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Solving the puzzle of autoimmunity: critical questions

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Abstract

Despite recent advances in delineating the pathogenic mechanisms of autoimmune disease, the puzzle that reveals the true picture of these diverse immunological disorders is yet to be solved. We know that the human leukocyte antigen (HLA) loci as well as many different genetic susceptibility loci with relatively small effect sizes predispose to various autoimmune diseases and that environmental factors are involved in triggering disease. Models for mechanisms of disease become increasingly complex as relationships between components of both the adaptive and innate immune systems are untangled at the molecular level. In this article, we pose some of the important questions about autoimmunity where the answers will advance our understanding of disease pathogenesis and improve the rational design of novel therapies. How is autoimmunity triggered, and what components of the immune response drive the clinical manifestations of disease? What determines whether a genetically predisposed individual will develop an autoimmune disease? Is restoring immune tolerance the secret to finding cures for autoimmune disease? Current research efforts seek answers to these big questions.

Introduction

Over the past several decades, much has been learned about the pathogenesis of autoimmune diseases, a diverse group of heterogeneous disorders that may be characterized by multi-organ or single-organ system involvement. Underlying these diverse clinical phenotypes is a dysregulated immune system with an enhanced capacity to respond against self. The immune system is normally designed to defend against foreign pathogens by using an array of T and B lymphocytes, which bear antigen receptors, and innate immune cells, which may be activated by pathogen- or damage-associated molecular patterns. These cells orchestrate a finely tuned immune response through tightly regulated cell-cell interactions and secretion of cytokines, chemokines, and other inflammatory mediators. The body's defense against foreign pathogens must occur without causing undue harm to self. To accomplish this feat, the bulk of self-reactive T and B lymphocytes are eliminated in the thymus and bone marrow through a process of negative selection. However, this process is imperfect, albeit purposely, and self-reactive lymphocytes that escape into the periphery must be kept under wraps by an array of peripheral tolerance mechanisms. When the balance of the effector and regulatory arms of an immune response is thrown off, self-reactive T and B cells become activated and promote autoimmunity [1]. What finally pushes the immune system out of balance is a black box.

When speaking of autoimmune diseases, we often consider those featuring immunity against self-antigens and those without detectable anti-self-responses in the same breath. Rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), multiple sclerosis, type 1 diabetes (T1D), and celiac disease are examples of autoimmune diseases associated with the production of autoantibodies and, in some cases, self-reactive T cells. On the other hand, immunity against self-antigens is not a feature of psoriasis, inflammatory bowel disease, or ankylosing spondylitis, although the adaptive immune system is clearly involved in their pathogenesis [2,3]. There are some similarities in disease mechanisms because they both respond favorably

Table 1. Critical questions about autoimmunity

Inciting events
How, when, and where is the autoimmune response triggered?
How does an immune response triggered at one site lead to an inflammatory response at a remote anatomically distinct site?
Genetic predisposition
What factors determine whether genetically predisposed individuals develop autoimmune disease or remain healthy?
Why do some autoimmune diseases cluster in individuals and families?
Why are some autoimmune diseases rarely observed together?
Gene-environment interactions
What are the roles of environmental influences and oxidative stress in autoimmunity?
How does the microbiome contribute to the development of autoimmune disease?
Disease mechanisms
How does the human leukocyte antigen (HLA) locus contribute to the development of autoimmunity?
How do self-reactive T cells escape normal immune tolerance mechanisms and cause autoimmunity?
Which components of the immune system drive the clinical phenotype in each autoimmune disease?
Treatment mechanisms
Why doesn't an effective therapy work for everyone with the same autoimmune disease?
Why do treatments for one group of autoimmune diseases inadvertently provoke or worsen other autoimmune diseases?
Can autoimmune disease be cured by restoration of immune tolerance?

to anti-tumor necrosis factor (anti-TNF) therapy. In contrast, the predominant genetic associations of seropositive and seronegative disease diverge in an important way, namely their relationships with class II and class I HLA risk alleles, respectively.

Despite a growing understanding about the pathogenesis of autoimmune disease, untangling the complex events that provoke autoimmunity, produce clinical disease, and perpetuate its chronicity has been a major challenge. The interrelationships between the causative factors of autoimmune disease—genetics and environment—are mostly a mystery. In most instances, elucidation of the relative contribution of T cells, B cells, myeloid cells, and dendritic cells, as well as other rare cell types, to disease pathogenesis is a work in progress. The mechanisms of tissue inflammation are complex and involve the interactions between multiple immune cell types and an array of mediators that are balanced to favor an effector response.

Arguably, much progress toward understanding disease mechanisms has been made through the discovery of effective therapies that target specific cytokines [4]. These results have revealed vulnerable nodes in the mechanisms of disease, such as TNF in RA, psoriasis, and inflammatory bowel disease. However, a substantial proportion of patients with RA and these other diseases are not responsive to TNF inhibitors, highlighting the heterogeneity of disease and the likely presence of disease subtypes. It has also proven difficult to modulate the immune system for sustained benefit. Therapy such as TNF inhibition that weakens host defense and increases malignancy risk must be maintained indefinitely. The gaps in knowledge about the pathogenesis and treatment of autoimmune disease are evident in this very complicated puzzle. In this article, we discuss some of the critical questions dogging the community of researchers invested in better understanding autoimmunity (Table 1). Others may ask different questions, but it all depends on who is touching what part of the elephant [5]. The geneticist searches for allelic variation in the genes regulating immune pathways that are the driving force behind disease. The epidemiologist focuses on the inciting events. Clinicians who must deal with sick patients regardless of their genetic risks and disease triggers seek biomarkers that can identify disease subtypes and predict treatment response.

The critical questions: pathogenesis of rheumatoid arthritis

Hypotheses related to the triggering of autoimmune disease are generally framed as a function of the interplay between heredity and the environment. Two critical questions emerge from this organizing principle: (1) Which components of the immune system drive the clinical phenotype in each autoimmune disease? (2) How, when, and where is the autoimmune response triggered? Questions about the disease-inducing immunity have focused on the adaptive immune response (for example, autoantibodies and self-reactive B and T cells). How, when, and where disease-inducing immunity is triggered pose further questions about the events leading to the breakdown in immune tolerance and disease perpetuation.

With reference to these questions, it is useful to describe a conceptual model of the pathogenesis of RA, which has

gained increasing support and implicates triggering immune events at extra-articular sites, such as the lung. This breach in turn promotes immunity against citrullinated proteins in the synovium, the articular cartilage and bone, and consequently the development of chronic joint inflammation [6]. Autoantibodies to post-translationally modified citrullinated proteins (ACPAs) define a clinical phenotype associated with the two major risk factors for RA, the HLA DRB1 shared epitopes and an environmental exposure, cigarette smoking. Together they interact to increase the risk of developing ACPA-positive RA [7]. The R620W allele of PTPN22, a non-HLA genetic risk factor, has a strong effect in models that combine it with these other two major risk factors [8]. Serum ACPAs develop many years before the onset of clinical disease, suggesting that immunity to citrullinated proteins first occurs outside the joints [9]. The initiating events in RA may take place in the lungs. Recent studies indicate that those with or at increased risk for ACPA-positive RA have evidence of local production of ACPA in their bronchoalveolar fluid and lung tissue [10]. The lungs are an attractive candidate for an initiating immune response because of their limited barrier to environmental exposures. Periodontal mucosa also has a limited barrier that could be the site of initiation or amplification of autoimmunity. Bacterial species associated with periodontal disease, such as *Porphyromonas gingivalis*, have been shown to express the enzyme peptidyl arginine deaminase, which citrullinates human peptides correlated with autoimmunity [11].

How does immunity to citrullinated antigens triggered in the lungs lead to synovial inflammation? Dual expression of the primary targets of ACPAs in the lungs and joints is a possible explanation. Although identical citrullinated proteins are present in bronchial and synovial tissue of patients with RA, it remains unclear how ACPA induces disease in the joints [12]. Indeed, marked hypercitrullination is a feature of the rheumatoid joint [13]. It also appears that the joint is a reservoir of locally produced ACPAs because it has been shown that synovial fluid from ACPA-positive patients contains high titers of these antibodies [14]. In addition, immunoglobulin G-positive B cells that secrete antibodies to citrullinated peptides have been isolated from synovial fluid [15]. Once present in the joint, how does ACPA promote joint inflammation? Antibodies to citrullinated vimentin, another antigen in the joint, have been shown to stimulate osteoclast activation, perhaps contributing to the early stages of joint inflammation (bone marrow edema) and later joint erosions (damage) [16,17]. Immunity to other citrullinated antigens may augment joint inflammation by unknown mechanisms. Citrullinated fibrinogen, for example, may increase the potency of the innate immune response by binding to Toll-like receptor-4 and Fcγ receptor [18]. Another study has shown that citrullination of a chemokine, known as ENA/CXCL5, is higher in serum and synovial fluid from patients with RA than healthy controls and that citrullinated CXCL5 may recruit more monocytes to the inflamed joint than the non-citrullinated chemokine [19].

Although the story remains incomplete, the evolving evidence suggests that a breakdown of immune tolerance in the lungs, potentially induced by cigarette smoking or other exposure, in a genetically predisposed individual with distinct HLA class II alleles and non-HLA alleles, leads to the production of antibodies to citrullinated proteins before disease onset. This hypothesis raises critical questions about the regulation of adaptive immunity in the lungs, its dynamic behavior in relation to external stimuli, and the role of the microbiome in shaping a local immune response. These events in the lungs are followed by unknown events in the joint (perhaps a "second hit") that trigger inflammation. What is the time course of these events? The immune response to citrullinated protein is initially restricted many years before disease onset and later expands with time to target multiple epitopes on different citrullinated proteins near the onset of clinical disease [20]. This feature of epitope spreading leading up to disease onset may be a general feature of autoimmune disease, as illustrated by the evolution of serum autoantibody specificity predating the onset of SLE [21]. In RA and SLE, increased serum levels of chemokines and cytokines also precede the development of symptoms [22,23]. Is epitope spreading a consequence of an evolving systemic inflammatory response prior to onset of clinical disease, or a factor contributing to the development of tissue inflammation, or both?

Environmental control of autoimmunity: epigenetics and the microbiome

What factors determine whether genetically susceptible individuals will develop an autoimmune disease? Twin studies show that the environment influences the development of autoimmunity [24], and many environmental agents have been linked to autoimmune diseases [25]. Some environmental factors may contribute to autoimmunity by epigenetic mechanisms. Epigenetic mechanisms are heritable changes in gene expression that are not encoded in the DNA sequence but that are replicated each time a cell divides. DNA methylation and histone modifications are epigenetic mechanisms that control gene expression at the transcriptional level and thus are central to lymphocyte subset development and effector function. Epigenetic mechanisms are susceptible to environmental influences such as drugs, toxins, oxidative stress, and hormones, which trigger or exacerbate autoimmune diseases such as SLE in genetically predisposed individuals [26,27].

DNA methylation typically represses gene expression, and factors that inhibit methylation can promote expression of genes which are normally silenced. Decreased DNA methylation secondary to environmental exposures and oxidative stress converts T cells into a pro-inflammatory self-reactive phenotype that causes a lupus-like syndrome in mice [28]. Patients with active SLE have T cells with similar epigenetic changes and overexpression of genes that are normally silenced by DNA methylation [29]. There is evidence that drugs such as procainamide and hydralazine cause SLE by this mechanism in genetically susceptible individuals [28]. Decreased DNA methylation due to oxidative stress and other environmental factors may also be relevant in SLE occurring in the absence of known triggers [27].

The concept of environment has expanded from exogenous triggers, such as infection and toxic exposures, to endogenous triggers harbored in the microbiome. The complex relationships between commensal bacteria and autoimmunity are not well understood, but increasing evidence suggests that the microbiome may play an important role in the development of autoimmune disease [30,31]. One unusual species, segmented filamentous bacteria, induces an intestinal pro-inflammatory subset of T lymphocytes known as T helper 17 (Th17) cells [32], which have been implicated in a number of autoimmune diseases [33]. This species of bacteria also promotes autoimmune arthritis and experimental autoimmune encephalomyelitis at sites remote from the intestine in animal models [34,35]. A recent study in humans examined fecal microbiota in new-onset RA by sequencing microbial 16S ribosomal RNA genes [36]. An impressive 75% of patients with new-onset untreated RA had evidence of *Prevotella copri* colonization, compared with only 21% of healthy control subjects and 12% of subjects with treated chronic RA. These data raise new questions about the relationship between gut colonization and pathogenesis of RA as well as additional questions about how commensal bacteria influence autoimmunity in general.

Genetic susceptibility: human leukocyte antigen and the role of adaptive immunity

The first known autoimmune disease-associated gene complex was the HLA locus, which even today predominates over other genetic associations. Proteins encoded by HLA alleles are central to the adaptive immune response by binding antigenic peptide fragments which, in turn, recognize T lymphocytes. How does this normal function of HLA molecules contribute to autoimmune disease? The working hypothesis for this striking HLA disease association has been that allele-specific HLA molecules bind self-peptides, or peptides that mimic self, in a unique conformation that is recognized by self-reactive T cells. Such self-reactive T cells might ordinarily be eliminated by negative selection in the thymus or controlled by peripheral tolerance mechanisms, such as induction of anergy or suppression by T regulatory cells [1,37]. Why do self-reactive T cells escape normal immune tolerance mechanisms and cause autoimmune disease? This question remains unanswered today and likely involves disruption of the fine balance between effector and regulatory immune compartments.

Some self-peptides bind to HLA molecules weakly; preproinsulin peptides in T1D, for example, bind with low affinity to HLA-A2 [38]. Despite weak binding of the peptides to HLA, T cells in the peripheral blood of T1D subjects recognize these low-affinity peptide-HLA complexes, suggesting that low-affinity peptide-HLA interactions might trigger low-affinity T cells that are less subject to control by normal tolerance mechanisms such as negative selection and anergy. A recent study has shown that T-cell responses to a low-affinity insulin peptide are present in T1D subjects but not normal controls. Interestingly, these T cells also recognize microbial antigens, suggesting a mechanism by which infection could lead to disease [39]. Such low-affinity T cells could escape tolerance mechanisms, persist in the body, and when unchecked cause autoimmune disease.

Some self-antigens are created in response to environmental triggers in genetically susceptible individuals. A previously cited example is the citrullination of proteins that occurs in genetically susceptible smokers prior to the development of RA. Environmental factors can also trigger disease by inducing unexpected alterations of the peptide-HLA complex recognized by T cells, as recently demonstrated for beryllium-associated inflammatory lung disease. Chronic beryllium disease has been described as a T cell-mediated allergic hypersensitivity illness but shares a number of features with autoimmune diseases, including a close association with HLA. The immune response in chronic beryllium disease involves T-cell reactivity to self-peptide-HLA complexes in which the beryllium cation is bound deeply buried within the complex rather than at the surface of the complex. The beryllium cation thus does not contact the T-cell receptor itself but rather alters the surface conformation of self-peptide HLA complexes such that they are recognized as novel by T cells [40].

HLA class I and II molecules are the strongest genetic risk factors for many autoimmune diseases. In several cases, such as RA, disease-promoting class II HLA polymorphisms are located in the antigen-binding groove, implying that altered presentation of self-peptides underlies

disease pathogenesis [41]. HLA class I molecules present endogenous peptides from self-proteins and intracellular pathogens for binding by cytotoxic T cells (CTLs). The association between HLA-B27 and the spondyloarthropathies, including reactive arthritis, is the strongest genetic risk between an HLA class I allele and a human autoimmune disease; however, the mechanisms behind this genetic association remain to be defined. HLA-B27-restricted epitopes from *Chalmydia trachomatis* are recognized by specific CTLs from patients with reactive arthritis; these epitopes show high sequence homology with HLA-B27 self-peptides, suggesting that molecular mimicry is a possible mechanism by which class I HLA molecules may be linked with the pathogenesis of disease [42]. Another possibility relates to the observation that cytokine-induced HLA-B27 upregulation in macrophages results in a robust induction of the unfolded protein response (UPR) [43]. Although UPR activation has been associated with amplification of immune and inflammatory responses, direct evidence that these events act upstream of cell signaling and contribute to human disease pathogenesis is lacking [44].

Genetic elements and immune pathways that contribute to autoimmune disease

Which autoimmune disease will a genetically susceptible individual develop? In addition to HLA, a large number of autoimmune-predisposing genes have been identified [45,46]. Some genetic variants lead to severe autoimmune syndromes and are observed rarely in the population [47]. One such example is the genetic variant encoding the autoimmune regulator AIRE, a transcription factor required for the ectopic expression of tissue-specific antigens in the thymus. Loss-of-function variants of AIRE defect disrupt negative selection of self-reactive T lymphocytes, leading to autoimmunity at various sites [48,49].

The vast majority of the genetic loci associated with increased susceptibility to autoimmune disease are also found in healthy individuals. Most of these susceptibility genes have been identified by genome-wide association studies and Immunochip studies, and many are known to affect critical immune pathways [45,46]. There are also unaccounted-for genetic contributions known as "missing heritability". Some of this missing heritability may be due to relatively common loci with small effect sizes [50]. Alternatively, rare variants with large effect sizes may escape detection in genome-wide association studies [51]. Since risk alleles are found mostly in both healthy and autoimmune individuals, it is likely that the combination of many different loci interacting with environment factors promotes autoimmune disease.

Autoimmunity appears to be inherited as a trait. Individuals with one autoimmune disease are at higher risk of developing a second autoimmune disease, and multiple autoimmune diseases cluster within families [52]. Autoantibody-mediated (seropositive) diseases, such as RA, T1D, and autoimmune thyroid disease, occur in clusters. This clustering is likely explained by susceptibility gene variants in common among the diseases. One prominent example is the single-nucleotide polymorphism variant PTPN22, which is a risk allele for RA, T1D, SLE, and other autoimmune diseases [53–56]. PTPN22 encodes lymphocyte tyrosine phosphatase, a negative regulator of T-cell activation. Altered T-cell receptor (TCR) signaling has been reported in the setting of the autoimmunity-associated PTPN22 variant [57,58]. Lymphocyte tyrosine phosphatase is expressed not only in T cells but also in B cells and myeloid cells. The variant allele of PTPN22 has been associated with defective B-cell tolerance and abnormally high numbers of self-reactive B cells [59–61]. The PTPN22 variant of lymphocyte tyrosine phosphatase also regulates type I interferon production following Toll-like receptor-driven activation of myeloid cells, an observation that suggests a possible link between infections and autoimmune disease [62]. PTPN22-related defects in the T cell, B cell, and innate immune compartments could in theory contribute to disease in many different organ systems and, depending on the presence of other gene loci, produce a variety of clinical phenotypes [56].

Autoimmune diseases without autoantibodies (seronegative), such as psoriasis, inflammatory bowel disease, and ankylosing spondylitis, also show evidence of clustering. These seemingly unrelated diseases share an association with spondyloarthropathy, which is characterized by sacroiliitis and enthesitis (inflammation of tendons and ligaments at insertion sites) rather than the synovial inflammation typical of RA. Psoriasis and inflammatory bowel disease are associated with gene variants of the interleukin-23 (IL-23) receptor [63,64]. IL-23 is a cytokine that supports the development of Th17 cells, which produce the pro-inflammatory cytokine IL-17 as well as other IL-17-producing cell types [65]. Notably, there is much higher correlation between susceptibility genes for ankylosing spondylitis and psoriasis than between genes for ankylosing spondylitis and RA [45].

Unifying diseases into a single diagnostic category may not be particularly useful, since the molecular pathways contributing to disease pathogenesis are likely related in some cases but highly distinct in others. This concept has major implications for drug development and repurposing of approved medications for new indications [66]. Discordant sets of genetic susceptibility genes may

also explain why therapy for one autoimmune disease can inadvertently trigger another autoimmune disease. One example is a variant of the TNF receptor that is associated with an increased risk for multiple sclerosis but which is protective against developing ankylosing spondylitis [67]. Perhaps not surprisingly then, anti-TNF therapy, though effective for ankylosing spondylitis, has been shown to worsen multiple sclerosis and may induce a multiple sclerosis-like disease as an adverse consequence of treatment [68].

Disease heterogeneity: variable response to therapeutic intervention

When a therapy is effective in an autoimmune disease, why does it not work for everyone with that disease? Within a given autoimmune disease, there is heterogeneity in clinical phenotypes, implying differences among individuals in pathogenic mechanisms. Thus, it is not surprising that individuals within a diagnostic category vary in their response to therapy. In relapsing-remitting multiple sclerosis, for which type I interferon (interferon beta) is considered standard first-line therapy, 30% to 50% of patients do not respond to this intervention. Somewhat paradoxically, in multiple sclerosis, elevated expression of type I interferon response genes pre-treatment correlates with the absence of a beneficial response to type I interferontherapy [69,70]. It has been suggested that a high-interferon response gene signature identifies a subset of patients with multiple sclerosis who have a distinctly different pathogenic mechanism driving disease [71]. Similarly, in RA, an interferon response gene signature has been found to predict a lack of response to rituximab therapy [72–74]. "Personalizing" treatment of autoimmune disease on the basis of genetic factors, biomarkers, and lifestyle choices has the potential to dramatically improve the therapeutic approach for many autoimmune diseases [75].

Restoration of immune tolerance as a treatment for autoimmunity

Amelioration of clinical disease is not usually durable without ongoing therapy and often requires frequent switching of drugs to sustain disease control. To keep disease at bay, combined interventions may be needed to simultaneously check effector lymphocytes, potentiate regulatory elements, and control the innate immune response [76]. Even if such combinations are successful in remediating the manifestations of autoimmune disease, it remains to be proven whether durable immune tolerance and a healthy state can be restored in the autoimmune disease setting without changing genetic predisposition or reversing the environmental damage. Prevention may yet prove to be the optimal treatment strategy.

Conclusions

A complex picture of autoimmune pathogenesis is emerging in which genetic predisposition resides in a large number of loci encoding key immune pathway molecules. We now recognize that expression of these genes is under epigenetic control, which can be influenced by a number of environmental factors in susceptible individuals. The microbiome resides at the boundary of self and the environment and appears to also influence autoimmunity by mechanisms that are not yet understood. Although progress has been made in understanding the pathogenesis of autoimmune disease, a large number of questions remain unanswered (Table 1). Filling this gap in knowledge will require a greater understanding of the inciting events leading to autoimmunity and clinical disease, the role of the environment (including the microbiome) in triggering and perpetuating disease, the hierarchy of aberrantly regulated immune pathways involved in disease pathogenesis, and identification of the most vulnerable nodes in the immune system for therapeutic targeting. We expect too that the nosology for autoimmune disease will slowly evolve from its current framework of clinical and serologically defined states into an immune pathway-based scheme of overlapping phenotypes and sub-phenotypes that will underpin rationally designed therapy in the future. The pieces of the puzzle are slowly coming together.

Abbreviations

ACPA, autoantibody to citrullinated proteins; CTL, cytotoxic T cell; HLA, human leukocyte antigen; IL, interleukin; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; T1D, type 1 diabetes; Th17, T helper 17; TNF, tumor necrosis factor; UPR, unfolded protein response.

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