2005

Cutting Edge: CD4+CD25+ Regulatory T Cells Contribute to Gender Differences in Susceptibility to Experimental Autoimmune Encephalomyelitis

Jay Reddy
University of Nebraska-Lincoln, jayreddy@unl.edu

Hanspeter Waldner
Brigham and Women's Hospital, Boston, MA

Xingmin Zhang
Brigham and Women's Hospital, Boston, MA

Zsolt Illes
Brigham and Women's Hospital, Boston, MA

Kai W. Wucherpfennig
Harvard Medical School, Boston, MA, kai_wucherpfennig@dfci.harvard.edu

See next page for additional authors

Published in *The Journal of Immunology* 175:9 (2005), pp. 5591-5595; doi: 10.4049/jimmunol.175.9.5591

Submitted June 10, 2005; accepted September 1, 2005.

Cutting Edge: CD4$^+$CD25$^+$ Regulatory T Cells Contribute to Gender Differences in Susceptibility to Experimental Autoimmune Encephalomyelitis

Jay Reddy,[1] Hanspeter Waldner,[1] Xingmin Zhang,[1] Zsolt Illes,[1]
Kai W. Wucherpfennig,[2] Raymond A. Sobel,[3] and Vijay K. Kuchroo[1]

1. Center for Neurologic Diseases, Brigham and Women's Hospital, Boston, MA 02115
2. Department of Cancer Immunology and AIDS, Dana-Farber Cancer Institute, and Department of Neurology, Harvard Medical School, Boston, MA 02115
3. Department of Pathology, Stanford University School of Medicine, Stanford, CA 94305

Corresponding author — Dr. Vijay K. Kuchroo, Center for Neurologic Diseases, Brigham and Women's Hospital, Harvard Medical School, 77 Avenue Louis Pasteur, Boston, MA 02115, email vkuchroo@rics.bwh.harvard.edu

Abstract

Female B10.S mice are highly resistant to proteolipid protein (PLP) 139–151-induced experimental autoimmune encephalomyelitis (EAE) and depletion of PLP 139–151- reactive CD4$^+$CD25$^+$regulatory T (Treg) cells can slightly increase their EAE susceptibility. Although male B10.S mice are moderately susceptible to EAE, we report that depletion of Treg cells in male B10.S mice before immunization with PLP 139–151 renders them highly susceptible to severe EAE with more CNS neutrophil infiltrates than nondepleted controls. Increased susceptibility is associated with an enhanced PLP 139–151-specific T cell response and greater production of IFN-γ, IL-6, and IL-17. Male CD4$^+$CD25$^+$ effector cells depleted of Treg cells proliferate to a greater degree than those from females in response to either anti-CD3 or PLP 139–151. These data suggest that because of their capacity to regulate potent autoaggressive effector cells, Treg cells partly contribute to the resistance to autoimmunity in the male mice.

Abbreviations: EAE, experimental autoimmune encephalomyelitis; Treg, regulatory T; PLP, proteolipid protein; wt, wild type; tg, transgenic; TMEV, Theiler's murine encephalomyelitis virus; 7-AAD, 7-aminoactinomycin D; PMN, polymorphonuclear leukocyte

Autoimmune diseases are more prevalent in females than in males. This disparity also exists in autoimmune disease models (1) and appears to be partly hormone dependent. Sex steroids ameliorate experimental autoimmune encephalomyelitis (EAE)[3], an animal model of multiple sclerosis (2– 4), but the mechanism(s) by which they alter the disease are unclear. Because immune cells express estrogen and androgen receptors (5, 6), sex hormones may act directly on Ag-specific T cells to alter their functions. Some studies suggest that sex steroids induce immune deviation from a Th1 to a Th2 phenotype (2– 4, 7).

A subset of CD4$^+$ cells called CD4$^+$CD25$^+$ regulatory T (Treg) cells that expresses Forkhead box P3 (Fox P3) can control autoimmune responses (8 –10). Estrogen modulates the expression of Fox P3 mRNA and middle-aged male mice with EAE have diminished Treg cell activity (11, 12), but it is not known whether Treg cell functions are affected by gender. Immunization with myelin proteolipid protein (PLP) 139– 151 results in EAE in male and female SJL (I-A^s) mice with comparable severity, but only female mice show chronic relapsing- remitting disease (13, 14). In contrast, B10.S mice (H-2^5 congenic with SJL) are relatively resistant to PLP 139–151-induced EAE (15), but it is not known whether gender affects resistance. Using IAs tetramers for PLP 139–151, we found more myelin PLP-specific Treg cells in the naive periphery of female B10.S mice than in SJL mice (15). Depletion of Treg cells in vivo in female B10.S mice predisposed them moderately to EAE.

We report here that although male B10.S mice have fewer PLP 139–151 tetramer$^+$ CD4 cells than female mice, depletion of Treg cells in vivo makes them more susceptible to PLP 139–151-induced EAE with increased inflammatory cytokine production and more severe clinical and histological disease than nondepleted mice. Although male and female B10.S mice have comparable Treg cell functions, effector cells expand more in male than in female mice in response to TCR-mediated stimulation. These results indicate that Treg cells partly contribute to gender differences in EAE susceptibility.

Materials and Methods

Mice

B10.S (B10.S-H2^s/Sg) wild-type (wt) mice 4–8 wk old were obtained from McLaughlin Research Institute. B10.S/Sg-*Tg(TcrPLP)1858Hpw* mice (referred to as 5B6 transgenic (tg) B10.S mice) transgenic for the PLP139–151- specific TCR 5B6 were generated and maintained as described previously (16).

Peptide synthesis and immunization procedures

PLP 139–151 (HSLGKWLGHPDKF) and neuraminidase (NASE) 101–120 (EALVRQGLAKVAYVYKPNNT) were synthesized on Fmoc chemistry and used for immunizations as described previously (15).

Proliferation assays

To assess inhibitory effects of Treg cells, PLP 139–151-specific 5B6 tg T cells were fractionated into $CD4^+CD25^+$ and $CD4^+CD25^-$ cells by magnetic separation (Miltenyi Biotec). They were plated at a density of 1 x 10^6 cells/ml in the presence of APCs and PLP 139–151 (20 μg/ml) for 2 days in complete DMEM. After pulsing with 1 mCi of [^{3}H] thymidine (1 Ci = 37 GBq) for 16 h, proliferation was measured as counts per minute. $CD3^+$ T cells enriched by negative selection (R&D Systems) from immunized B10.S wt mice were stimulated with PLP 139–151 in the presence of APCs. Response of $CD4^+CD25^-$ T cells to anti-CD3 (1 μg/ml, clone, 145-2C11; BD Pharmingen) was tested with $CD11c^+$ cells as APCs.

Flow cytometry

To study cellular subsets in lymph nodes, various mAbs procured from BD Pharmingen. After staining, cells were analyzed by flow cytometry (FACSCalibur; BD Biosciences); data were analyzed using FlowJo software (Tree Star).

IAs tetramer staining

Frequencies of Ag-specific T cells were determined by flow cytometry using IA^s/PLP 139–151 and Theiler's murine encephalomyelitis virus (TMEV) 70–86 tetramers (negative control) as described previously (15). Four-color analysis included anti-CD25/FITC (clone, 7D4), IA^s tetramers (PE), 7-aminoactinomycinD(7-AAD), and anti-CD4-allophycocyanin (clone, RM4-5). Percentages of $tetramer^+$ cells in the live ($7\text{-}AAD^-$) $CD4^+$population were determined.

Cytokine analysis

$CD3^+$ T cells were enriched from anti-CD25- and control Ig-treated B10.S mice 10 days after immunization with PLP 139–151. They were stimulated with APCs loaded with PLP 139–151 (50 μg/ml) for 4–6 days. Supernatants on day 2 were tested for cytokines by ELISAs, and frequencies of cytokinesecreting cells were determined on day 4 by intracellular staining, as described previously (15).

Real-time (TaqMan) RT-PCR

Total RNA was extracted from $CD4^+CD25^-$ and $CD4^+CD2^+$ cells from a group of B10.S mice followed by treatment with DNase 1 (RNeasy kit; Qiagen). cDNA was synthesized using TaqMan RT reagents (Applied Biosystems). Fox P3 and GAPDH mRNA was measured by PCR in separate tubes in duplicates using probes labeled with FAM and VIC and with TAMRA as a quencher using TaqMan universal mix and the ABI Prism 7700 sequence detection system (Applied Biosystems). Primers and probe for GAPDH were purchased (Applied Biosystems). Fox P3 sequences were as described previously (17). A comparative threshold cycle (C_T) was used to determine mRNA expression of Fox P3 and GAPDH relative to no-template control (calibrator). C_T value was normalized for each sample using the formula: $\Delta C_T = C_{T\ (Fox\ P3)}$ - CT (GAPDH), and the relative expression of Fox P3 was then calculated using the formula 2^{-CT}.

Histopathology

Following induction of EAE, the mice were monitored for clinical signs of disease for 4 wk and scored as described previously (13). Mice were killed when their score reached ≥4 or when they began to recover. Brains and spinal cords were fixed in 10% phosphate-buffered formalin. Histologic disease was evaluated as previously described (13) and polymorphonuclear leukocyte (PMN) infiltration as described in Table I legend.

Statistics

Student's *t* test was used to determine significance of data except for rank correlations between inflammatory foci and PMN scores, which were determined by Wilcoxon signed rank test. A value of $p \leq 0.05$ was considered significant.

Table I. Clinical and histologic EAE in B10.S mice treated with anti-CD25 or control Ab[a]

Treatment	Clinical Disease[b]			Histopathology (mean ± SEM) (No. of Inflammatory Lesions)					
						PMN (neutrophil) score (%)			
	Incidence (%)	Mean day of onset[c]	Mean maximum score	Meninges	Parenchyma	Total	3+	2+	1+	0
Males										
Rat IgG control	3/12 (25)	14.3 ± 1.9	0.25 ± 0.1	10.3 ± 4.1	8.3 ± 5.6	18.6 ± 9.4	0	0	2/12 (16.6)	10/12 (83.3)
Anti-CD25	12/14 (85.7)	17.3 ± 1.3	1.8 ± 0.4	64.3 ± 11.8	60.6 ± 12.6	124.9 ± 23.2	2/14 (14.3)	6/14 (42.8)	2/14 (14.3)	4/14 (28.6)
p values			0.001	0.0002	0.0008	0.0003	0.0004			
Females										
Rat IgG control	0/8 (0)	0	0	2.3 ± 1.2	1.6 ± 1.1	3.9 ± 2.3	0	0	0	8/8 (100)
Anti-CD25	3/9 (33.3)	15.0 ± 4.6	1.2 ± 0.6	21.0 ± 12.4	23.8 ± 15.4	44.8 ± 27.7	1/9 (11.1)	0	1/9 (11.1)	7/9 (77.8)

a. Numbers are mean ± SEM.

b. B10.S male or female mice were treated with anti-CD25 or rat IgG Ab on days 5 and 3 prior to immunization with PLP 139–151 in CFA, and the disease was scored as described in *Materials and Methods* and compared between groups.

c. Represents only mice that showed clinical disease. PMN score: 3+, solid, abscess-like foci with mostly neutrophils; 2+, multiple foci with mixtures of neutrophils and mononuclear cells; 1+, few neutrophils; 0, no neutrophils but when inflammatory foci were present, the infiltrates were predominantly mononuclear cells.

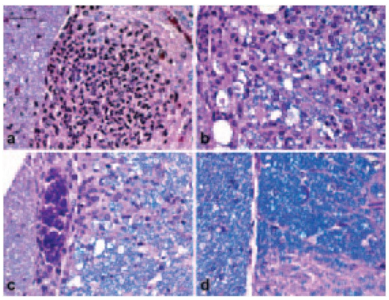

Figure 1. Effect of anti-CD25 treatment on CNS inflammatory infiltrates in PLP 139–151-immunized male and female B10.S mice. *a*, An inflammatory focus in an anti-CD25-treated male mouse with EAE contains a predominance of neutrophils intermixed with mononuclear cells (PMN score = 3+; see legend to Table I). *b*, Typical mononuclear infiltrates in an anti-CD25- treated female mouse (PMN score = 0). *c*, A small subpial mononuclear cell infiltrate in a control Ig-treated male mouse. *d*, No inflammatory cell infiltrates in a control Ig-treated female mouse. Each panel is from the spinal cord with a spinal nerve root on the left side of the field. Luxol fast blue-H&E stain. Original magnification, ×240. Bar in *a* = 25 μm.

Table II. Lymphocyte subsets in male and female B10.S mice[a]

Marker	Male	Female
CD3[b]	51.06 ± 0.99	44.68 ± 1.84
Gate: $CD3^+$ cells		
CD4	49.36 ± 0.77	50.52 ± 1.33
CD8	42.82 ± 0.43	40.52 ± 1.43
CD25[c]	7.70 ± 0.26	9.71 ± 0.29
Gate: $CD3^-$ cells		
CD11b	3.77 ± 0.55	3.23 ± 0.26
CD11c	0.97 ± 0.08	0.97 ± 0.05
B220	77.98 ± 1.50	80.6 ± 0.61

a. Lymph node cells from male and female B10.S mice were stained with the indicated markers. The percentages of individual subsets were then determined by flow cytometry. $n = 5$/group

b. $p \leq 0.007$

c. $p \leq 0.00047$

Results and Discussion

We report here that Treg cells partly contribute to gender differences in susceptibility to EAE. We previously showed that PLP 139–151-reactive Treg cells exist in the naive periphery of EAE-resistant B10.S female mice and that depletion of these in vivo before immunization with PLP 139–151 resulted in expansion of IFN-γ-producing cells and induction of EAE in ~30% of mice (15). Because autoimmune diseases are more common in females than males, we hypothesized that there may be gender differences in the function of the naturally occurring $CD4^+CD25^+$ Treg cell subset.

When we depleted Treg cells in vivo by administration of anti- CD25 Ab in male B10.S mice before immunization, 85% of the mice (12 of 14) developed moderate to severe EAE, whereas only 3 of 12 control mice (25%) developed mild EAE, i.e., loss of tail tonicity ($p \leq 0.001$) (Table I). In agreement with our previous results (15), a small number of female B10.S mice (three of nine, 33%) depleted of Treg cells but no control Abtreated mice developed disease, suggesting that genetic resistance to EAE in female B10.S mice does not lie entirely in the Treg population. In female B10.S mice, multiple factors may contribute to EAE resistance, i.e., Ag-specific defects in Th1 cytokine secretion and IL12-Rb2 expression (18, 19), a tendency to produce elevated amounts of Th2 cytokines (20), APCs (16) and the presence of a high proportion of

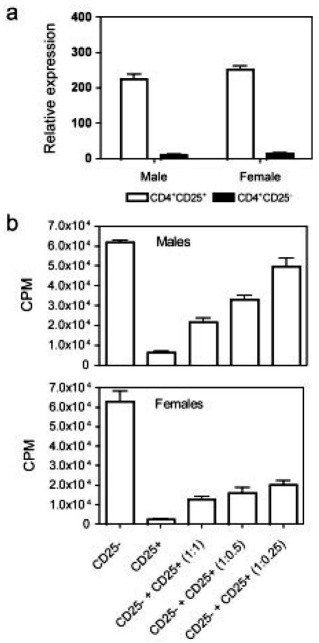

Figure 2. PLP 139–151-specific Treg cells are functional in both male and female B10.S mice. *a*, Fox P3 and GAPDH mRNA expression in Treg and $CD4^+CD25^-$ cells. Each bar represents relative Fox P3 mRNA mean ± SEM values. $n = 5$ (four to five mice per sample). *b*, Treg and $CD4^+CD25^-$ cells isolated from PLP 139–151-specific tg B10.S mice of either sex were stimulated with PLP 139–151 together at indicated ratios or separately and proliferation was measured as described in *Materials and Methods*. A representative experiment is shown.

Treg cells that react with PLP 139–151 tetramers in their naive periphery (15). Once males develop EAE following anti-CD25 treatment, there were no other clinical differences between males and females. Histologic disease in anti-CD25 Ab-treated male mice was significantly greater than in the controls ($p \leq 0.0003$) (Table I), and there were more PMNs in CNS infiltrates ($p \leq 0.0004$) (Table I, Figure 1). The histologic disease in males was also more severe than in anti-CD25-treated females (mean total inflammatory foci = 124.9 vs 44.8) ($p \leq 0.05$). The disease severity in females was enhanced when compared with control mice but inflammatory foci contained fewer neutrophils (Table I, Figure 1*b*).

To determine the mechanisms for greater incidence and severity of EAE in Treg-depleted male B10.S mice, we tested whether there are any differences in Treg cell function between male and female B10.S mice in vitro.

We first determined that male B10.S mice have a higher percentage of $CD3^+$ cells in their lymph nodes than age-matched female B10.S mice, but the percentage of Treg cells is lower in males (Table II). We then confirmed that Treg cells in male and female B10.S mice are equally effective in inhibiting expansion of effector cells using anti-CD3 as a stimulus (data not shown). FoxP3 mRNA, a marker for Treg cells, was also expressed in comparable amounts (Figure 2*a*). Therefore, we could identify no major differences in Treg populations between male and female B10S mice. We next tested whether Treg cells from male and female B10.S mice have any defect in their suppressive function in response to self-Ags using TCR tg mice specific to PLP 139– 151 (16). We found that Treg cells from male and female tg B10.S mice did not proliferate to PLP 139–151, whereas male and female effector cells responded equally well to PLP 139– 151. Treg cells inhibited the proliferative response of effector cells but there were differences; the inhibition of $CD4^+CD25^-$ cells mediated by Treg cells in female B10.S mice was maintained even at low ratios of regulatory: effector cells. However, in male mice as ratios of Treg cells to effectors were lowered, the effector cells expanded to a greater extent (Figure 2*b*). This suggests either that Treg cells from female mice are more efficient in their suppressive function than those in males or that the effector cells in males are capable of responding to autoantigens more vigorously than those in females. We quantified

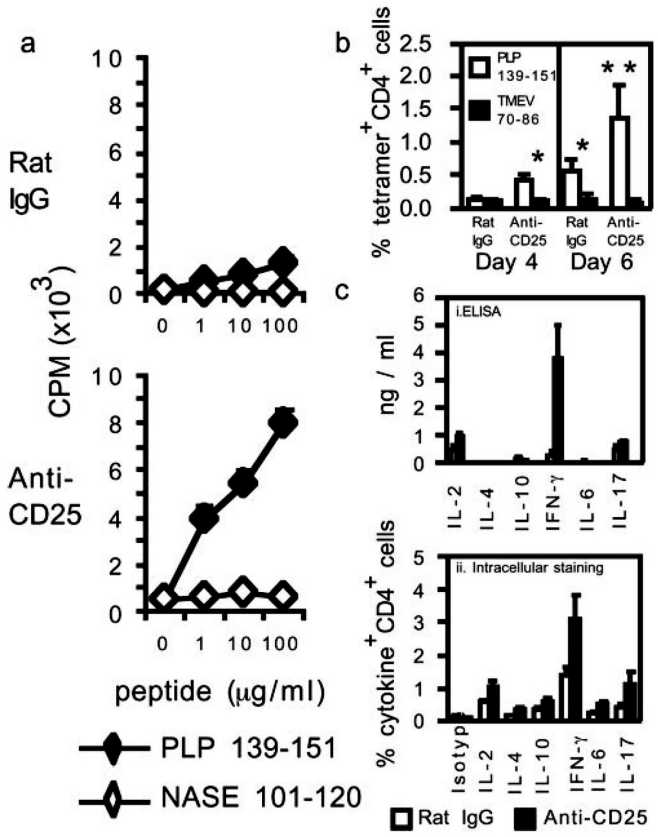

Figure 3. Depletion of Treg cells before immunization with PLP 139–151 enhances Ag-specific T cell proliferation and inflammatory cytokines in male B10.S mice. *a*, Male B10.S mice pretreated with anti-CD25 or isotype control on day -5 and day -3 were immunized with PLP 139–151. Ten days later, $CD3^+$ cells were stimulated with PLP 139–151 and proliferation was measured as described in *Materials and Methods*. A representative experiment is shown. *b*, Viable lymphoblasts harvested from the cultures stimulated with PLP 139–151 were stained with PLP 139–151 or TMEV 70–86 tetramers. Mean ± SEM values are shown (n = day 4, 6; day 6, 10). (*,$p \leq 0.02$; **, $p \leq 0.01$). *c*, Cytokine ELISA: supernatants from cultures stimulated with PLP 139–151 were examined for cytokine secretion by ELISA and compared between groups (n = 8–12 mice/group) (IL-2, $p \leq 0.01$; IL-10, $p \leq 0.02$; IFN-γ, $p \leq 0.006$, and IL-17, $p \leq 0.08$). Intracellular staining: viable lymphoblasts were harvested on day 4 from the cultures described above and restimulated with anti-TCR β and anti-CD28 Ab for 4–6 h and stained with anti-CD4 and 7-AAD. The frequency of cytokine-secreting cells was then determined by flow cytometry in the live (7-AAD^-) $CD4^+$ subset. Each bar represents mean ± SEM values (n = rat IgG, 9; n = anti-CD25, 10). (IL-2 and IL-10, $p \leq 0.01$; IL-4, $p \leq 0.0005$; IFN-≤, $p \leq 0.02$; IL-6, $p \leq 0.001$; and IL-17, $p \leq 0.056$).

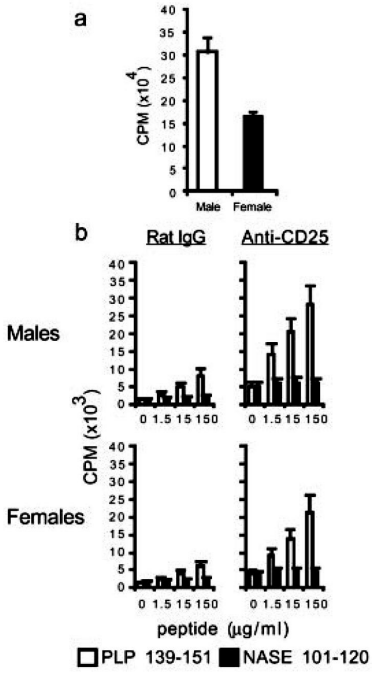

Figure 4. Ag-specific, $CD4^+CD25^-$ effector cells from male B10.S mice expand more vigorously than those derived from female mice. *a*, $CD4^+CD25^-$ cells isolated by magnetic separation from male and female B10.S mice were stimulated with anti-CD3 Ab (1 μg/ml) and $CD11^+c$ cells as APCs, and proliferation was measured as described in *Materials and Methods*. A representative experiment of three is shown. *b*, $CD3^+$ cells were enriched from male and female B10.S mice pretreated with anti-CD25 or IgG Ab and immunized with PLP 139–151. The cells were stimulated with PLP 139–151 and proliferation was measured as above. Mean ± SEM values are shown. (n = males, 8; females, 7). (1.5 μg/ml, $p \leq 0.08$; 15 μg/ml, $p \leq 0.09$).

mice to be 0.04% of CD4 cells (8 in 20,000). By contrast, the precursor frequency in female B10.S mice is 40 in 20,000 CD4 cells (15). We did not observe any significant shift in PLP 139– 151 tetramer reactivity in effector vs Treg cells, as previously reported in female B10.S mice (15) (data not shown). Therefore, the higher incidence and greater severity of EAE in male B10.S mice are unlikely to be due to an increased frequency or function of PLP 139–151-specific Treg cells.

We next compared T cell proliferation to PLP 139–151 between anti-CD25 and rat IgG-treated male mice. The PLP 139–151-specific responses, but not NASE responses, were significantly higher in the anti-CD25 Ab-treated than in the control group (Figure 3*a*). Consistent with this, percentages of PLP 139–151 tetramer$^+$CD4 cells were increased by ~3-fold more in the anti-CD25 Ab-treated males compared with controls (Figure 3*b*). This response was specific because there was negligible reactivity to TMEV 70–86 tetramers. These data suggest that the expansion of PLP 139–151-reactive cells in mice depleted of Treg cells contributed to the more severe disease. This expansion appeared to have resulted from the loss of regulation mediated by Treg cells, which can prevent the expansion of effector cells in an Ag nonspecific manner (21).

Previously, male SJL mice immunized with myelin basic protein or PLP were found to produce IL-4 and IL-10, while females produced more IFN-γ (2, 22). We tested whether a similar bias exists in immunized male B10.S mice, with or without Treg cell depletion. By ELISA, the production of both IL-2 ($p \leq 0.01$) and IFN-γ ($p \leq 0.006$) was significantly elevated, with a marginal increase in IL-17 production ($p \leq 0.08$) on day 2 in the cultures derived from $CD25^+$ cell-depleted male mice compared with controls (Figure 3*c*). As previously noted in female B10.S mice (15), IL-10 secretion was reduced in the anti-CD25 Ab-treated group ($p \leq 0.02$), suggesting that Treg cells may directly or indirectly regulate IL-10 production. However, by intracellular staining, the frequencies of cells secreting all of the cytokines tested (IL-2, IL-4, IL-6, IL-10, IL-17, and IFN-γ) were elevated in anti-CD25-treated male mice (Figure 3*c*). Among proinflammatory cytokines, IFN-γ-secreting cells were present at a higher frequency (3.09 vs 1.35) followed by IL-17 (1.09 vs 0.41) and IL-6 (0.5 vs 0.21) in the anti-CD25-treated vs control mice. The number of TNF-α-secreting cells did not vary between groups (data not shown). Recently, it was reported that IL-23-dependent production of inflammatory cytokines (IL-17, IL-17F, IL-6, and TNF-α) is essential for the development of CNS inflammation (23). In anti-CD25 Ab-treated mice, the numbers of cells that secrete IFN-γ, IL-6, and IL-17 was increased, implying that Treg cells regulate the expression of these cytokines. In the absence of Treg cells, the effector cells producing inflammatory cytokines may expand resulting in altered chemokines and increased proportions of PMNs in inflammatory foci (24).

We then sought to detect any fundamental difference in the expansion of effector cells between male and female B10.S wt mice. Using anti-CD3 as a stimulus, we observed that the effector cells from male B10.S mice expanded to a greater extent than those from female mice (Figure 4*a*). To verify that a similar differential response occurs to PLP 139–151, we immunized groups of male and female B10.S mice with PLP 139–151 after treating them with anti-CD25 or control Ab. We measured the PLP 139–151-specific response by [^{3}H]thymidine incorporation. As expected, mice depleted of CD25$^+$ cells responded to PLP 139–151 significantly more strongly than controls of either sex (Figure 4*b*). However, the T cell response to PLP 139– 151 in the male B10.S mice tended to be higher than in the female mice treated with anti-CD25 Ab, especially at lower Ag concentrations (Figure 4*b*). Similar trends were confirmed by using 5B6 tg T cells specific to PLP 139–151 (data not shown). These data suggest that the autoreactive effector cells in male B10.S mice can respond to autoantigens vigorously, but they are kept in check by Treg cells.

In summary, in male B10.S mice treated with anti-CD25 Ab, the incidence of PLP 139–151-induced EAE was enhanced significantly when compared with female B10.S mice, suggesting that Treg cells do not play as large of a role in mediating genetic resistance to EAE in female compared with male mice. Although there was no fundamental difference in Treg cell function between male and female mice, the effector cells from male B10.S mice in the absence of Treg cells tended to proliferate to a higher degree than those from females. This suggests that male Treg cells control the expansion of pathogenic autoreactive cells more effectively and that this contributes to their greater disease resistance.

Acknowledgments – This work is supported by National Institutes of Health Grants R01 NS30843, P01 NS38037, and R01 AI44880, NS 046414 (to R.A.S.), P01 A145757 and R01 NS44914 (to K.W.W.), and National Multiple Sclerosis Society Grant 2571-D9 (to V.K.K.). V.K.K. is the recipient of Javitz Neuroscience Investigator Award.

References

1. Yu, C. Y., and C. C. Whitacre. 2004. Sex, MHCand complement C4 in autoimmune diseases. *Trends Immunol.* 25: 694–699.
2. Bebo, B. F., Jr., A. Fyfe-Johnson, K. Adlard, A. G. Beam, A. A. Vandenbark, and H. Offner. 2001. Low-dose estrogen therapy ameliorates experimental autoimmune encephalomyelitis in two different inbred mouse strains. *J. Immunol.* 166: 2080–2089.
3. Voskuhl, R. R., and K. Palaszynski. 2001. Sex hormones in experimental autoimmune encephalomyelitis: implications for multiple sclerosis. *Neuroscientist* 7: 258–270.
4. Bebo, B. F., Jr., J. C. Schuster, A. A. Vandenbark, and H. Offner. 1999. Androgens alter the cytokine profile and reduce encephalitogenicity of myelin-reactive T cells. *J. Immunol.* 162: 35–40.
5. Benten, W. P., A. Becker, H. P. Schmitt-Wrede, and F. Wunderlich. 2002. Developmental regulation of intracellular and surface androgen receptors in T cells. *Steroids* 67: 925–931.
6. Sakazaki, H., H. Ueno, and K. Nakamuro. 2002. Estrogen receptor α in mouse splenic lymphocytes: possible involvement in immunity. *Toxicol. Lett.* 133: 221–229.
7. Liva, S. M., and R. R. Voskuhl. 2001. Testosterone acts directly on CD4$^+$ T lymphocytes to increase IL-10 production. *J. Immunol.* 167: 2060–2067.
8. Shevach, E. M. 2001. Certified professionals: CD4$^+$CD25$^+$ suppressor T cells. *J. Exp. Med.* 193: F41–F46.
9. Hori, S., T. Nomura, and S. Sakaguchi. 2003. Control of regulatory T cell development by the transcription factor Foxp3. *Science* 299: 1057–1061.
10. Sakaguchi, S., N. Sakaguchi, M. Asano, M. Itoh, and M. Toda. 1995. Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor α-chains (CD25): breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. *J. Immunol.* 155: 1151–1164.
11. Polanczyk, M. J., B. D. Carson, S. Subramanian, M. Afentoulis, A. A. Vandenbark, S. F. Ziegler, and H. Offner. 2004. Cutting edge: estrogen drives expansion of the CD4$^+$CD25$^+$ regulatory T cell compartment. *J. Immunol.* 173: 2227–2230.
12. Matejuk, A., C. Hopke, A. A. Vandenbark, P. D. Hurn, and H. Offner. 2005. Middle- age male mice have increased severity of experimental autoimmune encephalomyelitis and are unresponsive to testosterone therapy. *J. Immunol.* 174: 2387–2395.
13. Tuohy, V. K., Z. Lu, R. A. Sobel, R. A. Laursen, and M. B. Lees. 1989. Identification of an encephalitogenic determinant of myelin proteolipid protein for SJL mice. *J. Immunol.* 142: 1523–1527.
14. Bebo, B. F., Jr., A. A. Vandenbark, and H. Offner. 1996. Male SJL mice do not relapse after induction of EAE with PLP 139–151. *J. Neurosci. Res.* 45: 680–689.
15. Reddy, J., Z. Illes, X. Zhang, J. Encinas, J. Pyrdol, L. Nicholson, R. A. Sobel, K. W. Wucherpfennig, and V. K. Kuchroo. 2004. Myelin proteolipid protein-specific CD4$^+$CD25+ regulatory cells mediate genetic resistance to experimental autoimmune encephalomyelitis. *Proc. Natl. Acad. Sci. USA* 101: 15434–15439.
16. Waldner, H., M. Collins, and V. K. Kuchroo. 2004. Activation of antigen-presenting cells by microbial products breaks self tolerance and induces autoimmune disease. *J. Clin. Invest.* 113: 990–997.
17. Aluvihare, V. R., M. Kallikourdis, and A. G. Betz. 2004. Regulatory T cells mediate maternal tolerance to the fetus. *Nat. Immunol.* 5: 266–271.
18. Segal, B. M., and E. M. Shevach. 1996. IL-12 unmasks latent autoimmune disease in resistant mice. *J. Exp. Med.* 184: 771–775.
19. Chang, J. T., E. M. Shevach, and B. M. Segal. 1999. Regulation of interleukin (IL)-12 receptor β2 subunit expression by endogenous IL-12: a critical step in the differentiation of pathogenic autoreactive T cells. *J. Exp. Med.* 189: 969–978.
20. Maron, R., W. W. Hancock, A. Slavin, M. Hattori, V. Kuchroo, and H. L. Weiner. 1999. Genetic susceptibility or resistance to autoimmune encephalomyelitis in MHC congenic mice is associated with differential production of pro- and anti-inflammatory cytokines. *Int. Immunol.* 11: 1573–1580.
21. Thornton, A. M., and E. M. Shevach. 2000. Suppressor effector function of CD4$^+$CD25$^+$ immunoregulatory T cells is antigen nonspecific. *J. Immunol.* 164: 183–190.
22. Cua, D. J., D. R. Hinton, and S. A. Stohlman. 1995. Self-antigen-induced Th2 responses in experimental allergic encephalomyelitis (EAE)-resistant mice: Th2-mediated suppression of autoimmune disease. *J. Immunol.* 155: 4052–4059.
23. Langrish, C. L., Y. Chen, W. M. Blumenschein, J. Mattson, B. Basham, J. D. Sedgwick, T. McClanahan, R. A. Kastelein, and D. J. Cua. 2005. IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. *J. Exp. Med.* 201: 233–240.
24. Zehntner, S. P., C. Brickman, L. Bourbonniere, L. Remington, M. Caruso, and T. Owens. 2005. Neutrophils that infiltrate the central nervous system regulate T cell responses. *J. Immunol.* 174: 5124–5131.