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THE ROLE OF INFECTION IN AUTOIMMUNE DISEASES

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Abbreviations: major histocompatibility complex (MHC), juvenile idiopathic arthritis (JIA), T regulatory (Treg), human leukocyte antigen (HLA), cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4), heat shock protein (hsp), tumor necrosis factor alpha (TNF α), interleukin (IL)

Abstract

In order for the normal immune system to protect against foreign pathogens, it must be able to distinguish self from nonself. This is accomplished by a process of microbial pattern recognition in the innate immune system and by central and peripheral tolerance in the adaptive immune system. However, despite these mechanisms of tolerance, self-reactive T cells still exist and can cause autoimmune diseases via a number of mechanisms, including molecular mimicry, epitope spreading, and bystander activation. In this article we aim to explore the scientific data supporting these concepts of pathogenesis and how they can be applied to future therapies, such as epitope-specific immunotherapy which aims to induce tolerance with cross-reactive heat shock proteins conserved in both microbes and humans. Ultimately, the goal is to improve knowledge and understanding of the role of infectious pathogens in molecular mechanisms and genetic predispositions involved in autoimmune disease pathogenesis, so that more focused therapies can be developed.

Physiological Inflammation

The immune system exists to protect the host against foreign invaders. The normal host immune response, consisting of the innate and adaptive immune systems, elicits a powerful inflammatory response which is necessary to help control the spread of foreign pathogens. However, if left unchecked, this inflammatory response can lead to tissue destruction. The innate immune system, consisting of endothelial cells, phagocytes, natural killer cells, and antigen presenting cells such as macrophages and dendritic cells, responds to foreign invaders with an early inflammatory response by recognizing conserved

regions on microbes. Despite its prompt response, innate immunity does not provide lasting memory.

In contrast, the adaptive immune system, consisting of T and B cells that are stimulated by cytokines and antigen presenting cells from the innate immune system, produces a targeted cell-mediated immune response with memory that can facilitate clearance of the same microbial invader in the future [1]. In order for the immune system to protect against foreign invaders, it must be able to distinguish self from nonself. This is the challenge that both the innate and adaptive immune systems face on a daily basis.

Self versus Nonself: The Innate Response

The first line of defense against a foreign antigen is the innate immune system. While this arm of the immune system cannot produce immunological memory, it does begin the inflammatory process that works towards eradication of the pathogen, in addition to activating the adaptive immune response. For example, macrophages have a mannose receptor that recognizes a sugar residue commonly found on microbes and not on host cells, which when bound can lead to phagocytosis of the microbe [2]. In addition, cells such as endothelial cells, macrophages, dendritic cells, and neutrophils contain transmembrane evolutionarily-conserved proteins known as Toll-like receptors that recognize microbial conserved molecular structures [3,4]. These Toll-like receptors activate a transcription factor known as NF- κ B, which subsequently leads to production of cytokines and co-stimulatory molecules on antigen presenting cells needed to initiate an adaptive immune response [5].

Self versus Nonself: Central and Peripheral T Cell Tolerance

Just as with the innate immune system, it is crucial to the host that cells in the adaptive immune response be able to differentiate self from nonself. For T cells, this is initially achieved at the thymic level by positive selection for those cells that weakly recognize self antigens bound to a major histocompatibility complex (MHC) on an antigen presenting cell [6]. In addition, T cells whose

receptors bind too strongly to self antigens undergo cell death, a process known as negative selection [7]. These two forms of central selection strive to create a T cell repertoire that can respond to peptides bound to self MHC molecules while removing T cells that would lead to an autoimmune response.

But how does a T cell that will respond to a foreign antigen become positively selected in the thymus if only self antigens exist during thymic selection? One theory is that the host's carefully selected T cells are crossreactive, recognizing both microbial and self epitopes, a mechanism known as molecular mimicry [8,9]. A molecular basis for this crossreactivity is supported by the crystallography data showing large conformational changes in the T cell receptor upon antigen binding, therefore providing structural evidence that different peptides can bind to the same T cell receptor [10].

Recently it was shown in a non-transgenic mouse model that physiological positive selection can generate an oligoclonal population of T cells that recognize both self peptides and a bacterial homologue [11]. Such evidence supporting both the structural and physiological feasibility of molecular mimicry may explain how self antigens, the only antigens available during thymic development, are crucial in developing a normal T cell repertoire that can later respond to infectious agents.

Tolerance is not just an event that occurs in the neonatal period but one that must continue throughout life as we are faced daily with foreign antigens and the immune system must decide as to how strongly to respond to these antigens. Although negative selection in the thymus plays a major role in self tolerance, studies have shown that healthy individuals have self-reactive T cells in the periphery which have the potential for activation leading to autoimmune disease [12]. To decrease the probability of self-reactive T cells even further, tolerance occurs in the periphery via ignorance, deletion, or anergy.

In the case of ignorance, self antigens may be in such low doses that they do not trigger a T cell response or they may be sequestered and, therefore, are inaccessible to T cells [13,14]. Deletion, also known as activation-induced cell death, occurs when the Fas protein, involved in the regulation of apoptosis, binds

to its ligand on the surface of chronically stimulated T cells [15]. In one experiment, disruption of the signal cascade from the Fas-Fas ligand in a murine model led to defective activation-induced cell death, and consequently, autoimmune arthritis [16]. A third mechanism of peripheral T cell tolerance is encounter of a self-antigen leading to anergy. Originally thought to occur via lack of costimulation, it is now known that anergy requires *in vivo* ligation of the cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4), which inhibits activated T cells [17].

Peripheral tolerance can also be controlled by a distinct subset of T cells called regulatory T cells (Treg). Thymus-derived Treg cells which express both CD4 and CD25 surface markers were first shown to be important in peripheral tolerance when it was noted that inoculation of CD25⁺ T cells could prevent the development of autoimmune diseases seen in thymectomized neonatal mice [18]. One recent study showed a higher frequency of Treg cells in the synovial fluid of patients with a remitting form of oligoarticular JIA than in patients with extended oligoarticular JIA, which has a less favorable prognosis [19]. Another study has further identified that the functional T regulatory cells are more specifically CD4⁺CD25^{bright} cells, and can be found in the synovium of patient with rheumatoid arthritis [20]. These data appear to support the role of Treg cells in regulating inflammation, and consequently the severity of disease, in the target organ.

Mechanisms

Despite these methods of central and peripheral tolerance, self-reactive T cells which can be triggered to cause autoimmune diseases still exist. The mechanisms that have been found to show a link between infectious pathogens and autoimmune diseases include molecular mimicry, epitope spreading and bystander activation ([Table 1](#)).

Molecular mimicry

Molecular mimicry is the concept that T cells are activated that can recognize both microbial and self epitopes and an excessive inflammatory

process occurs. One of the most studied autoimmune diseases occurring via molecular mimicry is the heart disease noted in rheumatic fever. Both animal and human studies have shown that exposure to the dominant epitope of group A streptococcal polysaccharide, N-acetyl-glucosamine, leads to cross-reactive T cells and antibodies to cardiac myosin [21,22,23,24,25]. Further data supporting molecular mimicry is seen in the development of herpes stromal keratitis after herpes simplex virus-type 1 infection. In this condition, T cell clones respond to both a corneal self antigen and a peptide derived from herpes simplex virus-type 1 [26]. Mutant herpes simplex virus-type 1 that lacks this epitope did not induce keratitis [27]. Type 1 diabetes, an autoimmune disease that results from the destruction of pancreatic islet β cells, has been associated with rotavirus and cytomegalovirus. These viruses share sequence homology to the autoantibody glutamic acid decarboxylase found commonly in type 1 diabetics [28,29].

Another example of homology between a microbial peptide and self antigen is in inflammatory heart disease where the outer membrane of *Chlamydia* is homologous to the murine heart muscle myosin heavy chain. Immunization of a murine model with this homolog or infection with *Chlamydia trachomatis* via the respiratory tract led to a similar frequency of inflammatory heart disease as immunization with the myosin peptide [30]. Also in susceptible mouse strains expressing a particular MHC Class II molecule, immunization with myelin basic protein peptide can induce experimental autoimmune encephalomyelitis [31]. Supporting the role of molecular mimicry in this model is the fact that the herpes virus *Saimiri* peptide, which has limited homology to myelin basic protein, stimulates the myelin basic protein-specific T cell hybridomas and causes experimental autoimmune encephalomyelitis in mice [32].

As for the role of molecular mimicry in rheumatologic disorders, rheumatoid arthritis has shared epitopes between the human leukocyte antigen (HLA) -DRB1 chain found in 90% of patients and the dnaJ class of heat shock proteins in *Escheria coli*. While patients with early rheumatoid arthritis had proliferation of T cells with dnaJ peptide *in vitro* stimulation, normal subjects with

the same HLA-DR type, identical twins without disease, and those with other autoimmune diseases did not [33]. In oligoarticular juvenile idiopathic arthritis (JIA), T cell cytotoxic responses and production of proinflammatory cytokines, such as interferon gamma, were elicited *in vitro* in response to stimulation with Epstein-Barr virus proteins homologous to 9-mer peptides derived from HLA Class II molecules associated with oligoarticular JIA [34].

With regard to juvenile dermatomyositis, patients noted to relapse after a *Streptococcus pyogenes* pharyngitis led to the hypothesis that recognition of streptococcal proteins might trigger an autoimmune response to a muscle antigen [35]. Further supporting the role of molecular mimicry in juvenile dermatomyositis, self epitopes in the human skeletal myosin heavy chain were found to be homologous to peptides in the *Streptococcus pyogenes* M5 protein [36]. *In vitro* studies with the human skeletal myosin heavy chain and its M5 peptide homologues showed more elevated cytotoxic T cell responses to the M5 peptide in children with active juvenile dermatomyositis than in patients in remission, children with JIA, patients with post-streptococcal disease (such as glomerulonephritis, rheumatic fever, and reactive arthritis) or healthy controls [37,38]. In these same patients, production of tumor necrosis factor-alpha (TNF- α) was elevated in the peripheral blood mononuclear cells of patients with active juvenile dermatomyositis exposed to the M5 peptide compared to controls. Finally, this same study showed an overlap in the T cell receptor V β regions recognizing the human skeletal myosin heavy chain peptide and the M5 peptide, further supporting the role of molecular mimicry in the pathogenesis of juvenile dermatomyositis.

Epitope spreading

Besides molecular mimicry, two other theories are commonly used to explain how infectious agents can lead to autoimmune diseases. The first of these two, known as epitope spreading, focuses on microbially-induced T cell-mediated tissue damage that leads to release of self antigens from a sequestered site [39]. These self peptides are then presented to T cells by local antigen presenting cells, spreading the immune response to self antigens. One

such example is Theiler's murine encephalomyelitis virus which induces a T cell-mediated demyelinating disease in mice that clinically resembles chronic, progressive multiple sclerosis [40]. Kinetic and functional studies showed that T cell responses to myelin occurred because of new priming of self-reactive T cells to sequestered autoantigens from demyelination, rather than cross-reactivity, as occurs with molecular mimicry [41].

Bystander activation

Another alternative theory to molecular mimicry focuses on the effects of the microbially-induced inflammatory cascade rather than an antigen-specific response. This concept, known as bystander activation, is the nonspecific activation of self-reactive T cells stimulated by cytokines [42,43]. Coxsackie B4 virus has been associated with the development of type 1 diabetes and shared sequence similarities with the islet autoantigen glutamic acid decarboxylase found in these patients. However, rather than finding viral acceleration of diabetes as would be expected in molecular mimicry, mice with a transgenic T cell receptor specific for a different islet autoantigen developed diabetes, reflecting T cell receptor-independent bystander activation [44].

While molecular mimicry, epitope spreading and bystander activation support the role of pathogens in autoimmune disorders, it is unclear why of the many individuals exposed to these common pathogens, only a relatively small percentage develop autoimmune diseases. In fact, though healthy humans are exposed to killed or live microbes via vaccines, no conclusive data exists linking vaccinations with autoimmune diseases [45]. Therefore, though a pathogen may be part of the initial trigger, it is likely that genetic predisposition and environmental exposures are also strongly involved.

Genetics and Environmental Factors in Autoimmunity

Diabetes

Among all genetic components, human leukocyte antigen (HLA) class II genotype has been the most closely associated genetic factor linked to autoimmune diseases. In type 1 diabetes, allelic variations of three adjacent

class II genes, *HLA-DRB1*, *-DQA1*, and *-DQB1*, have been linked to an increased risk of developing disease [45,46]. Furthermore, differences in worldwide patterns of incidence of type 1 diabetes can be partially explained by genetic allelic differences between different ethnic groups [47]. In a country such as Finland which has the world's highest incidence of type 1 diabetes, the progression to diabetes in monozygotic twins was 50% within 7 years if the index twin was diagnosed at 10 years of age or younger [48]. In a study comparing North American monozygotic twins, the long-term risk of developing type 1 diabetes if the co-twin developed disease before 15 years of age was 44% [49]. The fact that disease penetrance was not 100% in these twins suggests that while genetic factors may play a substantial role in type 1 diabetes, nongenetic factors such as environment and the host's immune response also influence the expression of autoimmune disease.

Environmental factors that have been implicated in the development of type 1 diabetes include coxsackie virus, where polymerase chain reaction analysis of sera from diabetic children showed that 43% were positive for coxsackie B virus, compared to 4% of controls [50,51]. A similar correlation has been found between cytomegalovirus and type 1 diabetes, where 20% of patients had cytomegalovirus genomic DNA in their lymphocytes, compared to only 2% of controls [52]. In a murine model, infection with cytomegalovirus has also been shown to accelerate the onset of diabetes [53]. Besides viruses, chemicals such as alloxan and streptozotocin have been shown, at least in experimental animal models, to induce diabetes via cytotoxic action against pancreatic β cells mediated by reactive oxygen species [54,55].

Rheumatic diseases

In rheumatologic disorders, genetic and environmental associations have also been found to be important in the development of autoimmune disorders. In JIA, certain HLA class II alleles, namely HLA-A2 (isn't this a class I allele ??), HLA-DRB1*08, HLA-DRB1*11, and HLA-DPB1*02, are independently associated with increased risk of developing disease [56,57]. Furthermore, when stimulated with Epstein-Barr virus-derived peptides homologous to self HLA epitopes,

peripheral blood mononuclear cells from JIA patients with these above HLA alleles showed increased cytotoxic ability compared to those of control patients [58]. In contrast, data from Kuwaiti children with JIA showed an association with HLA-DRB1*0307 and HLA-DRB1*0308, indicating that HLA allelic associations may differ between ethnic groups [59].

Rheumatoid arthritis appears to be associated with HLA-DRB1 alleles which contain the QKRAA amino acid sequence [60,61]. In fact, humoral and cellular immune responses are triggered in early rheumatoid arthritis patients with these HLA-alleles when stimulated with microbial antigens that share this QKRAA sequence, such as the dnaJ class of heat shock proteins from *Escherichia coli*, *Brucella ovis*, *Lactobacillus lactis*, and Epstein-Barr virus [62,63]. While the HLA-DRB1 alleles and the frequency of these shared epitopes may vary between different ethnic groups, genotype does not necessarily correlate to disease severity, reflecting that factors besides genetics influence disease severity in rheumatoid arthritis [64]. In addition, data from monozygotic twins shows a concordance rate between 12 and 20% suggesting that environmental factors have substantial involvement in the development of rheumatoid arthritis [65]. The most consistently reported occupational exposure associated with rheumatoid arthritis is silica [66]. In addition, mineral oil, which acts as an adjuvant and induces arthritis in mice, has been suggested as a risk factor, although no human data exists [67].

Therefore, it appears that while there may be genetic predispositions to autoimmune diseases, disease incidence and severity is also shaped by the host's immune response and environmental factors. A better understanding of response to cross-reactive self-epitopes and genetic factors, as well as their links to disease severity, will help target therapies in the future.

Targeted Therapy in Autoimmune Diseases

Current therapy for many autoimmune diseases is non-specifically aimed at decreasing the inflammatory cascade. This approach can lead to deleterious side effects, including osteoporosis, infection, and immunodeficiencies. Therapy

directed at a specific part of the inflammatory cascade, such as anti-TNF α antibody, has shown promise by restoring the capacity of regulatory T cells to inhibit cytokine production, although CD4⁺CD25^{bright} cells that are most closely identified with regulation did not demonstrate this inhibition [68] ([Table 2](#)). However, even such targeted therapy has shown that TNF α is critical for protective immunity against infectious agents, and that anti-TNF α therapy can lead to higher incidence of *Mycobacterium tuberculosis* infection [69,70]. A better form of therapy would be one that aims to control the immune response more specifically, enhancing the mechanisms of central and peripheral T cell tolerance that naturally exist to prevent autoimmunity. One such example would be **epitope-specific immunotherapy** that aims to create tolerance to self-epitopes involved in a specific autoimmune disease.

In terms of how to best induce tolerance, previous work has demonstrated that oral consumption of a potent nonself antigen by humans can lead to systemic T cell tolerance, reducing T cell proliferation compared to controls when challenged with the same antigen by subcutaneous immunization [71]. As occurs naturally, experimental animal models have shown that higher doses of antigen favor anergy or deletion [72]. Conversely, it is thought that low doses of orally or mucosally administered antigens induce production of regulatory T cells from the gut-associated lymphoid tissue, which then migrate to the systemic immune system [73]. In fact, recent data in mice demonstrate that colonic CD4⁺CD25⁺ T cells are necessary in order to prevent an inflammatory response to enteric antigens [74]. Furthermore, animal experiments have shown that oral administration of a self-antigen leads to suppression of an autoimmune disease, as is seen in depletion of peripheral antigen-specific T cells in mice after oral administration of myelin basic protein, protecting the mice from experimental autoimmune encephalomyelitis [75].

The ideal antigen for epitope-specific therapy would be one that has strong antigenic potential, induces regulatory cytokines, is available at the site of inflammation, and is conserved across species so as to treat diseases involving molecular mimicry. One class of proteins that appears to fill these criteria are

heat shock proteins (hsp), which are evolutionarily conserved proteins present in prokaryotic and eukaryotic cellular organisms that are upregulated by stress [76].

Hsps, denoted by their weight in kilodaltons such as hsp60 or hsp70, have immunomodulatory properties both at the innate and adaptive immune response levels. In innate defense, human hsp60 has been shown to stimulate mouse macrophages to release the proinflammatory cytokine TNF α , which serves as a danger signal [77]. Further studies have shown that the proinflammatory response from several hsps are mediated through the innate immune Toll-like receptors, specifically TLR2 for chlamydial hsp60, and TLR2 and TLR4 for human hsp70 [78,79].

Despite this proinflammatory pathway activation in innate immunity, experimental autoimmune models have shown repeatedly that immunization with hsp antigens leads to resistance rather than autoimmune disease, as would be expected. For example, in rat adjuvant arthritis, immunization with self hsp60 or hsp65 was found to be protective against development of autoimmune arthritis [80,81]. Nasal administration of mycobacterial hsp65 and hsp70 peptides homologous to rat hsp sequences induced cross-reactive T cells that produced the inhibitory cytokine interleukin-10 (IL-10) [82,83]. These data are, therefore, consistent with a regulatory T cell activation by the adaptive immune response after hsp stimulation.

In children with remitting oligoarticular JIA where a higher frequency of CD4⁺CD25⁺ cells were found in the synovial fluid, significant *in vitro* T cell responses to human hsp60 in peripheral blood mononuclear cells and synovial fluid correlated with earlier disease remission [84,85,86]. Similarly, mycobacterial hsp70 has been shown to induce IL-10-secreting synovial cells of arthritic patients with subsequent decrease in the proinflammatory cytokine TNF α [87]. These data imply that immune modulation with hsps may be an effective method of treatment in JIA or rheumatoid arthritis.

In theory, one should be able to induce tolerance to proinflammatory epitopes or shift the immune system to produce regulatory T cells to self epitopes via bystander activation. This could be accomplished by presenting conserved,

cross-reactive peptides via the mucosal route. Application of such a theory led to considering dnaJp1, an *Escherichia coli* hsp-derived peptide that includes the QKRAA sequence known to be cross-reactive in rheumatoid arthritis, as a possible epitope-specific immunotherapy for rheumatoid arthritis. A phase I/IIa trial showed this treatment to be safe, in addition to oral therapy with dnaJp1 reducing the IL-2, TNF α , interferon gamma proinflammatory-producing cells and replacing them with IL-10 and IL-4 anti-inflammatory-producing cells [88]. Further data revealed that the total number of dnaJp1-specific T cells did not change, whereas the expression of CD4⁺CD25⁺ cells increased [89]. This data suggests that epitope-specific immunotherapy does not change the number of peptide-specific T cells by clonal deletion, but rather transforms their function into a regulatory phenotype. A similar outcome has been reported with *in vitro* incubation of oligoarticular JIA patients' T cells with human hsp dnaJ epitopes [90].

Another form of autoimmune therapy is geared towards interfering with the costimulatory pathway that is critical for activation of proinflammatory T cells. Most promising has been CTLA-4, which inhibits activated T cells by blocking the costimulatory molecule B7 on antigen presenting cells from binding to its T cell receptor. A murine model demonstrated that intraarticular injection of an adenovirus vector containing a gene that encoded for CTLA-4 immunoglobulin inhibited the development of collagen-induced arthritis [91]. The initial study in patients with rheumatoid arthritis showed that intravenous infusion of CTLA-4 immunoglobulin was well tolerated and that there was a trend towards clinical improvement [92,93]

As these newer forms of therapy demonstrate, the goal in the treatment of autoimmune diseases is to use the knowledge of genetic and cellular inflammatory mechanisms to create targeted therapy that does not disrupt the protective effects of the immune system.

Summary

- Via mechanisms of pattern recognition and tolerance, the innate and adaptive immune systems strive to differentiate self from non-self.
- Peripheral tolerance is influenced by the presence of regulatory T cells that produce the anti-inflammatory cytokine, IL-10.
- Mechanisms that show a link between infectious pathogens and autoimmune diseases include molecular mimicry, epitope spreading, and bystander activation.
- Environmental and genetic factors, most notably HLA class II genotype, affect autoimmune disease predisposition.
- Newer mechanisms of autoimmune disease therapy include anti-TNF α antibody, epitope-specific immunotherapy, and CTLA-4.
- Epitope-specific immunotherapy with heat shock proteins demonstrates regulatory T cell activation by the adaptive immune system.

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