Biological Sex As a Critical Variable in CD4\(^+\) Effector T Cell Function in Preclinical Models of Multiple Sclerosis

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Abstract

**Significance:** T cells play a pivotal role in maintaining adaptive immune responses against pathogens. However, misdirected T cell responses against self-tissues may lead to autoimmune disease. Biological sex has profound effects on T cell function and is an important determinant of disease incidence and severity in autoimmune diseases such as multiple sclerosis (MS).

**Recent Advances:** Many autoimmune diseases skew toward higher female incidence, including MS; however, it has become increasingly more accepted that men living with MS are more prone to developing a progressive disease course and to having worsened disease outcomes.

**Critical Issues:** In this review, we discuss what is known about the role of biological sex on T cell development and differentiation, examining evidence that male sex can augment T helper 17 (Th17) responses. Next, we outline what is known about sex differences in animal models of MS, and about the distinct roles played by sex hormones versus sex chromosomes in pathogenesis in these models. Finally, we discuss recent advances that examine the molecular basis for worsened disease outcomes in males, with a particular focus on the role played by Th17 cells in these models.

**Future Directions:** Better understanding the role of biological sex in T cell function may pave the way to effective personalized treatment strategies in MS and other autoimmune diseases. *Antioxid. Redox Signal.* 37, 135–149.

**Keywords:** sex differences, multiple sclerosis, experimental autoimmune encephalomyelitis, CD4\(^+\) T cell, sex hormones, sex chromosomes, Jarid1c

Introduction

CD4\(^+\) T cells are a critical component of the adaptive immune system, which governs antigen (Ag)-specific detection of, and response to, pathogens. CD4\(^+\) T cells rapidly proliferate and differentiate into effector subsets upon recognition of cognate Ag presented by major histocompatibility complex (MHC) class II complexes on the surface of antigen-presenting cells (APCs). They subsequently traffic to sites of inflammation, where they recruit immune effector cells such as CD8\(^+\) T cells, B cells, macrophages, neutrophils, or eosinophils, among others. Due to their great inflammatory potential, T cell responses are subject to tight regulatory control, most notably to avert reactions to self-tissues. Nevertheless, these mechanisms are imperfect, with \(~6\) in 100 individuals in the Western world having an autoimmune disease (108). Autoimmunity thus represents a major public health burden worldwide.

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Multiple sclerosis (MS) is a chronic neurodegenerative disease of the central nervous system (CNS) for which there is currently no cure. MS has a high rate of prevalence in Western countries: for example, in Canada, as many as 1 in 350 individuals are affected (84). MS is considered an autoimmune disease in which T and B cells cross the blood/brain barrier (BBB) and launch an inflammatory attack against CNS myelin. This inflammatory immune response affects both myelin sheath and axons leading to neuronal dysfunction (91). MS has a heterogeneous presentation of disease, manifesting in affected individuals as fatigue, pain, motor deficit, visual disturbances, and cognitive impairment (33). Around 80% of patients will initially present relapsing/remitting (RR) disease, and 30%–60% of these will transition to a secondary progressive form that is currently refractory to treatment (63).

As is the case for many autoimmune diseases, the failure of central, thymically enforced tolerance of T cells results in the presence of myelin-specific T cells in the mature immune repertoire; furthermore, peripheral immune activation of these myelin-specific T cells is important in MS pathogenesis. In RR-MS, activated T cells specific for myelin Ags, such as myelin basic protein (MBP) and myelin oligodendrocyte glycoprotein (MOG), have been identified in the blood and cerebrospinal fluid of MS patients. These activated T cells migrate to the CNS and are subsequently reactivated upon recognition of myelin Ag in situ. This leads to an inflammatory cascade of events resulting in MS symptoms in affected individuals (27).

While the central role of adaptive immune responses in RR-MS is well-established, its role in progressive MS is a matter of active debate. Those arguing against a role for lymphocytes in progressive MS have relied on evidence that many existing immunomodulatory MS disease-modifying therapies (DMT) are ineffective in this form of disease, and that progressive MS lesions tend to be immunologically silent as visualized by magnetic resonance imaging. On the contrary, it is now known that B cell- and T cell-rich ectopic lymphoid organs can form in the CNS meninges (70) and that they increase in frequency during secondary progressive MS (66, 92), where they can potentially secrete inflammatory mediators and trophic growth factors to support immune cells trapped in the parenchyma behind a closed BBB. Indeed, axonopathy in progressive MS correlates with T and B cell accumulation (42). Furthermore, the B cell depleting DMT ocrelizumab is the first therapy to show efficacy during the active phase of MS (57).

Sex is a critical variable in most physiological processes, with adaptive immune function being no exception. “Sex” refers to the biological attributes governed by the expression and function of sex hormones and/or sex chromosome components. While the terms are often used interchangeably in the literature, sex is distinct from “gender,” which refers to socially and/or culturally defined features. Sex differences have long been of interest to immunologists, in part, because many autoimmune diseases, including MS, show a striking bias toward female incidence. Indeed, MS affects three times as many women as men. However, it is now understood that the proportion of MS-affected men who develop a progressive disease course exceeds that of MS-affected women (37). Furthermore, there is a growing body of evidence suggesting that male sex enhances the function of inflammatory T helper 17 (Th17) CD4+ effector T cells that are pivotal in autoimmunity.

In this review, we outline what is known about the role of biological sex in effector CD4+ T cell function. We then describe evidence for the role of sex in distinct mouse models of MS, with a particular focus on models of progressive disease and on studies of the relative contribution of sex hormones/chromosomes to disease processes.

**Effector T Cell Subsets**

Upon recognition of cognate Ag in peripheral lymphoid tissue, T cells can differentiate into distinct effector subtypes that are dictated by the cytokine milieu present at the time of activation. T cell effector subsets are traditionally defined by master transcription factors (TFs) that drive each fate, as well as by their production of signature cytokines (51). The developmental cues, master TFs, and signature cytokines characteristic of the best-described CD4+ T cell effector subtypes (Th1, Th2, Th17, Thf, Treg) are depicted in Figure 1.

Th subtypes play distinctive roles in physiologic and maladaptive immune responses. The classic Th1/Th2 paradigm, proposed over 40 years ago by Mosmann et al. (74), held that inflammatory CD4+ Th1 cells induce cell-mediated immunity to intracellular pathogens, while Th2 cells promote humoral, antibody-mediated responses that are directed against extracellular invaders and that feature prominently in allergy/asthma. In the mid-2000s, the IL-17A-producing Th17 subset was discovered to be distinct from Th1 cells (49, 62). Both Th1 and Th17 cells drive cell-mediated immune responses: Th1 cells primarily attract macrophages to sites of infection/inflammation, while Th17 cells recruit neutrophils (106).

T follicular helper (Tfh) cells provide essential help to B cell responses by spurring the development of germinal centers while also providing essential cell-cell signals (28). T regulatory cells (Treg), which can arise both from central thymic selection and extrathymically, are characterized by their expression of the X-linked TF FoxP3 and they actively suppress inflammatory responses (77).

Th1, Th17, Tfh, and Treg have all been described as playing pivotal roles in CNS autoimmunity. In this review, we discuss the role of sex as a variable in the function of these subtypes. It is important to keep in mind, however, that the list of effector T cell subpopulations is continually expanding and that the search for novel populations is an active field of study. In addition, while we focus on CD4+ T cells in this review, we note that a similar paradigm exists for CD8+ (Tc) effector cells as well. In addition, it is now accepted that B cells also play an important role in MS pathogenesis, with B cell-depleting anti-CD20 therapeutic regimens being highly effective. Furthermore, as CD20 is not expressed on plasma B cells, it is probable that B cells in MS act via Ag-presentation or other functions not related to their ability to secrete antibody (109).
Role of Sex in Regulating T Cell Effector Function

Sex-mediated effects on Th1

Sex-specific differences in T cell function have been an area of intense research interest. CD4⁺ T cells express estrogen receptor (ER)α and ERβ (83) as well as the androgen receptor (65). Female murine T cells exhibit enhanced proliferative responses upon ex vivo rechallenge with an immunizing peptide (112); ER signaling appears crucial, as female CD4-Cre·ERα-fl/fl CD4⁺ T cells display defective proliferation in response to agonistic antibodies to CD3 and CD28 (72). Furthermore, in a human study, a greater proportion of lamina propria lymphocytes (LPL) from healthy women were positive for the proliferation marker Ki67 compared with LPL from healthy men (90).

Intriguingly, these studies also demonstrated that Th1 responses may be exacerbated in females (Fig. 2). Female mouse T cells showed upregulation of the Th1 signature cytokine interferon (IFN)γ upon Ag recall (112), while both peripheral CD4⁺ and LPL from healthy women had increased expression of IFNγ and the Th1-associated chemokine RANTES relative to men (90). Estrogen contributes to Th1 skewing in females, as exogenous in vivo administration of 17-β-estradiol increases production of IFNγ from CD4⁺ T cells in an ERα-dependent manner from two different mouse strains (67). Exogenous administration of estrogen in ovariectomized female mice also augments expression in T cells of the chemokine receptors CCR1–5 that are ligands for the Th1-associated chemokines RANTES and MIP1α; furthermore, estrogen sensitizes T cells to MIP1α-mediated downstream signaling (71). Curiously, while testosterone does not appear to directly inhibit IFNγ production from CD4⁺ T cells, it does induce IL-10 production (65), which can inhibit Th1 responses, when exogenously applied to both male and female T cells.

Overall, the literature thus indicates that Th1 responses are increased in females and that female sex hormones contribute directly to this phenomenon. Nevertheless, at least one report has suggested that the Th1:Th2 ratio is increased in men rather than women (45). Furthermore, the role of estrogens in regulating Th1 cell function may be nuanced, as it was shown that estriols can induce a shift toward a Th2 cytokine profile.
when exogenously administered to human T cells at concentrations similar to those seen during pregnancy (110).

**Sex-mediated effects on Tfh and Treg**

Tfh cells provide essential help to B cell responses. Choi and colleagues identified a molecular regulatory circuit that specifically controls their responses in females (59, 82). In the absence of the nuclear receptor peroxisome proliferator-activated receptor (PPAR)$\gamma$, female, but not male, mice show a Tfh-dependent increase in autoimmune pathology. Female sex hormones synergize with PPAR$\gamma$ to effect this regulation, as concomitant treatment of male mice with exogenous estradiol, plus a PPAR$\gamma$ agonist, reduced Tfh frequency (82). Furthermore, aged female, but not male, ER$\alpha$-knockout mice displayed an increased autoimmune phenotype characterized by elevated frequencies of Tfh and germinal center B cells (59).

Treg are considered critical in maintaining maternal tolerance to the allogenic fetus (88). Thus, it is perhaps unsurprising that female sex hormones are important regulators of Treg frequency and function. ER and progesterone receptor are expressed on CD4$^+$CD25$^+$ Treg (7), and estradiol enhances the suppressive capacity of both mouse (75) and human (86) Treg. Interestingly, the presence of FoxP3$^+$ cells correlates with serum estradiol during the menstrual cycle of fertile women, peaking at the late follicular phase (9). On the contrary, male sex has been implicated in maintaining/promoting Treg function in specific contexts. Transient chemical castration of male subjects caused a reduction in circulating CD4$^+$CD25$^+$ frequency (79), while in a spontaneous colitis model characterized by severe pathology in females, Treg were impaired in female but not male animals (47). Strikingly, an elegant recent study from the group of Kallies (100) showed that IL-10$^+$ visceral adipose tissue (VAT) Treg are increased in male mice, and that the phenotype relies on an androgen receptor-driven regulatory circuit that promotes secretion of the VAT Treg growth factor IL-33 from stromal cells. Altogether, these findings suggest that while female sex hormones are important in promoting Treg function under physiologic conditions, male sex may also positively influence the function of these cells in pathology- or tissue-specific contexts.

**Male sex may augment Th17 cell function**

Intriguingly, a number of studies have reported that male sex increases the frequency and activation of Th17 cells (Fig. 2). The first reports came from models of autoimmune heart disease, in which T cells from male mice immunized with class II-restricted myosin heavy chain-alpha peptide showed enhanced IL-17 production upon Ag recall. Passive transfer of these reactivated male T cells resulted in an IL-17-dependent heart phenotype, as in vivo IL-17 blockade ameliorated disease in recipient animals (56). In coxsackievirus B3-induced myocarditis, male mice developed more severe pathology, and presented a higher frequency of Th17 cells in the spleen and heart compared with females (64). Furthermore, in collagen Ag-induced arthritis, exogenous estradiol reduced disease severity while inducing an accumulation of Th17 cells in peripheral lymph nodes as opposed to the inflamed joints (5).

In a human study, microarray analysis of T cells from healthy men and women showed that while numerous inflammatory Th1-related genes such as IFNG and IL12RB were upregulated in female cells, IL17A was strongly upregulated in male cells (50). In another study, restimulated CD4$^+$ T cells from healthy men showed enhanced expression of both IL-17A mRNA and protein relative to female cells; mechanistically, this effect was dependent on the nuclear receptor PPAR$\gamma$1, as siRNA of PPAR$\gamma$1 reduced IL-17A expression from male cells (112). These findings are further supported by those in the context of allergic rhinitis, in which Th17 cells are increased in the peripheral blood of men (99). Expression of the IL-17 receptor β-subunit on CD4$^+$ T cells from male children correlates with their serum expression of immunoglobulin E (IgE), a marker of severe allergic pathology; such a relationship was not observed in females (53). It must be noted that not every analysis of human samples has identified an association between male sex and Th17 frequency/activation: one study found that female peripheral and gut CD4$^+$ T cells had higher intrinsic IL-17 expression relative to male cells (90). However, on the balance, these findings indicate that male sex may be an important positive regulator of Th17 cells. Indeed, our group has also recently published (35) that male sex exacerbates Th17 cell-mediated pathology in a model of chronic progressive CNS autoimmunity (discussed in the 1C6-Th17 adoptive transfer section).

**Sex As a Variable in Regulating MS Incidence and Severity**

MS is subject to striking sex dichotomies [extensively reviewed in Refs. (37, 38, 102)]. It is well established that the incidence of MS is higher in women compared with men (37). Curiously, however, this sex bias in MS incidence is only manifested after puberty (38), suggesting a central role for sex hormones in regulating disease processes. Indeed, testosterone is potentially protective in MS: lower circulating testosterone has been found to be correlate with worsened disease outcome in MS-affected men (21), and in a phase II trial, exogenous testosterone was shown to halt, and possibly reverse, neurodegeneration in men with MS (61, 93). This argues for a model in which male sex hormones reduce MS disease burden, while female sex hormones increase it. However, it is additionally known that pregnancy, an event characterized by high circulating levels of estrogen, can have a suppressive effect on MS disease burden (102).

While relapsing MS incidence favors women, the sex balance in progressive MS is closer to 1:1. Male sex is a risk factor for poor MS outcomes such as reaching an Expanded Disability Status Scale score of 6—requiring a walking aid to walk for 100 m (30, 60). Brain atrophy is also higher in men with MS, and male sex is also predictive of cognitive difficulties in the context of MS (103). The contribution of biological sex to MS incidence and outcomes is thus complex. In the next section, we detail what is known about the role played by biological sex in experimental autoimmune encephalomyelitis (EAE) models of MS.

**Sex As a Variable in Regulating MS-Like Disease in EAE**

EAE is an umbrella term referring to multiple animal (typically rodent) models of CNS autoimmunity. EAE is considered
to recapitulate the immune aspects of MS pathogenesis, with paralytic/vision disturbances being most readily modeled [reviewed in greater detail in Rangachari and Kuchroo (87)]. EAE is classically induced by active immunization of susceptible mouse strains with encephalitogenic proteins/peptides that are frequently derived from myelin components or from other CNS cells such as neurons or astrocytes. Active immunization protocols initiate a T cell-driven response, although they may also be used to interrogate APC or B cell function. Adoptive transfer of activated, autologous, encephalitogenic T cell clones, or of activated transgenic T cells with Ag specificity to encephalitogenic epitopes, is another commonly used approach. The latter is particularly useful in isolating the contributions of effector T cells to immune pathogenesis, as the initial steps in T cell activation are bypassed.

EAE can be subject to striking sex-specific differences in a strain- and protocol-dependent manner. In this section, we review what is known about the role of sex in disease outcomes in these models (Fig. 3).

**SJL/J models of EAE**

Active immunization of SJL/J mice with proteolipid protein (PLP)\textsubscript{139–151} induces an RR form of EAE that is reminiscent of the disease seen in ~80% of MS patients from onset (69). RRMS is more common in women, and thus, it is intriguing that EAE on the SJL/J strain tends to be more severe in female mice. Active immunization of male SJL/J mice resulted in an acute monophasic disease course that was not followed by relapses as was the case in female mice (15). Adoptive transfer of female PLP\textsubscript{139–151}-reactivated splenocytes induced EAE of increased severity relative to that caused by male cells, irrespective of the sex of the recipient animal (13). Furthermore, peptide-restimulated female splenocytes favored the production of IFN\textgamma over immunomodulatory IL-10, while male cells showed the opposite tendency (14). Thus, sex differences in the T cell compartment were likely the source of worsened disease in female mice. In addition, several lines of evidence implicate testosterone as an ameliorative factor in SJL/J EAE. Gonadectomy of male SJL/J rendered them susceptible to severe active immunization EAE (16). Furthermore, exogenous testosterone reduced the ratio of IFN\textgamma:IL-10 from female splenocytes (14) and in vivo administration of testosterone similarly reduced EAE severity and increased T cell-derived IL-10 in an MBP immunization model (29). Collectively, these findings indicated that female SJL/J are prone to worsened EAE, with their lower levels of testosterone being a potential factor.

The four-core genotype model as a tool to dissect the contribution of sex hormones versus sex chromosomes. The roles of androgens and estrogens/progesterone in autoimmunity have been extensively studied. However, sex-based

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**FIG. 3.** Sex as a variable in commonly used models of active immunization EAE. In C57BL6/J mice, no sex differences are observed upon active immunization with MOG\textsubscript{35–55} (80). In SJL/J mice, females develop a relapsing/remitting pattern upon active immunization with PLP\textsubscript{139–151}, while males show an acute non-remitting course with a lower overall disease burden (13, 15). MOG\textsubscript{35–55}-immunized NOD mice display a relapse/chronic disease pattern with no sex differences observed (80). Male B10.PL mice develop EAE of greater severity in the acute phase when immunized with whole MBP (80). MBP, myelin basic protein; MOG, myelin oligodendrocyte glycoprotein; NOD, nonobese diabetic; PLP, proteolipid protein. Color images are available online.
phenotypes may also arise from the contributions of sex chromosome complement (XX vs. XY). The elegant four-core genotype (FCG) mouse strain permits one to dissect the relative contributions of sex hormones and chromosomes to biological processes [reviewed in Arnold and Chen (8) and presented in Fig. 4]. FCG mice are double transgenics in which the testes-determining gene Sry is knocked out of the Y chromosome (Y^{SryKO}), but is ectopically expressed on chromosome 3 (Tg-Sry). In the presence of Sry, mammals are hormonally male; in its absence, they default to a female hormonal pathway. Thus, when hormonally and chromosomally male Tg-Sry XY^{SryKO} mice are crossed to XX females, the resultant progeny have one of four phenotypes: (i) hormonally and chromosomally male (Tg-Sry XY^{SryKO}O), (ii) hormonally and chromosomally female (XX), (iii) hormonally male and chromosomally female (Tg-Sry XX), (iv) hormonally female and chromosomally male (XY^{SryKO}O).

Use of the FCG model on the SJL/J background revealed unexpected roles for chromosomal factors in EAE outcomes. Upon active immunization, XX SJL/J mice developed disease of greater severity than XY^{SryKO} mice. Similarly, Tg-Sry XX developed more severe EAE than Tg-Sry XY^{SryKO}. Thus, worsened outcomes were associated with female sex chromosomes irrespective of the hormonal context. Adoptive transfer of XX T cells induced disease of greater severity than XY^{SryKO} T cells, indicating that these effects were localized in the T cell compartment (94).

Interestingly, by using bone marrow (BM) chimeric approaches on the FCG strain, Voskuhl and colleagues were able to show that the male SJL/J CNS was uniquely susceptible to injury during EAE, as XY^{SryKO} host mice developed EAE of greater severity relative to XX, regardless of whether XX or XY^{SryKO} BM was implanted (36). Importantly, both XY^{SryKO} and XX are hormonally female, leading them to conclude that the cause of increased disease susceptibility lay with male sex chromosomes. The phenotype was then linked to differential expression of TLR7 within the male and female CNS. It is probable that the increase in T cell inflammation in the XX immune system is dominant over the greater vulnerability of the XY^{SryKO} CNS to injury, thus explaining why intact female SJL/J develop more severe EAE relative to males upon adoptive transfer (94). The segregation of sex hormone and chromosome effects thus has deepened our understanding of pathology in this mouse strain.

Use of consomic strains to identify a role for Y chromosomal elements in EAE. When B10.S strain mice are intercrossed with SJL/J, the F2 progeny present a mixture of clinical courses upon active immunization with SJL/J spinal cord homogenate. These include acute progressive, relapse/ remitting, and chronic nonremitting disease (22). By analyzing large cohorts of these F2 mice, Teuscher and colleagues were able to identify a number of autosomal genetic loci that can regulate disease outcomes in a sex-specific manner (22, 23). Next, they pinpointed a curious parent-of-origin (POO) effect through which EAE pathology was selectively reduced in F2 females whose sires and grandsires had an SJL/J-origin Y chromosome. This suggested that male genetic regulatory elements could have important effects on female descendants. Crossing XX C57BL/6J (B6) female mice with B6 males that carried consomic Y chromosome variants with known associations with autoimmune disease, they showed again that female progeny of these crosses had differential severity of EAE depending on the Y chromosome possessed by their sires (98). Young SJL/J males bearing a B10.S-derived Y chromosome showed increased EAE severity relative to age-matched SJL/J (95), and male B6 mice with an SJL/J-derived consomic Y chromosome showed reduced EAE burden relative to WT B6 (26), indicating that genes on the SJL/J Y can directly repress pathogenicity. This effect is potentially due to SJL/J expressing fewer copies of the Sly and Rbm4 Y-linked multicopy genes (26). Thus, sex chromosomal factors may indeed collaborate with sex hormones to reduce EAE severity in SJL/J males.

FIG. 4. Mouse models for dissecting the role of sex hormones and sex chromosomes and for studying X chromosome dosage. (A) FCG mouse model. The testes-determining gene Sry is knocked out of the Y chromosome (Y^{SryKO}) and is crossed to a transgenic strain that expresses Sry on autosomal chromosome 3 (Tg-Sry). Upon crossing phenotypically male Tg-SryXY^{SryKO} with wild-type XX females, one can generate phenotypic males with XX and XY chromosomes and phenotypic females with XX and XY chromosomes. FCG mice allow one to separately interrogate the biological functions of sex chromosomes versus hormones. (B) XY* model. An aberrant Y chromosome rearrangement permits meiotic rearrangement of X and Y. Crossing XY* males to XX results in male mice with two X chromosomes (XXY*) and female mice with one X (XO), as well as males and females with one X apiece. This model permits one to study the effects of X chromosome dosage. FCG, four-core genotype. Color images are available online.
B6 EAE

The MOG[35–55]-dependent active immunization model on the B6 strain results in a relatively rapid (<30 days) disease course that can take an acute monophasic or stable chronic form, depending on the specific experimental parameters used (17). The B6 model is widely used, in part, because of its rapid disease course and also because of the wide array of genetically modified strains available on this background. However, there are no grossly observable differences in disease incidence or severity between male or female mice (80). Furthermore, disease development was similar between all four genotypes in FCG × B6 mice (94).

Nevertheless, careful analysis of specific immune marker genes has revealed sex-associated phenotypes in this model. In one study, deletion of the sodium channel Nav1.5 in astrocytes selectively worsened EAE in female but not male B6 mice, indicating that this molecule suppresses neuroinflammation in a sex-specific manner (81). Furthermore, the B6 model has been used to uncover important insights on the contribution of sex chromosomes to T cell function in EAE. For example, Voskuhl and colleagues found that the X chromosome “silencing escape” gene Kdm6a is expressed more highly in in vitro-activated XX B6 T cells compared with the XY xenK10 counterparts; conditional knockout of Kdm6a in T cells reduced the severity of EAE upon MOG[35–55] immunization (55).

The same group also found that five X-linked genes—Msl3, Prps2, Hccs, Tmsb4x, and Tlr7—were actually expressed more highly in XY xenK20 versus XX CD4+ T cells (46). Next, they used the XY* genetic model (Fig. 4) to identify a POO imprinting effect that could explain upregulation of X genes in male T cells. In this model, the Y chromosome (Y*) has an aberrant chromosomal rearrangement that permits the meiotic recombination of X and Y, which is normally not possible. Mating of XY* to WT XX generates four genotypes at F1 that differ in X chromosome dosage: (i) XX females, (ii) XY* males, (iii) XYxX (effectively XO) females, and (iv) XXxY* males (1). The dam-derived X chromosome of XYxX mice thus carries a maternal epigenetic imprint (XmO). By subsequently crossing XYxX into a wild-type XY male, one can obtain XYxXY mice in which X comes from the sire and thus they bear a paternal imprint (XPo). Analysis of the loci of the five differentially expressed X-genes revealed that they were methylated to a greater degree in XPo, suggesting that paternal POO effects may account for differences in X-linked gene expression in T cells (46).

Together, these findings indicate the pertinence of studying the role of sex even when differences between males and females may not be immediately apparent: here, biological sex may regulate specific inflammatory/regulatory pathways that can influence disease severity in the B6 model.

Nonobese diabetic/ShiLtJ EAE

As their name suggests, nonobese diabetic (NOD) mice spontaneously develop T cell-dependent insulitis and pancreatic beta-cell destruction. They are additionally susceptible to developing other autoimmune diseases such as thyroiditis and EAE (40). When immunized with MOG[35–55], NOD-EAE mice may develop relapses and remissions followed by a chronic worsening phase in a disease course that is reminiscent of relapsing/progressive MS (11, 68). In addition to the spinal cord, inflammatory lesions in NOD-EAE are also observed in midbrain/hemispheric structures such as the dentate gyrus, fimbria, and caudate putamen (31). This is important because other, less chronic, models of EAE have been criticized for implicating only the spinal cord and brain stem.

It should be noted that Baker et al. presented data in which they considered the disease pattern of individual MOG[35–55]-immunized NOD mice monitored over 60 days. Only a subset of these mice showed a progressive pattern of phenotype (10). They argued that when viewing the mean disease curve of multiple NOD-EAE mice considered together, the appearance is created of a late-stage progressive course when in actuality it may be the result of an increased frequency of relapse in individual mice at these later time points. Indeed, using an adoptive T cell transfer model on the NOD genetic background, we ourselves have found that a little over half of the mice show a progressive course when their disease courses are considered individually, and about one-fifth show a progressive course preceded by at least one relapse. The remainder of mice present relapses only, or a stable disease course marked by neither distinct relapses nor progression (35). We regard this heterogeneity as advantageous, as it permits us to interrogate the factors and mechanisms that may either augment or diminish the incidence of a progressive course.

Sex is an important regulator of diabetes in NOD mice, as a higher incidence is seen in females (58). Sex steroids appear crucial, as orchietomy of males increases the incidence of diabetes, while ovariectomy of females decreases it (41). By contrast, male and female MOG[35–55]-immunized NOD mice develop EAE of similar incidence and severity (80). However, we have recently shown that effector CD4+ T cells from MOG[35–55]-specific T cell receptor (TcR) transgenic “1C6” mice on the NOD genetic background display a striking sex difference in EAE severity upon adoptive transfer. Male 1C6 Th17 induce severe chronic disease upon adoptive transfer to NOD. Scid recipients (35). These data, discussed in greater detail in the 1C6-Th17 adoptive transfer section, suggest that active immunization of NOD mice may mask cell-specific sex differences.

129.S1/SvImJ (SV.129) EAE: evidence for sex-specific regulation of T cells by PPARα

SV.129 mice are, like B6 mice, H-2b-restricted, and similar to B6, show no overt differences in disease severity between males and females upon immunization with MOG[35–55]. However, Dunn and Steinman observed that deficiency in the nuclear receptor PPARα increased disease severity in males and not females (39). This sex differential was T cell dependent, as immunization of male KO CD4+ → Ragα/− resulted in more severe disease than that seen in male WT CD4+ → Ragα/−, while no differences between female KO CD4+ → Ragα/− and male WT CD4+ → Ragα/− were observed. Mechanistically, PPARα expression was greater in male CD4+ and was dependent on the presence of androgen (39). In a follow-up study, it was then found that PPARα preferentially inhibited Th1 cell differentiation by male CD4+ by limiting chromatin accessibility of the Ifng locus (111). These data add to the mounting evidence that the PPAR family of nuclear receptors may regulate effector T cell function in a sex-specific manner.
B10.PL mice develop EAE upon immunization with MBP determinants. While immunization with the encephalitogenic MBP_{1-11} (Ac1-11) resulted in no gross differences in EAE incidence and severity between the sexes (12), males immunized with whole MBP developed disease in the acute phase that was much more severe than that seen in females (80). In the case of the former, while no sex difference was initially seen, immunized males, but not females, showed improved recovery when receiving a tolerizing oral dose of Ac-11 (12). In the case of the latter, gonadectomy of female mice increased their susceptibility to severe outcomes, suggesting that estrogen may be protective in this model (80). Another work on the B10.PL strain has shown that estrogen may have an important role in synergizing with vitamin D3 (itself an important protective factor in human MS) to reduce EAE severity specifically in female mice (96).

Virally mediated models of demyelination

Intracerebral inoculation of Theiler's murine encephalomyelitis virus (TMEV) leads to CNS demyelination. In SJL/J mice, TMEV inoculation causes greater disease severity in males (3). Another group showed that B6 males also presented greater susceptibility and disease severity upon TMEV inoculation. Intriguingly, gonadectomized males developed disease of even greater severity, although treatment of these mice with estrogen reduced disease severity by reducing Th1 responses (43). Thus, both testosterone and estrogen reduce symptomology in this model; the underlying propensity for severe disease in males may be linked to sex chromosomes, a possibility that remains to be explored.

Sex Differences in T Cell Transgenic Models of Progressive EAE

One critique of many EAE models is that they rarely feature the relapse/progressive disease course that is so common...
in MS. Here, we describe two models that can help reveal the immune features of RR versus progressive CNS autoimmunity. Both exploit transgenic T cell specificities to myelin Ag, and notably, both present striking sex differences that are reminiscent of human pathology (Fig. 5).

**TCR1640 mice**

TCR1640 transgenic mice on the SJL/J background express an ectopic TcR directed against MOG

$\text{MOG}_{\text{222-234}}$ (85). These mice develop spontaneous EAE in the absence of an immunogen or adjuvant, with >85% of TCR1640 females developing RR disease, while >50% of males develop progressive disease (85). These differential outcomes are reminiscent of what is seen in humans, in which the RRMS incidence favors women, while men with MS are more likely to develop a progressive course. In a recent study, adoptive transfer approaches were used to show that sex differences in the TCR1640 strain were indeed driven by the sex of the T cells (34). When splenocytes from presymptomatic female TCR1640 mice were differentiated in vitro under mixed Th1/Th17 conditions and then transferred to SJL/J recipients, the resulting disease tended to take an RR course. By contrast, male TCR1640 splenocytes largely transferred progressive disease. These dichotomies were observed irrespective of the sex of the recipients. Annotated RNA-seq analysis indicated that transcripts associated with negative regulation of T cell immunity were upregulated in female TCR1640 cells. This suggested that in females such genes might repress the progressive phenotype induced by male cells, and implied that T cell activation pathways are essential to progressive disease.

**1C6-Th17 adoptive transfer**

1C6 transgenic mice bear a TcR specificity for MOG

$\text{MOG}_{\text{235-255}}$ on the NOD background. The transgenic TcR is derived from a class II-restricted CD4$^+$ T cell clone; however, it is expressed on both CD4$^+$ and CD8$^+$ T cells in these mice (4, 107). Thus, 1C6 mice represent a useful model to study myelin Ag-specific effector T cell responses in RR→progressive disease (54).

In a recent report (35), we found that adoptive transfer of in vitro-differentiated male 1C6 Th17 to lymphocyte-deficient NOD.Scid mice resulted in a severe, progressive EAE phenotype, with over half of recipients attaining ethi-

On the other hand, female 1C6 Th17 cells induced disease of greater severity compared with male Th17 cells. Thus, while it is possible that IFN$\gamma$ itself might play a contributing role in the extremely severe disease induced by male Th17 cells, it is additionally possible that it might mark a phenotype of Th17 functional plasticity that has been associated with worsened outcomes in autoimmune disease (2, 24, 97). Indeed, CNS-infiltrating male 1C6 Th17 cells from recipients that experienced poor outcomes tended to express higher amounts of TGF-$\beta$, a marker of Th17 plasticity (35). Interestingly, pathogenic Th17 cells have been characterized as positive for the Th1 transcription T-bet (105) as well as for the inflammatory cytokine granulocyte macrophage colony stimulating factor (104). By contrast, non-pathogenic Th17 tend to secrete IL-10 (76). It has also been found that CNS-infiltrating pathogenic Th17 cells can injure neurons via their release of glutamate, suggesting that they may be directly cytotoxic (19). Going forward, it will be interesting to investigate whether sex can regulate this cytotoxic function of Th17 cells.

Strikingly, gonadectomy of male or female 1C6 mice did not appear to affect disease outcomes upon Th17 generation and adoptive transfer. This indicated that sex hormones might be secondary to sex chromosomes in mediating differences in EAE severity. Direct comparison of Tg-Sry XY and Tg-Sry XX 1C6 Th17 on an NOD:B6 F1 background revealed that the former transferred disease of greater severity. In addition to confirming these findings in pure NOD-background mice, it will be interesting to also interrogate the phenotype of XY versus XX 1C6 Th17, to observe whether the pathogenetic effect of XY is also seen in the context of female sex hormones.

Jarid1c: a putative X-linked inhibitor of Th17 pathogenicity.

A key finding of the above work is that the X-linked lysine demethylase Jarid1c/KDM5c (Fig. 6) can repress pathogenic Th17 responses (35). We identified Jarid1c by first analyzing a published data set of pathogenic (CNS-infiltrating) versus nonpathogenic (peripheral immune tissue) Th17 cells from B6-EAE (44). We searched for X-linked genes that were downregulated in pathogenic Th17, reasoning that these genes might repress disease caused by female cells. We next compared these differentially regulated X genes with a list of splenic mouse genes that escape second-copy silencing of the X chromosome (18). Of the three remaining transcripts of interest (Jarid1c, Utp14a, and Pdcd1), only Jarid1c was downregulated in male CNS-infiltrating 1C6 Th17 cells. Overexpression and pharmacological inhibition approaches revealed that Jarid1c reduces the severity of 1C6 Th17-mediated EAE. Importantly, we also found that Jarid1c was sharply reduced in CD4$^+$ T cells from sex-aggregated MS-affected individuals as opposed to healthy controls (35). Further comparison of Jarid1c expression in CD4$^+$ T cells from men with MS and women with MS, we found it was downregulated in men with MS compared with women with MS (35).

Jarid1c is a member of the Kdm5 family of histone H3K4 demethylases that are recruited to both enhancer and promoter elements (89). Recent findings have linked the methylation state of lysines to the transcriptional state of genes (48, 89, 101). Jarid1c represses transcriptional activity at promoter sequences by removing methyl groups from permissive euchromatic H3K4me3 marks; in its absence, transcriptional activity is deregulated (78). We find that, in
addition to reducing the pathogenicity of male Th17 cells, overexpression of Jarid1c reduces their expression of IFNγ (35). It is thus tempting to speculate that Jarid1c could be recruited to the promoter region of the IFNγ gene to inhibit its transcription. Alternately, it may target upstream regulators of IFNγ such as T-bet or Stat1. However, recent findings indicate that the roles played by Jarid1c in transcriptional regulation may be more nuanced than previously suggested. For example, it can further repress gene transcription by complexing with the adaptor molecules SUV39H1 and DDB1 to actually augment repressive heterochromatic H3K9me3 marks (89). Interestingly, Jarid1c may augment gene expression by reducing the frequency of H3K4me3 relative to that of the permissive H3K4me1 marks that predominate at gene enhancer sites (78). Therefore, the precise mechanisms by which Jarid1c regulates transcription in phenotypically plastic Th17 cells remain to be elucidated.

More generally, the role of Jarid1c in immune function is recently starting to come into focus. In dendritic cells (DCs), Jarid1c associates with the transcriptional repressor PCGF6 to enforce a quiescent state. Knockdown of Jarid1c enhanced the expression of activation markers such as MHC class II,
CD80, and IL-12p40 in both baseline and activated DCs (20). These data, together with ours, could suggest that Jarid1c may act as a broad-scale repressor of inflammatory immune responses. However, more work is required to better understand the molecular basis for Jarid1c-mediated inhibition of pathogenic Th17 responses.

**Future Perspectives**

In an era of precision/personalized medicine, biological sex is a critical factor to consider, and it is one of the best recognized variables to affect MS incidence and outcomes. Remarkably, however, it has received limited consideration as a factor that can influence responses to MS therapy. Indeed, the majority of phase III clinical trials in MS either do not conduct baseline analyses of the role of sex, or lack sufficient power to sensitively detect sex differences (52). CD4⁺ T cells play critical roles in MS pathogenesis, and as discussed here, many of their functions are regulated in a sex-specific manner and could pave the way for new treatment approaches. For example, one could envisage strategies that target Th1-specific cell surface receptors, such as CD226 (32), in women, or Th17-specific receptors, such as CCR4 or CCR6 (6), in men. Thus, going forward, increased appreciation of the role of sex in pathogenic T cell responses may help us to design more effective individualized treatment modalities for MS-affected individuals.

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M.U., M.R.F., and M.R. contributed to writing the article. M.R. supervised and approved the final version.

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**References**

1. Aarde SM, Hrnčir H, Arnold AP, and Jentsch JD. Reversal learning performance in the XY* mouse model of Klinefelter and Turner syndromes. *Front Behav Neurosci* 13: 201, 2019.

2. Abromson-Leeman S, Bronson RT, and Dorf ME. Encephalotrophic T cells that stably express both T-bet and ROR gamma t consistently produce IFN gamma but have a spectrum of IL-17 profiles. *J Neuroimmunol* 215: 10–24, 2009.

3. Alley J, Khasabov S, Simone D, Beitz A, Rodriguez M, and Njenga MK. More severe neurologic deficits in SJL/J male than female mice following Thelier’s virus-induced CNS demyelination. *Exp Neurol* 180: 14–24, 2003.

4. Anderson AC, Chandwaskar R, Lee DH, Sullivan JM, Solomon A, Rodríguez-Manzanet R, Greve B, Sobel RA, and Kuchroo VK. A transgenic model of central nervous system autoimmunity mediated by CD4⁺ and CD8⁺ T and B cells. *J Immunol* 188: 2084–2092, 2012.

5. Andersson A, Stubbellius A, Karlsson MN, Engdahl C, Erlandsson M, Graham N, Lagerquist MK, and Isander U. Estrogen regulates T helper 17 phenotype and localization in experimental autoimmune arthritis. *Arthritis Res Ther* 17: 32–12, 2015.

6. Annunziato F, Cosmi L, Liotta F, Maggi E, and Romagnani S. Defining the human T helper 17 cell phenotype. *Trends Immunol* 33: 505–512, 2012.

7. Aristimuño C, Teijeiro R, Valor L, Alonso B, Tejera-Alhambra M, de Andrés C, Miñarro DO, López-Lazareno N, Faure F, and Sánchez-Ramón S. Sex-hormone receptors pattern on regulatory T-cells: clinical implications for multiple sclerosis. *Clin Exp Med* 12: 247–255, 2012.

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

9. Arruvito L, Sanz M, Banham AH, and 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.

10. Baker D, Nutma E, O’Shea H, Cooke A, Orian JM, and Amor S. Autoimmune encephalomyelitis in NOD mice is not initially a progressive multiple sclerosis model. *Ann Clin Transl Neurol* 6: 1362–1372, 2019.

11. Basso A, Frenkel D, Quintana FJ, Costa-Pinto FA, Petrovic-Stojkovic S, Puckett L, Monsonego A, Bar-Shir A, Engel Y, Gozin M, and Weiner HL. Reversal of axonal loss and disability in a mouse model of progressive multiple sclerosis. *J Clin Invest* 118: 1532–1543, 2008.

12. Bebo BF, Adlard K, Schuster JC, Unsicker L, Vandenberg AA, and Offner H. Gender differences in protection from EAE induced by oral tolerance with a peptide analogue of PLP 139-151. *Front Neuroendocrinol* 13: 162–169, 2012.

13. Bebo BF, Schuster JC, Vandenberg AA, and Offner H. Gender differences in experimental autoimmune encephalomyelitis develop during the induction of the immune response to encephalitogenic peptides. *J Neurosci Res* 52: 420–426, 1998.

14. Bebo BF, Schuster JC, Vandenberg AA, and Offner H. Androgens alter the cytokine profile and reduce encephalitogenicity of myelin-reactive T cells. *J Immunol* 162: 35–40, 1999.

15. Bebo BF, Vandenberg AA, and Offner H. Male SJL mice do not relapse after induction of EAE with PLP 139-151. *J Neurosci Res* 45: 680–689, 1996.

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

17. Berard JL, Wolak K, Fournier S, and David S. Characterization of relapsing-remitting and chronic forms of experimental autoimmune encephalomyelitis in C57BL/6 mice. *Glia* 58: 434–445, 2010.

18. Berletch JB, Ma W, Yang F, Shedjere J, Noble WS, Distech CE, and Deng X. Escape from X inactivation varies in mouse tissues. *PLoS Genet* 11: e1005079, 2015.
19. Birkner K, Wasser B, Ruck T, Thalman C, Luchtman D, Pape K, Schmaul S, Bitar L, Krämer-Albers E-M, Stroh A, Meuth SG, Zipp F, and Bittner S. f1-Integrin- and KV1.3 channel-dependent signaling stimulates glutamate release from Th17 cells. *J Clin Invest* 130: 715–732, 2020.

20. Boukhaled GM, Cordeiro B, Deblois G, Dimitrov V, Bailey SD, Holowka T, Domi A, Guak H, Chiu H-SC, Everts B, Pearce EJ, Lupien M, White JH, and Krawczyk CM. The transcriptional repressor polycomb group factor 6, PCGF6, negatively regulates dendritic cell activation and promotes quiescence. *Cell Rep* 16: 1829–1837, 2016.

21. Bove R, Musallam A, Healy BC, Raghaonkar K, Blaniz G, Bakshi R, Weiner H, De Jager PL, Miller KK, and Chitnis T. Low testosterone is associated with disability in men with multiple sclerosis. *Mult Scler* 20: 1584–1592, 2014.

22. Butterfield RJ, Blankenhorn EP, Roper RJ, Zachary JF, Doerge RW, Sudweeks J, Rose J, and Teuscher C. Genetic analysis of disease subtypes and sexual dimorphisms in mouse experimental allergic encephalomyelitis (EAE): relapsing/remitting and monophasic remitting/nonrelapsing EAE are immunogenetically distinct. *J Immunol* 162: 3096–3102, 1999.

23. Butterfield RJ, Blankenhorn EP, Roper RJ, Zachary JF, Doerge RW, and Teuscher C. Identification of genetic loci controlling the characteristics and severity of brain and spinal cord lesions in experimental allergic encephalomyelitis. *Am J Pathol* 157: 637–645, 2000.

24. Carbajal KS, Mironova Y, Ulrich-Lewis JT, Kulkarni D, Grifka-Walk HM, Huber AK, Shraga P, Giger RJ, and Segal BM. The Y chromosome as a regulatory element specifically expressed on the surface of Th1 cells and regulating their expansion and effector functions. *J Immunol* 195: 2552–2559, 2015.

25. Carrel L and Willard HF. X-inactivation profile reveals extensive variability in X-linked gene expression in females. *Nature* 434: 400–404, 2005.

26. Case LK, Wall EH, Dragon JA, Saligrama N, Kremenstov DN, Moussawi M, Zachary JF, Huber SA, Blankenhorn EP, and Teuscher C. The Y chromosome as a regulatory element shaping immune cell transcriptomes and susceptibility to autoimmune disease. *Genome Res* 23: 1474–1485, 2013.

27. Chitnis T. The role of CD4 T cells in the pathogenesis of multiple sclerosis. *Ann Neurol* 79: 43–72, 2007.

28. Crotty S. FoT follicular helper cell biology: a decade of progress. *Curr Opin Immunol* 23: 1474–1485, 2013.

29. Dalal M, Kim S, and Voskuhl RR. Testosterone therapy specifically increases susceptibility to experimental autoimmune encephalomyelitis with thymectomy, on the development of T cell-mediated autoimmunity. *J Exp Med* 204: 321–330, 2007.

30. Damasceno A, Glehn von F, Branda˜o CO, Damasceno BP, Damasceno BP, and Teuscher C. The Y chromosome as a regulatory element specifically expressed on the surface of Th1 cells and regulating their expansion and effector functions. *J Immunol* 175: 1558–1565, 2005.

31. Dang PT, Bui Q, D’Souza CS, and Orian JM. Modelling T cell recruitment in a chronic model of central nervous system autoimmunity. *Cell Rep* 34: 108833, 2021.

32. Dhaeeze T, Lachance C, Tremblay L, Grasmuck C, Bourbonnière L, Larouche S, Saint-Laurent O, Lécuyer M-A, Rébillard R-M, Zandee S, and Prat A. Sex-dependent factors encoded in the immune compartment dictate relapsing or progressive phenotype in demyelinating disease. *JCI Insight* 4, 2019.

33. Dhaeeze T, Lachance C, Tremblay L, Grasmuck C, Bourbonnière L, Larouche S, Saint-Laurent O, Lécuyer M-A, Rébillard R-M, Zandee S, and Prat A. Sex-dependent factors encoded in the immune compartment dictate relapsing or progressive phenotype in demyelinating disease. *JCI Insight* 4, 2019.

34. Doerge RW, Sudweeks J, Rose J, and Teuscher C. Genetic analysis of disease subtypes and sexual dimorphisms in mouse experimental allergic encephalomyelitis (EAE): relapsing/remitting and monophasic remitting/nonrelapsing EAE are immunogenetically distinct. *J Immunol* 162: 3096–3102, 1999.

35. Doss PMIA, Umair M, Bailleargeon J, Fazazi R, Fudge N, Akbar I, Yeola AP, Williams JB, Leclercq M, Joly Beauparlant C, Beauchemin P, Ruda GF, Alpagh M, Anderson AC, Brennan PE, Droit A, Lassmann H, Moore CS, and Rangachari M. Male sex chromosomal complement exacerbates the pathogenicity of Th17 cells in a chronic model of central nervous system autoimmunity. *Cell Rep* 34: 108833, 2021.

36. Du S, Itoh N, Askarimam S, Hill H, Arnold AP, and Voskuhl RR. XY sex chromosome complement, compared with XX, in the CNS confers greater neurodegeneration during experimental autoimmune encephalomyelitis. *Proc Natl Acad Sci U S A* 111: 2806–2811, 2014.

37. Dunn SE, Gunde E, and Lee H. Sex-based differences in multiple sclerosis (MS): part II: rising incidence of multiple sclerosis in women and the vulnerability of men to progression of this disease. *Curr Top Behav Neurosci* 26: 57–86, 2015.

38. Dunn SE, Lee H, Pavri FR, and Zhang MA. Sex-Based differences in multiple sclerosis (part I): biology of disease incidence. *Curr Top Behav Neurosci* 26: 29–56, 2015.

39. Dunn SE, Ousman SS, Sobel RA, Zuniga L, Baranzini SE, Youssel S, Crowell A, Loh J, Oksenberg J, and Steinman L. Peroxisome proliferator-activated receptor (PPARalpha) expression in T cells mediates gender differences in development of T cell-mediated autoimmunity. *J Exp Med* 204: 321–330, 2007.

40. Faria AMC and Weiner HL. Oral tolerance: therapeutic implications for autoimmunity diseases. *Clin Dev Immunol* 13: 143–157, 2006.

41. Fitzpatrick F, Lepault F, Homo-Delarche F, Bach JF, and Dardenne M. Influence of castration, alone or combined with thymectomy, on the development of diabetes in the nonobese diabetic mouse. *Endocrinology* 129: 1382–1390, 1991.

42. Frischer JM, Bramow S, Dal-Bianco A, Lucchinetti CF, Rauschka H, Schmidbauer M, Laursen H, Størensen PS, and Lassmann H. The relation between inflammation and neurodegeneration in multiple sclerosis brains. *Brain* 132: 1175–1189, 2009.

43. Fuller A, Yahikozawa H, So EY, Dal Canto M, Koh C-S, Welsh CJ, and Kim BS. Castration of male C57L/J mice increases susceptibility and estrogen treatment restores resistance to Theiler’s virus-induced demyelinating disease. *J Neurosci Res* 85: 871–881, 2007.

44. Gauhlomme JT, Yosef N, Lee Y, Gertner RS, Yang LV, Wu C, Pandolfi PP, Mak T, Satijia R, Shalek AK, Kuchroo VK, Park H, and Regev A. Single-cell genomics unveils critical regulators of Th17 cell pathogenicity. *Cell* 163: 1400–1412, 2015.

45. Giron-González JA, Moral FJ, Elvira J, García-Gil D, Guerrero F, Gavilán I, and Escobar L. Consistent production of a higher TH1:TH2 cytokine ratio by stimulated autoreactive CD4+ T cells in men compared with women. *Eur J Endocrinol* 143: 31–36, 2000.

46. Golden LC, Itoh Y, Itoh N, Iyengar S, Coit P, Salama Y, Arnold AP, Sawalha AH, and Voskuhl RR. Parent-of-origin differences in DNA methylation of X chromosome
genes in T lymphocytes. Proc Natl Acad Sci U S A 116: 26779–26787, 2019.

47. Goodman WA, Garg RR, Reuter BK, Mattioli B, Rissman EF, and Pizarro TT. Loss of estrogen-mediated immunoprotection underlies female gender bias in experimental Crohn’s-like ileitis. Mucosal Immunol 7: 1255–1265, 2014.

48. Greer EL and Shi Y. Histone methylation: a dynamic mark in health, disease and inheritance. Nat Rev Genet 13: 343–357, 2012.

49. Harrington LE, Hatton RD, Mangan PR, Turner H, Murphy TL, Murphy KM, and Weaver CT. Interleukin 17-producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. Nat Immunol 6: 1123–1132, 2005.

50. Hewagama A, Patel D, Yarlagadda S, Strickland FM, and Richardson BC. Stronger inflammatory/cytotoxic T-cell response in women identified by microarray analysis. Genes Immun 10: 509–516, 2009.

51. Hirahara K, Vahedi G, Ghoreschi K, Yang X-P, Nakayama S, Kanno Y, O’Shea JJ, and Laurence A. Helper T-cell differentiation and plasticity: insights from epigenetics. Immunology 134: 235–245, 2011.

52. Houtchens MK and Bove R. A case for gender-based approach to multiple sclerosis therapeutics. Front Neuroendocrinol 50: 123–134, 2018.

53. Hunninghake GM, Chu J-H, Sharma SS, Cho MH, Himes BE, Rogers AJ, Murphy A, Carey VJ, and Raby BA. The CD4+ T-cell transcriptome and serum IgE in asthma: IL17RB and the role of sex. BMC Palm Med 11: 17–8, 2011.

54. Ignatius Arokia Doss PM, Roy A-P, Wang A, Anderson AC, and Rangachari M. The non-obese diabetic mouse strain as a model to study CD8(+)- T cell function in relapsing and progressive multiple sclerosis. Front Immunol 6: 541, 2015.

55. Itoh Y, Golden LC, Itoh N, Matsukawa MA, Ren E, Tse V, Arnold AP, and Voskuhl RR. The X-linked histone demethylase Kdm6a in CD4+ T lymphocytes modulates autoimmunity. J Clin Invest 130: 3852–3863, 2019.

56. Jane-wit D, Altuntas CZ, Monti J, Johnson JM, Forshuber TG, and Tuohy VK. Sex-defined T-cell responses to cardiac self determine differential outcomes of murine dilated cardiomyopathy. Am J Pathol 172: 11–21, 2008.

57. Kappos L, Bar-Or A, Cree BAC, Fox RJ, Giovannoni G, Gold R, Vermersch P, Arnold DL, Arnould S, Scherz T, Wolf C, Wallström E, Dahlke F, EXPAND Investigators. Siponimod versus placebo in secondary progressive multiple sclerosis (EXPAND): a double-blind, randomised, phase 3 study. Lancet 391: 1263–1273, 2018.

58. Kikutani H and Makino S. The murine autoimmune diabetes model: NOD and related strains. Adv Immunol 58: 285–322, 1992.

59. Kim D-H, Park H-J, Park H-S, Lee J-U, Ko C, Gye MC, and Choi J-M. Estrogen receptor α and β contribute to T cell-mediated autoimmune inflammation by promoting T cell activation and proliferation. Sci Signal 11: eaar9415, 2018.

60. Koch M, Kingwell E, Rieckmann P, Tremlett H; UBC MS Clinic Neurologists. The natural history of secondary progressive multiple sclerosis. J Neurol Neurosurg Psychiatr 81: 1039–1043, 2010.

61. Kurth F, Luders E, Sicotte NL, Gaser C, Giesser BS, Swerdloff RS, Montag MJ, Voskuhl RR, and Mackenzie-Graham A. Neuroprotective effects of testosterone treatment in men with multiple sclerosis. Neuroimage Clin 4: 454–460, 2014.

62. Langrish CL, Chen Y, Blumenschein WM, Mattson J, Basham B, Sedgwick JD, McClanahan T, Kastelein RA, and Cua DJ. IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. J Exp Med 201: 233–240, 2005.

63. Larochelle C, Uphaus T, Prat A, and Zipp F. Secondary progression in multiple sclerosis: neuronal exhaustion or distinct pathology? Trends Neurosci 39: 325–339, 2016.

64. Li Z, Yue Y, and Xiong S. Distinct T17 inductions contribute to the gender bias in CVB3-induced myocarditis. Cardiovasc Pathol 22: 373–382, 2013.

65. Liva SM and Voskuhl RR. Testosterone acts directly on CD4+ T lymphocytes to increase IL-10 production. J Immunol 167: 2060–2067, 2001.

66. Magliozzi R, Howell O, Vora A, Serafini B, Nicholas R, Puopolo M, Reynolds R, and Aloisi F. Meningeal B-cell follicles in secondary progressive multiple sclerosis associate with early onset of disease and severe cortical pathology. Brain 130: 1089–1104, 2007.

67. Maret A, Coudert JD, Giraudou L, Fourcas G, Gourdy P, Krust A, Dupont S, Chambon P, Druet P, Bayard F, and Guéry J-C. Estradiol enhances primary antigen-specific CD4 T cell responses and Th1 development in vivo. Essential role of estrogen receptor alpha expression in hematopoietic cells. Eur J Immunol 33: 512–521, 2003.

68. Mayo L, Trauger SA, Blain M, Nadeau M, Patel B, Alvarez JI, Mascanfroni ID, Yeste A, Kivissäki P, Kallas K, Ellezam B, Bakshi R, Prat A, Antel JP, Weiner HL, and Quintana FJ. Regulation of astrocyte activation by glycolipids drives chronic CNS inflammation. Nat Med 20: 1147–1156, 2014.

69. McRae BL, Kennedy MK, Tan LJ, Dal Canto MC, Picha KS, and Miller SD. Induction of active and adaptive relapsing experimental autoimmune encephalomyelitis (EAE) using an encephalitogenic epitope of proteolipid protein. J Neuroimmunol 38: 229–240, 1992.

70. Michel L, Touil H, Pikor NB, Gommerman JL, Prat A, and Bar-Or A. B cells in the multiple sclerosis central nervous system: trafficking and contribution to CNS-compartmentalized inflammation. Front Immunol 6: 636, 2015.

71. Mo R, Chen J, Grolleau-Julius A, Murphy HS, Richardson BC, and Yang RL. Estrogen regulates CCR gene expression and function in T lymphocytes. J Immunol 174: 6023–6029, 2005.

72. Mohammad I, Staraka I, Nagy T, Guo J, Yatkin E, Väänänen K, Watford WT, and Chen Z. Estrogen receptor α contributes to T cell-mediated autoimmune inflammation by promoting T cell activation and proliferation. Sci Signal 11: eaar9415, 2018.

73. Montalban X, Hauser SL, Kappos L, Arnold DL, Bar-Or A, Comi G, de Seze J, Giovannoni G, Hartung H-P, Hemmer B, Lublin F, Rammohan KW, Selmaj K, Traboulsee A, Sauter A, Masterman D, Fontoura P, Belachew S, Garren H, Mairon N, Chin P, Wolinsky JS; ORATORIO Clinical Investigators. Ocrelizumab versus placebo in primary progressive multiple sclerosis. N Engl J Med 376: 209–220, 2017.

74. Mosmann TR, Cherwinski H, Bond MW, Giedlin MA, and Coffman RL. Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine
activities and secreted proteins. J Immunol 136: 2348–2357, 1986.
75. Offner H. Neuroimmunoprotective effects of estrogen and derivatives in experimental autoimmune encephalomyelitis: therapeutic implications for multiple sclerosis. J Neuroimmunol 78: 603–624, 2004.
76. Ogawa C, Bankoti R, Nguyen T, Hassanazadeh-Kiai N, Nadeau S, Porritt RA, Cousie M, Fan X, Dhali D, Eberl G, Ohnmacht C, and Martins GA. Bim-1 functions as a molecular switch to prevent inflammatory activity in Foxp3+RORγt+ regulatory T cells. Cell Rep 25: 19–28.e5, 2018.
77. Oukka M. Interplay between pathogenic Th17 and regulatory T cells. Ann Rheum Dis 66(Suppl 3): iii87–90, 2007.
78. Oukka M, Interplay between pathogenic Th17 and regulatory T cells. Cell Rep 3: 1071–1079, 2013.
79. Page ST, Plymate SR, Bremner WJ, Matsumoto AM, Hess DL, Lin DW, Amory JK, Nelson PS, and Wu JD. Effect of medical castration on CD4+CD25+ T cells, CD8+ T cell IFN-gamma expression, and NK cells: a physiological role for testosterone and/or its metabolites. Am J Physiol Endocrinol Metab 290: E856–63, 2006.
80. Papenfuss TL, Rogers CJ, Gienapp I, Yurrita M, McClain SM, Holstege FCP, Grosveld FG, and Timmers HTM. DNA methylation in autoimmune encephalomyelitis in multiple murine strains. J Neuroimmunol 150: 59–69, 2004.
81. Pappalardo LW, Samad OA, Liu S, Zwinger PJ, Black JA, J-M. Gender-specific differences in PPARγ regulation of follicular helper T cell responses with estrogen. Sci Rep 6: 28495–9, 2016.
82. Phipel KL, Henderson RA, Adelman SJ, and Elloso MM. Differential estrogen receptor gene expression in human peripheral blood mononuclear cell populations. Immunol Lett 97: 107–113, 2005.
83. Poppe AY, Wolfson C, and Zhu B. Prevalence of multiple sclerosis in Canada: a systematic review. Can J Neurol Sci 35: 593–601, 2008.
84. Pollinger B, Krishnamoorthy G, Berer K, Lassmann H, Boś MR, Damm R, Domingues HS, Holz A, Kurschus FC, and Wekerle H. Spontaneous relapsing-remitting EAE in the SJL/J mouse: MOG-reactive transgenic T cells recruit T cells by promoting their proliferation. Immunology 118: 58–65, 2006.
85. Rangachari M and Kuchroo VK. Using EAE to better understand principles of immune function and autoimmunity pathogenesis. J Autoimmun 45: 31–39, 2013.
86. Robertson SA, Care AS, and Moldenhauer LM. Regulatory T cells in embryo implantation and the immune response to pregnancy. J Clin Invest 128: 4224–4235, 2018.
87. Rondinelli B, Rosano D, Antonini E, Frenquelli M, Montanini L, Huang D, Segalla S, Yoshihara K, Amin SB, Lazarevic D, The BT, Verhaak RGW, Futreal PA, Di Croce L, Chin L, Cittaro D, and Tonon G. Histone demethylase JARID1C inactivation triggers genomic instability in sporadic renal cancer. J Clin Invest 125: 4625–4637, 2015.
88. Sankaran-Walters S, Macal M, Grishina I, Nagy L, Goulart L, Coolidge K, Li J, Fenton A, Williams T, Miller MK, Flamm J, Prindiville T, George M, and Dandekar S. Sex differences matter in the gut: effect on mucosal immune activation and inflammation. Biol Sex Differ 4: 10, 2013.
89. Schoonheim MM, Hulst HE, Brandt RB, Strik M, Wink AM, Uitdehaag BMJ, Barkhof F, and Geurts JIG. Thalamus structure and function determine severity of cognitive impairment in multiple sclerosis. Neurology 84: 776–783, 2015.
90. Serafini B, Rosicarrelli B, Magliozzi R, Stigliano E, and Aloisi F. Detection of ectopic B-cell follicles with germinal centers in the meninges of patients with secondary progressive multiple sclerosis. Brain Pathol 14: 164–174, 2004.
91. Sicotte NL, Giesser BS, Tandon V, Klutch R, Steiner B, Drain AE, Shattuck DW, Hull L, Wang H-J, Elashoff RM, Swerdloff RS, and Voskuhl RR. Testosterone treatment in multiple sclerosis: a pilot study. Arch Neurol 64: 683–688, 2007.
92. Smith-Bouvier DL, Divekar AA, Sasidhar M, Du S, Tiwari-Woodruff SK, King JK, Arnold AP, Singh RR, and Voskuhl RR. A role for sex chromosome complement in the female bias in autoimmune disease. J Exp Med 205: 1099–1108, 2008.
93. Spach KM, Blake M, Bunn JY, McElvany B, Noubade R, Blankenhorn EP, and Teuscher C. Cutting edge: the Y chromosome controls the age-dependent experimental allergic encephalomyelitis sexual dimorphism in SJL/J mice. J Immunol 182: 1789–1793, 2009.
94. Spach KM and Hayes CE. Vitamin D3 confers protection from autoimmune encephalomyelitis only in female mice. J Immunol 175: 4119–4126, 2005.
95. Suryani S and Sutton I. An interferon-gamma-producing Th1 subset is the major source of IL-17 in experimental autoimmune encephalitis. J Neuroimmunol 183: 96–103, 2007.
96. Teuscher C, Noubade R, Spach K, McElvany B, Bunn JY, Fillmore PD, Zachary JF, and Blankenhorn EP. Evidence that the Y chromosome influences autoimmune disease in male and female mice. Proc Natl Acad Sci U S A 103: 8024–8029, 2006.
97. Tsvetkova-Vicheva VM, Gecheva SP, Kompa-Penkova R, Velkova AS, and Lukanov TH. IL-17 producing T cells correlate with polysensitization but not with bronchial hyperresponsiveness in patients with allergic rhinitis. Clin Transl Allergy 4: 3, 2014.
98. Vasanthakumar A, Chisanga D, Blume J, Gloury R, Britt K, Henstridge DC, Zhan Y, Torres SV, Liene S, Collins N, Cao E, Sidwell T, Li C, Spallanzani RG, Liao Y, Beavis PA, Gebhardt T, Trevaskis N, Nott SL, Zajac JD, Davey RA, Febbraio MA, Mathis D, Shi W, and Kallies A. Sex-specific adipose tissue imprinting of regulatory T cells correlate with polysensitization but not with bronchial hyperresponsiveness in patients with allergic rhinitis. Clin Transl Allergy 4: 3, 2014.
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102. Voskuhl RR and Gold SM. Sex-related factors in multiple sclerosis susceptibility and progression. Nat Rev Neurol 8: 255–263, 2012.

103. Voskuhl RR, Patel K, Paul F, Gold SM, Scheel M, Kuchling J, Cooper G, Assever S, Chien C, Brandt AU, Meyer CE, and Mackenzie-Graham A. Sex differences in brain atrophy in multiple sclerosis. Biol Sex Differ 11: 49–10, 2020.

104. Wang X, Lennard Richard M, Li P, Henry B, Schutt S, Yu X-Z, Fan H, Zhang W, Gilkeson G, and Zhang XK. Expression of GM-CSF is regulated by Fli-1 transcription factor, a potential drug target. J Immunol 206: 59–66, 2021.

105. Wang Y, Godec J, Ben-Aissa K, Cui K, Zhao K, Pucsek AB, Lee YK, Weaver CT, Yagi R, and Lazarevic V. The transcription factors T-bet and Runx are required for the ontogeny of pathogenic interferon-γ-producing T helper 17 cells. Immunity 40: 355–366, 2014.

106. Wu X, Tian J, and Wang S. Insight into non-pathogenic Th17 cells in autoimmune diseases. Front Immunol 9: 1112, 2018.

107. Yeola AP, Ignatius Arokia Doss PM, Baillargeon J, Akbar I, Mailhot B, Balood M, Talbot S, Anderson AC, Lacroix S, and Rangachari M. Endogenous T cell receptor rearrangement represses aggressive central nervous system autoimmunity in a TcR-transgenic model on the non-obese diabetic background. Front Immunol 10: 3115, 2019.

108. Youinou P, Pers J-O, Gershwin ME, and Shoenfeld Y. Geo-epidemiology and autoimmunity. Front Immunol 10: 3115, 2019.

109. Youinou P, Pers J-O, Gershwin ME, and Shoenfeld Y. Geo-epidemiology and autoimmunity. J Autoimmun 34: J163–J167, 2010.

110. Zamvil SS and Hauser SL. Antigen presentation by B cells in multiple sclerosis. N Engl J Med 334: 378–381, 2011.

111. Zang YCQ, Halder JB, Hong J, Rivera VM, and Zhang JZ. Antagonizing peroxisome proliferator-activated receptor κB. J Neuroimmunol 206: 59–66, 2021.

112. Zhang MA, Ahn JJ, Zhao FL, Selvanantham T, Mallevaey K, Cui K, Zhao K, Pucsek AB, Lee YK, Weaver CT, Yagi R, and Lazarevic V. The transcription factors T-bet and Runx are required for the ontogeny of pathogenic interferon-γ-producing T helper 17 cells. Immunity 40: 355–366, 2014.

113. Zhang MA, Rego D, Moskhova M, Kebir H, Chrusicinski A, Nguyen H, Akkermann R, Stanczyk FZ, Prat A, Steinman L, and Dunn SE. Peroxisome proliferator-activated receptor (PPAR)γ and -γ regulate IFNγ and IL-17A production by human T cells in a sex-specific way. Proc Natl Acad Sci U S A 109: 9505–9510, 2012.

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Abbreviations Used

Ag = antigen
APC = antigen-presenting cell
B6 = C57BL6/J
BBB = blood/brain barrier
BM = bone marrow
CCR = C-C chemokine receptor
CD = cluster of differentiation
CNS = central nervous system
DC = dendritic cell
DDB1 = DNA damage-binding protein 1
DMT = disease-modifying therapy/therapies
EAE = experimental autoimmune encephalomyelitis
ER = estrogen receptor
FCG = four-core genotype
FoxP3 = forkhead box P3
IFN = interferon
IL = interleukin
Jarid = Jumonji AT-rich activating domain
LPL = lamina propria lymphocytes
MBP = myelin basic protein
MHC = major histocompatibility complex
MIP = macrophage inflammatory protein
MOG = myelin oligodendrocyte glycoprotein
MS = multiple sclerosis
NOD = nonobese diabetic
PLP = proteolipid protein
POO = parent-of-origin
PPAR = peroxisome proliferator-activated receptor
Rag = recombination-activating gene
RANTES = regulated on activation, normal T cell expressed and secreted
RR = relapsing/remitting
Scid = severe combined immunodeficiency
sRNA = short interfering ribonucleic acid
SJJ = Swiss James Lambert
Sry = sex-determining region Y
SUV39H1 = suppressor of variegation 3–9 homologue 1
SV.129 = C57BL6/J
Tc = cytotoxic T cell
TcR = T cell receptor
TFs = transcription factors
Tfh = T follicular helper
Tg = transgenic
Th = T helper cell
Th1 = T helper 1
Th2 = T helper 2
Th17 = T helper 17
TLR = Toll-like receptor
TMEV = Theiler’s murine encephalomyelitis virus
Treg = T regulatory cells
VAT = visceral adipose tissue
Xm = maternal X
Xp = paternal X