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Highlights

► We defined the probability of links between environmental factors and autoimmunity. ► Innate immunity mediated by toll-like receptors mediates tolerance breakdown. ► Abnormalities in B, Th17, and T regulatory cells lead to autoimmunity. ► Self antigens undergo post-translational modifications causing molecular mimicry. ► Epigenetic changes, not limited to DNA methylation, may predispose to autoimmunity. ► Mechanisms are in agreement with epidemiology and models discussed in the issue.

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Review

Mechanisms of environmental influence on human autoimmunity: A national institute of environmental health sciences expert panel workshop

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ABSTRACT

The mechanisms leading to autoimmune diseases remain largely unknown despite numerous lines of experimental inquiry and epidemiological evidence. The growing number of genome-wide association studies and the largely incomplete concordance for autoimmune diseases in monozygotic twins support the role of the environment (including infectious agents and chemicals) in the breakdown of tolerance leading to autoimmunity via numerous mechanisms. The present article reviews the major theories on the mechanisms of the environmental influence on autoimmunity by addressing the different degrees of confidence that characterize our knowledge. The theories discussed herein include (i) the role of innate immunity mediated by toll-like receptors in triggering the autoimmune adaptive response characterizing the observed pathology; (ii) changes in spleen marginal zone B cells in autoantibody production with particular focus on the B10 subpopulation; (iii) Th17 cell differentiation and T regulatory cells in the aryl hydrocarbon receptor model; (iv) self antigen changes induced by chemical and infectious agents which could break tolerance by post-translational modifications and molecular mimicry; and finally (v) epigenetic changes, particularly DNA methylation, that are induced by environmental stimuli and may contribute to autoimmunity initiation. We are convinced that these working hypotheses, in most cases supported by solid evidence, should be viewed in parallel with animal models and epidemiological observations to provide a comprehensive picture of the environmental causes of autoimmune diseases.

1. Introduction

The etiology of autoimmune diseases (AID) remains largely unknown despite numerous research efforts ranging from clinical studies to epidemiology to experimental models. The leading working hypothesis states that autoimmunity results from a susceptible genetic background and the impact of specific environmental factors. This is well epitomized by the most recent plethora of genome-wide association studies based on a large number of single nucleotide polymorphisms [1–5]. Nevertheless, proposed associations only account for disease susceptibility in subgroups of patients, thus suggesting that environmental factors are crucial to determine disease onset. The number of candidates proposed for specific autoimmune diseases is continuously growing as new evidence is reported for infectious agents and chemicals/xenobiotics [6–9]. Among the latter, we are particularly intrigued by the possibility that toxicological factors such as silica have been recently addressed as ideal candidates based on epidemiological associations and other experimental evidence. In general terms, two approaches can be utilized to evaluate the potential for environmental chemicals to contribute to autoimmunity and are summarized elsewhere in this *Journal* issue [9]: (i) epidemiological studies, which may associate chemical exposure with biologic markers of autoimmunity and (ii) laboratory studies which identify plausible biological mechanisms through which environmental agents could induce or enable activation of

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autoimmune clones. In this workshop, epidemiological and human data relevant to environmental agents and autoimmunity were thoroughly reviewed by different study groups. Over the past 10 years, the NIEHS has participated in trans-NIH committees and sponsored a number of workshops examining the role of the environment on the development of autoimmune disease. Despite the recommendations for research initiatives and the ongoing accumulation of research data, there are still numerous gaps in our knowledge. The goal of the workshop that was held in Durham, NC was to bring together experts from the environmental health science and autoimmune research communities to (i) review the findings from their diverse research disciplines concerning the role of the environment and the development of autoimmune disease; (ii) identify conclusions that can be drawn with confidence from existing data; (iii) identify critical knowledge gaps and areas of uncertainty; (iv) establish key elements of a coherent research agenda to help fill these gaps and resolve uncertainties.

The major categories of putative environmental agents are summarized in Table 1 along with the proposed mechanisms that will be discussed in the present article. We should note that the discussion of some of the proposed mechanisms is far from comprehensive of the literature available as numerous studies addressing the issues have been performed in the majority of autoimmune diseases. When appropriate, the discrepancies and common findings of these studies are discussed. Nevertheless, we are aware that some of the issues addressed in the specific sections will be skewed by the authors' areas of expertise. As in the other two associated report articles on epidemiology [Citation from same issue] and animal models [Citation from same issue], we herein summarized evidence for the specific exposure-disease associations reviewed that we considered could be classified in the "confident," "likely," or "unlikely" categories regarding the contribution of the agent to the development of the disease according to the Vallombrosa statement. A detailed definition of these categories is illustrated in the review article by Frederick W. Miller and Colleagues in the same *Journal* issue [9].

2. Effects on innate immunity

2.1. Toll-like receptor (TLR) activation by xenobiotics related to autoimmune disease

It has long been held that the innate immune response plays a key role in host protection from pathogens. This network of the innate immune system signaling includes the recognition of broad patterns or molecular motifs called pattern-associated molecular patterns (PAMPs) by germline-encoded "common" receptors called pattern recognition receptors (PRRs). Pattern recognition rather than specific epitope recognition allows for more rapid screening of molecules to determine "self" from "non-self" or pathogen, thereby enabling the fast response characterized by the innate immune system. PRRs are designed to recognize and bind to signals and structures more common to pathogens than to their hosts. Some PRRs recognize structures on the surface of pathogens such as lipopolysaccharide, flagellin, peptidoglycan, and lipoprotein. Other PRRs interact with patterns like single-stranded (ss) RNA and foreign methylation (or demethylation) patterns on DNA such as unmethylated CpG DNA.

Table 1
Proposed environmental factors and the putative mechanisms involved in the breakdown of immune tolerance.

Environmental factors that have been associated with autoimmune diseases
• Infectious agents (bacteria, viruses)
• Chemicals/xenobiotics
• Adjuvants
• Physical elements (ultraviolet radiation)
Possible mechanisms
• Polyclonal B cell activation;
• Direct effect impairing the immune response (*Th17 cells*);
• Effects on innate immunity (*TLR, adjuvants*);
• Direct interaction with regulatory cells (*T regulatory cells*);
• Modification of self antigens (*post-translational modifications*);
• Alterations of DNA methylation (*epigenetics*)

The mammalian toll-like receptors (TLR) are a family of PRRs which are evolutionarily conserved and include at least 12 different TLRs. Each of these TLRs recognize PAMPs characteristic of pathogenic microorganisms. Different combinations of TLRs are expressed on hematopoietic and non-hematopoietic cells and with various extra- and intra-cellular expression patterns. For example, TLR4 is expressed on the surface of macrophages and TLR7 is located in the wall of the endosome to recognize microbial components accessible after "digestion".

There is a constant interplay between the innate and adaptive arms of the immune system and TLRs play a key role in this interaction. More recent studies into the innate immune response and upregulation of proinflammatory autoimmune responses have provided a link between TLRs and autoimmune disease [10]. One hypothesis is that altered innate immune responses and dysregulated TLR signaling are a key step in triggering autoimmune diseases [11]. For example, in virus-induced animal models of type I diabetes, activation of TLR pathways exacerbates the disease [12] while the activation of the type I interferon pathway may drive autoantigen presentation and autoantibody production in primary Sjögren's syndrome [13,14]. Also in humans, genetic polymorphisms and functional changes of TLR9 have been associated with systemic lupus erythematosus (SLE) [15] and primary biliary cirrhosis (PBC).

The activation of the innate immune system via TLR predisposes to toxic-induced inflammation. In fact, the pre-exposure of macrophages to a number of stimulants including zymosan for TLR2, poly(I:C) for TLR3, LPS for TLR4, flagellin for TLR5, R848 for TLR7/8, ODN1826 for TLR9 can induce hyperexpression of inflammatory cytokines in experimental models [13,14]. The pertussis toxin functions as a surrogate for environmental factors to induce animal models of autoimmunity, such as experimental autoimmune encephalomyelitis. It has been reported that the primary mechanism by which the toxin induces the disease is mediated by TLR4.

Amidst this relatively strong body of work demonstrating a role for TLR dysregulation in autoimmune disease, few studies have examined the effects of exposures to xenobiotics within this system. One study showed that activation of TLR in macrophages predisposes the cells to toxin-induced inflammatory cytokine production. More recently, a study found that co-exposure to nickel and TLR2 agonists lead to synergistic effects on release of IL-6 by lung fibroblasts in a protein kinase-dependent pathway [16]. While not directly related to autoimmune disease in this study, the relationship between upregulated TLR signaling and inflammation was clear and could be involved in autoimmune endpoints in the long term.

In mouse strains susceptible to mercury-induced autoimmune disease, LPS exposure exacerbates the disease. *In vitro* studies on the response of human peripheral blood mononuclear cells to nanomolar concentrations of mercuric chloride demonstrated an unopposed proinflammatory cytokine response only when the cells were co-exposed to LPS [17].

2.2. Adjuvant effects and inflammatory responses

Compelling data from both animals and human studies suggest an adjuvant role of environmental agents in autoimmunity (Table 2). Adjuvants can be defined as agents that stimulate the

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Table 2
Data implicating the association of adjuvants with autoimmune manifestations in animals and humans [117].

Adjuvant	Phenotype	Species
Pristane, IFA, squalene	Chronic arthritis	Mice and rats
Pristane, IFA, squalene	Lupus-related anti-nRNP/Sm/Su Ab	Mice
Pristane, IFA, squalene, mineral oil	Anti-cytoplasmic Ab, anti-ssDNA/ chromatin Ab	Mice
MDP; LPS; Gram + CoxackieB3, IL1β, TNFα	Experimental thyroiditis; Myocarditis	Mice

Alum in vaccine (HBV, HAV, MS, tetanus) Chronic fatigue syndrome, polymyalgia rheumatica human.

immune system without any antigenic effect *per se*. In the context of autoimmunity, it can be envisioned that adjuvants possess the ability to activate innate and adaptive immunity and induce the release of chemokines and proinflammatory cytokines (Table 3). Adjuvant may (i) mimic conserved molecules e.g. LPS on bacterial cell wall and unmethylated CpGs, (ii) activate the innate immune system by augmenting dendritic cells or macrophage functions by binding to TLR, and (iii) modulate the release of chemokines and subsequent recruitment of immune cells in the adaptive arm. Thus, environmental adjuvants can induce early non-antigen-specific signals that initiate an innate immune response and also shape the later adaptive responses that are responsible for autoimmune pathology [18].

Adjuvants mediate non-specific stimulation of immune cells that is important for the initiation and perpetuation of autoimmune response in animal models of autoimmunity.

Studies in experimental animal models revealed the importance of the adjuvant effect in the induction of autoimmune disease. Immunization of antigen must be accompanied by a powerful adjuvant, e.g. complete Freund adjuvant, which includes the mycobacterium component. Incomplete Freund adjuvant results in the production of antibodies but without the occurrence of autoimmune diseases. Adjuvant induced key early proinflammatory cytokines is essential for disease manifestation.

Active infection or microbial products of the infection can provide the adjuvant effect necessary for the induction of many autoimmune disorders [19]. For example, it was reported that determinant mimicry between 65 kDa mycobacterial heat-shock protein and its homologues in microbial agents in the conventional environment, is involved in modulating the severity of adjuvant arthritis in rats. Radiation, chemical toxins, or microbial products may selectively activate or inhibit innate immune response pathways or selectively influence a single TLR pathway to influence the induction of autoimmunity.

Although accumulating data has implicated the significance of environmental agents such as microbial products, radiations and chemical compounds are potential adjuvants of immune responses and can lead to autoimmunity. We are convinced that more studies are needed, ideally with replication in different laboratories using standardized techniques, to identify the molecular motifs of adjuvants and their physiological receptors that are associated with clinical manifestation of autoimmunity. This should be accompanied by detailed studies on adjuvant effects on different immune cell populations to delineate their corresponding mechanisms in the initiation and/or perpetuation of the disease processes. One example of these controversies is the case against silicone that was first proposed over 20 years ago as a "human adjuvant disease" that failed to be subsequently validated [20]. Nevertheless, the conclusions of the available studies were that silicone cannot induce autoimmune disease in normal animals, but may exacerbate disease in predisposed strains, as well discussed in the review article on animal models found in this *Journal* issue.

Future findings will be useful in defining the interaction of pathogen-associated recognition pattern/motifs in host–microbe interaction and their downstream effect in autoimmunity. In addition, the lack of specific autoimmune associated biomarkers of adjuvant exposure warrant the need to develop improved technologies in their identification and designing functional studies of specific biomarkers of adjuvant exposure e.g. receptors of PAMPs and other novel biomarkers.

Table 3
Immunological players in the innate and acquired immunity associated with adjuvant induced autoimmunity [118–129].

Immunostimulant	Cellular interaction	Type of immune response
TLR ligands		
Bacterial lipopeptide, lipoprotein and lipoteichoic acid; mycobaterial lipoglycan; yeast zymosan, porin	TLR2, 1/2, 2/6	Th1, Ab, NK cell
Viral ds RNA	TLR3	NK cell
Lipopolysaccharide, Lipid A, MPL®, AGPs	TLR4	Strong Th1, Ab
Flagellin	TLR5	Th1, CTL, Ab
Viral ss RNA, imidazoquinolines	TLR7/8	Strong Th1, CTL
Bacterial DNA, CpG DNA, hemozoin	TLR9	Strong Th1, CTL, Ab; NK cell
Uropathogenic bacteria, protozoan profilin	TLR11	Th1
Others		
Saponins (Quil-A, QS-21, Tomatine, ISCOM, ISCOMATRIX™)	Antigen processing	Strong Th1, CTL, Ab, long term memory
Bacterial toxin (CT, LT)	ADP ribosylating factors Ab	

3. B cell activation

B lymphocytes, a major component of adaptive immunity, generate their pre-immune, anticipatory repertoire in the bone marrow via a gene recombination process known as V(D)J recombination. The pairing of rearranged heavy chains with rearranged light chains increases the pre-immune repertoire further and it is estimated that these processes generate a repertoire of 10^7–10^8 B lymphocytes each with unique surface receptors. This enormous diversity of the B lymphocyte repertoire comes with a cost. Since much of the B cell repertoire is somatically generated, evolutionary forces cannot efficiently purge potentially autoreactive Ig. Instead, several checkpoints in B cell development exist that ensure that a large portion of the autoreactive B lymphocytes is excluded from the immunocompetent peripheral lymphocyte populations. These checkpoints include central tolerance in the bone marrow and several checkpoints in the spleen germinal centers during the generation of high affinity memory B cells where autoreactive B cells are subjected either to deletion, receptor editing, or rendered anergic. Dysfunctions of these tolerance checkpoints have been directly correlated with autoimmune disease in murine models [21,22].

We are confident that B cells contribute to the development of autoimmune disease in several ways and environmental pathways to B cell activation are summarized in Table 4 and can be compared with T cell activation mechanisms in Table 5. The best-known role of B cells in autoimmunity is through the secretion of pathogenic autoreactive antibodies. They can secrete pathogenic autoreactive antibodies that can cause tissue damage, they can induce activation of autoreactive T cells in murine models of lupus, and they may secrete pro-inflammatory cytokines.

B cells generally are derived from three major subsets: B1 cells that can be further subdivided into B1a and B1b, marginal zone B

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Table 4
Environmental agents associated with B cell activation [130].

B cell feature	Environmental agent
Polyclonal activation with hypergammaglobulinemia	Epstein–Barr virus, HIV-1 gp120, influenza hemagglutinin, bacterial DNA or CpG ODN
Rheumatoid factor and cryoglobulin production	Hepatitis B and C viruses, HIV, bacteria, cotrimoxazole, interferon alpha, cocaine, intravenous radiographic contrast, influenza and HBV vaccination
Molecular mimicry- or neoantigen-mediated autoantibody production	Drugs; infectious agents; xenobiotics like tobacco smoke

cells, and follicular zone B2 cells [23]. Follicular zone B2 cells are the precursors to high affinity memory B cells during secondary immune responses but contribute to T-dependent primary immune responses as well. When activated by foreign antigens and after receiving a signal from CD4+ helper T cells, B2 cells undergo a process of somatic Ig hypermutation (SHM) in transiently formed structures known as germinal centers in the spleen, lymph nodes, and ileal Peyer's patches, where the immunoglobulin (Ig) receptors are further diversified by the introduction of mutations into the DNA encoding the rearranged variable exon. Coupled to antigen driven-selection by follicular dendritic cells for high affinity variants, SHM leads to the formation of high affinity memory B cells. B2 cells also readily undergo class switch recombination to generate switched antibodies such as IgG and IgA. Many of the pathogenic antibodies associated with autoimmune disease bear the hallmark of antibodies secreted by B2 cells: they are hypermutated, of high affinity to self antigen, and switched to IgG. However, while it is likely that these cells undergo a tolerance checkpoint prior to their differentiation into memory B cells in the germinal center, the mechanism underscoring this process is poorly defined. Also, there is evidence suggesting that when SHM occurs outside the germinal centers or when germinal centers are ectopic as in the joint of patients with rheumatoid arthritis (RA), B cells with high affinity to self-antigen develop. The events leading to the formation of ectopic germinal centers or to extrafollicular SHM are not understood.

The roles of B1 and marginal zone B cells in autoimmunity remain poorly defined. These cells occupy the peritoneal and pleural cavities and are a major source of natural antibodies. They participate in T-independent immune responses, and tend to secrete unmutated IgM antibodies with low avidity to self-antigens [23]. B1 cells are divided into B1a cells thought to originate from fetal liver and B1b cells that develop from the bone marrow. B1 cells are of limited diversity as they tend to have short CDR3 with little n-region addition, do not undergo SHM and are mostly IgM. They recognize antigens found in many common pathogens, participate early in the response and are thought to be a bridge between innate immunity and the adaptive immune response during the course of an infection. It is likely that the specificities encoded in the Ig genes of B1 cells have been under significant evolutionary pressure. Despite the polyreactivity of the secreted antibodies, to date, there is limited evidence that B1 cells contribute to autoimmune disease, as well illustrated by cold antibody autoimmune hemolytic anemias in which IgM appear to play a direct role in hemolysis and red cell sequestration, particularly when drug-induced and with obvious therapeutic implications [24]. They tend to secrete IL-10, an anti-inflammatory cytokine, and in animal models they have been tied to exacerbation of autoimmunity while in others, their increase in numbers actually reduced autoimmunity. One possibility is that occasionally these B cells secrete low avidity autoreactive IgM natural antibodies that help clear apoptotic debris and protect against the formation of IgG-containing immune complexes. It has been reported that low avidity anti-dsDNA IgM protects against lupus nephritis [25]. Using an animal model of autoimmune cholangitis in dnTGFβRII mice, Igμ-/-dnTGFβRII mice developed more severe cholangitis than dnTGFβRII mice and had a higher frequency of activated CD4+, CD8+ cells in the liver, suggesting that B cells may have a suppressive function in autoimmune response in dnTGFβRII mice [26]. It is clear that the role of B1 cells in autoimmunity is complex and may include both an exacerbating and an alleviating component.

Marginal zone B cells reside in the spleen marginal zone, an area rich in macrophages and dendritic cells and where there is slowed blood flow. This enables the cells in the marginal zone to trap particulate matter, including encapsulated bacteria. Because of this, marginal zone B cells are positioned to generate a quick humoral response against systemic blood-borne pathogens. They have a low threshold of activation compared to follicular B2 cells and respond to antigen in a T cell independent manner. Like B1 cells, they tend to secrete unmutated IgM with low self-reactivity; although recent work suggests that some marginal zone B cells can be significantly autoreactive. The role of these cells in autoimmunity remains poorly defined and like B1 cells, may involve both a negative regulatory component (through secretion of IL-10) and/or an exacerbating component (through the secretion of autoreactive antibody or even as self-antigen-presenting cells).

There is some evidence but more is needed suggesting that B1 cells and marginal zone B cells can modulate autoimmunity either by exacerbating it through secretion of autoreactive antibodies and/or by down-modulating it through secretion of anti-inflammatory cytokines. In addition, a better understanding of the role of low avidity autoreactive IgM as either pathogenic or protective in autoimmunity is needed. There are significant differences between murine and human B1 and marginal zone B cells that need to be addressed, including the fact that human marginal zone B cells can re-circulate and may undergo hypermutation [27]. Given the critical importance of central tolerance in the bone marrow to B cell-mediated autoimmunity, it is important to better define the role of specificity and affinity to self-antigen in deciding the fate of developing B cells in this critical step of B cell tolerance.

Recent developments suggest a potential novel splenic population of B cells with potent anti-inflammatory properties termed B10. These B cells that appear to exclusively secrete IL-10 may be functionally specialized to carry out a negative regulatory role in inflammation and autoimmunity [28]. The cellular origins of these cells are unclear but appear to be a phenotypically distinct population from other splenic B cell populations and it is likely they represent a distinct subset [29,30].

The role of B cells of all or any of the subsets as antigen presenting cells to other cells like follicular dendritic cells, macrophages, and T cells remains unclear although evidence clearly supports the notion. Targeting the survival/apoptotic pathways, which when dysregulated lead to expansion and survival of autoreactive B cells (such as the BAFF/BlyS receptor system and CD40), is an exciting area that is emerging for development of novel therapeutics. For example, overexpression of pro-survival components in these pathways in murine models leads to the expansion of

Table 5
Environmental agents associated with T cell activation [42,44,95].

T cell feature	Environmental agent
Inflammatory cytokine production	Smoke, allergens, xenobiotics, AhR, micronutrients, infections
Alterated self-antigen presentation/recognition	Infections; chemicals
T reg alterated balance	Sex hormones, AhR, chemicals, UV radiation

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autoreactive B cells. How this translates into potential therapies for autoimmune disease in humans remains unclear.

We know very little of the tolerance checkpoint mechanism regulating the formation of high affinity autoreactive B2 cells both in and outside the germinal center. Since many of the pathogenic antibodies are switched and hypermutated, and since extrafollicular hypermutation leads to the formation of autoreactive cells, it is likely that the germinal center reaction is under tight control warding against the formation of autoreactive B cells, that SHM is meant to occur within the confines of the germinal center, and that this is a key step in the prevention of antibody-mediated autoimmunity.

Recent data suggest that sex hormones like estrogen and prolactin can differentially activate autoreactive B cell populations from different subsets such as B2 cells, which is responsible for the majority of the high affinity pathogenic antibody production and marginal zone autoreactive B cells. In addition, it opens the door to a possible connection between B-cell mediated autoimmunity and endocrine disruptors, such as environmental estrogens [31]. There is suggestive evidence for this but more is needed. If true, it may provide a basis for the gender bias in most autoimmune disorders with a strong B cell component.

Finally, given the biology of B lymphocytes, one may expect certain classes of environmental factors to enhance B-cell mediated autoimmunity. These include the following: 1) agents that induce activation or expansion of autoreactive B cells (such as those that cause enhanced transcription of pro-survival molecules such as BAFF, or that cause relaxed negative selection against autoreactive lymphocytes, 2) agents that may induce secretion of pathogenic antibodies, such those that promote necrosis or apoptosis that may lead to the inappropriate presentation of nuclear components to lymphocytes, and 3) agents that induce secretion of pro-inflammatory cytokines such as endocrine disruptors. Agents with these effects have the potential to disrupt B cell function.

4. Direct effect impairing the immune function: T helper 17 (Th17) cells

Th17 cells are a relatively newly-defined CD4+ T cell subset characterized by the secretion of IL-17. Activation of Th17 cells is enhanced through TLR2 signaling [32] and in the experimental animal model of multiple sclerosis TLR2 agonists increased disease. In humans, Th17 cell activation has been associated with exacerbation of multiple sclerosis (MS) [33]. Th17 cells participate in the response to extracellular bacteria and fungi infections as Th17 cells in healthy subjects are mainly localized within mucosal surfaces [14]. The interactions with commensal flora are likely to modulate the finely tuned Th17-T regulatory (Treg) balance with Th17 cells acting against pathogens and Treg suppressing the immune response to normal microbial flora and environmental antigens. Nevertheless, dysregulated Th17 cell activity can lead to pathology, as in chronic inflammatory diseases such as asthma and inflammatory bowel diseases [34,35].

Dietary components and environmental toxins can also influence the Th17 response possibly leading to the development of autoimmune diseases in susceptible individuals. This role of Th17 has been investigated in most autoimmune diseases and data are particularly convincing in multiple sclerosis, rheumatoid arthritis, Crohn's disease, and psoriasis where they seem to be involved in the development and in the relapse of the diseases [36–38] and with putative therapeutic implications with biologics [39]. Vitamin A and vitamin D seem to exert their immune modulatory effects controlling the balance between Th17 and Treg, although the mechanisms have not been characterized in detail [40].

The high affinity aryl hydrocarbon receptor (AhR) is a ligand-dependent transcription factor ubiquitously expressed in vertebrate cells. Ligation of the AhR favors differentiation of Th17 cells and can exacerbate autoimmunity, as reported in animal models of MS [41,42]. In vitro studies have demonstrated that this occurs through a STAT1-mediated mechanism [43]. Ligands for the AhR include halogenated aromatic hydrocarbons and non-halogenated polycyclic aromatic hydrocarbons, with one of the best known environmental contaminant ligands being 2,4,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Smoking is an important environmental risk factor for RA, and nicotine stimulates the α7-nicotinic acetylcholine receptors which have immunomodulatory effects. In a rat adjuvant-induced arthritis model of human RA nicotine-pretreatment aggravates arthritis increasing IFN and IL-17 production, whereas post-treatment nicotine suppressed the disease [44]. On a different level, cockroach exposure is a major risk factor for the development of asthma. It has been demonstrated that the exposure of naïve mice to cockroach feces increases the levels of IL-17A and other inflammatory cytokines in whole lung cultures indicating a development of a mixed Th2/Th17 response as in asthma [45].

Some studies have demonstrated that AhR ligation promotes Th17 cell differentiation and activity to exacerbate autoimmune disease in animal models [42] while others have shown TCDD ligation to AhR promotes the expansion of regulatory T cell populations, decreases Th17 frequency, and ultimately limits symptoms of disease [41]. This dichotomy was nicely reviewed by Ho and Steinman [46] and summarized more recently by Marshall and Kerkvliet [47]. Moreover, the mechanisms linking nicotine, inflammation and IL-17 production require better clarification as previously illustrated. The involvement of environmental contaminants and exacerbation of autoimmune disease through Th17 cells remains a large data gap for the field.

5. T regulatory (Treg) cells

Treg cells are characterized by the expression of CD4, CD25 and the transcription factor forkhead box P3 (FOXP3) and are considered to play a major role in the maintenance of immune tolerance [48], a conclusion formulated following the first seminal reports of the development of autoimmune conditions in mice lacking Treg cells. Given the potential for environmental factors to influence the development of autoimmunity, it may reasonably be postulated that these environmental factors influence autoimmunity by altering production or activation of Treg cells, as supported by epidemiological studies [49–52]. This paucity of epidemiological data may reflect the difficulties in quantifying exposure to multiple environmental chemicals and in analyzing multiple Treg subsets and their functions in human populations. In contrast, an accumulating body of literature suggests plausible molecular mechanisms through which environmental factors could alter the balance between autoimmunity and immunosuppression through changes in Treg expression and/or function. These laboratory-based mechanistic studies are summarized below.

In 1971, Gershon *et al.* coined the term "infectious tolerance" to describe the ability to transfer immune suppression to an otherwise normal recipient with leukocytes from an immunosuppressed animal. In the following 10 years, dozens of laboratories confirmed and extended these studies by demonstrating that atypical T lymphocytes (suppressor T cells) were responsible for active immunosuppression of responses to foreign and self antigens, including responses mediating autoimmune disease. While their existence was challenged in the 1980s, hundreds of manuscripts now establish the existence of suppressor T cells, currently referred to as Treg cells, which actively dampen autoimmune responses

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[53–55]. Treg are mainly, but not exclusively [56] $CD4^+$ and can be subdivided into subpopulations based on their phenotype, mechanism of induction, and cytokine production [53,56]. Natural Treg cells (nTreg) develop in the thymus, inducible Treg cells (iTreg) are thought to be generated in the periphery following antigen and TGF-β stimulation, and IL-10-secreting T regulatory type 1 cells (Tr1) are produced after antigen, TGF-β and IL-27 exposure. Down-regulation of each of these Treg subsets has been associated with the onset of autoimmune disease. Given the pivotal role that all of these Treg subsets play in suppressing autoimmune disease, it is reasonable to postulate that any environmental chemical capable of altering their generation or activity has the potential to influence autoimmune responses.

Several studies demonstrate the ability of the AhR ligand TCDD and related but lower affinity AhR ligands to suppress the immune system at multiple levels from T and B lymphocyte development to effector cell function. While pre-T cells, thymic stromal cells or thymic T cells were first suggested to be among the immediate victims of TCDD exposure, more recent reports demonstrated that TCDD also directly alters mature T cell function. Studies by Kerkvliet *et al.* first demonstrated that TCDD induces immunosuppressive T cells expressing some Treg markers including GITR, high levels of CD25, and low levels of CD62L [47]. Subsequently, other laboratories demonstrated AhR expression in Tregs and the ability of AhR ligands to directly influence Treg induction [40,57–60]. AhR-driven reporter assays and AhR-specific chromatin immunoprecipitation demonstrated direct AhR interaction with and transactivation of *FoxP3*, a gene critical to the development of inducible and natural Tregs [41]. In these $FoxP3^+$ Tregs, a conventional AhR–ARNT heterodimer induces *FoxP3* transcription. In addition, AhR can dimerize with c-Maf, a master regulator of IL-10 secretion in IL-27 + TGF-β-induced, $Foxp3^-$ Tr1 cells [57]. Interestingly, it is these $FoxP3^-$ Tr1 cells that most closely resemble the TCDD-inducible Tregs initially described by Kerkvliet *et al.* in murine models [56,61,62]. The AhR similarly is involved in generating human Tr1 cells (in the absence of TGF-β) or inducible Tregs (in the presence of TGF-β) [58]. Collectively, these data provide strong evidence that environmental chemicals can modulate Tregs although, by themselves, they do not explain how environmental chemicals may induce or exacerbate autoimmunity.

AhR ligands also affect skewing of the T cell repertoire toward regulatory T cells via an indirect action on antigen presenting cells. It has been well established that the nature of interactions between APC and T cells dictates production of functionally disparate Th_1, Th_2, Th_{17} and Treg cell subsets [63,64]. Therefore, environmental chemical effects on APC could in turn alter the production of T cell subsets including Treg cells. Indeed, activation of dendritic cell AhR with a synthetic AhR agonist (VAF347) promotes production of Tregs which suppress allograft responses [64]. Interestingly, TCDD can promote transcription of indoleamine 2,3-dioxygenases (IDO) [65], enzymes which play a central role in tryptophan metabolism [66] and which, in dendritic cells, are associated with skewing of the T cell repertoire toward $FoxP3^+$ Tregs [63]. Although the mechanisms through which APCs regulate Treg production are unclear, it is tempting to speculate that AhR-control of IDO expression in APCs may alter production of tryptophan metabolites, some of which in turn activate AhR in APC-associated T cells. Several studies demonstrate that multiple tryptophan metabolites are AhR agonists [60,67].

While most of these studies suggest that AhR activation in T cells or in APC may increase Treg production and therefore decrease autoimmunity [68], the opposite outcome must be considered and evaluated. That is, it is well established that the outcome of AhR activation is highly variable and may be specific to the activating ligands and/or conditions of activation [61]. For example, AhR activation in human B cells with a metabolizable, environmental AhR ligand, benzo[*a*]pyrene, inhibits cell growth and differentiation into plasma cells while TCDD has no such affect [69]. In murine multiple sclerosis (MS) models, treatment with a metabolizable and potential endogenous AhR ligand, 6-formylindolo [2-b,3] carbazole (FICZ), interferes with Treg development and potentiates autoimmunity while TCDD, a persistent AhR ligand, increases Tregs and decreases autoimmunity [41]. Of note, the increase in autoimmunity observed following FICZ treatment is likely due in part to Th_{17} induction [40,70], as discussed elsewhere in this article. Furthermore, the time of exposure also may dictate whether environmental AhR ligands enhance or suppress autoimmunity. For example, alteration of thymic selection by neonatal exposure to low TCDD doses increases production of autoantibodies in NFS/sld mice, a model for human Sjögren's syndrome [71]. Although not directly demonstrated, changes in the thymic microenvironment in these TCDD-exposed animals could compromise natural Treg develop and facilitate autoantibody production. Therefore, we believe that the context-specific activation of the AhR may result in either increased or decreased Treg activity and that this possibility needs to be directly tested.

Given the critical role of several classes of receptors in T cell development, the possibility exists that multiple environmental ligands for several cellular receptors modulate Treg induction. In this vein, the PPARγ receptor, which recognizes a variety of environmental chemicals, including phthalate esters and organotins [72], is required for the induction of Tregs which dampen IFNγ and IL-12 production during inflammatory colitis [73]. PPARγ also influences production of $CD4^+$ $FoxP3^+$ Treg and $IL10^+$-producing $CD4^+$ T cells in experimental IBD [74]. Activation of the retinoic acid receptor (RAR), a PPARγ dimerization partner, promotes Treg induction from naïve cells [75], further suggesting the possible role of a PPARγ/RAR heterodimer in Treg production. Estrogens, and by inference the estrogen receptors, play an important role in Treg development [76]. Treatment of mice with low estrogen doses induces murine Tregs, reduces experimental MS symptoms in mice and potentiates human Treg function. While the literature is replete with data demonstrating the ubiquity of xenoestrogens, studies on the potential for these environmental chemicals to influence Treg induction or activity have either not been performed or have not been reported. This clearly represents an area worthy of more study. Interestingly, cross talk between the ER and PPARγ, PPARγ and AhR, RAR and AhR, and AhR and ER has been suggested, invoking the specter of complex chemical mixtures affecting development of Tregs in unpredictable ways. Receptor-independent environmental stressor-mediated effects also have been reported. For example, UVB light induces IL-10-producing Tregs capable of antigen-specific immunosuppression [77,78]. UV light-mediated Treg induction may represent an immunosuppressive response to UV-mediated epithelial cell death and autoantigen presentation by Langerhans cells [79].

Collectively, the data strongly suggest that a variety of environmental chemicals could alter Treg production or function through the activation, or inactivation, of multiple intracellular receptors. It is clear, then, that new studies must be designed and executed to test the hypothesis that exposure to environmental chemicals, capable of modulating intracellular receptor signaling, is associated with the risk or severity of autoimmunity in humans.

6. Modification of self antigens

It is estimated that 50–90% of the proteins in the human body are subject to post-translational modifications (PTM) and these modifications may well contribute to tolerance breakdown. Indeed, PTM (i.e. acetylation, lipidation, citrullination, glycosylation, etc.),

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either native or aberrant, may play a fundamental role for specific autoantibody recognition in autoimmune diseases. This has been hypothesized in autoimmune diseases such as celiac disease, Sjögren's syndrome, RA, and MS [80]. On the other hand, the lack of PTM is also capable of leading to the breakdown of tolerance as in the case of PBC, an autoimmune disease targeting the intrahepatic biliary epithelial cells [81]. Any change in an immunodominant epitope can affect its presentation by antigen presenting cells to T cells and can lead to different T cell activation states or to anergy, thus constituting a plausible mechanism to autoimmunity. These changes may well explain the tissue specificity and determine the transition between non-traditional and traditional antigens [82]. In the paradigmatic case of primary biliary cirrhosis (PBC), the lack of an expected PTM alters protein degradation leading to the accumulation and exposure of large amounts of autoantigens, as postulated for "traditional" autoantigens in organ-specific autoimmune diseases [83]. In most cell types, lysine-lipoylated sequences when released from mitochondria during apoptosis [84] are oxidized by glutathiones; the oxidated forms are not immunogenic and are not recognized by serum AMA because glutathionylation masks the autoantibody recognition site [84,85]. In a complementary fashion, MS pathogenesis includes PTM that increase the complexity of myelin proteins, i.e. the major MS autoantigens, either secondary to the autoimmune response or to the neurodegenerative process [86,87]. The current neurodegenerative hypothesis on MS onset is based on metabolic changes in myelin constituents that alter the PTM and destabilize the membrane structure leading to myelin degradation [87].

Several studies have indicated that mercury can modify fibrillarin. Mercury-induced cell death results in the formation of a unique 19 kDa cleavage fragment of fibrillarin that cannot be detected in cells that die from other causes. These mercury modifications of fibrillarin appear to render it more immunogenic. It is however unclear whether this process is limited to fibrillarin itself, i.e. whether non-targeted cellular proteins are left intact following mercury exposure. In support of a specific effect, autoantigens including lamin BI, SS-B/La, U1-70 kDa were first shown not to interact with mercury and were not part of the autoimmune response elicited by its exposure while later data reported the different outcomes of mercury-induced cell apoptosis in terms of autoantigen integrity. Indeed, these reports could not suggest any mercury-specific mechanism in apoptosis thus supporting that the differences observed with fibrillarin manifest some degree of specificity. Fibrillarin is modified by exposure to mercury, but antibodies in mercury-induced autoimmunity react better against the intact molecule than against mercury-modified fibrillarin [88]. Therefore, it is likely that the loss of tolerance to fibrillarin takes place at the T cell level rather than among B cells. Indeed, CD4+ T cells isolated from mercury-treated B10.S mice can be restimulated *in vitro* with mercury-complexed nuclei and expanded with IL-2. These cells proliferate to mercury-modified fibrillarin, but display a lower response to normal fibrillarin. In contrast, naïve T cells do not proliferate against mercury-treated fibrillarin.

Approximately 140 unique amino acids and amino acid derivatives from PTM can be incorporated into proteins. Enzymes mediating PTM are compartmentalized at particular sites, whether in intracellular organelles, such as vacuoles or the endoplasmic reticulum (ER), or in extracellular spaces. Most of the available data on the role of PTM in autoimmunity come from circumstantial evidence or from the study of the putative environmental factors and cofactors (including oxidative stress, infectious agents, or specific elements such as lead) inducing PTM. With regard to mercury, although T cell response is initially directed against mercury-modified fibrillarin, epitopes present on native fibrillarin can later be recognized during a process of epitope spreading [89]. After one week of mercury treatment, T cells display a strong response to mercury-complexed fibrillarin, but not to the native protein. After 8 weeks of mercury treatment, T cells respond equally well to both mercury-modified and native fibrillarin. This indicates that the autoimmune response to fibrillarin takes place in two stages. Initially, the mercury-modification of fibrillarin triggers the loss of self-tolerance. In a second stage, the autoimmune response becomes permanent and expands to native determinants.

First and most generically we are relatively confident that multiple self-protein modifications (phosphorylation, glycosylation, acetylation, deamidation) can lead to either T and/or B cell responses to the self-antigens and produce the breakdown of tolerance, although the specific mechanisms remain to be elucidated.

Second, we believe that autoantibodies to modified self-antigens tend to be promiscuous in their ability to bind either the modified or unmodified forms and may thus be crucial to the effector immune reaction against target tissues, as well represented in RA. Similarly, the autoimmune response can be directed to either the modified or unmodified protein.

In the case of RA, collagen type II is the potential candidate autoantigen involved in the pathogenesis of the disease and several studies have detected different B and T cell epitopes on collagen type II molecule [90]. In humans the most important collagen type II B cell epitope is the peptide 359–369 (CII359–369)-ARGLTGRPGDA, while T cells preferentially recognize the DR4/DR1-restricted collagen type II peptide 261–273 (CII261–273)-AGFKGEQGKGEP. Both peptides can undergo PTM *in vivo,* particularly citrullination and glycosylation. Such modifications have a complex impact on the presentation and ability to activate immune cells. Collagen type II contains a large number of lysine residues that are often post-translationally hydroxylated and, subsequently, glycosylated with a β-D-galactopyranosyl- or an α-D-glucopyranosyl-β-D-galactopyranosyl unit. In particular, the peptide CII263-271 contains two glycosylation sites, K264 and K270. T cells can recognize both glycosylated forms bound to DR4, but the β-D-galactopyranose unit located in hydroxylysine 264 seems to be dominant. The effect of these glycosylations has not been clarified yet, but they seem to enhance the peptide immunogenic potential while slowing down its processing rate. The peptide CII359-369 contains, instead, two citrullination sites: R360 and R365. Citrullination is a PTM in which a basic amino acid, the peptidyl arginine, is converted into the neutral, non-standard amino acid peptidyl citrulline. The reaction is catalyzed by calcium-dependent peptidyl arginine deiminase (PAD). Citrullination is an apoptotic PTM that seems to be helpful in opening protein conformation and in favoring the cleavage processes. Citrullination can change the protein charge distribution and the ability to form H-bond. *In vitro* the modification of more than 10% of arginines leads to denaturation of the protein. Vimentin, another autoantigen involved in RA pathogenesis, is citrullinated as an initial step during apoptosis. In addition, many other intracellular citrullinated proteins were found in the RA synovia. In inflamed tissue, such as during arthritis, PAD can be released and can, therefore, citrullinate extra-cellular proteins, like fibrinogen and collagen. This process contributes to the generation of neoantigens presented to the immune system.

Third, autoantigens can be modified or be left unchanged following apoptosis and this is well represented by recent data in PBC with cholangiocyte-specific mitochondrial antigen processing directly linked to disease. B cell immunodominant epitope is represented by the inner lipoylated domain of pyruvate dehydrogenase complex (PDC)-E2 subunit while other epitopes are localized within the outer lipoylated domain of the same complex, the E2 subunit of the branched chain 2-oxoacid dehydrogenase complex, and the E2 subunit of the 2-oxoglutarate dehydrogenase complex

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[91]. They all share a common motif in the N-terminal region containing lysine-lipoylated domains [85]. In most cell types lysine-lipoylated sequences are oxidized by glutathiones when released from mitochondria during apoptosis as the oxidated forms are not immunogenic and are not recognized by serum anti-mitochondria autoantibodies as the glutathionylation masks the autoantibody recognition site via potential mechanism [85]. Conversely, cholangiocytes fail to covalently link glutathione to lysine-lipoyl groups during apoptosis [92] with consequent accumulation and exposure of potentially self-reactive antigens as the reduced forms of PDC-E2 fail to undergo normal protease degradation. In cholangiocytes the cleavage of the immunodominant PDC-E2 epitope has not been detected *in vivo* either during apoptosis or during phagocytosis. Accordingly, the lack of putative post-translational modifications alters protein degradation leading to the accumulation and exposure of a great amount of self-reactive antigens. Most recent data support the mechanisms linking the peculiar apoptotic features of the target cells and the immune-mediated injury. These data included the experimental evidence that the intact epitopes are found in the apoptotic blebs only in the biliary epithelium compared to other epithelial cells [92] and, more importantly, the capacity of macrophages to uptake these blebs and present the autoantigen to resident lymphocytes [93] thus proving the pathogenetic connection between the antigen modification and the immune response [94].

Despite the numerous lines of evidence we are concerned that further approaches are needed to demonstrate the role of PTM in autoimmunity. First, both citrullination and glutathionylation are involved in the complex mechanisms of PTM taking place during apoptosis. Putative PTM or their lack during apoptosis can alter the immunogenicity of proteins and that mechanism has been also evocated in other organ-specific autoimmune diseases [83]. These may also include glycosylation, as reported in MS, and may well contribute to the organ specificity of autoimmune diseases. We submit that future efforts should be dedicated to identify if modified antigens are taken up by local antigen presenting cells and ultimately lead to the local or systemic immune system activation, similar to that observed for PBC. Similarly, a limiting factor in the study of mercury-induced autoimmunity is that no study has yet identified a fibrillarin epitope (either native or mercury-modified) that is recognized by T cells in this model. Clearly, this is a complex task, especially considering the extent of PTM of the fibrillarin molecule, but such identification would go a long way in helping us understand the role of T cells in this model.

Second and possibly preliminary to other experiments, we need to develop *in vitro* or *ex vivo* models to prove that autoantigens can be modified to increase their immunogenicity and be turned into 'traditional' antigens. This appears crucial to determine the plausibility of the proposed PTM. Third and finally, we need more translational approaches to aim for disease biomarkers to be tested in clinical practice. In particular, sera and T cells from a large number of well-defined patients and appropriate controls should be tested against modified antigens to estimate the pathogenetic and causative role of PTM-driven reactivities. Similarly, samples should be tested longitudinally at different time points of the disease natural history or treatment response over decades. This paradigm is well represented by the study of antigen glycosylation in MS as IgM autoantibodies to recombinant myelin MOG within the central nervous system were reported to be predictors of clinically definite MS. However, these results were not confirmed by other studies possibly because differently folded and modified forms of MOG have been used. Ultimately, specifically glycosylated synthetic peptides are proposed as MS biomarkers have been demonstrated to be powerful tools for increasing autoantibodies recognition in patient sera at different disease stages [95].

7. Modifications of DNA methylation

Epigenetic changes are an ideal interface in the environment/genetic interactions with environmental changes producing changes in gene expression [8], as well illustrated by the earlier study of the effects of a specific dietary regimen, i.e. foods rich in methyl donors, on the coat color in *agouti* pregnant rodents. The major mechanism of epigenetics is DNA methylation consisting of the addition of a methyl group to the fifth carbon of cytosine residues via enzymes called DNA methyltransferases (DNMTs) and a methyl group donor, S-adenosylmethionine (SAM). Altered CpG island methylation may, indeed, change chromatin structure, being typically able to modulate the gene transcription machinery. Specific epigenetic changes in the immune cells would be responsible for immune-tolerance breakdown through the hypo- or hypermethylation of gene promoters [96,97]. Recent data reported the association of DNA methylation with several environmental factors including exposure to prenatal tobacco smoke [98], alcohol consumption [99], and environmental pollutants [100], all of which have been advocated for autoimmunity incidence. Both *in vitro* and *in vivo* experimental models have shown that variation of the epigenome may lead to the onset of autoreactive T cell clones. Specific epigenetic defects have been associated with autoimmune disorders. As an example, the differentiation of T helper cells to Th1 subsets producing interferon γ (IFNγ) or Th2 subpopulations producing interleukin 4 (IL-4) and IL-13 cytokines is epigenetically regulated. Importantly, Th1 cells manifest an IFNγ demethylated promoter, opposite to what is observed in the Th2 cells. Most recently there have been numerous studies to support the importance of epigenetics in the initiation and perpetuation of autoimmunity, with most studies performed on peripheral blood mononuclear cells in specific conditions (Table 6). In some cases, findings were recapitulated in different conditions, thus supporting the theory of a common theme for autoimmune diseases [101,102] and providing fascinating bases for the geoepidemiology of autoimmunity [49,103,104]. The recent genome-wide association studies demonstrated that genomics significantly predispose to SLE onset [105–109], but additional complementary factors and in particular impaired T and B cell DNA methylation may be crucial for disease development [110]. We expect that the rapid development of genome-wide mapping for DNA methylation and histone

Table 6
Observed changes in DNA methylation in specific autoimmune diseases. Modified from [131].

Systemic lupus erythematosus	T and B cell global DNA hypomethylation with decreased DMNT1 transcription [132,133]; CD4+ T cell changes:-CD70 demethylation [134,135]; - CD40L demethylation (in women); B cell changes:-CD70 demethylation; - Perforin demethylation;
Rheumatoid arthritis	RA synovial fibroblasts (RASF) changes [136,137]: - Global DNA hypomethylation; - Hypomethylation of CpG islands in LINE-1 promoter; - Hypomethylation of death receptor 3 (DR-3) promoter; - Unmethylated CpG in IL-6 promoter
Systemic sclerosis	Methylation of CpG islands in FL1 promoter with reduced expression.
Type 1 diabetes mellitus	Global hypermethylation activity caused by altered metabolism [138]: - Glucose and insulin levels increase methylation by altering homocysteine metabolism; - Low protein diet decreases islet mass and vascularity;
Multiple sclerosis	*PAD2* hypomethylation in white matter cells;

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modifications [111] will soon overcome the current data to provide a more comprehensive picture of the epigenetics of autoimmunity lymphocytes and target cells despite the novel issues arising [112,113] particularly in terms of environmental epigenetics [114]. We foresee that the understanding of these mechanisms and the identification of target molecules are expected to lead to new classes of therapeutic molecules, coined "epigenetic therapies" [115,116].

8. Summary of conclusions and levels of confidence

8.1. Effects on innate immunity

Two major pathways (TLR, adjuvants) have been discussed to illustrate the role of innate immunity in linking environmental factors and autoimmunity as aberrant regulation of the innate immune system may contribute to the development of autoimmune diseases. However, data on the underlying mechanisms remain incomplete, with limited focus on the effect of microbial products on TLR. Future studies should be extended to include other PAMPs and environmental agents such as chemicals and radiations. Further studies defining gene predisposition with regard to adjuvant effect in autoimmunity and the mechanisms involved are expected to enhance our understanding of disease pathogenesis.

Based on existing evidence, we are confident of the following

- The interaction between xenobiotics and TLR is a major mechanism involved in the interaction of environmental factors with autoimmunity development;
- The activation of the innate immune system via TLR predisposes to toxic-induced inflammation;
- Adjuvants possess the ability to activate the innate and adaptive immunity and induce the release of chemokines and proinflammatory cytokines;
- Immunization of antigen must be accompanied by a powerful adjuvant, complete Freund adjuvant, which includes the mycobacterium component. Incomplete Freund adjuvant results in the production of antibodies but without the occurrence of autoimmune diseases.

Based on existing evidence, we consider the following likely but requiring conformation

- Altered innate immune responses and dysregulated TLR signaling are a key step in triggering autoimmune diseases as in virus-induced animal models of type I diabetes;
- TLR activation in macrophages may predispose cells to toxin-induced inflammatory cytokine production;
- Active infection or microbial products of the infection can provide the adjuvant effect necessary for the induction of many autoimmune disorders.

We believe the following broad themes should be pursued in future investigations

- The allergenicity, functional mimicry of environmental contaminants and physical/chemical elements resembling TLR ligands;
- The dysregulation of the regulatory B cell (IL-10 producing, CD5+ B cells) through modulation of TLR signaling;
- The molecular motifs of adjuvants and their physiological receptors that are associated with clinical manifestation of autoimmunity;
- Genomic predisposition to innate immunity dysfunctions.

8.2. B cells

It is well established that a breakdown in central tolerance (in the bone marrow) is a major contributor to autoimmunity in many autoimmune models. Because of the potential for different B cell subsets to either contribute to autoimmunity development or to ameliorate it, delineating the differential contributions of these subsets will be critical for the fine tuning of B cell depletion strategies in the treatment of autoimmune disease. Finally, recent developments implicate environmental factors in biasing the activation of various B cell subsets leading to autoimmune manifestations. There are limited data and clearly more is needed to examine the direct impact of environmental factors in B cell function.

Based on existing evidence, we are confident of the following

- Dysfunctions of B cell tolerance checkpoints are directly correlated with autoimmune disease in murine models;
- B cells modulate autoimmunity both positively and negatively as secretors of pathogenic antibodies and proinflammatory cytokines, as antigen presenting cells and autoreactive T cells and as secretors of anti-inflammatory cytokines such as IL-10;
- Follicular B cells (B2) are a major source (if not the predominant source) of autoreactive switched pathogenic antibodies;
- When somatic hypermutation occurs outside of the context of germinal centers, or when germinal centers occur ectopically, B cells secreting pathogenic autoantibodies can emerge;
- Sex hormones like estrogen and prolactin can differentially activate autoreactive B cell populations from different subsets such as B2 cells.

Based on existing evidence, we consider the following likely but requiring conformation

- B1 cells and marginal zone B cells can modulate autoimmunity either by exacerbating it through secretion of autoreactive antibodies and/or by down-modulating it through secretion of anti-inflammatory cytokines;
- B10 cells appear to exclusively secrete IL-10 may be functionally specialized to carry out a negative regulatory role in inflammation and autoimmunity.

We believe the following broad themes should be pursued in future investigations

- The roles of B1 and marginal zone B cells in autoimmunity;
- The role of the recently discovered B10 cell population in autoimmunity;
- The survival/apoptotic pathways that when dysregulated lead to expansion and survival of autoreactive B cells (such as the BAFF/BlyS receptor system and CD40);
- Tolerance checkpoint mechanisms regulating the formation of high affinity autoreactive B2 cells both in and outside the germinal center;
- Environmental (chemical) agents with the potential to disrupt B cell function.

8.3. T helper 17 (Th17) cells

The recurrent infections that afflict patients with impaired Th17 immunity strongly support the importance of Th17 cells as an immunity checkpoint. However, dysregulated Th17 activity can result in immunopathology and thus the generation of Th17 cells must be tightly controlled. The environment operates at multiple levels to control Th17 immunity involving not only Th17

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differentiation or IL-17 production but also antigen presenting cells and Treg. More importantly, increasing evidence suggests that a number of xenobiotics, allergens and micronutrients can influence IL-17 production and, possibly, immune diseases. We believe that modulation of the Th17 response will be a major therapeutic target in immune mediated diseases in particular, autoimmune diseases.

Based on existing evidence, we are confident of the following

- Dysregulated Th17 cell activity can lead to pathology, as in chronic inflammatory diseases such as asthma and inflammatory bowel diseases;
- Th17 cells are involved in MS, RA, Crohn's disease, and psoriasis where they seem to be involved in the development and in the relapse of the diseases.

Based on existing evidence, we consider the following likely but requiring conformation

- Smoking is an important environmental risk factor for RA and nicotine exerts immunomodulatory effects via Th17 cells;
- AhR binding by aromatic hydrocarbons and non-halogenated polycyclic aromatic hydrocarbons favors differentiation of Th17 cells and can exacerbate autoimmunity.

We believe the following broad themes should be pursued in future investigations

- The involvement of environmental contaminants and exacerbation of autoimmune disease through Th17 cells;
- Therapeutic modulation of Th17 cells.

8.4. T regulatory (Treg) cells

Laboratory-based studies demonstrate plausible molecular mechanisms through which environmental agents could affect Treg induction or function. Although most of these studies suggest the involvement of immunosuppressive Tregs, it is possible that Treg numbers and/or activity may be either increased or decreased by environmental agents depending on the context of the receptor-mediated signaling, the nature and strength of a stimulus (or complex stimuli), and the timing of exposure. Since studies on the role of environmental chemical receptors in Treg production and function is still in its infancy, more laboratory and population-based research are required to determine: (i) if environmental agents induce Tregs that suppress autoimmunity in humans and (ii) if conditions exist under which Treg induction or function is compromised by exposure to environmental agents, increasing the risk of autoimmunity.

Based on existing evidence, we are confident of the following

- Treg cell quantitative and qualitative changes are culprit for tolerance breakdown;
- The AhR ligand TCDD induces immunosuppressive T cells expressing specific Treg markers;
- AhR ligands also affect skewing of the T cell repertoire toward regulatory T cells via an indirect action on antigen presenting cells;
- TCDD induces IDO transcription to skew the T cell repertoire toward FoxP3^{+} Tregs;
- Activation of PPARγ promotes Treg induction from naïve cells;

Based on existing evidence, we consider the following likely but requiring conformation

- While most of these studies suggest that AhR activation in T cells or in APC may increase Treg production and therefore decrease autoimmunity, the opposite outcome is likely and possibly ligand-specific;
- The context-specific activation of the AhR by specific ligands may result in either increased or decreased Treg activity;
- Sex hormones play an important role in Treg development and may underlie female predominance of autoimmune diseases.

We believe the following broad themes should be pursued in future investigations

- Specific chemical, infectious, or physical agents capable to modulate Tregs;
- Environmental modulators of AhR stimulation;
- Mechanisms of sex-specific Treg changes.

8.5. Modification of self antigens

PTM may affect the immunogenicity of self-proteins, triggering an autoimmune response to lead to neoantigen development. Nevertheless, most evidence comes from cross-sectional clinical studies and may thus be burdened by structural defects that may limit the applicability of results to clinical practice and disease pathogenesis.

Based on existing evidence, we are confident of the following

- The majority of human proteins undergo PTM and these modifications or lack thereof may lead to tolerance breakdown;
- PTM may explain the tissue specificity of autoimmune diseases;
- MS pathogenesis includes PTM that increase the complexity of myelin proteins through the autoimmune response or the neurodegenerative process;
- Citrullination is an apoptotic PTM that seems to be helpful in opening protein conformation and in favoring the cleavage processes thus favoring RA development;
- Cholangiocytes do not covalently link glutathione to lysine-lipoyl groups during apoptosis with consequent accumulation and exposure of potentially self-reactive antigens account for the bile duct specific pathology in PBC development.

Based on existing evidence, we consider the following likely but requiring conformation

- Multiple self-protein modifications (phosphorylation, glycosylation, acetylation, deamidation) can lead to either T and/or B cell responses to the self-antigens;
- Serum autoantibodies to modified self antigens may bind either the modified or unmodified forms and thus be crucial to the effector immune reaction against target tissues;
- Mercury-induced cell death results in the formation of a unique 19 kDa cleavage fragment of fibrillarin which is more immunogenic.

We believe the following broad themes should be pursued in future investigations

- The mechanisms by which citrullination and glutathionylation lead to tolerance breakdown in genetically susceptible individuals;
- The role of glycosylation in MS and other autoimmune diseases;

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- Experimental models to prove that autoantigens can be modified to increase their immunogenicity and be turned into 'traditional' antigens;
- Technologies to reverse or induce PTM in animal models of autoimmunity.

8.6. Modifications of DNA methylation

The potential role of epigenetics in environmental/genetic interactions, where environmental changes produce modifications in gene expression, has been suggested by some intriguing experimental studies summarized in Table 5 and well illustrated by MZ twin discordance rates.

Based on existing evidence, we are confident of the following

- The association of DNA methylation profiles with several environmental factors including exposure to prenatal tobacco smoke, alcohol consumption, and environmental pollutants;
- DNA methylation is important in the regulation of the immune function in some congenital diseases (i.e., Silver–Russel and BeckwithWeidman syndromes);
- Changes in DNA methylation in specific peripheral cell types are associated with autoimmune diseases.

Based on existing evidence, we consider the following likely but requiring conformation

- Phenotypic differences significantly increased along with age of the twins in a trend coined as "epigenetic drift", which occurs during life according with the different exposures to environmental stressors and may well explain late-onset autoimmunity;
- Specific impairments in the regulation of epigenetic processes in immune cells would be responsible for immune-tolerance breakdown through both hypomethylation of genes or involvement of transcription repressors;
- Most recent genome-wide association studies demonstrated that genomics significantly predispose to SLE onset but experimental data indicate that epigenetic mechanisms, and in particular impaired T and B cell DNA methylation, may constitute one of these factors.

We believe the following broad themes should be pursued in future investigations

- The functional effects *in vivo* of DNA methylation changes under different environmental and genomic conditions;
- The development of new therapeutic molecules capable to prevent or counteract DNA methylation changes in a cell-specific manner;
- The DNA methylation changes in the target cells and not only in the rapidly accessible effector immune cells.

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