LUKA ABASHISHVILI, ANA GOGOLASHVILI, DIANA KERATISHVILI, MARIAM PESTVENIDZE

INFLUENCE OF GENDER IN DEVELOPING AUTOIMMUNE DISEASES (Review Article)

USMD program, Tbilisi State Medical University, Tbilisi, Georgia

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Abstract

Autoimmune diseases and their management are one of the most challenging problems of modern society. These types of diseases are far more commonly seen in women than in men. We believe that understanding the reasons for the sex-based differences in autoimmune diseases can play a significant role in managing the patients. This review summarizes the most important contributors to gender-based disparity, such as the X chromosome, sex hormones, micro-RNA, and microbiota.

Introduction

Autoimmune disorders are the result of an exaggerated immune response that is detrimental to multiple organs and tissues. Because of their chronic nature and associated comorbidities, these diseases are a major public health problem. They increase the societal burden in terms of healthcare costs, loss of work productivity, and reduced quality of life; the etiology of autoimmune diseases is still unknown, but based on the available evidence, an interaction between genetic, environmental and lifestyle factors play a key role in disease development.

The prevalence of autoimmune diseases is much higher in females than in males. 80% of autoimmune patients are women [1]. The women were found to have a higher quantity of immunoglobulins and many circulating CD4 T cells that lead to a more effective immune response to infections, allografts, and tumors. The downside of this fact is that females are more likely to mount their immune system against self-antigens [1,2]. For instance, systemic lupus erythematosus (SLE), Sjogren's syndrome, Grave's disease, and Hashimoto's thyroiditis are seven to ten times more common in women than men; multiple sclerosis (MS), rheumatoid arthritis (RA), and scleroderma are two to three times more common [2]. Moreover, odds of having female sex in patients with SLE was approximately 9 based on data from the Georgia (USA) Lupus registry 2002 [2]. Symptom severity, disease course, response to therapy, and overall survival may also differ between males and females with autoimmune diseases [3]. Understanding the basis of this variance can be vital for the development of future research on this topic.

Several experts speculate that the sex chromosomes, sex hormones, micro-RNA, and sex-specific environmental factors such as dimorphic microbiota are important mechanisms of gender bias in autoimmunity. In this review, we discuss current and foundational studies addressing the possible reasons behind these fundamental differences.

X chromosome and autoimmunity

Female and male karyotype differs from each other. Females have two X chromosomes whereas males have one X and one paternal Y chromosome. The Y chromosome contains approximately 100 genes including the SRY sex-determining gene, whereas the X chromosome has approximately 1,100 genes [4]. To avoid double X chromosome expression, females exhibit phenomena called XCI (X chromosome inactivation), which allows equal gene expression. The role of sex chromosomes in autoimmune diseases has been proposed based on several mechanisms including X chromosome inactivation patterns, fetal microchimerism, and X-chromosome monosomy and duplication [5]. X chromosome inactivation is not complete and about 15% of the genes escape inactivation, leading to over-expression of some X-linked genes in females [5]. A recent study using highly sensitive approaches to measure allele-specific gene expression found that no X-chromosome was 100% inactive in any of the female cells examined. Furthermore, the degree of XCI was heterogeneous between cells [6]. Numerous genes (such as CD40 ligand, chemokine receptor CXCR3, O linked N- acetylglucosamine transferase, Forkhead boxP3 (FOXP3), toll-like receptor (TLR)7, TLR8, IL-2 receptor gamma, tyrosine-protein kinase BTK, and IL-9 receptor) encoded by the X chromosome are shown to influence the immune response in a sex-dependent manner when over-expressed [7]. In this line, a single mutation in the interleukin-1 receptor-associated kinase 1 gene (IRAK1) contributes to an increased risk for lupus [8]. We believe that overexpression of

these genes due to incomplete inactivation can be an underlying cause of increased susceptibility to autoimmune diseases.

Fetal microchimerism was first suggested as a possible factor in autoimmunity based on the observation that most autoimmune diseases manifest their peak of incidence following the fertile period. These fetal cells are often hematopoietic and can differentiate into somatic cells in multiple organs, potentially acting as targets for autoimmunity and resembling graft-versus-host disease after stem-cell transplantation. Multiple studies suggest that karyotype abnormalities such as loss of X chromosome or monosomy of it may be an underlying cause of autoimmunity. It is highlighted that absence of a second X chromosome in females (Turner syndrome) is linked with increased susceptibility to autoimmune diseases compared to sex matching individuals in the general population. A Cohort study conducted in Denmark showed that the overall risk of autoimmune disease among women with Turner's syndrome was twice that among Danish women in general [9]. The idea, that those chromosomal abnormalities and especially X chromosome involvement in autoimmunity is further supported by a study result, which revealed that men who have Klinefelter's syndrome (47, XXY genotype) are 14 fold more susceptible to systemic lupus erythematosus, compared to the population with normal karyotype [5].

Thus, the X chromosome, its incomplete inactivation, monosomy, duplication, and fetal microchimerism can be one of the most significant contributors of multiple gene over-expression, which plays an important role in autoimmunity and influences the gender-based differences in the prevalence of autoimmune disorders.

Sex hormones

One of the significant differences between the female and male bodies is the sex hormonal composition. Women and men synthesize the same sex hormones (androgens, estrogens, progesterone) but at different levels, and their effects depend on their concentration levels and the type of target immune cell [10]. These differences may be the one contributing factor to gender dimorphism in immune responses and the reason why there is a significant difference in the incidence of autoimmune disease development between males and females.

The gender dimorphism in autoimmunity is more evident and apparent after puberty. For example, pre-pubertal onset multiple sclerosis (MS) is rare [11] and gender bias within these cases of MS are absent [12]. After the onset of puberty, however, incidence changes rapidly and pubertal girls are found to be at greater risk of developing MS than pre-pubertal. Moreover, the earlier onset of puberty in girls is also associated with an increased risk of developing MS. Similarly, in SLE, the adult male to female ratio of 1:9 may be as low as 1:2 before puberty [12]. However, many environmental factors also influence the development of autoimmune diseases.

The exact interaction between sex hormones and immune reactivity is incompletely understood, however, many studies investigate the impact of sex hormones on different constituents of our immune system. According to these reports, one of the targets of sex hormones is the autoimmune regulator gene (AIRE gene). Recent studies showed that the androgen/androgen-receptor complex directly binds to the promoter region of the AIRE gene and increases its transcription. This leads to escalated tissue self-antigen expression resulting in a more efficient negative selection of T cells [13]. As a consequence, mice administered dihydrotestosterone were protected from central nervous system autoimmunity. On the other hand, estrogen suppresses AIRE gene expression and gives the opposite result [13]. This different effect of sex steroids on the AIRE gene is a significant mechanism by which sex bias occurs in autoimmunity. In vitro studies claim that sex hormones control the production of a variety of immune cytokines, including interleukin (IL)-1 [14,15], IL-6, IL-2, IL-4, IL-5, interferon-gamma [16], and transforming growth factor-beta [8]. The IFNs are of obvious relevance to this subject because they are well known to be overexpressed in patients with certain autoimmune diseases [1]. The interferon-gamma promoter region has four estrogen response elements and there are odds that higher estrogen levels in females stimulate interferon-gamma production by T-cells, which may increase the susceptibility of developing interferon-gamma mediated autoimmune diseases in females [13].

Estrogens

The effects of estrogens on the immune system are very complex. There are three types of endogenous estrogens: estrogen (E1), estradiol (E2), and estriol (E3, produced only during pregnancy). Each of them has distinct action on intracellular estrogen receptors that are present in all cells of the immune system including T and B lymphocytes, and peripheral NK cells [17]. In particular, estradiol can regulate immune responses acting at multiple levels including cell development, proliferation, cytokine or antibody production, and apoptosis. Estradiol has two main receptors, estrogen receptor α, and estrogen receptor β. As mentioned above, all immune cells express intracellular estrogen receptors, but the proportion of one estrogen receptor subtype to another may be different that may alter the estrogen effect, either by aggravating or alleviating inflammation. Activation of estrogen receptor α results in immune system enhancement while the activation of β receptors has a slightly immunosuppressive effect [18]. The number of intracellular estrogen receptors does not change during the menstrual cycle, with age, or after menopause. Estradiol appears to favor the survival of high-affinity DNA-reactive B cells at both the immature and transitional B cell stages facilitating the maturation of a potentially pathogenic naive auto-reactive B cell [18].

In females, circulating levels of estrogens fluctuate because of the menstrual cycle, pregnancy, and menopause. This is significant because the varying concentration of estrogen may affect immunity differently. For example, a high level of estrogen during pregnancy or the periovulatory phase of the menstrual cycle inhibits pro-inflammatory pathways and stimulates anti-inflammatory ones. Conversely, at low levels (as seen after menopause), estrogen stimulates pro-inflammatory pathways [1].

Estradiol stimulates antibody synthesis from B cells independent of the circulating level. New investigations found out that estradiol promotes the expression of activation-induced deaminase in the B cells that drives antibody diversification and transforms benign antibodies into autoantibodies leading to autoimmunity.

Androgens and Progesterone

In men, androgen levels are higher and the incidence of autoimmune disease is low, which may give us a clue that androgens protect against immunity. Studies showed that orchiectomy of male mice leads to overt autoimmunity, whereas treatment with androgens in ovariectomized female mice reduced mortality [19]. In humans, treatment with testosterone had a positive effect on men with MS (slowed cognitive decline and brain atrophy) [20]. The possible explanation of this effect is that testosterone suppresses the expression of the pro-inflammatory cytokines TNF-α, interleukin (IL)-1β, and IL-6 and promotes the expression of the anti-inflammatory cytokine IL-10 [17,18]. It also down regulates Th1 differentiation by up regulating type 1 protein tyrosine phosphatase (Ptpn1) in both mice and humans, reduces the proliferation and differentiation of lymphocytes, and may suppress immunoglobulin production [17,18]. Overall, these data strongly support an immunosuppressive role for androgens although, since their effects may vary considerably depending on the level of exposure, the exact role of androgens is still unknown [17,18].

The presence of progesterone receptors in immune cells suggests that this hormone has an impact on immune responses. Activation of intracellular progesterone receptors by low physiologic concentrations of progesterone is thought to suppress antibody responses in both sex [17,18]. Understanding and analyzing the impact of sex steroids on immune-mediated diseases could lead to the identification of innovative and readily available therapeutic interventions, such as hormone antagonists or agonists, to manage autoimmune diseases.

Autoimmunity and miRNA

Multiple studies demonstrate the importance of miRNA expression in immune cells. Zhou et al. (2008) observed that dicer-deficient Tregs lost the ability to suppress an immune response [21]. These mice developed an autoimmune disease that closely resembles IPEX syndrome (FOXP3 knockout phenotype). This suggests the importance of miRNA in maintaining a balanced adaptive immune response. Having said that, this article emphasizes the major difference between a female and male expression of miRNA in autoimmune diseases. There are three major contributing factors to sexual dimorphism of miRNA expression: sex chromosome, hormones, and external (environmental) stimuli.

Firstly, according to the miRBase microRNA archive (www.miRBase.org2013), the X chromosome contains approximately 113 miRNA genes, while the Y chromosome only has 2. Skewed XCI may also contribute to the disparity of X-linked genes expressed in females. There has been ongoing research to identify exactly which miRNA is involved in the pathogenesis of autoimmune diseases like systemic lupus erythematosus and rheumatoid arthritis. Dai and Ahmed (2014) were the ones who tested the splenocytes of female and male NZB/WF1 mice. The NZB/WF1 model closely resembles human lupus. They found a significant increase in expression of the miR-182 cluster, miR-155, miR-31, miR-148a, miR-127, and miR-379 after the onset of lupus in female NZB/WF1 mice [22]. Another example of altered miRNA expression in different diseases is miRNA-223, coded by the X chromosome. There was increased expression of miRNA-223 in rheumatoid arthritis and type 2 diabetes patients, but decreased activity in systemic lupus erythematosus [22].

Secondly, evidence suggests the influence of sex hormones like estrogen and androgens on expressing miRNA. Sex hormones bind to nuclear hormone receptors and alter gene expression [23]. This can be both direct and indirect. The genes influenced by sex hormones may directly contain the miRNA molecule genes or have the promoter regions embodied in them [23]. Moreover, sex hormones alter post-transcriptional modification and expression of miRNA by inducing or inhibiting molecules like export 5, Dosha, or Dicer [22]. Dai et. al (2008) discovered increased miR146a and miR-223 in estrogen-treated splenic lymphocytes which then enhanced the response of the cells to lipopolysaccharide (LPS) [24]. This further supports the hypothesis of different miRNA expressions between genders.

Last but not least, the response to external stimuli differs in females and males. For example, sex-specific miRNAs were down-regulated in female mice but not in male mice after exposure to ionizing radiation [22]. In addition, there has been some difference in the metabolism of different drugs between sex. Females have been shown to have increased activity of Cyp2b9, a subclass of the cytochrome P450 superfamily [22]. Scientists have found a negative correlation between Cyp2b9 and miRNA expression [22]. This may contribute to decreased expression of these miRNA genes in females compared to males. Thus, environmental influences can also contribute to dimorphic miRNA gene expression between the two groups.

To sum up, there has been an increasing investigation to discover the role of miRNA in causing autoimmunity. There is some evidence showing that miRNA can be a culprit in causing lupus, rheumatoid arthritis, and other autoimmune diseases. All - chromosomal, hormonal, and environmental influences on miRNA are vital. However, we need more investigation to finally understand what is the role of miRNA in causing autoimmunity.

Gut Microbiota

Gut microbiota is the assembly of all the microorganisms, such as bacteria, viruses, fungi, protozoa that live in the digestive tract of humans and other animals. It has long been known about their importance on various levels for the human body [25]: they help us with digestion, ferment dietary components, fight against harmful pathogens, produce vitamins, and immunologically active molecules such as short-chain fatty acids, and many more. However, it is only recently that studies demonstrated that disbalance in the intestinal microbiota is associated with the pathogenesis of some autoimmune diseases. Moreover, the difference in microbiota composition also contributes to sexually dimorphic immunity.

The interaction between gut flora and the immune system involves two main components: molecular mimicry and molecular complementarity. Since the intestinal microbiota significantly contributes to the normal functioning of the human body, it has the utmost significance to protect them from pathogenic bacteria and the host immune system. Through evolutionary processes, these microbes have evolved to look like host antigens, to mimic them. On the other hand, the immune system has developed to make a simplified “body double” [26] of the host and minimal distinctions between “self” and “non-self”. Using molecular mimicry, these bacteria effectively avoid immunological detection and processing. It is well known that complementarity between specific proteins on the cell surface of the microbe and receptors or transporters on the cell surface of host cells is essential for the identification of host cells by microbes. That’s why many commensal microbes produce mimics of receptor ligands, suggesting molecular complementarity.

Based on molecular mimicry and molecular complementarity, many microbial antigens look like host antigens so much that an active immune response to the microbe may cause cross-reaction with the host, causing autoimmune disease. Or in the opposite, if an autoimmune disease is induced against the host, any microbiome components expressing antigens similar to those targeted by the autoimmune disease will also be affected. Anyways, either or both mechanisms could contribute to autoimmune disease development.

Knowing that the microbiome differs in individuals by sex, it is not surprising that the sex bias in autoimmune diseases is also affected by the microbiome and vice versa. Though gender differences in microbiota composition are found both in mice and in human studies, the lack of standardization in human studies may mask the sexual dimorphism in microbiota composition. The reason is simple - many factors such as age, genetic background, BMI, diet, and sex hormones appear to interfere with the sexual dimorphism in microbiota composition. This is the reason why studies have been performed on rodents.

The classic example is considered to be the study performed on NOD [27] (non-obese diabetic) mice. It showed that female NOD mice with normal microbiota were several times more likely to develop type 1 diabetes (T1D) compared to male NOD mice with normal microbiomes. But after comparing female and male germ-free NOD mice, this difference disappeared. It was further confirmed by the microbiota transfer study by Markle et al. according to which, transplantation of microbiota from conventional NOD males to germ-free NOD females resulted in the protection of the female mice against T1D. There can be three models [28] explaining these results: A - suggesting that either through immune or metabolic mechanisms hormones regulate the microbes and that microbes then activate the protective effector mechanisms, B - microbes are regulators of hormonal metabolism and the hormones are the actual effectors, and C - both microbiota and hormones contribute in an additive fashion.

Understanding the contribution of gut microbiota in gender dimorphism of autoimmune diseases, in addition to many other possible causes, can be crucial for the future treatment of these types of conditions. Developing certain approaches to microbiome manipulations and treatment of microbiome dysbiosis using probiotic replacement therapies can be rather helpful.

Conclusions

In conclusion, this paper has discussed the reasons for the abnormal functioning of the immune system causing autoimmunity and the possible contributors to the sex-based disparity. The evidence suggests that the most important factors, from the huge list of influencers that may underlie this striking gender difference, are the X chromosome, sex hormones, micro-RNA, and microbiota. Analyzing these findings and implementing them in modern therapeutic interventions could become a cornerstone of managing autoimmune diseases.

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