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EDITORIAL

The long and latent road to autoimmunity

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Autoimmune diseases affect nearly 5% of the general population, yet etiology remains poorly understood. Genomic factors are clearly necessary but remain insufficient to explain the loss of tolerance; environmental and stochastic factors fill this gap. This paradigm is indicated by the concordance rates (ranging between 4 and 63%) for autoimmune diseases among monozygotic twins, who share an identical genome, compared to the invariably lower rates in dizygotic twins. The term "exposome" cumulatively refers to the non-hereditary (i.e., environmental) factors that account for the remaining susceptibility and include endogenous factors such as hormones. Accumulating evidence suggests that exposure to infections, drugs, vaccines, and chemicals may contribute to the loss of tolerance. The mechanisms by which environmental factors can shape the immune system to generate autoimmunity include molecular mimicry, self-antigen modification, bystander activation, and immune reactivity modulation. In all cases, we should first consider the prolonged time between an environmental trigger and the appearance of autoreactivity and subsequent clinical disease. In this special issue, several excellent reviews discuss the potential mechanisms linking environmental factors and autoimmunity.

The concept of autoimmunity, or an immune response directed toward the self, dates back to 1890 and coincides with the beginning of the modern era of immunology. Before then, it was already known that the body can protect from the dangers of the outer world (the environment) and can learn from the experience, i.e., adaptive immunity. Nonetheless, at the beginning of the twentieth century, serum sickness, blood group reactions, and anaphylaxis were reported. In 1901, autoantibodies were described by Paul Ehrlich; however, it took several decades to develop modern technologies (i.e., electrophoresis, radioactivity, and chromatography) that could be used to identify specific serum reactivities. In 1930s and 1940s, Eric Waaler and Harry Rose simultaneously described what we now coin rheumatoid factors in rheumatoid arthritis (RA), and in 1948, antibodies directed toward cells and nuclei were described in systemic lupus erythematosus (SLE). In 1960s–1970s, autoantibodies were recognized as the first manifestation of autoimmune diseases.

Clinical epidemiology has linked infections and autoimmunity. The increasing incidence of autoimmune and inflammatory diseases during the last century led to the suggestion of the "hygiene hypothesis", linking immune dysregulation to sanitation. The impact of environmental factors in autoimmunity was the focus of a National Institute of Environmental Health Sciences (NIEHS) expert panel workshop in 2012; this panel has critically re-evaluated the epidemiology and mechanistic studies associated with autoimmunity in the scientific literature.[1] The concept of the "exposome" was introduced to collate and measure the effects of environmental factors—both exogenous and endogenous. With respect to endogenous exposure, the microbiota that constitute the ecological community of commensal, symbiotic and pathogenic microorganisms living on our mucosal surfaces has been identified as an environmentally induced influencer of autoimmunity, which is elegantly reviewed in this special issue (Bo Li, CMI, 2017). Despite significant progress in genetics, clinical epidemiology, and technical and analytical methodology, it remains unclear why autoimmunity affects as many as 5% of the general population. Autoimmunity remains a hot topic in immunology, as exemplified by the continuously growing number of publications (Invernizzi P., CMI; Lu Q., CMI, Bo Li, CMI, 2017, Shoenfeld Y., CMI).[2–4] Although we should not overlook the possible role of stochastic factors, the intriguing relationship between autoimmunity and the environment may help us understand the mechanisms of disease development and lead to the improvement of therapeutic strategies.

Environmental factors, particularly infections, can trigger an exaggerated immune response in genetically predisposed individuals, but several stimuli may be necessary for the establishment of an autoimmune disease; this is well exemplified by epigenetic changes that can alter the immune response (Lu Q., CMI, 2017).[5] How a system that is designed to recognize and eliminate pathogens can revert its action and cause destructive self-responses, i.e., break tolerance, is a key question that remains unanswered. There are several mechanisms by which environmental factors can shape the immune system to generate autoimmunity. These mechanisms include molecular mimicry, self-antigen modification, bystander activation, and immune reactivity modulation.

Molecular mimicry occurs when foreign antigens share sequences or structural similarities with self-antigens (Shoenfeld Y., CMI, 2017). A classic example of a disease that is involved in molecular mimicry is rheumatic fever, in which T cells respond to a specific peptide epitope of *Streptococcus pyogenes* and stimulate the generation of the B cells of a cross-reactive antibody to human cardiac myosin, resulting in acute rheumatic fever-associated carditis. Such processes may occur during the pathogenesis of other autoimmune diseases, e.g., *Escherichia coli* and primary biliary cholangitis (PBC), in which cross-reactivity occurs between *E. coli* and E2-PDC, triggering the anti-mitochondrial immune response in PBC.[6] An additional example is related to reactivity to *Campylobacter* in the induction of Guillain-Barrè syndrome.

Pathogens may induce the release of intracellular self-antigens during a chronic autoimmune or inflammatory response and also at the mucosal surface with the induction of neutrophil extracellular traps (NETs), which develop in response to bacteria or inflammation. This mechanism has been observed in RA and SLE.[7] In the case of RA, intracellular antibacterial and citrullinated proteins are externalized and can trigger mucosal anti-cyclic citrullinated peptide antibody (ACPA) responses.

Post-translational modifications play an important role in autoimmune diseases during pathogenesis. It is estimated that 50–90% of proteins are subjected to post-translational modifications, and these changes may contribute to tolerance breakdown. Post-translational modifications include acetylation, lipidation, citrullination, and glycosylation, among others, and are crucial for specific autoantibody recognition of autoimmune diseases, i.e., RA and multiple sclerosis. Conversely, altered protein degradation that leads to the accumulation and exposure of large amounts of autoantigens may likewise be important (Invernizzi P., CMI, 2017).

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Some pathogens are able to modify self-proteins or expose microbial antigens that resemble self-proteins, thereby creating neoantigens. For example, *Porphyromonas gingivalis* expresses a peptidylarginine deaminase (PAD) enzyme capable of citrullinating self-proteins (fibrinogen and enolase); these self-proteins act as neoantigens and can bind with high affinity to the major histocompatibility complex class II HLA-DR4 shared epitopes, leading in turn to anti-citrullinated peptide antibodies and rheumatoid arthritis development. Autoantibodies directed toward modified proteins bind both the native and the modified forms of collagen type II in the pathogenesis of RA; recent data have revealed different B and T cell epitopes on type II collagen. B and T cell epitopes may undergo citrullination and glycosylation in vivo, thus inducing immune activation in genetically predisposed subjects.[8]

Although the study of autoimmune diseases has long centered on the adaptive immune system, the discovery that innate immune cells express sensors for foreign and self-ligands has shifted the focus toward the first defense from the environment, which precedes the adaptive response. The innate immune system recognizes broad patterns or molecular motifs called pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) by germline-encoded "common" receptors called pattern recognition receptors (PRRs). These mechanisms allow a more rapid screening of self from "non-self" molecules. Toll-like receptors (TLR) are a family of PRRs that recognize PAMPs that are characteristic of pathogenic microorganisms. TLRs play a key role in the interplay between the innate and adaptive immune systems and are associated with the pathogenesis of autoimmune diseases.[9] By inducing the production of type I interferons (IFNs) and pro-inflammatory cytokines, these sensors are both endosomal and cytosolic, and their activation in dendritic cells (DCs) represents the initiating factor of several autoimmune diseases; in turn, this phenomenon can activate T and B cells and autoantibody production.[10] Interestingly, environmental factors such as viral infections, stress, injury, and UV light are sufficient for exposing endogenous PAMPs to the innate immune system via active/passive release; these PAMP molecules subsequently interact with PRRs such as TLRs to activate NF-kB-like transcription factors.[11]

Bystander activation occurs when a pathogen stimulates TLRs and other PRRs on antigen-presenting cells (APC), leading to the production of pro-inflammatory mediators, which in turn lead to tissue damage. In this context, the release of both tissue and bacterial antigens can stimulate bacterial-specific T cells and non-specific autoreactive T cells. Alternatively, some bacteria may express superantigens that can activate T cells in a T cell receptor-independent manner. Bystander activation is hypothesized to play a role in the pathogenesis of SLE, since bacterial and viral DNA can signal through TLRs, which can lead to excessive type I IFN responses and polyclonal B cell activation. Another example is the association between *Staphylococcus aureus* and antineutrophil cytoplasmic antibody (ANCA) vasculitis, since *S. aureus* DNA contains methylation motifs that can activate ANCA-producing B cells via TLRs in patients with ANCA-associated vasculitis.[12]

Nucleic acid sensors, such as the cationic peptide LL37, which contributes to the antimicrobial defense mechanism of damaged skin, have been implicated in this response. In psoriasis, LL37 forms complexes with extracellular self-nucleic acids present in the affected skin as the result of the inflammatory process and associated cell damage, and LL37 allows entry of these nucleic acids into intracellular compartments containing TLR7/8/9 or cytosolic DNA sensors.[13] Moreover, by circulating T cells, LL37 acts as an autoantigen and induces IL-17 production in psoriasis patients, and LL37 is correlated with disease severity.[14] Other nucleic acid sensors include IFI16, which is overexpressed in several autoimmune diseases, and autoantibodies directed toward IFI16 have also been reported.[15,16]

Th17 cells participate in the response to extracellular bacterial and fungal infection;[17] Th17 cells are localized mainly at mucosal sites of healthy subjects and are activated via TLR2 signaling. Microbiota mucosal interaction is thought to modulate Th17 cell activation, while dietary components and environmental toxins also influence the Th17 response[18] (Bo LI, CMI, 2017). In particular, commensal segmented filamentous bacteria (SFB) induce pro-inflammatory Th17 cells in the small intestine *lamina propria*, probably due to the adherent colonization of the intestinal epithelium by SFB, which enter the mucosal layer and adhere tightly to the terminal ileum epithelial cells.[19] SFB are believed to also exert an effect on autoimmune diseases. Indeed, several studies using animal models have demonstrated that SFB colonization promotes RA and multiple sclerosis but is protective against diabetes in non-obese diabetic (NOD) mice.[20,21] The effect of SFB on immune cells depends on the genetic background, as exemplified by increased gut permeability and altered gut microbial communities in HLA-DRB1*0401-susceptible RA mouse models.[22] The importance of Th17 cells is of particular interest in psoriasis and in psoriatic arthritis, since these cells represent a crucial mediator of chronic inflammation; in addition, biologics targeting IL-17 cells have been approved to treat these diseases.[23]

Tregs are T cells characterized by the expression of CD3, CD25, and the transcription factor forkhead box P3 (FOXP3); Tregs play a major role in the maintenance of our immune system.[24] Epidemiological studies suggest that environmental factors influence the number or activation of Treg cells.[25] Moreover, sex hormones play an important role in Treg development, which may underlie the female predominance.[1]

B cells represent a crucial mediator of autoimmune diseases, as exemplified by autoantibody production and the hypergammaglobulinemia found in SLE, SSc, and PBC[4] (Invernizzi P., CMI, 2017). B cells generate their pre-immune inventory in the bone marrow via a genetic recombination process known as V(D)J recombination; this recombination leads to an inventory of 10^7–10^8 B cells, each with unique surface receptors. This recombination leads to autoreactive B cells. In fact, early immature B cells in 55–75% of cases display autoreactivity, which leads to 20% autoreactive mature B cells. Several checkpoints exist to ensure that autoreactive B cells are excluded from immunocompetent peripheral lymphocytes. Despite these checkpoints, polyspecific autoreactive B cells are found in the periphery and produce polyspecific natural autoantibodies.[26] These natural autoantibodies are usually germline-encoded, of the IgM isotype, and non-pathogenic; however, polyspecific B cells may undergo somatic hypermutation and class switching to produce high-affinity IgG pathogenetic autoantibodies.[27] Recently B regulatory cells (Bregs), a subpopulation of B cells producing IL-10, have been extensively studied with respect to autoimmune diseases and are reduced in autoimmune diseases, both in number and functionality.[28,29] Moreover, IL-35 and TGF-β have also been associated with B cell-mediated immunosuppression in *Salmonella* infection in mice.[30] In fact, via IL-10, Bregs may also have a role in infectious diseases, particularly with respect to viral infections such as HIV and hepatitis B virus (HBV). In HBV, Bregs regulate antigen-specific $CD8^+$ T cells during HBV infection.[31] Regarding HIV, via T cell impairment, Bregs contribute to immune dysfunction associated with HIV infection; this contribution occurs specifically by the expression of IL-10 and possibly programmed death (PD)-L1, a member of the B7-H1 family. The suppressive properties of Bregs in HIV infection are associated with the prevalence of TLR ligands and CD40L.

The mechanisms leading to autoimmunity are reviewed in this issue of *Cellular and Molecular Immunology*. We hypothesize that more than one of these pathways lead to the onset of autoimmune diseases. We foresee that the application of new high-throughput technologies and data mining will allow finer recognition of disease-specific pathways and the development of

specific cell types or molecules to suppress autoimmunity and restore immune balance. These processes are expected to take a long time, and the treatment of autoimmune diseases remains a major challenge.

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