLA-DR molecules of thyrocytes induced by interferon-γ. This effect of selenium was in the inverse correlation with anti-oxidative capacity. Inhibitory effect of Se on HLA-DR molecule expression and antioxidative capacity is involved into therapy of autoimmune thyroiditis. Our in vitro study provided evidence that the free radical scavenging effect of Se plays an important role in the therapy and the prevention of autoimmunity.

Abbreviations

AIT: Autoimmune thyroiditis
ATD: Autoimmune thyroid disease
HLA: Human leucocyte antigen DR locus
IFN-γ: Interferon-γ
MHC: Major histocompatibility complex
Se: Selenium
TAS: Total antioxidant state
TPO: Thyroid peroxidase
APCs: Antigen presenting cells.

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Review Article

Genetic Factors of Autoimmune Thyroid Diseases in Japanese

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Autoimmune thyroid diseases (AITDs), including Graves' disease (GD) and Hashimoto's thyroiditis (HT), are caused by immune response to self-thyroid antigens and affect approximately 2–5% of the general population. Genetic susceptibility in combination with external factors, such as smoking, viral/bacterial infection, and chemicals, is believed to initiate the autoimmune response against thyroid antigens. Abundant epidemiological data, including family and twin studies, point to a strong genetic influence on the development of AITDs. Various techniques have been employed to identify genes contributing to the etiology of AITDs, including candidate gene analysis and whole genome screening. These studies have enabled the identification of several loci (genetic regions) that are linked to AITDs, and, in some of these loci, putative AITD susceptibility genes have been identified. Some of these genes/loci are unique to GD and HT and some are common to both diseases, indicating that there is a shared genetic susceptibility to GD and HT. Known AITD-susceptibility genes are classified into three groups: HLA genes, non-HLA immune-regulatory genes (e.g., CTLA-4, PTPN22, and CD40), and thyroid-specific genes (e.g., TSHR and Tg). In this paper, we will summarize the latest findings on AITD susceptibility genes in Japanese.

1. Introduction

Autoimmune thyroid diseases (AITDs) are common autoimmune endocrine diseases [1], and according to one study, AITD are the commonest autoimmune diseases in the USA [2]. Even though the hallmark of AITD is infiltration of the thyroid with thyroid reactive lymphocytes, the end result is two clinically opposing syndromes: Hashimoto's thyroiditis (HT) manifesting by hypothyroidism and Graves' disease (GD) manifesting by hyperthyroidism. In HT, the lymphocytic infiltration of the thyroid gland leads to apoptosis of thyroid cells and hypothyroidism [3]. In contrast, in GD, the lymphocytic infiltration of the thyroid leads to activation of TSH-receptor- (TSHR) reactive B cells that secrete TSHR-stimulating antibodies causing hyperthyroidism [4]. GD and HT are complex diseases, and their etiology involves both genetic and environmental influences [1]. Up until 15 years ago, the only known gene for AITD was HLA-DR3 haplotype (DRB1*03-DQB1*02-DQA1*0501) in Caucasians. However, with the advent of new genomic tools and the completion of the human genome and the HapMap projects, new non-HLA genes have been identified and their functional effects on disease aetiology started to be dissected as well. This paper will summarize the recent advances in our understanding of the genetic contributions to the etiology of AITD in Japanese population. Since most of the studies were performed in relatively small size samples recruited from Japanese population, the results have some limitations.

2. A Brief Overview of AITD Genes Identified in Caucasians

In Caucasians, the first locus shown to be associated with AITDs was the HLA-DRB1 locus (reviewed in [5]). HLA-DR3 (DRB1*03) haplotype has been consistently shown to be associated with GD, with an odds ratio (OR) of 2.0–3.0 [6–8]. The literature regarding HT is less consistent with reports of associations with DR3 and DR4 in Caucasians, as well as a negative association with DR 1 and 8, suggesting a protective role [9]. Recently, Zeitlin et al. [10] investigated DRB1-DQB1-DQA1 in the largest UK Caucasian HT case control cohort to date comprising 640 HT patients and 621 controls. A strong association between HT and DR4

haplotype (DRB1*04-DQB1*03-DQA1*03) was detected, and protective effects were detected for DR13 haplotype (DRB1*13-DQB1*06-DQA1*01) and DR7 [10]. It was recently shown that arginine at position 74 of the DRβ1 chain (DRβ1-Arg74) is important for the development of GD in a significant proportion of patients [11, 12]. A study from England provided evidence of a primary association of HLA-C, and, to a lesser extent HLA-B, with GD. Other genes have also been shown to influence the expression of GD in Caucasians [13]. These include the genes for cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) [14, 15], CD40 [16], protein tyrosine phosphatase-22 (PTPN22) [17], thyroglobulin (Tg) [18, 19], and TSH-receptor (TSHR) genes [20].

3. HLA Genes in Japanese

Located on chromosome 6p21 is the major histocompatibility complex region that encodes for HLA glycoproteins. The HLA region is a highly polymorphic region that contains many immune response genes and has been found to be associated with various autoimmune disorders. The HLA molecule binds a peptide antigen (autoantigen in the cause of autoimmunity). It presents the antigen for recognition by the T-cell and as such the T-cell then determines if the antigen is self (and no immune response is mounted) or nonself and an immune response is mounted [21].

The HLA associations are with different alleles in Japanese. In previous studies, HLA-B35 is associated with GD and HLA-DRw53 with HT in the Japanese population (reviewed in [22]). HLA-Bw46 is associated with GD and HLA-DR9 with HT in the Chinese population (reviewed in [22]). The European GD-associated HLA haplotype (HLA-B*08-DRB1*03-DQA1*0501-DQB1*02) is virtually absent in Japanese [23]. Dong et al. previously reported that HLA-A*02 and DPB1*0501 are associated with Japanese GD [24]. Recently, they also demonstrated that HLA-A*02 and DPB1*0202 showed association with thyroid-stimulating hormone-binding inhibitory immunoglobulins- (TBII) negative GD, indicating that TBII-negative GD may be genetically distinct from TBII-positive GD [25]. In addition, Wan et al. reported that HLA-A*02 and DPB4*0101 are associated with Japanese HT [26].

4. Non-HLA Immune-Regulatory Genes in Japanese

4.1. The CTLA-4 Gene. The cytotoxic T-lymphocyte-associated protein 4, CTLA-4, gene is located on chromosome 2q. It is a highly polymorphic gene that was first discovered to be associated with risk for AITD by the candidate gene approach. Under normal circumstances, the CTLA-4 protein acts to suppress T-cell activation during normal immune response in order to prevent T-cell overactivity [27]. CD4+CD25-T-cells only express CTLA-4 on their surface after the T-cell receptor is activated, and its engagement with its ligand suppresses the ongoing immune response. Decreased or absent CTLA-4 activity permits uninhibited T-cell activity and a prolonged, unregulated immune response [28], making CTLA-4 an attractive candidate gene for autoimmunity. Indeed, the CTLA-4 gene has been found to be associated with many other autoimmune diseases.

A microsatellite in 3′UTR of CTLA-4 has been linked to AITD in Caucasians (reviewed in [27]); the longer the AT repeat at this site is, the less inhibitory activity CTLA-4 has. It has also been associated with AITD in Japanese [29]. Other variants of CTLA-4 gene have been linked to AITD in Caucasians (reviewed in [27]); a G allele substitution at an A/G single nucleotide polymorphism (SNP) at position 49 was also found to be associated with AITD in Japanese [30]. Recently, an A/G SNP downstream from the 3′UTR, designated CT60, was found to be associated with GD in Caucasians and has been suggested as the causative variant, albeit this has not been conclusively demonstrated [15]. It was also found to be associated with AITD in Japanese [31, 32]. Interestingly, another SNP (rs231779) is more likely the susceptibility variant for GD in Chinese Han population, suggesting that the susceptibility variants of the CTLA-4 gene varied between the different geographic populations with GD [33]. Additionally, most recent stratification analyses suggested a possible synergistic interaction of CTLA-4 CT60 with HLA-A*02 and -DPB1*0501 in the susceptibility to TBII-positive GD [34].

4.2. The CD40 Gene. The CD40 molecule, located on chromosome 20q, is crucial to both innate adaptive immune responses. It is present on the surface of antigen presenting cells (APCs) including B cells. The T-cell-APC interaction results in activation of CD40 as a costimulatory molecule. CD40 also plays a critical role in activating B lymphocytes allowing them to terminally differentiate and secrete antibodies (reviewed in [35]). It is no surprise that the CD40 gene has been linked to many autoimmune disorders. Whole genome linkage scanning has identified strong linkage of CD40 to GD. The causative variant predisposing to GD is a C/T polymorphism in the Kozak sequence (dbSNP accession number rs1883832), a nucleotide sequence that is essential for the initiation of translation of the CD40 molecule. Specifically, the CC genotype has been identified in Caucasians to be associated with GD [16]. Indeed, functional studies demonstrated that the C-allele of this SNP increased CD40 mRNA translation by ~20–30% when compared with the protective T allele [27]. We and others have also confirmed an association between the rs1883832 and GD in Japanese [36, 37].

4.3. The PTPN22 Gene. The protein tyrosine phosphatase-22 (PTPN22) gene encodes for the lymphoid tyrosine phosphatase (LYP), a molecule that, similar to CTLA-4, functions to inhibit T-cell activation [38]. A nonsynonymous SNP in the PTPN22 gene, R620 W (dbSNP accession number rs2476601), was found to be associated with GD, as well as other autoimmune diseases. This substitution results in a functional change in the LYP protein resulting in activation of T-cell, but the mechanism is unclear [35]. Indeed, this association seems specific for Caucasians and was not found in the Japanese GD population [39].

Table 1: PTPN22 haplotype structure and frequencies[a].

	SNP ID					Haplotype comparison[b]						
Haplotype	1	2	3	4	5	AITD	GD	HT	Controls	AITD versus Controls P value	GD versus Controls P value	HT versus Controls P value
1	C	T	T	G	A	0.59	0.59	0.59	0.60	0.69	0.75	0.69
2	C	C	T	G	C	0.20	0.20	0.20	0.17	0.24	0.29	0.28
3	C	C	C	A	A	0.13	0.13	0.13	0.12	0.54	0.66	0.47
4	T	C	C	G	A	0.067	0.066	0.070	0.060	0.63	0.72	0.61
5	C	C	T	G	A	0.013	0.017	0.0061	0.051	<0.0001	<0.0001	0.0004

[a]The program, SNPAlyze ver. 7.0 Standard, was used to estimate common (frequencies > 0.01) haplotypes for the five SNPs genotyped.
[b]Each haplotype was compared with the other haplotypes combined.
SNP: single nucleotide polymorphism; AITD: autoimmune thyroid disease; GD: Graves' disease; HT: Hashimoto's thyroiditis.

Recently, the rs2488457 SNP in the promoter region was reported to be associated with acute onset T1D in a Japanese population [40]. However, there was no association of the rs2488457 SNP with GD [41]. Furthermore, the rs3789604 SNP of the PTPN22 gene was found to be associated with RA, independently of rs2476601 [42]. The rs3789604 SNP lies 1496 bases downstream of PTPN22 at the 50 end of the round spermatid basic protein 1 gene (RSBN1), where it encodes either a silent mutation or putative transcription factor-binding sites (TRBS), depending on the transcript. Recently, the AA-genotype and A-allele frequencies of the rs3789604 were significantly higher in GD patients than in control subjects [43], suggesting that the rs3789604 or a gene with linkage disequilibrium may be relevant to susceptibility to GD in Japanese populations. Therefore, we further analyzed five other SNPs including rs12760457, rs2797415, rs1310182, rs2476599, and rs3789604, to clarify whether a susceptibility locus for AITD exists at another location within the PTPN22 gene. Our results showed no association with disease of any of the individual SNPs [44].

Because of the strong LD between five variants, haplotype analysis was undertaken using the computer program SNPAlyze version 7.0. Five haplotypes were identified, three of which (haplotypes 1, 2, and 4) were correlated with haplotypes 1, 4, and 5 identified in the report by Carlton et al. (Table 1) [42]. Four haplotypes (haplotypes 1–4) were relatively common, and 1 haplotype was rare. Distribution of the haplotype is significantly different between AITD and control by permutation procedure ($P = 0.0036$) [44]. A novel protective effect of a haplotype containing five SNPs was observed ($P < 0.0001$ for AITD, $P < 0.0001$ for GD, and $P < 0.0001$ for HT, resp.) (Table 1) [44].

4.4. The Zinc-Finger Gene in the AITD Susceptibility Region (ZFAT) Gene. Shirasawa et al. [45] identified a novel zinc-finger gene, designated ZFAT, as one of the AITD susceptibility genes in 8q23-q24 through an initial association analysis using the probands in their previous linkage analysis [46]. The distance between thyroglobulin and ZFAT genes is about 1.8 M bp. A subsequent association analysis of the samples from a total of 515 affected individuals and 526 controls in Japanese [45]. The T allele of the SNP located in the intron 9 of ZFAT (Ex9b-SNP10) is associated with increased risk for AITDs (dominant model: OR = 1.7, $P = 9.1 \times 10^{-5}$) [45]. The Ex9b-SNP10 is located in the 3′-UTR of truncated-ZFAT (TR-ZFAT) and the promoter region of the SAS-ZFAT [45]. The human ZFAT gene encodes a 1,243-amino acid residue protein containing one AT-hook and 18 C2H2 zinc-finger domains. ZFAT is also highly conserved among species from fish to human [44]. The ZFAT protein is expressed in the B and T lymphocytes in mice, and ZFAT regulates the genes involved in immune responses [47]. Furthermore, ZFAT is an anti-apoptotic molecule that is critical for cell survival in human leukemic MOLT-4 cells [48].

4.5. The FCRL Genes. Fc receptor-like 3 (FCRL3) is one of five FCRL genes that are preferentially expressed on B lymphocytes and have a highly structural homology with Fc receptors [49]. The 1p21-23 cytoband, in which the FCRL family resides, has been identified as a candidate locus for multiple autoimmune disorders in both human and murine models [50]. Kochi et al. [51] identified a strong association of SNPs in this region with GD susceptibility in Japanese and concluded that the origin of the association was a regulatory SNP in the promoter region of FCRL3. This susceptibility gene of GD was first identified from Japanese population. This SNP (−169C/T) (dbSNP accession number rs7522061) alters the binding affinity of NFκB and regulates gene expression, and high FCRL3 expression on B lymphocytes is observed in individuals with the disease-susceptible genotype. The SNP rs7522061 in the FCRL3 gene was also reported to be associated with AITD in Caucasians [52] and two other autoimmune diseases, rheumatoid arthritis (RA), and systemic lupus erythematosus (SLE) [51]. More recently, SNP rs3761959, which tags rs7522061 and rs7528684 (previously associated with RA and GD), was associated with GD in the extended cohort, confirming the original result. In total, three of the seven FCRL3 SNPs showed some evidence for association ($P < 0.05$), with SNP rs11264798 showing the strongest association of the tag SNPs ($P = 4.0 \times 10^{-3}$) [53]. SNP rs6667109 in FCRL5, which tagged SNPs rs6427384, rs2012199, and rs6679793, all found to be weakly associated in the original study, showed little evidence of association in the extended cohort [53].

4.6. Other Immune-Regulatory Genes. Other immune-regulatory genes tested for association with AITD in Japanese

include Interleukin-23 receptor (IL-23R) [54], Interferon-induced helicase (IFIH1) [55], FOXP3 [56], and interleukin-2 receptor alpha (IL-2RA) genes [44].

Interleukin-23 (IL-23) is a recently discovered heterodimeric cytokine. IL-23 acts primarily on CD4+ T cells that have already been exposed to antigens, sustaining long-term cellular immunity by promoting survival and the effector cytokine production of T helper 1 (Th1) memory cells [57]. Several SNPs in the IL-23R gene have recently been shown to be associated with autoimmune and inflammatory conditions, including Crohn's disease [58], rheumatoid arthritis (RA) [59], and psoriasis [60]. More recently, an association was reported between these SNPs and GD and Graves' ophthalmopathy (GO) in North American Caucasians [61]. SNP rs11209026 is specific for Crohn's disease [58], while rs10889677 and rs2201841 have been shown to confer risk for both Crohn's disease [58] and rheumatoid arthritis (RA) [59]. Intriguingly, these latter two SNPs are the same SNPs Huber et al. [61] found to be associated with GO; however, rs7530511 was associated with GD, but not specifically with GO [61]. In contrast, the rs11209026 SNP did not show an association with GD or GO in their cohort [61], but this SNP has not been proven to be the causative SNP in Crohn's disease [58]. Thus, different variants in the IL-23R gene may predispose individuals to different autoimmune conditions. We did not find an association between the IL-23R gene and AITDs in the Japanese population, perhaps due to ethnic differences, environmental factors, or a very small effect that cannot be detected in our dataset [54].

Interferon-induced helicase 1 (IFIH1) is thought to have a role in protecting the host from viral infection by sensing viral nucleic acids in the cytoplasm and triggering a cellular antiviral and apoptotic response [62, 63]. Because Coxsackie and other enteroviral infections are epidemiologically linked to type 1 diabetes (T1D) incidence [64], it was suggested that IFIH1 polymorphism acts as a molecular link between the specific viral trigger and the autoimmune response in T1D [65]. Recently, using preliminary results from large-scale association analyses of nonsynonymous SNPs, a novel locus for T1D was identified; IFIH1, which is also known as the melanoma a differentiation-associated 5 (MDA-5), or Helicard, gene [65]. Using logistic regression analysis, the most strongly associated marker in IFIH1 was identified as SNP rs1990760, which encodes an alanine to threonine amino acid change at codon 946, with an odds ratio for association with T1D of 1.16 (5%–95% confidence interval, 1.11–1.22) for the major allele [65]. More recently, the association of IFIH1 alleles with GD in Caucasians was found to be stronger than that with T1D, with an OR for association of 1.47, in contrast to the 1.16 seen for T1D [66]. However, this was not found when IFIH1 was also investigated in a larger independent GD dataset [67]. The IFIH1 gene encodes transcripts that have widespread expression in lymphoid and other tissues, thus suggesting that they could have a role in numerous autoimmune conditions. However, our data did not show significant differences in allele or genotype frequencies for the rs1990760 SNP between AITD patients and control subjects in Japanese [55].

Two whole genome scans for linkage in GD have shown evidence for linkage at putative X-chromosome loci, Xq21 [68], and Xp11 [69], and these loci have also been identified in localized linkage scans of the X-chromosome, Xq21 [68] and Xp11 [70], although one of the two genome wide screen increased their numbers and performed an enlarged genome wide screen and no evidence for Xp21 as a region of linkage to GD [71]. In terms of broader relevancy to autoimmunity in general, Xp11 has also been linked to other autoimmune disorders, T1D, multiple sclerosis, and RA, thus suggesting the presence of common susceptibility polymorphism(s) [72–74]. The FOXP3 gene is located at Xp11.23 within this area of autoimmune disease linkage and is therefore an excellent positional candidate gene for autoimmunity at this locus. Indeed, Bassuny et al. [75] reported an association between a functional microsatellite polymorphism, (GT)n, located in the promoter/enhancer region of FOXP3, and T1D in a Japanese population. However, a subsequent study could not confirm the FOXP3 association with T1D in an Italian population [76]. A recent study from the UK tested several FOXP3 polymorphisms for associations with GD and found no robust evidence that those polymorphisms contributes to the susceptibility to GD [77]. We tested the FOXP3 gene locus for associations with AITDs in two cohorts of US Caucasians and Japanese AITD patients [56]. Our study demonstrated a weak association between polymorphisms of the FOXP3 gene and AITD in US Caucasians but not in the Japanese. However, one group from Japanese reported that the −3279A/C SNP of the FOXP3 gene is related to the development and intractability of GD and the −2383CC genotype to the severity of HT [78]. These results, if replicated, may suggest that inherited abnormalities of Treg function may contribute to the etiology of AITD.

The interleukin-2 receptor-α (IL-2RA) encodes the α-chain of the IL-2 receptor (IL-2R) complex (also known as CD25), which is central to immune regulation as an important modulator of self-tolerance and immunity [79], and the IL-2RA association with type 1 diabetes was originally identified by Vella and coworkers [80]. The IL-2RA gene has also been associated with GD [81], RA [82], and multiple sclerosis [83] in Caucasians, which implies that this locus may have a general effect on predisposition to autoimmunity. Recently, two SNPs in intron 1 of the IL-2RA, rs706778, and rs3118470, were associated with T1D in the Japanese population [84]. However, we did not find an association between the IL-2RA gene and AITDs in the Japanese population [44]. It may be due to the effect size being smaller than that seen in Caucasians or that there dataset may not be powerful enough to detect such an effect.

5. Thyroid-Specific Genes in Japanese

5.1. The Thyroglobulin Gene. The thyroglobulin (Tg) protein is the major thyroidal protein antigen and is a precursor to thyroid hormones. Tg is also a key antigen in AITD as evidenced by the fact HT is characterized by antithyroglobulin antibodies which are detected in 75% of patients [35]. Whole genome linkage studies identified a locus on chromosome

8q24 that was linked with AITD; this locus contained the Tg gene [71]. Sequencing of the Tg gene identified several nonsynonymous SNPs that were associated with AITD [85]. In our dataset of Japanese AITD patients we found an association between a Tg microsatellite polymorphism and HT [86].

5.2. TSH Receptor (TSHR) Gene. The TSHR gene is located on chromosome 14q. It was found to be associated with GD both by the candidate gene approach and by whole genome linkage studies [71]. The TSHR gene was a prime candidate gene for GD since GD is caused by autoantibodies that bind to and stimulate the TSH-receptor. Several TSHR SNPs have been tested for association with GD, including nonsynonymous SNPs in the extracellular TSH-receptor domain and in the intracellular domain of the TSHR; all of these gave conflicting results [87]. However, linkage studies demonstrated significant evidence for linkage of GD with a locus on chromosome 14q harbouring the TSHR gene and many other genes [71]. It was later found that noncoding SNPs in intron 1 of the TSHR confer the association with GD [88]. Recently, association within the TSHR intron 1 in Caucasians was narrowed down to two SNPs (rs179247 and rs12101255) within intron 1 [89]. The disease-associated genotypes of rs179247 (AA) and rs12101255 (TT) showed reduced mRNA expression ratios of flTSHR relative to two alternate TSHR mRNA splice variants [89]. Płoski et al. [90] validated association of TSHR intron 1 SNPs with GD in three independent European cohorts and demonstrated that the etiological variant within the TSHR is likely to be in strong linkage disequilibrium with rs12101255. In Japanese, multiple SNPs in intron 7 of TSHR gene were associated with GD [91].

6. Conclusions and Future Direction

Through the genetic studies undertaken to date, we now know that substantial ethnic differences exist in AITD genetic predisposition between populations of Caucasian and Japanese ancestries. Beside the HLA-DRB1 alleles as described, the most evident ethnic difference is seen in the nonsynonymous coding polymorphism of the PTPN22 gene in Caucasian populations. This polymorphism is rarely found in Japanese population [39] and thus specifically contributes to AITD in populations of Caucasian descent. The absence of a disease-risk allele in a population, as in the case of PTPN22, can easily explain the genetic heterogeneity between populations [92]. However, the situation is more complex in cases where the disease-risk allele is shared among different populations and the results of association tests are not. This could occur when (1) a positive association in the primary report represents a false positive due to sampling biases, (2) a negative association observed in a replication study is a false negative due to a lack of statistical power, or (3) true genetic heterogeneity exists (genetic contribution of the gene polymorphisms is zero in a population, or lower than that of the population originally reported) [92].

The most recent step in the evolution of genetic studies in common disease sees association analysis performed at a genome-wide level to produce genome-wide association studies [93–95]. These studies, now beginning to emerge in some common diseases, bring together the latest advances in our genetic maps, large-scale automated genotyping technologies, and large national DNA collections [96–98]. Genotyping technology is now being utilized which is able to produce information on over 500000 SNPs in cohorts of between 2000 and 3000 samples. These studies will not only help identify novel loci but should also provide insights into the different technical, analytical, methodological, and biological aspects of genome-wide association analysis. Assuming that these new approaches deliver some novel loci, an important next step will be the validation and replication of loci that will then justify more detailed fine mapping and functional analyses. Recently, one group from China did the first >500 k published SNP screen in a large Chinese population [99]. They identified two new susceptibility loci for atopic dermatitis in the Chinese Han population [99]. Since genetic heterogeneity of disease susceptibility variants between ethnic groups is common in complex diseases, following up this work will be important in the future.

Genetic analyses undertaken in the last decade have revealed a completely new picture of AITD pathogenesis and made us aware of heterogeneity among individuals and populations. Our final goal is to establish new treatments for AITD, based on the pathogenesis and prognosis of individuals, which could lead to the development of tailor-made therapies for AITD. To reach this goal, we should continue to uncover unknown genetic predispositions and clarify differences in roles among ethnicities. Upcoming genome-wide scans for additional populations worldwide and the meta-analysis of these studies may elucidate the complete picture of AITD.

Conflict of Interests

The author declares that they have no competing financial interests.

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Review Article

How Does Age at Onset Influence the Outcome of Autoimmune Diseases?

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The age at onset refers to the time period at which an individual experiences the first symptoms of a disease. In autoimmune diseases (ADs), these symptoms can be subtle but are very relevant for diagnosis. They can appear during childhood, adulthood or late in life and may vary depending on the age at onset. Variables like mortality and morbidity and the role of genes will be reviewed with a focus on the major autoimmune disorders, namely, systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), multiple sclerosis (MS), type 1 diabetes mellitus (T1D), Sjögren's syndrome, and autoimmune thyroiditis (AITD). Early age at onset is a worst prognostic factor for some ADs (i.e., SLE and T1D), while for others it does not have a significant influence on the course of disease (i.e., SS) or no unanimous consensus exists (i.e., RA and MS).

1. Introduction

Autoimmune diseases (ADs) affect approximately 5% of the world population [1, 2]. The age at onset varies widely depending on the disease. For example, sixty-five percent of patients with systemic lupus erythematosus (SLE) start manifesting their symptoms between ages 16 and 55 [3]. Another 20 percent manifest them before age 16 and the remaining 15 percent after age 55 [4]. Rheumatoid arthritis (RA) can begin at any age but has its peak between ages 30 and 55 [5]. Juvenile idiopathic arthritis (JIA) is a term used to describe the autoimmune, inflammatory joint condition that develops in children. Another prevalent AD is Sjögren's syndrome (SS) which is considered to be more prevalent in women between ages 45 and 50 [6]. Multiple Sclerosis (MS) usually appears between ages 20 and 40, and it is very rare during adolescence [7]. Type 1 diabetes mellitus (T1D) is considered a childhood and adolescent disease with two peaks of onset, one between ages 5 and 9 and a second between ages 10 and 14 [8]. On the other hand, an adult onset would be considered to be in a range of 25–61 years old [9]. Finally, autoimmune thyroiditis (AITD) is thought to be a disease that can appear in childhood but is more prevalent during adulthood [10]. Herein, we analytically reviewed the effect of age at onset on the most prevalent ADs, their clinical differences (Table 1), and their genetic and immunologic relationships (Table 2).

2. Systemic Lupus Erythematosus

SLE is a chronic AD that affects individuals of every age. It is more common in adults, but it may be diagnosed during childhood as well [11]. Childhood disease onset is characterized by a high degree of morbidity compared with adult SLE populations [12]. It is associated with a higher mean in the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) scores at presentation. The patients have a higher frequency of renal disease, malar rashes, pericarditis, hepatosplenomegaly, and hematologic alterations [13]. In treatment, they usually have a higher use of prednisone and the need for additional immunosuppressive therapies [14]. Patients at this age are susceptible to a longer lifetime of damage from the disease flares and the treatment side effects and a mortality rate that is 2 times higher [11, 12]. Other associations found in childhood onset are growth failure, delayed puberty, and fibromyalgia. In contrast, adult onset

Table 1: Clinical differences between early and adult onset.

AD	Early onset	Late onset	
SLE	Higher degree of morbidity [12] Higher (SLEDAI) score at presentation Higher incidence of renal disease Malar rashes Pericarditis Hepatosplenomegaly Hematologic alterations (Leucopenia) Higher use of prednisone Additional immunosuppressive therapies Greater lifetime of damages from the disease flares and the treatment side effects [13] 2 times higher mortality rate Growth failure Delayed puberty Fibromyalgia [11] Higher odds of presenting proteinuria Haemolytic anaemia Arthritis [16]	Increased rate of pulmonary disease [11] Increased rate of simultaneously developing another AD [12]	
RA	Proximal Interphalangeal, metacarpophalangeal, elbow, metatarsophalangeal, and ankle joints Classical rheumatoid hand deformities Interstitial lung disease Associated SS [21] Shorter morning stiffness [19]	Acute onset in large and small joints (specially shoulders) PMR-like symptoms [20] Constitutional features Weight loss Myalgia Rheumatic nodules Neuropathy [21] Longer morning stiffness [19]	*Positive RF PJIA** Rapid onset of inflammation in multiple joints Proximal interphalangeal, metacarpophalangeal, elbow, metatarsophalangeal, and ankle joints Effects of the disease in a growing skeleton: Growth retardation Accelerated growth of an affected joint [24]
SS	Recurrent parotid gland enlargement Milder extraglandular manifestations [32]	Sicca symptoms [31]	
T1D	Ketosis and ketoacidosis Higher mean glycated hemoglobin [38, 40]	Better preserved B-cell function Longer symptomatic period before diagnosis Less frequency of insulin autoantibodies and HLA class II susceptibility alleles [40, 41] Milder signs of metabolic decompensation and a lower glycated hemoglobin level at diagnosis [39, 42]	
MS	Mainly relapsing remitting disease onset Frequent presentation with brainstem-cerebellar dysfunction Pyramidal symptom Optic neuritis [52]	Primary progressive course Motor symptoms are predominant	
AITD	Often transient [10] Delayed growth Bradykinesia Delayed pubertal development [57, 58]	Permanent [10]	

AD: autoimmune disease; RA: rheumatoid arthritis; SS: Sjögren's syndrome; T1D: type 1 diabetes; MS: multiple sclerosis; AITD: autoimmune thyroiditis; PJIA: polyarticular juvenile idiopathic arthritis; RF: rheumatoid factor.

*In RA, early age at onset is considered ≥16 years old. Positive RF PJIA is considered a comparable disease with a childhood onset (<16 years old).

Table 2: Genetic and immunological factors related to age at onset.

Autoimmune disease	Population	Immunologic	Genetic
SLE early onset*	African Americans and Gullah		Odds of developing the disease increased by 48% per risk allele in Gullah patients and 25% in African-American patients [16]
	Caucasians Hispanics African Americans and Gullah	Higher odds of presenting Anti-dsDNA antibody [16]	
	Caucasians		Association with *MBL2* gene [17]
Positive RF PJIA	Caucasians	Higher frequency of Anti-CCP antibodies	HLA-DR4 alleles [26]
RA early onset	Hispanics	Higher frequency of Anti-CCP antibodies	HLA-DRB1 *DERAA* sequence [29]
SS early onset	Asians	Higher prevalence of anti-M3R Antibodies [35]	
T1D early Onset	Caucasians		Association with high Risk HLA DQB1*02/*0302 [44] Association with *PTPN22* C1858T [45]
	African Americans		Association with absence of DQB1*0602 and increase in DQB1*0201 [42]
	Asians		Association with STAT4 polymorphism [46]
T1D late onset	Caucasians	Less frequency of insulin autoantibodies	
MS susceptibility	Hispanics		High risk DQB1*0602 susceptibility allele is the same that protects in T1D [55]
AITD early onset	Asians		Coexistence of HLA-B*46 and HLA-Cw*01 [60]

AD: autoimmune disease; RA: rheumatoid arthritis; SLE: systemic lupus erythematosus; SS: Sjögren's syndrome; M3R: muscarinic 3 receptor; T1D: type 1 diabetes; MS: multiple sclerosis; AITD: autoimmune thyroiditis; PJIA: polyarticular juvenile idiopathic arthritis; RF: rheumatoid factor.
*Defined as ≤20 years old.

patients are more likely to develop pulmonary disease [11] and may have an increased rate of simultaneously developing another AD such as SS [15]. Additionally, Webb et al. showed that childhood onset had higher odds of presenting proteinuria, haemolytic anaemia, arthritis, leucopenia, and anti-dsDNA antibody. They also showed that early adult onset (≤50 years) had greater odds of having proteinuria, cellular casts, seizures, anti-nRNP antibody, anti-Sm antibody, and anti-dsDNA antibody than late-adult-onset patients (>50 years) [16]. Hersh et al. stated that rates for clotting disorders, myocardial infarction, and neurological disorders (e.g., seizures) are similar in both of them [11]. In both cases, better disease outcome can be achieved if an early diagnosis is made by a better recognition of age-specific manifestations and the use of a good treatment algorithm [15].

As other ADs, SLE is a complex disease in which several polymorphic genes and environmental factors over time influence the onset and course of disease. Among the genes found to be associated with SLE, *MBL2* gene has been suggested to influence the age at onset [17]. Webb et al. showed that odds of developing SLE during childhood (<18 years) increased by 48% per risk allele in Gullah people with SLE and 25% in African-American patients. No association between the number of SLE risk alleles and age at onset in Hispanic patients and European American was found [16].

3. Rheumatoid Arthritis and Juvenile Idiopathic Arthritis

RA is a chronic, systemic, and destructive inflammatory AD that involves both small and large diarthrodial joints. It usually develops in middle-aged adults but may also appear during childhood or late in life [18]. Patients who are diagnosed between ages 16 and 65 are considered young onset and after 65, late onset with each of them having different semiologic characteristics. Pease et al. found that late-onset patients had longer stiffness in the morning [19]. In contrast, Deal et al. reported no difference in morning stiffness between young- and late-onset patients [5]. It has been reported that older patients have more acute onset in both large (specially the shoulders) and small joints and usually present polymyalgia

rheumatica-like symptoms [20]. Turkcapar et al. reported that proximal interphalangeal, metacarpophalangeal, elbow, metatarsophalangeal, and ankle joints are more associated with young-onset AR. Classical hand deformities, interstitial lung disease, and SS presented less commonly at late onset. However, these patients can present more constitutional features like weight loss, myalgia, rheumatic nodules, and neuropathy [21]. Anti-CCP seropositivity and elevated inflammatory markers at onset are associated with poor radiological outcome in both early and late onset [22, 23]. Studies differ when talking about prognosis and report favorable, similar, or worse outcome. Most of them conclude that treatment should be instituted equally on both.

Juvenile idiopathic arthritis (JIA) is a term that describes a group of disorders that share the clinical manifestation of chronic joint inflammation from unknown causes that begins before 16 years of age [24]. It is divided into seven groups being polyarticular JIA (PJIA), the one most similar to RA and the most related to rheumatoid factor positivity [25]. PJIA is defined by the presence of more than four affected joints during the first six months of illness. There is a bimodal distribution of the age at onset: from 2 to 5 years and 10 to 14. Children with polyarticular disease with onset after 10 years of age are divided into negative rheumatoid factor (RF) polyarthritis and positive RF polyarthritis. Positive RF polyarthritis tends to be associated with anti-CCP antibodies and a more severe disease than their adult counterpart [26]. They usually have a rapid onset of inflammation in multiple joints, especially hands, wrists, elbows, and feet. A big difference between it and the adult form of the disease is the effect of the disease on their growing skeleton that can lead to growth retardation or accelerated growth of an affected joint [24].

The HLA-DRB1 gene has been associated with RA susceptibility, especially with those alleles carrying the shared epitope [27]. Anti-CCP antibodies can be detected at early disease stages and may be used as indicators of RA progression and prognosis [28]. Diaz et al. reported that anti-CCP antibodies and the HLA-DRB1 DERAA sequence influence the age at onset of RA [29]. This finding may be useful to predict early RA onset in genetically predisposed patients [29]. In JIA, RF-positive polyarthritis with anti-CCP antibodies is associated with the presence of HLA–DR4 alleles and an aggressive disease course [26].

4. Sjögren's Syndrome

SS is a chronic AD characterized by xerophthalmia and xerostomia caused by a progressive lymphocytic and plasma cell infiltration in the exocrine glands and may also have systemic involvement. It is very rare during childhood [30]. Drosos et al. showed that recurrent parotid gland enlargement was more common in early age onset while sicca symptoms were more common in adults [31]. Ostuni et al. reported that the clinical manifestations were similar but extraglandular manifestations were milder [32]. Anaya et al. implied that the clinical symptoms in children do not fulfill the classical diagnostic criteria which are successfully applied to adults [33]. Botsios et al. found that young, adult and elderly patients had similar sensitivity of diagnostic test positivity [34]. In contrast, Nakamura et al. showed the prevalence of anti-muscarinic 3 receptor (anti-M3R) antibodies is higher in early-onset SS than in late-onset SS patients [35]. However, this findings warrant further replication and confirmation.

HLA-DRB1*0301-DQB1*0201 haplotype has been associated with SS [36]. No age at onset relationship was found.

5. Type 1 Diabetes

T1D is a chronic AD resulting from progressive destruction of the pancreatic B cells. B-cell damage may be induced at any age [37]. Childhood- and late-onset patients are characterized by symptoms like polydipsia, polyuria, and weight loss, but younger patients suffer more from diabetic ketoacidosis and ketosis as the initial presentation [38, 39]. Studies indicate that late-onset patients have better preserved B-cell function than early-onset patients. They are also characterized by a longer symptomatic period before diagnosis and a lower frequency of insulin autoantibodies and HLA class II susceptibility alleles [40, 41]. Adult onset can be associated with milder signs of metabolic decompensation and a lower glycated hemoglobin level at diagnosis [39, 42].

Associated HLA-Class II alleles for development of the disease are identified. DRB1*0301-DQA1*0501-DQB1*0201 is considered risk alleles and DRB1*1301-DQB1*0603-DQB1*0602, protective alleles [43]. The high-risk HLA DRB1*0301 and DQB1*02/*0302 alleles are commonly associated with young onset as well as absence of DQB1*0602. Adult patients carry high-risk DQB1*02/0302 less frequently [39, 42, 44]. The *PTPN22* C1858T polymorphism has also been associated with higher risk for development of ADs, specifically T1D, RA, SLE, and Graves disease. This polymorphism could be associated with development of T1D at an early age [45]. Lee et al. showed that *STAT4* polymorphism is associated with early-onset T1D and not with late onset in Asian population and also suggested a dosage effect of risk alleles on the age of onset of disease [46].

6. Multiple Sclerosis

Age at onset of MS as in most of ADs is defined as the age when the first symptoms appear, although the disease process may have begun earlier [47]. Duquette et al. reported an early-onset prevalence of 2.7% with respect to the entire MS population [48]. Studies are consistent with the idea that, in early onset, mild and severe disability levels are reached after a longer time than in the case of adult onset. However, these disability levels are also reached at a lower age in comparison with adult cases. This can be translated into more disability in early-onset patients than their corresponding adult counterpart at the same age. Bad prognostic factors for a worse outcome include disability in the first year, high relapse rate, short interattack intervals, the relapsing progressive course, or a shift of the progressive phase [7, 49–51]. Deryck et al. showed specific clinical characteristics in patients with early onset such as mainly relapsing remitting disease

onset and frequent presentation with brainstem-cerebellar dysfunction, pyramidal symptom, and optic neuritis [52]. Kis et al. showed that primary progressive course and motor symptoms are more characteristic of late-onset patients [53]. Trojano et al. state that current age, together with duration of disease and apart from age at onset, influences MS progression [54].

Associated HLA-class alleles of the disease are also identified. DRB1*1501 and DQB1*0602 were found to be high risk factors for MS [55]. No onset relationship was described, but high risk factor allele DQB1*0602 is thought to be protective in T1D.

7. Autoimmune Thyroiditis

AITD is an inflammatory state of the thyroid gland that results from interaction between genetic and environmental factors. Hashimoto thyroiditis is the most frequent form of chronic autoimmune thyroiditis. This is the most common cause of hypothyroidism in children [56]. Some of its manifestations at this age are effects on growth, bad school performance, bradykinesia, and delayed pubertal development. A goiter is the main symptom that indicates AITD. Patients that receive proper treatment at this age will probably experience normal growth and puberty [57, 58]. Both young and adult onset patients may show symptoms of lethargy, intolerance to cold, constipation, dry skin, brittle hair, and muscle pain. Late-onset hypothyroidism, once it begins, is permanent but, in some young onset patients and postpartum women, it is often transient [10]. Young patients with other ADs are at increased risk of having AITD. A good example is T1D. Riley et al. showed that approximately 20 percent of T1D patients have high serum antithyroid antibody concentrations and 5 percent have abnormalities in thyroid function [59]. That is why they have to be screened annually.

Genetic susceptibility is linked to the HLA DR3 group [57] and to polymorphisms at *CTLA-4* gene, among others. Cho et al. suggested that coexistence of HLA-B*46 and HLA-Cw*01 may be a genetic marker for early-onset AITD in Koreans [60].

8. Conclusions

Age at onset varies among ADs and so do their manifestations. Some of them, for example, MS and SS, are rare during childhood but others such as T1D primarily occur during this period. Early age at onset cannot always be associated with a worse prognosis. Early age at onset is a worst prognostic factor for some ADs (i.e., SLE and T1D), while for others it does not have a significant influence on the course of disease (i.e., SS) or no unanimous consensus exists (i.e., RA and MS). Knowledge of the early-age symptoms will help physicians to provide better treatment which, coupled with educational and transition support, might improve outcome. Understanding of genetic influences and association studies between diseases are required to determine the role of genes in age at onset. Studies with a larger number of people with nontypical ages at onset would bring further insights.

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Review Article

Local Cartilage Trauma as a Pathogenic Factor in Autoimmunity (One Hypothesis Based on Patients with Relapsing Polychondritis Triggered by Cartilage Trauma)

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In the recent years, it has been of great interest to study the binding mechanism between the innate and adaptive immune responses as interrelated processes for the development of multiple autoimmune diseases. Infection has been a well-known trigger of autoimmunity and trauma has been related as well too. Cryptogenic antigens release, recognition of pathogenic structure, and metabolic changes generated by both stimuli begin an inflammatory process which in turn activates the immune system amplifying T and B cell responses. The development of relapsing polychondritis after trauma may have a direct association with these events and in turn probably trigger autoimmune phenomena.

1. Introduction

Proteins that are hidden in the tissues and by different events such as trauma begin to be recognized and attacked by immune system are called cryptogenic antigens, so it starts a process initially with innate and later with acquired immunity mechanisms. An example of this condition is the sympathetic ophthalmia (SO) where breaching of systemic ocular barriers compromises the relative immune privilege of the eye and causes sensitization to previously sequestered uveoretinal antigens [1]. A similar mechanism is observed in relapsing polychondritis (RP) started by local cartilage trauma triggering an immune response against cartilage in distant sites of the body and even in noncartilaginous tissues [2].

2. Lessons Learned of SO

This disease is precipitated by ocular trauma to one eye, followed by destructive inflammation in the nontraumatized or "sympathizing" eye [3]. It is thought that antigens released from the traumatized eye find their way into the draining lymph node and generate systemic immune response [4]. An accompanying infection may provide an adjuvant effect, although severe endophthalmitis, which quickly destroys the injured eye and eliminates the source of antigen, may actually lessen chances of developing the disease [5]. In uveitic disease, it is believed that T cells are capable of recognizing retinal antigens by microbial stimuli that may be immunologically similar in structure to their cognate retinal antigen (antigenic mimicry). Microbial components also interact with innate pattern recognitionreceptors (PRRs) on antigen-presenting cells, generating "danger" signals that are necessary to elicit inflammatory reactions. Following exposure to an uveitogenic stimulus, circulating retinal antigen-specific cells become activated and acquire effectors function [5]. In the above examples, it is postulated that the retinal antigen recognition distance may be submitted by local effects, but it is obvious to assume that there is not a similar type of tissue throughout the body. Therefore, we cannot rule out that it can presented by a systemic effect.

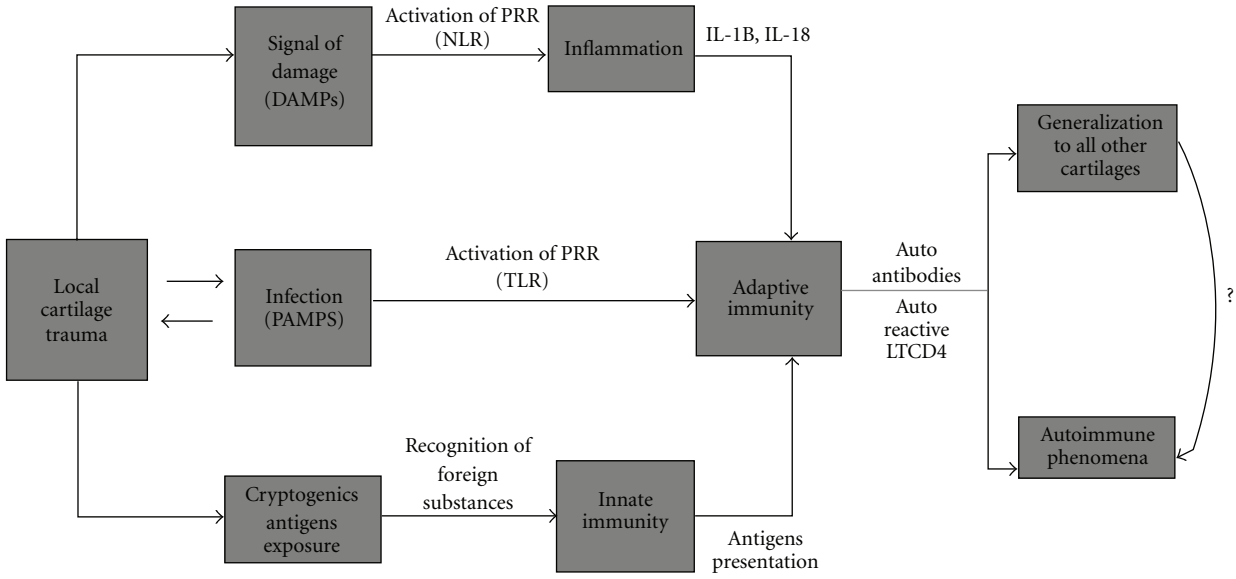

Figure 1: Local cartilage trauma initiates an inflammatory process with or without infection as coadjuvant as well as activation of the innate and adaptive immunity through induction of PAMPs, DAMPs, and cryptogenic antigens which are recognized by PRR (TLR, NLR) that results in the production of IL-1B and IL-18. These interleukins amplify T and B cell responses and might serve as a crucial link into adaptive immune responses. PRR, pattern recognition receptors; TLR, Toll-like receptors; NLR, NOD-like receptors; LTCD4, lymphocytes T CD4.

3. Local Cartilage Trauma as a Triggering Factor of RP

RP is a rare immune-mediated disease of unknown etiology which is associated with inflammation in cartilaginous, especially hyaline cartilage throughout the body.

Possible mechanisms involved are an autoimmune background and a trigger factor such as cancer, infection as hepatitis C virus [6], or drugs (e.g., anti-TNF) [7]. In some cases, recently reported cartilage trauma may be a trigger of the disease in a susceptible person [8–10]. Puncture or other type of trauma with or without presence of foreign material in the cartilage and often associated with infection could trigger an autoimmune disorder by exposing unusual cartilage matrix protein antigens [11].

Also in this case like SO, probably the proteins that are hidden in the tissues would be exposed by factors such as trauma and begin to be recognized by the immune system as foreign, triggering processes for their elimination initially with innate and later with acquired immune mechanisms. This process has not been clearly defined; however, several reported cases of RP have shown an association between mechanical insult to cartilage and the development of more local cartilage inflammation and even the involvement of distant cartilaginous tissues [2].

One hypothesis comes out from these conditions and interrelate innate immune response, and the activation of the adaptive system may be mediated by PRR of the innate immune cells such as Toll-like receptors (TLRs) and NOD-(nucleotide-binding oligomerization domain-)like receptors (NLRs). TLR family consists of 10 receptors that are divided into membrane and endosomal localization and which allow for recognition and response to pathogen-associated molecular patterns (PAMPs) representing diverse microbial products (lipopolysaccharide, flagellin) of most pathogens [12, 13]. This could explain the significant role of infection as a coadjuvant factor in OS and in our cases of RP. Infection is a well-known contributing factor for the development of autoimmunity [14, 15]. NLR family members are cytosolic sensors of microbial components and danger signals such as damage-associated molecular patterns (DAMPs) which represent common metabolic consequences of infection and inflammation released during the tissue damage and cell lysis and include high mobility group box 1 protein, alarmins, heat shock proteins, and uric acid [16, 17].

These two ways of pattern recognition contribute to making the link between innate and adaptive immune response through the production and expression of IL-1B and IL-18 generating an inflammatory response and acting on lymphocytes in several pathways including upregulating IL-2 receptor expression, which in turn prolongs survival of T cells and increases B cell proliferation and production of antibodies [18, 19]. IL-1B and IL-18 also play a crucial role in driving the differentiation and amplification of Th17 and Th1 cells, respectively [20, 21]. Thus, in general, these interleukins amplify T and B cell responses and might serve as a crucial link translating into adaptive immune responses [18]. In the RP triggered by local trauma, the inflammatory reaction may lead to the production of autoantibodies and autoreactive T lymphocytes against cartilage structures (e.g., collagen type II) which in turn could develop chondritis at other sites [22].

4. RP and Systemic Autoimmune Phenomena

It is well known and reported in the literature that about 30% of the patients with RP have additional autoimmune or hematological diseases, most frequently systemic vasculitis, rheumatoid arthritis, myelodysplastic syndromes, or systemic lupus erythematous [23].

Recently, we reported a comparative analysis of 18 patients diagnosed with RP with or without tissue trauma (7 versus 11 patients, resp.) and evaluated the development of the systemic autoimmune phenomena. The results showed that the patients with previous local trauma lead to a greater systemic autoimmune response shown by the development of antinuclear antibodies, rheumatoid factor, antineutrophil cytoplasmic antibodies, autoimmune thyroiditis, rheumatoid arthritis, and vasculitis [2]. Also included in this last group, the description of an RP case induced by local cartilage trauma in the ankle with later development of central nervous system compromise manifested as a hypertrophic pachymeningitis, PR3 ANCAs-positive vaculitis outside of the complete clinical expression of a Wegener's granulomatosis with request for aggressive treatment with cyclophosphamide and rituximab given severity of the condition and poor initial response to treatment [24].

Figure 1 outlines the hypothetic steps in the pathogenesis of autoimmunity in patients with local cartilage trauma and development of RP.

5. Conclusions

Local cartilage trauma with or without infection triggers an immune processes directed against cryptogenic antigens that conduce to inflammation of cartilage both local and later in other sites of the body. By diverse mechanisms which may be implicated by PRR such as TLR and NLR, a systemic autoimmune phenomena is induced. The presentation of RP after local cartilage trauma may influence the presence of this systemic autoimmunity.

Conflict of Interests

The authors declare that they have no conflict of interests.

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