European Journal of Immunology

HIGHLIGHTS

REVIEW

Sex differences regulate immune responses in experimental autoimmune encephalomyelitis and multiple sclerosis

Lucy Ryan and Kingston H. G. Mills

School of Biochemistry and Immunology, Trinity Biomedical Science Institute, Trinity College Dublin, Dublin 2 , Ireland

MS is an autoimmune disease of the CNS that afflicts over 2.5 million people worldwide. There are striking sex differences in the susceptibility to and progression of this disease in humans. Females are twice as likely to develop MS than males, whereas disease progression and disability is more rapid in males compared with females; however, the latter is still controversial. There is growing evidence, mainly from animal models, that innate and adaptive immune responses are different in males and females, and that this can influence the outcome of a range of diseases including infection, cancer, and autoimmunity. Since MS is an immune-mediated disease, sex differences in pathogenic immune responses may account for some of the differences in susceptibility to and progression seen in men versus women. Indeed, data from the mouse model of MS, EAE, have already provided some evidence that female mice have earlier disease onset associated with stronger Th17 responses. This review will discuss the possible immunological basis of sex differences in susceptibility and disease outcome in EAE and MS and how a better understanding of sex differences in the responses to disease-modifying therapies may lead to improved patient treatment.

Keywords: multiple sclerosis · experimental autoimmune encephalitis · sex differences · Th17 cells · autoimmune disease

Introduction

MS is a disabling neurological and autoimmune disease of the CNS, where the immune system attacks the myelin sheath that surrounds nerve fibers. CD4 T cells that secrete IL-17 (Th17 cells), B cells, and other immune cells cross the blood-brain–barrier (BBB) and mediate pathology in the CNS. It has been known for many years that women are more susceptible to MS than men, with the female-to-male disease incidence ratio ranging from 2:1 to 3:1, depending on geographical region [1–3]. There is some evidence that men show quicker disease progression, especially those with relapsing-remitting MS (RRMS), with a more rapid accumulation of disability compared with women [4–6]. However, definitive evidence and a mechanism for more severe disease in men are still lacking and are discussed in more detail below.

Correspondence: Prof. Kingston H. G. Mills
e-mail: kingston.mills@tcd.ie

[Correction added on 3 December 2022, after first online publication: Peer review history statement has been added.]

It has become increasingly clear that the immune response in males and females differs in many aspects. This is consistent with the higher prevalence of autoimmune diseases in females and the higher risk of mortality from malignant cancer in males. Females generally mount stronger immune response to vaccines and usually generate higher levels of circulating antibodies compared with males [7]. Furthermore, female, but not male C57BL/10 mice survive infection with *Plasmodium chabaudi* and this was related to testosterone suppression of protective immunity to the parasite [8]. The recent COVID-19 pandemic has also revealed differences in immune responses between males and females. Men develop more severe COVID-19 symptoms and are at a higher risk of mortality compared with women. However, women show higher levels of activated T cells during SARS-CoV-2 infection compared with men [9].

This review will discuss sex differences in the immune system that are relevant to MS. Understanding these differences is not only important for elucidating the mechanism of disease but could help to inform the discovery of novel therapeutic targets and disease-modifying molecules.

Disease severity in EAE is gender dependent

Studies in the mouse model of MS, EAE have attempted to explain differences in disease severity between males and females. Some studies have shown no difference in the course of disease in myelin oligodendrocyte protein (MOG)-induced EAE in C57BL/6 mice [10–12]. One study reported stronger proinflammatory responses, greater lymphocyte infiltration and demyelination in the spinal cord in males and higher regulatory responses in female C57BL/6 mice, but no difference in disease severity between sexes [10]. Nevertheless, it has been reported that endogenous oestrogens can inhibit CNS inflammation and the development of EAE in female C57BL/6 mice through estrogen receptor-α (ER-α) expression on nonhematopoietic cells [13]. Furthermore, it has been demonstrated that female SJL mice have enhanced EAE disease severity compared with males [12, 14]. While female SJL mice immunized with myelin proteolipid protein (PLP) or MOG had enhanced clinical scores compared with males, male B10.PL and PL/J mice developed more severe disease than females and no sex differences were found in the C57BL/6 or NOD strains [12]. Furthermore, the transfer of myelin basic protein-specific T cells to naive SJL mice resulted in EAE with earlier onset and more acute disease in female recipient mice [15]. One of the most in-depth and convincing studies showed greater expression of spingosine-1-phosphate receptor-2 (S1PR2) and vascular permeability in cerebella of female compared with male SJL mice [14]. Treatment with a S1PR2 antagonist ameliorated EAE in female, but not in male, SJL mice. Furthermore, *S1pr2*$^{-/-}$ mice had less severe EAE, and this was associated with reduced BBB permeability, fewer inflammatory foci and less demyelination in the spinal cord [14]. Furthermore, the same study also showed that female patients with MS had higher S1PR2 in the cerebellum compared with male patients. This study nicely links the data from EAE and MS, but did not address differences in CNS infiltration of Th17 cells between female and male mice, which may be the key to sex differences in these diseases.

Sex and the immune response

Sex is defined by sex chromosomes, reproductive organs, and levels of sex hormones. Additionally, social behavior and cultural norms reflect gender differences, which may also influence disease susceptibility and progression [16]. Sex chromosomes and levels of sex hormones are inextricably linked; therefore, it can be difficult to decipher the precise role of one independent of the other. The "four core genotype" (FCG) mouse model has been useful for separating the phenotypic effects arising from sex chromosomes and gonadal hormones [17]. This model produces XX and XY mice with testes and XX and XY mice with ovaries through the deletion or insertion of the *Sry* testis-determining gene. A phenotypic difference between XX and XY mice with the same gonadal type is caused by sex chromosomes. Conversely, a phenotypic difference between mice with the same sex chromosome complement but a different gonadal type is due to gonadal hormones [17]. This separation is important, as sex differences in the immune system can arise from both sex chromosome complement and levels of sex hormones.

The human X chromosome codes for approximately 2000 genes, many of which are involved in the regulation of immune function [18]. Genes for PRRs, cytokine receptors, and transcription factors are present on the X chromosome. The X chromosome also contains many microRNAs that have roles in modulating immune responses [16]. In females, a process of X-inactivation occurs where one X chromosome is silenced to ensure the expression of a single set of X chromosome genes. However, an estimated 15% of genes escape X-inactivation in humans. This results in higher gene expression in females compared with males [18]. Disparate immune gene expression can directly cause differential immune responses in males and females.

Sex hormones have various immune-modulating functions. Sex hormones can directly influence immune responses through sex hormone receptors, which are present on many immune cells. Additionally, several immune genes have androgen or estrogen response elements in their promoter region [16]. Glucocorticoid hormones have potent anti-inflammatory effects and are commonly prescribed for autoimmune and inflammatory disorders [19]. Sex hormones influence glucocorticoid concentrations by modulating the hypothalamic-pituitary-adrenal axis [20], thereby, indirectly modulating immune responses.

The severity of many autoimmune diseases, including MS, is suppressed during late pregnancy [21]. Studies in the EAE model have suggested that pregnancy-associated attenuation of disease is associated with enhancement of the frequency of Treg cells, and this was mediated by the binding of progesterone to the glucocorticoid receptor on T cells [22]. Although they did not examine effector T cells, it has been reported that progesterone can inhibit differentiation of naïve human cord blood cells into Th17

cells [23]. However, the study in mice showed that T-cell-specific deletion of glucocorticoid receptor did not augment the number of Treg cells or protection against EAE during pregnancy [22]. Conversely, other studies in the EAE model showed that adoptive transfer of autoreactive immune cells from XX compared with XY mice into naïve mice enhanced disease susceptibility and outcome [24]. Therefore, some aspects of sex differences in EAE and possibly MS may be independent of sex hormones.

Sex differences in innate immune responses

Innate immune sensors

IFN-β is a widely used therapy for RRMS. IFN-β is a type 1 interferon that signals through the type 1 interferon receptor (IFNAR) complex. The mechanism of action behind the therapeutic effect of IFN-β in MS is poorly understood [25]. However, it has been reported that IL-27 produced by DCs mediates the response to IFN-β by inhibiting Th17 cells [26]. It has also been suggested that IFN-β treatment of MS patients has distinct effects on CD4 T-cell responses by differentially influencing IL-6 and IFN-γ production in males versus females [27]. However, this study was based on mitogen-activated T cells and was not followed up with more physiological measures of pathogenic T-cell responses.

There is also some evidence of a role for IFN-α in the pathogenesis of MS and this too may have a sex difference. Activation of TLR7, encoded on the X chromosome, results in IFN-α production via interferon regulatory factor 5 (IRF5). As well as its antiviral effect, IFN-α is involved in the maturation of DCs and differentiation of Th1 cells [28, 29]. TLR7-activated peripheral blood leucocytes from females produce significantly higher concentrations of IFN-α than leucocytes from males [29]. Plasmacytoid DCs (pDCs) express high levels of IRF5 and are a major source of IFN-α. pDCs from female mice deficient in ER-α show lower expression of IRF5 mRNA and reduced production of IFN-α in response to TLR7 stimulation [28]. This suggests that IFN-α production is at least partially regulated by estrogen signaling.

However, the possible role of TLR7 signaling in MS is still controversial. Dzopalic et al. demonstrated that activation of TLR7 in human monocyte-derived DCs results in differentiation of Th17 and Th1 cells, with accompanying increases in IL-17 and IFN-γ production [30]. It has been shown that activation of TLR7 in pDCs promotes differentiation of Th17 cells [31]. Th17, as well as Th1 cells, are pathogenic in EAE and MS [32]. However, Ye et al. show that TLR7 activation in T cells inhibits differentiation of Th17 cells, reducing IL-17 production, thereby protecting against the development of EAE [33].

TLR9-induced IL-10 production by human PBMCs is higher in males compared with females and this correlates with plasma levels of the male sex hormone dehydroepiandrosterone sulfate. [34]. Interestingly, IL-10 production by female PBMCs inversely correlated with estradiol levels. This suggests IL-10 production by innate immune cells may be regulated by male sex hormones. IL-10 is an important anti-inflammatory cytokine known to be protective in EAE [32].

Expression of TLR4 on human neutrophils and murine macrophages is higher in males compared with females [35, 36]. Consistent with this, cells from males produced higher concentrations of TNF, IL-6, IL-1β, and IP-10 following stimulation with LPS [35, 36]. These proinflammatory cytokines are pathogenic in MS [32]. However, treatment of macrophages with testosterone prior to LPS stimulation decreases TLR4 expression and TNF production [37]. These findings highlight the limitations of certain in vitro studies.

Putative estrogen response elements have been identified in the promoters of *Tlr7*, *Tlr3*, *Myd88*, *Stat3*, and *Ifnar1*. Furthermore, putative androgen response elements were found in the promoters of *Irf7* and *Mapk3* [38]. These findings suggest that innate immune responses are influenced by sex chromosomes, sex hormone receptor signaling, and sex hormone regulation of gene transcription. These sex differences should be considered in future studies on the immunopathology of MS.

Innate immune cells

APCs from females are more efficient at presenting antigen to primed lymphocytes than APCs from males [39]. Activation of ER-α by estradiol promotes the maturation of conventional DCs and pDCs, with increased expression of MHC class II and costimulatory molecules, CD40 and CD86 [40]. Therefore, female DCs may be more efficient at presenting autoantigen to encephalitogenic T cells. However, further research is needed to elucidate sex differences in DC function.

A study in the EAE model using SJL mice with mutant c-kit has indicated a male-specific protective effect of innate lymphoid cell 2 (ILC2) [41]. c-kit is a stem cell factor involved in the survival of many hematopoietic lineages. c-kit-deficient male mice had a lower number of ILC2 precursors in the draining LNs and CNS compared with WT male mice and this resulted in increased peripheral Th17 responses and exacerbated EAE. ILC2 is an important source of IL-5, IL-9, and IL-13 and drives Th2 responses. The authors hypothesize that c-kit signaling is required for ILC2 precursor survival and that loss of these cells removed an attenuating influence on the development of EAE. This study reported that female WT mice have lower number of CNS-infiltrating ILC2 on day 10 of EAE compared with male WT mice. However, other studies focused on respiratory tissue have shown no differences in number of ILC2 in the lung of male versus female mice and suggested that ILC2 from female mice are more readily activated than ILC2 from male mice [42]. It has also been reported that female mice have significantly higher number of ILC2 in the lung compared with male mice [43]. Conversely, it has been demonstrated that male mice had more ILC2 progenitors and a greater number of mature ILC2 in peripheral tissues than female mice and this was associated with reduced susceptibility to allergic airway inflammation [44]. Furthermore, androgen receptor signaling in hematopoietic cells was found to play

a protective role in type 2 airway inflammation by limiting differentiation of ILC2 precursors into mature ILC2 [44]. However, studies in humans showed that women have an increased number of circulating ILC2 compared to men and that testosterone decreased the number of ILC2 in the lungs and attenuated airways inflammation [45]. Although there is no clear explanation for the discrepancies between studies, it may reflect differences between tissues or diseases under investigation. Nevertheless, it is possible that ILC2 may reduce susceptibility to EAE by promoting a Th2 response, especially in male mice [41]. Whether the protective effect of ILC2 against EAE is mediated by sex hormones or due to chromosomal complement and the extrapolation of these findings to MS requires further research. However, a study on human cord blood found that newborn boys have higher number of ILC2 compared with newborn girls [46].

Sex differences in adaptive immune responses

CD3 T cells from women have a stronger response to in vitro stimulation with phytohemagglutinin, when compared with T cells from men [47]. In addition, T cells from females have higher expression of proinflammatory and cytotoxic gene transcripts including included *Ifng*, *Gzma*, *Ili2rb2*, *Ltb*, and *Gnly* [48]. Some of these transcripts contain an estrogen response element in their promoters, suggesting that sex hormones may in part explain differences in adaptive immune responses between males and females.

Th1 cells

Th1 cells are known to play a role in the pathology of MS and EAE [49, 50]. Adoptive transfer of activated myelin-specific Th1 cells from mice with EAE induces disease in naïve mice [50] Th1 cells are a major source of IFN-γ and GM-cerebrospinal fluid (CSF). IFN-γ is associated with relapse in MS patients [49] and plays a pathogenic role at the induction phase of EAE [51]. GM-CSF, produced by both Th1 and Th17 cells, also has a pathogenic role in EAE [52, 53]. Following immunization with BCG, Th1 cytokine production is higher in females compared with male mice [54]. However, studies with coxsackievirus B3-induced myocarditis have shown that male mice have stronger Th1 responses, whereas Th2 responses are stronger in females [55].

It has been known for some time that disease in female MS patients is attenuated during late pregnancy [21]. Many female sex hormones are elevated during pregnancy. The female sex hormone estriol is only present in pregnant women and reaches high concentrations during the third trimester. Estriol binds to ER-β which is highly expressed in the brain [56]. Treatment with estriol attenuates EAE in male and female mice and this was associated with reduced production of proinflammatory cytokines, including IFN-γ, by autoantigen-specific spleen cells [57]. Treatment of mice with an ER-β agonist attenuated EAE by inhibiting activation of microglia as well as T cells [58]. During pregnancy, Th2 are enhanced over Th1 responses. This suggests estriol may have a protective effect in MS and in other immune-mediated diseases by inhibiting Th1 cells [56].

Th17 cells

IL-17-producing CD4 T cells (Th17 cells), as well as IL-17-producing γδ T cells, have well-established pathogenic roles in EAE [59]. Furthermore, the results of early clinical trials have shown that neutralization of IL-17 can reduce active lesions in RRMS patients [60]. IL-23 and IL-1β play a crucial role in the activation of IL-17-secreting γδ T cells and Th17 [61–63]. Mice deficient in the p19 chain of IL-23 are resistant to the development of EAE [64]. Furthermore, IL-17$^{-/-}$ mice are resistant to induction of EAE [65].

Although data are emerging on sex differences in Th17 cell responses, there are conflicting reports in the literature. It has been reported that IL-17A gene expression is significantly higher in polyclonally activated peripheral T cells from men compared with women [48]. However, Sankaran et al. reported the IL-17-associated transcriptional factors, RORγ and STAT3, are significantly higher in T cells from women compared with men [66]. Treatment of mice with estradiol suppressed IL-17 production and attenuated EAE [67], suggesting that IL-17 production may be regulated by sex hormones. It has been shown that the protective effect of estradiol in EAE is mediated through ER-α on T cells, leading to inhibition of the development of Th1 and Th17 cells [68]. Deletion of ER-α in T cells reduces their activation and enhances expression of Foxp3 [69]. An elegant study by Garnier et al. showed that estrogen signaling in CD4^{+} T cells suppressed differentiation of Th17 cells through a trans-acting mechanism involving the immune checkpoints PD-1/PD-L1 [70]. Since anti-IL-17A was found to be effective in early clinical trials in the treatment of RRMS patients [60], the differences in IL-17A production between males and females could be important for therapeutic outcomes.

γδ T cells

γδ T cells infiltrate the CNS after induction of EAE and are a major source of IL-17A, especially early in the course of EAE [63]. γδ T cell-derived IL-17A has a role in mobilizing IL-1β-producing innate cells to the draining LN in EAE [65]. These innate cells promote the further differentiation of IL-17A-producing encephalitogenic Vγ4^{+} γδ T cells and Th17 cells. Furthermore, a novel population of T cells expressing both an αβ and γδ TCR are an important source of IL-17 in the LN and CNS early in EAE [71]. γδ T cells have also been found in brain lesions and CSF of MS patients [72, 73]. Caccamo et al. reported that women have higher levels of circulating Vγ9/Vδ2 γδ T cells compared with men [74]. Further investigations on sex differences in γδ T-cell populations and function in EAE and MS are needed. It will be

important to elucidate the source of IL-17A early in MS disease and whether this differs between males and females.

Treg cells

Treg cells are important modulators of inflammation and impaired Treg cell function, leading to unconstrained autoreactive T cells, is a common feature of many autoimmune diseases including MS [32]. Treg cells exert their immunosuppressive function by inhibiting APCs and by secreting the immunosuppressive cytokines IL-10 and TGF-β [75]. Patients with RRMS have reduced levels of the transcriptional regulatory factor Foxp3, which is crucial for the development of natural Treg [76]. Interestingly, Foxp3 is encoded on the X chromosome [16]. One study reported that women have significantly lower numbers of peripheral Treg cells compared with men [77]. Treg cell numbers are reduced, or their function is defective, in many autoimmune diseases, including MS [78, 79], but there have been limited reports on sex difference in Treg cells in human autoimmune diseases. However, a study on patients with undifferentiated arthritis found lower numbers of Treg cells in females compared with male patients [80]. In the relapsing-remitting EAE model, male mice did not relapse, but relapse was induced by orchidectomy and this was associated with enhanced infiltration of Th1 cells into the CNS [81], suggesting that testosterone modulates immune responses that mediate pathology in EAE. Studies with human T cells have shown that testosterone can induce expression of Foxp3 on T cells via direct interaction with an androgen receptor binding site in the Foxp3 locus, leading to epigenetic changes that promote differentiation or maintenance of Treg cells [82]. These findings suggest a role for testosterone in preventing development of autoimmune diseases. However, the extent of sex differences in Treg cell function in MS remains unclear. Further investigation on the frequency and function of Treg numbers and expression of immune checkpoints in females and males are needed.

B cells

Although most of the focus has been on the pathological role of T cells, evidence has emerged in recent years that B cells may have a role in the pathogenesis of MS. Antimyelin autoantibodies have been identified in MS patients [83]. However, there is no clear evidence that these antibodies are confined to MS patients and their relevance, if any, to disease progression is controversial [84, 85]. Alternatively, B cells may promote pathology in MS by presenting autoantigen to pathogenic T cells or by producing proinflammatory cytokines that contribute to neuroinflammation [86]. The most striking evidence in support of a pathogenic role for B cells in MS is provided by the demonstration that Rituximab, an anti-CD20 B cell-depleting antibody, reduces disease activity in RRMS and primary progressive MS (PPMS) patients [87]. Consistent with the findings that females mount stronger antibody responses to immunization [16], females tend to have higher numbers of B cells compared with males [47]. Antibody levels may be in part regulated by female sex hormones. A study by Lu et al. demonstrated that antibodies and antibody-secreting cells are highest before ovulation in females [88]. B cells express ER-α and ER-β and estradiol enhances survival and activation of immature B cells, and this can lead to the generation of mature autoreactive B cells [89]. This may in part explain the female predominance of systemic lupus erythematosus, but its relevance to MS remains to be examined. Further studies on the differences in numbers of B cells and antibody responses may provide insight into sex differences in the immune responses that mediate MS and may influence the response to B cell-directed therapies.

Synthesis of sex differences in adaptive immune responses in EAE and MS

Sex differences in susceptibility to and progression of MS may reflect differences in the balance between pathogenic Th1, Th17, and B cells versus protective Th2 and Treg cells (Fig. 1). There is indirect evidence for this from studies on sex differences in immune responses, in general, but limited evidence from studies in MS patients. Studies in mice with the EAE model are beginning to provide some clues including evidence of more CNS infiltrating lymphocytes and, therefore, potentially pathogenic T and B cells in female mice. However, conclusive studies showing stronger Th17 responses and weaker Treg response in women are still lacking, especially in MS patients. Future studies on sex differences in the response to therapeutics that target IL-17/Th17 cells or B cells should provide more information to address these knowledge gaps.

MS disease progression in males

Although women are more susceptible to MS, men with RRMS appear to show worse clinical outcomes than women. Males with RRMS accumulate disability and progress to secondary progressive MS (SPMS) significantly faster than females [4]. Additionally, men with MS show worse cognitive impairment and grey matter atrophy compared with women [56]. Earlier cognitive decline in men with RRMS was associated with enhanced disability score [6]. A study of over 5000 RRMS patients in Canada found that conversion to SPMS disease course occurred earlier and at a young age in males [5]. It has also been reported that male patients with RRMS or SPMS accumulate disabilities faster than females, but no sex differences in the rate of disability accumulation were found in in PPMS patients [4]. Furthermore, another study did not find a higher risk of secondary progression in male RRMS patients [90]. Therefore, the findings on faster disease progression in male MS patients remain controversial and there is no clear mechanism to explain quicker progression in men but higher incidence of MS in women.

The FCG mouse model has been used to examine sex differences in EAE associated with sex chromosome complement in the

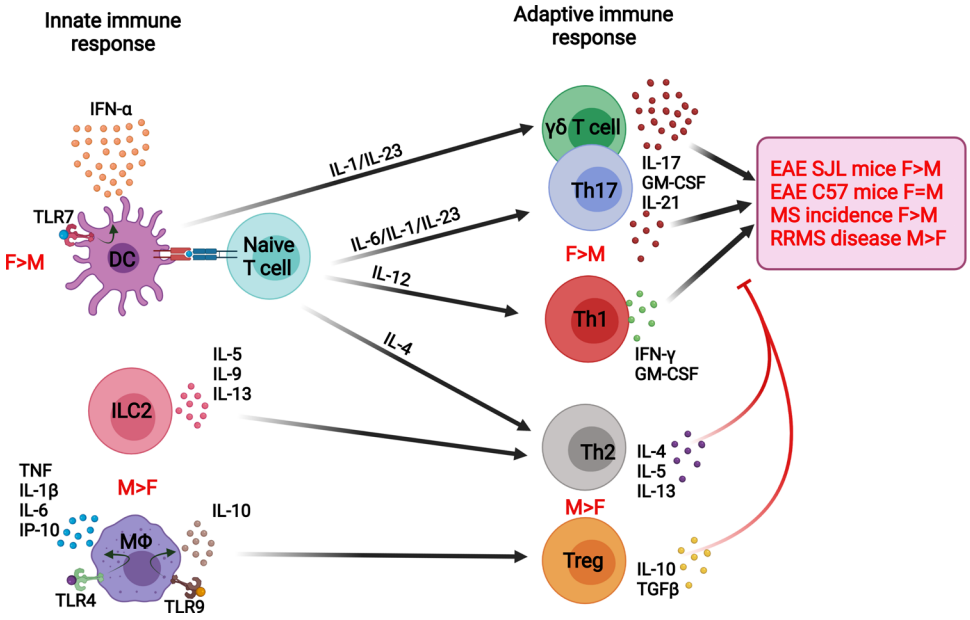

Figure 1. Schematic diagram illustrating the hypothetical role of innate and adaptive effector and regulatory immune responses that mediate EAE and MS and possible sex differences in these responses. Current evidence suggests that IL-17 secreted by Th17 cells and γδ T cells plays a major role in disease pathology in EAE and MS. The role of Th1 cells is less clear. However, GM-CSF secreted by Th1 as well as Th17 cells is considered to have a pathogenic role in EAE. In general, these effector immune responses are stronger in females compared with males. This may reflect stronger innate immune responses that promote induction or expansion of Th1, Th17, and γδ T cells. It may also reflect weaker anti-inflammatory macrophages, ILC2, or regulatory T cell responses in female mice. Dendritic cell, DC; type 2 innate lymphoid cell, ILC2; macrophage, MΦ; regulatory T cell, Treg; experimental autoimmune encephalomyelitis, EAE; multiple sclerosis, MS, relapsing remitting multiple sclerosis; RRMS; C57BL/6 (mice), C57; female, F; male, M. Created with BioRender.com.

CNS, independent of sex differences in the immune system. Using BM chimeras, it was demonstrated that EAE is more severe in mice with an XY sex chromosome complement in the CNS [91]. These mice showed greater neuropathology compared with mice with an XX sex chromosome complement in the CNS [91]. The next step will be to identify the genes on sex chromosomes that contribute to greater neurodegeneration in males. It will also be important to untangle the relationship between neurodegeneration and inflammation and how this may differ between the sexes.

Conclusion and future perspective

Immune responses differ in many aspects between males and females. However, it is only in recent years that sex differences in the immune system have been properly appreciated and investigated. The vast majority of studies in animal models have been conducted in one sex under the assumption that the findings would hold true for the opposite sex [56]. Studies using the EAE model show a clear female bias, with one report stating that 85% of studies using EAE were conducted on female rodents only [92]. The paucity of sex-based biological differences in biomedical research is no longer acceptable given the clear sex differences in immune responses and in the prevalence and progression of many diseases. The importance of studying disease models in both sexes is becoming more evident, with research funding bodies, including National Institutes of Health, now requiring the inclusion of sex as a biological variable in grant applications [18].

MS has striking sex differences in susceptibility and disease progression. These clinical observations should be considered in preclinical experiments to facilitate the discovery and development of novel and more effective disease-modifying therapies. This has been termed the "bedside-to-bench-to-bedside" approach to drug development [56]. Additionally, sex may be an important factor to consider for patient stratification, as the efficacy, optimal dose and side-effects of drugs can differ between males and females [56].

There is already some evidence from the EAE model that drugs licensed for the treatment of MS may have distinct effects in males and females. Dimethyl fumarate (DMF), a treatment currently used for MS, suppresses Th1/Th17 inflammatory responses and promotes a Th2 type response which has been shown to protect against the development of severe EAE [93]. Treatment with DMF was more effective in male compared with female rats with EAE and this was associated with enhanced anti-inflammatory myeloid cells and reduced IL-17 production by CD4 T cells [94]. Given the sex differences in IL-17 production and neurodegeneration, DMF and other disease-modifying therapies may have differential effects in males and females. However, evidence for this from MS patients is still lacking [95] and many studies on the responses to disease-modifying therapies in MS patients did not stratify the data based on gender.

Understanding the basis of sex differences in the immune response, whether due to sex chromosome complement or sex hormone signaling, will be important for elucidating MS pathogenesis and for the discovery of novel therapeutic targets. Further research committed to answering these questions will improve the current understanding of MS and will ultimately lead to better patient treatment.

Acknowledgments: This work was supported by grants from Science Foundation Ireland (16/IA/4468 and 12/RI/2340).

Conflict of interest: The authors declare no commercial or financial conflict of interest.

Peer review: The peer review history for this article is available at https://publons.com/publon/10.1002/eji.202149589

References

1 **Ahlgren, C., Odén, A. and Lycke, J.**, High nationwide prevalence of multiple sclerosis in Sweden. *Mult. Scler.* 2011. **17**: 901–908.

2 **Celius, E. G. and Vandvik, B.**, Multiple sclerosis in Oslo, Norway: prevalence on 1 January 1995 and incidence over a 25-year period. *Eur. J. Neurol.* 2001. **8**: 463–469.

3 **Orton, S. M., Ramagopalan, S. V., Brocklebank, D., Herrera, B. M., Dyment, D. A., Yee, I. M., Sadovnick, A. D. et al.,** Effect of immigration on multiple sclerosis sex ratio in Canada: the Canadian Collaborative Study. *J. Neurol. Neurosurg. Psychiatry* 2010. **81**: 31–36.

4 **Ribbons, K. A., McElduff, P., Boz, C., Trojano, M., Izquierdo, G., Duquette, P., Girard, M. et al.,** Male sex is independently associated with faster disability accumulation in relapse-onset MS but not in primary progressive MS. *PLoS One* 2015. **10**: e0122686.

5 **Koch, M., Kingwell, E., Rieckmann, P. and Tremlett, H.**, The natural history of secondary progressive multiple sclerosis. *J. Neurol. Neurosurg. Psychiatry* 2010. **81**: 1039–1043.

6 **Savettieri, G., Messina, D., Andreoli, V., Bonavita, S., Caltagirone, C., Cittadella, R., Farina, D. et al.,** Gender-related effect of clinical and genetic variables on the cognitive impairment in multiple sclerosis. *J. Neurol.* 2004. **251**: 1208–1214.

7 **Furman, D., Hejblum, B. P., Simon, N., Jojic, V., Dekker, C. L., Thiébaut, R., Tibshirani, R. J. et al.,** Systems analysis of sex differences reveals an immunosuppressive role for testosterone in the response to influenza vaccination. *Proc. Natl Acad. Sci. U S A* 2014. **111**: 869–874.

8 **Benten, W. P., Wunderlich, F. and Mossmann, H.**, Testosterone-induced suppression of self-healing Plasmodium chabaudi malaria: an effect not mediated by androgen receptors? *J. Endocrinol.* 1992. **135**: 407–413.

9 **Takahashi, T., Ellingson, M. K., Wong, P., Israelow, B., Lucas, C., Klein, J., Silva, J. et al.,** Sex differences in immune responses that underlie COVID-19 disease outcomes. *Nature* 2020. **588**: 315–320.

10 **Wiedrick, J., Meza-Romero, R., Gerstner, G., Seifert, H., Chaudhary, P., Headrick, A., Kent, G. et al.,** Sex differences in EAE reveal common and distinct cellular and molecular components. *Cell. Immunol.* 2021. **359**: 104242.

11 **Rahn, E. J., Iannitti, T., Donahue, R. R. and Taylor, B. K.**, Sex differences in a mouse model of multiple sclerosis: neuropathic pain behavior in females but not males and protection from neurological deficits during proestrus. *Biol. Sex Differ.* 2014. **5**: 4.

12 **Papenfuss, T. L., Rogers, C. J., Gienapp, I., Yurrita, M., McClain, M., Damico, N., Valo, J. et al.,** Sex differences in experimental autoimmune encephalomyelitis in multiple murine strains. *J. Neuroimmunol.* 2004. **150**: 59–69.

13 **Lélu, K., Delpy, L., Robert, V., Foulon, E., Laffont, S., Pelletier, L., Engelhardt, B. et al.,** Endogenous estrogens, through estrogen receptor α, constrain autoimmune inflammation in female mice by limiting CD4+ T-cell homing into the CNS. *Eur. J. Immunol.* 2010. **40**: 3489–3498.

14 **Cruz-Orengo, L., Daniels, B. P., Dorsey, D., Basak, S. A., Grajales-Reyes, J. G., McCandless, E. E., Piccio, L. et al.,** Enhanced sphingosine-1-phosphate receptor 2 expression underlies female CNS autoimmunity susceptibility. *J. Clin. Invest.* 2014. **124**: 2571–2584.

15 **Voskuhl, R. R., Pitchekian-Halabi, H., MacKenzie-Graham, A., McFarland, H. F. and Raine, C. S.**, Gender differences in autoimmune demyelination in the mouse: implications for multiple sclerosis. *Ann. Neurol.* 1996. **39**: 724–733.

16 **Klein, S. L. and Flanagan, K. L.**, Sex differences in immune responses. *Nat. Rev. Immunol.* 2016. **16**: 626–638.

17 **Arnold, A. P. and Chen, X.**, What does the "four core genotypes" mouse model tell us about sex differences in the brain and other tissues? *Front. Neuroendocrinol.* 2009. **30**: 1–9.

18 **Voskuhl, R. R.**, The effect of sex on multiple sclerosis risk and disease progression. *Mult. Scler.* 2020. **26**: 554–560.

19 **Bereshchenko, O., Bruscoli, S. and Riccardi, C.**, Glucocorticoids, sex hormones, and immunity. *Front. Immunol.* 2018. **9**: 1332.

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

21 **Confavreux, C., Hutchinson, M., Hours, M. M., Cortinovis-Tourniaire, P. and Moreau, T.**, Rate of pregnancy-related relapse in multiple sclerosis. Pregnancy in multiple sclerosis Group. *N. Engl. J. Med.* 1998. **339**: 285–291.

22 **Engler, J. B., Kursawe, N., Solano, M. E., Patas, K., Wehrmann, S., Heckmann, N., Lühder, F. et al.,** Glucocorticoid receptor in T cells mediates protection from autoimmunity in pregnancy. *Proc. Natl Acad. Sci. U S A* 2017. **114**: E181–e190.

23 **Lee, J. H., Ulrich, B., Cho, J., Park, J. and Kim, C. H.**, Progesterone promotes differentiation of human cord blood fetal T cells into T regulatory cells but suppresses their differentiation into Th17 cells. *J. Immunol.* 2011. **187**: 1778–1787.

24 **Smith-Bouvier, D. L., Divekar, A. A., Sasidhar, M., Du, S., Tiwari-Woodruff, S. K., King, J. K., Arnold, A. P. et al.,** A role for sex chromosome complement in the female bias in autoimmune disease. *J. Exp. Med.* 2008. **205**: 1099–1108.

25 **Rudick, R. A. and Goelz, S. E.**, Beta-interferon for multiple sclerosis. *Exp. Cell Res.* 2011. **317**: 1301–1311.

26 **Sweeney, C. M., Lonergan, R., Basdeo, S. A., Kinsella, K., Dungan, L. S., Higgins, S. C., Kelly, P. J. et al.,** IL-27 mediates the response to IFN-beta therapy in multiple sclerosis patients by inhibiting Th17 cells. *Brain Behav. Immun.* 2011. **25**: 1170–1181.

27 **Contasta, I., Totaro, R., Pellegrini, P., Del Beato, T., Carolei, A. and Berghella, A. M.**, A gender-related action of IFNbeta-therapy was found in multiple sclerosis. *J. Transl. Med.* 2012. **10**: 223.

28 **Griesbeck, M., Ziegler, S., Laffont, S., Smith, N., Chauveau, L., Tomezsko, P., Sharei, A. et al.,** Sex differences in plasmacytoid dendritic cell levels of IRF5 drive higher IFN-alpha production in women. *J. Immunol.* 2015. **195**: 5327–5336.

29 **Berghofer, B., Frommer, T., Haley, G., Fink, L., Bein, G. and Hackstein, H.,** TLR7 ligands induce higher IFN-alpha production in females. *J. Immunol.* 2006. **177**: 2088–2096.

30 **Dzopalic, T., Dragicevic, A., Vasilijic, S., Vucevic, D., Majstorovic, I., Bozic, B., Balint, B. et al.,** Loxoribine, a selective Toll-like receptor 7 agonist, induces maturation of human monocyte-derived dendritic cells and stimulates their Th-1- and Th-17-polarizing capability. *Int. Immunopharmacol.* 2010. **10**: 1428–1433.

31 **Yu, C. F., Peng, W. M., Oldenburg, J., Hoch, J., Bieber, T., Limmer, A., Hartmann, G. et al.,** Human plasmacytoid dendritic cells support Th17 cell effector function in response to TLR7 ligation. *J. Immunol.* 2010. **184**: 1159–1167.

32 **Fletcher, J. M., Lalor, S. J., Sweeney, C. M., Tubridy, N. and Mills, K. H.,** T cells in multiple sclerosis and experimental autoimmune encephalomyelitis. *Clin. Exp. Immunol.* 2010. **162**: 1–11.

33 **Ye, J., Wang, Y., Liu, X., Li, L., Opejin, A., Hsueh, E. C., Luo, H. et al.,** TLR7 signaling regulates Th17 cells and autoimmunity: novel potential for autoimmune therapy. *J. Immunol.* 2017. **199**: 941–954.

34 **Torcia, M. G., Nencioni, L., Clemente, A. M., Civitelli, L., Celestino, I., Limongi, D., Fadigati, G. et al.,** Sex differences in the response to viral infections: TLR8 and TLR9 ligand stimulation induce higher IL10 production in males. *PLoS One* 2012. **7**: e39853.

35 **Aomatsu, M., Kato, T., Kasahara, E. and Kitagawa, S.,** Gender difference in tumor necrosis factor-alpha production in human neutrophils stimulated by lipopolysaccharide and interferon-gamma. *Biochem. Biophys. Res. Commun.* 2013. **441**: 220–225.

36 **Marriott, I., Bost, K. L. and Huet-Hudson, Y. M.,** Sexual dimorphism in expression of receptors for bacterial lipopolysaccharides in murine macrophages: a possible mechanism for gender-based differences in endotoxic shock susceptibility. *J. Reprod. Immunol.* 2006. **71**: 12–27.

37 **Rettew, J. A., Huet-Hudson, Y. M. and Marriott, I.,** Testosterone reduces macrophage expression in the mouse of toll-like receptor 4, a trigger for inflammation and innate immunity. *Biol. Reprod.* 2008. **78**: 432–437.

38 **Hannah, M. F., Bajic, V. B. and Klein, S. L.,** Sex differences in the recognition of and innate antiviral responses to Seoul virus in Norway rats. *Brain Behav. Immun.* 2008. **22**: 503–516.

39 **Weinstein, Y., Ran, S. and Segal, S.,** Sex-associated differences in the regulation of immune responses controlled by the MHC of the mouse. *J. Immunol.* 1984. **132**: 656–661.

40 **Seillet, C., Rouquie, N., Foulon, E., Douin-Echinard, V., Krust, A., Chambon, P., Arnal, J. F. et al.,** Estradiol promotes functional responses in inflammatory and steady-state dendritic cells through differential requirement for activation function-1 of estrogen receptor alpha. *J. Immunol.* 2013. **190**: 5459–5470.

41 **Russi, A. E., Walker-Caulfield, M. E., Ebel, M. E. and Brown, M. A.,** Cutting edge: c-Kit signaling differentially regulates type 2 innate lymphoid cell accumulation and susceptibility to central nervous system demyelination in male and female SJL mice. *J. Immunol.* 2015. **194**: 5609–5613.

42 **Mathä, L., Shim, H., Steer, C. A., Yin, Y. H., Martinez-Gonzalez, I. and Takei, F.,** Female and male mouse lung group 2 innate lymphoid cells differ in gene expression profiles and cytokine production. *PLoS One* 2019. **14**: e0214286.

43 **Kadel, S., Ainsua-Enrich, E., Hatipoglu, I., Turner, S., Singh, S., Khan, S. and Kovats, S.,** A major population of functional KLRG1(−) ILC2s in female lungs contributes to a sex bias in ILC2 numbers. *Immunohorizons* 2018. **2**: 74–86.

44 **Laffont, S., Blanquart, E., Savignac, M., Cénac, C., Laverny, G., Metzger, D., Girard, J. P. et al.,** Androgen signaling negatively controls group 2 innate lymphoid cells. *J. Exp. Med.* 2017. **214**: 1581–1592.

45 **Cephus, J. Y., Stier, M. T., Fuseini, H., Yung, J. A., Toki, S., Bloodworth, M. H., Zhou, W. et al.,** Testosterone attenuates group 2 innate lymphoid cell-mediated airway inflammation. *Cell. Rep.* 2017. **21**: 2487–2499.

46 **Forsberg, A., Bengtsson, M., Eringfalt, A., Ernerudh, J., Mjosberg, J. and Jenmalm, M. C.,** GATA binding protein 3(+) group 2 innate lymphoid cells are present in cord blood and in higher proportions in male than in female neonates. *J. Allergy Clin. Immunol.* 2014. **134** (1):228–230.

47 **Abdullah, M., Chai, P. S., Chong, M. Y., Tohit, E. R., Ramasamy, R., Pei, C. P. and Vidyadaran, S.,** Gender effect on in vitro lymphocyte subset levels of healthy individuals. *Cell. Immunol.* 2012. **272**: 214–219.

48 **Hewagama, A., Patel, D., Yarlagadda, S., Strickland, F. M. and Richardson, B. C.,** Stronger inflammatory/cytotoxic T-cell response in women identified by microarray analysis. *Genes Immun.* 2009. **10**: 509–516.

49 **Frisullo, G., Nociti, V., Iorio, R., Patanella, A. K., Marti, A., Caggiula, M., Mirabella, M. et al.,** IL17 and IFNgamma production by peripheral blood mononuclear cells from clinically isolated syndrome to secondary progressive multiple sclerosis. *Cytokine* 2008. **44**: 22–25.

50 **O'Connor, R. A., Prendergast, C. T., Sabatos, C. A., Lau, C. W., Leech, M. D., Wraith, D. C. and Anderton, S. M.,** Cutting edge: Th1 cells facilitate the entry of Th17 cells to the central nervous system during experimental autoimmune encephalomyelitis. *J. Immunol.* 2008. **181**: 3750–3754.

51 **Dungan, L. S., McGuinness, N. C., Boon, L., Lynch, M. A. and Mills, K. H.,** Innate IFN-gamma promotes development of experimental autoimmune encephalomyelitis: a role for NK cells and M1 macrophages. *Eur. J. Immunol.* 2014. **44**: 2903–2917.

52 **Codarri, L., Gyulveszi, G., Tosevski, V., Hesske, L., Fontana, A., Magnenat, L., Suter, T. et al.,** RORgammat drives production of the cytokine GM-CSF in helper T cells, which is essential for the effector phase of autoimmune neuroinflammation. *Nat. Immunol.* 2011. **12**: 560–567.

53 **Grifka-Walk, H. M., Giles, D. A. and Segal, B. M.,** IL-12-polarized Th1 cells produce GM-CSF and induce EAE independent of IL-23. *Eur. J. Immunol.* 2015. **45**: 2780–2786.

54 **Huygen, K. and Palfliet, K.,** Strain variation in interferon gamma production of BCG-sensitized mice challenged with PPD II. Importance of one major autosomal locus and additional sexual influences. *Cell. Immunol.* 1984. **85**: 75–81.

55 **Huber, S. A. and Pfaeffle, B.,** Differential Th1 and Th2 cell responses in male and female BALB/c mice infected with coxsackievirus group B type 3. *J. Virol.* 1994. **68**: 5126–5132.

56 **Golden, L. C. and Voskuhl, R.,** The importance of studying sex differences in disease: the example of multiple sclerosis. *J. Neurosci. Res.* 2017. **95**: 633–643.

57 **Palaszynski, K. M., Liu, H., Loo, K. K. and Voskuhl, R. R.,** Estriol treatment ameliorates disease in males with experimental autoimmune encephalomyelitis: implications for multiple sclerosis. *J. Neuroimmunol.* 2004. **149**: 84–89.

58 **Wu, W. F., Tan, X. J., Dai, Y. B., Krishnan, V., Warner, M. and Gustafsson, J.A,** Targeting estrogen receptor β in microglia and T cells to treat experimental autoimmune encephalomyelitis. *Proc. Natl Acad. Sci. U S A* 2013. **110**: 3543–3548.

59 **McGinley, A. M., Edwards, S. C., Raverdeau, M. and Mills, K. H. G.,** Th17cells, gammadelta T cells and their interplay in EAE and multiple sclerosis. *J. Autoimmun.* 2018. **S0896-8411**: 30007–30016

60 **Havrdova, E., Belova, A., Goloborodko, A., Tisserant, A., Wright, A., Wallstroem, E., Garren, H. et al.,** Activity of secukinumab, an anti-IL-17A

antibody, on brain lesions in RRMS: results from a randomized, proof-of-concept study. *J. Neurol.* 2016. **263**: 1287–1295.

61 **Sutton, C., Brereton, C., Keogh, B., Mills, K. H. and Lavelle, E. C.**, A crucial role for interleukin (IL)-1 in the induction of IL-17-producing T cells that mediate autoimmune encephalomyelitis. *J. Exp. Med.* 2006. **203**: 1685–1691.

62 **Langrish, C. L., Chen, Y., Blumenschein, W. M., Mattson, J., Basham, B., Sedgwick, J. D., McClanahan, T. et al.,** IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. *J. Exp. Med.* 2005. **201**: 233–240.

63 **Sutton, C. E., Lalor, S. J., Sweeney, C. M., Brereton, C. F., Lavelle, E. C. and Mills, K. H.**, Interleukin-1 and IL-23 induce innate IL-17 production from gammadelta T cells, amplifying Th17 responses and autoimmunity. *Immunity* 2009. **31**: 331–341.

64 **Cua, D. J., Sherlock, J., Chen, Y., Murphy, C. A., Joyce, B., Seymour, B., Lucian, L. et al.,** Interleukin-23 rather than interleukin-12 is the critical cytokine for autoimmune inflammation of the brain. *Nature* 2003. **421**: 744–748.

65 **McGinley, A. M., Sutton, C. E., Edwards, S. C., Leane, C. M., DeCourcey, J., Teijeiro, A., Hamilton, J. A. et al.,** Interleukin-17A serves a priming role in autoimmunity by recruiting IL-1beta-producing myeloid cells that promote pathogenic T cells. *Immunity* 2020. **52**: 342–356.E6.

66 **Sankaran-Walters, S., Macal, M., Grishina, I., Nagy, L., Goulart, L., Coolidge, K., Li, J. et al.,** Sex differences matter in the gut: effect on mucosal immune activation and inflammation. *Biol Sex Differ* 2013. **4**: 10.

67 **Wang, C., Dehghani, B., Li, Y., Kaler, L. J., Vandenbark, A. A. and Offner, H.**, Oestrogen modulates experimental autoimmune encephalomyelitis and interleukin-17 production via programmed death 1. *Immunology* 2009. **126**: 329–335.

68 **Lélu, K., Laffont, S., Delpy, L., Paulet, P. E., Périnat, T., Tschanz, S. A., Pelletier, L. et al.,** Estrogen receptor α signaling in T lymphocytes is required for estradiol-mediated inhibition of Th1 and Th17 cell differentiation and protection against experimental autoimmune encephalomyelitis. *J. Immunol.* 2011. **187**: 2386–2393.

69 **Mohammad, I., Starskaia, I., Nagy, T., Guo, J., Yatkin, E., Väänänen, K., Watford, W. T. et al.,** Estrogen receptor α contributes to T cell-mediated autoimmune inflammation by promoting T cell activation and proliferation. *Sci. Signal* 2018. **11**.

70 **Garnier, L., Laffont, S., Lélu, K., Yogev, N., Waisman, A. and Guéry, J. C.**, Estrogen signaling in bystander Foxp3(neg) CD4(+) T cells suppresses cognate Th17 differentiation in trans and protects from central nervous system autoimmunity. *J. Immunol.* 2018. **201**: 3218–3228.

71 **Edwards, S. C., Sutton, C. E., Ladell, K., Grant, E. J., McLaren, J. E., Roche, F., Dash, P. et al.,** A population of proinflammatory T cells coexpresses alphabeta and gammadelta T cell receptors in mice and humans. *J. Exp. Med.* 2020. **217**: e20190834.

72 **Shimonkevitz, R., Colburn, C., Burnham, J. A., Murray, R. S. and Kotzin, B. L.**, Clonal expansions of activated gamma/delta T cells in recent-onset multiple sclerosis. *Proc. Natl Acad. Sci. U S A* 1993. **90**: 923–927.

73 **Wucherpfennig, K. W., Newcombe, J., Li, H., Keddy, C., Cuzner, M. L. and Hafler, D. A.**, Gamma delta T-cell receptor repertoire in acute multiple sclerosis lesions. *Proc. Natl Acad. Sci. U S A* 1992. **89**: 4588–4592.

74 **Caccamo, N., Dieli, F., Wesch, D., Jomaa, H. and Eberl, M.**, Sex-specific phenotypical and functional differences in peripheral human Vgamma9/Vdelta2 T cells. *J. Leukoc. Biol.* 2006. **79**: 663–666.

75 **Wing, K. and Sakaguchi, S.**, Regulatory T cells exert checks and balances on self tolerance and autoimmunity. *Nat. Immunol.* 2010. **11**: 7–13.

76 **Venken, K., Hellings, N., Hensen, K., Rummens, J. L., Medaer, R., D'Hooghe, M. B., Dubois, B. et al.,** Secondary progressive in contrast to relapsing-remitting multiple sclerosis patients show a normal CD4+CD25+ regulatory T-cell function and FOXP3 expression. *J. Neurosci. Res.* 2006. **83**: 1432–1446.

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

78 **Fletcher, J. M., Lonergan, R., Costelloe, L., Kinsella, K., Moran, B., O'Farrelly, C., Tubridy, N. et al.,** CD39+Foxp3+ regulatory T cells suppress pathogenic Th17 cells and are impaired in multiple sclerosis. *J. Immunol.* 2009. **183**: 7602–7610.

79 **Viglietta, V., Baecher-Allan, C., Weiner, H. L. and Hafler, D. A.**, Loss of functional suppression by CD4+CD25+ regulatory T cells in patients with multiple sclerosis. *J. Exp. Med.* 2004. **199**: 971–979.

80 **Niu, H. Q., Yuan, C., Yan, C., Li, N., Lei, Y. S., Li, X., Ru, J. et al.,** Decreased numbers and sex-based differences of circulating regulatory T cells in patients with seropositive undifferentiated arthritis. *Ther. Adv. Chronic Dis.* 2021. **12**: 2040622320986721.

81 **Bebo, B. F., Jr., Zelinka-Vincent, E., Adamus, G., Amundson, D., Vandenbark, A. A. and Offner, H.**, Gonadal hormones influence the immune response to PLP 139–151 and the clinical course of relapsing experimental autoimmune encephalomyelitis. *J. Neuroimmunol.* 1998. **84**: 122–130.

82 **Walecki, M., Eisel, F., Klug, J., Baal, N., Paradowska-Dogan, A., Wahle, E., Hackstein, H. et al.,** Androgen receptor modulates Foxp3 expression in CD4+CD25+Foxp3+ regulatory T-cells. *Mol. Biol. Cell* 2015. **26**: 2845–2857.

83 **Zhou, D., Srivastava, R., Nessler, S., Grummel, V., Sommer, N., Bruck, W., Hartung, H. P. et al.,** Identification of a pathogenic antibody response to native myelin oligodendrocyte glycoprotein in multiple sclerosis. *Proc. Natl Acad. Sci. U S A* 2006. **103**: 19057–19062.

84 **Polman, C. H. and Killestein, J.**, Anti-myelin antibodies in multiple sclerosis: clinically useful? *J. Neurol. Neurosurg. Psychiatry* 2006. **77**: 712.

85 **Kuhle, J., Lindberg, R. L., Regeniter, A., Mehling, M., Hoffmann, F., Reindl, M., Berger, T. et al.,** Antimyelin antibodies in clinically isolated syndromes correlate with inflammation in MRI and CSF. *J. Neurol.* 2007. **254**: 160–168.

86 **Negron, A., Robinson, R. R., Stuve, O. and Forsthuber, T. G.**, The role of B cells in multiple sclerosis: current and future therapies. *Cell. Immunol.* 2019. **339**: 10–23.

87 **Scotti, B., Disanto, G., Sacco, R., Guigli, M., Zecca, C. and Gobbi, C.**, Effectiveness and safety of Rituximab in multiple sclerosis: an observational study from Southern Switzerland. *PLoS One* 2018. **13**: e0197415.

88 **Lu, F. X., Abel, K., Ma, Z., Rourke, T., Lu, D., Torten, J., McChesney, M. et al.,** The strength of B cell immunity in female rhesus macaques is controlled by CD8+ T cells under the influence of ovarian steroid hormones. *Clin. Exp. Immunol.* 2002. **128**: 10–20.

89 **Grimaldi, C. M., Cleary, J., Dagtas, A. S., Moussai, D. and Diamond, B.**, Estrogen alters thresholds for B cell apoptosis and activation. *J. Clin. Invest.* 2002. **109**: 1625–1633.

90 **Koch, M., Uyttenboogaart, M., van Harten, A. and De Keyser, J.**, Factors associated with the risk of secondary progression in multiple sclerosis. *Mult. Scler.* 2008. **14**: 799–803.

91 **Du, S., Itoh, N., Askarinam, S., Hill, H., Arnold, A. P. and Voskuhl, R. R.**, XY sex chromosome complement, compared with XX, in the CNS confers greater neurodegeneration during experimental

autoimmune encephalomyelitis. *Proc. Natl Acad. Sci. U S A* 2014. **111**: 2806–2811.

92 **Beery, A. K. and Zucker, I.**, Sex bias in neuroscience and biomedical research. *Neurosci. Biobehav. Rev.* 2011. **35**: 565–572.

93 **Bruck, J., Glocova, I., Geisel, J., Kellerer, C., Rocken, M. and Ghoreschi, K.**, Dimethyl fumarate-induced IL-17(low) IFN-gamma(low) IL-4(+) Th cells protect mice from severe encephalomyelitis. *Eur. J. Immunol.* 2018. **48**: 1588–1591.

94 **Stojic-Vukanic, Z., Kotur-Stevuljevic, J., Nacka-Aleksic, M., Kosec, D., Vujnovic, I., Pilipovic, I., Dimitrijevic, M. et al.,** Sex bias in pathogenesis of autoimmune neuroinflammation: relevance for dimethyl fumarate immunomodulatory/anti-oxidant action. *Mol. Neurobiol.* 2018. **55**: 3755–3774.

95 **Li, R., Sun, X., Shu, Y., Mao, Z., Xiao, L., Qiu, W., Lu, Z. et al.,** Sex differences in outcomes of disease-modifying treatments for multiple sclerosis: a systematic review. *Mult. Scler. Relat. Disord.* 2017. **12**: 23–28.

Abbreviations: **BBB**: blood—brain–barrier · **DMF**: dimethyl fumarate · **ER**: estrogen receptor estrogen receptor-α (ER-α) · **CSF**: cerebrospinal fluid · **FCG**: four core genotype · **IFNAR**: type 1 interferon receptor · **ILC2**: innate lymphoid cell 2 · **IRF5**: interferon regulatory factor 5 · **MOG**: myelin oligodendrocyte protein · **pDC**: plasmacytoid dendritic cell · **PLP**: myelin proteolipid protein · **PPMS**: primary progressive multiple sclerosis · **RRMS**: relapse-remitting multiple sclerosis · **SPMS**: secondary progressive multiple sclerosis · **S1PR2**: spingosine-1-phosphate receptor-2

Full correspondence: Prof. Kingston Mills, School of Biochemistry and Immunology, Trinity Biomedical Sciences Institute, Trinity College Dublin, Dublin, D02 R590, Ireland.
e-mail: kingston.mills@tcd.ie

Received: 20/8/2021
Revised: 30/9/2021
Accepted: 29/10/2021
Accepted article online: 2/11/2021