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Is autoimmunity a matter of sex?

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ABSTRACT

Autoimmune diseases include several conditions that cumulatively are estimated to affect over 5% of the US population with a striking female predominance reported for most of them. The cause and mechanisms of this sex bias remains unknown despite multiple proposed hypotheses. Indeed, it is well established in several experimental settings that the human immune system exhibits sexual dimorphism with basic immune responses differing between females and males. Among candidate factors to explain these differences we note that particular attention has been primarily devoted to sex hormones, yet data have been inconclusive or have not been confirmed. The same seems to apply to the hypothesis of fetal microchimerism. Most recently, sex chromosome abnormalities and skewed X chromosome inactivation have been suggested as novel players, particularly in later-onset diseases. We review herein the most recent data on the mechanisms proposed for the female predominance. We also attempt to determine whether observed sex ratios are in fact the result of sex-biased awareness in case-finding studies.

Contents

1. Introduction

Autoimmune diseases (AID) include more that 70 different disorders affecting over 10 million patients in the United States. These disorders manifest a wide variability in terms of targeted tissues, age of onset, and response to immunosuppressive treatments. The one feature that is shared by the majority of these conditions, however, is the predominance in the female sex with over 80% of patients with AID being women [1,2].

Even though the female predisposition to AID has been known for over a century, the precise cause of this bias remains unknown and relatively few hypotheses have been proposed. A susceptible genetic background is considered to be necessary,

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yet not sufficient, to explain both AID onset and the female predominance, while several environmental factors have been suggested as additional players in tolerance breakdown.

Among sex-related factors that have been proposed, we note that (i) hormones and reproductive history; (ii) fetal microchimerism; (iii) X chromosome inactivation; and (iv) X chromosome abnormalities have collected the most data. However, none of these hypotheses has thus far gathered enough convincing evidence and in most cases data are conflicting. The effects of sex hormones on the immune function was first based on the reported role of estrogens in lymphocyte maturation, activation, and synthesis of antibodies and cytokines [3–5]. Moreover, studies on the immune responses in normal subjects and AID reported that estrogen receptor (ER) ligands are capable to modulate both the innate and adaptive immunity components, alter antigen presenting cell (APC) numbers or functions in vivo and in vitro, and regulate dendritic cell (DC) differentiation [6]. When these observations were used to compare sex hormonal profiles between patients with AID and controls, data were disappointing and weak. Furthermore, the occurrence of AID in men, the onset at different times of the reproductive history, and the lack of consistent effects on disease course also militate against the role of sex hormones. Sex chromosomes have then been widely studied in the past decade, yet the role of fetal microchimerism and X chromosome inactivation awaits confirmation as a severely skewed X chromosome inactivation pattern was found in women with systemic sclerosis [7]. We then demonstrated that women with PBC, systemic sclerosis, and thyroid autoimmune disorders manifest an enhanced rate of X monosomic cells in peripheral blood compared with healthy age-matched women [8,9].

This review will illustrate the available evidence and proposed theories on sex differences in AID and discuss their potential implications and limitations. Table 1 illustrates the major mechanisms proposed to influence female predominance in AID.

2. Sex differences and the immune system

The human immune system manifests some degree of sexual dimorphism with basic immune responses differing between females and males. In general terms, women have an enhanced antibody production and increased cell-mediated responses following immunization [10] while men produce a more intense inflammatory response to infectious organisms [11]. Further, women have higher CD4+ T cell counts than men which contributes to an increased CD4/CD8 ratio [12], higher levels of plasma IgM [13], and greater Th1 cytokine production. The significance of these changes remain poorly defined since, with the obvious exception of AID, there does not appear to be significant differences in susceptibility to infection or inflammation degrees between sexes.

3. Sex ratios in autoimmunity

The most striking sex differences in autoimmune diseases are observed in Sjogren's syndrome, SLE, PBC, autoimmune thyroid disease and scleroderma in which 80% of the patients are women. On the other hand, rheumatoid arthritis (RA), multiple sclerosis (MS) and myasthenia gravis have a lower female prevalence but still 60–75% of the patients are women. A third group, which includes inflammatory bowel diseases and immune-mediated (type 1) diabetes, is characterized by a female:male ratio that is approaching 1:1 with a slight predominance of the male sex [14]. Finally, putatively autoimmune disorders such as primary sclerosing cholangitis (PSC) are characterized by male predominance [1].

4. Sex hormones

Estrogens, androgens, and prolactin, have been the first proposed candidates to have important roles in the sex bias observed in autoimmunity, due to their capacity of modulating the immune response via androgen and estrogen receptors

Table 1
Proposed mechanisms influencing female predominance in autoimmune diseases

Autoimmune disease	Female:male ratio	Sex hormones	X chromosome	Ref
Systemic lupus erythematosus	9:1	Estrogen allows survival of autoreactive B cells and skews their maturation toward marginal zone phenotype Prolactin allows survival of autoreactive B cells and skews their maturation toward marginal zone phenotype, leads to the production of IFN-γ	Triplication of PAR1 region Duplication of TRL7	[5,21,37,38]
Primary biliary cirrhosis	10:1	Use of hormone replacement therapies is significantly associated with increased risk of PBC	Increased frequency of X chromosome monosomy in peripheral T and B lymphocytes	[8,23]
Autoimmune thyroid diseases	8:1	Hyperprolactinemia (?)	Increased frequency of X chromosome monosomy in peripheral T and B lymphocytes and skewed XCI	[9,21,34]
Systemic sclerosis	5:1	Estrogens induce fibroblast dysfunction Hyperprolactinemia (?)	Increased frequency of X chromosome monosomy in peripheral T and B lymphocytes and skewed XCI	[7,9,21]
Rheumatoid arthritis	4:1	Estrogen activates synovial cell proliferation, including macrophages and fibroblasts. TNFα blockers affect estrogen synovial levels		[21,39]
Multiple sclerosis	3:1	Estrogen determines disease improvement; progesterone seems to have an effect on myelinating and remyelinating the nervous system.		[21]
Myasthenia gravis	2:1	Estrogen promotes AChR-specific Th1 cell expansion		[40]

(ER). Indeed, progenitors and mature cells express both receptors and suggest that sex hormones can directly modulate the development of immune cells. Sex hormones may also directly influence the homing of lymphocytes to a target organ and the process of antigen presentation [6], thus influencing the organ specificity of AID as well as the breakdown of tolerance. In further details, IFN-γ, IL-1 and IL-10 production is enhanced in vitro by estrogens whilst IL-4 and IL-5 decrease in the presence of androgens [15]. These differences may determine distinct immune environments in males and females with women more likely to develop a Th1 response, except during pregnancy when Th2 response is predominant.

The modulatory effect of estrogens is different in normal conditions and during AID with a biphasic effect. Indeed, lower levels facilitate the immune response while higher levels suppress it. It has been demonstrated, for example, that IFN-γ secretion is generally stimulated by estrogens but the peak enhancement at lower estrogen concentrations is higher [16]. The effect of estrogens on the secretion of TNF-αβ is also biphasic, with enhancement occurring at low and inhibition at high concentrations [15]. These data indicate that estrogen is capable of modulating both pro- and anti-inflammatory activities of CD4+ T cells and thus has the potential to influence the outcome of CD4+ T cell-mediated immune responsiveness. Sex hormones also modulate the hypothalamic–pituitary–adrenal (HPA) axis and are thus capable to modulate the stress response [17]. Indeed, women have higher corticosterone–cortisol concentrations compared to men and glucocorticoids suppress the production of sex hormones and the action of these hormones in tissues [17]. These mechanisms are central to the regulation of the balance of Th1/Th2 cytokines within sites of inflammation, and to the appropriate or inappropriate termination of the inflammatory response in infections, tolerance development, or AID [18].

When specific AID were studied, increased plasma levels of estrogens were reported in patients with SLE while the high incidence of the disease during the female reproductive years and the presence of frequent flares during pregnancy also supported a role for sex hormones. Since SLE is characterized by an enhanced Th2 response, the above mentioned observation that estrogens stimulate IL-4, IL-5, IL-6 and IL-10 secretion by Th2 lymphocyte is of obvious interest. These cytokines are also potent stimulators of B lymphocyte maturation and promote the survival and activation of high affinity autoreactive B cells. Interestingly, estrogens and prolactin *per se* share the potential to break tolerance and lead to the appearance of DNA-reactive B cells [19]. The administration of estradiol to SLE animal models increases total IgG production, induces specific anti-DNA antibodies secretion, and accelerates the onset of immune complex glomerulonephritis. In the same scenario, ovariecomized females have better survival rates [20]. Similar to estrogen levels, hyperprolactimemia has been observed in 25% of patients with SLE [21]. It is already well accepted a correlation between apoptosis and AID possibly through the ineffective removal of apoptotic cells. Both estrogens and prolactin may regulate immune cell survival through the Fas/FasL system which is influenced in terms of FasL expression in monocytes [22], yet whether these two hormones play a direct role remains to be determined.

Several studies have attempted to determine the impact of sex hormones on PBC. These have included epidemiological studies in which a negative association with parity was first denied and ultimately confirmed [23]. Of interest, taking hormonal replacement therapies following menopause was found in this latter study to be significantly associated with PBC [23]. Furthermore, the differences in plasma estrogen levels between women with PBC and controls observed in earlier studies may be secondary to long-standing cholestasis or may account for the wide variability of their measurements during the reproductive cycle.

5. Fetal microchimerism in autoimmunity

It has been hypothesized that AID pathogenesis and female predominance may be secondary to the presence in affected women of allogenic male fetal cells decades after pregnancy (i.e. fetal microchimerism). Microchimeric cells were first found in peripheral blood mononuclear cells from patients with scleroderma and it was suggested that nonautologous cells may be mediating a graft-versus host disease-like reaction in these patients [24,25]. Other studies have failed to recapitulate these findings [26]. Several studies found no significant difference in frequency of male microchimerism in female PBC and controls [27] Cumulatively, available data on the role of fetal microchimerism in AID are still controversial and studies have also shown that naturally acquired fetal and maternal microchimerism are common in healthy individuals.

6. The genetics of sex chromosomes

The importance of genetic factors as possibly the major determinants in AID susceptibility is supported by familiar clustering , variable prevalence in different ethnic groups, associations with HLA haplotypes or single nucleotide polymorphisms, and concordance rates in monozygotic and dizygotic twins [14]. Quite surprisingly, however, few studies have investigated the genetics of sex chromosomes in AID despite major clues such as the observation that several genes crucial for the maintenance of immune function and tolerance map on the X chromosome. In fact, specific mutations of X chromosome genes cause immunodeficiency syndromes characterized by different degrees of severity [28]. Foxp3, which gene localizes in the short arm of the X chromosome, is essential for regulatory T cells and its deficiency or mutation lead to an early onset, highly aggressive, and often fatal multiorgan autoimmune disease [29]. Further, constitutive X monosomy or major structural abnormalities of the X chromosome as observed in Turner's syndrome [30] manifest common autoimmune features and in some cases chronic cholestasis [31].

The X chromosome inheritance displays a peculiar pattern compared to autosomal chromosomes, since women are functional mosaics for X-linked genes. In female, most genes on one X chromosome are silenced as a result of X chromosome inactivation (XCI), although more recent data have mined this dogma by demonstrating that several genes can escape XCI in physiologic conditions. The result of this process is to achieve equivalent levels of X-linked gene products between males and females [32]. As mentioned

above, however, at least 15% of X-linked genes are capable of escaping XCI in healthy women and are expressed from both X chromosomes. Among these, up to 10% of total X-linked genes manifest variable XCI patterns in different individuals [32].

As a result of these observations, a role for the X chromosome in AID was first proposed based on the fact that women with AID have a significantly higher frequency of peripheral blood cells with a single X chromosome (i.e. X monosomy) compared to healthy women. Importantly, this was observed in diseases with different organ specificities such as scleroderma and autoimmune thyroid disease [9] or PBC [8]. The later finding that the lost X chromosome is preferentially one parentally inherited [33] also supports the critical involvement of X chromosome gene products in the female predisposition to AID. Other authors have suggested that women affected with specific female-preponderant AID manifest a skewed XCI pattern in their peripheral white blood cells, as supported by data in scleroderma and autoimmune thyroid diseases [7,34] while in PBC we failed to demonstrate such preferential inactivation [33]. It should be noted, however, that only the latter study accounted for more than one locus while previous reports only investigated the androgen receptor methylation which, as explained previously, poorly represents XCI.

7. Are we overlooking autoimmunity in men?

Is it possible that men are being diagnosed less with AID for other reasons than true differences in prevalence rates with sex? When considering AID with subtle onset and progression, this issue is obviously difficult to address but should not be overlooked. Indeed, we cannot currently rule out the possibility that physician awareness plays a role in determining the probability of a possible AID diagnosis. To support this hypothesis, we should consider the opposite example of male-predominant coronary heart disease in which a video-simulated test was performed. This study demonstrated that women and men evaluated by their general practitioner for typical symptoms are subjects to significantly different care [35]. In fact, women are asked less questions and are ordered less diagnostic tests which result in less frequent specialist referral, correct diagnosis, and appropriate treatment.

We should also note that few AID have non invasive diagnostic markers that allow the identification of all cases in population-based studies. Accordingly, most epidemiological studies are based on case-finding methods and account only for already diagnosed cases. Is it possible that this mechanism is more likely to identify female cases? The case of PBC is quite paradigmatic since no population-based study is available (also due to the lack of sensitivity for serum autoantibodies). As illustrated in Fig. 1, sex ratios differ significantly between case-finding studies are considered (upper panel) and screening studies on large numbers of sera (lower panel), although numbers in this latter case are significantly smaller. This possible flaw is also supported by the data from family studies in which the discrepancy between male and female first degree relatives is significantly of lower magnitude compared to the proposed figures in the clinical series [36].

8. Conclusions and future directions

The pathogenesis of all AID recognizes the necessary role of environmental factors and genetical susceptibility to lead to tolerance breakdown and females seem to be more prone to AID development based on both components. We have reviewed herein the proposed mechanisms but it appears clear that none of these has reached a definite consensus and some issues need to be addressed. First, all aspects have witnessed crucial developments, as discussed for the case of X chromosome silencing or well represented by the rise of new players such as microRNA or histone acetylation, and these cannot be overlooked. Second, we are convinced that the study of female autoimmunity cannot be complete without a careful evaluation of male cases which should be collected through a multicenter effort to achieve a sufficient number. Third, the role of sex hormones, particularly estrogens, should be re-evaluated with more modern research tools. Ultimately, the study of sex differences in autoimmunity will help

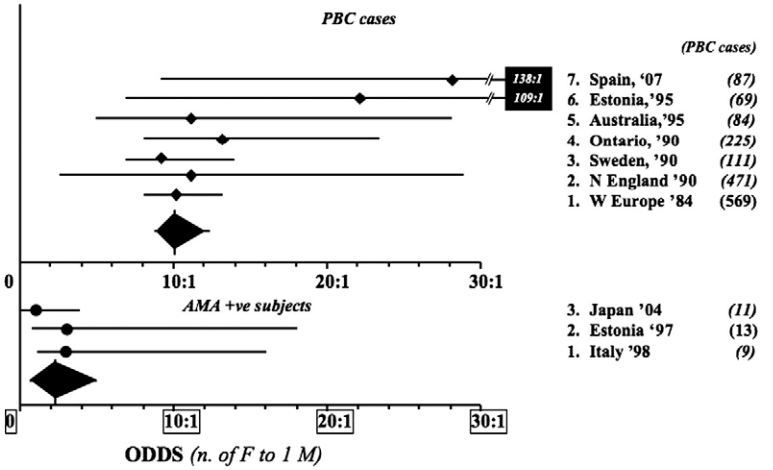

Fig. 1. Re-evaluation of available data on PBC sex ratios. The upper panel illustrates confidence intervals and mean sex ratios from population-based studies in which at least 60 cases were identified. The lowel panel depicts confidence intervals and mean sex ratios from case-finding studies based on sera screening. As shown, observed female to male ratios were 10:1 and 2.1:1, respectively.

towards a better definition of the mechanisms leading to the widely different AID clinical features, in particular allowing a clear definition of cases more likely to progress or to present major complications and to develop novel therapeutic approaches.

Take-home messages

- Data from the epidemiology of autoimmune diseases, particularly those ensuing later in life, support a variable female predominance in most conditions;
- The human immune system exhibits sexual dimorphism and basic immune responses differ between females and males in terms of antibody production and cellular responses;
- Sex hormones were first suggested for inducing female susceptibility due to their effects on cytokine production, B cell maturation, homing of lymphocytes, and antigen presentation and the reproductive differences between women with and without autoimmunity; data on sex hormones in autoimmunity are, however, inconclusive;
- The persistence of fetal genetic material (termed fetal microchimerism) may induce autoimmunity in women but previous observations in systemic sclerosis were not confirmed;
- Major defects of the X chromosome are more frequent in women with late-onset autoimmune diseases while data on X inactivation patterns are inconsistent;
- Clinicians should not rule out the possibility that autoimmune diseases are being overlooked in men.

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