Modulatory effects of estrogen in two murine models of experimental colitis

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Received 31 October 2001; accepted in final form 4 February 2002

Verdú, Elena F., Yi Kang Deng, Premysl Bercik, and Stephen M. Collins. Modulatory effects of estrogen in two murine models of experimental colitis. Am J Physiol Gastrointest Liver Physiol 283: G27–G36, 2002. First published February 20, 2002; 10.1152/ajpgi.00460.2001.—The association between oral contraceptives or pregnancy and inflammatory bowel disease is unclear. We investigated whether 17β-estradiol modulates intestinal inflammation in two models of colitis. Female mice were treated with 17β-estradiol alone or with tamoxifen, tamoxifen alone, 17α-estradiol, or placebo. Dinitrobenzene sulfonic acid (DNB)- or dextran sodium sulfate (DSS)-induced colitis were assessed macroscopically, histologically, and by myeloperoxidase (MPO) activity. Malondialdehyde and mRNA levels of intercellular adhesion molecule-1 (ICAM-1), interferon-γ (IFN-γ), and interleukin-13 (IL-13) were determined. In DNB colitis, 17β-estradiol alone, but not 17α-estradiol plus tamoxifen, or 17α-estradiol reduced macroscopic and histological scores, MPO activity and malondialdehyde levels. 17β-Estradiol also decreased the expression of ICAM-1, IFN-γ, and IL-13 mRNA levels compared with placebo. In contrast, 17β-Estradiol increased the macroscopic and histological scores compared with placebo in mice with DSS colitis. These results demonstrate anti-inflammatory and proinflammatory effects of 17β-estradiol in two different models of experimental colitis. The net modulatory effect most likely reflects a combination of estrogen receptor-mediated effects and antioxidant activity and may explain, in part, conflicting results from clinical trials.

CLINICAL DATA ON THE ASSOCIATION between inflammatory bowel disease (IBD) and female sex hormones are conflicting. On one hand, IBD is believed to flare during a first pregnancy and postpartum and, on the other hand, to be better controlled during subsequent pregnancies (14, 43, 44). Others have proposed that the course of IBD during pregnancy depends on disease activity at conception (42, 64). Oral contraceptives have been suggested as a risk factor for relapse in Crohn’s disease (60), but this is not supported by other studies (13). A recent report cited female gender, but not oral contraceptive use, as a risk factor for relapse in ulcerative colitis (5). Although study design, inclusion criteria, and different formulations of oral contraceptives may explain some of the discrepancies between studies, a causal relationship between sex hormones and IBD has not been clearly established. It also remains unknown whether sex hormones and, in particular, estrogen, possess immunomodulating effects in the gastrointestinal system.

Females have stronger humoral and cell-mediated immune responses than males (3, 18, 22, 59) and, in general, have a higher incidence of autoimmune diseases (31, 33, 40, 48, 57). Although estrogen has been reported to enhance immunity, estrogen therapy has been shown to attenuate inflammation in carrageenan-induced pleurisy in rats and myocardial reperfusion injury and to improve outcome after cerebral ischemia (15, 47, 56). This discrepancy may reflect recently described divergent effects of estrogen based on dose, tissue specificity, and cellular environment (63).

Most of the effects of estrogen are mediated by binding of the hormone to specific estrogen receptors (ERs) that act as nuclear transcriptor activators (25). T cells, B cells, and macrophages are known targets of estrogen (24, 62). Classical ERs have been described in vascular endothelium, fibroblasts, smooth muscle cells, and gastrointestinal mucosa, including epithelial cells (8, 25, 27, 54). Thus it is reasonable to expect that estrogen modulates inflammation in the gastrointestinal system.

In this study, we examined the effect of supraphysiological doses of 17β-estradiol (those achieved during pregnancy) on colitis induced in female mice. In light of the conflicting clinical data regarding sex hormones and Crohn’s disease and ulcerative colitis, we investigated whether the effect of 17β-estradiol differed according to the manner in which colitis is induced. For this purpose, we used two different murine models of colitis: 1) a lymphocyte-dependent model by intracolonic administration of dinitrobenzene sulfonic acid (DNB) and 2) a lymphocyte-independent model by oral administration of dextran sodium sulfate (DSS). We found that supraphysiological doses of 17β-estradiol have anti-inflammatory effects in DNB colitis and proinflammatory effects in DSS colitis, demonstrating complex immunomodulatory effects of estrogen during intestinal inflammation.

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MATERIALS AND METHODS

Animals

Female C57Bl/6 mice were obtained from Taconic and kept under specific pathogen-free conditions. Female, macrophage colony-stimulating factor (M-CSF-1) deficient mice (op/op) and heterozygotes (op+/+) were obtained from a colony maintained at McMaster Animal Care Facility as described previously (21). All experimental protocols were approved by the McMaster Animal Care Committee and the Canadian Council on the Use of Laboratory Animals.

DNB Colitis

Study design and treatment groups. Female C57Bl/6 mice were divided into five groups (n = 5–13 per group) that received 17β-estradiol alone (0.5 mg/pellet) or with tamoxifen (5 mg/pellet), tamoxifen alone (5 mg/pellet), 17α-estradiol (0.5 mg/pellet), or placebo via 21-day release pellets implanted subcutaneously (IRA, Sarasota, Florida). The dose of 17β-estradiol used in this study markedly elevates the plasma hormone level in mice (~1,000 pg/ml) by 7 days after implantation, and these levels remain constant for 4 wk (49, 50).

DNB colitis was induced on day 18 after pellet implantation, and mice were killed 3 days postcolitis. Additional mice received 17β-estradiol alone (0.5 mg/pellet) or with tamoxifen (5 mg/pellet), or placebo via 21-day release pellets implanted subcutaneously. DNB colitis was induced on day 7 after pellet implantation, and mice were killed 14 days postcolitis.

Induction of colitis. Mice were anaesthetized with enflurane. A 10-cm long PE-90 tubing (Clay Adams, Parsippany, NJ), attached to a tuberculin syringe, was inserted 3.5 cm into the distal colon. Colitis was induced by the administration of 100 μl of a 4-mg DNB solution (ICN Biomedicals, Aurora, Ohio) in 50% ethanol. Control mice (without colitis) received saline administered as above. Mice with colitis were supplied with 8% sucrose and 0.1% saline in drinking water until assayed. All experiments were performed within 1 wk of tissue collection. The cytokines that characterize DNB colitis are usually detected during later phases, interferon-γ (IFN-γ) and interleukin-13 (IL-13), were only measured in tissue obtained 14 days post-DNB colitis.

Total cellular RNA was isolated using the single-step method (10). The concentration of RNA was determined measuring absorbance at 260 nm, and its purity was assessed using the absorbance ratio of A260/280 spectrophotometrically. RNA was stored at −70°C until the RT-PCR. mRNA was reversely transcribed as described previously (21) to yield cDNA, which was amplified by PCR using gene-specific primers.

PCR reactions were performed in a total volume of 50 μl in the presence of Taq DNA polymerase (GIBCO-BRL), 10 nM 2-deoxynucleotide 5′-triphosphate (GIBCO-BRL), and 15 pg of 5′ and 3′ primers. Amplification was performed by 25 cycles for the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH), 39 cycles for IFN-γ and IL-13, and 35 cycles for ICAM-1, consisting of denaturation at 94°C for 45 s, primer annealing at 55°C for 45 s, and primer extension at 72°C for 60 s with the use of a Perkin-Elmer thermal cycler (480, Branchburg, NJ). The following primers specific for GAPDH and cytokines were used. GAPDH: up 5′-CCATGGAAAGGCTGGG-3′, down 5′-CAGATGTTCATGATTACCT-3′ (21); IFN-γ: up 5′-CATGGCTGTTCGTTGCTGTTAC-3′, down 5′-TCGGATGCTACTGATGGC-3′ (23); IL-13: up 5′-CTTCTGGCTGTTGGTGTCG-3′; down 5′-GATGGCAATTTGAGTGGTTAC-3′ (38); ICAM-1: up 5′-GTAGAGGTGACTGAGGTT-3′, down 5′-ATACAGCAGTGCACTTCC-3′ (34).

To exclude the amplification of genomic DNA contaminating the samples, experiments were also performed using RNA as the substrate for PCR. After amplification of 15 ul of PCR, products were separated electrophoretically in 2% agarose gel, visualized by ethidium bromide staining, and photographed using a Polaroid Land film type 55 (Kodak, Rochester, NY). The negatives were used for densitometrical quantification of hand intensity using the Kodak Digital Science 1D 2.0 Image Analysis Software. Results were normalized to the housekeeping gene and expressed as ratio of cytokines to GAPDH mRNA expression.

DSS Colitis

In contrast to DNB colitis, acute DSS colitis does not require the presence of T cells to induce damage (17). DSS damage seems to be more dependent on the perpetuation of inflammation by translocation of intestinal flora (2). Macrophages have also been involved in DSS-induced damage (17). To investigate some of the possible mechanisms whereby estradiol can affect severity of DSS colitis, we used op/op mice that totally lack the population of macrophages dependent on M-CSF-1, in addition to C57Bl/6 mice.

Treatment groups and induction of colitis. Female C57Bl/6 op/op and op/+ mice (n = 5 to n = 10) received 17β-estradiol alone (0.5 mg/pellet) or placebo via 21-day release pellets implanted subcutaneously (IRA).

Colitis was induced on day 16 after pellet implantation by DSS 5% mol wt 40,000 (ICN Biomedicals) in drinking water.
Mice were killed 5 days postcolitis. Control mice (without colitis) received normal drinking water.

**Disease severity and histological scores.** The colon was removed and opened longitudinally, and macroscopic damage was immediately assessed. Tissue was obtained from the sigmoid colon and processed as above for histological assessment. Macroscopic and histological scores were performed using a previously described scoring system for DSS colitis (12) slightly modified to score separately for rectal and gross colonic bleeding.

**TNF-α measurement.** Serum samples were obtained from C57Bl/6 mice with and without DSS colitis and stored at −20°C until TNF-α protein measurement by ELISA using a commercial kit (Quantikine M murine; R&D Systems, Minneapolis, MN). MCSF-1 mutant mice lack the population of macrophages that are the main source for proinflammatory cytokines, such as TNF-α. Because a number of other cells may also produce this cytokine, we used RT-PCR to measure mRNA as described above and ELISA to measure TNF-α protein in colonic tissue.

The following amplification cycles and primers specific for housekeeping gene β-actin and TNF-α were used. β-actin: 27 cycles, up 5’-CTTCTCTGGCATGAGTCTCG-3’, down 5’-GGAGCAATGATCTTGATCTTC-3’ (31); TNF-α: 35 cycles, up 5’-GCCCTGGCTTCTTCTCTG-3’, down 5’-GGCAATT-CAGTTCCAGGTGCT -3’ (29).

For TNF-α protein measurement, colonic tissue was homogenized using a Bronkmann polytron for 15 s in 1 ml of a solution containing 100 μM of PMSF and aprotinin (10 μg/ml) (11). Homogenates were then centrifuged at 12,000 rpm for 10 min at 4°C. Supernatants were stored at −20°C until assayed by ELISA as above.

**Statistical Analysis**

Nonparametric data are presented as medians and 95% confidence intervals and parametric data as means ± SD as stated in the table legends. Box plots depict 5th, 25th, 50th (median), 75th, and 95th percentiles. On the basis of data distribution, statistical testing was performed using the Mann-Whitney U-test for unpaired nonparametric data or Student’s t-test for unpaired parametric data. Multiple comparisons were performed using the Friedman test followed by Wilcoxon-Wilcoxon.

**RESULTS**

**Effects of Estrogen on Mice Without Colitis**

In mice without colitis, estrogen pretreatment resulted in more similar macroscopic and histological scores and MPO activities than in placebo-treated mice (data not shown).

**Effects of Estrogen on Body Weight**

Eighteen days after pellet implantation and before the induction of colitis with DNB or DSS, treatment with 17β-estradiol alone or with tamoxifen increased body weight compared with placebo, tamoxifen alone, and 1α-estradiol (Table 1). This effect was more evident in mice in which colitis was induced 18 days rather than 7 days after pellet implantation (data not shown).

**Effects of Estrogen at Day 3 Post-DNB-Induced Colitis**

In mice with DNB colitis, treatment with 17β-estradiol alone reduced the macroscopic scores by 50% compared with placebo or 17α-estradiol, and by 40% compared with tamoxifen plus 17β-estradiol or tamoxifen alone. Consistently, 17β-estradiol treatment reduced the histological scores by 50% compared with placebo, 17α-estradiol or tamoxifen alone, and by 30% compared with tamoxifen plus 17β-estradiol. However, 17β-estradiol treatment alone did not prevent all damage induced by DNB/ethanol, because scores remained higher than in mice without colitis, and was reflected by edema, gland proliferation, and mild architectural distortion (Figs. 1 and 2, A and B).

17β-Estradiol treatment decreased PMN infiltration in mice with DNB colitis as reflected by a 15-fold reduction in MPO activity compared with placebo-treated mice with colitis (Fig. 1C). This marked reduction in MPO activity, suggested a direct antioxidant effect of 17β-estradiol in addition to the histological finding of a reduced PMN infiltration. The antioxidant effect was confirmed by a reduction in lipid peroxidation from 580 (420–640) nmol MDA/g in placebo-treated mice with DNB colitis, to 282 (75–388) nmol MDA/g in 17β-estradiol-treated mice with DNB colitis (P < 0.04).

The ICAM-1-to-GAPDH ratios 3 days post-DNB colitis are shown in Table 2. ICAM-1 mRNA expression was decreased in 17β-estradiol-treated mice compared with placebo-treated mice with DNB colitis. ICAM-1 mRNA expression was similar in 17β-estradiol-treated mice with DNB colitis and in mice with no colitis.

**Effects of Estrogen at Day 14 Post-DNB-Induced Colitis**

Figure 2, C and D, depicts minimal inflammation in a mouse treated with 17β-estradiol alone and severe

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**Table 1. Influence of hormonal treatment on body weight before and after induction of colitis with DNB and DSS**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>3 Days Post-DNB</th>
<th>16 Days After Pellet (DNB)</th>
<th>5 Days Post-DSS</th>
<th>16 Days After Pellet (DSS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>15.7±1.20†</td>
<td>18.3±1.2</td>
<td>18.0±1.2</td>
<td>19.0±1.4</td>
</tr>
<tr>
<td>17β-Estradiol</td>
<td>21.0±1.60</td>
<td>21.5±1.4*</td>
<td>19.0±1.0†</td>
<td>21.0±1.0</td>
</tr>
<tr>
<td>Tamoxifen + 17β-estradiol</td>
<td>18.4±1.10†</td>
<td>22.5±1.5*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tamoxifen</td>
<td>16.6±1.52†</td>
<td>19.4±0.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17α-Estradiol</td>
<td>16.8±0.82†</td>
<td>19.4±1.4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are mean grams body weight ± SD. DNB, dinitrobenzene sulfonic acid; DSS, dextran sodium sulfate. †P < 0.05 vs. placebo; ‡P < 0.05 vs. before colitis
Estradiol alone decreased MPO activity significantly (**P < 0.001 vs. 17β-estradiol; #P < 0.01 vs. placebo).

**Effects of Estrogen on DSS-Induced Colitis**

**C57Bl/6 mice.** Table 3 shows the disease severity and histological scores at day 5 in mice with DSS colitis. 17β-Estradiol treatment had opposite effects on the severity of DSS colitis when compared with DNB colitis. In mice with DSS colitis treated with 17β-estradiol, the disease severity score and histological scores increased by 100 and 67%, respectively, with respect to placebo-treated mice with DSS colitis (P = 0.01).

**MCSF-1 deficient mice (op/op).** Although there was a trend toward lower disease severity scores and histological scores in op/op mice with DSS colitis as a whole, these differences did not achieve statistical significance.

17β-Estradiol treatment in op/+ mice increased disease severity scores by 86% and histological scores by 56% after induction of DSS colitis, in a similar way as it did in C57Bl/6 mice (Fig. 5). However, mice lacking macrophages dependent on MCSF-1 and treated with 17β-estradiol also had increased disease severity scores by 150% and increased histological scores by...
80% when compared with op/op mice treated with placebo (Fig. 5).

**TNF-α in C57Bl/6 Mice and MCSF-1 Deficient Mice (op/op) Mice**

Placebo-treated C57Bl/6 mice with DSS colitis and C57Bl/6 mice without colitis had undetectable TNF-α plasma levels below the lowest standard of the assay. In contrast, in estradiol-treated C57Bl/6 mice with DSS colitis, TNF-α plasma levels were detected in all mice [130 pg/ml (95–230)].

Low levels of mRNA for TNF-α and protein TNF-α measured by ELISA were detected in op/op mice treated with DSS colitis. Overall, op/op mice treated with 17β-estradiol tended to have higher cytokine levels in colonic tissue than mice treated with placebo (Fig. 6).

**DISCUSSION**

In light of the conflicting clinical data regarding sex hormones and IBD, we examined the effect of supra-physiological doses of 17β-estradiol on DNB- and DSS-induced colitis, two experimental models with different underlying pathophysiological mechanisms. Three days after induction of DNB colitis, 17β-estradiol reduced the severity of colitis, the infiltration of colonic tissue with PMN cells (assessed both by histology and MPO activity), the degree of lipid peroxidation, and the level of mRNA expression of ICAM-1. 17β-Estradiol also reduced the severity of tissue damage, the infiltration of tissue with mononuclear cells, and the mRNA expression of IFN-γ, IL-13, and ICAM-1, 14 days postcolitis. In contrast, 17β-estradiol increased the disease severity and histological scores in DSS colitis in C57Bl/6 and in MCSF-1-deficient mice (op/op).

Our results indicate that the anti-inflammatory effect of 17β-estradiol in DNB colitis was mediated by ERs. Tamoxifen is a selective ER modulator with both agonist and antagonist activities depending, in part, on the target tissue and the estrogenic milieu (29). Because the effect of tamoxifen as an ER antagonist in the gastrointestinal tract is unknown and because we wished to evaluate the role of ER, we also used 17α-estradiol, an inactive estrogen that binds to ERs without activating transcription. Tamoxifen alone or with 17β-estradiol inhibited the protective effect of 17β-estradiol on macroscopic and histological scores 3 days postcolitis. At later stages, tamoxifen with 17β-estradiol significantly inhibited the protective effect of 17β-

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**Table 2. ICAM-1-to-GAPDH mRNA ratio in colonic tissue from mice 3 days post-DNB colitis**

<table>
<thead>
<tr>
<th>Condition</th>
<th>No Colitis</th>
<th>Placebo/DNB-Colitis</th>
<th>17β-Estradiol/DNB Colitis</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICAM-1-to-GAPDH mRNA ratio</td>
<td>0.7(0.61–0.78)</td>
<td>0.9(0.85–0.93)*†</td>
<td>0.6(0.65–0.76)</td>
</tr>
</tbody>
</table>

Data are medians (95% confidence interval). ICAM-1, intercellular adhesion module-1; GAPDH, glyceraldehyde-3-phosphate dehydrogenase. *P = 0.04 vs. no colitis, †P = 0.006 vs. 17β-estradiol.
estradiol on histological scores, but there was only a trend toward inhibiting the protective effect in macroscopic scores. This may be because, in contrast to histological damage, macroscopic damage 14 days post-DNB colitis is minimal, and differences between groups are difficult to detect. The absence of a protective effect in mice with DNB colitis treated with tamoxifen or 17β-estradiol supports the fact that the anti-inflammatory effect of 17β-estradiol involves ERs.

Sex steroids have antioxidant properties that are not blocked by ER antagonists (4, 15) and that could contribute to the protective effect of 17β-estradiol in DNP colitis.

Table 3. Disease severity and histologic scores in C57B1/6 mice post-DSS colitis treated with 17β-estradiol or placebo

<table>
<thead>
<tr>
<th>C57B1/6</th>
<th>Placebo</th>
<th>17β-Estradiol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disease severity</td>
<td>2.7(2–3)</td>
<td>5.5(5–6)*</td>
</tr>
<tr>
<td>Histologic score</td>
<td>1.2(1–2)</td>
<td>2.0(2–4)†</td>
</tr>
</tbody>
</table>

Data are medians (95% confidence interval); n = 10. Estrogen treatment increased disease severity and histologic scores compared to placebo. *P < 0.01 vs. placebo; †P = 0.001 vs. placebo.

DNB colitis is minimal, and differences between groups are difficult to detect. The absence of a protective effect in mice with DNB colitis treated with tamoxifen or 17α-estradiol supports the fact that the anti-inflammatory effect of 17β-estradiol involves ERs.

Sex steroids have antioxidant properties that are not blocked by ER antagonists (4, 15) and that could contribute to the protective effect of 17β-estradiol in DNP colitis.

Fig. 3. A: macroscopic scores in mice 14 days post-DNB-colitis and in mice without colitis. Fourteen days postcolitis, treatment with 17β-estradiol alone led to lower scores than placebo (P = 0.03 vs. 17β-estradiol). B: histological scores in mice 14 days post-DNB-colitis and in mice without colitis. Fourteen days postcolitis, treatment with 17β-estradiol alone led to lower scores than placebo and than tamoxifen plus 17β-estradiol. (P < 0.02 vs. 17β-estradiol). C: MPO activity (units/g tissue) in mice 14 days post-DNB-colitis and in mice without colitis. Treatment with 17β-estradiol alone decreased MPO activity significantly (P < 0.03 vs. 17β-estradiol). Mice with colitis, treated with tamoxifen plus 17β-estradiol had higher MPO activity than 17β-estradiol-treated mice, but lower than placebo-treated mice (P < 0.05 vs. placebo).

Fig. 4. A: cytokine-to-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA ratios 14 days post-DNB-colitis. In mice with colitis, treatment with 17β-estradiol decreased cytokine ratios of interferon-γ (IFN-γ), interleukin-13 (IL-13), and intercellular adhesion module-1 (ICAM-1). IFN-γ: P = 0.01 vs. 17β-estradiol and vs. without colitis; #P < 0.05 vs. 17β-estradiol and vs. without colitis. IL-13: P < 0.05 vs. 17β-estradiol; #P = 0.05 vs. tamoxifen plus 17β-estradiol. ICAM-1: P = 0.01 vs. 17β-estradiol and vs. without colitis. B: examples of bands for IFN-γ, IL-13, and ICAM-1 in 2 mice treated with 17β-estradiol and in 2 mice treated with placebo.
tribute to the low MPO activity and lipid peroxidation seen during 17β-estradiol treatment. Our results suggest that a pure antioxidant effect is not, however, the sole anti-inflammatory mechanism of 17β-estradiol. 17β-Estradiol did not decrease the severity of DNB colitis, although it possesses antioxidant activity. We believe that the major anti-inflammatory effect of 17β-estradiol in DNB colitis is the inhibition of neutrophil recruitment during the early stages of colitis. This is supported by the following findings. Three days post-colitis, ICAM-1 mRNA expression was lower in mice treated with 17β-estradiol, and histological examination revealed a marked decrease in PMN infiltration. Also, MPO activity was close to zero and lipid peroxidation levels were markedly reduced in 17β-estradiol-treated mice compared with placebo-treated mice. Our results are in accordance with previous reports in systems other than the gastrointestinal tract, where an inhibitory effect of estrogen on PMN infiltration has also been suggested. 17β-Estradiol was shown to reduce PMN chemotaxis and PMN infiltration at several sites of inflammation (7, 15, 56, 66) and to decrease the level of adhesion molecules, such as sICAM-1 and sVCAM-1 in cardiovascular disease (9, 61).

Immune response in DNB colitis results from a delayed-type hypersensitivity (DTH) response reaction against haptenized colonic proteins and is characterized by a T helper (Th1) cytokine response (19). In contrast, the mechanism by which DSS induces acute colitis is unclear. It seems to result from an alteration in the colonic epithelium with resulting bacterial translocation that perpetuates inflammation rather than from an alteration of B and T cell responses because acute DSS colitis develops in SCID mice. In vitro immune and macrophages have, therefore, been suggested to play a role (17). Estrogen has been reported to suppress T cell-dependent DTH and activation of inflammatory cells producing TNF-α and IFN-γ (51–53, 58). Our data suggest a downregulation of the Th1 response associated to DNB colitis as reflected by decreased IFN-γ mRNA expression 14 days postcolitis in 17β-estradiol-treated mice. The finding that IL-13 mRNA expression was also decreased in mice treated with 17β-estradiol alone was somewhat unexpected. On the basis of a recent report that estrogen has biphasic effects on immune responses (63), 17β-estradiol at high doses would suppress Th1 responses and

Fig. 5. A: disease severity score in macrophage colony-stimulating factor-1 (MCSF-1)-deficient mice (op/op) and their heterozygous controls (op/+). Treatment with 17β-estradiol increased disease severity score in both op/op and op/+ mice (*P < 0.01 vs. placebo). There was a trend toward higher scores in 17β-estradiol-treated op/+ mice compared with op/op mice. B: histologic score in MCSF-1 deficient mice (op/op) and their heterozygous controls (op/+) after DSS colitis. Treatment with 17β-estradiol increased histology scores in both op/op and op/+ mice (*P < 0.01 vs. placebo).

Fig. 6. A: tumor necrosis factor (TNF)-α-to-actin mRNA ratios post-DSS-colitis in MCSF-1 deficient mice (op/op). B: TNF-α measurements by ELISA post-DSS-colitis in MCSF-1 deficient mice (op/op). Cytokine ratios and cytokine levels tended to be higher in mice treated with 17β-estradiol but this did not achieve statistical significance (P = 0.4, P = 0.09, respectively).
promote Th2 responses, and this could also occur in the gut during an episode of inflammation. It is possible, however, that Th3 or Th2 cytokines, other than IL-13, not measured in this study, are increased by high doses of estrogen. We believe that in this study, the effect of 17β-estradiol on mRNA expression of Th1 and Th2 cytokines is a consequence of the lower initial acute inflammation, rather than a direct effect of estradiol on Th cells. This could also explain the fact that ICAM-1 expression on lymphoid cells may differ from the effect of estradiol on integrin expression on lymphoid cells. This matter should be further investigated.

A number of studies have suggested that estrogen modifies macrophage function and the production of inflammatory mediators (16, 26, 39, 55). However, we found an increase in disease severity score and histological scores in both op/+ and op/op mice with DSS colitis treated with 17β-estradiol compared with mice with DSS colitis treated with placebo. The enhanced colitis severity seen in 17β-estradiol-treated op/op mice, excludes a role for MCSF-1 macrophages and implicates other mechanisms. Although there was a clear increase in disease severity score and histological scores, in 17β-estradiol-treated mice with DSS colitis, MPO activity was low (data not shown). In fact, in placebo-treated mice, MPO activity was overall 10-fold lower in mice with DSS colitis than in mice with DNB colitis. This suggests that the antioxidant effect and/or the inhibition of neutrophil infiltration by 17β-estradiol would be less prominent in DSS colitis than in DNB colitis. Op/op mice have resident MCSF-1-independent macrophages with preserved antigen presentation and phagocytosis (65). Estradiol has been shown to sensitize immune cells to lipopolysaccharide (LPS), and females treated with pharmacological doses of estradiol, are more sensitive to liver damage by LPS (20, 30, 37). Because translocation of luminal bacteria may play an important role in perpetuating DSS colitis, it is possible that the increased severity may relate to the sensitizing effects of estradiol to bacterial endotoxins. Interestingly, TNF-α plasma levels were increased in estradiol-treated C57Bl/6 mice at day 5 post-DSS colitis compared with placebo-treated mice with colitis and mice without colitis. Consistent with previous reports on plasma TNF-α levels in op/op mice (65), we detected low levels of TNF-α in colonic tissue from op/op mice. Despite the low levels, TNF-α tended to be higher in estradiol-treated op/op mice with DSS colitis than in placebo-treated op/op mice with colitis. Natural killer cells, lymphocytes, mast cells, basophils, and eosinophils may be an alternative source of TNF-α in these mice.

Estradiol treatment alone, or with tamoxifen, led to a 15% increase in body weight before the induction of colitis compared with mice treated with placebo. The effect on body weight was more pronounced if estrogen pretreatment was more prolonged (18 instead of 7 days). However, it is very unlikely that this increase in body weight could have affected the severity of colitis significantly. First, when estradiol was administered with tamoxifen, an increase in body weight was also noted. Still, colitis severity was increased compared with 17β-estradiol treatment alone, and significant weight loss was observed after the induction of colitis. Second, despite an increase in body weight before the administration of DSS in 17β-estradiol-treated mice, severity of DSS colitis and weight loss were increased when compared with placebo-treated mice with DSS colitis.

In conclusion, this is, to our knowledge, the first demonstration that estrogen has complex effects on intestinal inflammation. In DNB colitis, supraphysiological doses of 17β-estradiol decreased recruitment of PMN cells at early stages of colitis, probably through an ER-mediated mechanism. An antioxidant effect of 17β-estradiol also contributed to the reduced tissue damage. At later stages of DNB colitis, a downregulation of the DNB-associated Th1 response was observed. Our data do not support the hypothesis that 17β-estradiol-induced increased severity of DSS colitis involves activation of MCSF-1 macrophages. Other mechanisms whereby estrogen may affect intestinal inflammation remain to be investigated. These include a possible effect on neuropeptide release and transmission, effects on smooth muscle contractility, and effects on the healing and repair process. Recent reports on the genetic changes that may induce susceptibility for IBD suggest a link between a defective innate immune response to bacterial components and the development of chronic inflammation (28, 44). The NOD2 gene encodes for a protein that makes nuclear factor-κB responsive to bacterial LPS. It could be hypothesized that the effect of sex hormones could also depend on the genetic background or specific mutations that underlie the development of IBD in a given individual. Some mutations may involve mechanisms modulated by sex hormones and some may not. Taken together, our results indicate that the effect of estrogen on colitis depends on the manner in which inflammation is induced. This may explain the apparent conflicting clinical literature on gender, pregnancy, and sex hormones in IBD.

This study was supported, in part, by a grant from the Canadian Institutes for Health Research (to S. M. Collins).

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