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Review

Sex Differences in Autoimmune Disease from a Pathological Perspective

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Autoimmune diseases affect ~8% of the population, 78% of whom are women. The reason for the high prevalence in women is unclear. Women are known to respond to infection, vaccination, and trauma with increased antibody production and a more T helper (Th)2-predominant immune response, whereas a Th1 response and inflammation are usually more severe in men. This review discusses the distribution of autoimmune diseases based on sex and age, showing that autoimmune diseases progress from an acute pathology associated with an inflammatory immune response to a chronic pathology associated with fibrosis in both sexes. Autoimmune diseases that are more prevalent in males usually manifest clinically before age 50 and are characterized by acute inflammation, the appearance of autoantibodies, and a proinflammatory Th1 immune response. In contrast, female-predominant autoimmune diseases that manifest during the acute phase, such as Graves' disease and systemic lupus erythematosus, are diseases with a known antibody-mediated pathology. Autoimmune diseases with an increased incidence in females that appear clinically past age 50 are associated with a chronic, fibrotic Th2-mediated pathology. Th17 responses increase neutrophil inflammation and chronic fibrosis. This distinction between acute and chronic pathology has primarily been overlooked, but greatly impacts our understanding of sex differences in autoimmune disease. *(Am J Pathol 2008, 173:600–609; DOI: 10.2353/ajpath.2008.071008)*

Autoimmune diseases are the third most common category of disease in the United States after cancer and cardiovascular disease, affecting ~5 to 8% of the population or 14.7 to 23.5 million people.[1] Conservative estimates indicate that ~78% of the people affected with autoimmune diseases are women.[2–4] For some time it has been known that the basic immune response differs between men and women. Women respond to infection, vaccination, and trauma with increased antibody production, whereas inflammation is usually more severe in men resulting in an increased mortality in men and protection against infection in women.[5–10]

Antibodies provide critical protection against infection, and are the key protective response induced by vaccination.[11] Naturally occurring autoantibodies are frequently found in the serum of normal humans and are important in clearing cellular debris induced by inflammation or physical damage.[11,12] However, autoantibodies may induce damage by binding self-antigens and activating the complement cascade, resulting in direct cytotoxicity or an immune complex (IC)-associated pathology. The number of different autoantibodies present in an individual is a good predictor of the risk of developing an autoimmune disease. For example, estimates based on first degree relatives show that the likelihood of a child developing type 1 diabetes within 5 years is 10% in the presence of one autoantibody, 30% for two autoantibodies, and 60 to 80% if three autoantibodies are present.[13] Thus, the risk for developing an autoimmune disease increases as the number of autoantibodies increases, and the number of autoantibodies increases as we age, regardless of sex (Figure 1).[14,15] So even though an increased antibody response protects women from infections, it also increases the risk of developing an autoimmune disease.

In a similar manner, immune cells may damage tissues directly by killing cells or indirectly by releasing cytotoxic cytokines, enzymes, or reactive nitrogen/oxygen intermediates. Cytokines and other mediators released by resident mast cells (MCs) and macrophages recruit inflammatory cells, such as neutrophils, macrophages, and T cells, to the site of damage. $CD4^+$ T cells have been

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Incidence	Acute Pathology (< 50 yrs)	Chronic Pathology (> 50 yrs)	Target
	Inflammation	Fibrosis	
	Autoantibodies	Increased # of Autoantibodies	
	Males: Cell-mediated/Th1 (blue)	**Fibrosis/Th2/Th17 (pink)**	
	Females: Ab-mediated/Th2 (pink)		
Increased incidence in Males (M) during acute and/ or chronic phase:			
	Myocarditis*	Dilated cardiomyopathy (DCM)	Heart
	Wegner granulomatosis	Wegner granulomatosis	Arteries
	Idiopathic pulmonary fibrosis (IPF)	**IPF, M 11:7**	Lung
	Gastritis	Pernicious anemia	Stomach
	Diabetes	Diabetes	Pancreas
M 2:1	**Ankylosing spondylitis** (AS)	AS	Joints
Increased incidence in Females (F) during acute and chronic phase:			
F 2:1	**Multiple sclerosis** (MS)	MS	Myelin
F 2:1	Dermatomyositis	**Dermatomyositis**	Muscle
F 3:1	**Thrombocytopenia purpura** (ATP)	ATP	Platelets
F 3:1	**Myasthenia gravis** (MG)	MG	Receptor
F 3:1	**Rheumatoid arthritis** (RA)	RA	Joints
F 4:1	Systemic sclerosis	**Systemic sclerosis**	Collagen
F 4-6:1	Autoimmune hepatitis (AIH)	**AIH**	Liver
F 7:1	**Graves' disease**	Graves' disease	Receptor
F 9:1	**Systemic lupus erythematosus** (SLE)	SLE	Nucleus
F 9:1	Sjogren syndrome	**Sjogren syndrome**	Glands
F 3-5:1	Hashimoto's thyroiditis	**Hashimoto's, F 10-20:1**	Thyroid

***Bold = represents age when signs and symptoms of autoimmune disease usually manifest clinically**

Figure 1. Incidence of autoimmune diseases in men and women categorized by age, sex, and immunopathology. Most male-predominant autoimmune diseases manifest clinically (ie, show signs and symptoms of clinical disease) before 50 years of age and are characterized by acute cell-mediated pathology. Acute autoimmune diseases with an increased incidence in women have a clear antibody (Ab)-mediated pathology, whereas those appearing later in life are associated with chronic inflammation, fibrosis, increased numbers of autoantibodies, and a Th2-type immune response. Th17 responses increase acute neutrophil inflammation and chronic fibrosis. Autoimmune diseases in bold represent the age when the autoimmune disease manifests clinically. Ratios represent the incidence of a particular autoimmune disease in females (F) compared to males (M). Blue shading depicts a Th1 response and pink shading a Th2 response and fibrosis. Incidence data were obtained from References 49 and 50.

classified as T helper (Th)1, Th2, or Th17 cells depending on the release of interferon (IFN)-γ, interleukin (IL)-4, or IL-17, respectively. IFN-γ and IL-17 are proinflammatory cytokines associated with inflammatory organ-specific autoimmune diseases such as myocarditis, in which IFN-γ has an important role in recruiting monocytes/macrophages and neutrophils and IL-17 in recruiting neutrophils and activating fibroblasts.[16–19] IL-17 is involved in both autoimmune and allergic diseases and consists of six family members including IL-17 (also called IL-17A), IL-17B, IL-17C, IL-17D, IL-17E (also called IL-25), and IL-17F.[19] IL-17 can act synergistically with tumor necrosis factor (TNF)-α and IL-1β or IFN-γ to increase fibrosis or Th1 responses, respectively.[17–19] IL-4, on the other hand, recruits B cells and eosinophils and activates B cells to produce autoantibodies associated with IC-mediated autoimmune diseases such as Graves' disease and systemic lupus erythematosus (SLE) (Figure 1).[8,10,11]

Regulatory T cells (Tregs) in peripheral tissues downregulate Th1, Th2, and Th17 responses and decrease acute inflammation in autoimmune diseases.[15,20,21] Tregs inhibit inflammation via several mechanisms including cell-to-cell contact-induced apoptosis and/or production of anti-inflammatory cytokines such as IL-10 and transforming growth factor (TGF)-β. Tregs have been shown to prevent the development of autoimmune diseases in animal models and to reduce ongoing disease.[22] Data are still emerging on the relationship between IL-4, IL-17, IFN-γ, and autoimmune diseases. So far IL-4 and IFN-γ have been found to inhibit IL-17 responses, but the precise role of IL-17 in the pathogenesis of many autoimmune diseases remains to be determined.

Generation of Th Responses by Pattern Recognition Receptors

The immune response to infection, adjuvants, physical injury, or self-tissues is principally identical. That is because the immune system recognizes the presence of infectious organisms and damage to tissues using pattern recognition receptors such as Toll-like receptors

(TLRs). Damage to tissues caused by physical or microbial agents releases extracellular matrix (self) proteins such as hyaluronan and fibronectin, which stimulate TLR4 on macrophages similar to bacterial or viral peptides.[23–26] Recognition of infectious or self-antigen(s) by TLR on antigen-presenting cells such as MCs, macrophages, or dendritic cells initiates a proinflammatory cascade involving TNF-α and IL-1β and the transcription factors MyD88 and nuclear factor (NF)-κB resulting in an acute inflammatory response. TLR signaling generates a Th1 response because of transcriptional induction of IFN-γ by MyD88, NF-κB, IL-12-induced STAT4, and/or caspase-1 activation of IL-18.[26–29] IL-17 is generated by activation of TLR/MyD88 and NOD-like receptors (NLRs) after *Mycobacterium* infection.[30,31] We have shown that TLR4 signaling after infection with coxsackievirus B3 (CVB3) induces a Th1 response in male BALB/c mice by an IL-18-induced mechanism rather than the classical IL-12/STAT4-induced IFN-γ pathway.[23,29,32] However, female BALB/c mice respond to CVB3 infection with an increased Th2 response (Figure 2) and increased numbers of $CD4^+Foxp3^+$ Tregs by up-regulating a receptor on MCs and macrophages called T-cell immunoglobulin mucin-3 (Tim-3).[32,33] Tim-3 reduces TLR4 expression in females during innate immunity, thereby inhibiting the proinflammatory response and increasing CTLA-4 expression in T cells and the development of a $CD4^+Tim\text{-}3^+CTLA4^+$ Treg population.[32,33] A similar anti-inflammatory role for Tim-3 has been found for other models of autoimmune disease including diabetes and experimental autoimmune encephalomyelitis (EAE), a murine model of multiple sclerosis (MS), in which Tim-3 was shown to induce apoptosis of Th1 cells via receptor-mediated mechanisms.[34,35] Thus, males and females respond to infection or adjuvant inoculation by increasing TLR/NLR expression, but the proinflammatory response is attenuated in females by inhibition of TLR4 expression by Tim-3 and increased Tregs.

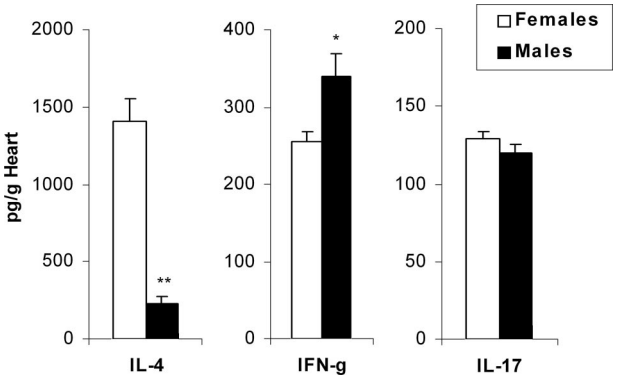

Figure 2. Males produce more IFN-γ (Th1 response) and females more IL-4 (Th2 response) during acute myocarditis. There is no significant difference in IL-17 (Th17 response) between males and females in three of four experiments, but IL-17 was significantly increased in males in one of four experiments. Female and male BALB/c mice were infected with CVB3 on day 0, and cytokine levels in the heart were assessed during acute myocarditis at days 10 or 12 after infection. Data show the SEM of 7 to 10 mice per group at day 12. Similar results were obtained in at least three separate experiments. $^*P < 0.05$, $^{**}P < 0.01$.

TLR expression on antigen-presenting cells is up-regulated (surface or intracellular) in response to infection with bacteria, viruses, or inoculation with adjuvants such as complete Freund's adjuvant (CFA) and/or pertussis toxin, which are used with self-antigens to induce autoimmune disease in animal models.[24,27] Although agents such as CVB3 and pertussis toxin lead to a predominant Th1 response because of TLR4 activation, autoimmune disease models using CFA generate a predominant Th17 response because of the *Mycobacterium* component of the adjuvant.[17] CFA is used to induce autoimmunity in several autoimmune disease models including EAE, collagen-induced arthritis (CIA) [a model of rheumatoid arthritis (RA)], and experimental autoimmune myocarditis (a model of acute myocarditis that progresses to dilated cardiomyopathy). TLR signaling not only induces Th1-directed immunity in response to infection but also provides a potent negative signal preventing the development of Th2 cells.[36] One exception is TLR2 signaling, which increases Th2 responses and IL-10 thereby inhibiting Th1 immune responses.[37,38] However, TLR2 engagement can increase IFN-γ production from already differentiated Th1 cells.[39] Although T cells gradually shift to a predominantly Th1 or Th2 response because of transcriptional silencing of IL-4 by T-bet/Runx3 or inhibition of IFN-γ transcription by site-specific methylation, respectively,[40,41] IFN-γ, IL-4, and IL-17 may all be present during adaptive responses (Figure 2).[29,32] Thus, all arms of the immune response (ie, the IL-4-driven B cell/antibody-mediated response and the IL-17/IFN-γ-driven cell-mediated response) are required for effective clearance of infection and repair of damaged tissues.[15]

Acute versus Chronic Pathology in Autoimmune Disease

Acute inflammation is a rapid response to infection or tissue injury that delivers leukocytes and plasma proteins to the site of injury.[42] From a pathological perspective, acute inflammation follows a sequence of events, an immediate edema produced by mediator products of resident MCs and macrophages, followed by an influx of neutrophils and monocytes/macrophages to the injured site throughout the next few days, followed by an adaptive T- and B-cell response in the first week or two. Acute inflammation can involve a predominantly Th1 (macrophage/neutrophil) and/or Th17 (neutrophil) response as occurs after viral or bacterial infections or injury, or a predominantly Th2 response (eosinophils) as occurs for asthma and allergy. Most acute inflammatory responses do not manifest clinically as autoimmune diseases and resolve once the infection has been cleared or the damaged tissue healed; that is, in most cases acute pathology heals without apparent permanent damage to tissues. This explains why, for many autoimmune diseases, the early acute phase is often silent—there are no clinical signs or symptoms of disease unless inflammation persists.[24] Several mechanisms are responsible for resolving acute inflammation including Tregs, anti-inflammatory cytokines such as IL-4, IL-10, or TGF-β, and apoptosis of

inflammatory cells.[42] If acute inflammation cannot be resolved or tissues are incapable of regeneration, then the acute response may progress to a chronic inflammatory state characterized by a mononuclear infiltrate (macrophages, lymphocytes, and plasma cells), tissue destruction/necrosis, and fibrosis depending on the nature of the tissue or organ involved. Fibrosis is the hallmark of chronic pathology. Fibroblast proliferation and collagen deposition have been shown to be increased by TNF, IL-1β, IL-4, IL-13, IL-17, and TGF-β1 in most organs examined.[18,19,43,44] For autoimmune diseases, chronic pathology is characterized by fibrosis, increased numbers of autoantibodies, and features of a Th2- or Th17-type immune response (Figure 1). In males, it is possible that a gradual shift from a Th1 to Th2 response with age increases the progression to fibrosis in susceptible individuals or that a proinflammatory Th17 response leads to chronic fibrosis (Figure 1). Additionally, a heightened TNF/IL-1, TLR-driven innate immune response in susceptible individuals or mice could increase the risk of developing chronic autoimmune disease because of the proinflammatory and profibrotic nature of TNF and IL-1β, regardless of sex.[24,43,45] A heightened TNF/IL-1 proinflammatory response is characteristic of most animal models of autoimmune disease induced by CFA and self-peptides, such as EAE, experimental autoimmune myocarditis, and CIA.[10,24,45,46]

Autoimmune diseases that are more prevalent in males, such as myocarditis and ankylosing spondylitis, usually manifest clinically (ie, show signs and symptoms of clinical disease) early in life and are characterized by acute inflammation (ie, macrophages, neutrophils, and T cells) (Figure 1, see bold), the appearance of autoantibodies, and a proinflammatory immune response (Figure 2).[47–50] One exception is idiopathic pulmonary fibrosis, which manifests later in life with a higher incidence in males (Figure 1). In idiopathic pulmonary fibrosis, the early acute phase of disease does not manifest clinically, but signs and symptoms of disease appear once lung fibrosis is established. Female-predominant autoimmune diseases that manifest clinically during the early, acute phase include autoimmune thrombocytopenia purpura, myasthenia gravis, Graves' disease, and SLE (Figure 1, see bold). Interestingly, these are autoimmune diseases in which a clear antibody-mediated pathology has been elucidated.[49,50] Autoantibodies to platelets induce thrombosis in autoimmune thrombocytopenia purpura, autoantibodies to the acetylcholine receptor block transmission at the neuromuscular junction resulting in myasthenia gravis, autoantibodies and ICs mediate tissue injury in SLE, and autoantibodies to the thyrotropin receptor stimulate thyroid cells resulting in Graves' disease. Generation of an antigen-specific autoantibody, such as an antibody that can bind the acetylcholine receptor, is all that is necessary to trigger the pathology so that these diseases manifest clinically early in life once autoantibody is produced in sufficient quantities. In contrast, autoimmune diseases that manifest clinically later in life in females are associated with chronic pathology, fibrosis, and increased numbers of autoantibodies (Figure 1). Thus, male-predominant autoimmune diseases that manifest during the early, proinflammatory phase are associated with acute inflammation, whereas female-predominant diseases that manifest during the early, acute phase are associated with primarily antibody-mediated pathology (ie, autoimmune thrombocytopenia purpura, myasthenia gravis, SLE, and Graves' disease) (Figure 1). This pattern is consistent with studies examining the acute immune response to infection or trauma of males (Th1, inflammatory) and females (Th2, antibody).[6,8,51]

On the other hand, autoimmune diseases with an increased incidence in females that manifest clinically later in life (ie, past age 50) are characterized by chronic inflammation, fibrosis, increased numbers of autoantibodies, and a Th2-type immune response (Figure 1, see bold).[49,50,52] And so why are autoimmune diseases more prevalent in females? Two factors may work together to increase the prevalence of disease in females. First, the Th2-type immune response to infection or trauma in females accentuates both acute and chronic antibody-mediated pathology (Figure 1). Second, males die at an earlier age from heart disease (including atherosclerosis and myocarditis), diabetes, and cancer, diseases with a higher prevalence in males.[53] An IL-4-mediated Th2 response protects females from severe acute inflammation by transcriptional inhibition of IFN-γ production and by increasing anti-inflammatory Tim-3 and Treg cell populations (ie, $CD4^+Tim\text{-}3^+CTLA4^+$ and classical $CD4^+Foxp3^+$ Treg).[29,32,33,54] Thus, the heightened proinflammatory Th1 response to infection made by males increases the severity of acute inflammation and the risk of early death so that males susceptible to develop chronic autoimmune diseases might not survive to develop disease. This distinction between acute and chronic pathology in autoimmune diseases has been primarily overlooked but greatly impacts our understanding of the pathogenesis of disease and provides a framework for understanding differences in the prevalence of autoimmune diseases between men and women.

Regulation of Inflammation by Sex Hormones

Sex hormones, such as estrogen, testosterone, and progesterone, are believed to mediate many of the sex-based differences in the immune response and to account for sex differences in the prevalence of autoimmune diseases.[8,10,52,55–57] Estrogens and androgens directly influence the immune response by interacting with hormone receptors on immune cells.[14] Likewise, cytokine receptors (eg, IL-1R, IL-18R) are found on hormone-producing tissues, indicating bi-directional regulation of the immune response. Another factor that must be taken into consideration when examining sex differences is the effect of sex hormones on target organs or tissues (Figure 1). For example, estrogen receptors and/or androgen receptors in the heart are found not only on infiltrating immune cells but also on/in cardiac muscle, smooth muscle, and endothelial cells.[10,58]

The precise interaction between hormones and the innate immune response after infection is just beginning to be understood. Although most autoimmune diseases

exhibit a strong female bias,[55–57] studies of estrogen's effects on immune function have been contradictory. It is clear that estrogen stimulates antibody (and autoantibody) production by B cells. Estrogen has also been shown to increase IL-4, IL-10, and TGF-β levels and to increase CD80 and Foxp3 expression, which increase $CD4^+Tim\text{-}3^+CTLA4^+$ and classical $CD4^+CD25^+Foxp3^+$ Treg populations.[10,32,33,51,59,60] Although estrogen at high periovulatory to pregnancy levels inhibits human and mouse T cells, it has the opposite effect at low doses.[10] Furthermore, estrogen inhibits TNF-α production from human peripheral blood mononuclear cells obtained from men or women only if it is administered with lipopolysaccharide, a ligand for TLR4, but has the opposite effect if estrogen is administered without lipopolysaccharide.[10] Similar results have been obtained in mice immunized with MOG/CFA in EAE or in murine cells treated with lipopolysaccharide.[46,60] Even though numerous studies have shown that estrogen can stimulate T-cell proliferation,[10] in most cases the Treg component of the CD3 or CD4 cell compartment was not examined. We and others have found that female mice develop increased numbers of $CD4^+Foxp3^+$ Treg populations after infection or adjuvant treatment and that the induction of this protective response is dependent on TLR signaling that occurs during innate immunity.[32,33,61,62] These results agree with studies showing that estrogen, via estrogen receptor-α, directly down-regulates NF-κB and Th1 responses in various human and murine cell types.[63–66] Estrogen is particularly potent at inhibiting lipopolysaccharide/TLR4-induced proinflammatory pathways in human cells[64,67] and is effective at inhibiting Th1 responses in both male and female mice.[68,69] How can we reconcile these seemingly contradictory findings? One answer may lie in the finding that estrogen stimulates IFN-γ production from T cells but inhibits IFN-γ from macrophages and dendritic cells.[10,59] Thus, estrogen's inhibitory role would be particularly important during innate immunity when autoimmune diseases are initiated, as occurs for EAE.[46] In addition, estrogen has been found to increase fibrosis because of its ability to stimulate IL-4, TGF-β, and fibroblast growth factor.[10,70] Overall, these studies suggest that at high doses and/or during innate immune responses to infection or adjuvant estrogen generates an anti-inflammatory, profibrotic Th2 response.

Far less research has been conducted on the role of androgens on immunity. Several studies have found that androgens stimulate a Th1 response in humans or rodents.[6,29,32,51,56,71–74] In a rat heart ischemia model, testosterone has been shown to decrease cardiac function after acute injury by increasing TNF-α, IL-1β, IL-6, and caspase-1.[75] However, other animal studies have found that androgens reduce autoimmune disease.[76] One of the difficulties in studying androgens is the fact that testosterone activates both androgen receptors and estrogen receptors by aromatase conversion of testosterone to estrogen.[77] Thus, effects measured by testosterone treatment may be attributable to either sex hormone.[78] In both sexes testosterone levels decline with age, which may contribute to the increasing Th2 response observed past 50 years of age in men (Figure 1). Although some studies suggest that androgens increase Th1 responses, more research is needed to establish their affect on the immune response.

Sex Differences in Autoimmune Disease

Several factors could account for discrepancies in the role of sex hormones observed between human studies and animal models of autoimmune diseases. Most animal studies do not distinguish acute from chronic phases of disease or acute versus chronic pathology. This results in confusion regarding the role of Th1, Th2, and Th17 responses and the effects of hormones on disease pathogenesis. Because acute and chronic phases of disease are regulated differently in males and females, distinguishing these two phases in animal models and patients could lead to more effective treatments. Additionally, adjuvants such as CFA/pertussis toxin favor a Th1/Th17 response regardless of sex. Thus, many autoimmune disease models such as EAE and CIA examine the effect of estrogen on acute cell-mediated disease (Figure 1). Yet, chronic pathology in autoimmune disease models is disproportionally less studied and very little is known about the effects of sex hormones on chronic pathology. Importantly, TLR-driven immune responses have been shown to overcome natural tolerance,[79,80] which could account for the Th1-type immune responses observed in females in various autoimmune disease models. The finding that estrogen treatment decreases Th1 responses in both males and females in adjuvant-induced models further supports this idea.[68,69] Additionally, humans are exposed to numerous infections/toxins throughout their lifetime, which is not adequately modeled in animal studies. The timing of infections and changes in hormone status in patients could alter the disease outcome. And finally, some differences are likely to be attributable to altered regulation of the immune response in genetically diverse mouse strains.[81] For example, female nonobese diabetic (NOD) mice spontaneously develop a Th1 response during the progression to diabetes, but the relevance of this model to human disease has recently been questioned.[82]

SLE: Acute Antibody-Mediated Disease

A clear connection exists between high estrogen levels and increased numbers of autoreactive B cells in SLE (Figure 1). Pregnancy increases disease in patients, and high-dose estrogen treatment of mice in lupus-prone models accelerates IC-mediated kidney damage.[10,83] Estrogen has also been shown to increase autoreactive B-cell survival, autoantibodies, and kidney disease in BALB/c mice.[84–86] In addition, male patients with SLE have higher estrogen to androgen ratios and lower levels of testosterone in their sera.[87] In lupus-susceptible C57BL/6 mice, males have lower levels of autoantibodies than female mice.[88] Thus, SLE is a disease in which antibody almost exclusively mediates pathology, and estrogen has a virtually undisputed role in increasing autoantibody-mediated pathology. However, studies in pa-

tients with SLE have found elevated Th1 to Th2 ratio and IL-18 to IL-4 levels in plasma correlate positively with disease activity,[89,90] suggesting a role for Th1 responses. One possible explanation is that when ICs bind tissues they stimulate TLRs (ie, TLR9) via the autoantigen (ie, DNA) component of the IC generating a proinflammatory Th1 response.[91] Furthermore, not all clinical studies of SLE patients agree on the role of Th1 or Th2 profiles. One study found that SLE patients had significantly fewer IFN-γ-secreting cells and increased levels of serum estrogen and progesterone.[92] In another study, TIM-1 expression on peripheral blood mononuclear cells from SLE patients (associated with Th2 responses) correlated significantly with disease activity whereas TIM-3 expression (associated with Th1 responses) did not.[93]

Rheumatoid Arthritis: Acute Mixed Cell and Antibody-Mediated Disease

The pathology of RA includes a mixture of cell-mediated and antibody-mediated damage.[51] Clinically, RA is more prevalent in women before age 50 but disease severity is greater in women after 50 years of age, suggesting a Th2, antibody-mediated pathology (Figure 1).[10,49] However, estrogen has been shown to protect against CIA in DBA/1LacJ mice and Lewis rats.[94,95] What could account for this discrepancy? One possibility is that the acute inflammation including macrophages and neutrophils that is induced by CFA/collagen in the CIA model is inhibited by estrogen, as discussed in the previous section. In support of this idea, male mice are more susceptible to CIA than females.[96] Thus, the Th1/Th17 response induced by CFA in CIA models may be responsible for differences between animal and human studies. Interest in the role for IL-17 found in CIA has led to recent clinical studies examining the role of IL-17 and IL-23 (a cytokine that supports Th17 responses) in the joints of RA patients. Although the p19 component of IL-23 has been detected in patients with RA, a recent study found that the p40 subunit of IL-23 was not expressed.[97] In another study, Th1 cells were more abundant than Th17 cells in the joint.[98] In support of a role for estrogen in clinical RA, one study found that free estrogen levels in the synovial fluid of men with RA were increased two-fold compared to controls, similar to estrogen levels in women with RA.[99] In addition, incidence rates of RA in men increase with age as androgen levels decrease and Th2 responses increase.[100] Overall, these findings suggest that estrogen increases RA-mediated pathology. Although women are protected from RA during pregnancy,[49] pregnancies usually occur when women are <50 years old. Thus, the high estrogen levels present during pregnancy may reduce acute cell-mediated pathology (ie, inhibit macrophages and T cells) helping to alleviate symptoms.

Diabetes: Acute Cell-Mediated Disease

Although female NOD mice are more likely to develop diabetes than males, autoimmune diabetes in humans occurs slightly more often in males.[52] More recently, the question of whether NOD mice represent an accurate pathological picture of type I diabetes has arisen.[82] The disease phenotype in humans and mice is different. In female NOD mice the lymphocytic infiltrate is extensive, whereas in human insulitis few leukocytes are detectable in the islets. Furthermore, infections such as CVB3 prevent disease in NOD mice but are thought to be primary triggering events for diabetes, pancreatitis, and other autoimmune diseases in humans.[24,101,102] More appropriate animal models for diabetes are urgently needed. Because neutralizing antibody is known to be critical for reducing CVB3 infection in females,[103] males may develop worse viral-induced pancreatic disease.

Systemic Sclerosis: Chronic Fibrotic Disease

Systemic sclerosis is regarded as the prototypic fibrotic disease (Figure 1). Although a relatively uncommon disease, it has the highest case-specific mortality of any of the autoimmune rheumatic diseases because of organ-based vascular and fibrotic complications, highlighting that most of the mortality because of chronic inflammatory conditions is attributable to fibrosis.[44] Pathological characteristics of human disease and animal models include overexpression of the profibrotic cytokine TGF-β, autoreactivity against extracellular matrix proteins, such as collagen, and increased numbers of MCs, eosinophils, and basophils—features associated with chronic pathology. Some of the clinical and immunological aspects of the disease resemble dermatomyositis and RA, diseases that are severe later in life, have an increased prevalence in females, and are associated with a Th2 response (Figure 1).

MS: Acute Mixed Cell and Antibody-Mediated Disease

MS is typically thought of as a Th1/Th17-mediated disease because its animal model, EAE, has a Th1/Th17 phenotype.[104] Most patients develop MS when they are <50 years old suggesting a cell-mediated pathology (Figure 1). Estrogen treatment decreases EAE and TNF-α levels if administered before disease starts in murine models, but it has no significant affect once disease has begun.[46,105] Estrogen has been found to increase Treg numbers in EAE in C57BL/6 mice, which is associated with its suppressive ability.[106] The possibility that EAE has a Th1-mediated acute pathology and a Th2-mediated chronic pathology is supported by the observation that male SJL mice only develop acute EAE whereas female SJL mice develop chronic disease.[96] Additional evidence that MS is a Th1-mediated disease comes from studies in patients in which clinically defined relapsing-remitting MS was exacerbated when patients were treated with IFN-γ.[107] Furthermore, men develop more severe inflammation than women with MS, whereas pregnancy in humans and mice decreases disease.[108]

If MS is an acute Th1-mediated disease, why is there an increased prevalence in females (Figure 1)? One possible reason is the existence of several subgroups of patients with different pathogeneses.[104] Although some patients display a more Th1-mediated disease (fulminate acute disease) involving macrophage-mediated demyelination (similar to EAE), a subset of patients develop antibody-mediated demyelination associated with a Th2-type response.[104] Even in a subset of patients with Th1-mediated pathology, there can be an abundance of granulocytes and eosinophils indicative of a mixed Th1/Th2 response.[104] This could explain both the early appearance of disease (<50 years) and the increased incidence in women.

Hashimoto's Thyroiditis: Antibody-Mediated and Fibrotic Disease

In Hashimoto's thyroiditis there is an extensive infiltration of the thyroid gland with lymphocytes, plasma cells (antibody-producing B cells), and macrophages as well as germinal center formation.[50] Thyroid follicles are progressively destroyed by IC deposition and complement attack resulting in necrosis, fibrosis, and hypothyroidism. The acute and chronic phase of Hashimoto's occurs predominantly in women, and female animals also develop more severe experimental autoimmune thyroiditis.[50] Increased disease in experimental autoimmune thyroiditis is dependent on sex hormones, with estrogen increasing and testosterone decreasing severity in mice.[109] Although disease in experimental autoimmune thyroiditis is Th1-mediated, this may be attributable to the effect of using CFA as an adjuvant in the animal model, as discussed earlier, because only modest evidence for the role of cell-mediated injury has been shown for patients.[50]

Myocarditis/Dilated Cardiomyopathy: Acute Cell-Mediated to Chronic Fibrotic Disease

Myocarditis and atherosclerosis are more prevalent in men.[110] Women respond to infection or trauma with less inflammation in the heart compared to men.[5,9] Likewise, animal studies have consistently shown that females are protected from acute myocardial injury during ischemia, burn, and sepsis.[9] In CVB3-induced myocarditis, male mice develop significantly increased acute inflammation compared to females, yet there is no difference in viral replication in the heart.[29,32] It is interesting to note that two outbreaks of CVB3 infection in humans have demonstrated no sex difference in the rate of infection,[111,112] even though a clear increase in incidence and mortality of heart disease occurs in men,[110,113] indicating that CVB3 increases heart disease by acting as an adjuvant.[24]

In experimental autoimmune myocarditis, a Th17 response has been shown to be necessary for the development of disease in mice.[114,115] Again, the use of CFA as an adjuvant may be responsible for this profile. In CVB3-induced myocarditis we observe Th17 cells in the heart during acute myocarditis, but IL-17 levels are generally not increased in male mice (Figure 2) indicating that a Th17 response does not account for sex differences in acute inflammation. However, IL-17 was found to be important in amplifying the chronic, fibrotic stage of experimental autoimmune myocarditis in IFN-γR-deficient mice,[115] similar to the increased fibrosis observed in IFN-γ-deficient mice in CVB3-induced myocarditis.[43] Thus, IL-17 may increase the $CD11b^+$ neutrophil infiltrate during acute myocarditis and contribute to chronic pathology by increasing fibrosis leading to dilated cardiomyopathy. In CVB3-induced myocarditis, TLR4 signaling increases proinflammatory cytokines and acute inflammation in both sexes (Figure 3).[29,32] Similarly, CVB3 infection increases Tim-3 signaling and numbers of $CD4^+Foxp3^+$ and $CD4^+Tim\text{-}3^+CTLA4^+$ Tregs, which decrease Th1 inflammation in both sexes.[32,33,54] However, an increased Th2 response in females reduces the acute inflammatory response compared to males (Figure 2). Activation of the immune response by TLR and regulation by Tim-3 and Treg occur not only in heart disease but also in other autoimmune diseases. For example, Tim-3 reduces inflammation in EAE and diabetes animal models,[35] whereas TLR-mediated signaling increases inflammation.[34,36] Tim-3 expression may be associated with Th2 responses in females because of its location in the IL-4 gene complex (estrogen increases IL-4 in females) (Figure 3).[8,32,35] Testosterone is known to increase MC and macrophage numbers and may also increase TLR4 levels on antigen-presenting cells (Figure 3).[32,116] Estrogen receptor signaling not only decreases Th1 responses but also reduces MC and macrophage numbers (Figure 3).[8,32,116,117] Thus, sex hormones alter expression of pro- and anti-inflammatory signaling pathways that determine the severity of acute inflammation. However, the effect of sex hormones on chronic pathology is primarily unknown. Myocarditis progresses from

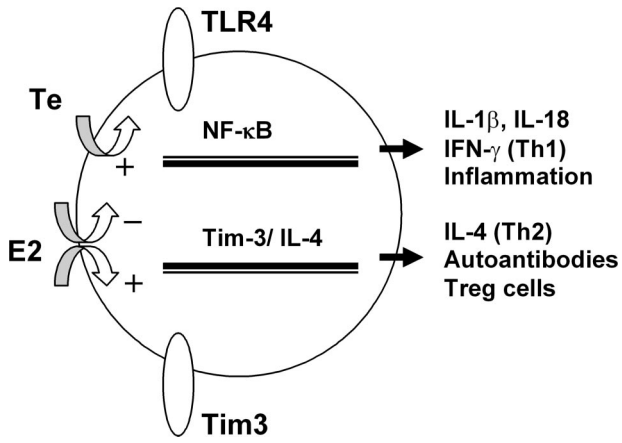

Figure 3. Role of sex hormones in regulating inflammation after infection. Testosterone (Te) increases MC and macrophage numbers, TLR4 expression, and NF-κB signaling in males resulting in increased levels of IL-1β, IL-18, and IFN-γ, increased inflammation, and a Th1-type immune response. Estrogen (E2) inhibits NF-κB signaling, reduces Th1 responses, and increases IL-4 transcription resulting in increased Th2 response, B-cell proliferation, autoantibody production, and Tim-3 expression in females. Tim-3 signaling increases Treg cell populations that dampen the TLR4-induced Th1 inflammatory response to infection.

an acute Th1 response to chronic Th2-mediated fibrosis and dilated cardiomyopathy (Figure 1).[43,48] Thus, the increased prevalence of autoimmune diseases in women may be attributable to an increased Th2 response after infection that promotes autoantibody production, chronic inflammation, and fibrosis, possibly explaining why women given estrogen replacement therapy after menopause develop worse heart disease.[118]

Conclusions

Understanding the mechanisms behind the increased incidence of autoimmune diseases in women has remained elusive. Our recent findings that cross talk between TLR4 and Tim-3 signaling determines the severity of inflammation between sexes in heart disease has led us to examine other autoimmune diseases for Th1- versus Th2-mediated pathology. Based on our understanding of the pathogenesis of disease, we have examined autoimmune diseases according to age and sex and found that the incidence of autoimmune diseases falls into a male/female pattern based on pathology. Male-predominant autoimmune diseases usually manifest clinically (ie, show signs and symptoms of clinical disease) before age 50 and are characterized by acute inflammation and a Th1-type response, whereas autoimmune diseases with an increased incidence in females that occur early in life have a clear antibody-mediated pathology. Autoimmune diseases with an increased incidence in females appear clinically later in life when chronic pathology, fibrosis, and increased numbers of autoantibodies are present. This distinction between acute and chronic pathology in autoimmune diseases, which has been primarily overlooked in both animal models and the clinical setting, greatly impacts our understanding of the pathogenesis of disease and provides a framework for understanding differences in the prevalence of autoimmune diseases between men and women. Because acute and chronic phases of disease are regulated differently in males and females, distinguishing these two pathological phases in animal models and patients could lead to more effective treatments.

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