Estradiol Treatment Redirects the Isotype of the Autoantibody Response and Prevents the Development of Autoimmune Arthritis

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A number of clinical and experimental observations have been made relating elevated estrogen levels with the amelioration of autoimmune diseases, yet questions remain about the levels required for efficacy as well as the mechanism of disease inhibition. Using the collagen-induced arthritis (CIA) model, we have studied the effects of physiological, sustained levels of 17β-estradiol in preventing the development of autoimmune arthritis and analyzed the changes in the autoimmune response. Using time-release pellets of 17β-estradiol, arthritis development was significantly inhibited in three different strains of CIA-susceptible mice compared with the effect of placebo treatment, and serum estradiol levels similar to those of mice in estrus were found to be equally effective as higher estradiol concentrations. Analysis of the autoimmune response in the estradiol-treated mice indicated that T cell production of IFN-γ was markedly decreased, and significant decreases were also observed in levels of IL-10 and GM-CSF produced by lymph nodes cells from estradiol-treated mice. Although the total IgG anti-CII response was only minimally affected by estrogen treatment, a significant reduction in the levels of IgG2a anti-CII Abs and an increase in the levels of IgG1 anti-CII Abs were observed in estradiol-treated mice. These data indicate that estradiol treatment altered the Th profile of the autoimmune T cell response, which, in turn, altered the production of IgG Abs to an isotype that is poor at fixing complement, an important component in the immunopathogenesis of CIA.


The relationship between elevated estrogen levels and the amelioration of autoimmune diseases has been described in a number of clinical and experimental observations. Although studies of susceptibility to autoimmunity frequently focus on the function of specific MHC genes and the cytokines associated with the autoimmune response, it is also clear that estrogen can play an important role in regulating the onset, severity, and progression of an autoimmune disease. Some of the strongest clinical evidence for the relationship between estrogen and autoimmunity comes from observations made with patients with autoimmune diseases who become pregnant. For example, in females with rheumatoid arthritis, it has been noted that elevated estrogen levels that occur during pregnancy are often associated with amelioration of the disease (1). Conversely, when estrogen levels fall during the postpartum period, disease flares are a common occurrence. Similar observations have been made in patients with other T cell-mediated autoimmune diseases, such as multiple sclerosis (2–4). A number of mechanisms have been proposed by which estrogen modifies the autoimmune responses, including direct effect on the pathogenic T cells as well as altered function of regulatory T cells, yet it remains unclear how elevated levels of estrogen alter the course of autoimmunity, especially in light of the fact that females are generally more susceptible to autoimmune diseases than males.

Animal models of autoimmunity have proven useful in studying the effects of estrogen on the autoimmune response (5–9). In experimental autoimmune encephalomyelitis (EAE), an experimental model of multiple sclerosis, pregnancy has been shown to suppress the disease symptoms, and treatment with estrogen at levels equal to or less than those achieved in pregnancy significantly alters the course of the disease (6). At least part of the mechanism by which estrogen regulates the autoimmunity of EAE appears to involve alteration of the T cell response and potentially a shift from a pathogenic Th1-type cytokine response to a Th2 response (10). Although the changes in T cell cytokine production demonstrated by these studies in EAE were not dramatic, they are consistent with observations of altered cytokine production during pregnancy (11, 12). Similar to EAE, the development of collagen-induced arthritis (CIA) has been shown to be inhibited by the administration of estrogen (8, 9, 13). CIA is a well-established autoimmune arthritis model that shares many characteristics with rheumatoid arthritis. CIA is induced by immunization with type II collagen (CII), and the autoimmune response involves both CII-specific T and B cells (14). Although CIA is T cell dependent, the immunopathogenesis is primarily mediated by complement-fixing autoantibodies to murine CII (mCII) (15, 16), which, in turn, initiates an inflammatory response involving IL-1β and TNF-α in the

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3 Abbreviations used in this paper: EAE, experimental autoimmune encephalomyelitis; SCII, bovine CII; CIA, collagen-induced arthritis; CII, type II collagen; mCII, murine CII; RPA, RNase protection assay; Tg, transgenic.
synovial joints. Like EAE, CIA is considered a Th1-mediated autoimmune disease based on the production of high levels of IFN-γ and a predominant IgG2a response (17). However, since the primary mechanism of pathogenesis in CIA is the production of complement-fixing Ab specific for mCII, it remains to be determined whether estrogen affects CIA and EAE by similar or different mechanisms.

In the studies described here we have examined the effect of maintaining elevated, but physiological, levels of 17β-estradiol on the development of CIA and have studied the effect of 17β-estradiol on the immunopathogenic T and B cell responses to the immunogen, bovine CII (bCII), and the autoantigen, mCII. Because the immunopathogenesis of CIA is different from that in CIA, we sought to determine whether the mechanism by which the autoimmunity of CIA was altered by estrogen differed from that of EAE. Similar to results that have been reported in studies of EAE, we determined that both sustained serum levels of 4000 pg/ml of estradiol, a concentration roughly equivalent to 50% of that found during pregnancy, as well as serum levels of estradiol similar to those observed in female mice in estrus, were sufficient to block the development of CIA in several susceptible strains. Analysis of the autoimmune response indicated that at least part of the mechanism by which arthritis development was inhibited in CIA involved alterations in cytokine production by autoimmune T cells that, in turn, redirected the isotype of the pathogenic autoantibody response. These data serve to further demonstrate the intricate relationship between estrogen and the susceptibility and progression of autoimmune diseases, and the delicate balance that exists between T cell cytokine expression and the outcome of an immune response.

Materials and Methods

Animals

DBA/1LacJ mice were obtained from The Jackson Laboratory (Bar Harbor, ME) and were maintained in our animal facility. The CII-specific TCR transgenic (Tg) DBA/1 mice have been previously described (18). The HLA-DQ8 Tg mice were a gift from Dr. C. David (Mayo Clinic, Rochester, MN) (19) and were housed in the animal resource facility at Portland Veterans Affairs Medical Center. All mice were housed in microisolators in accordance with institutional guidelines and were provided with food and water ad libitum.

Collagen and collagen peptide preparation

Native bCII and mCII were purified from articular (bovine) and sternal (murine) cartilage as previously described (20). The human type II collagen peptide 257–274 was synthesized by F-moc chemistry using an automated peptide synthesizer (model 430; PE Applied Biosystems, Foster City, CA).

Estrogen treatment and arthritis induction

Mice were treated with 60-day estrogen pellets containing a total of either 2.5 or 0.36 mg of 17β-estradiol (Innovative Research of America, Sarasota, FL) or with a placebo pellet implanted s.c. above the shoulders using a 10-gauge trochar. Seven days later the mice were immunized with an emulsion composed of equal parts of CFA containing 4 mg/ml of heat-killed M. tuberculosis (Central Veterinary Laboratory, Surrey, U.K.) and bCII solubilized at 4 mg/ml in 50 mM acetic acid. DBA/1LacJ mice received an injection of 100 μl of emulsion (200 μg of bCII) at the base of the tail, whereas DQ8 Tg mice received an injection of 50 μl (100 μg of bCII) at the base of the tail plus a boost of 100 μg of bCII/IFA 21 days later. The development of arthritis was evaluated by grading each paw on a scale of 1–4, with 4 signifying the most severe arthritis (20). Mice were examined for incidence and severity of arthritis two or three times per week. Serum estrogen levels were measured by RIA as previously described (6).

Ab ELISA

Serum samples were collected at the intervals indicated postimmunization with CII/IFA, and CII-specific Ab was measured using a solid phase ELISA as previously described (21). The ELISA were performed in 96-well vinyl plates (Costar, Cambridge, MA) that were coated with a solution of 5 μg/ml of either bCII or mCII and were incubated overnight at 4°C. After three washes with a solution of 0.15 M saline and 0.05% Tween 20, serum samples were added at an initial dilution of 1/2,000 and seven 2-fold serial dilutions were made down the plate. Following an overnight incubation and three washes, a 1/5,000 dilution of peroxidase-conjugated goat polyclonal anti-mouse Ab specific for IgG, IgA, and IgM (Sigma-Aldrich, St. Louis, MO) or a 1/10,000 dilution of peroxidase-conjugated goat anti-mouse isotype Abs specific for IgG1, IgG2a, IgG2b, or IgG3 (Southern Biotechnologies, Birmingham, AL) was added to each well (100-μl volume/well). The plates were then incubated for 2 h at 4°C. washed three times, and developed with o-phenylenediamine dihydrochloride. Color development was stopped after ~30 min by addition of 50 μl of 2.5 N H2SO4. Absorbance was measured using a spectrophotometer at 490 nm, with the background absorbance at 650 nm subtracted.

Cytokine ELISA

Spleen cells from estradiol- or placebo-treated mice were resuspended at 4 × 106 cells/ml in IL-1 medium supplemented with 50 μM 2-ME, 2 mM glutathione, 100 U/ml of penicillin, 50 μg/ml of streptomycin, and 0.1% BSA (fraction V, IgG free, low endotoxin; Sigma-Aldrich). One hundred microliters of cells and 100 μg of CII peptide 257–274 were added to each well of a 96-well microtiter plate. Forty-eight hours later, 75 μl of supernatant was recovered from each well and frozen at −80°C. Cytokine analyses were performed using the Bio-Plex assay system (Bio-Rad, Hercules, CA). In brief, 50 μl of culture supernatant was added to 50 μl of Ab-conjugated beads in a 96-well filter plate (Bio-Rad). After a 30-min incubation, the plate was washed, and 25 μl of an anti-cytokine Ab solution was added to each well, followed by another 30-min incubation. The plate was then washed, and 50 μl of streptavidin-conjugated PE was added to each well. Following a final wash, each well was resuspended in 125 μl of the assay buffer (Bio-Rad) and analyzed by the Bio-Plex Protein Array System (Bio-Rad). Cytokine concentrations were calculated with a standard curve derived using various concentrations of cytokine standards in the assay.

IL-1β expression in the synovial tissue was measured by ELISA using extracts from tissue homogenates. Synovial tissues were carefully dissected from the ankle joints of the hind limbs, weighed, and stored frozen at −80°C. In some cases the hind limbs were removed and stored frozen before dissection. The tissue was then thawed in 200 μl of PBS containing 0.1% Tween 20 and a 1/100 dilution of protease inhibitor mixture (Sigma-Aldrich). The samples were homogenized using a polypropylene pestle and strained through a 400-μm mesh. Ten microliters of the homogenate was added to each well of a 96-well plate (Costar) and was incubated overnight at 4°C. In brief, 50 μl of culture supernatant was added to 50 μl of Ab-conjugated beads in a 96-well filter plate (Bio-Rad). After a 30-min incubation, the plate was washed, and 25 μl of an anti-cytokine Ab solution was added to each well, followed by another 30-min incubation. The plate was then washed, and 50 μl of streptavidin-conjugated PE was added to each well. Following a final wash, each well was resuspended in 125 μl of the assay buffer (Bio-Rad) and analyzed by the Bio-Plex Protein Array System (Bio-Rad). Cytokine concentrations were calculated with a standard curve derived using various concentrations of cytokine standards in the assay.

RNase protection assay (RPA)

Splenocytes from individual animals were frozen, subsequently thawed, and evaluated for the expression of cytokines and chemokines by RPA. Total RNA was extracted using the STAT-60 reagent kit (Tel-Test, Friendswood, TX). Cytokine mRNA expression was determined using the RiboQuant RPA kit (BD PharMingen, San Diego, CA) according to the manufacturer’s instructions. Sample loading was normalized to the quantity of the housekeeping gene, L32. RPA analysis was performed using 10 μg of total RNA hybridized with probes labeled with [32P]UTP. After digestion of ssRNA, the pellet of hybridized RNA was solubilized and resolved on a 5% sequencing gel. For quantification, gels were exposed to phosphor imaging (Bio-Rad), and radioactivity in individual bands was quantitated using Quantity One software (Bio-Rad).

Statistical analyses

Data were analyzed using Macintosh JMP statistics software (SAS Institute, Cary, NC). Statistical significance was determined by Student’s t test. Statistical significance was established at p < 0.05.

Results

Effect of estradiol on arthritis development

To determine the efficacy of sustained administration of 17β-estradiol in inhibiting the development of CIA, mice were implanted...
s.c. with pellets designed to deliver a constant dose of estrogen for a 60-day period. Following implantation, mice were given 7 days to recover from the implantation surgery and to stabilize their elevated serum estradiol levels before they were immunized with bCII emulsified in CFA. As shown in Fig. 1, the implantation of pellets containing 2.5 mg of estradiol in CIA-susceptible mice significantly decreased the incidence of arthritis compared with that in animals that received placebo pellets. This was true for CIA-susceptible DBA/1 mice (Fig. 1A), HLA-DQ8 Tg mice (Fig. 1C), and DBA/1 mice expressing a Tg CII-specific TCR (18) (Fig. 1D). Although nearly 100% of the mice in the DBA/1, DQ8, and TCR Tg placebo groups developed arthritis, only 10, 20, and 0%, respectively, of the mice receiving estradiol developed CIA, and in the DQ8 mice this occurred at a much later time point compared with arthritis development in the control group (Fig. 1C). Of the estrogen-treated mice that developed arthritis, no significant difference in severity of disease was noted, although because of the low incidence, these mice constituted a very small sample size. To determine whether a lower dose of estradiol would also confer protection from CIA, DBA/1 mice were implanted with pellets containing only 0.36 mg of estradiol, and after 7 days they were immunized with bCII and CFA. As shown in Fig. 1D, despite being a 7-fold lower dose, this amount was as effective as the 2.5-mg dose in preventing the development of autoimmune arthritis.

The levels of estrogen achieved in the sera of mice receiving the pellets containing 2.5 mg of estradiol (Table I) were similar to levels reported in female mice during pregnancy (6), while the mice treated with the 0.36-mg pellets had levels approximately 2-fold higher than estrous levels. Diestrous female mice were reported to have estradiol levels of 20–30 pg/ml that increase to 100–200 pg/ml during estrus and to 5,000–10,000 pg/ml during pregnancy (6). The levels measured in our placebo group (Table I) agree well with a mixture of estrous and diestrous mice, while the mean estradiol levels in the treated mice (Table I) are similar to or significantly lower than those observed in pregnant mice (6). Thus, this approach to estrogen therapy appears to achieve physiological levels of estradiol, although it is maintained in these mice for at least 3–4 wk longer than would normally occur during an average mouse gestation.

As shown in Fig. 2, analysis of the joints of the mice for the presence of IL-1β indicated no evidence of an inflammatory response in the estradiol-treated mice. IL-1β plays a major role in the inflammatory response in CIA and can be found in abundance in affected limbs (22). Each mouse tested from the placebo group contained high levels of IL-1β in the arthritic joints, while generally none could be detected in the joints of the estradiol-treated mice. The one mouse in the estradiol group that had high IL-1β levels in the synovial joint analyzed (Fig. 2) had also developed severe arthritis in that joint.

Analysis of the autoimmune CII-specific IgG response

To determine how estrogen treatment altered the development of arthritis, the autoimmune Ab response to CII was analyzed in DBA/1 and DQ8 mice. CII-specific Abs, especially IgG isotypes that bind complement, are an essential component of the immunopathogenesis of the CIA model (15, 23, 24). When sera from DBA/1 mice treated with estradiol or placebo and immunized with CII were analyzed, similar amounts of Ab to the immunogen (bCII) and the autoantigen (mCII) were found in placebo- and estradiol-treated groups of mice at an early time point that coincides with the initial development of arthritis (Fig. 3). At 6 wk

<table>
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<th>Treatment</th>
<th>Dose (mg)</th>
<th>Serum Concentrationa</th>
<th>n</th>
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<tbody>
<tr>
<td>Estradiol</td>
<td>2.5</td>
<td>4122.8 ± 409.3</td>
<td>5</td>
</tr>
<tr>
<td>Estradiol</td>
<td>0.36</td>
<td>425.7 ± 126.6</td>
<td>5</td>
</tr>
<tr>
<td>Placebo</td>
<td>Placebo</td>
<td>44.19 ± 3.9</td>
<td>5</td>
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a Pellets containing 2.5 or 0.36 mg of estradiol or placebo pellets were implanted s.c. using a trochar as described in Materials and Methods.

b Serum samples were collected 38 days after pellet implantation, and levels of estradiol were measured by RIA. Data are expressed as the means ± SEM.
treated with estradiol (2.5-mg pellets; n

FIGURE 2. Analysis of IL-1β production in the synovial tissues of mice treated with 17β-estradiol. Synovial tissue was collected from the paws of estradiol- or placebo-treated DQ8 mice, and the tissue was homogenized and extracted to recover soluble protein as described in Materials and Methods. IL-1β levels were measured using an ELISA, and quantity was calculated using a standard curve. Error bars indicate the SEM.

postimmunization (49 days of estradiol treatment), estradiol-treated mice had produced less bCII- and mCII-specific Ab compared with the placebo group. Although the decrease in bCII Ab levels in DBA/1 mice at this later time point was statistically significant, the decrease in mCII Ab levels was not. Similar data were obtained in experiments using DQ8 mice (Fig. 3C).

The fact that the estrogen-treated mice produced quantities of IgG anti-CII Ab similar to those produced by placebo-treated mice, yet did not develop arthritis, indicated that perhaps the isotypes of the CII-specific IgG differed between these groups. Isotype analysis of the mCII-specific Abs produced by these treated mice indicated significant differences among the Ig subclasses produced by the estradiol-treated mice compared with those produced by the placebo group (Fig. 4). Placebo-treated mice produced significantly more IgG2a than estradiol-treated mice at both time points, while estradiol-treated mice produced more IgG1 than mice in the placebo group. Although both groups produced similar amounts of IgG2b, mice from the placebo group produced significantly higher levels of IgG3 than estradiol-treated mice at the first time point analyzed. However, since only very low levels of IgG3 were detectable compared with the other isotypes measured, it is difficult to assess the role of IgG3 in the pathogenesis of CIA. Given the fact that IgG2a Abs are efficient at complement fixation, while IgG1 Abs are not, this set of experiments suggests that among estrogen’s protective effects is the ability to alter the autoimmune response from a proinflammatory Th1-type T cell response that promotes the production of the IgG2a isotype toward a Th2-type response that promotes the production of IgG1.

Effect of estradiol on the autoimmune T cell response

Because cytokines are known to regulate the expression of Ig isotypes, the effects of estradiol treatment on T cell stimulation and cytokine production were investigated. DBA/1 mice were implanted with either estradiol pellets (2.5 mg) or placebo pellets, and 7 days later they were immunized with CII. Ten days postimmunization their T cells were tested for the ability to proliferate and produce cytokines when stimulated with a CII peptide. No significant differences were observed in the proliferative response of T cells from placebo- and estradiol-treated DBA/1 and DBA/1 TCR Tg mice (Fig. 5). For analysis of cytokine production by these T cells, seven different cytokines were measured, focusing on four Th1 cytokines (IFN-γ, IL-2, GM-CSF, and TNF-α) and three Th2 cytokines (IL-4, IL-5, and IL-10). Of the seven cytokines analyzed, estrogen treatment had a significant effect on the production of IFN-γ, IL-10, and GM-CSF. The quantities of these cytokines produced by T cells from estradiol-treated mice were consistently and significantly lower than those produced by T cells from placebo-treated mice. The decrease in the amount of IFN-γ produced is consistent with the Ig isotype data described above, as

FIGURE 3. Measurement of CII-specific Ab by ELISA. Sera from mice treated with estradiol (2.5-mg pellets; n = 10 for DBA groups; n = 6 for DQ8 groups) or placebo were collected on days 21 and 42 postimmunization and analyzed for the presence of total bCII-specific (A) and mCII-specific (B) Ab by solid phase ELISA. The quantity of Ab detected is expressed as relative units based on a CII-specific reference serum. Error bars indicate the SEM. *, p < 0.05.

FIGURE 4. Analysis of isotype expression in the mCII-specific Ab response. Murine CII-specific isotypes were quantitated using the sera described in Fig. 3 and a solid phase ELISA in which the detection Abs were specific for the isotypes listed. The quantity of Ab detected is expressed as relative units based on a CII-specific reference serum. Error bars indicate the SEM. *, p < 0.05; **, p < 0.01.
IFN-γ plays an important role in promoting the isotype switch to IgG2a. Estradiol-treated DBA/1 T cells also produced significantly less GM-CSF (Fig. 6A), a cytokine whose role appears to be linked more closely to arthritis severity than to arthritis incidence (25). Although IL-2 and TNF-α levels were detectable, all levels measured were very low (<40 pg/ml) and approached the sensitivity of the assay. Although a decrease in Th1 cytokines might be predicted on the basis of the Ig isotype data presented above, there was no compensatory increase in the Th2 cytokines. In fact, the production of IL-10 by Tg T cells treated with estradiol or placebo pellets were implanted 7 days before immunization.

FIGURE 5. Analysis of CII-specific T cell proliferation using lymph node cells from estradiol- and placebo-treated mice. A, Tg CII-specific T cells were obtained from naive (unimmunized) mice treated with estradiol or placebo pellets 7 days previously. B, T cells were harvested from DBA/1 mice immunized 10 days previously with CII/CFA. Estradiol or placebo pellets were implanted 7 days before immunization.

FIGURE 6. Analysis of Th1 and Th2 cytokine expression by T cells from estradiol- or placebo-treated mice. Th1 (A) and Th2 (B) cytokine production was measured by ELISA using culture supernatants from CII-stimulated T cells harvested from DBA/1 mice treated with estradiol or placebo. Mice were implanted with pellets, and 7 days later they were immunized with CII. Ten days after immunization draining lymph node cells were recovered and stimulated in vitro with a CII peptide. Supernatants were harvested 48 h after culture and analyzed using a Bio-Plex-based assay as described in Materials and Methods. *, p < 0.02.

Discussion

A number of clinical studies have demonstrated a relationship between elevated levels of estrogen and the down-regulation of autoimmunity (27, 28). Paradoxically, most autoimmune diseases in which estrogen has been shown to have a therapeutic effect occur at a much higher incidence in females (29). For instance, both rheumatoid arthritis and multiple sclerosis have a higher incidence in females, yet both diseases are often ameliorated by pregnancy and frequently flare during the postpartum period when estrogen levels return to normal. Studies in the EAE and CIA animal models of autoimmunity have confirmed that elevated levels of estrogen can inhibit the development of autoimmunity (6, 9). In both of these models, the development of autoimmunity has been shown to be inhibited by pregnancy (30–32) as well as by the administration of estrogen in doses that mimic pregnancy (9, 33–35). Subsequent studies in the EAE model have revealed that sustained therapeutic estrogen levels similar to those found during estrus are effective in reducing disease severity (6). Thus, the evidence seems compelling that estrogen can play a significant role in regulating the autoimmune response and much of the research emphasis in this area has shifted to understanding the mechanisms by which the autoimmune response is affected.

In the studies described here, the CIA model was used to investigate the efficacy of sustained, low dose estradiol therapy on the development of autoimmune arthritis and to determine how the immunopathogenesis of this model is altered by elevated estrogen levels. Not only was sustained estradiol therapy very effective at preventing the development of arthritis in three different CIA-susceptible strains, but very low sustained serum levels of estradiol
similar to those observed during estrus were found to be as effective as higher doses. Studies in EAE revealed that treatment with low doses of estradiol that resulted in sustained estrous or diestrous levels of estrogen in the sera of mice were very effective at reducing the severity of disease, although pregnancy levels of estradiol were necessary for preventing the development of disease (6). When we examined the effect of low doses of estradiol in CIA, we found that subpregnancy levels of estradiol were very effective at inhibiting the development of arthritis. Because these physiological doses of estrogen were effective in down-regulating the autoimmune response, it is likely that the duration of these sustained estrogen levels played a significant role in their effect. The vehicle used for estradiol therapy in both of these studies was a constant release pellet capable of sustaining these estradiol levels for 60 days. Although no significant differences were observed in the T cell proliferative responses of estradiol-treated mice compared with controls, changes in the production of IFN-γ by T cells were quite apparent. Estradiol treatment reduced the production of IFN-γ by T cells in culture at both the protein and mRNA levels. Although the decrease in IFN-γ production did not appear to appreciably affect the total quantity of IgG anti-CII Ab produced, it did significantly alter the IgG isotype of the CII-specific response. Estradiol-treated mice produced higher levels of IgG1 than placebo-treated mice, whereas placebo-treated mice produced high levels of IgG2a compared with the lower levels produced by the estradiol-treated mice. These data are significant in that the immunopathogenesis of CIA is dependent on the production of mCII-specific Ab isotypes, such as IgG2a and IgG2b, that are capable of activating the complement cascade. Given that total IgG and IgG2b levels were not appreciably different between the estradiol and placebo groups, it was somewhat surprising that the estradiol-treated mice did not develop arthritis. However, IgG2a is a more potent activator of complement than IgG2b (23) and is more proficient at activation of macrophages, which, in turn, are more likely to up-regulate their IgG2a FcR than their IgG2b receptors in response to activation (36). These facts suggest two routes by which IgG2a might produce the arthritic response seen in placebo-treated animals and conversely support our hypothesis that the shift to the IgG1 isotype seen in estradiol-treated mice is responsible for the lack of arthritis in these animals, a possibility that can be tested by passive transfer of sera from treated animals to untreated mice (15, 37). However, it is equally plausible that the switch to IgG1 may simply be an indicator of estrogen-induced changes in T cell function or the generation of regulatory T cells that control the production of inflammatory mediators. In the EAE model, estrogen treatment has been shown to down-regulate the expression of TNF-α, an inflammatory mediator important to development of EAE. Although TNF-α has also been shown to play an important role in the pathogenesis of CIA (38, 39), we observed only modest changes in TNF-α expression in estrogen-treated mice immunized with CII.

A number of factors associated with pregnancy have been described that alter the immune response. Among those that have been examined for their roles in the amelioration of rheumatoid arthritis symptoms are the presence of fetal cells in maternal circulation (microchimerism) (40), the presentation of fetal peptides derived from HLA molecules by maternal HLA molecules (41), and increases in levels of steroid hormones, including cortisol, progesterone, and estrogen (29). Estrogen has received considerable attention and has been shown to be effective in down-regulating autoimmunity in a number of autoimmune models (6, 7). Several studies have demonstrated that estrogen can directly affect both the innate and adaptive immune system through receptor-specific interactions (42–45). Medina et al. (46) reported that elevated levels of estrogen can cause a decrease in the numbers of B cell precursors, although we and others (6) have not observed this effect. Given the fact that total levels of anti-CII Abs remained essentially unchanged as a result of estrogen treatment in our studies, an estrogen-driven suppression of B cell development appears not to have been a factor in the prevention of arthritis that we observed. It may be that estrogen treatment only 7 days before immunization may not be a sufficient amount of time to cause a decrease in the B cell population before it is stimulated by immunization with CII. Other B cell-related changes that may occur as a result of estrogen treatment include alterations in Ab affinity maturation. However, preliminary analysis of Ab affinity based upon isotype-specific titrations of anti-CII sera indicated no significant differences in the affinities of Abs produced by different treatment groups. Estrogen receptors have also been found on T cell populations (47), suggesting that estrogen inhibition of autoimmunity may occur as a result of a direct effect on the T cells. Benten et al. (48) reported that both CD8+ and CD4+ T cells have an novel estrogen signaling pathway, and estrogen has been shown to directly affect the cytokine expression of human T cell clones (49, 50). These data support a possible direct influence of estradiol on CD4+ T cells mediating the autoimmune response. However, estrogen receptors are present on many other nonimmune cell types that are also capable of altering T cell function.

Based on studies of the therapeutic effect of estrogen in EAE (5, 6, 9) and in the CIA studies described here, it is clear that T cell function is altered by estrogen, but it has been difficult to identify significant specific changes. Although T cell proliferation appears to be minimally affected, cytokine production appears to be the best indicator that changes in T cell function have occurred. In
Estradiol inhibits autoimmune arthritis

Estradiol (E2) is a major female sex hormone that plays a critical role in modulating immune responses. In the context of autoimmune arthritis, E2 has been shown to suppress the development and progression of experimental arthritis in animal models. This suppression is thought to be mediated through the regulation of cytokine production, particularly by shifting the balance from Th1 to Th2 cytokines.

**References**


