Preventive Effects of *Escherichia coli* Strain Nissle 1917 on Acute and Chronic Intestinal Inflammation in Two Different Murine Models of Colitis

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Received 16 July 2003/Accepted 11 December 2003

*Escherichia coli* strain Nissle 1917 (EcN) is as effective in maintaining remission in ulcerative colitis as is treatment with mesalazine. This study aims to evaluate murine models of acute and chronic intestinal inflammation to study the antiinflammatory effect of EcN in vivo. Acute colitis was induced in mice with 2% dextran-sodium sulfate (DSS) in drinking water. EcN was administered from day −2 to day +7. Chronic colitis was induced by transfer of CD4+ CD62L+ T lymphocytes from BALB/c mice in SCID mice. EcN was administered three times/week from week 1 to week 8 after cell transfer. Mesenteric lymph node (MLN) cytokine secretion (of gamma interferon [IFN-γ], interleukin 5 [IL-5], IL-6, and IL-10) was measured by enzyme-linked immunosorbent assay. Histologic sections of the colon were analyzed by using a score system ranging from 0 to 4. Intestinal contents and homogenized MLN were cultured, and the number of *E. coli*-like colonies was determined. EcN was identified by repetitive extragenic palindromic (REP) PCR. EcN administration to DSS-treated mice reduced the secretion of proinflammatory cytokines (IFN-γ, 32,477 ± 6,377 versus 9,734 ± 1,717 [*P = 0.004*]; IL-6, 231 ± 35 versus 121 ± 17 [*P = 0.02*]) but had no effect on the mucosal inflammation. In the chronic experimental colitis of the transfer model, EcN ameliorated the intestinal inflammation (histology score, 2.7 ± 0.2 versus 1.9 ± 0.3 [*P = 0.02*]) and reduced the secretion of proinflammatory cytokines. Translocation of EcN and resident *E. coli* into MLN was observed in the chronic colitis model but not in healthy controls. Administration of EcN ameliorated acute and chronic experimental colitis by modifying proinflammatory cytokine secretion but had no influence on the acute DSS-induced colitis. In this model, preexisting colitis was necessary for translocation of EcN and resident *E. coli* into MLN.

Probiotic microorganisms are defined as viable nutritional agents conferring benefits to the health of the human host (21). In human disease, the beneficial effects of various probiotics were demonstrated for the treatment of a variety of disorders such as infectious rotavirus-induced diarrhea in infants (15), amelioration of the side effects of antibiotic therapy (11), and prevention of atopic disease (6). Clinical trials have also suggested a potential role of different probiotic preparations in the treatment of inflammatory bowel disease (IBD) (36). The most striking data exist for *Escherichia coli* strain Nissle 1917 (EcN) in the maintenance of remission of ulcerative colitis (17, 18, 33). Treatment with EcN demonstrated beneficial effects equal to those of mesalazine (17). However, the mechanisms by which EcN mediates its effects are not fully understood. Alteration of the intestinal microflora (40), and other effects, including local and systemic immunomodulation (20, 25, 35) and barrier enhancement (22, 23), have been described previously. In vitro studies that used epithelial cell lines have demonstrated that EcN effectively inhibits the invasion of enteroinvasive *E. coli* and *Salmonella enterica* serovar Typhimurium (8, 28). Several animal models for acute or chronic intestinal inflammation have been established, and most of them are essentially associated with the commensal intestinal flora, as demonstrated by the lack of intestinal inflammation in germfree animals, while other rodents of the same genetic background, raised in an conventional setting, developed severe colitis (34). The majority of probiotic preparations have been already tested in gut flora-associated animal models of colitis (12, 23, 24, 37). However, no murine model of colitis has been employed so far to study the antiinflammatory mechanism of EcN in detail.

This study aims to evaluate the antiinflammatory effect of EcN in murine models of acute and chronic intestinal inflammation.

**MATERIALS AND METHODS**

*Animals.* BALB/c and immunodeficient C.B-17 SCID mice were purchased from Charles River Laboratories (Sulzfeld, Germany). All animals were held in standard caging conditions and received standard rodent chow and drinking water ad libitum. All animal experiments were approved by the local institutional review board.

**Acute colitis.** (i) **Experimental design.** BALB/c mice (body weight [BW], approximately 20 g) were divided into three groups. The treatment group (*n* = 4) with dextran-sodium sulfate (DSS)-induced acute colitis received EcN; one control group (*n* = 4) with DSS-induced acute colitis received plain water; and the other control group (*n* = 5) with DSS-induced acute colitis received antibiotic therapy with vancomycin-imipenem (50 mg/kg of BW), a treatment regimen known to effectively prevent acute DSS-induced colitis as shown previously (31).
Mice were sacrificed by asphyxiation, and intestinal tissues and mesenteric lymph nodes (MLN) were removed for further evaluation.

(ii) Induction of acute colitis. Acute colitis was induced by the addition of 2% DSS (INC Biomedicals, Inc., Aurora, Ohio) to the drinking water for 7 days. Control mice received plain drinking water.

(iii) Administration of EcN. The treatment group received 5 \times 10^{10} CFU of EcN (Ardeypharm, Herdecke, Germany) per ml resuspended in 200 ml of sterile saline by gastric gavage from day –2 (before the onset of colitis) until day 7, when the experiment was terminated. Antibiotic therapy with vancomycin-imipenem (50 mg/kg of BW) was administered in drinking water from day –2 until day 7.

Chronic colitis. (i) Experimental design. C.B.-17 SCID mice (BW, approximately 20 g) after adoptive T-cell transfer were divided into two groups. The treatment group (n = 9) received EcN, and the control group (n = 9) received plain water. Healthy BALB/c mice (n = 3) treated with EcN served as negative controls. At the end of the experimental period, mice were sacrificed, and intestinal tissues, MLN, and cecal contents were removed aseptically for further evaluation.

(ii) Induction of chronic colitis. Splenic CD4^+ CD62L^- T lymphocytes from BALB/c mice were isolated as described previously (26, 27) with slight modifications. In brief, CD4^+ T cells were purified from spleen mononuclear cells of healthy mice by negative depletion using anti-CD8, anti-major histocompatibility complex II, anti-B220, and anti-CD11b antibodies (BD Biosciences Pharmingen, San Diego, Calif) followed by anti-rat immunoglobulin G immunomagnetic microbeads (Miltenyi Biotech GmbH, Bergisch Gladbach, Germany). The resulting CD4^- T-cell population was further separated by immunomagnetic microbeads into CD62L^- and CD62L^- T cells. CD62L^- cells (purity, >95%) showed high expression of CD45RB by fluorescence-activated cell sorter analysis. A total of 0.25 \times 10^9 CD62L^- T cells were resuspended in 200 ml of sterile phosphate-buffered saline (PBS) and injected intraperitoneally (i.p.) into recipient C.B.-17 SCID mice. As a control, we used i.p. injection of 200 ml of PBS without cells.

(iii) Administration of EcN. EcN (5 \times 10^{10} CFU/ml in 200 ml of sterile saline [Ardeypharm]) was administered by gastric gavage two times/week from week 1 posttransfer to the end of the experiment at week 8.

Clinical assessment of colitis. At the end of the experiment, animals were clinically assessed for six clinical signs of intestinal inflammation, including general mobility and agility, stool texture, fur color and texture, anal prolapse, and rectal bleeding. One point was given for the presence of each sign. Points were added up, and a clinical score ranging from 0 to 6 was determined (Table 1).

Histological grading of colitis. Colonic tissues were removed and embedded into paraffin for histological analysis. The tissues were cut into 3- to 4-μm sections, stained with hematoxylin and eosin, and scored by two investigators (F.O. and M.S.) who were unaware of the source of tissue. For histological sections, stained with hematoxylin and eosin, and scored by two investigators (F.O. and M.S.) who were unaware of the source of tissue. For histological analysis, Sigma Stat version 2.03 (SPSS Inc., Chicago, Ill.) software was used. For analysis of numeric values, the one-way ANOVA and the Mann-Whitney rank-sum test were used. A P value of <0.05 was considered significant.

### RESULTS

#### Acute colitis. (i) Clinical signs. Acute colitis was induced in mice via administration of DSS in the drinking water. At the end of the experiment, no difference was seen between the groups in terms of body weight and length of the colon (data not shown).

(ii) Histology. Histologically, DSS-induced colitis revealed a patchy pattern of severe mucosal damage that included a loss of crypts, necrosis, and focal influx of inflammatory cells next to unaffected areas of mucosal architecture. No beneficial effect was observed after gastric administration of EcN (histology score, 3.0 ± 0.8 versus 3.1 ± 0.4 [not statistically significant]). Animals treated with vancomycin-imipenem, however, developed no histological signs of colitis, as previously documented (3.1 ± 0.4 versus 0.2 ± 0.1 [P ≤ 0.001]) (31).

(iii) Cytokine secretion. Despite the lack of an effect of EcN administration on the histological appearance of the mice with acute colitis, levels of the proinflammatory cytokines IFN-γ (Fig. 1) and IL-6 (Fig. 2) were significantly reduced, while the

#### TABLE 1. Clinical score system for the overall appearance of mice following induction of colitis by transfer of naive CD4^+ CD62L^- splenic T lymphocytes into immunodeficient SCID mice

<table>
<thead>
<tr>
<th>No. of points assigned</th>
<th>Appearance or presence of:</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Soft, liquid</td>
</tr>
<tr>
<td>0</td>
<td>Formed</td>
</tr>
<tr>
<td>1</td>
<td>Aglity</td>
</tr>
<tr>
<td>1</td>
<td>Fur color</td>
</tr>
<tr>
<td>1</td>
<td>Fur texture</td>
</tr>
<tr>
<td>1</td>
<td>Anus</td>
</tr>
<tr>
<td>1</td>
<td>Anal prolapse</td>
</tr>
<tr>
<td>3</td>
<td>Present</td>
</tr>
<tr>
<td>4</td>
<td>Not present</td>
</tr>
</tbody>
</table>

#### TABLE 2. Histological grading of intestinal inflammation

<table>
<thead>
<tr>
<th>Score</th>
<th>Characteristic(s)</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td>No inflammation</td>
</tr>
<tr>
<td>1</td>
<td>Slightly infiltrating cells in lamina propria</td>
</tr>
<tr>
<td>2</td>
<td>Infiltration with mononuclear cells</td>
</tr>
<tr>
<td></td>
<td>leading to separation of crypts, mild mucosal hyperplasia</td>
</tr>
<tr>
<td>3</td>
<td>Massive infiltration with inflammatory cells</td>
</tr>
<tr>
<td></td>
<td>leading to disturbed mucosal architecture, loss of goblet cells, marked mucosal hyperplasia</td>
</tr>
<tr>
<td>4</td>
<td>Crypt abscesses, ulceration</td>
</tr>
</tbody>
</table>
secretion of IL-5 and IL-10 by cells from MLN was not changed by probiotic or antibiotic treatment (data not shown).

**Chronic colitis.** (i) **Clinical signs.** Chronic murine colitis was induced by transfer of naïve CD4⁺ CD62L⁺ splenic T lymphocytes into immunodeficient SCID mice, and recipient animals were treated regularly with EcN immediately after cell transfer. At the end of the 8-week treatment period, mice receiving EcN appeared healthier than did untreated mice. This result was reflected by a significantly lower clinical score (Fig. 3). However, changes in body weight were unaffected by the probiotic treatment, as mice given EcN lost weight simultaneously with untreated animals (data not shown).

(ii) **Histology.** SCID mice injected i.p. with CD4⁺ CD62L⁺ T-lymphocytes developed severe intestinal inflammation (Fig. 4). Histologically, the colitis was characterized by a thickening of the mucosa due to edema and an influx of inflammatory cells (Fig. 4A). As shown in Fig. 4B, the intestinal inflammation was ameliorated by oral administration of EcN, a finding reflected by a reduction of the histological score (2.7 ± 0.2 versus 1.9 ± 0.3 [P = 0.02]).

(iii) **Cytokine secretion.** Lymphocytes from MLN from untreated CD4⁺ CD62L⁺ T-cell-injected SCID mice secreted high levels of proinflammatory cytokines (Fig. 5). Administration of EcN led to a significant reduction of IFN-γ (Fig. 5A) (P = 0.03), IL-5 (Fig. 5B) (P = 0.02), and IL-6 (Fig. 5C) (P = 0.02), while the level of secretion of the antiinflammatory cytokine IL-10 remained unchanged (data not shown).

(iv) **Analysis of the impact of EcN administration on the resident E. coli population in chronic colitis.** REP-PCR demonstrated successful colonization with EcN in mice treated with EcN (data not shown). Incubation of cecal contents from animals who were fed EcN revealed significantly higher concentrations of enterobacterial colonies than those of untreated controls (Fig. 6) (P = 0.001), suggesting that no replacement of the resident E. coli flora with EcN occurred. Furthermore, EcN, together with other Enterobacteriaceae, was grown from the MLN of colitic mice treated with EcN, indicating translocation of viable microorganisms (Fig. 7). No bacteria were cultivated from the MLN of healthy BALB/c mice after 8 weeks of EcN administration.

**DISCUSSION**

The exact etiology of chronic IBD is still unknown but seems complex and multifactorial. From human studies of and animal models for experimental colitis, increasing evidence is generated that the resident intestinal flora plays a critical role in the development of the intestinal inflammation on a given genetic
IL-2- and IL-10-knockout mice as well as HLA-B27 transgenic rats developed colitis when raised in specific-pathogen-free conditions, but they remained almost disease free under germfree conditions (32, 38, 39). Furthermore, clinical observations demonstrate beneficial effects of probiotic microorganisms in the treatment of IBD. EcN especially was shown to be as effective as mesalazine in the maintenance of remission in patients with ulcerative colitis (17). So far, however, no animal model of experimental colitis has been tested for further investigation of the mechanisms by which EcN may exert its beneficial effects.

Using a preventive setup in the present study, we demonstrated that EcN has immunomodulatory properties in acute and chronic experimental colitis, possibly mediated by translocation into MLN and alteration of the proinflammatory cytokine secretion profile—but EcN has no short-term effect on acute toxic mucosal damage.

We have chosen two different models of colitis to test the efficacy of EcN in acute and chronic experimental intestinal inflammation. The modified transfer model (26, 27), based on the CD4+ CD62Lhigh T-cell model, first described by Powrie et al. (29), is characterized by a massive influx of mononuclear cells into the colonic mucosa, elevated levels of proinflammatory cytokines, and a chronic, severe wasting syndrome. The degree of colitis in this model correlates with the extent of luminal bacterial growth (1) and therefore makes it suitable for experimental probiotic therapy. On the other hand, administration of DSS in drinking water induces acute colitis in mice (10) with patchy mucosal damage, including focal crypt loss, followed by acute transmural infiltration with inflammatory cells. It has been shown that neither T nor B cells are required for the induction of acute DSS-induced colitis, as this model can also be used for SCID mice (13). However, DSS colitis also seems to be dependent on the intestinal microflora (2, 3, 31).

FIG. 4. (A) Histological appearance of colitis induced by transfer of naïve CD4+ CD62L+ T lymphocytes in SCID mice. The colitis is characterized by a thickening of the mucosa due to edema and an influx of inflammatory cells (magnification, ×100). (B) Administration of EcN led to a significant reduction of the severity of chronic colitis in this model (magnification, ×100). (C) Histological score of the colonic inflammation in mice following the induction of colitis by transfer of naïve CD4+ CD62L+ T lymphocytes and administration of EcN compared to that of untreated colitic mice. *, P < 0.02 versus CD62L.

VOL. 11, 2004 EcN AMELIORATES EXPERIMENTAL MURINE COLITIS 375
The use of the probiotic microorganism EcN in SCID mice following transfer of CD4<sup>+</sup> CD62L<sup>-</sup> T lymphocytes and administration of EcN compared to the results seen with untreated colitic mice. Values given are means ± SEM (pg/µl). (A) IFN-γ (*, P < 0.03); (B) IL-6 (*, P = 0.02); (C) IL-5 (*, P = 0.02). All P values shown are versus those of CD62L.

The use of the probiotic microorganism EcN in SCID mice following transfer of CD4<sup>+</sup> CD62L<sup>-</sup> T cells ameliorated the development of intestinal inflammation, as was demonstrated by a healthier clinical appearance of the animals, lower histological scores, and changes in cytokine secretion profiles of mesenteric lymphocytes. A marked reduction in the secretion of the proinflammatory cytokine IFN-γ was observed, which is thought to contribute predominantly to the perpetuation of intestinal inflammation, as seen in many previous murine models of colitis (14). Additionally, MLN cells from EcN-treated colitic SCID mice secreted less IL-5 and IL-6. IL-6 seems to play a major role in intestinal inflammation, since blockage of the IL-6 receptor in a murine model of colitis successfully prevented both wasting disease and the development of macroscopic and histologic lesions (16). On the other hand, there was no effect on the secretion of IL-10, an antiinflammatory cytokine secreted predominantly by regulatory T cells.

Both the CD4<sup>+</sup> CD45RB<sup>high</sup> and the CD4<sup>+</sup> CD62L<sup>-</sup> models are characterized by chronic intestinal inflammation and a
severe wasting syndrome. While the intestinal inflammation is significantly ameliorated, treatment with EcN had no effect on the weight loss in this model. It can be hypothesized that probiotic therapy influences the intestinal inflammation but does not have an impact on the multiorgan pathology, thus contributing to the wasting syndrome as described previously by Powrie and Mason (30). Whether a longer observation period would be needed to record recovery of the weight loss in EcN treated mice remains unclear.

Different results were seen when EcN was administered in a model of acute colitis. In contrast to the effects of the probiotics on clinical and histopathological scores in chronic intestinal inflammation, application of EcN did not ameliorate the histological appearance of the acute DSS-induced colitis in mice. Histology in the acute DSS colitis model reveals a characteristic patchy pattern with noninflamed mucosa appearing next to necrotic tissue. However, the dominant parameter for the histology score in the acute phase is destruction of the mucosal architecture rather than the influx of inflammatory cells, which is most likely due to a primarily toxic effect of DSS. The beneficial effect of probiotic administration, however, is obviously limited to the immunologic modification of colitis, as demonstrated by a pronounced reduction of proinflammatory cytokine secretion, which is more relevant in the chronic phase of intestinal inflammation (13).

The microbial analysis of cecal contents of mice from the transfer model revealed that after 8 weeks of oral administration, EcN did not replace the resident E. coli population but rather led to an increase in the total E. coli concentration. Furthermore, subcultures of homogenized MLN showed a translocation of viable members of Enterobacteriaceae. Via REP-PCR, these colonies were identified as resident Enterobacteriaceae from the intestinal flora and as colonies from the orally administered EcN. This finding is even more interesting in context with our observation that bacteria could not be isolated from MLN derived from healthy BALB/c mice following administration of EcN for 8 weeks.

Intestinal inflammation leading to a breakdown of the intestinal epithelial barrier function that would subsequently allow microorganisms from within the gut lumen to translocate to mucosal lymphatic tissue was always suggested (4, 19, 45). There are previous reports that bacterial translocation occurs also in healthy mice but is limited to 10 to 20% of mice analyzed (5, 44). In as yet unpublished observations (for which healthy BALB/c mice were used), we were able to detect a peak concentration of translocated microorganisms within MLN and Peyer’s patches at 6 to 12 h post-oral administration, followed by a steady decline until no bacteria could be isolated from lymphoid tissue at 48 h post-oral administration (unpublished data).

The fact that neither translocated EcN nor translocated resident E. coli bacteria could be isolated from MLN of healthy BALB/c mice after 8 weeks of treatment confirms the hypothesis that under healthy conditions only a minor translocation of luminal intestinal bacteria to mesenterical lymphatic tissue takes place. These are then cleared rapidly by phagocytosis (45). However, under inflammatory conditions, the epithelial barrier is disrupted, and luminal bacteria, including the orally administered EcN, are able to continuously translocate in very large quantities to Peyer’s patches and MLN, as seen in the chronic model of colitis. A Th1-mediated T-cell response is induced, and proinflammatory cytokines secreted by antigen-presenting cells and effector T lymphocytes perpetuate the inflammation (9). The oral administration and subsequent translocation of EcN might positively influence this systemic reaction by an induction of regulatory T-cell responses. It has been shown in vitro that probiotic bacteria, in contrast to other bacterial strains, are able to reduce antigen-presenting cell activation after phagocytosis (7). This could be an explanation for the reduction of proinflammatory cytokine secretion seen in both of our in vivo models after oral EcN administration. In this context, it is likely that this mechanism of action would have no immediate effect on acute colitis but would rather change the severity of inflammation in chronic colitis.

In summary, we have shown that EcN is effective in modifying intestinal immunologic responses which result in the amelioration of chronic experimental murine colitis but have no clinical effect on the severity of acute mucosal damage. These findings are in accordance with clinical observations in which probiotic therapy was beneficial only in the maintenance of remission of chronic pouchitis and ulcerative colitis but had no effect for acute IBD. However, despite the successful use of probiotic therapy in cases of human pouchitis and ulcerative colitis, our suggestions of possible underlying antiinflammatory mechanisms, deployed by probiotic bacteria, cannot be fully extrapolated to the human situation since these experiments were performed under standardized conditions that are not feasible for human trials.

The analysis of intestinal microflora in this experimental setting was limited to Enterobacteriaceae, and the impact on the composition of the microflora by probiotic therapy therefore remains elusive. Note that no final conclusion can be drawn regarding a direct impact of EcN on cytokine production that reduces inflammatory activity as demonstrated previously in other studies (35, 41). On the other hand, an alteration in cytokine levels because of reduced inflammatory activity that is mediated by a modulation of the gut flora can also be anticipated (42). However, as demonstrated in this report, EcN is able to translocate into MLN and to modulate the proinflammatory cytokine secretion profile of mesenterical lymphocytes in both models of murine colitis, without significant amelioration of the acute DSS colitis. It can therefore be speculated that EcN directly modulates the immune response.

ACKNOWLEDGMENTS

We thank Ardeypharma, Herdecke, Germany for providing E. coli strain Nissle 1917.

M.S. is supported by a research grant from the University of Regensburg, Regensburg, Germany, as part of the ReForM program.

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