Beneficial effects of estrogen treatment in the HLA-B27 transgenic rat model of inflammatory bowel disease

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Harnish, Douglas C., Leo M. Albert, Yelena Leathurby, Amy M. Eckert, Agnes Ciarletta, Marion Kasaian, and James C. Keith, Jr. Beneficial effects of estrogen treatment in the HLA-B27 transgenic rat model of inflammatory bowel disease. Am J Physiol Gastrointest Liver Physiol 286: G118–G125, 2004. First published September 4, 2003; 10.1152/ajpgi.00024.2003.—A well-established model of bowel inflammation is the HLA-B27 transgenic rat that exhibits a spontaneous disease phenotype resulting in chronic diarrhea caused by immune cell activation. Estrogens have previously been shown to modulate the immune system, and both estrogen receptors (ERα and ERβ) are present in the intestine and cells of the immune system. Therefore, the ability of estrogen to ameliorate disease progression in the HLA-B27 transgenic rat was determined. HLA-B27 transgenic rats with chronic diarrhea were treated with 17α-ethynyl-17β-estradiol (EE) for 5 days. EE treatment dramatically improved stool scores after only 3 days. Histological scores of the degree of ulceration, inflammatory cell infiltration, fibrosis, and lesion depth of the colon were also improved by EE treatment. Because neutrophil infiltration into the colon is involved in the development and propagation of disease, myeloperoxidase (MPO) activity was measured. MPO levels were reduced by 80% by EE treatment. Cotreatment with the pure ER antagonist ICI-182780 (ICI) blocked the effects of EE on stool character, MPO activity, and histology scores, strongly suggesting that the activity of EE is mediated through ER. Mast cell proteases in colonos from estrogen-treated rats. In addition, a direct effect of estrogen on bone marrow-derived mast cell activity was demonstrated, suggesting that ER-mediated inactivation of mast cells may contribute to the improvement in the clinical sign and histological scores in this model.

mast cells; estrogen receptor

INFLAMMATORY BOWEL DISEASE (IBD) is a broad term encompassing four diseases: Crohn’s disease, ulcerative colitis, indeterminate colitis, and infectious colitis. A well-established model of chronic bowel inflammation is the HLA-B27 transgenic rat that exhibits a spontaneous inflammatory disease phenotype resulting in chronic diarrhea (28). This model represents a chronic intestinal inflammation with associated arthritis induced by the human class I major histocompatibility allele HLA-B27 that is strongly associated with human disease. Microscopic indications of inflammation include neutrophil infiltration, as measured by myeloperoxidase (MPO) activity, and various changes in colonic architecture such as the development of lesions, ulcerations, fibrosis, and crypt abscesses.

In both the HLA-B27 transgenic rat and in humans, bacteria and gut inflammation play a predominant role in the progression of the disease. A large number of studies have demonstrated that the enhanced secretion of proinflammatory cytokines by mast cells, T cells, dendritic cells, and macrophages appears to be an important factor in the initiation and perpetuation of intestinal inflammation (reviewed in Ref. 22). These cytokines recruit immune cells, thereby increasing leukocyte trafficking as well as intestinal permeability (42, 60). One of the key transcription factors responsible for cytokine expression and inflammatory gene expression in general is NF-κB. Current therapies for IBD, such as glucocorticoids, 5-amino-salicylic acid, and recombinant anti-TNF-α, are thought to be effective in part due to their ability to block NF-κB activation (4, 47, 48, 62). NF-κB is a dimeric transcription factor composed of homodimeric and heterodimeric complexes of the Rel family of proteins: p65 (Rel A), p50/105, c-Rel, p52/100, and Rel B. Binding of inhibitory protein-κB (IκB) to NF-κB masks the NF-κB nuclear localization signal and sequesters NF-κB in a nonactivated form in the cytoplasm. Cell activation by a variety of extracellular signals such as oxidative stress, cytokines, and lipopolysaccharide induces a cascade of events that lead to degradation of IκB, and activated NF-κB then translocates to the nucleus where it binds to DNA elements in the promoters of a number of proinflammatory gene families (51). Activated NF-κB has been detected in colonic biopsy samples from patients with Crohn’s disease and ulcerative colitis, suggesting a pathological role for this transcription factor (45, 61, 63). This is supported in experiments in which specific targeting against the p65 subunit of NF-κB by antisense or peptide therapy in mouse models of IBD abrogates the clinical and histological signs of the disease (23, 45). These data suggest that targeting NF-κB may be a viable strategy for treating IBD (reviewed in Ref. 44).

Estrogen has been demonstrated to have anti-inflammatory activity in a number of experimental models (14, 19, 21, 39) as well as numerous reports in cell-based assays (1, 20, 73). This anti-inflammatory activity of estrogen has been attributed to interference with NF-κB activity (56, 66). Two estrogen receptors have been identified (ERα and ERβ), and both are present in most tissues, including the intestine (10, 11) and cells of the immune system (33, 59, 72). These two receptors have a nearly identical DNA binding domain, both activate transcription through binding to identical ER response elements (13, 50), and both can antagonize NF-κB functional activity (6, 53).

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In light of this potential NF-κB antagonism, we decided to test estrogen action in the HLA-B27 transgenic rat model of IBD because this model develops intestinal inflammation that is linked to NF-κB activation in the enterocytes (27). Our results demonstrate that after only 3 days of treatment estrogen can reverse the chronic diarrhea in maximally diseased rats. The effect of estrogen is blocked by coadministration of the pure ER antagonist ICI-182870 (ICI), demonstrating the necessity of the ER and that the activity is not due to the antioxidant properties associated with estrogen. The beneficial effect of estrogen results in an improvement in colonic histology scores as well as a reduction in colonic MPO activity after 5 days. Mast cell-specific protease genes were decreased in the estrogen-treated rat colons compared with vehicle, suggesting that estrogen may exert its activity in part through modulation of mast cell activity. This was supported with experiments in bone marrow-derived mast cells (BMMC) in which cytokine production and degranulation were inhibited in the presence of 17β-estradiol (E2).

MATERIALS AND METHODS

Experimental design. Male HLA-B27 transgenic rats were obtained from Taconic and provided unrestricted access to food (PMI Lab diet 5001) and water. The details of treatment, assessment of clinical signs, and evaluation of colonic histological lesions of the HLA-B27 transgenic rat by our laboratory have been described previously (54). Briefly, 22- to 26-wk-old rats were placed into three groups with four rats per group and were dosed orally once per day for 5 days with estradiol (EE; 10 μg/kg po), EE plus ICI (10 mg/kg po), or EE plus ICI (10 mg/kg po) for 5 days. Stool scoring was as follows: diarrhea = 3; soft stool = 2; normal stool = 1. At the end of the study, sections of colon were prepared for histological analysis and additional sections were analyzed for MPO activity.

Histological analysis. Colonic tissue was immersed in 10% neutral buffered formalin. Each specimen of colon was separated into four samples for evaluation. The formalin-fixed tissues were processed in a Tissue Tek vacuum infiltration processor (Miles, West Haven, CT) for paraffin embedding. The samples were sectioned at 5 μm and then stained with hematoxylin and eosin for blinded histological evaluation using a scale modified after Boughton-Smith (7). Sections of colonic tissue were evaluated for several disease indicators and given relative scores. Four parameters were taken into account: ulceration, 0 to 2; inflammation, 0 to 3; depth of lesion, 0 to 3; and fibrosis, 0 to 2 (Table 1). The total histology score reflects the sum of all four parameters analyzed. After the scores were completed, the samples were unblinded and data were tabulated and analyzed by ANOVA linear modeling with multiple mean comparisons. Histological results are presented as means ± SE, and differences are considered significant if P < 0.05.

MPO activity. MPO activity was assessed as a measure of neutrophil infiltration into the colon (8). MPO levels were determined as previously described (54). The data are reported as means ± SE, and differences are considered significant at P < 0.05 as assessed by ANOVA testing with multiple mean comparisons.

NF-κB DNA binding experiments. Colonic nuclear extracts were prepared as described previously (29). Protein-DNA complexes were analyzed by TransAM NF-κB ELISA-based kit from Active Motif.

Mast cell protease gene expression. Colon total RNA was prepared using TRIzol reagent (GIBCO BRL). Mast cell protease gene regulation was determined by real time RT-PCR using an ABI PRISM 7700 Sequence Detection System according to the manufacturer’s protocol (Applied Biosystems) and using the Qiagen QuantiTect Probe RT-PCR kit. The following primers were used: mast cell protease 1 (RMC-1), 5′-GCACACTGCAAGGAAGAGAA, 3′-TTGTGTTTATCTGCGATCTCA, probe-CACAGTCACCCTCGGAGCTCATGAC; mast cell protease 2 (RMC-2), 5′-CGATTCCGTATAAAATGCGAG, 3′-GTGTGTGTTGATTTGCTGCTTCTCTTCT, probe-CGCTCAGCGGCGCATCTGCTGG; mast cell protease 3 (RMC-3), 5′-GACGTGAGGAATTCTCGGTGTG, 3′-CGATCTTCAGAGGGCCATG, probe-TGGAGTGTATCTTTCTCCTGGC; mast cell protease 4 (RMC-4), 5′-GCACACTGATGGAAGGGGA, 3′-TTGTGTGATTCTCTGCTATCA, probe-TACTTGTCACCCCTCGGACCTGAC; and carboxypeptidase A3 (CPA): 5′-AAACGGCAAGAAGACCCAA, 3′-AAACCCGTTCAGACCCCAA, probe-TCAAACCTGCACTGGCATTGCAGCCTCA. The data were analyzed by using Sequence Detector version 1.7 software (Applied Biosystems) and were normalized to GAPDH by using the Applied Biosystems primer set. The data shown represent the means ± SE for each group after ANOVA linear modeling with multiple mean comparisons.

BMMC culture experiments. Mast cells were derived from femurs and tibias of 8- to 12-wk-old C57BL/6 mice. From cultures of total bone marrow cells grown in DMEM with 10% FCS, mast cells were selectively expanded by using murine IL-3 (20 ng/ml; R&D Systems) and stem cell factor (50 ng/ml; R&D Systems). By wk 4 of culture, cells were >95% mast cells. BMMC were then maintained in murine IL-3 (50 ng/ml) in the absence of stem cell factor and used in experiments from 4 to 12 wk of culture.

Table 1. Histological lesion scoring

<table>
<thead>
<tr>
<th>Criterion</th>
<th>Severity-Determined Scoring</th>
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<tbody>
<tr>
<td></td>
<td>Severity</td>
</tr>
<tr>
<td>Ulceration</td>
<td>None</td>
</tr>
<tr>
<td>Small ulcers</td>
<td>1</td>
</tr>
<tr>
<td>Large ulcers</td>
<td>2</td>
</tr>
<tr>
<td>Inflammation</td>
<td>None</td>
</tr>
<tr>
<td>Mild</td>
<td>1</td>
</tr>
<tr>
<td>Moderate</td>
<td>2</td>
</tr>
<tr>
<td>Severe</td>
<td>3</td>
</tr>
<tr>
<td>Depth of lesion</td>
<td>None</td>
</tr>
<tr>
<td>Submucosa</td>
<td>1</td>
</tr>
<tr>
<td>Muscularis propria</td>
<td>2</td>
</tr>
<tr>
<td>Serosa</td>
<td>3</td>
</tr>
<tr>
<td>Fibrosis</td>
<td>None</td>
</tr>
<tr>
<td>Mild</td>
<td>1</td>
</tr>
<tr>
<td>Severe</td>
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For use in degranulation or cytokine production assays, BMMC were pretreated with E2 for 3 days. IgE anti-dinitrophenyl (DNP) (0.2 mg/ml; Sigma) was added for the final 24 h of this incubation. For degranulation, cells were washed into PACM buffer (in mM: 25 PIPES, pH 7.2, containing 110 NaCl, 5 KCl, 5 CaCl2, and 2.5 MgCl2), then challenged with DNP-BSA (Molecular Probes) for an additional 30 min in the presence of E2. Supernatants were assayed for β-hexosaminidase by incubation overnight with an equal volume of 1.3 mg/ml p-nitrophenyl N-acetyl-β-D-glucosaminide (Sigma) in 0.08 M citrate, pH 4.5, addition of NaOH to stop the reaction, and absorbance reading at 405 nM. Maximum release was determined by lysing the cells with Triton X-100. For cytokine production assays, cells pretreated with E2 for 3 days were challenged with DNP-BSA for 24 h in the presence of E2. Cytokines were assayed by using specific ELISA kits (R&D Systems). The data shown represent the means ± SE for each group after ANOVA linear modeling with multiple mean comparisons.

Fig. 3. Myeloperoxidase (MPO) activity in HLA-B27 transgenic rat colons treated with estrogen. MPO activity (U/g tissue) in rats 5 days after vehicle, EE, or EE plus ICI administration is shown. Treatment with EE alone significantly decreased MPO activity, which was antagonized by ICI coadministration (*P < 0.05 vs. vehicle).

RESULTS

Estrogen treatment improves stool character. Gastrointestinal inflammation resulting in clinical signs of diarrhea may be manifested through the activation of NF-κB, and blockade of its signaling pathway can result in improved signs and symptoms of disease (23, 45). Since estrogen has been shown to interfere with NF-κB in various systems (29), we investigated whether it would improve disease symptoms in the HLA-B27 transgenic rat model of IBD. The 22-wk-old HLA-B27 transgenic rats demonstrating chronic diarrhea were treated with EE (10 mg/kg po) for 5 days. After 3 days of treatment, normal stool character was achieved, but no resolution of the disease was observed in vehicle-treated rats throughout the course of the study (Fig. 1). Cotreatment with the pure ER antagonist ICI (10 mg/kg) resulted in a complete abrogation of the beneficial effects of EE, suggesting that the ER is required for the observed improvements in stool character.

Estrogen treatment improves colon histology. To determine whether the improvement in stool scores by EE resulted in improved colon histology, a variety of disease parameters were assessed, including the degree of ulceration, the number of inflammatory cell infiltrates, depth of lesions, and the amount of fibrosis observed. When the lesion scores were unblinded, tabulated, and analyzed, reductions in all aspects of colonic damage were seen in the specimens from EE-treated animals (Fig. 2A). Representative photomicrographs of colon specimens are seen in Fig. 2B. Although the degree of ulceration tended to be decreased, this change did not reach significance. With crypt disruption, an increased amount of fibrous, stromal tissue is seen between the crypts. With EE treatment, the normal crypt structure returns, inflammatory cells are decreased, and abundant goblet cells are seen. With the addition of ICI, these effects are lost.

Estrogen treatment reduces MPO activity. Immune cell infiltration into the colon is believed to be involved in the development and propagation of IBD (22), and the histological findings showed a reduced number of inflammatory cell infiltrates in the mucosal layer of the colon. In support of this, MPO results demonstrate that, consistent with the clinical sign of stool character, colon lesions were reduced and epithelial integrity was enhanced by EE treatment after only 5 days.
an 80% inhibition of MPO activity. This effect was not due to the antioxidant properties of EE (41) because cotreatment with ICI blocked the effect of EE on MPO activity. These results suggest that EE treatment may restore colon function by interfering with the recruitment and subsequent activation of leukocytes in the colon.

Estrogen reduces expression of mast cell-specific genes. In an attempt to determine whether the beneficial effect of EE was due to a direct inhibition of NF-κB activity, NF-κB DNA binding experiments were performed. A key step in NF-κB activation is its translocation to the nucleus and subsequent binding to NF-κB elements in the promoters of its target genes. Nuclear extracts were prepared from colonic tissue of vehicle-and EE-treated rats and analyzed for the presence of activated NF-κB. As shown in Fig. 4A, EE treatment did not interfere with NF-κB DNA binding compared with vehicle-treated rat nuclear extracts. The binding of NF-κB was specific because competition experiments with wild-type NF-κB oligonucleotide interfered with binding activity but the mutant consensus oligonucleotide had no effect (Fig. 4B). Therefore, the potential regulation of NF-κB activity by liganded ER does not involve direct inhibition of NF-κB nuclear translocation or DNA binding.

Since EE action may involve inhibition of NF-κB at the transcriptional level, a gene array experiment was performed to identify EE-regulated genes in the colon. One family of genes that was globally downmodulated by EE was the mast cell protease family. It has been shown that the activation of mast cells may be important in promoting leukocyte infiltration (71, 75), and their regulation by EE would be consistent with both the histology and MPO data. To verify whether EE treatment influences mast cell protease gene expression in the rat colon, mRNA analysis was performed by real time RT-PCR. As shown in Fig. 5, the expression of a number of mast cell serine protease genes were determined. RMCP-1, RMCP-3, and RMCP-4, and carboxypeptidase A3 (CPA) were determined from nondiseased Fisher rats and HLA-B27 transgenic rats exhibiting chronic diarrhea and treated with vehicle or EE for 5 days. Total RNA was isolated from the rat colon, and gene expression analysis was performed by real-time RT-PCR and plotted as means ± SE.
RMCP-4 mRNA levels were all dramatically induced in colons from diseased rats compared with nondiseased controls, whereas no induction of RMCP-2 or CPA was observed. Interestingly, RMCP-3 and -4 are not detected in normal rat tissue and have been shown to be expressed only during parasitic infections (19, 37). Here we demonstrate that they are also highly induced by colonic inflammation and that EE administration resulted in their reduced gene expression and reduced expression of RMCP-1. These results suggest that the beneficial effects of estrogen treatment on stool and histology scores as well as MPO activity may in part be attributed to inactivation of mast cells.

Estrogen blocks mast cell degranulation and cytokine expression. To confirm that estrogen could have a direct effect on mast cells, the ability of E2 to inhibit mast cell degranulation and inflammatory gene expression was determined in BMMC, a mucosal mast cell type analogous to those resident in the colon. For the degranulation experiments, the BMMC were pretreated with 1 μM E2 for 3 days and then challenged with various concentrations of DNP-BSA for 30 min. As shown in Fig. 6, E2 treatment resulted in a 50% reduction in mast cell degranulation as assessed by β-hexosaminidase release. Cytokine production of TNF-α, IL-6, and IL-13 was also repressed by the E2 pretreatment, consistent with its potential regulation of NF-κB transcriptional activity. These results demonstrate that E2 can impact directly on mast cell activity that may contribute to the anti-inflammatory action of estrogen observed in this model.

DISCUSSION

Estrogens have been increasingly demonstrated to have anti-inflammatory activity through their ability to interfere with cytokine signaling in a number of diverse disease settings. Previously, we demonstrated the ability of estrogen treatment to inhibit inflammatory gene expression in the liver in mice fed a high-fat diet (19) or after acute exposure to IL-1β (21). The best-characterized anti-inflammatory effect of estrogen treatment is its regulation of IL-6 expression due to ER inhibition of NF-κB and NF-IL6 activation of the IL-6 promoter (32, 55, 66). Further reports have implicated ER inhibition of NF-κB activity as a predominant mechanism of estrogen action and one that can occur through multiple mechanisms, including direct protein-protein interactions (56, 66), inhibition of NF-κB DNA binding (15, 57), induction of IκB expression (69), or coactivator sharing (29, 65).

Here we demonstrate an anti-inflammatory activity of EE in improving disease symptoms in the HLA-B27 transgenic rat model of IBD. Treatment of HLA-B27 transgenic rats exhibiting chronic diarrhea with EE resulted in a restoration of normal bowel scores within 3 days. In addition, histological analysis of the colon at the conclusion of the experiment showed pronounced improvements in the degree of ulceration, the number of infiltrating mast cells, the depth of the lesions, and the amount of fibrosis observed after only 5 days. This effect of estrogen was mediated through the ER because cotreatment with the pure ER antagonist ICI resulted in a complete abrogation of the beneficial effects of estrogen on both the clinical signs of disease and on histology scores.

However, no loss of NF-κB nuclear localization or DNA binding was observed with nuclear extracts from the rat colons treated with EE. This is consistent with previous observations (19) suggesting that ER is targeting NF-κB at the transcriptional level. The current hypothesis is that liganded ER binding of coactivators shared by NF-κB (i.e., CREB binding protein, CBP) results in transcriptional inhibition of NF-κB (29, 65). No difference in CBP expression was observed between the rats treated with vehicle compared with EE (data not shown), consistent with the possibility that CBP may become limiting when both ER and NF-κB are activated.

In a mouse dinitrobenzene sulfonic acid (DNB) colitis model, a 16-day pretreatment with E2 resulted in a similar improvement in the macroscopic and histology scores. However, worsening of disease severity scores occurred with E2 administration in a mouse dextran sodium sulfate (DSS)-induced colitis model (74). Whereas the inflammatory disease in the HLA-B27 transgenic rat and DNB mouse model appear to be T cell dependent (9, 18), the DSS colitis model appears to be T cell independent because disease can develop in SCID mice (16). The mechanistic differences between these models may in part explain the different activity of estrogen observed. Estrogen has been shown to regulate T cell activity (79) and is efficacious in other classic T cell-mediated disease animal models, including the rat adjuvant-induced arthritis model and the mouse collagen-induced arthritis model (5, 31). Although T cells are likely to be an important component in disease
progression in the HLA-B27 transgenic rat, they are probably not acting alone.

We present evidence that liganded ER activity in mast cells may also contribute to the improvement of disease symptoms in the HLA-B27 transgenic rat. Recent studies have suggested that mast cells may play a role in the pathogenesis of IBD. Increased numbers of mast cells and evidence of mast cell degranulation have been found at the sites of inflammatory lesions in Crohn’s disease (17, 25) and IBD (70, 78). A large number of studies have demonstrated that local production of proinflammatory cytokines, such as IL-1, IL-6, IL-8, and TNF-α, which are produced in activated macrophages and mast cells, are increased in IBD (36, 58). The mediators released by mast cells can, independently and in synergy with macrophage and T cell-derived cytokines, induce much of the inflammatory pathology seen in IBD.

The histology and the MPO data indicate that estrogen treatment resulted in a decrease in neutrophil infiltration, which is the same conclusion drawn from the experiments in the DNB colitis model. The authors concluded that the major anti-inflammatory effect of estrogen was the inhibition of neutrophil recruitment, as evidenced through the reduction of intracellular adhesion molecule-1 expression and MPO activity (74). The regulation of mast cell activity would be consistent with these conclusions because they are known to promote neutrophil adhesion and infiltration into the colon through their release of histamine and multiple cytokines (3, 26). It is possible to assess mast cell activity in vivo through monitoring the regulation of mast cell-specific protease genes. Although these serine proteases are a specific marker for mast cell activation (reviewed in Ref. 68), they are also pathological because their release during IgE-mediated hypersensitivity responses are also associated with permeability changes and leukocyte infiltration (64, 75). Here we demonstrate that mast cell proteases were induced in the HLA-B27 transgenic rats compared with nondiseased control Fisher rats and that estrogen treatment resulted in a reduction of gene expression of all three genes. Neither RMCP-3 nor RMCP-4 is expressed in normal rat tissue, and they have only been detected in rat intestines undergoing a parasitic infection (37), suggesting that they are expressed only during periods of severe duress. Here we demonstrate their potential role in IBD progression in this model. Although no induction of RMCP-1 or CPA gene expression was detected in the HLA-B27 transgenic rats compared with nondiseased control Fisher rats, these gene expression data suggest the possibility that estrogen’s ability to block neutrophil recruitment may result in part through inactivation of mast cells. A direct effect of estrogen on mast cell activation was confirmed with mouse BMMC. Human mast cells have been shown to contain ERα by immunocytochemistry (46, 52, 80), and we have confirmed the presence of ERα mRNA in the mouse BMMC, but no ERβ mRNA was detected by quantitative real-time RT-PCR (data not shown). Direct inhibition of mast cell activation by IgE as assessed by β-hexosaminidase release was observed with E2 treatment, and similar results were observed with mouse peritoneal connective tissue mast cells as well (data not shown). In addition, the expression of the cytokines TNF-α, IL-6, and IL-13 was also inhibited by E2, consistent with its potential regulation of NF-κB transcriptional activity. This effect of estrogen on mast cell cytokine expression does not appear to be unique either to BMMC cells or to IgE-mediated activation, because similar results were demonstrated with the human mast cell line HMC-1 stimulated with PMA or the calcium ionophore A23187 (34). In fact, in vivo activation of mast cells in this IBD model is most likely IgE independent, because no specific allergen challenge was involved.

These results confirm that estrogen can impact directly on mast cells and that this may in part be responsible for the dramatic improvement in the clinical and histology scores observed in this model. A pathological role for mast cells in intestinal bowel inflammation has been demonstrated in experiments using either rats treated with mast cell stabilizers (49, 67, 77) or in DSS-induced experimental colitis in mast cell-deficient rats (2). Interestingly, when colitis is induced in these same mast cell-deficient rats with 2,4,6-trinitrobenzene sulfonic acid, no role for mast cells was observed (24). Mast cells appear not to be involved in disease progression in mouse colitis experiments (12, 30, 38), whereas T cells are necessary for DNB-mediated colitis but not DSS-induced colitis in the mouse (16). Clearly, mast cell involvement in these various models of IBD may vary according to how the disease symptoms are stimulated and in what species the studies are conducted.

Clinical data on the association between IBD and estrogen are conflicting. Women do show changes in their immune responses during pregnancy when plasma estrogen levels are high (43, 76), but there appears to be no significant difference between the course of Crohn’s disease or ulcerative colitis during pregnancy (40). Understanding the basic mechanism of estrogen action in the various colitis models may help to comprehend what impact estrogen has on the immune system and aid in the design of synthetic ER ligands that may be beneficial in treating IBD patients.

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REFERENCES

Estrogen receptor alpha and beta expression and their isoforms in colon cancer. 

The role of endogenous estrogens in the protective role of endogenous estrogens in the development after estrogen loss: mediation by interleukin-6. 

Nikolaus S, Folscn U, and Schreiber S. 

The role of mast cells in dextran sulfate sodium-induced experimental inflammatory bowel disease. 


Hammer RE, Maika SD, Richardson JA, Tang JP, and Taurog JD. 


Nelson JL. 


Lutzelschwab C, Lunderius C, Enerback L, and Hellman L. 

The role of mast cells in the development of inflammatory disease of HLA-B27 transgenic rats by bone marrow engraftment. 


Evans MJ, Richardson JA, and Taurog JD. Transfer of the inflammatory disease of HLA-B27 transgenic rats by bone marrow engraftment. 


Higa A, Ishikawa N, Eto T, and Nawa Y. Evaluation of the role of mast cells in the progression of acetic acid-induced colitis in mice. 


Jansson L and Holmdahl R. Enhancement of collagen-induced arthritis in female mice by estrogen receptor blockage. 


Miyamoto N, Mandai M, Suzzuima I, Suzuki M, Kobayashi K, and Honda Y. Estrogen protects against cellular infiltration by reducing the expressions of E-selectin and IL-6 in endotoxin-induced uveitis. 


Minocha A, Thomas C, and Omar R. Lack of crucial role of mast cells in pathogenesis of experimental colitis in mice. 


Moordanid AD. Antioxidant properties of steroids. 


Mullin JM, and Snock KV. Effect of tumor necrosis factor on epithelial tight junctions and transepithelial permeability. 


Nelson JL. Pregnancy immunology and autoimmune disease. 


Neurath MF, Becker C, and Barbulescu K. Role of NF-kappaB in immune and inflammatory responses in the gut. 


Neurath MF, Pettersson S, Meyer zum Buschenfelde KH, and Strober W. Local administration of antiestrogen phosphorothioate oligonucleotides to the p65 subunit of NF-kappa B abrogates established experimental colitis in mice. 


Nicoloni S and Rudolph MI. Estrogen receptors in mast cells from arterial walls. 


Oda T. Role of mast cells in dextran sulfate sodium-induced experimental colitis in rats. 


Pace P, Taylor J, Suntharalingam S, Coombs RC, and Ali S. Human estrogen receptor beta binds DNA in a manner similar to and dimerizes with estrogen receptor alpha. 


Pahl HL. Activators and target genes of Rel/NF-kappaB transcription factors. 


Pang X, Cotreau-Bibbo MM, Sant GR, and Theodorides TC. Bladder mast cell expression of high affinity oestrogen receptors in patients with interstitial cystitis. 


