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IL-13-Mediated Gender Difference in Susceptibility to Autoimmune Encephalomyelitis

Sushmita Sinha,*† Laurie J. Kaler,*† Thomas M. Proctor,* Cory Teuscher,‡ Arthur A. Vandenbark,*†§ and Halina Offner2*†‖

Females tend to have stronger Th1-mediated immune responses and are more prone to develop autoimmune diseases, including multiple sclerosis. Macrophages are major effector cells capable of mediating or modulating immune responses in experimental autoimmune encephalomyelitis (EAE). IL-13 and estrogen have opposing roles on macrophages (the former enhancing and the latter inhibiting) in terms of MHC class II (MHC II) up-regulation and, thus, these factors might influence susceptibility to EAE differently in females vs males. In accordance with this hypothesis, females lacking IL-13 displayed lower incidence and milder EAE disease severity than males after immunization with myelin oligodendrocyte glycoprotein (MOG)-35–55 peptide/CFA/pertussis toxin. Female IL-13 knockout (KO) mice with restored expression after IL-13 pretreatment of female but not male macrophages from IL-13KO mice, with restored expression after IL-13 pretreatment of female but not male macrophages. APCs from IL-13KO females induced less proliferation by MOG-35–55-reactive T cells, and splenocytes from MOG peptide-immunized females had lower expression of IL-12, IFN-γ, MIP-2, and IFN-γ-inducible protein 10 than males. In contrast, these splenocytes had higher expression of anti-inflammatory factors, IL-10, TGF-β1, and Foxp3, a cytokine pattern typical of regulatory type II macrophages. These data suggest that the difference in EAE susceptibility in females is strongly influenced by gender-specific proinflammatory effects of IL-13, mediated in part through up-regulation of Th1-inducing cytokines and MHC II on CD11b+ macrophages. The Journal of Immunology, 2008, 180: 2679–2685.

Females tend to have stronger Th1-mediated immune responses and are more prone to develop autoimmune diseases, including multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, and thyroiditis. The gender bias of autoimmune diseases is currently unclear and is attributed to more aggressive proinflammatory Th1-like autoimmune responses in females. However, the molecular mechanism behind female prevalence of autoimmune diseases is poorly understood. IL-13 is produced by activated T cells but does not affect resting or activated T cells. It has been shown to inhibit the production of inflammatory cytokines, up-regulate MHC class II (MHC II) expression on monocytes (1), and induce anti-inflammatory CD40-dependent IgG/E class switch and IgG and IgM synthesis in B cells (2–6). On binding with one of its three receptors, IL-13Rα1 and IL-13Rα2, IL-13 mediates signal transduction through JAK STAT pathways (7). IL-13 plays a major role in several biological processes including airway hyperresponsiveness, allergic inflammation, tissue cosinophilia, parasite elimination, mast cell hyperplasia, IgE Ab synthesis, tissue remodeling, and fibrosis.

Its role in experimental autoimmune encephalomyelitis (EAE) has been of an anti-inflammatory cytokine and has been shown to be capable of suppressing EAE in Lewis male rats (8). What makes it interesting to study in the context of gender susceptibility is the possibility that IL-13 and estrogen (E2) have opposite effects on macrophages in terms of MHC II expression, with IL-13 up-regulating and E2 down-regulating the expression. Moreover, both molecules are believed to mediate their effects through APCs. Due to the pivotal role of macrophages in the effector phase of EAE and the recent possibility raised by Polanczyk et al. (9) that the protective effect of E2 might be mediated by nonlymphocytic cells such as macrophages and dendritic cells, together with the fact that IL-13 is a potent regulator of macrophages, a detailed study of the possible role of this cytokine in gender susceptibility to EAE is warranted.

Materials and Methods

Mice

Male and female IL-13 knockout (KO) mice on the C57BL/6 background were obtained from Dr. M. S. Duthe (Infectious Disease Research Institute, Seattle, WA). Original 129 × B6 IL-13KO mice were made by McKenzie et al. (10). These mice were then backcrossed with WT C57BL/6 (for five generations) by T. Hoshino (School of Medicine, Kurume University, Fukuoka, Japan) to generate IL-13KO mice on the B6 background. The 129P2/OlaHsd congenic interval encompassing the disrupted IL-13 allele was defined using informative microsatellite markers distinguishing 129P2/OlaHsd alleles from C57BL6J and C57BL/6J strain alleles (www.informatics.jax.org/searches/polymorphism_form.shtml). DNA was isolated from multiple IL-13KO males and females and genotyping was carried out as previously described (11).

Wild-type (WT) male and female C57BL/6 mice were purchased from The Jackson Laboratory. Animals were housed and cared for according to federal and institutional guidelines in the Animal Resource Facility at the
Veterans Affairs Medical Center (Portland, OR). The study was conducted in accordance with National Institutes of Health guidelines for the use of experimental animals, and the protocols were approved by the institutional animal care and use committee.

**Induction of EAE**

IL-13KO and C57BL/6 mice were immunized with 200 μg of myelin oligodendrocyte glycoprotein (MOG)-35–55 peptide in 400 μg of CFA. On the same day and day 2 after immunization, mice were injected (i.p.) with pertussis toxin (Ptx; 75 and 200 ng/mouse, respectively; List Biological Laboratories). The mice were assessed daily for signs of EAE according to the following scale: 0, normal; 1, limp tail or mild hind limb weakness; 2, moderate hind limb weakness or mild ataxia; 3, moderately severe hind limb weakness; 4, severe hind limb weakness or mild forelimb weakness or moderate ataxia; 5, paraplegia with no more than moderate hind limb weakness; and 6, paraplegia with severe forelimb weakness or severe ataxia or moribund condition. The cumulative disease index (CDI) is the sum of the daily EAE scores for each mouse for the entire duration of the experiment. The CDI is presented as mean ± SD for each group. Mice were monitored for disease until they were sacrificed for ex vivo analyses.

**Flow cytometry**

Four-color (FITC, PE, propidium iodide, allophycocyanin) fluorescence flow cytometry analyses were performed to determine the phenotypes of splenocytes following standard mAb-staining procedures. After staining, cells were washed with staining medium and analyzed immediately with a FACSCalibur using CellQuest (BD Biosciences) software. Data represent 50,000 events, unless otherwise noted. All Abs were purchased from BD Pharmingen. Cells were stained with a combination of the following mAbs: CD3, CD4, CD8, CD45, CD11b, CD11c, MHC Ia/II. Absolute numbers of cells were calculated from live-gated pooled splenocytes or CNS cells.

**Histopathology**

Intact spinal cords were removed from mice on day 30 of clinical disease and fixed in 10% formalin. The spinal cords were dissected after fixation and embedded in paraffin before sectioning. The sections were stained with H&E to assess inflammatory lesions and analyzed by light microscopy.

**Immunohistochemistry**

Four mice per IL-13KO male and female group with active EAE were perfused with 0.9% saline followed by cold 4% paraformaldehyde. Spinal cords were removed on day 30 after immunization and postfixed in 4% paraformaldehyde overnight for 2 h. Four sections of the lumbar spinal cord region per mouse were stained with each Ab. The sections were permeabilized with 0.2% Triton X-100 in PBS for 45 min followed by blocking with fish skin gelatin (0.5%) and BSA (3%) in BSA for 2 h at room temperature (RT). The sections were then incubated with primary Ab in blocking solution overnight at 4°C. The next day, the sections were washed with PBS and incubated with appropriate secondary Ab for 2 h at RT. After washing with PBS, the sections were mounted in Prolong Gold anti-fade and visualized on fluorescence microscope. Primary Abs were as follows: anti-CD11b (BD Bio sciences) and anti-MHC II (Santa Cruz Biotechnology). Primary and secondary Abs were used at dilutions of 1/75 and 1/200, respectively. White matter of the dorsal, lateral, and ventral spinal cords was photographed under ×20 magnification (for determining the percentage of area covered by an Ab stain) by fluorescence microscope. Digital images were saved in 12-bit TIF format (1024 × 1024 pixels). Data were generated from 20 raw images (four mice each in IL-13KO male and female groups). All image processing and analysis was done using BITPLANE Imaris software version 5.7.1. Background fluorescence was determined on the sections incubated only with the secondary Ab. An average fluorescence was also determined from several unlabeled regions of the spinal cords. The threshold was set two times above the background signal/noise. All the images were taken with exactly the same acquisition settings and all the data analyses were done in a blinded manner.

**Cytokine determination by the Multiplex Luminex kit**

Lymph node (LN) and spleen cells were cultured at 4 × 10^6 cells/well in a 24-well flat-bottom culture plate in stimulation medium (RPMI 1640, 1% sodium pyruvate, 1% l-glutamine, 0.4% 2-2-ME, 10% FBS) with 25 μg/ml MOG-35–55 peptide at 48 h. Supernatants were then harvested and stored at −80°C until tested for cytokines. Culture supernatants were assessed for cytokine levels using a Luminex Bio-Plex mouse cytokine assay kit (Bio-Rad) following manufacturer’s instructions. The following cytokines were determined in a single assay in three separate experiments: IL-1β, IFN-γ, TNF-α, IL-2, IL-4, IL-5, IL-6, IL-12, IL-13, and IL-17.

**RNA isolation and RT-PCR**

Total RNA was isolated from splenocytes using the RNeasy mini kit protocol (Qiagen) and then converted to cDNA using oligo(dT), random hexamers, and Superscript RT II enzyme (Invitrogen Life Technologies). Real-time PCR was performed using Quantitect SYBR Green PCR master mix (Qiagen) and primers (synthesized by Applied Biosystems). Reactions
were conducted on the ABI Prism 7000 Sequence Detection System (Applied Biosystems) to detect the following genes: L32 (forward), GGA AAC CCA GAG GCA TTG AC; reverse, TCA GGA TCT GGC CCT TGA AC; IL-10 (F, GAT GCC CCA AGA AGA A; R, CAC CCA GG3 AAT TCA AAT GC) and GAPDH (F, GGA ACC TCC CCC TGG CTT). IL-13 (F, ACT GCT CAG CAT ACA AAA GCA ACT; R, TGA GAT GCC CAGGGA TGG T; IL-4 (F, GGA GAT GGA TGT GCC AAA CG; R, CGAGCT CAC TCT CTT TAT T); IFN-γ (F, GCC CCT TCT CCA GACAG A; R, GCT GAT CAT GGC TGG GTT GT); IL-10 (F, GGC CCT TCT CCA GGACAG A; R, GCT GAT CAT GGC TGG GTT GT); RANTES (F, CCT CAC CAT CAC CTT CAT GC A; R, TCT CCT CTG GTG TGG CAC ACA C); MIP-2 (F, TGG GCT GCT GCT CAA; R, CCC GGG TGC TTT TTG TTT T); IFN-γ-inducible protein 10 (IL-10) (F, CGA TGA CGG GCC AGT GA; R, CGC AGG GAT GAT TTC AAG CT).

Generation of MOG-35–55-specific T cell lines

C57BL/6 mice were immunized with an emulsion of 200 μg of MOG-35–55 peptide in 400 μg of CFA. On day 10 after immunization, a single-cell suspension from LNs was prepared and stimulated in vitro with 25 μg/ml MOG-35–55 in 10% stimulation medium. After 4 days of culture, viable cells were propagated in culture medium containing 50–100 U/ml IL-2. These lymphocytes were cyclically restimulated for 2 days in the presence of 50 × 10^6 irradiated syngeneic APCs and 10 μg/ml peptide followed by expansion for 4 days in the presence of IL-2.

Ag-presentation assay

A single-cell suspension was prepared from spleens of male and female IL-13KO mice with active EAE (peak of disease). APCs were isolated from spleens using CD90 microbeads from Miltenyi Biotec. Briefly, splenocytes were incubated on ice for 15 min with CD90 microbeads. Cells were washed, and then sorted using the deplete program on an AutoMACS magnetic cell sorter. The negative fraction was washed, resuspended at 1 × 10^7/ml in stimulation medium containing 10% FBS. The overall purity of T cell-depleted APCs for all studied groups was similar and was over 95%. The APCs included similar proportions of CD19+ B cells and CD11b+ monocytes. APCs from both the groups were cocultured at various ratios together with 1 × 10^6 CD4+ T cells from a MOG-35–55-specific cell line. The cells were incubated for 3 days at 37°C in 7% CO2 and were then pulsed with 0.5 μCi [ methyl-3H] thymidine (PerkinElmer) for the final 18 h of incubation. The cells were harvested onto glass-fiber filters, and tritiated thymidine uptake was measured by a liquid scintillation counter. Means and SDs were calculated from triplicate wells. Net cpm was calculated by subtracting control cpm from Ag-induced cpm.

Bone marrow–derived macrophages (BMDM)

Bone marrow cavity from femora and tibias of 8-wk-old WT and IL-13KO male and female mice were flushed with RPMI 1640. The cells were washed and cultured in the presence of 500 U/ml MCSF-1 (Invitrogen Life Technologies). The cells were fed periodically and cultured for 7 days. After washing, 1 × 10^6 cells/ml from each group were stimulated either with 1 μg/ml LPS alone for 24 h and subjected to FACS staining. Alternatively, 1 × 10^6 cells/ml from IL-13KO male and female BMDM were pretreated with either 10 or 250 ng of IL-13 (BD Pharmingen) for 16 h followed by LPS stimulation for 24 h. The cells were then stained with a combination of the following Abs: CD45, CD11b, MHC Ia/II (MHC II), CD80, and CD86 using standard mAb staining procedures. After staining, cells were washed with staining medium and analyzed immediately with a FACSCalibur using CellQuest (BD Biosciences) software.

Statistical analysis

Statistical differences between disease scores of vehicle and treatment groups were determined by either the Mann-Whitney U test or the Student t test. Difference in incidence of EAE was determined using the χ^2 test. A p value < 0.05 was considered significant.

Results

Gender differences in susceptibility to autoimmune diseases are well-documented and it has long been known that the incidence of MS in females is two to three times higher than in males. This gender difference is also reflected in SJL/J mice with EAE, an animal model for MS. In these mice, females have stronger humoral and cell-mediated immune responses than males and a higher incidence and severity of EAE (12). However, the mechanisms underlying enhanced autoimmune disease susceptibility in females are poorly understood. While working with IL-13KO mice on the B6 background, we observed that female IL-13KO mice were either resistant or developed EAE with reduced severity compared with

<table>
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<th>Table I. Reduced number of CD11b+ cells in CNS of IL-13KO females</th>
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^a Absolute number of cells from brain and spinal cords of females vs males, p < 0.05.
IL-13 KO males or WT females or males. We thus explored the contribution of IL-13 expression to this marked gender difference.

IL-13 KO female mice have reduced incidence and clinical severity to EAE compared with IL-13 KO males and WT controls

The mean disease course (combined from three experiments) of male and female IL-13 KO mice immunized with MOG-35–55 peptide/CFA/Ptx is shown in Fig. 1A. After immunization, both male and female WT mice and all IL-13 KO males developed EAE with no mortality (Fig. 1B). In contrast, IL-13 KO females were significantly less susceptible to EAE induction. In three separate experiments, female IL-13 KO mice showed a highly significant reduction in EAE incidence (15 of 26 females vs 26 of 26 males, p < 0.001, Fig. 1B), with delayed onset in those with disease. Moreover, the IL-13 KO females had a lower peak disease score (p < 0.01) and CDI (p < 0.01, Fig. 1B) compared with male IL-13 KO mice in each of the three separate experiments.

Female IL-13 KO mice show reduced infiltration of CD11b+ cells and up-regulation of MHC II in the CNS

Mononuclear cells isolated from spleens, spinal cords, and brains of IL-13 KO males and females with EAE were subjected to staining with several cell surface markers. On day 30 postimmunization, female IL-13 KO mice had a significantly lower absolute number (female vs male, 12.6 × 10^6 ± 2 vs 25.9 × 10^6 ± 1; p < 0.05) and a lower percentage (Fig. 2, top panel) of CD11b+ macrophages in spleens than males. Similarly, the absolute number (Table I) and percentage (Fig. 2, middle panel) of resident and infiltrating CD11b+ macrophages in CNS (CD45+ intermediate and high cells) were significantly lower in IL-13 KO females vs males, as were the absolute number (Table I) and percentage (Fig. 2, bottom panel) of CD11b+ cells that expressed MHC II. CD45+ low CD11b+ cells represent CNS microglia, and CD45+ intermediate and high CD11b+ cells represent resident and infiltrating macrophages (13). Immunohistochemical staining for CD11b and MHC II also demonstrated significantly less infiltration of CD11b+ cells (Fig. 3A, percentage area of staining for CD11b+ cells in females vs males, 6.7% vs 24.5%, p < 0.05) and significantly less up-regulation of MHC II (Fig. 3B, percentage area of staining for MHC II in females vs males, 0.94 vs 10.3%, p < 0.05) in the spinal cords of IL-13 KO females as compared with males. Similarly, significantly reduced numbers of CD11b+ cells and reduced expression of MHC II in female IL-13 KO CNS were also observed in spinal cord sections taken from mice at the peak of disease (data not shown).

The reduction of cell numbers and expression of MHC II by infiltrating cells in the CNS of female vs male IL-13 KO mice appeared to be selective for CD11b+ macrophages. Thus, as is also shown in Table I, there were no significant differences between IL-13 KO females vs males in the absolute numbers of CD4+, CD8+, or CD11c+ cells infiltrating the CNS.

BMDM from male and female IL-13 KO mice display inherent differences in MHC II up-regulation

Due to the fact that IL-13 is a potent regulator of macrophage function and IL-13 KO female mice had consistently reduced numbers of CD11b+ cells in brains and spinal cords, we studied the functional differences in the CD11b+ cell populations in male vs female IL-13 KO mice. BMDM were stimulated with LPS for 24 h, with or without a 16-h pretreatment with IL-13, and the cells were evaluated for expression of various activation markers, including MHC II, CD80, and CD86. When stimulated with LPS, a significantly lower percentage of CD11b+ macrophages from female IL-13 KO BMDM cultures expressed I-A/E (MHC II) compared with IL-13 KO males or WT females (Fig. 4A). However, after pretreatment with IL-13, I-A/E expression was significantly increased on female BMDM compared with males or compared with LPS-stimulated BMDM without IL-13 pretreatment (Fig. 4A). In contrast, under similar conditions, I-A/E expression on male BMDM did not change significantly after pretreatment with IL-13.
To evaluate the cytokine milieu in IL-13KO mice, mononuclear IL-13KO mice were presented by APCs from female vs male IL-13KO mice at all ratios of APC:T cells. Proliferation of MOG-35–55-specific T cells cocultured at different ratios with APC from male vs female IL-13KO mice with EAE was assessed at the peak of disease (days 18–20) in the presence of MOG-35–55 peptide. Data are presented as net cpm relative to medium alone controls. Significant differences between male and female responses were determined using the Student t test (*, p < 0.05).

FIGURE 5. Differential ability of IL-13KO male and female APCs to present exogenous myelin Ag for the activation of MOG-35–55-specific T cells. Proliferation of MOG-35–55-specific T cells cocultured at different ratios with APC from male vs female IL-13KO mice with EAE was assessed at the peak of disease (days 18–20) in the presence of MOG-35–55 peptide. Data are presented as net cpm relative to medium alone controls. Significant differences between male and female responses were determined using the Student t test (*, p < 0.05).

To study whether the phenotypic differences in male and female APCs might also affect their Ag presentation, APCs from male and female IL-13KO mice at the peak of disease were cocultured with a MOG-35–55-specific T cell line and stimulated with MOG-35–55 peptide. As shown in Fig. 5, MOG-35–55-specific T cells proliferated significantly less when the Ag was presented by APCs from female vs male IL-13KO mice.

APCs from female IL-13KO mice present Ag with reduced efficiency compared with males

To evaluate the cytokine milieu in IL-13KO mice, mononuclear cells were isolated from spleens and cultured in the presence of 25 μg/ml MOG-35–55 peptide for 48 h, or were snap-frozen and mRNA evaluated for expression of cytokines and chemokines. At onset of EAE, the levels of IL-12 and the IL-12-induced cytokine, IFN-γ, were similar in both the sexes (data not shown). However, at the peak of disease (data not shown) and at day 30 after immunization (Fig. 6A), IL-12 and IFN-γ were significantly reduced in MOG-35–55-activated culture supernatants of female splenocytes as compared with males. None of the other cytokines measured, including IL-4 and IL-5, were significantly different between the two groups at any time point. IL-12 is a prototypic Th1 cytokine produced by macrophages and dendritic cells that strongly influences the development and differentiation of autoreactive Th1 cells (14). IL-12 is also important in the induction of the T cell-dependent and -independent activation of macrophages (15). Abs to IL-12 prevent the development of EAE in Lewis rats and spontaneous and superantigen-induced relapses (16, 17). Similarly, exogenous IL-12 has been shown to exacerbate EAE in both mouse and rat models (18, 19).

In addition to reduced levels of MOG peptide-induced IL-12 and IFN-γ, unmanipulated splenocytes from IL-13KO females had strongly decreased mRNA expression of the EAE-associated chemokines, MIP-2 (~9.8-fold) and IP-10 (~2.7-fold), compared with IL-13KO males (Fig. 6B). Conversely, splenocytes from the IL-13KO females had increased mRNA expression of the anti-inflammatory cytokines, IL-10 and TGF-β1 (+2.8- and +4-fold, respectively, Fig. 6B), and the regulatory T cell marker, FoxP3 (+2.8-fold, Fig. 6B). Taken together, these data indicate that the immune environment is substantially less inflammatory in IL-13KO female vs male spleens.

Anti-inflammatory environment in the spleens of female IL-13KO mice

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Discussion

Gender bias in autoimmune diseases is a well-known and as yet unexplained fact. Disproportionate numbers of women suffer from autoimmune diseases such as diabetes, arthritis, and multiple sclerosis. Several factors, including stronger humoral and cell-mediated immune responses in females, are thought to contribute to this discrepancy. However, the molecular mechanisms behind the inherent differences in male and female immune responses remain to be identified. IL-13 is an immunoregulatory cytokine produced by activated Th2 cells and is a key mediator in the pathogenesis of allergic hypersensitivity. Administration of IL-13 resulted in allergic inflammation, and tissue-specific overexpression of IL-13 in the lungs of transgenic mice caused airway inflammation and mucus hypersecretion (20). Its role in EAE has been of an anti-inflammatory cytokine. Young et al. (21) showed that IL-13 inhibited the adoptive transfer of proteolipid protein-induced EAE in female SJL mice, and
our previous study demonstrated increased levels of IL-13 in SJL mice after successful treatment of passive EAE with a rTCR ligand (22). Our unexpected observation that female IL-13KO mice have a lower incidence and reduced severity of EAE than males, prompted us to compare gender differences of immune cells from these mice. In preliminary studies, we found that myeloid-reactive T cells from both male and female IL-13KO mice were equally efficient at transferring passive EAE, and mice of either sex were equally susceptible to passive EAE. These observations led us to study the role of APCs in modulating EAE in these mice.

It has been shown previously that although T cells initiate EAE, the actual effector mechanisms leading to inflammation and demyelination in the CNS are largely provided by infiltrating macrophages and endogenous microglia (23). Brosnan et al. (24) demonstrated that depletion of macrophages delayed the onset and reduced the severity of EAE in Lewis rats, and in a separate study, Huitinga et al. (25) showed that elimination of macrophages completely prevented the clinical manifestations of EAE. This group later demonstrated that microglia were not appropriately activated in the absence of bloodborne macrophages, suggesting that interactions between the infiltrating macrophages and T cells were responsible for microglial activation (26). Cash et al. (8) showed that IL-13 can suppress cytotoxic and inflammatory functions of monocytes and macrophages, as well as clinical signs of EAE in male rats. In contrast, we here demonstrate that in the absence of IL-13, female mice were resistant to EAE and had significantly reduced CNS infiltration and inflammatory function of CD11b+ macrophages compared with males. Moreover, females had significantly reduced MHC II expression on the CD11b+ population in the CNS compared with males, a more limited capacity of these cells to present relevant Ags to encephalitogenic T cells, and an altered cytokine/chemokine profile during EAE induction. These results suggest that differences in IL-13-mediated effects on APC function could be a crucial factor in disease susceptibility. These opposing immune-mediated effects of IL-13 in EAE may be influenced by patterns of its receptor expression. It has been suggested that IL-13 can play a dual role in type II inflammatory diseases depending upon the activation of either the cell-bound IL-4Rα/IL-13Rα1 receptor or the decoy IL-13Rα2 type II receptor, which, respectively, promote or inhibit type II inflammation (27). A tight balance between IL-13 and IL-13Rα2 receptor expression is thought to be required for the establishment of an optimal healing response.

IL-13 has been shown to moderately enhance the expression of MHC II on macrophages from male rats (8) and in human blood (1, 28). These observations might predict that there would be less MHC expression by macrophages from IL-13KO mice. This was the case, in fact, only in female IL-13KO mice that had less severe EAE but not in male IL-13KO mice that developed EAE comparable to WT mice. Thus, CD11b+ BMDM from female IL-13KO mice did not up-regulate MHC II to the same extent as males when stimulated with LPS (Fig. 4). Moreover, spinal cord sections from IL-13KO female mice with EAE had less MHC II staining than male IL-13KO mice upon pretreatment with LPS compared with males. IL-13KO female mice with EAE had less MHC II staining than male IL-13KO mice. This was the more pronounced up-regulation of MHC II on CD11b+ BMDM from IL-13KO females upon pretreatment with IL-13 compared with males which did not exhibit this change. However, as mentioned above, T cells from male and female IL-13KO mice were equally capable of transferring EAE. Passive transfer involves the in vitro stimulation of T cells (from MOG-35–55/CFA-immunized mice) with MOG-35–55 in the presence of syngeneic irradiated APCs before their transfer to naive recipient mice. This result suggests that APC from both genders of IL-13KO mice were equally capable of initial T cell-priming events. Therefore, the difference seems to lie in downstream APC functions and in their subsequent trafficking to CNS. This notion is supported by the fact that IL-12 and IFN-γ levels were similar in male and female IL-13KO mice at disease onset but were decreased in female mice at the peak of disease and at day 30 postimmunization. These observations support the belief that IL-13 is more important in the effector phase of the immune response than in the initial differentiation of CD4+ T cells into Th2-type cells (20).

It is noteworthy that BMDM from WT female mice displayed significantly more MHC II upon LPS stimulation compared with WT males (Fig. 4). In the absence of other ameliorating factors, this might predict that WT females would mount a stronger immune response than males and perhaps develop more severe EAE. However, the in vivo severity of EAE was the same in WT females and males, possibly implicating other regulatory factors. The unexpected finding by Verdu et al. (12) that IL-13 mRNA expression was decreased in mice treated with 17β-estradiol finds relevance to this issue. This would lead us to speculate that IL-13 and estrogen have opposing effects on macrophage activation and MHC II expression. Importantly, native levels of estrogen that normally balance the effects of IL-13 in WT female mice might have more pronounced regulatory effects on macrophages in the absence of IL-13.

In fact, our own studies clearly demonstrate that exogenous E2 can protect against EAE and induce a nearly identical APC activation and cytokine profile as we observed in IL-13KO females, including down-regulation of IL-12, IFN-γ, MIP-2, and IP-10, and up-regulation of FoxP3 that is a marker of regulatory T cells (29). IL-12 drives the development of Th1 cell responses and has been shown to be of central importance in gender differences in EAE. Kim et al. (30) demonstrated that deficient IL-12 production by macrophages in male SJL mice resulted in reduced encephalitogenicity of myelin basic protein-specific T lymphocytes compared with females. In their study, LN cells from male SJL mice secreted reduced amounts of both IL-12 and IFN-γ upon Ag stimulation, similar to our results in splenocytes from IL-13KO females. IL-12 can further lead to increased IFN-γ production by NK cells and T cells which can in turn positively regulate IL-12 production by macrophages (31). This positive feedback amplification of IL-12 production by IFN-γ can be a driving force for uncontrolled inflammation and maintenance of a Th1 cell response. In contrast, IL-12 production and the response of T and NK cells to IL-12 are under negative regulation of IL-10 and TGF-β1 (32). Interestingly, mRNA of both of these cytokines was increased significantly in splenocytes from female IL-13KO mice compared with males. Therefore, reduced production of IL-12 and IFN-γ and increases in IL-10 and TGF-β1 in female IL-13KO mice may account for their reduced incidence and severity of EAE compared with males.

Finally, our study observed an increase in FoxP3 mRNA in IL-13KO splenocytes from EAE-resistant female mice. A recent study by Weber et al. (33) provides evidence for the existence of “type II monocytes” which are capable of modulating EAE by directing differentiation of Th2 cells and CD4+ CD25+FoxP3+ cells. The anti-inflammatory type II monocytes were characterized by increased secretion of IL-10 and TGF-β1 and decreased production of IL-12 and TNF-α. From our data, it would appear that in the absence of IL-13, the female CD11b+ cells transform to become type II monocytes leading to the increases in Th2 and regulatory T cell population concomitant with the recovery from EAE.

In summary, we have identified IL-13 as a regulatory cytokine, and our data for the first time provide evidence for the possible role...
of IL-13 in mediating gender susceptibility to an autoimmune disease, EAE, with possible implications for MS. Specifically, we have demonstrated that absence of IL-13 confers lower susceptibility to EAE in females vs males or WT females with normal levels of IL-13. However, we do acknowledge the fact that multiple immunoregulatory genes, including EAE susceptibility loci, are linked to IL-13 at ~53.4 Mb on central chromosome 11 (11, 34–37). Our microsatellite results showed that D19P20OahAs alleles are present at D11Mit271, D11Mit310, D11Mit153, D11Mit156, D11Mit278, D11Mit120, D11Mit285, D11Mit359, and D11Mit166 from ~45.6 to ~105.2 Mb (www.informatics.jax.org). Consequently, we cannot rule out formally that some of the differences we see between male and female IL-13KO mice do not derive from the effects of 129 alleles at these linked loci. Overall, this study warrants revisiting the role of IL-13 and its receptors in EAE. Additional experiments may reveal preferential gender usage of the IL-13Rα2 decoy receptor which might also influence serum IL-13 levels in both sexes. Interplay of estrogen and IL-13 on APCs might also be an interesting area of future research.

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Disclosures

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References