RIJKSUNIVERSITEIT GRONINGEN

Sexual dimorphism in the immune response:

endocrinologic and genetic insights

Author: Lisa Wanders
Supervisor: Dr. M.M. Faas
Master-essay
29.05.2016

Sexual dimorphism in the immune response:

endocrinologic and genetic insights

Author: Lisa Wanders
Supervisor: Dr. M.M. Faas
Group: Medical Biology

Abstract

Differences in the immune response exist between men and women. While females have a stronger adaptive immune response, including the cellular and humoral response, males have a stronger and more sensitive innate immune response. This essay reviews these differences and focuses on sex hormones and genetics as possible underlying causes. The implications for a variety of conditions are discussed. Females have a higher predisposition to acquire autoimmune diseases and are more susceptible to acute graft rejections but are at the same time more resistant to infections and gain better protection from vaccinations. Males on the other hand have a higher risk to develop multiple organ failure after trauma and severe infections after surgery. Moreover, higher rates of sepsis are observed in males compared to females. Despite extensive research in this field, many of the mechanisms underlying the sexual dimorphism in the immune response are still unknown.

Table of contents

1. Introduction

Gender differences in hormone levels and genetics do not only influence sexual differentiation and physical appearance but are also known to affect the immune system. Females are sometimes said to be immune privileged, as they amount a stronger cellular and humoral immune response and are therefore more resistant to for instance bacterial, viral or parasitic infections[1]. However, they are also at higher risk to develop certain autoimmune diseases, such as systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), multiple sclerosis (MS), Sjögren's syndrome and scleroderma[2,3].

Part of this bias can be explained by differences in sex hormone levels between males and females (estrogens, androgens and progestins), as these hormones can act on most immune cells[4]. Therefore, the female immune response differs further during the different stages of the menstrual cycle, influenced by fluctuating hormone levels[5].
Besides the influence of hormones, genetics also contribute to the sexual dimorphism in the immune response. While women have two X chromosomes, of which one is randomly inactivated during early development, males only have one X chromosome but also a Y chromosome. The X chromosome encodes many genes involved in the immune response and failure to inactivate these on the second X chromosome results in higher gene expression levels in females[6,7]. Furthermore, the Y chromosome is thought to epigenetically regulate gene expression genome wide in males, thereby contributing to sex-based differences in the expression of autosomal genes[8].

Due to the existence of sex-based differences in the immune system it is crucial to incorporate this knowledge into the development of therapeutic medications, in order to provide both sexes with optimal treatments. The past has shown that sex differences have not always been properly taken into account. Women of childbearing potential have been excluded from early phase clinical trials by the Food and Drug administration (FDA) for decades[9]. Fortunately, the need to include women in clinical trials has been recognized recently, which led to the development of policies to ensure female representation[9]. However, to determine sex-based differences in pharmacokinetics and pharmacodynamics precisely, it would be ideal to not only include women in general but to include women in different hormonal phases (e.g. during different phases of the menstrual cycle and menopause). Unfortunately this would also require much larger clinical trials in order to archive statistically relevant results, which would therefore be very costly[10].
This essay will review the most important sex-specific differences in the human immune response and their implications for males and females, with a focus on the influence of sex hormones and genetics.

2. Sex-based differences in the immune response

2.1 The immune system and action of sex hormones

Upon stimulation of the immune system, the innate immune response is the first line of defense. Its cellular components are monocytes, macrophages, natural killer (NK) cells, dendritic cells and granulocytes, the latter including neutrophils, eosinophils and basophils. The production of cytokines by these cells, together with antigens presented by antigen presenting cells (APCs), then activate the adaptive immune response where the cellular players are T and B lymphocytes[11,12]. T lymphocytes can be further subdivided into cytotoxic T cells (Tc cells, CD8+) which lyse cells expressing the respective antigen, T helper cells (Th cells, CD4+) which provide help for other lymphocytes, and regulatory T cells (Tregs). Tregs have an important role in the induction of tolerance, prevention of autoimmune conditions and dampening of the immune response. APCs, as for example dendritic cells (DCs), play a major role in the activation of T cells and in determining the direction of the T helper response. Depending on which cytokines they secrete they can induce type 1 (Th1), type 2 (Th2) or type 17 (Th17) T helper cells (figure1)[13]. While Th1 cells mediate cellular immunity, Th2 cells stimulate humoral immunity and can activate B cells to secrete antibodies. Th17 cells are thought to be proinflammatory and to be involved in the mediation of autoimmune responses[13–15].

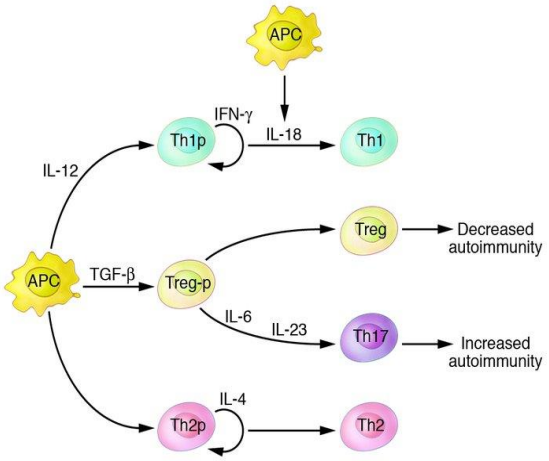

Figure 1. T helper cell subsets[13].

Sex hormones have long been known to influence the immune response. The three major sex hormones are androgens (e.g. testosterone) in men, and estrogens (mainly 17β- estradiol, abbreviated E2) and progesterone in women. Most immune cells express receptors for sex hormones and it is therefore likely that these hormones can directly affect the immune response. Estrogen receptors are expressed by monocytes, dendritic cells (DCs), natural killer cells (NKs), B and T lymphocytes, neutrophils and macrophages[16–18]. Progesterone receptors have been found on human T lymphocytes, peripheral blood leukocytes, mast cells and NK cells, while effects of progesterone on macrophages and plasmacytoid dendritic cells have been described[19–23]. Androgen receptors are expressed by human B and T lymphocytes, as well as by murine monocytes and macrophages[24–26].
As lipophilic steroid hormones, sex hormones can diffuse through the plasma membrane and bind to receptors in the cytoplasm but plasma membrane receptors have also been described[19]. Estrogen has two different estrogen receptors (ERs), namely ERα and ERβ, which mediate different responses[27]. For instance, while estrogen binding to ERα had a predominantly immunostimulatory role in a mouse model of SLE, binding to ERβ had a slightly immunosuppressive effect[27]. Different expression levels of ERα and ERβ on immune cells could in part explain how estrogen can have different effects on different immune cell types. While CD4+ cells mostly express ERα mRNA, with lower levels of ERβ mRNA, B cells expressed high ERβ mRNA levels but low ERα[28]. In contrast, CD8+ T cells and monocytes where found to express similar levels of both[28]. Classically, estrogen binds to intracellular receptors, resulting in nuclear import of the complex and binding to estrogen response elements (EREs), which controls target gene expression. On the other hand, ERs can also regulate gene expression by binding to other transcription factors, coactivators or corepressors, such as nuclear

factor-kB (NFκB)[29,30]. It has been shown that not only ERs can bind NFκB but also progesterone receptors (PRs) and androgen receptors (ARs)[31]. In contrast to receptor mediated genomic changes, signaling through membrane receptors allows for a more rapid response to changing hormone levels[32].

2.2 Sexual dimorphism in the immune response and the contribution of sex hormones

Differences between the female and male immune response have received much attention, with the general perception being that women mount a stronger cell mediated and humoral response to infections or vaccination, while men have a stronger innate immune response[33].

As dendritic cells are major antigen presenting cells and have therefore a large impact on the immune response, it is possible that differential activation in males and females contributes to gender differences in the immune response. Exposure of human immature DCs to estrogen was shown to increase their production of IL-6, IL-8 and monocyte chemoattractant protein 1 (MCP-1), suggesting a role for estrogen in the promotion of the inflammatory process[34]. Moreover, pretreatment of mature DCs with E2 increased their migration ability as well as their capacity to stimulate T cells[34]. In contrast, testosterone was shown to inhibit the capacity of DCs to stimulate CD4+ cells and to inhibit the immune response in parasite infected mice[35]. This shows that testosterone and estrogen might regulate T cell activation by affecting the stimulatory ability of DCs, thereby contributing to the stronger adaptive response in females and the relative inhibition in males.

The stronger cellular immune response is reflected by increased numbers of T lymphocytes in females[33,36]. In general, estrogen seems to have a cytoprotective effect on human T cells as[37] E2 was shown to reduce TNF-α induced cytotoxicity in peripheral T lymphocytes in a dose dependent manner[37]. Interestingly, this effect was shown to be mediated through ER signaling induced NFκB activation and caspase inhibition[37]. TNF-α can activate two opposing pathways, depending on which signaling cascade is triggered. The anti-apoptotic pathway is mediated by NFκB and results in cell survival, while FADD recruitment results in apoptosis via caspase activation[37]. E2 seems to inhibit this apoptotic pathway of TNF-α in T cells, which could contribute to the higher number of T cells observed in females as compared to males. In addition, estrogen also seems to promote T cell activation[38]. Treatment of ovariectomized mice with estrogen resulted in an increased frequency of antigen-specific Th1 cells and the production of IFN-y in response to stimulation, which was shown to be mediated by ERα[38]. Taken together, estrogen has a protective effect on T cells and enhances their activation, possibly contributing to the stronger cellular immune response in females.

On the other hand, the stronger humoral immune response is reflected by the higher immunoglobulin levels in females at baseline, as well as after stimulation with exogenous antigens. The strongest differences have been reported for the immunoglobulins M (IgM) and G (IgG)[39–44]. Besides higher levels of immunoglobulins, females are also said to have higher counts of B lymphocytes as compared to males[36,45]. Estrogen is thought to contribute to higher levels of IgM and IgG in females, by promoting the polyclonal activation of B cells[39,40,42–44]. *In vitro* studies confirmed that estrogen stimulates IgG and IgM production from female and male derived PBMCs, while testosterone inhibits it[43,46]. A more recent study suggested that the estrogen mediated stimulation of B cells might be brought about by downregulation of the B cell receptor CD80[47]. This receptor can engage with the inhibitory molecule CTLA-4 on T cells, wherefore its downregulation could increase B

cell activation[47]. It was further found that E2 enhances total IgG production in mouse splenocytes but without affecting B cell differentiation or proliferation. In addition, estrogen treatment protected B cells from apoptosis, possibly mediated by upregulation of the anti-apoptotic gene *Bcl-2*[47]. Results show that estrogen has a stimulating and protective effect on B cells, promoting their activation and the production of antibodies.

While males have a weaker adaptive immune response, it has been suggested that they have a stronger innate immune response than females[33,48]. It was found that males have significantly higher numbers of monocytes[33,36], which were also found to be more sensitive to proinflammatory stimuli[33]. This was concluded from the higher percentage of male-derived monocytes producing TNF, IL-1 and IL-12 after *in vitro* stimulation with endotoxin[33]. In the same study, counts of other immune cells (besides monocytes and lymphocytes) did not differ between males and females. However, a very recent study, probably with the largest dataset in Europe, also reported elevated NK cell numbers in males[36]. The stronger innate immune response in men might in part be due to anti-inflammatory effects of estrogen on cells of the innate immune system. For example, pretreatment of human peripheral monocytes with E2 decreased LPS- induced expression of the proinflammatory cytokine CXCL8 dose dependently[49]. A direct correlation between ERα levels and the suppression of CXCL8 expression was found and estrogen pretreated monocytes performed worse at the mobilization of neutrophils[16,49]. Furthermore, ERα signaling was shown to inhibit LPS induced NFκB mediated inflammatory pathways in macrophages[50]. This shows a possible mechanism of how E2 exerts its anti-inflammatory action on these cells. Specifically, E2 binding to ERα was shown to inhibit the translocation of p65, a subunit of NFκB, into the nucleus, thereby preventing the transcription of proinflammatory target genes[50].

LPS induced production of TNF *in vitro* can be used as a tool to assess innate immunity[48]. Upon whole blood stimulation by LPS, TNF levels were significantly higher in male than in female cells[48,51]. When looking at TNF production by stimulated immune cells, male derived monocytes showed a trend towards higher TNF production while its production by leukocytes was significantly higher for males than for females. Interestingly, stimulated TNF production by leukocytes and monocytes was also higher for postmenopausal woman compared to premenopausal women[51]. Even though correlations with sex hormone levels were not found, the authors do not exclude that estrogen could play a role in this by mechanisms other than total plasma levels[51]. It was concluded by the authors that women have an almost 30% lower innate immune response than males[48].

Differences in the immune response between males and females are summarized in table 1.

♀ **Females: Stronger adaptive immune response (IR)**	♂ **Males**: **Stronger innate IR**
■ Dendritic cells - **E** → DC → T cell activity - **T** --‖ DC → T cell activity ■ T cells - Higher numbers ♀ - **E**: cytoprotective, Th1 responses ↑ ■ B cells - Higher numbers ♀ - Higher Ig levels ♀ **E**: Ig ↑, B cell activation ↑ via CD80 ↓, apoptosis ↓ via BCL-2 ↑ **T**: Ig ↓	■ Monocytes - Higher numbers ♂ - Higher sensitivity to stimulation ♂ - **E** --‖ NFκB, anti-inflammatory ■ Higher NK cell numbers ■ Stronger neutrophil mobilization ■ Higher levels of proinflammatory cytokines (TNF, IL-1, IL-12)

Table 1. Summary of the main differences in the immune response between males and females. T = testosterone; E = estrogen; --‖ = inhibition; ⟶ = stimulation; Ig = immunoglobulin; ↑ = increase/increased expression, ↓= decrease/decreased expression.

2.3 Implications

The benefits females gain form a stronger adaptive immune response are a higher resistance to viral, bacterial or parasitic infections and a more efficient response to vaccines[52–55]. Gender differences in the immune response are also thought to contribute to a higher risk for acute allograft failure but a relative protection against chronic rejections in females[56–59]. Moreover, autoimmune diseases are more prevalent among females[3,60]. For instance, multiple sclerosis and rheumatoid arthritis show a female to male ratio of 2:1, Grave´s disease and Hashimoto´s thyroiditis a ratio of 7-10:1 and SLE shows a ratio of 9:1[3,60]. Males on the other hand are thought to be more susceptible to innate conditions such as sepsis, multiple organ failure (MOF) and infections after trauma[61–64].

Vaccinations

The stronger humoral immune response leads to the production of higher levels of antibodies by B cells, resulting in a better protection of females in response to many vaccines[52–55]. This effect is thought to be at least partly mediated by sex hormones. Testosterone seems to suppress the immune response in reaction to vaccination[52]. In a recent study, men with higher testosterone levels exhibited lower antibody responses to trivalent inactivated seasonal influenza vaccine (TIV) than men with lower testosterone levels[52]. It was suggested that testosterone can change expression levels of certain transcription factors, thereby altering the expression of genes involved in immune functions. Specifically, testosterone was proposed to upregulate a cluster of genes involved in lipid synthesis, including LTA4H (leukotriene A4 hydrolase) and MIF (macrophage inhibitory factor), which can potentially suppress inflammatory responses and decrease the production of inflammatory cytokines[52]. Higher levels of testosterone correlated with higher expression levels of these genes and a lower response to vaccination[52]. This suggests that testosterone might prevent a strong antibody response after vaccination by activating anti-inflammatory genetic programs. Unfortunately, this study did not distinguish in which cell subsets these programs were activated but used whole blood for gene expression analysis.

In contrast, estrogen was found to stimulate the immune response to vaccines[54,55]. In a postmenopausal mouse model, ovariectomized mice showed a decreased production of antibodies in response to influenza vaccination, while treatment of ovariectomized mice with E2 increased the production of virus-specific antibodies to levels obseved in normal female mice[54]. A similar effect of

estrogen on influenza vaccine induced antibody production was reported in a recent study in humans, where a direct correlation was found between E2 plasma levels and fold increase in IgG production in postmenopausal women receiving estrogen therapy[65].

Another study showed that the titer of neutralizing antibodies was significantly higher in mice receiving a Herpes Simplex Virus 2 (HSV-2) vaccine in combination with estradiol treatment compared to mice that were only vaccinated[55]. Estrogen is not only thought to influence antibody production by B cells in reaction to vaccination but also T cell responses. A very recent study showed that mice treated with E2, vaccinated against HSV-2 and challenged after six weeks with the wild type HSV-2 virus, are better protected than mice that did not receive E2 treatment[66]. It was shown in this study that estradiol primes DCs to induce Th17 and Th1 respnses. Especially the induction of the Th17 response seemed to be responsible for E2 induced enhancement of anti-viral T cell activity. The production of IL-1β by DCs was found to be primarily responsible for the Th17 response observed in E2 treated mice[66].
Taken together, estrogen seems to enhance T cell and B cell responses in reaction to vaccination, resulting in a better protection of females. Testosterone on the other hand inhibits the immune response upon vaccination. Results are summarized in table 2.
However, despite a better vaccine efficiency in females, their stronger immune response might also contribute to the increased occurrence of adverse events in response to many vaccinations, including the measles-mumps-rubella (MMR) vaccine[53,67].

Females: Higher vaccine efficiency: stronger antibody response
■ Influenza vaccine - **T** → anti-inflammatory genes (LTA4H, MIF) ↑; --‖ antibody production - **E** → antibody production ↑ ■ HSV-2 vaccine - **E** → DCs → Th1, Th17 response - **E** → neutralizing antibodies ↑

Table 2. Gender differences in vaccine efficiency. T = testosterone; E = estrogen;
--‖ = inhibition; → = stimulation; ↑ = increase/upregulation.

Allografts

In humans, female gender has been established as a risk factor for acute rejection of kidney and liver transplants[56,57]. However, besides this increased risk for acute rejection, females were also shown to have a lower risk for chronic allograft failure, with a higher 8-year graft and patient survival[57]. A recent study moreover reported male gender as an independent prognostic factor for poor 5-year transplant survival in the case of kidney transplants[58].
Yet, most studies on gender effects on transplantation success have been carried out in animal models. In the case of allogenic skin transplantations in rats for instance, females rejected grafts faster than males, in line with the stronger acute rejection found in humans[59]. Also here, sex hormones are thought to influence the gender biased outcome. Estradiol treatment was shown to reverse the beneficial immunosuppressive effects of cyclosporine and led to enhanced graft rejection in both, males and females. In contrast, testosterone enhanced the effect of cyclosporine and graft survival in male and female recipients[59]. This would be in line with the reported immunosuppressive role of testosterone and the immuno-stimulatory role of estrogen[68]. Similar results were found for

cardiac transplantations. Female mice treated with cyclosporine A (CsA) rejected grafts much faster than males treated with CsA, possibly a result of the strong lymphocyte infiltration observed in females[69]. Furthermore, administration of estradiol decreased graft survival in male and female recipients treated with CsA, while treatment with the estrogen antagonist tamoxifen and CsA improved allograft survival in females[69]. It could be possible that estrogen enhances lymphocyte infiltration into grafts, leading to fast acute rejections.

As seen in humans, studies in rats also show that females are less susceptible to chronic rejections. Interestingly, the roles of sex hormones in acute rejections seem to be reversed in chronic rejections. A protective effect of estrogen on chronic renal allograft rejection was proposed, while testosterone was found to have negative effects, increasing glomerulosclerosis and proteinuria[70,71]. Results were found to be solely dependent on sex hormones and independent of gender[70,71]. Effects are likely to be due to sex hormonal regulation of immune cell infiltration and production of growth factors and vasoactive agents by surrounding tissues. For kidney transplantations in mice, testosterone was related to poor outcome due to stimulation of TGF-β (transforming growth factor beta) and PDGF (platelet derived growth factor) production, while estradiol suppressed their production. TGF-β and PDGF are involved in fibrosis and smooth muscle cell proliferation, respectively, explaining their negative effect on kidney health[70,71]. Furthermore, estradiol was shown to significantly suppress graft infiltration by lymphocytes, monocytes and macrophages, while testosterone promoted graft infiltration by macrophages and CD5+ lymphocytes[72,73]. As the expression of the adhesion molecule ICAM-1 was reduced in estradiol treated mice, estrogen might decrease the infiltration of immune cells by altering the expression of adhesion molecules[70]. Recently, estradiol was shown to inhibit VCAM-1 and ICAM-1 expression in cytokine stimulated cultured human endothelial cells[74]. Also for cardiac transplantations, estrogen has a protective effect on chronic rejection, which was proposed to be mediated by ERβ rather than ERα[75].
Results suggest that estrogen protects from chronic allograft rejection by suppressing the expression of adhesion molecules and thereby graft infiltration by immune cells, and by modulating the expression of growth factors like PDGF and TGF-β. Results are summarized in table 3.

Females: opposite susceptibility to acute and chronic allograft failure
Stronger acute allograft rejections - Stronger lymphocyte infiltration - **E**: acute rejection ↑ - **T**: graft survival ↑
Lower susceptibility to chronic allograft rejections - **T:** glomerulosclerosis ↑, proteinuria ↑, TGF-β ↑, PDGF ↑, graft infiltration ↑ (lymphocytes, macrophages) - **E:** TGF-beta ↓, PDGF ↓, ICAM-1 ↓, graft Infiltration ↓

Table 3. Gender differences in transplantation efficiency. T = testosterone; E = estrogen; ↑ = increase; ↓ = decrease.

Autoimmune diseases

Autoimmune diseases are caused by the breakdown of self-tolerance which allows the immune system to attack components of the self, leading to tissue damage and dysfunction[76]. Many of the sex-based immune differences are thought to contribute to the relative prevalence of autoimmune diseases in females as compared to males and sex hormones are thought to play an important role. For instance, in women with SLE, estrogen seems to enhance T cell activation via ERs[77]. T cell mediated stimulation of B cells could then lead to an increased production of antibodies and exacerbate the disease[77]. This is in line with results of earlier studies which showed that estrogen stimulates antibody production from PBMCs from SLE patients, while testosterone inhibits it[78,79]. Furthermore, a meta-analysis evaluating multiple studies on sex hormone concentrations in SLE patients found significantly higher levels of estrogen and progesterone in women with SLE compared with healthy controls[80]. This suggests that high levels of estrogen and progesterone could contribute to the disease, which is in line with the relative increase in disease severity observed during pregnancy[81]. In general, the severity of many autoimmune diseases changes with the menstrual cycle and pregnancy, suggesting a strong role for sex hormones in autoimmune diseases[5,82]. Furthermore, expression levels of ERβ were found to be altered in T cells of woman with lupus compared with healthy controls, further supporting this idea[83].

By affecting the expression levels of certain genes, sex hormones are thought to contribute to the differential predisposition to autoimmune diseases[84]. The autoimmune regulator (AIRE) is expressed in medullary thymic epithelial cells (mTECs) where it functions as a transcriptional regulator of tissue specific antigens (TSAs)[84].The expression of these antigens on mTECs in the thymus plays a major role in the induction of central tolerance and negative selection of potentially autoreactive T cells. mTECs are thought to be directly involved in antigen presentation and induction of regulatory T cells, however, transfer of antigens to dendritic cells also contributes to tolerance induction[85]. AIRE expression was found to be lower in the thymus of female mice and humans as compared to males[84]. Interestingly, AIRE expression in females decreased strongly with the onset of puberty, suggesting a role for regulation by sex hormones[84]. This was confirmed as the treatment of cultured human and mouse TECs with estrogen decreased AIRE and TSA expression, while treatment with DHT (dihydrotestosterone) increased the expression of both[84]. The suppressive effect of estrogen on AIRE expression was further confirmed in experiments where human thymic fragments were transplanted into immunodeficient mice treated with estrogen. It is thought that estrogen acts epigenetically by increasing the methylation sites on the *AIRE* reporter, thereby repressing its transcription[84]. A link between AIRE expression levels and autoimmune diseases was established in a mouse model of experimental autoimmune thyroiditis (EAT), where estrogen treatment increased the production of anti-thyroglobulin antibodies[84]. However, this effect was abolished in thymectomized mice, showing that estrogen acts on the thymus to increase the autoimmune response. Furthermore, reduction of AIRE expression in male mice using miRNAi increased CD8+ T cell infiltrates in the thyroiditis model. Taken together, results suggest that lower levels of AIRE in mTECs in females might predispose to autoimmune diseases[84]. Supporting this idea is the observed infiltration of lymphocytes into multiple organs and the production of autoantibodies with reactivity to multiple organs in a model of *Aire* deficient mice[86].

Besides affecting AIRE expression, sex hormones are thought to play a major role in T and B cell involvements in autoimmune diseases. In mice, estrogen promoted the survival and maturation of autoreactive B cells by suppressing their negative selection[87,88]. The underlying mechanism was

proposed to be the estrogen induced activation of a set of genes that promotes the survival and activation of B cells[88,89]. E2 induced upregulation of the anti-apoptotic protein BLC-2 was thought to inhibit the induction of tolerance in naïve autoreactive B cells and predispose mice, which are transgenic for the heavy chain of an anti-DNA antibody, to an SLE-like phenotype[89]. Other B cell genes that were found to be upregulated by estrogen, include *Shp-1* and *Cd22*, which are also thought to promote autoreactivity of B cells[88]. It seems that estrogen activates genetic programs in B cells that promote the survival of autoreactive B cells, thereby predisposing to autoimmune diseases[88]. This idea is supported by a recent study that found gender differences in the global gene expression of 358 genes from B cells isolated from human PBMCs, possibly regulated by estrogen[90].

It further seems that men have higher and more stable levels of T regulatory cells than women[91,92]. Treg numbers fluctuate with the menstrual cycle, peaking during the late follicular phase where numbers are comparable between males and females[92]. High levels of estrogen promote Treg differentiation and their suppressive activity in human blood cells, which is important for fetal tolerance and could explain fluctuation in Treg numbers during the menstrual cycle[92,93]. Possibly, the higher and constant levels of regulatory T cells in men protect them from autoimmune diseases, while fluctuating and lower numbers in females might put them at risk and explain at least partly disease severity fluctuations during the female cycle[91].
Interestingly, a recent study found a 1,7 fold higher number of autoantigen specific T cells in the blood of healthy female donors compared to males, however, the exact reasons for this were not known[94]. Possibly lower levels of regulatory T cells and decreased negative selection of potentially autoreactive T cells could offer an explanation.
Results suggest that sex hormones play an important role in determining not only the severity of autoimmune diseases but also in establishing the higher predisposition of females.

T helper cell responses have also been shown to play a role in the susceptibility to autoimmune diseases[95]. In a mouse model for EAE, females are susceptible to disease induction while males are not[96]. Resistant males were shown to have developed a Th2 type response while susceptible females showed a Th1 response. Furthermore, transfer of female antigen specific Th1 cells induced EAE in the recipient while transfer of male derived Th2 cells did not. Interestingly, transfer of female Th1 and male Th2 cells together did induce EAE but the severity of the disease was significantly reduced[96]. This shows that sex-based differences in the T helper cell response could also contribute to differences in autoimmune disease prevalence and severity.
In line with this, different cytokine responses in males and females are also thought to contribute. Males have a stronger TNF response, which is known for its proinflammatory actions[48,51]. However, it has also been found to have immunosuppressive effects[97]. TNF is therefore a double-edged sword, which can promote autoimmune diseases due to its proinflammatory nature but can also be protective[97]. For example, anti-TNF therapy is used to ameliorate RA but is suspected to cause neurological adverse events and MS in a minority of patients[98–100]. A possible underlying mechanism is that besides its proinflammatory effects it can also stimulate Treg cells and inhibit proinflammatory Th17 cells[101–103]. In a collagen-induced arthritis mouse model of RA, blockage of TNF did reduce disease severity but also expanded Th1 and Th17 populations[103]. Interestingly, this expansion was found in the lymph node but not the joint, which explains why disease severity was still reduced. A study on humans provided insights into why this might have been the case. Treatment of RA patients with anti-TNF increased Th17 cells and IL-17 production, as well as Th1 cells and IFN-γ production[102]. However, CCR6 was shown to be downregulated on Th17 cells in RA patients who were successfully

treated with anti-TNF. As CCR6 is involved in the recruitment of Th17 cells to the synovium this might explain why the increased numbers of these cells did not exacerbate RA[102]. However, higher numbers of these proinflammatory cells could potentially contribute to the neurological adverse events observed in some patients. TNF has further been shown to promote Treg cell expansion via the TNF receptor 2 (TNFR2), which is highly expressed by these cells. Tregs express higher levels of TNFR2 than effector T cells but respond slower. Therefore, a delayed immunosuppressive effect of TNF has been suggested[101]. In this case, TNF could first activate effector T cells before its activation of Tregs would limit the immune response. Possibly, higher TNF levels in males lead to stronger proinflammatory signals but also limit the immune response, thereby protecting from autoimmune diseases. Results are summarized in table 4.

Genetic factors independent of sex hormones are also thought to play a role in the predisposition of females to autoimmune diseases, which will be discussed later in more detail.

Females: Stronger susceptibility to autoimmune diseases
■ Sex hormonal regulation of gene expression - **E** --‖ AIRE and TSA expression in mTECs => negative selection of B and T cells ↓ - **T** → AIRE and TSA expression in mTECs - **E** → BCL-2 ↑, SHP-1 ↑, CD22 ↑ in B cells → survival of autoreactive B cells ■ Lower and fluctuating Treg numbers ■ Higher numbers of autoreactive T cells ■ Different T helper cell and cytokine responses in males and females e.g. lower levels of TNF ♀ ○ Proinflammatory: TNF → effector T cells ○ Anti-inflammatory: TNF → Tregs ; TNF --‖ Th17

Table 4. Gender differences in autoimmune diseases. T = testosterone; E = estrogen; ↑ = increase; ↓ = decrease; --‖ = inhibition; → = stimulation; mTEC = medullary thymic epithelial cell; TSA = tissue specific antigen; ♀= in females.

Sepsis, multiple organ failure and infections after surgery

It is thought that the stronger innate immune response might contribute to lower survival rates of males compared to females in case of endotoxic shock and sepsis and put them at increased risk for the systemic inflammatory response syndrome (SIRS) and for multiple organ failure (MOF) after trauma[49,61–64]. Besides this, males are also more susceptible to developing post-surgery complications[104]. Detrimental effects of the innate immune response in these cases are mediated by leukocyte infiltration, especially neutrophils, and excessive production of inflammatory cytokines (including TNF-α and IL-6). The male inflammatory response to microbial stimuli is much stronger and more aggressive, and can therefore result in tissue damage[104,105]. Besides amounting stronger TNF responses, males are also thought to have higher levels of IL-6, as estrogen was shown to decrease IL-6 levels[106,107]. These inflammatory cytokines seem to be involved in the higher susceptibility of men for the conditions named above. For instance, in the case of sepsis, a study found higher levels of the anti-inflammatory cytokine IL-10 in women but higher levels of the proinflammatory cytokine TNF-α in males, which was related to the better outcome in women[61]. Furthermore, persistently higher IL-6 levels in males after severe injury or trauma were associated with higher rates of MOF[64] and higher susceptibility to SIRS and sepsis in males[63].

Sex hormones might play a direct role in the relative protection of females as it was shown to have anti-inflammatory effects on monocytes and macrophages and to dampen neutrophil recruitment during inflammation[49]. E2 is thought to downregulate the expression of CD16 on macrophages and monocytes, thereby inhibiting the expression of the proinflammatory cytokines IL-6, IL-1β and TNF-α[108,109]. Another study similarly found an increased percentage of IL-12, IL-1β and TNF-α producing monocytes in males compared to females after *in vitro* stimulation[33]. When looking at neutrophil function and activity, estrogen was shown to decrease their chemotactic activity, mediated by ERα[110,111]. A possible mechanism for the estrogen induced decrease in neutrophil chemotaxis was shown in a human study, where E2 reduced CXCL8 expression in monocytes stimulated with LPS. CXCL8 is a chemokine which attracts neutrophils to sites of inflammation[49].
Testosterone in contrast might have a proinflammatory effect on the innate immune response, as incubation of stimulated monocytes with physiological levels of testosterone increased the percentage of IL-12 and IL-1β producing monocytes. However, this effect was not observed for TNF-α[112]. Taken together, this suggests that estrogen dampens the innate immune response by inhibiting the production of pro-inflammatory cytokines by monocytes and macrophages and by suppressing neutrophil infiltration.

Results from a mouse study suggest that differences in resident immune cells could also play a role in the higher susceptibility of males to innate conditions[104]. Female mice had higher number of T cells, B cells and macrophages in peritoneal and pleural cavities which showed different behavior than in males. Resident macrophages portrayed higher levels of intracellular antibacterial NADPH oxidase activity, enhanced phagocytic capacity and greater toll-liker receptor (TLR) expression. However, macrophage cytokines (TNF-α, IL-6) were not elevated in females and IL-6 levels were even significantly lower. Furthermore, the infiltration of neutrophils in response to stimulation was less in females as compared to males. It was proposed and shown that tissue resident CD4+ T cells can regulate the expression of inflammatory cytokines by monocytes, contributing to lower levels of proinflammatory cytokines in females and decreasing neutrophil tissue infiltration[104]. The ability of T cells to down regulate the innate immune response has been described earlier[113,114].

In conclusion, the relative hyper-activation of the innate immune system in males could be a possible explanation for their higher susceptibility to endotoxic shock, sepsis, infections after surgery and multiple organ failure. Estrogen and differences in tissue resident cells seem to dampen the potentially tissue damaging innate response in females. Results are summarized in table 5.

Males: Higher susceptibility to endotoxic shock, sepsis, MOF, infection
■ Stronger activation of innate immune cells => tissue damage - **E** --‖ CD16 expression on monocytes/macrophages => proinflammatory cytokines (IL-6, TNF-α, IL-1β) ↓ - **E** --‖ CXCL8 expression ↓ => neutrophil chemotaxis ↓, - **T** → monocyte proinflammatory cytokines (IL-12, IL-1β) ↑ **!** In females: tissue resident T cells - suppress IL-6 and TNF-α production by monocytes - inhibit neutrophil infiltration

Table 5. Susceptibility to innate conditions. T = testosterone; E = estrogen; ↑ = increase; ↓ = decrease; => = leads to; --‖ = inhibition; → = stimulation.

3. Genetics influences

Next to hormonal influences, genetic factors are also thought to contribute to the sex-based differences in the immune response. The X and Y chromosomes (ChX, ChrY), as well as gender differences in autosomal gene expression, are thought to be influencing factors. However, it should be noted that sex hormones are involved in the regulation of genes and can therefore contribute to gene expression differences between males and females.

3.1 Sex chromosomes

In contrast to men, females have two X chromosomes which would lead to a double dosage of X-linked genes, if both were to be expressed. However, random inactivation of one of the X chromosomes during early embryonic development ensures equal expression levels in both genders. Due to the randomness of this process, approximately half of the female cells inactivate the maternal and the other half the paternal ChX, resulting in cellular mosaicism. However, X inactivation is not always complete[115]. It has been reported that 5-15 % of X-linked genes escape X-inactivation, with another 10% being expressed with high variation between female individuals[116,117]. The X chromosome encodes many genes involved in the immune response, as for example the toll-like receptors 7 and 8 (TLR7, TLR8), CD40 ligand (CD40L), forkhead box P3 (FOXP3) and Interleukin-2 receptor gamma (IL2RG)[115]. Escape of these genes from inactivation would lead to higher expression levels in females and could therefore contribute to differences in the immune response.

Next to escape from inactivation, skewed X-inactivation is also possible. In this case, either the maternal or paternal X chromosome gets preferentially inactivated. In result, some X- linked diseases are much more prevalent in males, as female skewing of X-activation allows the non-disease gene to become dominant, if it is a heterozygous mutation. For example, skewed X-inactivation in female carriers of Wiskott-Aldrich syndrome (WAS) leads to preferential expression of the normal gene in immune cells, giving females an immunological advantage[118]. However, exceptions to this skewing in WAS have been reported, in which case the disease also presents in females[118].

Disturbed X inactivation is thought to contribute to the higher prevalence of autoimmune diseases in females. In the normal situation, approximately half of the females' cells express the maternal and half the paternal X chromosome. This also counts for DCs, which are crucial for inducing self-tolerance in the thymus. In the normal situation these thymic DCs present paternal as well as maternal X-linked self-antigens to lymphocytes that pass the thymus. However, during extreme skewing, DCs will mostly present self-antigens from the X chromosome of one parent. For instance, if the inactivation of the maternal X-chromosome is strongly increased, DCs will mostly present paternal self-antigens. Resultantly, immune cells will be negatively selected only against these, allowing potentially autoreactive lymphocytes specific for maternal self-antigens to escape negative selection in the thymus[119,120]. It has been suggested that this mechanism contributes to the higher prevalence of SLE in females[120].

Reactivation of genes from the inactivated X chromosome has received increasing attention during the past years and might also predispose to some autoimmune diseases. As X inactivated genes are methylated, demethylation allows for reactivation of initially silenced genes. For instance, it has been found that CD40LG, which is a B cell costimulatory molecule that is expressed on the X chromosome, is demethylated on the inactivated ChrX in women with SLE[6,7]. Expression of CD40LG from both X chromosomes leads to higher protein levels in the cells and possibly to hyper-activation and increased antibody production by B cells[6,7].

It has also been proposed that higher protein levels, due to X-reactivation in general, could lead to aggregate formation, which could in turn trigger an immune response against these self-aggregates[121].

Besides the X chromosome, also the Y chromosome has recently gained more attention as a possible contributor to sexual dimorphism in the immune response[8]. For a long time it was thought that the Y chromosome does not have many functions besides sex determination, due to the low number of protein coding genes[8]. However, starting with *Drosophila,* a role for the Y chromosome in the epigenetic regulation of gene expression was shown. A recent study has evaluated the role of the Y chromosome in the regulation of gene expression in mice[8]. It was found that ChY can epigenetically regulate genome wide gene expression in CD4+ T cells and macrophages, by altering chromatin states and modulating the access of transcription factors. Furthermore, preliminary evidence was provided that this mechanism could be conserved in humans. It was concluded that the Y chromosome acts as an expression quantitative trait locus (eQTL), i.e. a genomic locus that influences the expression levels of other genes[8]. As this mechanism of gene expression regulation is not available in female immune cells, some differences in the immune response and in the expression of autosomal genes could be explained by it. The genetic influences on the immune response are summarized in table 6.

3.2 MicroRNAs

MicroRNAs have recently gained more interest for their possible role in establishing gender differences in the immune response. These microRNAs are small, non-protein-coding RNAs that are involved in the regulation of gene expression by affecting mRNA translation into proteins. Interestingly, the X chromosome has been shown to encode a much higher density of microRNAs than autosomes do on average[122]. 113 microRNAs were found to be expressed from the X chromosome[123]. In contrast, only two microRNAs have been identified on the Y chromosome[124]. This could affect gender differences in the immune response as microRNAs from the X chromosome regulate immune functions. For instance, they have been found to be involved in granulopoiesis, monocyte differentiation, the proliferation and survival of CD4+ T cells, and the regulation of DC development, apoptosis and IL-12 production[125–129]. Furthermore, aberrant expression of microRNAs is observed in autoimmune diseases such as lupus or rheumatoid arthritis[130,131].

There is evidence that microRNAs are expressed differentially between males and females in multiple organs[132–135]. Yet, limited research is available for gender differences of microRNA expression in immune cells. Therefore, a major finding was that estrogen seems to be involved in the regulation of microRNA expression in murine splenic lymphocytes[136]. In the respective study, estrogen treatment of mice affected the expression levels of multiple microRNAs, including those involved in the immune response[136]. For example, miR-146a, a negative regulator of toll-like receptors, was downregulated in lymphocytes of estrogen treated mice, which was related to higher production of LPS induced IFN-γ and NO by these cells. Importantly, an earlier study implicated this microRNA as negative regulator of inflammation and autoimmunity and miR-146a knockout in mice led to loss of peripheral T cell tolerance[137]. Possibly, estrogen induced suppression of miR-146a could contribute to the predisposition of females to autoimmune disease.
In contrast, miR-223, which is involved in granulocyte function[138], was found to be upregulated by estrogen[136,138]. Yet, this similarly resulted in higher IFN-γ production by LPS-stimulated mouse lymphocytes as compared to cells from placebo treated mice[136]. Interestingly, granulocytes of miR-

223 null mice were found to be hyper-mature and hypersensitive to stimulation. Furthermore, these mice suffered from exaggerated tissue destruction after stimulation with endotoxin[138]. If miRNA-223 was not only upregulated by estrogen in lymphocytes but also in granulocytes, this could possibly contribute to the lower susceptibility of females to innate conditions (e.g. endotoxic shock), as described earlier. However, more research is needed to determine this.
Results show that estrogen can regulate the expression of immune response regulating microRNAs in lymphocytes, which affects the production of cytokines at the post-transcriptional level.

Another study in beetles (*Tribolium castaneum*) found strong differences in gender-specific changes in immune gene and microRNA expression upon stimulation[139]. The number of microRNAs induced by stimulation with peptidoglycan (PGN) was much higher in females than in males; specifically, 28% of the microRNAs showed gender specific expression. Interestingly, even without stimulation, 11 microRNAs had different expression levels in females and males[139]. This gender-specific expression provides further evidence that they could play an important role in establishing gender based differences in the immune response.

Further evidence for microRNA involvement in autoimmune diseases comes from a recent study which identified a number of X-linked genes, including 5 X-linked microRNAs, which were overexpressed in experimentally demethylated CD4+ T cells from women compared to men, as well as in females with active lupus[140]. Among these were miR-188-3p and miR-98, which play an important role in T cell function. Both microRNAs suppress CBL, a E3 ubiquitin-protein ligase, which inhibits T cell activation[140,141]. Overexpression of these micro-RNAs could decrease CBL levels in T cells, thereby lowering T cell inhibition and possibly contributing to autoimmune responses[140]. The same study also suggests that those overexpressed genes where demethylated on the second X chromosome, again showing a possible role for X reactivation in the predisposition to autoimmune diseases[140]. MicroRNA dysregulation has not only been found in SLE but also in other autoimmune diseases such as MS, again showing their possible contribution to the higher prevalence of autoimmune disease in females[142–145]. Results are summarized in table 6.

Genetic influences on the IR and susceptibility to autoimmune diseases

- X-inactivation escape/ X-reactivation
  - of immune genes => higher expression in females
    - e.g. CD40LG reactivation => B cell activity ↑
  - Protein expression ↑ => aggregate formation => IR against self-aggregates
- Y chromosome: genome wide regulation of gene expression in male immune cells
- X-skewing
  - Lower susceptibility of females to certain X-linked diseases (e.g. WAS)
  - Self-antigen presentation by DCs ↓ => negative selection of autoreactive cells ↓
- MicroRNAs
  - **E** --‖ miRNA-146a expression (lymphocytes) => IFN-γ ↑, inflammation and autoimmunity ↑
  - **E** → miRNA-223 expression (lymphocytes) => IFN-γ ↑
  - Higher expression of X-linked microRNAs ♀
    - miRNA-188-3p
    - miRNA-98

    } --‖ CBL => T cell activation ↑

Table 6. Genetic influences on the IR and susceptibility to autoimmune diseases. IR= immune response; E = estrogen; --‖ = inhibition; → = stimulation; ↑ = increase, ↓= decrease, WAS = Wiskott-Aldrich syndrome, ♀= in females.

3.3 Distinguishing effects of sex hormones from genetic effects

Effects of sex hormones on the immune response are often hard to distinguish from purely genetic contributions, for example from the presence of an extra X or a Y chromosome, as sex chromosomes are also involved in the regulation of gene expression. In humans, this distinction can be made in *in vitro* studies, where immune cells can be treated with sex hormones and the effects on cell activation and secretory profile can be determined. Furthermore, studies on hormone replacement therapy in transsexuals have been helpful in the past[146]. In animal models in contrast, direct treatment with sex steroids allows for the determination of *in vivo* effects of sex hormones. While these methods allow for the investigation of the effects of sex hormones, pure genetic effects are more difficult to elucidate.

The discovery that sex chromosome number abnormalities, such as Klinefelter´s syndrome (XXY), increase the risk for autoimmune diseases, supports the idea that the X and Y chromosomes themselves might play a role in the establishment of gender differences in the immune response[147]. For example, the prevalence of SLE in XXY males was found to be 14% higher than in normal XY men and was predicted to be similar to the risk in women[147]. In humans it is difficult to distinguish hormone actions from pure sex chromosome effects but a mouse model has been developed that allows for exactly this distinction[148]. In this model, the testes determining region *Sry* is removed from the Y chromosome of male mice and inserted into an autosome. Crossing of these mice with normal XX mice gives rise to four different types of mice: gonodal female mice with two X chromosomes (XX) and gonodal females with one X one Y chromosome (XY^-), as well as gonodal males with an XY genotype (XY^- *Sry*) and gonodal males with a XX genotype (XX *Sry*). Gonadectomy and castration during early development then allows for the distinction between the action of sex hormones and chromosomal complement. Interestingly, it was found that the X and Y chromosomes and not sex hormones determine the predisposition to lupus and EAE in mice[149]. Mice with the XX chromosome complement, regardless of whether female or male, showed a higher susceptibility to EAE and lupus compared to XY^- *Sry* and XY^- mice. This was thought to be related to the Th2 profile observed in XY mice: ex-vivo stimulated lymph node cells (LNCs) produced significantly higher levels of IL-5 and IL-13 and showed a higher trend for IL-10 production. On the other hand, XX mice showed a significant upregulation of IL-13Rα2, which is located on the X chromosome. It was suggested that IL-13Rα2 could act as a decoy receptor to limit the Th2 response. Higher expression levels could be due to failure of inactivation or reactivation from the second X chromosome, resulting in a dosage effect of an X-linked gene[149].

Another study using this model revealed unexpected opposing effects of androgens and the Y chromosome on the immune response[150]. It was found that immune stimulation led to a stronger immune response (LNC proliferation; IFN, TNF, IL-10 production) in gonodal females (XX, XY^-) than in gonodal males (XX *Sry*, XY^- *Sry*). Importantly, gonadectomized XY^- mice showed a stronger immune response than gonadectomized XX mice, implying that the Y chromosome heightens the immune response more than the X chromosome and that under normal conditions testosterone would inhibit this immuno-stimulatory effect of the Y chromosome. This was confirmed as testosterone treatment of gonadectomized XY^- mice decreased the immune response to similar levels as observed in untreated gonadectomized XX mice, even with a lower trend for XY^-. It seems that the Y chromosome and androgens have opposite but compensatory effects on the immune response[150]. It has been suggested that these two opposing mechanisms have evolved to decrease the immune system differences between males and females[150,151].

Results show that chromosomal complement itself can affect the immune response independently of sex hormones and it is evident that this mouse model can help understanding the roles of sex hormones and genes in the immune response better.

4. Discussion/Conclusion

It has long been an accepted view that the immune response differs between males and females but many of the underlying mechanisms are still unknown. As many studies cannot be carried out in humans due to ethical concerns, *in vitro* studies in human cell lines and studies in animals are combined to gain further insights into the complex issues on hand. However, the use of different animal models and different hormone concentrations makes it harder to compare results. Some aspects are generally accepted, including the stronger cellular and humoral immune responses in females and their greater predisposition to autoimmune diseases. The view that testosterone has an immunosuppressive role, while non-pregnancy levels of estrogen are immunostimulatory, is also widely represented. However, it seems that the immunostimulatory effects of estrogen are focused on the adaptive immune response, as many studies show anti-inflammatory properties of estrogen on cells of the innate immune system. Testosterone on the other hand suppresses the adaptive immune response, while it seems to have a stimulatory effect on innate immune cells, resulting in a stronger innate immune response in men compared to women. This might explain why males are more susceptible to conditions such as endotoxic shock, sepsis and MOF. In general, sex hormones affect many aspects of the immune system and play an important role in the establishment of gender differences in the immune response. These differences can be used to explain at least in part the gender bias observed in allograft rejections, vaccination efficiency and prevalence of autoimmune diseases.

Besides sex hormones, also genetic differences between males and females are known to influence the immune response. It has long been known that the X chromosome encodes many genes involved in the immune response and that differences in the dosage of these X-linked immune genes could contribute to sexual dimorphism. However, the Y chromosome has only recently gained attention for its possible role in the establishment of sex-based differences in gene expression. Preliminary results in humans suggest that the Y chromosome could epigenetically regulate immune cell genes genome wide, making it a valuable target for further research.
Recently, also microRNAs have gained more attention for their possible contribution to gender differences in the immune response and female predisposition to autoimmune diseases. The number of microRNAs encoded by the X chromosome is much higher compared to the Y chromosome and many of them are involved in immune functions. Mechanisms like X-reactivation could result in different expression levels of these between males and females. Micro RNAs have gained increasing attention for possible therapeutic applications in many fields of medicine[152]. Further research into gender differences in microRNA expression and regulation could therefore be beneficial. For instance, findings that certain microRNAs are involved in autoimmune diseases in women could allow for the development of anti-miRNA drugs which could specifically target their expression in females.

While effects of sex hormones can be evaluated in *in vitro* studies in human or animal cells and in *in vivo* animal models, it is often difficult to clearly identify purely genetic contributions to sex-based differences in the immune response. Therefore, the development of a mouse model that allows for the distinction between the effects of sex hormones and chromosomal complement gives a major advantage in this field. For example, the finding that androgens and the Y chromosome have opposite but complementary effects on the immune response, possibly to diminish gender differences, could not have been made without such a model. However, it will be difficult to determine whether results obtained with this method are conserved in humans.

Many of the gender differences in the immune response are thought to contribute to the higher prevalence of autoimmune diseases in females, which shows that gender-based treatment of these conditions could be beneficial. The emerging field of microRNA therapeutics could possibly bring major advances in gender-specific treatment of autoimmune diseases in the future. However, this still requires much more research into the exact involvements and expression differences of specific microRNAs. The importance of further research in the field of gender immunology is also strengthened by the discovery that drug pharmacokinetics and pharmacodynamics can be affected by gender differences in the immune response, leading to different drug actions in males and females[9]. Even though much research has already been done, many results are still controversial, which makes it difficult to translate findings into medical applications. Especially because the underlying mechanisms are still largely unknown, it is crucial to include adequate numbers of females in clinical trials, which has not been the case in the past. In this way, more data on gender differences in the immune response can be collected which can hopefully lead to a better understanding and prevent gender-based adverse events. Taking into account gender differences during vaccine design for instance could not only decrease the risk of adverse events in females but potentially also decrease health care costs, as women are likely to require lower doses.

5. References

1. Giefing-Kröll, C., Berger, P., Lepperdinger, G. & Grubeck-Loebenstein, B. How sex and age affect immune responses, susceptibility to infections, and response to vaccination. *Aging Cell* **14,** 309–321 (2015).

2. Whitacre, C. C. Sex differences in autoimmune disease. *Nat. Immunol.* **2,** 777–780 (2001).

3. Lockshin, M. D. Sex differences in autoimmune disease. *Lupus* **15,** 753–756 (2006).

4. Bouman, A., Heineman, M. J. & Faas, M. M. Sex hormones and the immune response in humans. *Hum. Reprod. Update* **11,** 411–423 (2005).

5. Oertelt-Prigione, S. Immunology and the menstrual cycle. *Autoimmun. Rev.* **11,** A486–A492 (2012).

6. Lu, Q. *et al.* Demethylation of CD40LG on the Inactive X in T Cells from Women with Lupus. *J. Immunol.* **179,** 6352–6358 (2007).

7. Zhou, Y. *et al.* T cell CD40LG gene expression and the production of IgG by autologous B cells in systemic lupus erythematosus. *Clin. Immunol.* **132,** 362–370 (2009).

8. Case, L. K. *et al.* The y chromosome as a regulatory element shaping immune cell transcriptomes and susceptibility to autoimmune disease. *Genome Res.* **23,** 1474–1485 (2013).

9. Liu, K. A. & DiPietro Mager, N. A. Women's involvement in clinical trials: historical perspective and future implications. *Pharm. Pract. (Granada).* **14,** 708 (2016).

10. Fish, E. N. The X-files in immunity: sex-based differences predispose immune responses. *Nat. Rev. Immunol.* **8,** 737–744 (2008).

11. Rabb, H. The T cell as a bridge between innate and adaptive immune systems: Implications for the kidney. *Kidney Int.* **61,** 1935–1946 (2002).

12. Hansson, G. K., Libby, P., Schönbeck, U. & Yan, Z. Innate and Adaptive Immunity in the Pathogenesis of Atherosclerosis. *Circ. Res.* **91,** 281–291 (2002).

13. Gutcher, I. & Becher, B. APC-derived cytokines and T cell polarization in autoimmune inflammation. *J. Clin. Invest.* **117,** 1119–1127 (2007).

14. Boniface, K., Blom, B., Liu, Y.-J. & de Waal Malefyt, R. From interleukin-23 to T-helper 17 cells: human T-helper cell differentiation revisited. *Immunol. Rev.* **226,** 132–146 (2008).

15. Bettelli, E. & Kuchroo, V. K. IL-12– and IL-23–induced T helper cell subsets: birds of the same feather flock together. *J. Exp. Med.* **201,** 169–171 (2005).

16. Murphy, A. J., Guyre, P. M., Wira, C. R. & Pioli, P. A. Estradiol Regulates Expression of Estrogen Receptor ERα46 in Human Macrophages. *PLoS One* **4,** e5539 (2009).

17. Molero, L. *et al.* Expression of estrogen receptor subtypes and neuronal nitric oxide synthase in neutrophils from women and men: regulation by estrogen. *Cardiovasc Res* **56,** 43–51 (2002).

18. Kovats, S. Estrogen receptors regulate innate immune cells and signaling pathways. *Cell.*

Immunol. **294,** 63–69 (2015).

19. Dosiou, C. *et al.* Expression of membrane progesterone receptors on human T lymphocytes and Jurkat cells and activation of G-proteins by progesterone. *J. Endocrinol.* **196,** 67–77 (2008).

20. Zhao, X. J. Expression of oestrogen and progesterone receptors by mast cells alone, but not lymphocytes, macrophages or other immune cells in human upper airways. *Thorax* **56,** 205–211 (2001).

21. Arruvito, L. *et al.* NK Cells Expressing a Progesterone Receptor Are Susceptible to Progesterone-Induced Apoptosis. *J. Immunol.* **180,** 5746–5753 (2008).

22. Jones, L. A. *et al.* Toll-like receptor-4-mediated macrophage activation is differentially regulated by progesterone via the glucocorticoid and progesterone receptors. *Immunology* **125,** 59–69 (2008).

23. Hughes, G. C., Thomas, S., Li, C., Kaja, M.-K. & Clark, E. a. Cutting Edge: Progesterone Regulates IFN- Production by Plasmacytoid Dendritic Cells. *J. Immunol.* **180,** 2029–2033 (2008).

24. Kissick, H. T. *et al.* Androgens alter T-cell immunity by inhibiting T-helper 1 differentiation. *Proc. Natl. Acad. Sci. U. S. A.* **111,** 9887–9892 (2014).

25. Benten, W., Stephan, C. & Wunderlich, F. B cells express intracellular but not surface receptors for testosterone and estradiol. *Steroids* **67,** 647–654 (2002).

26. Huang, C.-K. *et al.* New Therapy via Targeting Androgen Receptor in Monocytes/Macrophages to Battle Atherosclerosis. *Hypertension* **63,** 1345–1353 (2014).

27. Li, J. & McMurray, R. W. Effects of estrogen receptor subtype-selective agonists on autoimmune disease in lupus-prone NZB/NZW F1 mouse model. **123,** 219–226 (2007).

28. Phiel, K. L., Henderson, R. A., Adelman, S. J. & Elloso, M. M. Differential estrogen receptor gene expression in human peripheral blood mononuclear cell populations. *Immunol. Lett.* **97,** 107–113 (2005).

29. Biswas, D., Singh, S., Shi, Q., Pardee, A. & Iglehart, J. Crossroads of estrogen receptor and NF-kappaB signaling. *Sci STKE* **288,** (2005).

30. Jakacka, M. *et al.* An Estrogen Receptor (ER)α Deoxyribonucleic Acid-Binding Domain Knock-In Mutation Provides Evidence for Nonclassical ER Pathway Signaling in Vivo. *Mol. Endocrinol.* **16,** 2188–2201 (2002).

31. McKay, L. I. & Cidlowski, J. A. Molecular Control of Immune/Inflammatory Responses: Interactions Between Nuclear Factor-κB and Steroid Receptor-Signaling Pathways. *Endocr. Rev.* **20,** 435–459 (1999).

32. Pierdominici, M. *et al.* Estrogen receptor profiles in human peripheral blood lymphocytes. *Immunol. Lett.* **132,** 79–85 (2010).

33. Bouman, A., Schipper, M., Heineman, M. J. & Faas, M. M. Gender difference in the non-specific and specific immune response in humans. *Am. J. Reprod. Immunol.* **52,** 19–26 (2004).

34. Bengtsson, Å. K., Ryan, E. J., Giordano, D., Magaletti, D. M. & Clark, E. A. 17beta -Estradiol (E2) modulates cytokine and chemokine expression in human monocyte-derived dendritic cells.

Blood **104,** 1404–1410 (2004).

35. Hepworth, M. R., Hardman, M. J. & Grencis, R. K. The role of sex hormones in the development of Th2 immunity in a gender-biased model of Trichuris muris infection. *Eur. J. Immunol.* **40,** 406–416 (2010).

36. Melzer, S. *et al.* Reference intervals for leukocyte subsets in adults: Results from a population-based study using 10-color flow cytometry. *Cytom. Part B Clin. Cytom.* **88B,** 270–281 (2015).

37. Takao, T., Kumagai, C., Hisakawa, N., Matsumoto, R. & Hashimoto, K. Effect of 17beta-estradiol on tumor necrosis factor-alpha-induced cytotoxicity in the human peripheral T lymphocytes. *J. Endocrinol.* **184,** 191–197 (2005).

38. Maret, A. *et al.* Estradiol enhances primary antigen-specific CD4 T cell responses and Th1 development in vivo. Essential role of estrogen receptor α expression in hematopoietic cells. *Eur. J. Immunol.* **33,** 512–521 (2003).

39. Butterworth, M., McClellan, B. & Allansmith, M. Influence of sex in immunoglobulin levels. *Nature* **214,** 1224–1225 (1967).

40. Gundbacher, F. Human X Chromosome Carries Quantitative Genes for Immunoglobulin M. *Science (80-.).* **176,** 311–312 (1972).

41. Michaels, R. M. & Rogers, K. D. A sex difference in immunologic responsiveness. *Pediatrics* **47,** 120–123 (1971).

42. Weetman, A., McGregor, A., Rees Smith, B. & Hall, R. Sex hormones enhance immunoglobulin synthesis by human peripheral blood lymphocytes. *Immunol. Lett.* **3,** 343–346 (1981).

43. Kanda, N. & Tamaki, K. Estrogen enhances immunoglobulin production by human PBMCs. *J. Allergy Clin. Immunol.* **103,** 282–288 (1999).

44. Eidinger, D. & Garrett, T. J. Studies of the regulatory effects of the sex hormones on antibody formation and stem cell differentiation. *J. Exp. Med.* **136,** 1098–116 (1972).

45. Abdullah, M. *et al.* Gender effect on in vitro lymphocyte subset levels of healthy individuals. *Cell. Immunol.* **272,** 214–219 (2012).

46. Kanda, N., Tsuchida, T. & Tamaki, K. Testosterone inhibits immunoglobulin production by human peripheral blood mononuclear cells. *Clin. Exp. Immunol.* **106,** 410–415 (1996).

47. Fu, Y. *et al.* Estrogen promotes B cell activation in vitro through down-regulating CD80 molecule expression. *Gynecol. Endocrinol.* **27,** 593–596 (2011).

48. Moxley, G. *et al.* Sexual dimorphism in innate immunity. *Arthritis Rheum.* **46,** 250–258 (2002).

49. Pioli, P. A. *et al.* Estradiol Attenuates Lipopolysaccharide-Induced CXC Chemokine Ligand 8 Production by Human Peripheral Blood Monocytes. *J. Immunol.* **179,** 6284–6290 (2007).

50. Ghisletti, S., Meda, C., Maggi, A. & Vegeto, E. 17 -Estradiol Inhibits Inflammatory Gene Expression by Controlling NF- B Intracellular Localization. *Mol. Cell. Biol.* **25,** 2957–2968 (2005).

51. Moxley, G. *et al.* Premenopausal sexual dimorphism in lipopolysaccharide-stimulated production and secretion of tumor necrosis factor. *J. Rheumatol.* **31,** 686–694 (2004).

52. Furman, D. *et al.* Systems analysis of sex differences reveals an immunosuppressive role for

testosterone in the response to influenza vaccination. *Proc. Natl. Acad. Sci.* **111,** 869–874 (2014).

53. Klein, S. L., Jedlicka, A. & Pekosz, A. The Xs and Y of immune responses to viral vaccines. *Lancet Infect. Dis.* **10,** 338–349 (2010).

54. Nguyen, D. C. *et al.* 17β-Estradiol restores antibody responses to an influenza vaccine in a postmenopausal mouse model. *Vaccine* **29,** 2515–2518 (2011).

55. Pennock, J. W. *et al.* Estradiol Improves Genital Herpes Vaccine Efficacy in Mice. *Vaccine* **27,** 5830–5836 (2009).

56. Brooks, B. *et al.* Influence of Donor and Recipient Gender on the Outcome of Liver Transplantation. *Transplantation* **62,** 1784–1787 (1996).

57. Meier-Kriesche, H. U. *et al.* Gender differences in the risk for chronic renal allograft failure. *Transplantation* **71,** 429–432 (2001).

58. Chen, P.-D. *et al.* Gender differences in renal transplant graft survival. *J. Formos. Med. Assoc.* **112,** 783–788 (2013).

59. Hirasawa, K. & Enosawa, S. Effects of sex steroid hormones on sex-associated differences in the survival time of allogeneic skin grafts in rats. *Transplantation* **50,** 637–641 (1990).

60. Beeson, P. B. Age and sex associations of 40 autoimmune diseases. *Am. J. Med.* **96,** 457–462 (1994).

61. Schröder, J., Kahlke, V., Staubach, K.-H., Zabel, P. & Stüber, F. Gender Differences in Human Sepsis. *Arch Surg.* **133,** 1200–1205 (1998).

62. Couto, D. D. O. *et al.* Gender and mortality in sepsis: do sex hormones impact the outcome? *Rev. Bras. Ter. intensiva* **23,** 297–303 (2011).

63. Mörs, K. *et al.* Influence of gender on systemic IL-6 levels, complication rates and outcome after major trauma. *Immunobiology* (2016). doi:10.1016/j.imbio.2016.03.005

64. Sperry, J. L. *et al.* Male Gender is Associated With Excessive IL-6 Expression Following Severe Injury. *J. Trauma Inj. Infect. Crit. Care* **64,** 572–579 (2008).

65. Engelmann, F. *et al.* Impact of Estrogen Therapy on Lymphocyte Homeostasis and the Response to Seasonal Influenza Vaccine in Post-Menopausal Women. *PLoS One* **11,** e0149045 (2016).

66. Anipindi, V. C. *et al.* Estradiol Enhances CD4+ T-Cell Anti-Viral Immunity by Priming Vaginal DCs to Induce Th17 Responses via an IL-1-Dependent Pathway. *PLoS Pathog.* **12,** e1005589 (2016).

67. Shohat, T. *et al.* Gender Differences in the Reactogenicity of Measles-Mumps-Rubella Vaccine. *Isr. Med. Assoc. J. IMAJ* **2,** 192–195 (2000).

68. Ortona, E., Delunardo, F., Maselli, A., Pierdominici, M. & Malorni, W. Sex hormones and gender disparity in immunity and autoimmunity. *Ital. J. Gender-Specific Med.* **1,** 45–50 (2015).

69. Zou, Y. *et al.* Estradiol enhances murine cardiac allograft rejection under cyclosporin and can be antagonized by the antiestrogen tamoxifen. *Transplantation* **74,** 354–357 (2002).

70. Müller, V. *et al.* Sex hormones and gender-related differences: Their influence on chronic

renal allograft rejection. *Kidney Int.* **55,** 2011–2020 (1999).

71. Antus, B. *et al.* Opposite effects of testosterone and estrogens on chronic allograft nephropathy. *Transpl Int* **15,** 494–501 (2002).

72. Antus, B. *et al.* Contribution of androgens to chronic allograft nephropathy is mediated by dihydrotestosterone. *Kidney Int.* **60,** 1955–1963 (2001).

73. Antus, B. *et al.* Effects of progesterone and selective oestrogen receptor modulators on chronic allograft nephropathy in rats. *Nephrol. Dial. Transplant* **20,** 329–35 (2005).

74. Hou, X. & Pei, F. Estradiol Inhibits Cytokine-Induced Expression of VCAM-1 and ICAM-1 in Cultured Human Endothelial Cells Via AMPK/PPARα Activation. *Cell Biochem. Biophys.* **72,** 709–717 (2015).

75. Savolainen, H., Frösen, J., Petrov, L., Aavik, E. & Hayry, P. Expression of estrogen receptor sub-types alpha and beta in acute and chronic cardiac allograft vasculopathy. *J. Hear. Lung Transplant.* **20,** 1252–1264 (2001).

76. Ngo, S. T., Steyn, F. J. & McCombe, P. A. Gender differences in autoimmune disease. *Front. Neuroendocrinol.* **35,** 347–369 (2014).

77. Rider, V. & Abdou, N. I. Gender differences in autoimmunity: molecular basis for estrogen effects in systemic lupus erythematosus. *Int. Immunopharmacol.* **1,** 1009–1024 (2001).

78. Kanda, N., Tsuchida, T. & Tamaki, K. Testosterone suppresses anti-DNA antibody production in peripheral blood mononuclear cells from patients with systemic lupus erythematosus. *Arthritis Rheum.* **40,** 1703–1711 (1997).

79. Kanda, N., Tsuchida, T. & Tamaki, K. Estrogen enhancement of anti-double-stranded DNA antibody and immunoglobulin G production in peripheral blood mononuclear cells from patients with systemic lupus erythematosus. *Arthritis Care Res.* **24,** 328–337 (1999).

80. McMurray, R. W. & May, W. Sex hormones and systemic lupus erythematosus: Review and meta-analysis. *Arthritis Rheum.* **48,** 2100–2110 (2003).

81. Clowse, M. E. B. Lupus Activity in Pregnancy. *Rheum. Dis. Clin. North Am.* **33,** 237–252 (2007).

82. Adams Waldorf, K. M. & Nelson, J. L. Autoimmune Disease During Pregnancy and the Microchimerism Legacy of Pregnancy. *Immunol. Invest.* **37,** 631–644 (2008).

83. Maselli, A. *et al.* Low expression of estrogen receptor β in T lymphocytes and high serum levels of anti-estrogen receptor α antibodies impact disease activity in female patients with systemic lupus erythematosus. *Biol. Sex Differ.* **7,** 3 (2016).

84. Dragin, N. *et al.* Estrogen-mediated downregulation of AIRE influences sexual dimorphism in autoimmune diseases. *J. Clin. Invest.* **126,** 1525–1537 (2016).

85. Aschenbrenner, K. *et al.* Selection of Foxp3+ regulatory T cells specific for self antigen expressed and presented by Aire+ medullary thymic epithelial cells. *Nat. Immunol.* **8,** 351–358 (2007).

86. Anderson, M. S. *et al.* Projection of an immunological self shadow within the thymus by the aire protein. *Science* **298,** 1395–401 (2002).

87. Grimaldi, C. M., Jeganathan, V. & Diamond, B. Hormonal Regulation of B Cell Development: 17 -Estradiol Impairs Negative Selection of High-Affinity DNA-Reactive B Cells at More Than One

Developmental Checkpoint. *J. Immunol.* **176,** 2703–2710 (2006).

88. Grimaldi, C. M., Cleary, J., Selma Dagtas, A., Moussai, D. & Diamond, B. Estrogen alters thresholds for B cell apoptosis and activation. *J. Clin. Invest.* **109,** 1625–1633 (2002).

89. Bynoe, M. S., Grimaldi, C. M. & Diamond, B. Estrogen up-regulates Bcl-2 and blocks tolerance induction of naive B cells. *Proc. Natl. Acad. Sci. U. S. A.* **97,** 2703–2708 (2000).

90. Fan, H. *et al.* Gender differences of B cell signature in healthy subjects underlie disparities in incidence and course of SLE related to estrogen. *J. Immunol. Res.* **2014,** 814598 (2014).

91. Afshan, G., Afzal, N. & Quereshi, S. CD4+CD25(hi) regulatory T cells in healthy males and females mediate gender difference in the prevalence of autoimmune diseases. *Clin. Lab.* **58,** 567–571 (2012).

92. Arruvito, L., Sanz, M., Banham, A. H. & Fainboim, L. Expansion of CD4+CD25+and FOXP3+ Regulatory T Cells during the Follicular Phase of the Menstrual Cycle: Implications for Human Reproduction. *J. Immunol.* **178,** 2572–2578 (2007).

93. Aristimuño, C. *et al.* Sex-hormone receptors pattern on regulatory T-cells: clinical implications for multiple sclerosis. *Clin. Exp. Med.* **12,** 247–255 (2012).

94. Su, L. F., Kidd, B. A., Han, A., Kotzin, J. J. & Davis, M. M. Virus-Specific CD4+ Memory-Phenotype T Cells Are Abundant in Unexposed Adults. *Immunity* **38,** 373–383 (2013).

95. Charlton, B. & Lafferty, K. J. The Th1/Th2 balance in autoimmunity. *Curr. Opin. Immunol.* **7,** 793–798 (1995).

96. Cua, D. J., Hinton, D. R. & Stohlman, S. A. Self-antigen-induced Th2 responses in experimental allergic encephalomyelitis (EAE)-resistant mice. Th2-mediated suppression of autoimmune disease. *J. Immunol.* **155,** 4052–4059 (1995).

97. O'Shea, J. J., Ma, A. & Lipsky, P. Cytokines and autoimmunity. *Nat. Rev. Immunol.* **2,** 37–45 (2002).

98. Sicotte, N. L. & Voskuhl, R. R. Onset of multiple sclerosis associated with anti-TNF therapy. *Neurology* **57,** 1885–1888 (2001).

99. Titelbaum, D. S., Degenhardt, A. & Kinkel, R. P. Anti-Tumor Necrosis Factor Alpha-Associated Multiple Sclerosis. *Am. J. Neuroradiol.* **26,** 1548–1550 (2005).

100. Andreadou, E. *et al.* Demyelinating Disease following Anti-TNFa Treatment: A Causal or Coincidental Association? Report of Four Cases and Review of the Literature. *Case Rep. Neurol. Med.* **2013,** 671935 (2013).

101. Chen, X., Baumel, M., Mannel, D. N., Howard, O. M. Z. & Oppenheim, J. J. Interaction of TNF with TNF Receptor Type 2 Promotes Expansion and Function of Mouse CD4+CD25+ T Regulatory Cells. *J. Immunol.* **179,** 154–161 (2007).

102. Aerts, N. E. *et al.* Increased IL-17 production by peripheral T helper cells after tumour necrosis factor blockade in rheumatoid arthritis is accompanied by inhibition of migrationassociated chemokine receptor expression. *Rheumatology* **49,** 2264–2272 (2010).

103. Notley, C. a *et al.* Blockade of tumor necrosis factor in collagen-induced arthritis reveals a novel immunoregulatory pathway for Th1 and Th17 cells. *J. Exp. Med.* **205,** 2491–2497 (2008).

104. Scotland, R. S., Stables, M. J., Madalli, S., Watson, P. & Gilroy, D. W. Sex differences in resident

immune cell phenotype underlie more efficient acute inflammatory responses in female mice. *Blood* **118,** 5918–5927 (2011).

105. Marriott, I. & Huet-Hudson, Y. M. Sexual Dimorphism in Innate Immune Responses to Infectious Organisms. *Immunol. Res.* **34,** 177–192 (2006).

106. Rachoń, D., Myśliwska, J., Suchecka-Rachoń, K., Wieckiewicz, J. & Myśliwski, A. Effects of oestrogen deprivation on interleukin-6 production by peripheral blood mononuclear cells of postmenopausal women. *J. Endocrinol.* **172,** 387–395 (2002).

107. Rogers, A. & Eastell, R. The effect of 17beta-estradiol on production of cytokines in cultures of peripheral blood. *Bone* **29,** 30–34 (2001).

108. Kramer, P. R., Winger, V. & Kramer, S. F. 17β-Estradiol utilizes the estrogen receptor to regulate CD16 expression in monocytes. *Mol. Cell. Endocrinol.* **279,** 16–25 (2007).

109. Kramer, P. R., Kramer, S. F. & Guan, G. 17beta-estradiol regulates cytokine release through modulation of CD16 expression in monocytes and monocyte-derived macrophages. *Arthritis Rheum.* **50,** 1967–1975 (2004).

110. Miyagi, M., Aoyama, H., Morishita, M. & Iwamoto, Y. Effects of Sex Hormones on Chemotaxis of Human Peripheral Polymorphonuclear Leukocytes and Monocytes. *J. Periodontol.* **63,** 28–32 (1992).

111. Malisorn, N., Morales, N. P., Sanvarinda, Y. & Wanikiat, P. Characterization of estrogen receptor mediating neutrophil function. *Charact. estrogen Recept. Mediat. neutrophil Funct.* **32,** 276–280 (2010).

112. Posma, E., Moes, H., Heineman, M. J. & Faas, M. M. The Effect of Testosterone on Cytokine Production in the Specific and Non-specific Immune Response. *Am. J. Reprod. Immunol.* **52,** 237–243 (2004).

113. Kipari, T. *et al.* Lymphocytes modulate peritoneal leukocyte recruitment in peritonitis. *Inflamm. Res.* **58,** 553–560 (2009).

114. Kim, K. D. *et al.* Adaptive immune cells temper initial innate responses. *Nat. Med.* **13,** 1248–52 (2007).

115. Libert, C., Dejager, L. & Pinheiro, I. The X chromosome in immune functions: when a chromosome makes the difference. *Nat. Rev. Immunol.* **10,** 594–604 (2010).

116. Carrel, L. & Willard, H. F. X-inactivation profile reveals extensive variability in X-linked gene expression in females. *Nature* **434,** 400–404 (2005).

117. Johnston, C. M. *et al.* Large-Scale Population Study of Human Cell Lines Indicates that Dosage Compensation Is Virtually Complete. *PLoS Genet.* **4,** e9 (2008).

118. Andreu, N. *et al.* Wiskott–Aldrich syndrome in a female with skewed X-chromosome inactivation. *Blood Cells, Mol. Dis.* **31,** 332–337 (2003).

119. Kast, R. Predominance of autoimmune and rheumatic diseases in females. *J. Rheumatol.* **4,** 288–292 (1977).

120. Stewart, J. J. The female X-inactivation mosaic in systemic lupus erythematosus. *Immunol. Today* **19,** 352–357 (1998).

121. Forsdyke, D. R. X chromosome reactivation perturbs intracellular self/not-self discrimination.

Immunol. Cell Biol. **87,** 525–528 (2009).

122. Guo, X., Su, B., Zhou, Z. & Sha, J. Rapid evolution of mammalian X-linked testis microRNAs. *BMC Genomics* **10,** 97 (2009).

123. Sharma, S. & Eghbali, M. Influence of sex differences on microRNA gene regulation in disease. *Biol. Sex Differ.* **5,** 3 (2014).

124. Yuan, Z., Sun, X., Liu, H. & Xie, J. MicroRNA Genes Derived from Repetitive Elements and Expanded by Segmental Duplication Events in Mammalian Genomes. *PLoS One* **6,** e17666 (2011).

125. Pulikkan, J. A. *et al.* Cell-cycle regulator E2F1 and microRNA-223 comprise an autoregulatory negative feedback loop in acute myeloid leukemia. *Blood* **115,** 1768–1778 (2010).

126. Fazi, F. *et al.* A minicircuitry comprised of microRNA-223 and transcription factors NFI-A and C/EBPα regulates human granulopoiesis. *Cell* **123,** 819–831 (2005).

127. Pinheiro, I., Dejager, L. & Libert, C. X-chromosome-located microRNAs in immunity: Might they explain male/female differences? *BioEssays* **33,** 791–802 (2011).

128. Lu, C. *et al.* miR-221 and miR-155 regulate human dendritic cell development, apoptosis, and IL-12 production through targeting of p27kip1, KPC1, and SOCS-1. *Blood* **117,** 4293–4303 (2011).

129. Grigoryev, Y. A. *et al.* MicroRNA Regulation of Molecular Networks Mapped by Global MicroRNA, mRNA, and Protein Expression in Activated T Lymphocytes. *J. Immunol.* **187,** 2233–2243 (2011).

130. Wang, H., Peng, W., Ouyang, X., Li, W. & Dai, Y. Circulating microRNAs as candidate biomarkers in patients with systemic lupus erythematosus. *Transl. Res.* **160,** 198–206 (2012).

131. Fulci, V. *et al.* miR-223 is overexpressed in T-lymphocytes of patients affected by rheumatoid arthritis. *Hum. Immunol.* **71,** 206–211 (2010).

132. Ziats, M. N. & Rennert, O. M. Identification of differentially expressed microRNAs across the developing human brain. *Mol. Psychiatry* **19,** 848–852 (2014).

133. Mujahid, S., Logvinenko, T., Volpe, M. V & Nielsen, H. C. miRNA regulated pathways in late stage murine lung development. *BMC Dev. Biol.* **13,** 13 (2013).

134. Cheung, L., Gustavsson, C., Norstedt, G. & Tollet-Egnell, P. Sex-different and growth hormone-regulated expression of microRNA in rat liver. *BMC Mol. Miology* **10,** 13 (2009).

135. Mishima, T. *et al.* MicroRNA (miRNA) cloning analysis reveals sex differences in miRNA expression profiles between adult mouse testis and ovary. *Reproduction* **136,** 811–822 (2008).

136. Dai, R. *et al.* Suppression of LPS-induced Interferon- and nitric oxide in splenic lymphocytes by select estrogen-regulated microRNAs: a novel mechanism of immune modulation. *Blood* **112,** 4591–4597 (2008).

137. Boldin, M. P. *et al.* miR-146a is a significant brake on autoimmunity, myeloproliferation, and cancer in mice. *J. Exp. Med.* **208,** 1189–1201 (2011).

138. Johnnidis, J. B. *et al.* Regulation of progenitor cell proliferation and granulocyte function by microRNA-223. *Nature* **451,** 1125–1129 (2008).

139. Freitak, D., Knorr, E., Vogel, H. & Vilcinskas, A. Gender- and stressor-specific microRNA expression in Tribolium castaneum. *Biol. Lett.* **8,** 860–863 (2012).

140. Hewagama, A. *et al.* Overexpression of X-Linked genes in T cells from women with lupus. *J. Autoimmun.* **41,** 60–71 (2013).

141. Naramura, M. *et al.* c-Cbl and Cbl-b regulate T cell responsiveness by promoting ligand-induced TCR down-modulation. *Nat. Immunol.* **3,** 1192–1199 (2002).

142. Zhao, S. *et al.* MicroRNA-126 regulates DNA methylation in CD4+ T cells and contributes to systemic lupus erythematosus by targeting DNA methyltransferase 1. *Arthritis Rheum.* **63,** 1376–1386 (2011).

143. Dai, Y. *et al.* Microarray analysis of microRNA expression in peripheral blood cells of systemic lupus erythematosus patients. *Lupus* **16,** 939–946 (2007).

144. Otaegui, D. *et al.* Differential micro RNA expression in PBMC from multiple sclerosis patients. *PLoS One* **4,** (2009).

145. Du, C. *et al.* MicroRNA miR-326 regulates TH-17 differentiation and is associated with the pathogenesis of multiple sclerosis. *Nat. Immunol.* **10,** 1252–1259 (2009).

146. Giltay, E. J. *et al.* In Vivo Effects of Sex Steroids on Lymphocyte Responsiveness and Immunoglobulin Levels in Humans. *J. Clin. Endocrinol. Metab.* **85,** 1648–1657 (2000).

147. Scofield, R. H. *et al.* Klinefelter's syndrome (47,XXY) in male systemic lupus erythematosus patients: Support for the notion of a gene-dose effect from the X chromosome. *Arthritis Rheum.* **58,** 2511–2517 (2008).

148. De Vries, G. J. *et al.* A model system for study of sex chromosome effects on sexually dimorphic neural and behavioral traits. *J. Neurosci.* **22,** 9005–9014 (2002).

149. Smith-Bouvier, D. L. *et al.* A role for sex chromosome complement in the female bias in autoimmune disease. *J. Exp. Med.* **205,** 1099–1108 (2008).

150. Palaszynski, K. M. *et al.* A Yin-Yang Effect between Sex Chromosome Complement and Sex Hormones on the Immune Response. *Endocrinology* **146,** 3280–3285 (2005).

151. De Vries, G. J. Sex steroids and sex chromosomes at odds? *Endocrinology* **146,** 3277–3279 (2005).

152. Broderick, J. A. & Zamore, P. D. MicroRNA therapeutics. *Gene Ther.* **18,** 1104–1110 (2011).