Lactoferrin reduces colitis in rats via modulation of the immune system and correction of cytokine imbalance

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Lactoferrin reduces colitis in rats via modulation of the immune system and correction of cytokine imbalance. Am J Physiol Gastrointest Liver Physiol 283: G187–G195, 2002. First published February 27, 2002; 10.1152/ajpgi.00331.2001.—Natural immunomodulator lactoferrin is known to exert an anti-inflammatory effect. However, there have been no studies that examine the mode of action of lactoferrin in reducing intestinal damage. We investigated the effect of lactoferrin on a trinitrobenzensulfonic acid (TNBS)-induced colitis model in rats. Bovine lactoferrin was given once daily through gavage, starting 3 days before (preventive mode) or just after TNBS administration (treatment mode) until death. The distal colon was removed to be examined. Colitis was attenuated by lactoferrin via both modes in a dose-dependent manner, as reflected by improvement in macroscopic and histological scores and myeloperoxidase activity. Lactoferrin caused significant induction of the anti-inflammatory cytokines interleukin (IL)-4 and IL-10, significant reductions in the proinflammatory cytokines tumor necrosis factor-α and IL-1β, and downregulation of the nuclear factor-κB pathway. We concluded that lactoferrin exerts a protective effect against colitis in rats via modulation of the immune system and correction of cytokine imbalance. Lactoferrin has potential as a new therapeutic agent for inflammatory bowel disease.

trinitrobenzenesulfonic acid; inflammatory bowel disease; tumor necrosis factor; interleukin; nuclear factor-κB

THE ETIOLOGY OF CROHN’S DISEASE and ulcerative colitis is still unknown. However, genetic factors in combination with environmental factors are suggested to be involved in the pathogenesis of Crohn’s disease (31). Inadequate or prolonged activation of the intestinal immune system plays an important role in the pathophysiology of chronic mucosal inflammation (11, 24, 31). Furthermore, cytokines play an important role in modulating the immune system. Cytokines are rapidly synthesized and secreted from inflammatory cells on stimulation and induce the production of adhesion molecules and other inflammatory mediators such as reactive oxygen metabolites, nitric oxide, and lipid mediators (1). Cytokines induce, amplify, prolong, and inhibit inflammation (3, 29). It has been reported that there is a disturbed balance between proinflammatory and anti-inflammatory cytokines in inflammatory bowel disease (3, 24, 29). Increased levels of proinflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, and IL-6 were detected in the colonic mucosa. They are secreted from macrophages, lymphocytes, and polymorphonuclear neutrophils (13, 21, 32). Synthesis of these cytokines is induced by the activation of nuclear factor (NF)-κB, a transcriptional factor that is involved in the regulation of many inflammation-associated genes (4).

On the basis of these observations, we speculated that appropriate modulation of the immune system by using natural immunomodulators might be useful in reducing the activity of inflammatory bowel disease. We focused our attention to lactoferrin, an iron-binding protein with a wide spectrum of biological activities. Lactoferrin exists in various biological fluids such as milk, tears, synovial fluid, seminal plasma, and pancreatic juice (22). Lactoferrin is stored in secondary granules of neutrophils and is released in response to activation of TNF-α, IL-6, and IL-8 (7). The serum level of lactoferrin, which is very low in the physiological state, increases significantly with infection (22). Receptors for lactoferrin were detected and isolated in activated B and T lymphocytes, monocytes/macrophages, intestinal brush-border cells, platelets, and neoplastic cells (22). Lactoferrin also influences the release of proinflammatory cytokines such as IL-1β, IL-2, IL-6, and TNF-α (7, 16, 18, 25, 37).

In this study, we examined the potential ability of lactoferrin to attenuate colitis using a 2,4,6-trinitrobenzenesulfonic acid (TNBS)-induced colitis model in rats. TNBS has been shown to induce colitis rapidly and reproducibly as a result of covalent binding of trinitrophenyl residues to autologous host proteins, leading to mucosal infiltration by polymorphonuclear...
cells (10), activation of the NF-κB pathway (27), and the production of TNF-α and IL-1β (28).

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats (7 wk of age, weighing 210–240 g) were obtained from Clea Japan (Tokyo, Japan) and used in the experiments. Rats were housed in hanging wire mesh cages in a temperature- (23 ± 2°C) and humidity- (50–70%) controlled room with a 12:12-h light-dark cycle. Animals were fed ad libitum with standard rat chow (Clea Japan) and had free access to tap water throughout the experimental period. The rats were allowed to adapt to our laboratory environment for 1 wk before beginning the experiment. This animal study was approved by the Ethics Committee of Yokohama City University.

Induction of Colitis

Colonic inflammation was induced by using a technique modified from that of Morris et al. (26). Briefly, rats were lightly anesthetized with ether after overnight food deprivation, and a polyethylene catheter was inserted 8 cm into the colon via the anus. TNBS (Wako Chemical, Tokyo, Japan) dissolved in 50% (vol/vol) aqueous ethanol (25 mg/ml) was injected into the colon (total volume of 1 ml/rat). The control rats received 1 ml of water intracolonically in place of TNBS and ethanol.

Experimental Procedures

Experiment 1: protective effects of lactoferrin on TNBS-induced colitis according to mode of administration. To evaluate the preventive effect of lactoferrin against TNBS-induced colitis, rats were divided into three groups: 1) TNBS-administered rats receiving 0.9% saline, 2) TNBS-administered rats receiving lactoferrin, and 3) nontreated controls (n = 8 in each group). Bovine lactoferrin (200 mg·kg⁻¹·day⁻¹, Morinaga Milk Industry, Tokyo, Japan) dissolved in 1 ml of 0.9% saline was administered once a day through gavage, starting from 3 days before colitis induction and continuing until death (preventive mode). The dose of lactoferrin was selected on the basis of the report that no adverse effect in rats was observed at the level of lactoferrin between 0 and 2,000 mg·kg⁻¹·day⁻¹ (39). Animals were weighed every day until death. Macroscopic and histological examinations, as well as determination of myeloperoxidase (MPO) activity, in the distal colon were conducted before, 7, and 14 days after TNBS administration. The distal colon was also measured. In addition, we examined the plasma and fecal concentrations of lactoferrin in this model 7 days after TNBS administration, and we investigated the changes in total number of colonic bacteria 7 days after TNBS administration.

Experiment 2: dose-response study of lactoferrin in TNBS-induced colitis. Dose-response relationships between lactoferrin and the degree of inflammation scored macroscopically and histologically and by measuring MPO activity in the distal colon were examined in four groups of animals 7 days after TNBS administration. Different doses of bovine lactoferrin (0, 50, 100, and 200 mg·kg⁻¹·day⁻¹) were administered once a day from 3 days before colitis induction until death.

Experiment 3: effects of lactoferrin on cytokines in the colonic tissue and the NF-κB pathway in TNBS-induced colitis. In other groups of TNBS-administered rats and control rats (n = 8 in each group) receiving lactoferrin in the preventive mode, the distal colon was resected. Proinflammatory cytokines, anti-inflammatory cytokines, and NF-κB were determined at 7 days after TNBS administration.

Macroscopic Assessment of Colonic Inflammation

The distal colon was quickly removed from the rats, opened longitudinally, and gently cleared of fecal material with the 0.9% saline solution. Colonic inflammation was evaluated macroscopically with the colon macroscopic scoring system of Wallace et al. (39) by an observer unaware of the treatment. This score is scaled from 0 to 10 based on criteria reflecting inflammation, such as hyperemia, bowel wall thickening, and extent of ulceration. After being scored, the distal 10 cm of the colon was blotted dry and weighed. The colon weight-to-body weight ratio was calculated as a marker of colonic inflammation (34).

Histological Assessment of Colonic Inflammation

Samples of the inflamed tissues were removed for histology. The colonic inflammation score was evaluated by using the histopathological grading system of Ameho et al. (2) by an observer blinded to the treatment. This grading, which takes into account the degree of infiltration, the presence of erosion, ulceration, or necrosis, and the depth and surface of the lesion, is scaled from 0 to 6.

Determination of the Plasma and Fecal Concentrations of Lactoferrin

Plasma concentration of lactoferrin was measured by an ELISA assay kit (Yagai, Yamagata, Japan) according to the manufacturer’s instructions. Fecal samples obtained from rectum were weighed, homogenized in buffer solution, and centrifuged, and lactoferrin in the supernatants was measured by ELISA by using the same kits.

Determination of the Total Number of Colonic Bacteria

Fresh fecal samples were collected from rectum, and the bacterial numbers in feces were assayed as described previously (17).

Determination of MPO Activity in the Colonic Tissue

Measurement of MPO activity is used as a biochemical marker of neutrophil infiltration into inflamed intestinal tissue (10, 26, 39). The MPO activity of the tissue was determined by the technique described by Bradley et al. (6).

Determination of Cytokine Concentrations in the Colonic Tissue

Samples of inflamed colon were weighed and homogenized for 1 min in 10 mM phosphate buffered saline, pH 7.4,
containing 1 mM EDTA, 5 mM dithiothreitol, and 10 μg/ml of each of aprotinin, leupeptin, and pepstatin A in an ice-chilled water bath, using a polytron homogenizer. The specimens were freeze-thawed four times. The homogenates were centrifuged at 10,000 g at 4°C for 5 min. TNF-α, IL-1β, IL-4, IL-6, and IL-10 in the supernatants were measured by ELISA by using assay kits (BioSource International, Camarillo, CA) with the quantitative immunometric sandwich enzyme immunoassay technique (2, 14, 23). An amplification system, with the streptavidin peroxidase reaction, was used to increase sensitivity. The sensitivities for TNF-α, IL-1β, IL-4, IL-6, and IL-10 were 4.0, 3.0, 2.0, 8.0, and 5.0 pg/ml, respectively. The interassay and intra-assay coefficients of variation were <10%. The assay was conducted according to the manufacturer's instructions.

Detection of Phosphorylated Inhibitor κB in Cytoplasm

The tissue sections were rehydrated in an ethanol series. Then sections were incubated with 0.5 μg/ml mouse monoclonal antibody raised against a peptide corresponding to an amino acid sequence containing phosphorylated Ser^32 of inhibitor κBα (IκBα; Santa Cruz Biotechnology, Santa Cruz, CA) at 4°C overnight. Sections were then incubated with a biotinylated secondary antibody at 25°C for 30 min and stained with Vector ABC reagent and diaminobenzidine (Vector Laboratories, Burlingame, CA). Slides were dehydrated in an ethanol series and counterstained with hematoxylin.

Statistical Analysis

All data are presented as means ± SE. Differences between groups were determined by analysis of variance followed by Fisher's protected least significant difference comparison test. With all statistical analyses, an associated probability (P value) of <0.05 was considered significant.

RESULTS

Experiment 1: Effects of Preventive vs. Treatment Mode of Lactoferrin Administration on TNBS-Induced Colitis

Body weight gain was significantly suppressed in the TNBS-administered rats in contrast to the control rats (P < 0.01) in the first 2 days. TNBS-administered rats exhibited severe watery diarrhea and hemorrhaging over the following days. Administration of lactoferrin (preventive mode) attenuated the TNBS inhibition on body weight (P < 0.01 vs. TNBS-only rats) in the first 2 days. In the following days, there was no difference in body weight gain among the three groups (Fig. 1).

Control rats killed 7 or 14 days after administration of saline showed no macroscopic lesions in the distal colon (Fig. 2A). In the TNBS-administered rats, very severe colitis with widespread hemorrhagic damage in the distal colon was observed 7 days after TNBS administration (Fig. 2B). Fourteen days after colitis induction, the lesions were still severe, although a tendency for improvement was observed. The macroscopic inflammation score was the highest at 7 days after TNBS administration and decreased subsequently, although it remained higher than in control rats until 14 days after administration (Table 1). Lactoferrin treatment in the prevention mode attenuated the macroscopic lesions observed 7 and 14 days after TNBS administration (Fig. 2C). Seven and fourteen days after colitis induction, a significant decrease in the macroscopic score (P < 0.01) was observed in rats that had received preventive lactoferrin compared with TNBS rats.

No histological abnormalities were detected in the control rats killed 7 and 14 days after saline administration (Fig. 3A). In TNBS rats, colonic inflammation extending deeply into the muscular layer was observed 7 days after administration. A polymorphic inflammatory infiltrate, rich in polynuclear neutrophils, and local ulceration of the mucosal layer were observed (Fig. 3B). Fourteen days after colitis induction, the...
lesions were still severe, with marked infiltration of neutrophils, and a tendency for improvement was observed. The histological inflammation score increased to a peak level at 7 days after TNBS administration and remained higher than in controls until 14 days after administration (Table 1). Lactoferrin dramatically reduced the inflammatory lesions, which consisted of smaller polymorphic infiltrates, limited edema, and small focal lesions indicative of repair of the mucosal layer (Fig. 3C). A significant decrease in histological score \( (P < 0.01) \) was observed in TNBS-administered rats given preventive lactoferrin compared with those given only TNBS.

The colon weight-to-body weight ratio, an index of colonic inflammation, also increased after TNBS administration, reached its peak level on day 7, and decreased subsequently (Table 1). Preventive lactoferrin significantly reduced the colon weight-to-body weight ratio \( (P < 0.01) \).

MPO activity in the colonic tissue was significantly higher in TNBS-administered rats compared with controls at 7 or 14 days after TNBS administration (Table 1).

Table 1. Time course of the changes in macroscopic inflammation score, histological score, colon weight-to-body weight ratio, and MPO activity after TNBS administration

<table>
<thead>
<tr>
<th></th>
<th>Day 0</th>
<th>Day 7</th>
<th>Day 14</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Macrosopic score</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control rats</td>
<td>0.0±0</td>
<td>0.0±0</td>
<td>0.0±0</td>
</tr>
<tr>
<td>TNBS rats</td>
<td>0.0±0</td>
<td>7.7±0.7*</td>
<td>5.5±2.5*</td>
</tr>
<tr>
<td>TNBS rats with lactoferrin</td>
<td>0.0±0</td>
<td>2.0±0.7†</td>
<td>1.3±1.3†</td>
</tr>
<tr>
<td><strong>Histological score</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control rats</td>
<td>0.0±0</td>
<td>0.0±0</td>
<td>0.0±0</td>
</tr>
<tr>
<td>TNBS rats</td>
<td>0.0±0</td>
<td>5.8±0.4*</td>
<td>5.8±0.4*</td>
</tr>
<tr>
<td>TNBS rats with lactoferrin</td>
<td>0.0±0</td>
<td>2.4±1.2†</td>
<td>2.8±1.4†</td>
</tr>
<tr>
<td><strong>Colon weight-to-body weight ratio</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control rats</td>
<td>3.0±0.3</td>
<td>2.4±0.3</td>
<td>2.5±0.3</td>
</tr>
<tr>
<td>TNBS rats</td>
<td>3.0±0.3</td>
<td>7.4±1.5*</td>
<td>5.4±1.0*</td>
</tr>
<tr>
<td>TNBS rats with lactoferrin</td>
<td>3.2±0.3</td>
<td>3.9±0.4†</td>
<td>3.6±1.1†</td>
</tr>
<tr>
<td><strong>MPO, mU/mg tissue</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control rats</td>
<td>0.29±0.43</td>
<td>0.19±0.52</td>
<td>0.36±0.43</td>
</tr>
<tr>
<td>TNBS rats</td>
<td>0.27±0.38</td>
<td>3.72±1.73*</td>
<td>2.20±1.11*</td>
</tr>
<tr>
<td>TNBS rats with lactoferrin</td>
<td>0.26±0.57</td>
<td>0.91±0.42†</td>
<td>0.54±0.37†</td>
</tr>
</tbody>
</table>

Values are means ± SE. Control rats received vehicle only; TNBS rats, trinitrobenzenesofoxidic acid (TNBS)-administered rats; TNBS rats with lactoferrin, TNBS-administered rats receiving lactoferrin (200 mg·kg⁻¹·day⁻¹) from 3 days before administration of TNBS until day 14 after the induction of colitis. MPO, myeloperoxidase. *P < 0.01 vs. control; †P < 0.01 for TNBS rats vs. TNBS rats with lactoferrin.

Fig. 3. Histological appearance of lesions in TNBS colitis treated with lactoferrin 7 days after TNBS administration. A: normal transparietal colon section from a control rat with a histological score of 0 (hematoxylin and eosin; original magnification, ×20 and ×200 for A-1 and A-2, respectively). B: transparietal colon section (histological score 6) from a TNBS-administered rat. Thickening of the colon wall, with a predominant inflammatory infiltrate extending deep into the muscular and serosal layers is evident (hematoxylin and eosin; original magnification, ×20 and ×200 for B-1 and B-2, respectively). C: transparietal colon section (histological score 1) from a TNBS-administered rat receiving preventive mode lactoferrin (200 mg·kg⁻¹·day⁻¹). Subepithelial edema with diastasis of the crypts and moderate inflammatory infiltration of the mucosa and submucosa are shown (hematoxylin and eosin; original magnification, ×20 and ×200 for C-1 and C-2, respectively).
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Table 2. Preventive vs. treatment mode of lactoferrin administration in terms of macroscopic inflammation score, histological score, colon weight-to-body weight ratio, and MPO activity in distal colon 7 days after TNBS administration

<table>
<thead>
<tr>
<th>Groups</th>
<th>Macroscopic Score</th>
<th>Histological Score</th>
<th>Colon Weight-to-Body Weight Ratio</th>
<th>MPO, mU/mg tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control rats</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>2.4 ± 0.3</td>
<td>0.19 ± 0.09</td>
</tr>
<tr>
<td>TNBS rats</td>
<td>7.7 ± 0.7</td>
<td>5.8 ± 0.4</td>
<td>7.4 ± 1.2</td>
<td>3.72 ± 1.73</td>
</tr>
<tr>
<td>TNBS rats with lactoferrin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preventive mode</td>
<td>2.0 ± 0.7</td>
<td>2.4 ± 1.2</td>
<td>4.0 ± 0.4‡</td>
<td>0.88 ± 0.42‡</td>
</tr>
<tr>
<td>Treatment mode</td>
<td>2.0 ± 1.2</td>
<td>2.9 ± 1.8‡</td>
<td>4.3 ± 1.6‡</td>
<td>1.36 ± 0.29‡</td>
</tr>
</tbody>
</table>

Values are means ± SE. Preventive mode, TNBS-administered rats receiving lactoferrin (200 mg·kg·day⁻¹) starting 3 days before administration of TNBS; treatment mode, TNBS-administered rats receiving lactoferrin (200 mg·kg·day⁻¹) daily after the administration of TNBS. *P < 0.01 and †P < 0.05 vs. control; ‡P < 0.01 for TNBS vs. TNBS rats with lactoferrin.

1). Lactoferrin administration caused a significant decrease in MPO activity compared with TNBS-administered rats at 7 and 14 days after TNBS administration (P < 0.01), consistent with decreased infiltration of neutrophils, which was observed in histology.

Table 2 shows the effects of the preventive and treatment modes of lactoferrin administration on TNBS-induced colitis. Administration of lactoferrin in the treatment mode also significantly reduced the macroscopic and histological inflammation scores, the colon weight-to-body weight ratio, and MPO activity at 7 days after TNBS administration. There were no significant differences in these parameters between the TNBS-administered rats in the treatment mode and those in the preventive mode of lactoferrin administration.

In TNBS-administered rats receiving lactoferrin, the plasma and fecal concentrations of lactoferrin 7 days after colitis induction were significantly increased (plasma concentration, 225 ± 40 ng/ml; fecal concentration, 12.2 ± 1.6 μg/g of feces). In control rats and TNBS-administered rats without lactoferrin, neither plasma nor fecal concentrations of lactoferrin were detectable.

With regard to the total bacterial numbers in feces 7 days after colitis induction, there were no significant differences between TNBS-administered rats receiving lactoferrin, TNBS-administered rats without lactoferrin, and control rats (9.28 ± 0.10, 9.39 ± 0.12, and 9.36 ± 0.15, respectively; data expressed as log₁₀ colony-forming units/g feces).

Experiment 2: Dose-Response Study of Lactoferrin in TNBS-Induced Colitis

Macroscopic and histological damage scores, the colon weight-to-body weight ratio, and tissue levels of MPO activity in TNBS-administered rats were reduced by lactoferrin treatment in a dose-dependent manner within 50–200 mg·kg⁻¹·day⁻¹ (Fig. 4). There was a highly significant linear correlation between the doses of lactoferrin and these inflammatory parameters.

Experiment 3: Effects of Lactoferrin Administration on Cytokines in the Colonic Tissue and NF-κB Pathway in TNBS-Induced Colitis

Seven days after colitis induction by TNBS administration, tissue concentrations of TNF-α, IL-1β, and IL-6 in TNBS rats were significantly higher than those of the control rats (P < 0.05–0.01). TNBS-induced increase in TNF-α and IL-1β concentrations in the colonic tissue were significantly suppressed (P < 0.01 vs. TNBS rats) by the administration of lactoferrin in the preventive mode (Fig. 5, A and B); however, TNBS-induced IL-6 production was not suppressed (Fig. 5C).

There were no significant differences in these three cytokine levels between the TNBS rats given lactoferrin and the control rats. Seven days after colitis induction, IL-4 and IL-10 concentrations in the colonic tissue showed no difference between TNBS rats and controls; however, IL-4 and IL-10 concentrations were significantly increased (P < 0.01 vs. TNBS rats or control rats) by administration of lactoferrin in the preventive mode (Fig. 5, D and E). There were no significant differences in these five cytokine levels between lactoferrin-fed control rats and the control rats.

Figure 6 shows immunohistochemical localization of phosphorylated IκB, an index of NF-κB pathway activation, in TNBS-induced colitis 7 days after colitis induction. Phosphorylated IκB was induced in rats 7
days after TNBS administration (Fig. 6B) compared with controls, which showed almost no staining (Fig. 6A). Administration of lactoferrin in the preventive mode attenuated phosphorylated IκB expression (Fig. 6C).

**DISCUSSION**

TNBS-induced colitis is a well-established model that is similar to human inflammatory bowel disease and is characterized by mucosal infiltration of neutrophils mediated, at least in part, by TNF-α and IL-1β activation (27) and activation of the NF-κB pathway (28). There are a number of studies that have shown that hapten reagent-induced colitis, induced by TNBS, favorably responds to some of the current therapies for inflammatory bowel disease such as sulfasalazine, glucocorticoids, cyclosporine (5), and anti-TNF-α antibodies (27). In this study, we examined the effects of lactoferrin on TNBS-induced colitis in Sprague-Dawley rats. Since it was reported that the lactoferrin showed anti-inflammatory and immunomodulatory effects (7, 22, 25, 37), lactoferrin is expected to attenuate exaggerated activation of proinflammatory cytokines in TNBS colitis. Oral administration of lactoferrin in TNBS-treated rats attenuated all of the inflammatory responses, such as increased colonic weight-to-body weight ratio, macroscopic signs of inflammation, increased histological inflammation score, and MPO activity, which suggest that the lactoferrin obviously suppressed TNBS-induced colitis. This is the first study to assess the therapeutic effect of lactoferrin on TNBS-induced colitis.

In this study, we observed that activation of proinflammatory cytokines, such as TNF-α, IL-1β, and IL-6,
in TNBS colitis was suppressed by lactoferrin administration. Many studies have suggested that TNF-α and IL-1β have an important pathogenic role in the development of TNBS-induced colitis through aggregation of polymorphonuclear cells in the colonic tissue (30). It was reported that these cytokines were activated in TNBS-induced colitis and that lethal pancolitis was developed by TNBS administration in TNF-α transgenic mice, whereas neutralization of TNF-α and IL-1β improved the colitis and chronic TNBS-induced colitis was absent in TNF-α-knockout mice (27). From these observations, it is suggested that the anti-inflammatory effect of lactoferrin takes place by suppression of TNF-α and IL-1β, which were activated in TNBS-induced colitis.

In contrast to TNF-α, IL-1β, and IL-6, IL-4 and IL-10 in the colonic tissue were activated by lactoferrin. IL-10 was reported to suppress IL-2 and interferon-γ production and to inhibit the synthesis of proinflammatory cytokines such as TNF-α, IL-1β, and IL-6 (9, 33). In addition, it was reported that IL-10-deficient mice developed chronic enterocolitis (20). IL-4 also has anti-inflammatory actions. It inhibits the activation of TNF-α, IL-1β, and IL-6 (37) and suppresses the growth and migration of lymphocytes and macrophages in the colonic tissue (36). These observations imply that IL-4 and IL-10 have an inhibitory effect on colonic inflammation. Our results showed for the first time that, in inflammatory conditions, oral administration of lactoferrin significantly increased IL-4 and IL-10 in the colonic mucosa, whereas in physiological conditions it did not. The activation of IL-4 and IL-10 by lactoferrin is also considered to contribute to the anti-inflammatory effect of lactoferrin on TNBS-induced colitis.

Furthermore, NF-κB has been demonstrated to play important roles in colonic inflammation in TNBS-induced colitis and spontaneous colitis of IL-10-deficient mice (28). It has been proven that cytokines are extremely important regulators of cellular infiltration, tissue damage, ulceration, secretion/diarrhea, motility, and fibrosis (19). The results of our study provide evidence that complex interactions among cytokines in the colonic inflammation exceed superficial response and that the immunomodulatory effect of lactoferrin on colonic inflammation is very attractive for treatment of colitis.

It was recently demonstrated that lactoferrin, administered intravenously or orally, inhibits the production of TNF-α and IL-6 in a murine model stimulated by lipopolysaccharides in the spleen (37) and in a rat model of carrageenan-induced inflammation of the footpad (42). It was also demonstrated that locally administered lactoferrin inhibits the production of TNF-α in a murine model of epidermal inflammation in the ear (8) and that lactoferrin administered in vitro inhibits the release of TNF-α and IL-1β from mixed lymphocyte cultures (7). However, there have been no reports that indicate that administration of lactoferrin modulates proinflammatory or anti-inflammatory cytokines in the colon. In this study, we demonstrate for the first time that administration of lactoferrin modulates proinflammatory and anti-inflammatory cytokines in the colon, resulting in attenuation and prevention of colitis.

It was recently suggested that NF-κB plays an important role in development of TNBS colitis on the basis of the observation that the transcription factor from NF-κB was excessively activated in colitis and that an antisense oligonucleotide to NF-κB abrogated the clinical and histological signs of colitis associated with the TNBS model (28). It has been revealed that the signal transduction pathways activated in response to TNF-α and IL-1β initiated NF-κB activation through the activation of IkB kinase, which phosphorylated IkB, the dissociation of IkB and NF-κB, and then the translocation of NF-κB to the nucleus, allowing NF-κB to play its role in colitis (12, 28, 35). In this study, our observations provided evidence that the activated pathway of NF-κB in TNBS-induced intestinal inflammation in rats was attenuated by lactoferrin and resulted in a fundamental block to developing colitis.

The results that showed an inhibitory effect of lactoferrin on TNF-α and IL-1β suggest that lactoferrin released from secondary granules of activated neutrophils at an inflammatory site may provide an inhibitory feedback mechanism to prevent excessive neutrophil aggregation and activation (7). Further study is required to explore the role of internal lactoferrin on modulating colonic inflammation.

Our results showed that the plasma concentration of lactoferrin was significantly increased after oral administration of lactoferrin. This is consistent with the report that, after administration of bovine lactoferrin through stomach tube, the plasma concentration significantly increased in piglets (15). But lactoferrin administered with enema was less effective (data not shown). Therefore, the attenuating effect on colitis by orally administered lactoferrin is mainly due to lactoferrin directly absorbed into plasma.

On the other hand, our results showed that, after oral administration of lactoferrin, the fecal concentration was also significantly increased, and lactoferrin is reported to have bacteriostatic activity (38); therefore, it remains possible that the decreased inflammation by orally administered lactoferrin is partly due to a decrease in the colonic bacterial numbers. However, we showed that oral administration of lactoferrin at the dose (200 mg·kg⁻¹·day⁻¹) did not have a significant effect on the total number of colonic bacteria in this model. This result indicates that bacteriostatic activity of lactoferrin is not the main cause of anti-inflammatory effects in colonic inflammation.

In this study, oral administration of lactoferrin, whether in the preventive or treatment mode, attenuates the inflammatory reaction of the colon induced by instillation of TNBS. This observation implies the possibility that lactoferrin, in clinical practice, might contribute not only to maintaining remission but even to inducing remission in patients with inflammatory bowel disease.
In conclusion, the results obtained from the experiments with TNBS-induced colitis in rats, an animal model that has similarities with human inflammatory bowel diseases, support and extend the existence of an important anti-inflammatory action of lactoferrin. Although it is not possible to extrapolate findings from animal models to the clinical situation, these data further suggest that lactoferrin is potentially attractive as a therapeutic strategy for the treatment of inflammatory bowel disease.

We thank Yoshiko Nagoshi for her excellent technical assistance and helpful discussions.

REFERENCES


