Suppression of experimental autoimmune encephalomyelitis using estrogen receptor-selective ligands

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Abstract

Estrogens have been shown to modulate disease activity in experimental autoimmune encephalomyelitis (EAE), the mouse model for multiple sclerosis. Consistent with these findings, the severity of disease is reduced in pregnant women with multiple sclerosis when levels of estrogens are high. Estrogens bind to two known estrogen receptors (ER), ERα and ERβ. The relative contribution of these receptors to estrogen-mediated suppression of EAE was explored using ERα-selective ligands. The ER antagonist ICI 182,780 reversed the suppressive effects of 17β-estradiol (E2), demonstrating that the protective effects of E2 on disease are dependent upon ER signaling. Treatment of SJL mice with the ERα-selective agonist proteolipid protein (PPT) prior to the induction of disease resulted in suppression of clinical symptoms of disease, whereas treatment with an ERβ-selective agonist (WAY-202041) had no effect. Treatment of mice with PLP peptide 139–151 (PPT) was also associated with decreased immune responses associated with disease. Consistent with its lack of effect on disease, the ERβ agonist had minimal effects on immune responses. The use of selective estrogen receptor modulators (SERMs) in this model was also explored, and we show that raloxifene and WAY-138923 were also effective in suppressing disease. These results demonstrate the beneficial effects of estrogen receptor ligands, in particular ERα-selective ligands, and may have implications in the development of therapeutic strategies for multiple sclerosis.

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Introduction

Multiple sclerosis (MS) is an autoimmune disease of the CNS in which the immune system mounts an inappropriate response to components of myelin, such as myelin basic protein or proteolipid protein. It is characterized by inflammation of the CNS and myelin damage. Autoreactive CD4+ T-helper-1 (Th1) cells and their products (for example, tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ), and metalloproteinases) mediate much of the immunopathology, resulting in the destruction of the myelin sheath and subsequent neurological dysfunction (reviewed in Steinman (2001)).

As with a number of autoimmune diseases, the incidence of multiple sclerosis is higher (2 to 3 times) in females compared with males (Whitacre 2001). Although these gender differences are not fully understood, it is thought that sex hormonal influences on autoimmune disease may be involved. The disease modulating effects of estrogens in MS have been described. For example, clinical disease is ameliorated during pregnancy, when estrogen levels are high, and worsens during the post-partum period, when sex hormone levels are reduced (Korn–Lubetzki et al. 1984, Birk et al. 1990, Abramsky 1994, Confavreux et al. 1998). Pregnancy is associated with an increase in many hormones in addition to estrogen, such as cortisol and progesterone. These hormones appear to have immunosuppressive properties during pregnancy (Stites et al. 1983) and alter cytokine production by T cells isolated from MS and normal patients (Correale et al. 1998), suggesting that these hormones individually or in concert may act to modulate immune responses and autoimmune disease during pregnancy. The protective effects of pregnancy on MS have been reproduced in humans as well as in animal models of MS by the administration of exogenous estrogens. The pregnancy hormone estriol (E3) has been shown to decrease the number and volume of lesions in the brains of relapsing–remitting MS patients, as well as decrease IFN-γ levels in peripheral blood mononuclear cells (Sicotte et al. 2002). It has also been demonstrated that estrogens can suppress disease in murine experimental autoimmune encephalomyelitis (EAE), a well-defined model for multiple sclerosis (Jansson et al. 1994, Kim S et al. 1999, Offner et al. 2000,
Estrogens appear to directly affect the function of T cells. Modulation of cytokine production by T cell clones from MS patients has been reported, where it was demonstrated that estrogen has biphasic effects on cytokine secretion (Gilmore et al. 1997). These results suggest that estrogens could potentially alter disease through direct binding to estrogen receptors (ERs) on pathogenic T cells.

Estrogen action is largely mediated through nuclear hormone receptors. To date two receptor isoforms have been identified, ERα and ERβ, and selective compounds for each have been developed (Green et al. 1986, Kuiper et al. 1996, Stauffer et al. 2000, Meyers et al. 2001, Harris et al. 2003, Muthyala et al. 2003). For example, propyl-pyrazole triol (PPT) is an ERα-selective ligand that has been shown to have over 400-fold selectivity for ERα over ERβ (Stauffer et al. 2000). This compound is a potent ERα-selective agonist during in vitro gene transcription assays, while demonstrating no activity on ERβ (Stauffer et al. 2000, Harris et al. 2002). Moreover, PPT is as efficacious as 17α-ethinyl-17β-estradiol in mediating several well-known estrogenic responses when evaluated in vivo, including stimulation of uterine weight increase as well as preventing both hot flushes and ovariectomy-induced bone loss (Harris et al. 2002). In contrast, WAY-202041 is an ERβ-selective agonist with 200-fold selectivity for ERβ (Harris et al. 2003). As expected, this compound does not possess uterotrophic activity since ERβ is not the dominant ER expressed in uterus (Harris et al. 2003). It is also inactive in other estrogen target tissues such as mammary glands. It was, however, shown to have salutary effects in adjuvant-induced arthritis and in the HLA-B27 transgenic rat model of inflammatory bowel disease (Harris et al. 2003).

In addition to ER–selective ligands, compounds that have tissue-specific effects functioning as estrogen agonists in some tissues while behaving as estrogen antagonists in others have been developed (SERMs), which act via both ERα and ERβ (reviewed in Riggs et al. (2003)). For instance, raloxifene, a SERM that is approved for the treatment of osteoporosis, behaves as an estrogen in bone, whereas it acts as an estrogen antagonist in breast tissue and in the uterus (Shang et al. 2002). In addition, WAY-138923 is a SERM with efficacy in preclinical breast cancer models (Greenberger et al. 2001). Thus, SERMs may retain the beneficial effects of estrogens while reducing or eliminating unwanted side effects. To further our understanding of the effects and mechanisms involved in estrogen action on the immune response and autoimmune disease, we used the adoptive transfer model of EAE and examined the effects of raloxifene and WAY–138923, and ER–selective ligands on the development of disease.

Materials and Methods

Induction of EAE by the adoptive transfer of proteolipid protein-primed effector cells

Induction of EAE was performed using a modification of methods described previously (Leonard et al. 1995). Intact and ovariectomized female SJL mice (6–8 weeks old) were purchased from Jackson Laboratories (Bar Harbor, ME, USA) and were used as donor and recipient mice, respectively, in the adoptive transfer model of EAE. Mice were maintained in our animal facilities under pathogen-free conditions according to Wyeth Animal Care and Use Committee guidelines. Donor mice were immunized with proteolipid protein peptide139–151 (PLP) emulsified in complete Freund’s adjuvant (CFA). Each animal received 150 µg PLP in a volume of 0.2 ml CFA that contained 4 mg/ml heat killed and dried Mycobacterium tuberculosis (H37/RA strain; BD Diagnostics Systems, Sparks, MD, USA). The PLP/CFA emulsion was injected s.c. into two sites (on the back, and at the base of the tail; 0.1 ml/ injection site). Ten days later, the mice were euthanized and the spleens were collected. Single cell suspensions were made from the spleens. After lysis of red blood cells, the cells were cultured at a concentration of 5 × 10⁶ cells/ml for 3 days in 75 cm² tissue culture flasks in RPMI-10 (RPMI medium containing 10% heat-inactivated fetal bovine serum, 100 U/ml penicillin, 100 µg/ml streptomycin, 2 mM glutamine, 50 µM 2-mercaptoethanol; Invitrogen, Carlsbad, CA, USA). PLP was added at a final concentration of 5 µg/ml. The cells were incubated at 37 °C in 5% CO₂. After the incubation, PLP-stimulated effector cells were harvested, washed with phosphate-buffered saline and injected i.p. (1.5 × 10⁷ cells/mouse) into 6–8 weeks old ovariectomized female SJL mice. Mice were scored daily as follows: 0, no overt signs of disease; 1, limp tail; 2, limp tail/hind limb weakness; 3, partial hind limb paralysis; 4, complete hind limb paralysis; 5, complete hind limb paralysis with mild fore limb weakness; 6, complete hind limb paralysis with severe fore limb weakness or paralysis or moribund (euthanize).

For compound administration, ovariectomized recipient mice were treated with daily s.c. injections of compound beginning up to 1 week prior to the induction of EAE. Compounds were delivered in vehicle consisting of 10% ethanol/90% corn oil. Control mice received vehicle only. Treatment continued until termination of the experiment. The doses of compound used (10 mg/kg/day) were chosen based on previous studies with the ER–selective ligands where doses of 15 mg/kg/day PPT were required.
to be efficacious on brain endpoints (hot flushes) as well as preserving bone density (Harris et al. 2002). Doses as low as 1 mg/kg/day WAY-202041 suppressed disease in the adjuvant-induced arthritis model and HLA-B27 transgenic rat model of inflammatory bowel disease (Harris et al. 2003). 17β-estradiol (E2) was used as a positive control at 10 µg/kg/day. A similar dose (6 µg/kg/day) of E2 was proven to have long-term effects on body and uterine weights and bone mineral density when dosed for 6 weeks (Harris et al. 2002). A 1000-fold excess (10 mg/kg/day) of the ER antagonist ICI 182 780 compared with E2 was used based on previous published studies (Jansson & Holmdahl 2001). These authors found that a 500-fold excess of ICI only partially blocked the effect induced by E2 and therefore used a 1500-fold higher dose.

Analysis of PLP-specific recall responses: cytokine production

To examine the effect of compounds administered in vivo on cytokine production by splenocytes from mice with EAE, mice were euthanized with CO2 at peak disease (14 days post-transfer of PLP-specific cells) and spleens were collected. Spleens were individually processed into single cell suspensions. After lysis of red blood cells, the cells were resuspended in RPMI-10 and were cultured in 24-well tissue culture plates at a concentration of 5 × 10^6 cells/ml. Cells were stimulated with 5 µg/ml PLP. Culture supernatants were collected after 3 days and frozen until use at –20 °C. Cytokines (TNF-α, IFN-γ, interleukin (IL)-5, IL-4 and IL-2) were measured in the supernatants using a commercially available flow cytometry kit (Cytometric Bead Array, Becton Dickinson BioSciences, San Diego, CA, USA). Samples were acquired with a FACSCalibur flow cytometer and analyzed using CELLQUEST software (Becton Dickinson Immunocytometry Systems, San Jose, CA, USA). IL-10 was measured using an IL-10-specific ELISA kit (Becton Dickinson BioSciences).

Effect of compounds on proliferation of effector T cells upon antigen stimulation

Proliferation of PLP-specific CD4⁺ T cells in response to PLP stimulation was examined by flow cytometry using the following assay: SJL mice were immunized with PLP emulsified in CFA. After 10 days, spleens were collected and single cell suspensions were made. After lysis of red blood cells, splenocytes were labeled with carboxy fluorescein succinimidy ester (CFSE; Molecular Probes, Eugene, OR, USA) as described previously (Wells et al. 1997). CFSE-labeled cells were then incubated with PLP for 3 days at 37 °C, 5% CO₂ in RPMI-10. Compounds were added at a final concentration of 1 µM. To determine the percentage of CD4⁺ cells that divided, the CFSE-labeled cells were stained with phycoerythrin (PE)-labeled antibodies specific for the CD4 marker prior to flow cytometric analysis. Propidium iodide (1 µg/ml; Sigma Chemical, St Louis, MO, USA) was added to the samples just prior to acquisition in order to exclude dead cells from analysis. Samples were acquired with a FACSCalibur flow cytometer and analyzed using CELLQUEST software.

Statistics

Results were analyzed by one-way ANOVA (Microsoft SAS/Excel). Results were expressed as mean ± S.E.M. The difference was considered statistically significant when P<0.05.

Results

Effect of an ER antagonist on EAE induced by the adoptive transfer of PLP-primed effector cells

To examine the effects of estrogen and ER ligands on EAE, the adoptive transfer model was employed in which ovariectomized recipient mice were treated with compounds beginning up to 1 week prior to the adoptive transfer of PLP-primed splenocytes. Treatment continued daily throughout the course of the disease. As shown previously, treatment with 17β-estradiol (E2) resulted in a delay in onset as well as decreased incidence and severity of disease (Fig. 1, Table 1) (Jansson et al. 1994, Offner et al. 2000, Bebo et al. 2001, Ito et al. 2001). To determine whether the protective effects of estrogen in this model were estrogen receptor-mediated, mice were treated with both E2 and the estrogen receptor antagonist ICI 182 780. ICI abolished the effect of E2 on disease (Fig. 1, Table 1).
Table 1 Effect of the ER antagonist ICI 182,780 on estrogen-mediated suppression of disease

<table>
<thead>
<tr>
<th>Incidence</th>
<th>Mean day of onset</th>
<th>Peak disease score (affected mice)</th>
<th>CDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>19/25 (76%)</td>
<td>10·5 ± 0·55**</td>
<td>2·36</td>
</tr>
<tr>
<td>E2</td>
<td>12/25 (48%)</td>
<td>12·8 ± 0·98*</td>
<td>6·14</td>
</tr>
<tr>
<td>E2+ICI</td>
<td>20/25 (80%)</td>
<td>9·55 ± 0·49**</td>
<td>17·7</td>
</tr>
</tbody>
</table>

Incidence, number of affected mice/total number of mice used (% incidence is given in parentheses); Mean day of onset, mean ± S.E.M. onset of disease in days; Peak disease score, mean ± S.E.M. clinical disease score of all mice at peak (d 14) disease (mean clinical disease score of only affected mice at peak disease is given in parentheses); Cumulative disease index, the mean ± S.E.M. of the sum of daily disease scores; *, P<0·05 compared to vehicle control; **, P<0·01 compared to E2 group.

Effect of ER-selective agonists on EAE induced by the adoptive transfer of PLP-primed effector cells

To determine the relative contribution of ERα and ERβ to the suppressive effects of estrogens in the EAE model, ovariectomized female recipient mice were treated with ER-selective ligands prior to the adoptive transfer of PLP-primed splenocytes. Dosing of recipient mice with these compounds continued throughout the course of disease. PPT is an ERα-selective ligand that has been shown to have over 400-fold selectivity for ERα over ERβ (Staufer et al. 2000). WAY-202041 is an ERβ-selective ligand with 220-fold selectivity for ERβ (Harris et al. 2003). Treatment with E2 or PPT resulted in a delay in onset as well as decreased incidence and severity of disease (Figure 2, Table 2). In contrast, little or no effect was observed with an ERβ-selective ligand.

Effect of ER-selective agonists on PLP-specific recall responses

Like MS, EAE is associated with the production of pro-inflammatory cytokines (for example, TNF-α and IFN-γ). CD4+ Th cells that produce these cytokines mediate much of the pathology. To determine whether the reduced severity of disease in PPT-treated mice was associated with suppression of cytokine production, immune responses were examined at peak disease (day 14). Consistent with the effect of the ERα-selective agonist PPT on disease, treatment of mice with PPT resulted in decreased cytokine production upon stimulation of splenocytes with PLP in vitro (Figure 3). Both Th1/pro- (TNF-α, IFN-γ, IL-2) and Th2/anti-inflammatory (IL-4, IL-5, IL-10) cytokines were suppressed by in vivo treatment with PPT. In contrast, the ERβ-selective agonist (WAY-202041) only suppressed the anti-inflammatory cytokines IL-4 and IL-10.

Effect of SERMs on EAE

SERMs are compounds with tissue-specific estrogen agonist/antagonist activity that are in clinical use for the treatment of diseases such as breast cancer and osteoporosis. However, their use in the treatment of inflammatory diseases is largely unexplored. We examined the effect of the SERMs raloxifene and a raloxifene analog, WAY-138923, on the development of EAE. Treatment with E2, raloxifene or WAY-138923 resulted in a delay in onset as well as decreased incidence and severity of disease (Fig. 4, Table 3). Unlike the effects of PPT treatment on cytokine production, in vivo administration of either raloxifene or WAY-138923 had no effect on the production Th1 or Th2 cytokines (data not shown).

Effect of SERMs on PLP-induced proliferation

Treatment of mice with SERMs had no effect on the capacity of PLP-specific splenocytes to produce cytokines,
Table 2: Effect of the ER-selective ligands on clinical disease

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Incidence</th>
<th>Mean day of onset</th>
<th>Peak disease score (affected mice)</th>
<th>CDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>31/33 (94%)</td>
<td>10.7 ± 0.31</td>
<td>2.18 ± 0.26 (2.40 ± 0.25)</td>
<td>12.1 ± 1.63</td>
</tr>
<tr>
<td>E2</td>
<td>10/35 (29%)</td>
<td>13.3 ± 0.86*</td>
<td>0.29 ± 0.12* (1.43 ± 0.35)</td>
<td>2.14 ± 0.84*</td>
</tr>
<tr>
<td>ERα ligand</td>
<td>12/36 (33%)</td>
<td>10.6 ± 0.94</td>
<td>0.85 ± 0.24* (2.42 ± 0.40)</td>
<td>4.27 ± 1.43*</td>
</tr>
<tr>
<td>ERβ ligand</td>
<td>27/36 (75%)</td>
<td>10.0 ± 0.36</td>
<td>1.86 ± 0.25 (2.68 ± 0.19)</td>
<td>11.2 ± 1.50</td>
</tr>
</tbody>
</table>

Incidence, number of affected mice/total number of mice used (% incidence is given in parentheses); Mean day of onset, mean ± S.E.M. onset of disease in days; Peak disease score, mean ± S.E.M. clinical disease score of all mice at peak (d 14) disease (mean clinical disease score of only affected mice at peak disease is given in parentheses); Cumulative disease index, the mean ± S.E.M. of the sum of daily disease scores; *, P<0.05 compared to vehicle control.

Figure 3: Effect of ER-selective ligands on PLP-specific immune responses. Mice were euthanized at peak disease (day 14 post-transfer). Splenocytes from these mice were stimulated in vitro with PLP for 72 h. Supernatants were collected and measured for the presence of TNF-α, IFN-γ, IL-2, IL-4, IL-5 and IL-10 (*, P<0.05 compared with vehicle control group). The mean ± S.E.M. from two experiments are shown (n=8–10/group).
However, it is possible that other functions of these cells may be affected. We therefore examined whether SERMs could affect the capacity of PLP-specific cells to proliferate in response to stimulation with PLP. PLP-primed cells were stimulated in vitro with PLP in the presence of E2, WAY-138923 or PPT. The effect of these compounds on the proliferation of CD4+ cells was examined by flow cytometry. Treatment of E2, WAY-138923 or PPT resulted in decreased proliferation of CD4+ T cells upon stimulation with PLP (Fig. 5). These results suggest that each of these compounds may potentially act, in part, by limiting the clonal expansion of antigen-specific T cells.

Discussion

The effect of estrogens on EAE is well documented. In the present study, we examined the role of ERs in the modulation of disease by estrogen with the use of pharmacological agents. The protective effect of E2 was

![Figure 4](image-url)

**Figure 4** Effect of SERMs on disease in the adoptive transfer model of EAE. Ovariectomized recipient mice were treated daily with vehicle, E2 (10 μg/kg), Raloxifene, or WAY-138923 (both at 10 mg/kg) beginning on day −7. Mice were scored daily for clinical signs of disease. Shown are the combined results of four experiments. The data are summarized in Table 3.

![Figure 5](image-url)

**Figure 5** Effect of compounds on proliferation of CD4+ T cells upon antigen stimulation. Splenocytes from PLP-primed mice were labeled with CFSE prior to stimulation with PLP in the presence of E2, WAY-138923, Raloxifene or PPT. Cells were harvested after 3 days and surface stained with PE-labeled anti-mouse CD4. The percentage of cells that divided was determined with CELLQUEST software using a CD4+ gate (*, P<0.0001 compared with PLP stimulation alone). The results of two experiments are shown.

<table>
<thead>
<tr>
<th>Table 3 Effect of SERMs on clinical disease</th>
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<tbody>
<tr>
<td><strong>Incidence</strong></td>
</tr>
<tr>
<td>Vehicle</td>
</tr>
<tr>
<td>E2</td>
</tr>
<tr>
<td>Raloxifene</td>
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<tr>
<td>WAY-138923</td>
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</tbody>
</table>

Incidence, number of affected mice/total number of mice used (% incidence is given in parentheses); Mean day of onset, mean ± S.E.M. onset of disease in days; Peak disease score, mean ± S.E.M. clinical disease score of all mice at peak (if 14) disease (mean clinical disease score of only affected mice at peak disease is given in parentheses); Cumulative disease index, the mean ± S.E.M. of the sum of daily disease scores; *, P<0.05 compared to vehicle control.
reversed by the ER antagonist ICI 182 780, suggesting that the effects of E2 in the EAE model are ER-mediated. By comparing the effects of ER-selective ligands on susceptibility to EAE, we found that an ERα-selective agonist suppressed disease, resulting in delayed onset and reduced incidence and severity compared with mice treated with vehicle. In contrast, an ERβ-selective agonist had little to no effect on disease. This suggests that the effects of estrogens on disease in the EAE model are mediated primarily through ERα and are consistent with the recently reported findings that the immunosuppressive effects of E2 and estriol are dependent upon ERα (Liu et al. 2003, Polanczyk et al. 2003). Further, we show that SERMs, which are non-steroidal ER ligands that have tissue-selective mixed agonist/antagonist activity, also suppressed EAE. These results suggest that SERMs and compounds that selectively bind to ERα may be useful as therapeutic agents for the treatment of MS.

It should be noted that while the aforementioned studies by Liu et al. and Polanczyk et al. demonstrated that ERα but not ERβ mediates the protective effect of exogenous estrogen administered to female mice in the EAE model, a recent study investigating the relative roles of ERα and ERβ signaling and function in susceptibility of male mice to EAE reported that ERα (Esr1)-deficient male mice develop less severe disease than their wild-type counterparts (Polanczyk et al. 2004a). With respect to the role of ERβ (Esr2) signaling in male mice with EAE, Esr2+/− but not Esr2−/− mice developed more severe disease compared with wild type Esr2+/+ mice. This effect appeared to be mediated by nonhematopoietic cells as demonstrated with the use of radiation bone marrow chimera studies (Polanczyk et al. 2004a). Thus, both receptors appear to be involved in endogenous estrogen signaling that regulates the severity of disease in male mice.

Our observation that ER ligands can inhibit the proliferation of PLP-specific CD4+ T cells would suggest that these compounds have the capacity to limit the expansion of autoreactive T cells in vivo, thereby reducing or preventing subsequent disease development, and may indicate that SERMs or ERα-selective ligands can act on immune responses directly. It has been suggested that T cells have the capacity to respond to estrogens since the expression of both ERs on lymphocytes has been reported (Tornwall et al. 1999, Rider et al. 2000, Kassi et al. 2001, Suenaga et al. 2001, Phiel et al. 2005). Indeed, Polanczyk et al. recently showed that E2-mediated suppression of certain genes (for example, IFN-γ and RANTES) in the EAE model is dependent upon the presence of ERα (Esr1)-expressing T cells, since inhibition of these genes by E2 was attenuated in E2-treated mice in which EAE was induced by the adoptive transfer of Esr1−/− T cells compared with mice that received Esr1+/+ T cells (Polanczyk et al. 2004b). This suggests that E2 may directly modulate the activity of T cells. However, despite these observed effects of E2 on T cells, the beneficial effects of E2 treatment on EAE were still demonstrated in recipients of Esr1−/− T cells, which suggests that T cells are not required for the protective effects of E2 (Polanczyk et al. 2004b). These results are consistent with the findings that pregnancy as well as estrogen treatment during EAE induces a population of CD45+VLA-4+ regulatory cells that appear to be non-T cells but confer protection from EAE (Matejuk et al. 2004). Additional studies using bone marrow chimeras further support these observations and provide evidence that E2 may act on nonhematopoietic cells such as endothelial cells, or on resident cells of the CNS (for example, microglia) to prevent disease (Garidou et al. 2004). Modulation of cells such as these by E2 may in turn suppress the activation and/or function of T cells.

Previous studies examining immune responses in estrogen-treated mice in the EAE model have produced variable results, demonstrating a suppression of Th1 cytokine production with or without a concomitant increase in Th2 cytokines that was associated with disease suppression (Kim et al. 1999, Offner et al. 2000, Bebo et al. 2001, Ito et al. 2001, Matejuk et al. 2001). Our results show that treatment with PPT is associated with a decrease of both Th1 and Th2 cytokines, suggesting that suppression of disease by ERα-selective agonists may not involve immune deviation to a Th2 response, but a global suppression of PLP-specific T cell activation. We were unable to observe a consistent suppression of cytokine production in SERM-treated mice, which may indicate that SERMs and PPT may have differential effects on PLP-specific immune responses. It is also possible that the disease-modulating effect of ER-selective ligands and SERMs in this model may be, in part, due to protective effects on the CNS. The neuroprotective activities of estrogens and SERMs are well documented, where treatment of animals has been shown to reduce ischemic stroke damage (reviewed in Dhandapani et al. (2002)). Further, E2 protects oligodendrocytes, the myelin forming cells in the CNS, from cytotoxicity-induced cell death in vitro, and the ER antagonist ICI 182 780 blocks this protective effect (Takao et al. 2004). These results demonstrate that the protective effect of E2 was mediated by the activation of ERs and are consistent with our findings in vivo. However, SERMs also have been shown to have beneficial therapeutic effects in lupus, an autoimmune disease in which the primary affected organ is not the CNS, which may suggest that SERMs may have beneficial effects on target organs other than the CNS (Duvic et al. 1978, Sturgess et al. 1984, Stoeger et al. 1994, Apelgren et al. 1996, Wu et al. 2000).

E2 has been shown to have biphasic effects on immune responses. Low doses appear to stimulate pro-inflammatory cytokine production, whereas higher doses are generally immunosuppressive (Gilmore et al. 1997). For example, it is thought that the disease-modulating effects of estrogens and pregnancy on autoimmune disease are due to a Th2 environment associated with increased
hormone production during pregnancy (Wegmann et al. 1993). This may explain why estrogens appear to have differential effects on the severity of some autoimmune diseases. A predominant Th2 response induced in pregnancy would inhibit autoreactive Th1 responses involved in the pathology of multiple sclerosis and rheumatoid arthritis (Carlsten et al. 1992, Wilder 1998). In contrast, it would potentially promote pathogenic autoantibody production in systemic lupus erythematosus (lupus) and therefore exacerbate disease. Despite the known immunosuppressive effects associated with treatment with high doses of estrogen, it has been shown that immunosuppressive and protective effects of E2 on EAE can be achieved with relatively low doses. Bebo et al. demonstrated that a dose as low as 80 µg/kg/day, which mimics the levels of E2 measured during the diestrus phase of the hormone cycle (20–30 pg/ml), was effective in suppressing disease (Bebo et al. 2001). The levels of hormone achieved with this dose of E2 are 100–200 fold lower than those measured during pregnancy in mouse serum. In the present study, we were able to achieve similar protective effects using even lower doses (10 µg/kg/day) of ER-selective ligand or SERMs. Thus, varied doses of estrogen do not completely explain the apparent disparate effects of estrogens on autoimmune disease severity.

Understanding the ways in which estrogens modulate immune responses and autoimmune diseases is complicated by the fact that although estrogens appear to improve some autoimmune diseases, paradoxically, the incidence of a number of autoimmune diseases is higher among females compared with males. Specifically, the female-to-male susceptibility ratio is 9:1 for systemic lupus erythematosus (SLE), 3:1 for MS, 4:1 for rheumatoid arthritis (RA), and 9:1 for Sjögren’s syndrome (SS) (Whitacre 2001). It has been proposed that estrogens could influence the frequency of autoimmune disease among females by interfering with tolerance induction and subsequently permitting the survival of potentially autoreactive lymphocytes (Bynoe et al. 2000). Alternatively, non-hormonal mechanisms such as skewed X chromosome inactivation have been suggested to contribute to loss of immune tolerance and therefore, increased frequencies of autoimmune diseases in females (Lockshin 2001). A better understanding of the immunomodulatory effects of estrogens is required to reconcile some of the contradictions observed.

Our results suggest that SERMs, which are in clinical use currently, may have value as a potential therapy for MS. A pilot study examining the effect of menopause and hormone replacement therapy on MS reported an improvement in symptoms in postmenopausal women who had received hormone replacement therapy (Smith & Judd 1992). The results from a recent phase I clinical trial demonstrate that estriol can significantly reduce brain lesions in nonpregnant women with relapsing remitting MS (Sicotte et al. 2002). Together, the results of these studies in humans suggest that treatment of autoimmune disorders with hormones or estrogen receptor ligands is possible.

In summary, the results presented herein demonstrate that the protective effects of estrogens against susceptibility to EAE are dependent upon ER signaling. Further, an ERα- but not ERβ-selective ligand suppressed disease, indicating that ERα mediates the disease modulating activity of estrogen in this model. The SERMs raloxifene and WAY-138923 also suppressed disease. SERMs are non-steroidal compounds that retain beneficial properties of estrogens but have reduced adverse (for example, uterotrophic) effects. They are currently approved and prescribed widely for the treatment of a number of diseases, including breast cancer and osteoporosis. Since they have relatively fewer side effects than classical estrogens, they may prove to be a favorable alternative to estrogens as a potential therapy for MS and other known autoimmune diseases that are ameliorated by estrogens.

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