



Deconstructing the sex bias in allergy and autoimmunity: From sex hormones and beyond

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Abstract

Men and women differ in their susceptibility to develop autoimmunity and allergy but also in their capacity to cope with infections. Mechanisms responsible for this sexual dimorphism are still poorly documented and probably multifactorial. This review discusses the recent development in our understanding of the cell-intrinsic actions of

biological factors linked to sex, sex hormones and sex chromosome complement, on immune cells, which may account for the sex differences in the enhanced susceptibility of women to develop immunological disorders, such as allergic asthma or systemic lupus erythematosus (SLE). We choose to more specifically discuss the impact of sex hormones on the development and function of immune cell populations directly involved in type-2 immunity, and the role of the X-linked Toll like receptor 7 (TLR7) in anti-viral immunity and in SLE. We will also elaborate on the recent evidence demonstrating that TLR7 escapes from X chromosome inactivation in the immune cells of women, and how this may contribute to endow woman immune system with enhanced responsiveness to RNA-virus and susceptibility to SLE.



1. Introduction

The decreased level of infectious diseases in developed country is associated with the concomitant increase in the prevalence of immunological disorders, including autoimmune and allergic diseases (Bach, 2002). Although the increased incidence of these diseases might be in part attributed to better diagnosis and improved access to medical facilities, this cannot explain the marked raise in autoimmune disease prevalence that occurred shortly in those countries in offspring of immigrants originating from a country with low incidence (Bach, 2002). This has been well illustrated by the raise in prevalence of type 1 diabetes (T1D) or multiple sclerosis (MS) in families of immigrants from Pakistan or from India moving to the United Kingdom or the United States, respectively (Okada, Kuhn, Feillet, & Bach, 2010). Likewise, the prevalence of systemic lupus erythematosus (SLE) is much lower in West African populations compared to African Americans. The hygiene hypothesis, purely based on the reduction in the diversity and magnitude of microbial burden in early life, first proposed to explain the observed increase in allergic disease (Strachan, 1989, 299:1259), was subsequently extended to the field of autoimmune diseases (Bach, 2002). Beside the disappearance of chronic infectious diseases, it is likely that the immunology of hygiene hypothesis is complex and involves loss of cellular and humoral immunoregulatory circuits with the adoption of Western life style, alterations of microbiome diversity thereby contributing to the rising incidence of allergic and autoimmune diseases (Bach, 2018; Lambrecht & Hammad, 2017).

Although, genetic and environmental factors, infection and western life-style, are likely key factors contributing to the development of allergy and autoimmunity, individual-intrinsic biological factors linked to sex are also

important triggers of these immunological disorders. Indeed, allergic and autoimmune diseases differentially affect women and men. In general, women develop strongest immune responses and thus the proportion of infected individuals and the severity of many viral, bacterial or parasitic infections are increased in men. However, heightened immunity comes at a steep price, which is the high incidence of autoimmune and allergic diseases in women. It is therefore likely that the immunological mechanisms shaping sex bias in infectious diseases and acquired immunity to pathogens are also the same that contribute to the higher incidence of autoimmune and allergic diseases in women. Understanding these pathways may therefore have implications not only for the treatment of these immunopathological disorders but also to promote optimal protective immunity in response to pathogens or to enhance vaccine efficacy.



2. General mechanisms regulating sex bias in autoimmunity and allergy

The reason why women have stronger immunity and higher incidence of autoimmunity and allergic diseases are starting to emerge (Klein & Flanagan, 2016; Markle & Fish, 2014). Although a strong effect of sex hormones, particularly estrogens, has long been suspected to mainly contribute to the reported female predominance of these diseases, recent data demonstrate that female sex hormones are not the only cause of the female predisposition. Below, we will summarize some important mechanisms presumably involved in the sexual dimorphism in autoimmune and allergic diseases.

2.1 X chromosome gene dosage and X chromosome inactivation escape

Sex-specific immune response can be influenced by both sex hormones produced by the gonads and X-linked genes, both factors may act in additive or independent manner to regulate sex-specific responses of particular cell subset, as we have shown for the TLR7 driven-response of human pDCs (Laffont et al., 2014). Alternatively, X chromosome dosage may affect susceptibility to autoimmunity in a sex-hormone independent manner (Smith-Bouvier et al., 2008). XY mice lacking the Y chromosome-specific Sry gene developed with ovaries instead of testis, and shared a female hormonal background similar to XX mice. Backcrossing these females to males expressing a Sry transgene on autosome resulted in XX Sry and XY⁻ Sry testes-bearing

animals. Thus, it was possible to compare XX and XY⁻ mice irrespective of gonadal sex. In both model, the XX sex chromosome carriers developed enhanced susceptibility to autoimmunity, such as SLE, compare to XY mice (Sasidhar, Itoh, Gold, Lawson, & Voskuhl, 2012; Smith-Bouvier et al., 2008). This effect of X chromosome complement was independent of the presence of sex hormones (Sasidhar et al., 2012; Smith-Bouvier et al., 2008). A strong indication that the number of X chromosomes, not just sex itself, is important in lupus comes with the observation that males with Klinefelter syndrome, who carry one or more extra X chromosomes, develop lupus and Sjögren's syndrome with a risk similar to females (Harris et al., 2016; Scofield et al., 2008; Seminog, Seminog, Yeates, & Goldacre, 2015). Men with SLE have been described expressing the extremely rare 46, XX male karyotype (prevalence of 1 in 20,000 live male birth) with one X chromosome expressing the Sry gene due to abnormal cross-over during meiosis. A comprehensive evaluation of X chromosome aneuploidy identified such a rare case in a cohort of 316 men with SLE, supporting further the hypothesis that two X chromosomes impart risk of SLE compared to the presence of only one X chromosome (Dillon et al., 2012). In contrast, lupus prevalence is low in women with 45,X Turner Syndrome, though these patients developed various autoimmune disorders (Cooney et al., 2009). Together, these observations led to the hypothesis that candidate genes on the inactive X chromosome (Fig. 1) could potentially contribute to the development of autoimmunity, particularly SLE and Sjögren's syndromes.

In female mammals, one of the two X chromosomes is randomly inactivated to equalize dosage of gene expression between sexes. The process of X chromosome inactivation (XCI) results in cellular mosaicism, where about one-half of the cells in a tissue express the maternal X chromosome and the other half the paternal one's. X inactivation is established at an early stage of female embryogenesis but 15–23% of X-linked human genes escape XCI and are thus expressed from both the active (Xa) and inactive (Xi) X chromosome in certain tissues or individuals (Carrel & Willard, 2005; Tukiainen et al., 2017). The X chromosome encodes many immunity-associated genes, including FOXP3, IL2RG, CXCR3, CD40L, BTK, IRAK1, OGT and the ssRNA sensing TLRs, TLR7 and TLR8, some of them have been shown to escape XCI to variable levels (Fig. 1). Among these X-linked genes, TLR7 and TLR8, most likely represent the best candidate genes, as studies demonstrated that expression of two copies of either TLR7 or TLR8 alone is sufficient to induce

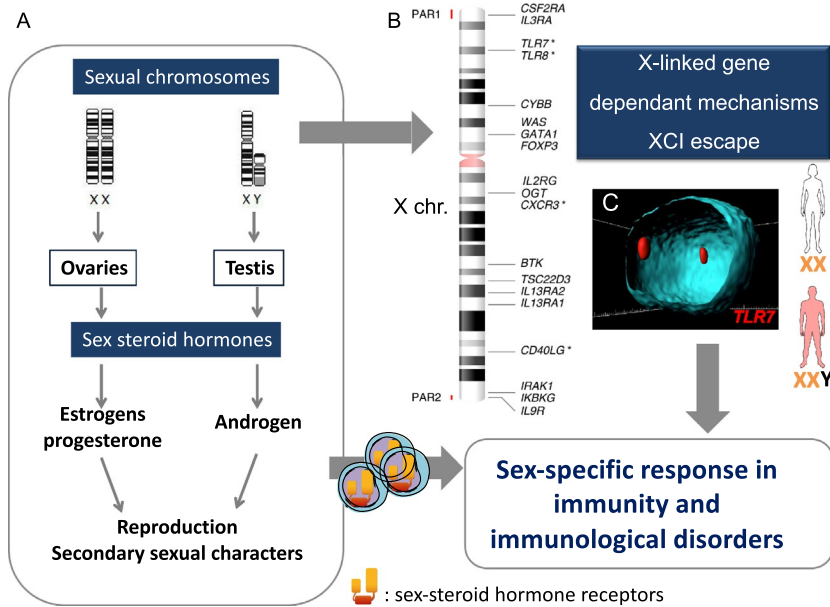


Fig. 1 Sex chromosome and sex hormones in the regulation of immunity. (A) The biological factors that govern the differences between female and male immune systems are the sex steroid hormones produced by the ovaries and testis and acting through nuclear receptor family members, and sex chromosomes, the presence of two X chromosomes versus one X and one Y chromosome. Sex hormones and sex chromosomes may act in concert or independently to govern directly or indirectly a number of factors in the host immune cells, through cell-intrinsic signaling or sex-specific regulation of X-linked gene expression thereby directly regulating immune cell development and functions. (B) The X chromosome contains some 1100 genes, some of them involve in adaptive or innate immunity (Fish, 2008; Libert, Dejager, & Pinheiro, 2010). The gene encoding for the ssRNA-sensing TLRs, *TLR7* and *TLR8*, are located on the short arm of the X chromosome within a region reported to escape XCI (Carrel & Willard, 2005). Asterisks denote genes reported or suspected to escape XCI, according to references (Lu et al., 2007; McDonald et al., 2015; Souyris et al., 2018; Wang et al., 2016). (C) 3-D visualization of *TLR7* primary transcripts by FISH RNA showing biallelic expression of *TLR7* in a B cell obtained from a 47, XXY Klinefelter syndrome man (Souyris et al., 2018). XCI, X chromosome inactivation escape.

autoimmunity in otherwise wild type animals (Deane et al., 2007; Guiducci et al., 2013), whereas *Tlr7*-deficient mice of a lupus-prone genetic background are significantly protected from autoimmunity (Green & Marshak-Rothstein, 2011). Consequently, it has been hypothesized that gene-dose effects due to escape from XCI of immune related X-linked genes, such as *TLR7* or *CD40L*, could contribute to the sex bias in SLE susceptibility (Fig. 1).

2.2 Sex and microbiome composition

Sex-specific determinants, like sex hormones, have also been recently reported to determine microbiome composition and to potentially influence susceptibility to autoimmunity (Markle et al., 2013; Yurkovetskiy et al., 2013). In these two studies, changes in microbiome composition were reported in NOD mice after puberty, and evidence were provided that male-associated changes of gut microbiota were prevented by castration and were associated with strong protection of T1D (Markle et al., 2013; Yurkovetskiy et al., 2013). Indeed, adoptive transfer of male commensal bacteria to young females causes systemic hormonal and metabolic changes together with protection from T1D (Markle et al., 2013; Yurkovetskiy et al., 2013). These protective effects of male-specific microbiota were ablated by inhibition of androgen receptor signaling in vivo (Markle et al., 2013).

Microbiota dysbiosis has also been observed in SLE patients; however, it is not clear whether the altered microbiota directly contributes to disease onset and progression or whether it is a result of disease process (Christou, Banos, Kosmara, Bertsias, & Boumpas, 2019). A candidate pathobiont has recently been identified with *E. Gallinarum*. *E. Gallinarum* has been shown to translocate from the gut to the liver and other systemic tissues to promote autoimmunity in lupus-prone (NSW x BXSB) F1 male mice (Manfredo Vieira et al., 2018). The interactions between bacteria and sex were not addressed in this model as both sexes in (NZW x BXSB)F1 mice develop an early SLE-like disease (Hang, Izui, & Dixon, 1981). In males, the disease resembled that in the BXSB male parent and is not affected by sex hormonal manipulation unlike in female mice (Hang et al., 1981). The abnormal male sex bias in BXSB mice was subsequently shown to arise from the translocation of 16 genes from the X chromosome onto the Y chromosome, with a major contribution of *Tlr7* gene, endowing all male immune cells with 2 copies of this gene (Deane et al., 2007; Pisitkun et al., 2006). Whether *E. Gallinarum* which was found in liver biopsies from SLE patients (most female), participate to the female predisposition to SLE is still unknown (Manfredo Vieira et al., 2018).

Although, hormonal shift at puberty may contribute to divergence in microbiome composition between male and female (Markle et al., 2013; Yurkovetskiy et al., 2013), whether this mechanism dominantly contributes to the sex bias in the susceptibility to autoimmunity in Humans remains to be established. For instance, unlike in NOD mice, there is no major sex bias in T1D in Humans (Whitacre, 2001), and the susceptibility to develop SLE

or Sjögren's syndrome is equivalent between women and men with Klinefelter syndrome, suggesting a role for non-hormonal related genetic factors (Dillon et al., 2011; Scofield et al., 2008).

Thus, despite evidence showing that sex hormones may influence autoimmunity through indirect effects on environmental factors such as microbiome composition (Markle et al., 2013; Yurkovetskiy et al., 2013), the precise mechanism orchestrating the bidirectional interactions between the microbiome and sex hormones are still unclear and will require further investigations. Besides this indirect effect of sex-specific determinants, evidence also exist that sex hormones may act directly to modulate phenotype and functions of immune cells involved in the immunopathological processes (Markle & Fish, 2014).

2.3 Sex steroid hormones signaling

Sex hormones mediate their effects through estrogen receptors (ER α and ER β), androgen receptor (AR), and progesterone receptors (PR-A and PR-B), which are ligand inducible transcription factors. ERs, PRs, and AR regulate gene transcription through direct binding to specific response elements at specific DNA sites leading to epigenetic modifications of chromatin and direct transcription of target genes. Moreover, multiple receptors can bind to the same cis-elements on chromatin. For instance, AR and glucocorticoid receptor ChIP-seq studies have shown that AR and GR are capable of binding to the same sites on chromatin and the shared AR- and GR-binding events are significantly associated with genes regulated by both androgen and glucocorticoid (Pihlajamaa, Sahu, & Janne, 2015). Alternatively, after ligand activation, sex steroid hormone receptors can bind to other transcription factors, such as Fos/Jun AP-1 complex or SP-1 for ER α , and regulate gene expression through indirect DNA binding (Arnal et al., 2017). Beside the "genomic/nuclear" actions, estrogens and androgens can induce the activation of several kinases, as well as changes in calcium level. These Membrane Initiated Steroid Signaling (MISS) actions are mediated by a pool of intracellular receptors localized at the plasma membrane (Arnal et al., 2017; Pihlajamaa et al., 2015)

Immune cells, including innate cells, express sex-steroid hormone receptors (Fig. 1), including ERs (Esr1, Esr2) and AR to various degrees depending on their microenvironment (Kadel & Kovats, 2018). For instance, group 2 innate lymphoid cells express Esr-1 when they are isolated from the uterus (Bartemes, Chen, Iijima, Drake, & Kita, 2018), whereas

Esr-1 is barely detected in bone marrow ILC2 progenitor and tissue-resident ILC2 populations, which selectively express high level of Ar transcripts (Laffont, Blanquart, & Guery, 2017; Laffont, Blanquart, Savignac, et al., 2017; Laffont, Seillet, & Guery, 2017) (see below Fig. 2). Of note, elegant studies have defined the role of sex hormone receptor signaling in the direct regulation of innate and adaptive components of the immune systems in various pathophysiological contexts in vivo (Calippe et al., 2010; Garnier et al., 2018; Griesbeck et al., 2015; Kim et al., 2017; Laffont, Blanquart, & Guery, 2017; Laffont, Blanquart, Savignac, et al., 2017; Laffont, Seillet, & Guery, 2017; Lelu et al., 2011; Mohammad et al., 2018; Seillet et al., 2012; Zeng et al., 2018).

In this review, we will cover the current understanding of the impact of sex-linked factors in allergic inflammation and autoimmune diseases, with a particular focus on the role of sex steroid hormones on the development and function of immune cell populations directly involved in type 2 immunity ((Laffont, Blanquart, & Guery, 2017; Laffont, Blanquart, Savignac, et al., 2017; Laffont, Seillet, & Guery, 2017), together with the recent evidence demonstrating that the X-linked Toll like receptor 7 (TLR7) escapes from X chromosome inactivation in immune cells, and its possible implication in the enhanced susceptibility of women and 47, XXY Klinefelter syndrome men to systemic autoimmunity (Souyris et al., 2018).

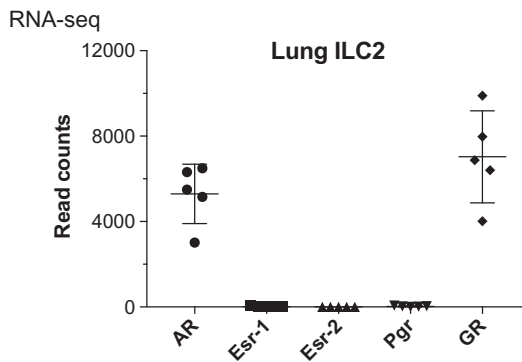


Fig. 2 Tissue-resident ILC2 selectively express the androgen receptor. ILC2 from steady state mice expressing the *Il5* reporter allele Red5 (R5) were sorted and analyzed by RNA sequencing as shown (Ricardo-Gonzalez et al., 2018). The figure shows the read counts obtained for the indicated sex hormone receptors Esr-1 (estrogen receptor α), Esr-2 (estrogen receptor β), Pgr (progesterone receptor) and the glucocorticoid receptor (GR) from R5+ ILC2 isolated from the lung. Data were obtained from Ricardo-Gonzalez, R. R., Van Dyken, S. J., Schneider, C., Lee, J., Nussbaum, J. C., Liang, H. E., et al. (2018). Tissue signals imprint ILC2 identity with anticipatory function. *Nature Immunology*, 19, 1093.



3. Biological role of sex-linked factors in allergic asthma

3.1 Gender bias in asthma prevalence and severity

Asthma is one of the most common chronic long-term health conditions in Europe, affecting over 30 million people aged 45 years and under in Europe (Edwards et al., 2017). Asthma is a heterogeneous, complex disease with clinical phenotypes that incorporate persistent symptoms and acute exacerbations. Asthma is a chronic inflammation of the airways principally mediated by exacerbated type 2 immune responses (Lambrecht & Hammad, 2015; Lloyd & Snelgrove, 2018). Sex differences in asthma prevalence and phenotypes have been well described (Almqvist et al., 2008; Carey, Card, Voltz, et al., 2007; Holgate et al., 2015). Asthma is more common in males from birth until puberty (Peroni, Piacentini, Bodini, & Boner, 2009) (Holgate et al., 2015) but becomes more prevalent (Carey, Card, Voltz, et al., 2007; Zein & Erzurum, 2015) and more severe (Almqvist et al., 2008; Fu et al., 2014; Tantisira et al., 2008) in females after puberty.

Patients with severe and difficult to treat asthma are more represented in women above 25 years of age (Edwards et al., 2017). Although this switch was attributed to a late incidence of asthma among girls (Almqvist et al., 2008; Nicolai, Pereszlenyiova-Bliznakova, Illi, Reinhardt, & von Mutius, 2003), higher rate of asthma remission has been reported in boys compared to girls (Arshad et al., 2014). High-resolution time course analysis of the natural progression of asthma symptoms through childhood and adolescence have shown that greater severity in boys before age 7 years is followed by a continuous decline of symptom severity in both sexes at ages 7–9 years (Fu et al., 2014). Whereas symptom severity remained low in boys at ages 10–17 years, female sex dominance in severity started precisely at the onset of puberty, and continues to worsen at late puberty and at age 17 years (Fu et al., 2014). Moreover, in a longitudinal study the shift in asthma prevalence during the pubertal period was associated with a higher incidence and lower rate of remission of asthma in female compared with male subjects (Vink, Postma, Schouten, Rosmalen, & Boezen, 2010). Together, these observations suggest a positive impact of female sex hormones on asthma. Indeed, changes in asthma outcomes have been observed in women in and around menstruation (Rao et al., 2013), pregnancy (Schatz et al., 1988; Virchow, 2012), menopause (Foschino Barbaro et al., 2010; Troisi, Speizer, Willett, Trichopoulos, & Rosner, 1995) and oral contraceptive use (Tan, McFarlane, & Lipworth, 1997). By contrast, the drop in asthma

incidence observed in and around the time of puberty in males, as well as the increased numbers of remission observed in males is strongly suggestive of a protective action of male sex hormones (Almqvist et al., 2008; Carey, Card, Voltz, et al., 2007). These observations fit with the commonly accepted immunosuppressive role attributed to androgens, which generally suppress immune cell reactivity (Klein & Flanagan, 2016). Although, one cannot exclude that sex hormones may play a role in the pathogenesis of asthma through direct regulation of lung development and function (Townsend, Miller, & Prakash, 2012), we and others have hypothesized that these changes from male to female predominance are thought to be due to biological processes by which sex hormones directly modulate the immune system (Fuseini & Newcomb, 2017; Keselman & Heller, 2015; Laffont, Blanquart, & Guéry, 2017). Below, we will summarize the recent findings pointing to a major role of sex hormones in regulating the immunological cellular actors involved in allergic asthma with a focus on the emerging evidence of the protective action of male sex steroid hormone androgens, through the negative regulation of group 2 innate lymphoid cells (ILC2).

3.2 Immunological mechanisms of allergic asthma

Asthma is a complex and chronic inflammation characterized for most patients by allergic inflammation of the airway and associated with type 2 immune responses (Holgate et al., 2015; Lambrecht & Hammad, 2015). The immunological mechanisms of allergic asthma have been described in many papers including an excellent review by Lloyd and Snelgrove (Lloyd & Snelgrove, 2018). Herein, we will mainly focus on the key events leading to the disease and type 2 lung inflammation. Almost 10 years ago now, the discovery of ILC2 as a potent innate source of type 2 cytokines (IL-5, IL-13, IL-9) within tissue adds a new early effector in the cascade of events leading to asthma development. Activation of ILC2 is typically driven by alarmins (IL-33, IL-25 and TSLP) released by damaged epithelial cells following allergen exposure. It has been recently shown that a broad array of environmental allergens (fungi, pollen, HDM) possesses a common intrinsic proteolytic activity leading to cleavage and fully activation of IL-33 (Cayrol et al., 2018; Cayrol & Girard, 2018). After activation, ILC2s proliferate and secrete large amount of IL-5 and IL-13. IL-5 promotes differentiation and recruitment of eosinophils to the lung, whereas IL-13 plays pleiotropic role among which increasing mucus production and bronchoconstriction. IL-9 favors mast cell proliferation and activation.

Model of ILC2 deficiency has demonstrated not only the key role of ILC2 in innate cell activation but also their contribution of Th2 cell response to allergen protease in the lung (Halim et al., 2014). ILC2-derived IL-13 licenses lung DCs to migrate to the draining lymph node and to prime Th2 cells which are necessary to induce IgE production by B lymphocytes (Halim et al., 2014). Th2 then will migrate back to the lung and promote IgE secretion and contribute to inflammation through type 2-cytokine secretion. ILC2 can also promote directly Th2 responses either through their capacity to express MHC class II molecules (Oliphant et al., 2014), or through the delivery OX40L-mediated instructive signals thereby promoting IL-33-driven expansion of Th2 and Treg cells (Halim et al., 2018). Thus, ILC2 are likely to contribute at multiple and sequentially distinct levels to enhance adaptive Th2 immunity (Lloyd & Snelgrove, 2018).

In Human, numerous studies have also demonstrated that the numbers and the activation status of ILC2 are increased in samples (sputum, blood) from pediatric and adult asthmatic patients compared with controls (Doherty & Broide, 2018). In addition, GWAS studies identify some of the top susceptibility genes in asthma to be linked with ILC2 biology (*IL33*, the IL33 receptor *IL1RL1*, *RORA*, *IL2RB*, and *IL13*) (Moffatt et al., 2010; Torgerson et al., 2011). Furthermore, a recent study has demonstrated that genetic polymorphisms associated with asthma localized with gene regulatory elements in ILC2 (Stadhouders et al., 2018). Together, these studies in Human suggest that ILC2 most probably play a pathogenic role in patients with allergic asthma.

3.3 Impact of sex hormones in type 2-mediated allergic airway inflammation

Similarly to what is observed in human disease, female mice are also more susceptible to asthma compared to males. Indeed, cardinal features of asthma (IgE production, lung eosinophilia, type 2 cytokines production in tissues, airway hyperresponsiveness) are higher in females either in the OVA or the HDM model of allergic asthma (Blacquiere et al., 2010; Fuseini et al., 2018; Melgert et al., 2005; Takeda et al., 2013).

This sex bias in allergic asthma raised the question of the respective contribution of sexual hormones (estrogens, androgens). Estrogen effects on asthma seem complex with studies being difficult to reconcile. Indeed, on one hand it has been shown, either in the OVA or the HDM model of asthma, that ovariectomized mice had reduced eosinophilia, IgE production and airway hyperreactivity (Fuseini et al., 2018; Riffó-Vasquez, Ligeiro

de Oliveira, Page, Spina, & Tavares-de-Lima, 2007), suggesting that ovarian hormones increased lung inflammation. On the other hand, ER α deficiency was associated with enhanced airway responsiveness at steady-state and upon allergen sensitization without any impact on inflammation. However, in this model, ovariectomy or estrogen-supplementation of WT mice failed to recapitulate the phenotype of constitutive ER α -deficient mice, suggesting a ligand-independent mechanism of action. These results suggest that ER α signaling could down-regulate airway hyperresponsiveness by acting on airway smooth muscle and nerves, rather than in immunocompetent cells (Carey, Card, Bradbury, et al., 2007). This is contrary to expectation and goes against the epidemiological and experimental studies showing exacerbated incidence and severity of asthma in females. Therefore, studies are still needed to firmly establish if and how estrogen-mediated cell-intrinsic signaling of ER α in immune cells could contribute to the enhanced asthma severity in females. On the contrary, studies aimed at analyzing the role of androgens and androgen-receptor signaling on asthma have concluded to a protective role of the male hormone on lung inflammation. Indeed, while castration of male abolished sex bias in asthma (Fuseini et al., 2018; Hayashi, Adachi, Hasegawa, & Morimoto, 2003), administration of 5 α -dihydrotestosterone (DHT) (Becerra-Diaz, Strickland, Keselman, & Heller, 2018) or dehydroepiandrosterone (DHEA), a natural steroid hormone secreted by the adrenal gland and converted into androgens or estrogens, limited OVA-induced asthma (Liou & Huang, 2011). Furthermore, in a mouse model with a mutation in the *Ar* gene rendering mice unresponsive to androgen, it has been shown that AR signaling limits HDM-associated eosinophilia (Fuseini et al., 2018). Taken together, estrogens and androgens seem to mainly play opposite role in asthma susceptibility. Using genetic and pharmacological approaches a clearer picture is now emerging of the cellular targets that could be responsible for the sex hormone mediated regulation of allergic asthma, particularly regarding the role of androgens.

3.4 Regulation of ILC2 by androgen receptor signaling

Based on the central role of ILC2 in asthma pathophysiology and the evidence of sex bias in the disease, ILC2 were suspected to be differently regulated between males and females. Indeed, at steady state, the frequency and absolute number of ILC2s were increased in the lungs of female mice compared to males, as well as in adipose tissue and mesenteric lymph nodes

(Laffont, Blanquart, & Guery, 2017; Laffont, Blanquart, Savignac, et al., 2017; Laffont, Seillet, & Guery, 2017). This higher proportion of ILC2 in female lung was also observed during lung inflammation induced by IL-33 (Laffont, Blanquart, & Guery, 2017; Laffont, Blanquart, Savignac, et al., 2017; Laffont, Seillet, & Guery, 2017) or *Alternaria* extract administration (Cephus et al., 2017). These differences in ILC2 number and phenotype were associated with an increased capacity of ILC2 from females to produce IL-5 and IL-14 (Kadel et al., 2018; Warren et al., 2017). In female, a major subset of ILC2 expressing high level of KLRG1 was lacking (Cephus et al., 2017; Kadel et al., 2018; Laffont, Blanquart, & Guery, 2017; Laffont, Blanquart, Savignac, et al., 2017; Laffont, Seillet, & Guery, 2017). Whether the absence of KLRG1 expression on ILC2 plays a role in lung inflammation, as it has been reported for human skin ILC2 in atopic dermatitis (Salimi et al., 2013) remains to be evaluated. Altogether, these works suggested that naturally produced androgens, via activation of AR in ILC2, were responsible for the sex differences observed in lung ILC2 number and phenotype by acting as a powerful negative regulator of their in vivo development and expansion (Laffont, Blanquart, & Guery, 2017). Indeed, using mix bone-marrow chimeras we could further demonstrate that androgen, via the cell-intrinsic activation of its receptor *Ar* in ILC2 was responsible for the sex differences observed in lung ILC2 number and phenotype. Moreover, using an in vitro model of IL-33-driven ILC2 differentiation, the pure AR agonist 5 α -DHT or a selective androgen receptor modulator were reported to regulate the in vitro development of ILC2, while increasing KLRG1 expression through *Ar*-signaling (Laffont, Blanquart, & Guery, 2017; Laffont, Blanquart, Savignac, et al., 2017; Laffont, Seillet, & Guery, 2017), see patent WO/2018/185030). Indeed, like ILC2p (Laffont, Blanquart, & Guery, 2017; Laffont, Blanquart, Savignac, et al., 2017; Laffont, Seillet, & Guery, 2017; Ricardo-Gonzalez et al., 2018), lung ILC2 selectively expressed the *Ar* gene among other sexual hormone receptors (Fig. 2), in agreement with previous observations describing *Ar* as a prototypical signature gene of tissue-resident ILC2 (Robinette et al., 2015). The absence of estrogen receptor gene expression in lung ILC2 is consistent with the lack of effect of ovariectomy, estrogen administration or *Esr-1* gene deletion in lung ILC2 numbers (Cephus et al., 2017; Laffont, Blanquart, & Guery, 2017; Laffont, Blanquart, Savignac, et al., 2017).

In Human, and in agreement with data obtained in murine model of lung inflammation, women with asthma have increased numbers of circulating

ILC2 compare with men (Cephus et al., 2017). Studies are now needed to determine whether androgens are also key inhibitors of human ILC2 and to consider the modulation of AR as future therapeutics in asthma. Androgen-mediated regulation of ILC2 could therefore contribute to the switch of asthma susceptibility between sexes around puberty.



4. Biological role of sex-linked factors in TLR7-driven antiviral responses and in systemic autoimmunity

A growing body of data shows that sex hormones and sex-chromosome loci regulate biological pathways common to autoimmune and infectious diseases (Libert et al., 2010; Markle & Fish, 2014). This is particularly clear for TLR7, a single-stranded RNA receptor encoded by an X-linked gene. The response initiated by TLR7-mediated sensing of ssRNA by innate immune cells and B cells is an essential line of defense against RNA viruses. However, TLR7 can also respond to endogenous ssRNA containing ligands, potentially leading to autoimmunity or inflammation if not properly controlled (Theofilopoulos, Kono, & Baccala, 2017).

4.1 Role of TLR7 in the germinal center (GC) responses to ssRNA viruses and virus-like particles (VLP)

B cell-intrinsic Myd88-TLR7 signaling is essential to the germinal center responses to exogenous retrovirus (Browne, 2011; Browne & Littman, 2009). Likewise, TLR7 is critical for the antibody-dependent control of endogenous retroviruses (ERV), and lack of TLR7 results in retroviremia and ERV-induced tumor development (Young et al., 2012; Yu et al., 2012). In chronic lymphocytic choriomeningitis virus (LCMV) infection, B cell-intrinsic TLR7 signaling was also required for optimal B cell responses and GC formation (Clingan & Matloubian, 2013; Walsh et al., 2012). Together, these results indicate that TLR7 signaling is critical to promote plasma cell output by enhancing GC selection of antigen-specific B cells (Rawlings, Schwartz, Jackson, & Meyer-Bahlburg, 2012). Bacterial phage Q β virus-like particles (VLP) had emerged as a useful model to study the TLR-dependent activation and differentiation of Ag-specific B cells under physiological context in vivo (Hou et al., 2011; Tian et al., 2018). Q β -VLP can package host bacterial ssRNA and are therefore equipped with unique feature of natural pathogens, which can serve as a potent TLR7 ligand to promote Ab production and GC responses (Hou et al., 2011; Tian et al., 2018). Indeed, it has been established that Myd88-signaling in B cells was

critical for the anti-Q β -VLP GC response (Hou et al., 2011; Tian et al., 2018). This model likely represents the antiviral response more closely than the T cell-dependent (TD) antibody responses to soluble protein antigens and pointed to a critical non-redundant role of TLR7-driven B cell activation in GC reaction (Hou et al., 2011; Tian et al., 2018). Indeed, using this model it has been recently reported that B cells are the dominant Ag-presenting cells inducing naïve CD4 T cell activation and differentiation in response to Q β -VLP or influenza virus vaccination (Hong et al., 2018). Again, B cell-intrinsic Myd88-signaling was required for this process illustrating further the critical function of TLR7 activated B cells in the generation of T cell dependent GC response to RNA viruses, as opposed to the role of DC in the GC response to soluble protein Ag (Hong et al., 2018). Of note, female mice have been reported to mount greater humoral and cell-mediated immune responses to influenza infection and vaccination than males (Fink, Engle, Ursin, Tang, & Klein, 2018). In addition, female mice immunized with the influenza trivalent inactivated vaccine or infected with live H1N1 or H3N2 viruses develop greater neutralizing antibody titers than males (Lorenzo et al., 2011; Zivkovic et al., 2015). Interestingly, deletion of Tlr7 reduces sex differences in vaccine-induced antibody responses and protection, suggesting that sex-based differences in expression of Tlr7 in B cells may contribute to greater antibody production in females than males (Fink et al., 2018). Indeed, it has been recently reported that TLR7 escapes XCI in human B cells and that biallelic B cells show enhanced capacity to differentiate into plasmablasts and to class switch toward IgG (Souyris et al., 2018), see Fig. 3. In agreement with previous hypothesis by others, better TLR7-driven responses in females might have been positively selected during evolution in mammals as it would favor qualitatively and quantitatively better antibody responses in females against ssRNA viruses, thereby improving greater survival of offspring through the vertical transfer of protective antibody (Fink & Klein, 2018). It is therefore likely that a direct cost of this evolutionary benefit is the higher susceptibility of women to TLR7-driven antibody mediated autoimmune diseases, such as SLE or Sjögren syndrome.

4.2 Role of TLR7 dosage in break-down of B cell tolerance in SLE

SLE is a chronic disorder characterized by high-affinity autoantibodies against macromolecular complexes with a nucleic acid component: RNA associated with the U1 small nuclear RNP or the Ro60 autoantigen, and double-stranded DNA (dsDNA) (Theofilopoulos et al., 2017). The disease

is clinically heterogeneous in its manifestations and course, and affects many organs and tissues including the kidney, skin, joints, and central nervous system (Petri et al., 2012).

The first observation of a key role of TLR7 came from the analysis of SLE mechanism in the BXSB mouse models, in which males are more susceptible to spontaneous development of lupus-like disease, along with high-titer anti-cardiolipin and anti-Sm/RNP autoantibodies and glomerulonephritis compared to female mice. The higher incidence of disease in males is due to a translocation of a cluster of X-linked genes onto the Y chromosome (Pisitkun et al., 2006; Subramanian et al., 2006). In this model, male BXSB animals carry a supernumerary copy of the *Tlr7* gene on their Y chromosome. This extra copy of *Tlr7* was associated with twofold increase in *Tlr7* transcription and protein expression, and was found dominantly responsible of this phenotype, emphasizing the relationship between TLR7 dosage, autoantibodies targeting RNA-associated proteins and disease pathogenesis (Deane et al., 2007; Fairhurst et al., 2008; Santiago-Raber et al., 2008). Likewise, four- to eightfold overexpression of *Tlr7* is sufficient for the spontaneous onset of SLE in non-autoimmune strains (Deane et al., 2007), and selective suppression of *Tlr7* expression in lupus-prone mice is, conversely, protective against the disease (Christensen et al., 2006).

Although, TLR7-signaling may control SLE development at multiple levels, for example through TLR7 expressed in pDCs (Crow, 2014), neutrophils (Garcia-Romo et al., 2011; Lande et al., 2011), or monocytes (Jacquemin et al., 2015), so far the critical non-redundant role of TLR7 has only been demonstrated in B cell responses (Jackson et al., 2014; Soni et al., 2014). Cell-intrinsic activation of TLR7 has been shown to directly contribute to the functional responses of B cells, in particular the production of antibodies against the Sm/RNP autoantigen in SLE-prone mouse models (Hung et al., 2015; Hwang et al., 2012; Jackson et al., 2014; Lau et al., 2005; Soni et al., 2014). In a BAC transgenic system, subtle upregulation of *Tlr7* expression was sufficient to drive SLE when combined with the *Sle1* locus (Hwang et al., 2012). Specific deletion of the additional copies of *Tlr7* encoded in the transgene specifically in B cells abolished the development of antibodies to RNA-protein complexes and protected from SLE disease (Hwang et al., 2012). Elegant studies subsequently established the essential role of endogenous TLR7, but not TLR9, as a critical B cell-intrinsic sensor for the development of spontaneous GC response

in both autoimmune and non-autoimmune responses (Jackson et al., 2014; Soni et al., 2014).

Together, these studies firmly established the direct causal link between intrinsic expression of *Tlr7* within B cells and the development of many autoimmune traits associated with SLE (Hwang et al., 2012; Jackson et al., 2014; Soni et al., 2014), underscoring the importance of tight regulation of TLR7 expression to prevent spontaneous autoimmunity. Thus, as for B cell responses to RNA virus, B cell activation through endosomal TLR7 is a critical component of the GC response in the context of SLE.

4.3 Role of TLR7 in the development of age-associated B cells (ABC) and in the extrafollicular B cell response in SLE

Sex-specific differences in leukocyte population have been surveyed in young and adult mice. Although, no major sex- or age-associated bias were observed for T cells or dendritic cells, a particular population of B cells bearing CD11b and CD11c, and lacking CD21, was specifically identified in aged non-autoimmune prone female mice. These age-associated B cells (ABCs) were also found upregulated in lupus-prone mice at the time of disease onset (Rubtsov et al., 2011). Spontaneous ABC development was lost in Myd88- or TLR7-deficient mice, suggesting a critical role for B cell-intrinsic TLR7 signaling in their development, although this was not directly established (Rubtsov et al., 2011).

The appearance of CD11b⁺/CD11c⁺ ABC-like cells was found to be critical for effective viral clearance, and was dependent on T-bet expression in B cells, which was synergistically driven through both TLR7- and IFN- γ receptor signaling (Rubtsova, Rubtsov, van Dyk, Kappler, & Marrack, 2013). Interestingly, conditional deletion of T-bet in B cells protected from lupus-like autoimmunity and abrogated spontaneous GC formation (Rubtsova et al., 2017), in agreement with this observation, IFN- γ R is needed for spontaneous GC appearance in lupus-prone mouse models (Domeier et al., 2016; Jackson et al., 2016). Given, the spontaneous appearance of ABCs selectively in female, the contribution of ABCs in TLR7-driven autoimmunity and the strong requirement of TLR7-signaling for their development, the contribution of X chromosome dosage effects due to TLR7 bi-allelic expression in ABC development warrants further investigation.

A human equivalent of the T-bet⁺ CD11c^{bright} mouse ABC has been recently characterized in humans (Jenks et al., 2018). These cells called

double negative (DN2) because they do not express memory (CD27) nor naïve (IgD) markers, lack CD21 and CXCR5 expression and expressed T-bet and CD11c, were classified as extrafollicular B cells. They were found to expand in SLE patients, particularly those with severe disease, strongly correlating with nephritis and anti-Sm/RNP autoantibodies. Like, their mouse ABC counterpart, DN2 were found to be hyper responsive to TLR7 ligands and were poised to generate robust PC differentiation in the presence of IL-21 and IFN- γ , even in the absence of BCR stimulation. In vitro, BCR-activated naïve B cells in the presence of IL-21/IFN- γ and TLR7 ligands were shown to differentiate into DN2 and PC lineage B cells, suggesting that DN2 may derived from activated naïve B cells in vivo. Altogether, these results indicate that TLR7 could also contribute to the extrafollicular pathogenic B cell responses in SLE.

4.4 TLR7 escapes from X chromosome inactivation

The X chromosome encompasses 155 Mb and over 1000 genes (Ross et al., 2005). The pseudoautosomal regions of the chromosome, PAR1 and PAR2, carry a minority of X-linked genes with functional homologs on the Y chromosome, and these genes (e.g., CSF2RA SLC25A6 and IL3RA on PAR1 or IL9R on PAR2) are not subjected to XCI (Fig. 1). Indeed, these telomeric regions are the only portion of the sex chromosomes that can recombine during male meiosis (Graves, Wakefield, & Toder, 1998). Most of the genes located outside the pseudoautosomal regions, however, are unique to the X chromosome and therefore carried in two copies in women but only one in men. These genes include many protein-coding and miRNA genes known to shape the immune response (Fish, 2008; Libert et al., 2010; Pinheiro, Dejager, & Libert, 2011). Loss-of-function mutations in some of these genes (e.g., *BTK*, *WAS*, *IL2RG*, *FOXP3*) cause X-linked primary immunodeficiencies (Libert et al., 2010). The genes of two RNA-sensing endosomal TLRs, *TLR7* and *TLR8*, are carried on a non-pseudoautosomal region of Xp and are maintained under strong purifying selection, which attests to non-redundant vital functions (Barreiro et al., 2009) (Fig. 1).

In female mammals, one of the two X chromosomes undergoes random inactivation to equalize expression of X-linked genes between XX females and XY males. However, XCI is incomplete in humans. Using primary fibroblast cell lines, early studies estimated that approximately 15% of

X-linked genes escape inactivation (Carrel & Willard, 2005). Of these, most of the genes in the PAR1 and PAR2 regions escape XCI constantly, while other genes escape randomly or in a tissue-specific manner (Carrel & Willard, 2005; Vermeesch et al., 1997). Interestingly, the proportion of genes escaping inactivation was found to differ dramatically between different regions of the X chromosome, most of the genes that escape XCI were enriched in clusters in the short arm of the Xp, a region containing the *TLR7* locus (Carrel & Willard, 2005). However, additional escapees were found distributed throughout the X chromosome (Carrel & Willard, 2005; Cotton et al., 2011). More recently, Tukiainen et al. extended the analysis of XCI to cells obtained directly from human tissues and determined that XCI could affect at least 23% of X-linked genes, with heterogeneity between tissues, persons and cell types (Tukiainen et al., 2017). Although XCI escape has been reported in mice, previous surveys of XCI in various mouse cell lines showed that only 3% of mouse genes partially or completely escape XCI (Deng et al., 2015; Pinter et al., 2012; Yang, Babak, Shendure, & Disteche, 2010).

Focusing on lymphocyte populations, a recent study reported that some genes on the inactive X chromosome (Xi) are predisposed to become partially reactivated in the lymphocytes of women (Wang et al., 2016). Biallelic expression of X-linked immune-related genes such as *CXCR3* and *CD40LG* was documented in T cells and in EBV-transformed B cells with frequencies ranging from 4% to 5%. Likewise, biallelic expression of *TLR7* was also reported in subpopulations of EBV-transformed B cell lines. Although the number of subjects was too low to draw any conclusions, no marked differences in *TLR7* biallelic cell frequencies were observed in EBV-B cells between SLE sufferers and healthy women (Wang et al., 2016). Several studies have suggested that epigenetic modifications accompanying X inactivation persist in EBV-B cell lines and clones. Nevertheless, it is unclear whether the active *TLR7* allele on the Xi arose from EBV-transformation to generate the lymphoblastic B cell lines, or was a property persisting from the parental primary lymphocytes.

Recent work from our laboratory directly investigated whether the human *TLR7* gene escapes XCI in primary immune cell populations from women (Souyris et al., 2018). Using a single-cell RT-PCR approach, we profiled the mono- or biallelic expression of *TLR7* by measuring the relative expression of *TLR7* transcripts from both X chromosome in females heterozygous for *TLR7* exonic SNP markers. We showed that *TLR7* does escape

X chromosome inactivation in distinct populations of immune cells, pDCs, B cells and monocytes from women. A proportion of cells exhibiting biallelic expression of *TLR7* was observed in all donors tested, with frequencies ranging from 7% to 45%. Using RNA-FISH approaches, we documented the presence of primary *TLR7* transcripts on the inactive, XIST-painted X chromosome of B lymphocytes, monocytes and pDCs from women. Importantly, biallelic expression of *TLR7* was similarly observed in immune cells from 47,XXY Klinefelter syndrome males (Souyris et al., 2018). In support of an enhanced dosage of *TLR7* expression in women immune cells, a greater expression of *TLR7* protein in the leukocytes from women than in the male cells was also observed. The elevated *TLR7* protein dose in woman PBMCs correlated with an enhanced *TLR7*-driven proliferative response of PC lineage-committed B cells in women compared to men. By contrast, no sex bias was found when a ligand of *TLR9* (which is autosomally encoded) was used to promote B cell proliferation. In women, B cells with biallelic *TLR7* expression were enriched within the CD27^{hi} proliferating PC populations following treatment with *TLR7*-specific ligands. In support of causality, an increase in biallelic cell frequency was only found in *TLR7*-stimulated CD27^{hi} plasmablasts, whereas counter-selection of biallelic cells was instead observed in B cells that responded to a *TLR9* ligand. This demonstrated that the increase in *TLR7*-biallelic B cells was not secondary to cell proliferation, and supported the conclusion that biallelic memory B cells are endowed with enhanced responsiveness to a *TLR7* ligand. Importantly, functional consequences of *TLR7* biallelic expression was also documented in naïve B cell responses as well. Compared to monoallelic ones, naïve B cells expressing *TLR7* from both X chromosomes had enhanced expression *TLR7* mRNA at the single cell level and displayed over twofold greater propensity to IgG class switch relative to monoallelic B cells, whereas no association was found when B cells were stimulated through *TLR9* (Souyris et al., 2018).

Because increased *TLR7* is a known risk factor in lupus, we propose that higher *TLR7* dosage arising from X-inactivation escape connects the presence of two X chromosomes in females and individuals with Klinefelter syndrome with the greater risk of developing lupus (Fig. 3). Our hypothesis is that an escape of *TLR7* from XCI in B cells from women or KS men will reduce their activation threshold in response to *TLR7*-specific ligands. We posit that *TLR7* biallelic B cells are more responsive than monoallelic cells at specific checkpoints that involve signaling through *TLR7*, like IgG class

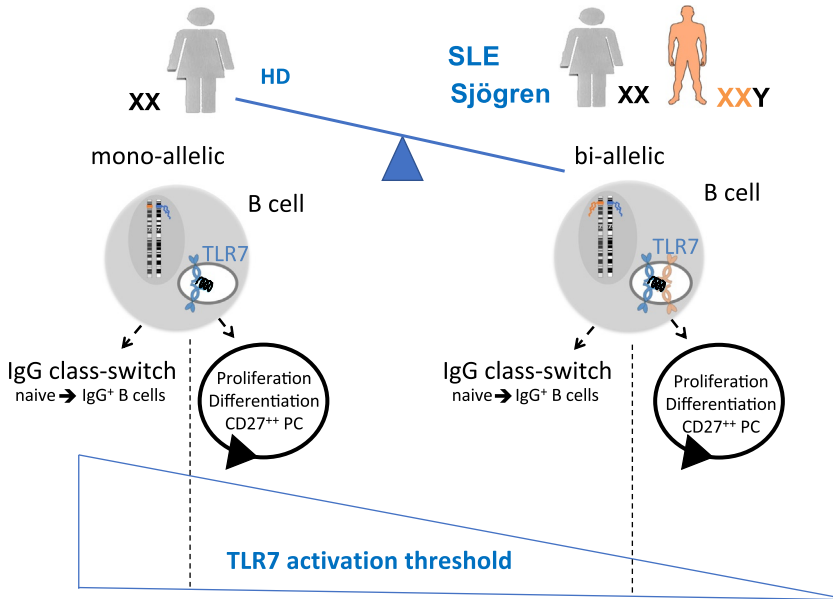


Fig. 3 Threshold hypothesis to explain the enhanced functional responses of TLR7 biallelic B cells in women or KS men. Biallelic expression of *TLR7* in B cells from women may contribute to disease pathogenesis by lessening the threshold of TLR7-signaling. B cells expressing TLR7 biallelically were more responsive than monoallelic cells at specific checkpoints of B cell differentiation that involve signaling through TLR7, such as CD27^{hi} PC lineage cell differentiation from memory B cells and naïve B cell differentiation into IgG class-switched B cells (Souyris et al., 2018). Whether female patients with rheumatic diseases deviate from healthy women regarding the frequency of biallelic cells will need further investigation.

switching, CD27^{hi} plasmablast differentiation from memory B cells, and the GC response to ssRNA-virus (Fig. 3). According to the threshold hypothesis, we expect no selective advantage of biallelic cells in the presence of high concentrations of ligands. This could be easily tested in model using ssRNA-loaded VLPs to measure GC responses and antibody production, and we could predict a lack of sex-bias in response to high concentrations of ligands, whereas lowering the doses should reveal enhanced female-specific responsiveness to TLR7-specific ssRNA-VLPs. Besides regulating adaptive B cell responses, we cannot exclude that such mechanism may also occur in innate cells, such as monocytes or pDCs, endowing TLR7 biallelic innate cells with exacerbated responsiveness to low doses of ssRNA containing ligands, further fueling inflammation and promoting chronic disease development.



5. Concluding remarks

Sex may affect susceptibility to AID and allergy through indirect effects on gut microbiota or regulation of target tissue specific factors, however so far the most striking evidence emerging actually point to cell-intrinsic effects of sex-linked factors on immune cells themselves. Enhanced immunity in female has often been attributed to the effects of sex hormone, like estrogens. Although there is no doubt that estrogens are important regulator of innate immunity (Laffont, Seillet, & Guery, 2017; Zeng et al., 2018), evidence also exist for immunosuppressive properties of the male sex hormone androgens in some situations (Kissick et al., 2014; Klein & Flanagan, 2016). This could be particularly relevant in the context of asthma or parasite infection where ILC2 play a central role in the initiation of type 2 inflammation (Tait Wojno & Artis, 2016).

Besides sex hormones, evidence are emerging for a key role of X-linked genetic factors also contribute to this gender bias. Enhanced dosage of X-linked gene contributing to adaptive and innate immunity to nucleic acid like TLR7 through XCI escape in immune cells. Although, this will require further direct demonstration in experimental models, this mechanism is likely to explain why females and males with Klinefelter syndrome are similarly susceptible to TLR7-dependent autoimmune diseases. The cumulative effect of biallelic TLR7 dosage on B cell differentiation and PC proliferation during antigen-specific development of the B cell response could enhance the antibody response to ribonucleoprotein in women with SLE, and also the normal antibody response to RNA viruses (Fink & Klein, 2018). In this functional context, TLR7 biallelic expression in females may carry an adaptive evolutionary advantage over strict monoallelic expression as observed in men. While endowing females with greater fitness in the fight against microbial pathogens and the control of ERVs, it would come at the cost of increased risk of autoimmunity.

Author contributions

All authors have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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Conflict of interest statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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