Review

Searching for the "X factor" in Sjögren's syndrome female predilection

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ABSTRACT

Sjögren's syndrome is typified by a strong female predilection which is also observed in other systemic autoimmune diseases. Although many factors may be contributing to this phenomenon, the exact underlying mechanisms remain unclear. Apart from the traditionally considered hormonal and environmental factors, lately the role of sex chromosomes and especially of the X chromosome has drawn much attention. In the current review, we focus on the inherent genetic imbalance between the sex chromosomes and their influence and role on gender-discordant disease presentation. To compensate for this imbalance, nature has created a defective epigenetic mechanism to silence the second rich in immune related genes X chromosome. Genes escaping silencing, transfer the genetic imbalance into the transcriptional and protein level, contributing to gender differences as reflected in functions of the innate and adaptive immunity. Under this prism, recent research data on SS, regarding specific immune X-linked loci are being presented and analysed. The "X Factor" in the search for an explication of women's predilection in autoimmunity, may lie behind these unique properties of the X chromosome.

Introduction

Autoimmune disorders (ADs) represent a range of organ specific or systemic diseases characterised by aberrant immune responses against self-antigens, leading to damage of tissues or organs. Even though single autoimmune diseases are rare, as a group they affect up to 6% of the industrialised general population (1) and are among the leading causes of morbidity and mortality in middle aged women (2, 3). Importantly, around 78% of those with an autoimmune disease are women (4). The magnitude of female predilection correlates with disease's prevalence since common diseases show a higher female skewing. For example, autoimmune thyroiditis, the most common organ specific autoimmune disorder, as well as systemic lupus erythematosus (SLE) and Sjögren's syndrome (SS), the prototypical systemic autoimmune disorders, share the most striking female sex biases. Earlier epidemiologic studies supported a female to male ratio in SLE and SS of 9-11:1, but recent data shows the gender ratio in SS to be even higher at 20:1 (5). Therefore, SS constitutes the most female predominant systemic autoimmune disease, and it presents the best research model to investigate and elucidate the implicated mechanisms favouring a women bias (Table I).

Growing scientific evidence shows that the adult females mount stronger innate and adaptive immunological responses, reflected by a lower risk for serious infections and a better vaccine antibody response, although the pathophysiology has not been thoroughly worked out (6-11). The ongoing worldwide severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic, showing a case mortality ratio of 1,7 males *versus* 1 female, is another pertinent example (12). In this line, when it comes to immune deregulation, the precise pathogenetic processes that govern autoimmune disorders' significant sexual dimorphism and predilection are still undetermined, concealing a crucial step both in understanding the fundamental underlying mechanisms of autoimmunity and tailoring a true precision medicine therapeutic approach. It is commonly believed that inherent differences between genders might contribute to this phenomenon. For decades, the unique female hormonal milieu was considered

Table I. Female to male ratio between systemic and organ specific autoimmune diseases.

Autoimmune disease	Female: Male ratio	Reference
Systemic autoimmune disease		
Sjögren's syndrome	20:1	[5]
Systemic lupus erythematosus	9:1	[110]
Systemic sclerosis	3:1	[111]
Rheumatoid arthritis	2:1	[112]
Vasculitides		
Giant cell arteritis	3:1	[113]
ANCA vasculitis	1:1	[114]
Takayasu's arteritis	11:1	[115]
Inflammatory myopathies	2:1	[116]
Seronegative spondyloarthropathies		
Ankylosing spondylitis	1:3	[117]
Psoriatic arthritis	1:1.5	[117]
Reactive arthritis	1:1.2	[117]
Organ specific systemic disease		
Hashimoto thyroiditis	10:1	
Grave's disease	4:1	[118]
Multiple sclerosis	3:1	[119]
Vitiligo	1:1	[120]
Addison's disease	1:1.7	[121]
Diabetes mellitus type I	1:1.8	[122]

the sole contributing factor, though offering only partial interpretation, since female predilection exists before puberty or after menopause, and disease course remains mostly unaffected in patients on oral contraceptives or oestrogen replacement therapy (13). Recently, the inherent genetic imbalance caused by the unequal dosages of genes on the sex chromosomes has been implicated as well. Females carry two X chromosomes and males one X and one Y, a clamant but until recently overlooked discrepancy between genders. Supportive evidence for the contribution of the X chromosome in the pathogenesis of autoimmune diseases emerges from chromosomal disorders such as the Turner syndrome (X, O), where patients have a very low risk of developing SLE, and the Klinefelter syndrome (XXY), where patients have a 14-fold risk of developing SLE or SS, almost equal to that of euploid women (XX) (14-16). Epigenetic changes related to X chromosome inactivation and skewing as well as maternal microchimerism, a maternal-embryo trafficking of circulating cells during embryogenesis, are also under investigation as contributing factors to the phenomenon. The notion that the second, rich in immune related genes, X chromosome in women, instead of the much smaller but sex determining Y chromosome in males is accountable, partly, for the female predilection in autoimmunity is compelling and emphasises once more the fate-shaping power of genetics and epigenetics. In the present review we highlight the role of the X chromosome as an important sex biased determinant, the X factor, using Sjögren's syndrome as a systemic autoimmune disease model.

Dissecting the X and Y chromosomes

Both X and Y chromosomes, even though vastly different in structure, size, function, and number of genes, originate from the same pair of homologous chromosomes, known as autosomes (17). Approximately 200 million years ago a male determining mutation occurred in one of the autosomal genes triggering various stepwise processes that hindered recombination, thus allowing the 2 sex chromosomes to evolve independently (18, 19). Despite evolutionary divergence, 2 regions at both termini of X and Y chromosome, called pseudo-autosomal regions (PAR 1 and PAR 2) containing at least 29 genes, retained homology and the ability to recombine during meiosis (20, 21). The X chromosome encodes around 1100 annotated genes, with few if any implicated in sex determination, compared to the Y chromosome that contains 78 male specific genes crucial for germ cell differentiation, fertility, and masculinization (20, 22). Evidenced by the high number of X related genes implicated in in the development of immune cells (Bruton tyrosine kinase, IL2RG), in the innate (TLR 7, TLR 8, IRAK) and adaptive (CD40L, FOXP3) immune responses, is the instrumental role X chromosome imparts in regulating immune homeostasis and maintaining tolerance (23). In this line, mutations in specific genes of the X chromosome may result in immunodeficiency syndromes sharing autoimmune manifestations, including the hyper IgM syndrome, the immunodysregulation polyendocrinopathy enteropathy X-linked (IPEX), and the X-linked recessive severe combined immunodeficiency (XSCID) syndromes caused by mutations in the CD40 ligand, the forkhead box P3 (FOXP3) and the IL-2 receptor genes respectively, all residing in different parts of the X chromosome (24-27). Another recently discovered novel X chromosome-based mutation is that of SAM and SH3 domain-containing 3 (SASH3), which is postulated to orchestrate signal transductions downstream pathways in lymphocytes that, when dysfunctional, result in a clinical phenotype that combines susceptibility to various infections and autoimmune cytopenias (28). Similarly, a limited number of reports have linked specific Y located genes with genome wide regulatory properties, such as the Sly and Rbmy genes charged with immune modulation and conferring susceptibility to autoimmune diseases (29).

The X chromosome accounts for more than 5% of the total DNA in each cell, approximately 3 times more than the Y chromosome, an imbalance even more apparent in the gene apparatus, since the former contains more than 100 times the number of transcriptionally active genes leading to an obvious genetic inequity between females (XX) and males (XY). To relieve those imbalances, in female mammals, early in embryogenesis, either paternal or maternal X chromosome gets epige-

netically inactivated randomly by a process called X chromosome inactivation (XCI), formatting a heterochromatin nucleolar satellite in contact with the nucleus membrane, called Barr body (30-32). Failure of X chromosome silencing leads to lethality due to the overabundance of X gene related products that interfere with normal cell function (33, 34). In each embryonic cell the assortment of X chromosome rendered inactive is random and irreversible, resulting in a mosaic pattern of expression in cells originating from different germ layers. However, the supposedly random delegation process is not always perfect, and skewed patterns in which more than 75% of cells preferentially inactivate the maternal or paternal X chromosome have been observed. Cross-sectional studies have supported a possible influence of aging on the degree of skewing, though without confirmation by more recent longitudinal studies (35). Interestingly, patients with autoimmune diseases have been related to extreme skewing (>90%), despite several conflicting reports (36). A proposed pathogenetic explanation connecting chromosome skewing with the ignition of the autoimmunity cascade, implicates the X linked self-antigens derived from the residual cell population after non-random skewing. In this case, these self-autoantigens behave as cryptoantigens that may escape central thymic T cell tolerance, resulting in autoantigen presentation and generation of pathologic autoreactive T cell clones later in life (37-40). It is known, however, that in females harbouring structural alterations on one X chromosome, a preferential expression of the other normal chromosome occurs. The ability of the cell to deliberately silence the harmful chromosome, as it happens in X-linked agammaglobulinaemia and Wiskott-Aldrich syndrome, is an evolutionary phenomenon, clearly offering a survival advantage in women carriers. A complete explanation for skewing when both chromosomes are structurally sound remains elusive (41-43). Except for the extraordinary epigenetic transcriptional silencing mechanism of skewed X-chromosome inactivation, a post transcriptional crucial regulatory role has been also assigned to the X chromosome, since it affords the second largest number of genes among all chromosomes transcribing non-coding RNA molecules called (miRNAs). A number of these genes have been associated with a tendency to develop autoimmune diseases, uncloaking an additional epigenetic immune modulating role of the X chromosome (44, 45).

The most intriguing property of XCI process, however, is that it is an incomplete process, lacking a telomere-to-telomere chromosomal wide approach, opening the pandoras box of genetic material imbalance between genders. Evaluation of sex gene expression in humans has revealed that 15 to 23% of X resident genes escape inactivation and are expressed seamlessly from both the active and the inactive chromosome, creating at least for some antigens an active protein abundance in females. Pervasive heterogeneity exists in the loci of the escape genes in the X chromosome [except for the telomeric regions belonging to the pseudo-autosomal regions (PAR 1 and 2), which by definition are not susceptible to inactivation], between female individuals, and even among cells from different tissues of the same individual, since complete biallelic expression in only one tissue appears to be quite rare, at around 6%) (46, 47). Despite the emergence of novel biotechnologies currently available, it is predominantly the use of single cell RNA seq analysis that has enabled scientists to identify repeatedly established escaping genes, but a consensus on the complete list of those genes which evade silencing has not been reached, as several reports are not concordant (31, 48, 49). To be enlisted, a gene requires a biallelic read in a single cell or a tissue, provided that XCI is completely skewed. Genes that escape epigenomic silencing might explain phenotypic variability among females heterozygous for X-linked conditions, gene deletions that occasionally lead to disease because of dosage sensitivity (theory proposed by Miyake *et al*. referring to Kabuki syndrome) as well as various clinical manifestations in patients with X-chromosome aneuploidy (50). So, the towering question of why autoimmunity privileges females might be answered by the immune related genes escaping inactivation, a fact that sets those genes at the centre of scientific inquiry. Below we will briefly elaborate on the X chromosome immune genes that have been shown to escape silencing and display an established or putative role in SS pathogenesis, building in that way a probable association with the striking SS female predilection. A selection of genes that play a significant role in various cell processes and/or in the immune system operation that have been shown to escape XCI silencing but have been meagerly or not at all studied in SS are shown in Table II.

Sjögren's syndrome related XCI escapees' profile

Toll-like receptor 7 (TLR 7)

Toll like receptors (TLRs) are type I transmembrane proteins in charge of recognising evolutionary conserved molecular patterns from microbes or cell damage debris, initiating an immune response. Among the pattern recognition receptors (PRRs), phylogenetic analysis has shown that TLRs are the most ancient class (especially TLR3), bearing the broadest recognition repertoire (51, 52). In humans, 10 TLR subtypes have been identified, classified into 2 categories according to their location: i) located in the plasma membrane (TLR1, TLR2, TLR4, TLR5, TLR6) and ii) intracellular TLRs (TLR3, TLR7, TLR8, TLR9, TLR 10) (53). TLR 7 has the ability to sense single stranded RNA (ssRNA) and upon ligation, to produce many proinflammatory cytokines (54, 55). Among the downstream inflammatory pathways, TLR 7 induces the production of type I interferons mainly by plasmacytoid dendritic cells (pDCs) via the interferon regulatory factor 7 (IRF7) transcription factor. In SS patients, type I interferon (IFN)-inducible genes, have shown a remarkable overexpression, at both the inflamed salivary glands and peripheral blood mononuclear cells (PBMCs), suggesting a pivotal role in the pathogenesis of the disease. Among the IFN-induced genes, a special attention should be given to B-cell activat-

Table II. A selection of immune related genes that escape XCI silencing and have not been studied extensively in Sjögren's syndrome.

Gene Nomeclature	Function
Lysosome-associated membrane protein 2 (LAMP 2)	Crucial role in chaperone-mediated autophagy
DEAD-Box Helicase 3 X-Linked (DDX3X)	RNA helicase mediating INF signaling
Methyl-CpG Binding Protein 2 (MECP2)	Epigenetically regulates methylation sensitive genes, important role in immune homeostasis
Cluster of differentiation 99 (CD99)	Important transmembrane protein for leukocyte trafficking and T cell adhesion
Interleukin 13 Receptor Subunit Alpha 1 (IL13RA1)	Important for type II inflammatory responses
Ubiquitin Specific Peptidase 27 X-Linked (USP27X)	Deubiquitinating enzyme related to increased cell apoptosis
Ubiquitin Specific Peptidase 9 X-Linked (USP9X)	Deubiquitylating enzyme with important role in neurodevelopment
Ornithine carbamoyltransferase (OTC)	Located in liver mitochondria and functions as part of the urea cycle
Ribosomal Protein S6 Kinase A3 (RPS6KA3)	A serine/threonine kinase involved in the MAPK pathway
Lysine-specific demethylase 6A (KDM6a)	Functions as histone demethylase
The eukaryotic translation initiation factor 2 complex (EIF2S3)	A part of the protein synthesis cascade

ing factor (BAFF) which serves as a biomarker and therapeutic target in SS, and can be produced by salivary gland epithelial cells (SGECs) after ssRNA stimulation (56, 57). In accordance, TLR 7 has been found upregulated in blood samples, PMBCs and cultured salivary gland epithelial cells (SGECs) of patients with SS compared to gender matched controls (58-60). In addition, a recent study by Davies *et al*. showed that SS patients' PBMC TLR7 stimulation significantly alters the downstream molecular phosphorylation profile compared to healthy controls (61). Another mechanism through which TLR 7 activation might contribute to Sjögren's pathogenesis is via age-associated B cells (ABCs). ABCs is a newly discovered B cell subset that expresses the transcription factor T-bet and accumulates with normal aging. Interestingly ABCs also display a premature expansion in various autoimmune diseases, including SS, having the capacity to produce harmful autoantibodies (62-64). It has also been shown that TLR 7 is the driving force behind the proliferation and inflamed tissue accumulation of autoantibody secreting ABCs involved in lupus manifestations, seen in an autoimmune prone mice model (65). Evidently, TLR 7 is involved in the pathogenesis of SS, but whether a biallelic expression plays a role in the female sex bias observed in autoimmune diseases remains unknown. Souyris *et al*. showed that B lymphocytes, monocytes, and interferon producing plasmacytoid dendritic cells, not only in females but also in males with Klinefelter syndrome, express elevated levels of TLR7 and exhibit a propensity for class switch toward IgG producing plasma cells in contrast to the behaviour of immune cells from euploid males (XY) (66). Moreover, a recent study focusing on pDCs confirmed that TLR7 escapes inactivation in this cell line as well, resulting in higher mRNA transcription of IFNα and IFNβ that is further augmented substantially by IFNα exposure (67), creating a vicious cycle. Interestingly, a gain of function polymorphism mutation in TLR 7 gene predisposes male individuals to develop SLE (65).

CD40 ligand

CD 40 ligand (CD40L), also known as CD154, is a transmembrane protein type II located primarily on T helper cells. Its primary target is CD40 on the surface of B cells. When a CD40-CD40L interaction occurs, it acts as a second co-stimulatory signal, vital for B cell activation, isotype class switching and germinal centre formation (68, 69). Further studies have shown that CD40 and its ligand is also expressed in non-lymphoid cell types including monocytes, basophils, eosinophils, dendritic cells, megakaryocytes, platelets, fibroblasts, smooth muscle, and endothelial cells, mediating both humoral and cellular immunity (70, 71). SS is a disease characterised by the presence of germinal centre-like regions in the lip biopsy of more than 20% of patients. Even though being ectopic (outside of the secondary lymphoid organs), these structures are fully functional and equipped with all the required molecular tools, such as CD40L and activation-induced cytidine deaminase (AID), to activate B cells and produce large quantities of (auto) antibodies (72-74). The presence of ectopic germinal centre in the lip biopsy has been associated with an increased risk for future lymphoma development, a higher focus score, more systemic manifestations, and enriched autoantibody profile (75, 76). It is important that this knowledge should be guiding clinicians with their patients' follow up plans (77). As expected, reports dating from the early 2000, showed increased expression of CD40L in lip biopsies of 17 SS patients and their cultured SGECs compared to healthy donors (78, 79), indicating a putative role of T and B cell interactions in the pathogenesis of the disease (80). In addition, T cells carrying CD40L might mediate epithelial gland dysfunction through interaction with the CD40 molecule expressed on epithelial cells membrane (81). The first therapeutic initiative to block the CD40 pathway in lupus patients ended in early discontinuation of the trial, because of an increase in

thromboembolic events caused by the cross-linking of anti-CD40L antibodies to platelet CD40L and Fc gamma Receptor IIa on adjacent platelets (82). However, newer therapeutic anti-CD40 agents conferring different amino acid sequence in the Fc region preventing platelet aggregation have completed phase II studies, with promising preliminary results (83, 84).

Given the aforementioned crucial role of CD40 in the pathogenesis of Sjogren's syndrome, the fact that a number of women's T cells have biallelic CD40L expression may provide a mechanistic explanation for the sexual divergence (85). Supporting evidence comes from murine models in which CD40 overexpression resulted in high titres of autoantibodies (86) and from human subjects with CD40L gene duplication who develop autoimmune manifestations and lymphopenia (87). Finally, a recent study revealed that stimulated PBMCs from normal women and males with Klinefelter syndrome harboured higher expression of CD40L on T cells, compared to euploid males and females with Turner syndrome (88).

C-X-C motif chemokine receptor 3

The only chemokine receptor gene encoded by the X chromosome is C-X-C motif chemokine receptor 3 (CXCR3), a crucial Th1 response regulator (89). Its expression is ubiquitous among all immune cell lineages, but its role has been especially studied in activated T cells, serving as a receptor for interferon (IFN)-γ inducible chemokines CXCL9, CXCL10, and CXCL11. All these chemokines are not constitutively expressed unless induced by an inflammatory stimulus and mainly regulate immune cell migration, differentiation, and activation (90). Given its immune cell expression, selective ligation, and prominent function, CXCR3 has been implicated in autoimmune disease pathogenesis (91). Especially in the salivary and lacrimal glands of SS patients, CXCR3 and its ligands have been shown to be upregulated (92, 93). In addition, CXCR3 mRNA levels in $CD4^+$ T cells of female donors have shown increased levels compared to males, despite the fact that this difference abated at the protein level, suggesting additional post-transcriptional regulation (94). Initial studies on XCI escape genes failed to recognise CXCR3 as an escapee. A recent study, however, exploiting more sensitivity single-cell RNA FISH analysis exhibited biallelic expression patterns for CXCR3 a finding that was confirmed by *in vivo* fluorescence in a specially designed dual reporter mouse model (85, 95). Conclusively, CXCR3 seems to play a role in the pathogenetic mechanisms of SS among other autoimmune diseases and its biallelic expression and ample protein production if confirmed in future studies, could become a potential contributor to the observed preponderance of the female sex.

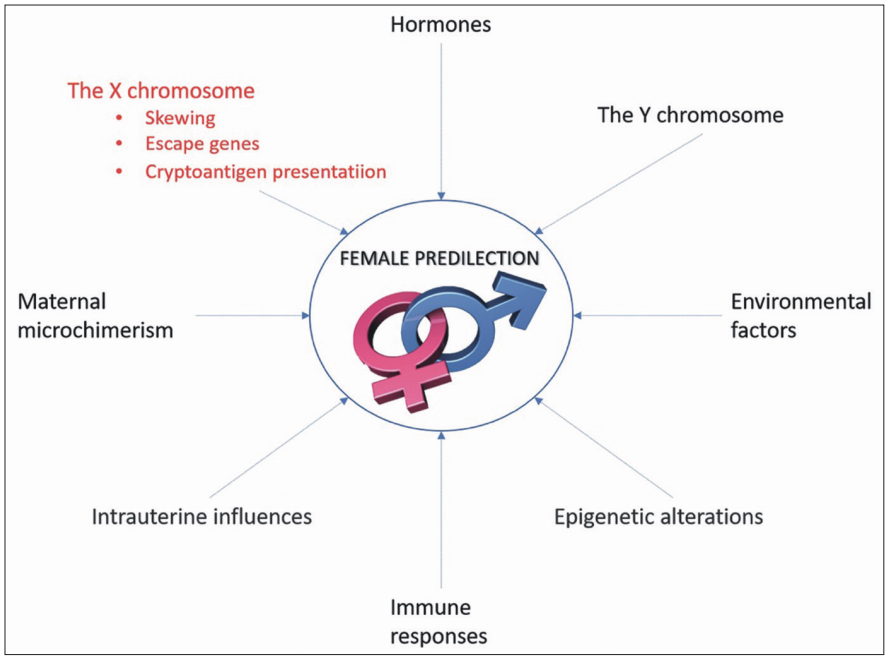

Fig. 1. Female predilection in autoimmunity may be the end result of complex interactions between several confounding factors, with the X chromosome's unique features constituting a key contributor.

Interleukin-1 receptor associated kinase

Interleukin-1 receptor associated kinase (IRAK-1) contributes to a promiscuous innate immunity proinflammatory signal transduction, relaying signals from Toll/IL-1 receptor (TIR) family (96). Upon ligation, these membrane receptors dimerize and recruit MYD88 (except for TLR 3), an adaptor protein that interacts with IRAK-4 and then phosphorylates IRAK-1 and 2 (97). Upon its activation, IRAK-1 phosphorylates TRAF-6 in a proline–serine–threonine-rich domain and activates several downstream pathways, including NF-kB, leading to various gene expression of cytokines (IL-1, IL-6, IL,12, TNF-α), chemokines and proliferative factors (G-CSF, M-CSF). Understanding the role of IRAK-1 in SS awaits further studies, as there is only a single report investigating its expression in SS patients' PBMCs, demonstrating decreased expression compared to healthy donors (98). In lupus, however, human and murine studies have established IRAK-1 as a prominent protein involved in its pathogenesis. Indeed, IRAK-1 deficient SLE mice models eschew all lupus-associated manifestations with concurrent reversal of any abnormal dendritic cell function, while in humans, polymorphisms of IRAK-1 gene have been correlated with increased lupus incidence and increased IRAK-1 levels in $CD4^+$ T cells from SLE patients (99-101). Clearly, further studies investigating its role in the pathophysiology of SS via biallelic expression of specific immune cell population or salivary gland epithelial cells, are the next core steps to uncloak a potential link that might prove fruitful in the elucidation of the remarkable sex disparity among SS patients.

Chromosome X open reading frame 21 (CXorf21)

Until fairly recently, the function of the protein encoded by CXof21 was a mystery, even though GWAS studies had already identified CXorf21 among the SLE and SS susceptibility genes and its levels had been shown to parallel the disease activity (102-104). In 2020, Heinz *et al*. renamed the protein as "TLR adaptor interacting with SLC15A4 on the lysosome' (TASL) and highlighted its key function as a gatekeeper in the recruitment and activation of the transcription factor IRF5 after endosomal TLR 7, 8 and 9 ligation (105, 106), resembling innate immune adaptor proteins STING, MAVS and TRIF. CXorf21 gene may variably escape X chromosome inactivation and its levels are increased in monocytes, B cells and lymphoblastoid cell lines of females compared to males (107). On this, functional studies on SS and SLE patients revealed sexual dimorphism in their lysosomal cell pH (lower pH in female compared to males' cells), possibly disrupting normal endolysosomal antigen processing owing to higher levels of CXorf21. Thus, CXorf21 gene combined with the aforementioned TLR 7 is a duo of closely related inactivation evading genes, since their product proteins are constantly collaborating in innate immune signalling (108). Both genes display a female gender predominant protein expression and have a proven crucial role in the pathogenesis of autoimmune diseases and as such, they hold the promise that their further study might shed light to our understanding of the factors and processes underpinning the observed sex bias among autoimmune patients including SS. (107).

Conclusion

Autoimmune diseases favour women in a striking and enigmatic manner. To date, many contributing factors have been incriminated in this phenomenon, creating a largely unexplored pathogenetic tapestry that mediates women's predilection for autoimmunity. Components of the tapestry that define gender bias in autoimmune diseases encompass the hormonal milieu and sex chromosomes on the one hand and innate and acquired immunity on the other, through a dynamic and complex interplay (Fig. 1). For many years, hormones took a central role when scientists attempted to clarify natures' gender discrimination, with either inconclusive or even conflicting results. To fill this knowledge gap, the scientific community turned to nature's own imperfection regarding the homeostatic process of balancing the genetic material between the sexes. Indeed, a failure to completely silence the second, rich in immune related genes, X chromosome may engender an environment that renders women particularly vulnerable to autoimmune disorders. The multilevel regulation of gene expression also involves sex chromosomes (epigenetic, transcriptional, post-transcriptional regulation), highlighting the difficulty to identify those key genes and their effector protein products, driving the female predilection in autoimmunity. Apart from the well-established loci with immune functions, there are also loci with still ill-defined functions which may indirectly interfere with both innate and acquired immunity. In addition, the underlying immune deregulation may involve the effector, memory, or regulatory component of acquired immunity. Thus, an overly complex network of interactions among the key contributors may eventually define the female predilection in autoimmunity.

Availability of a suitable disease model in humans that might allow promising lines of research to be further explored in depth, would be a considerable advance and perhaps act as the holy grail in our quest to illuminate sex bias in autoimmunity. Such a model would encompass a) a robust female predominance, b) a high prevalence, c) a wide age distribution with disease onset before puberty as well as after menopause and d) a practical histologic access to the autoimmune disorder's main target organ. Sjögren's syndrome, exhibiting a female predominance more pronounced than in any other systemic autoimmune disease, a wide age gamut and an easily obtainable target tissue specimen by the safe and highly informative minor salivary gland biopsy, is indubitably the disease candidate of choice (109). So far, studies in SS are limited in number and mainly involve sporadic reports of biallelic expression of certain XCI escape genes in the peripheral blood, as described above. No studies have been performed in cultured salivary gland epithelial cells or immune cells infiltrating the salivary glands. Such studies could shed light on the different protein apparatus and its subsequent downstream effects between genders and prove in a mechanistic order whether X chromosome is the true "X factor" underlying sexual divergence in autoimmunity.

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