TNF-α is crucial for the development of mast cell-dependent colitis in mice

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Rijnierse, Anneke, Andries S. Koster, Frans P. Nijkamp, and Aletta D. Kraneveld. TNF-α is crucial for the development of mast cell-dependent colitis in mice. Am J Physiol Gastrointest Liver Physiol 291: G969–G976, 2006. First published June 22, 2006; doi:10.1152/ajpgi.00146.2006.—Inflammatory bowel disease (IBD) describes chronic inflammatory conditions of the gastrointestinal tract, and TNF-α plays a pivotal role in mediating the response. The proinflammatory cytokine TNF-α is rapidly released by mast cells after degranulation. In the present study, we hypothesized TNF-α to be an important player in our recently described mast cell-dependent murine model for IBD. The effect of neutralizing anti-TNF-α MAb was studied on colonic hypersensitivity in mice induced by a skin application of dinitrofluorobenzene (DNFB) followed by an intrarectal challenge with dinitrobenzene sulfonic acid. Features of the colonic hypersensitivity response included diarrhea, mast cell infiltration and activation, infiltration of inflammatory cells in the colon, colonic patch hypertrophy, and increased mast cell-derived TNF-α levels in the colon. Anti-TNF-α MAb could effectively abrogate diarrhea in DNFB-sensitized mice 72 h after the challenge. The numbers of colonic patches and total tissue damage scores were reduced by anti-TNF-α MAb treatment in DNFB-sensitized mice 72 h after the challenge. Mast cell infiltration and activation remained unaffected by neutralizing anti-TNF-α MAb. Treatment with the corticosteroid dexamethasone, a frequently used therapeutic treatment in IBD, resulted in a reduction of diarrhea, cellular infiltration, and total tissue damage scores to the same extent as anti-TNF-α MAb. Additionally, dexamethasone treatment could also reduce total TNF-α levels in the colon, mast cell numbers, and mast cell activation in both vehicle- and DNFB-sensitized mice 72 h after the challenge. These findings suggest that TNF-α can play an instrumental role in causing inflammatory responses in the present murine model for IBD downstream from mast cell activation.

In our laboratory, we have developed and characterized a chemically induced immunological murine model for colonic hypersensitivity. Hypersensitivity is evoked by skin sensitization of mice with the low-molecular-weight compound 2,4-dinitrofluorobenzene (DNFB) followed by a local intrarectal challenge with the hapten. Features of this colonic hypersensitivity model are diarrhea, hypertrophy of lymphoid structures, recruitment of inflammatory cells, and infiltration and activation of mast cells in the colon (31). In this model, TNF-α levels in colon tissue were shown to be elevated after the induction of colonic hypersensitivity. Moreover, the increase of TNF-α in colonic lymphoid structures was demonstrated to be mainly of mast cell origin (31). To investigate the importance of TNF-α for the development of colonic hypersensitivity, we carried out anti-TNF-α MAb treatment in this murine model. Treatment with the corticosteroid dexamethasone was conducted to assess the therapeutic efficacy in DNFB-induced colonic inflammation. Consecutive treatment with both anti-mouse TNF-α Ab and dexamethasone resulted in improved stool consistency, reduced infiltration of inflammatory cells, and decreased hypertrophy of colonic lymphoid structures after DNFB sensitization and local challenge with the hapten. Mast cell infiltration and activation as well as total TNF-α levels in the colon were not affected by treatment with anti-TNF-α Ab. Dexamethasone, however, could significantly reduce mast cell numbers, mast cell activation, and total TNF-α levels in both vehicle- and DNFB-sensitized mice 72 h after the challenge. These findings demonstrate that TNF-α plays an important role in this colonic hypersensitivity model downstream from mast cell activation.

As a major mediator of inflammation, TNF-α is of importance in (auto)immune diseases like inflammatory bowel disease (IBD), and increased levels can locally be found at the site of inflammation (20, 32). IBD is a chronic inflammatory disorder of the gastrointestinal tract, and TNF-α has been shown to be elevated in blood, stool, and intestinal tissue of IBD patients (7, 26, 27). Not only are increased numbers of mast cells found in the mucosa of IBD patients both in inflamed and noninflamed areas (11, 29), but the mast cell has also been described to be an important source for TNF-α in the human intestine (6). This was further confirmed by the observation that mast cells in IBD patients contain a higher density of TNF-α and increased expression of TNF-α mRNA (6, 22).

Moreover, therapy with infliximab, an IgG1 murine-human chimeric anti-TNF-α MAb, in patients suffering from Crohn’s disease and ulcerative colitis, the major representatives of IBD, was shown to be effective in suppressing the inflammatory response (32, 36).

TUMOR NECROSIS FACTOR (TNF)-α is a proinflammatory cytokine with a wide variety of biological functions in inflammation like tissue remodeling, alteration of epithelial barrier permeability, increasing vascular permeability, activation of macrophages, recruitment of inflammatory cells, and upregulation of adhesion molecules (4, 6, 36). TNF-α can be produced and released by activation by monocytes, macrophages, T cells, and mast cells (4, 25). More importantly, mast cells are the only cell type capable of storing presynthesized TNF-α in the granules together with de novo synthesis of this cytokine (14). Indeed, during IgE-mediated allergic responses and bacterial infection, mast cells secrete TNF-α within minutes after challenge (34, 37). This unique ability of mast cells provides evidence that mast cells are the only readily available source of TNF-α in the early onset of infection and inflammation in peripheral tissues.
MATERIALS AND METHODS

Animals. Male BALB/c mice were obtained from Charles River Laboratories (Maastricht, The Netherlands). Mice were 6–8 wk of age and weighed 20–25 g by the time of use. Animals were housed in groups not exceeding 8 mice/cage. The experimental groups consisted of 9–10 mice/group. All mice were observed for stool consistency, colonic patches, and mast cell activation. Of these groups, 6–7 mice/group were used to assess mast cell infiltration and TNF-α levels in the colon, whereas in a number of 3 mice/group, the colon was harvested for histological purposes. Tap water and chow food were allowed ad libitum; there was a 12:12 h day-night cycle. All experiments were conducted in accordance with The Animal Care Committee of Utrecht University (Utrecht, The Netherlands).

Induction of colonic hypersensitivity. Mice were sensitized on day 0 by an application of either DNFB (0.6% in acetone-olive oil, 4:1) or vehicle (acetone-olive oil, 4:1) epicutaneously on the shaved abdomen (50 μl) and paws (50 μl divided over 4 paws). On day 1, mice received a boost of DNFB or vehicle on the abdomen only (50 μl). All animals were challenged intrarectally with 0.6% dinitrosulfonic acid (DNS) dissolved in 10% ethanol on day 5. The sensitization, boost, and challenge took place under light inhalation anesthesia (3% halothane). Mice were macroscopically scored on days 5–8 for stool consistency. Thereafter, mice were killed with an overdose of pentobarbital sodium to determine in vivo mast cell activation, mast cell infiltration into colonic tissue, colonic vascular permeability, cytokine production in the colon, colonic damage and cellular infiltration, and the presence and numbers of colonic patches.

Treatment regimes. Both vehicle- and DNFB-sensitized mice were treated intraperitoneally with 0.45 mg/mouse anti-TNF MAb (clone XT 22, rat IgG1, purified in our laboratory) or rat control IgG 10 min before and 24 and 48 h after challenge. This concentration of anti-TNF-α Ab has been previously shown to be successful in different murine models for experimental intestinal inflammation (16, 19, 23).

Dexamethasone treatment was accomplished in vehicle- and DNFB-sensitized mice with an intraperitoneal administration of 10 mg/kg 10 min before and 24 and 48 h after the challenge. PBS served as a control for dexamethasone. This concentration of dexamethasone has been shown to be successful in a whole variety of animal models and species (3, 19).

Clinical scoring of the disease. Clinical characteristics of the inflammatory response were obtained by assessing stool consistency. The stool consistency was scored every day at the same time point by placing the mice separately in cages without bedding. They were left in the cages until they relieved enough feces to establish the consistency till a maximum of 15 min to score no stool. Feces were taken out in the cages until they relieved enough feces to establish the consistency. Thereafter, mice were killed with an overdose of pentobarbital sodium to determine in vivo mast cell activation, mast cell infiltration into colonic tissue, colonic vascular permeability, cytokine production in the colon, colonic damage and cellular infiltration, and the presence and numbers of colonic patches.

Macroscopic scoring of the disease. After animals were killed 72 h after the challenge, the colon was carefully dissected from the anus until the cecum and placed in saline. The colon was opened longitudinally over the mesenteric border and washed gently in saline. It was placed on a rubber mat with the mucosal side up, and the numbers of colonic patches, which appear like bulges in the tissue, were counted with the naked eye.

Histology and immunohistochemistry. After careful dissection, the colon was placed in saline. The colon was then opened longitudinally over the mesenteric line, and feces were removed by gently washing in saline. The colon then was placed with the serosal side up and dissected free from fat. The making of Swiss rolls was accomplished by rolling the colon from the distal to proximal end. The roll was immediately placed in ice-cold 4% paraformaldehyde in PBS (pH 7.4) for at least 24 h and routinely embedded in paraffin for further use. Serial sections of 5 μm were cut using a microtome (Leica) and routinely stained with hematoxylin-eosin to observe damage and cellular infiltration. Per mouse, three different longitudinal sections of the colon tissue were stained and microscopically analyzed. Pictures shown are representatives of the different treatment groups.

To detect mast cells, 5-μm sections were immunohistochemically stained for mouse mast cell protease (mMCP)-1 as previously described (31). Per mouse, three different longitudinal sections of the colon tissue were stained and analyzed. The numbers of mMCP-1-positive cells were quantified by microscopical visualization and manual counting. Results are expressed as median average numbers of cells (minimum – maximum) per colon section.

Neutrophils were identified by peroxidase staining. Paraffin section of 5 μm were immersed in saline. Subsequently, sections were incubated with 0.25 mg/ml dianisobenzidine (DAB) solution for 20 min, and, after the addition of 0.1% peroxidase, the incubation was followed by another 20 min. Sections were counterstained by hematoxylin. The infiltration of neutrophils was scored as described previously (31). In brief, 0 was no infiltration; 1 was infiltration between the mucosa and submucosa but not around colonic patches; 2 was extensive infiltration, especially around colonic patches (cluster formation). Results are expressed as median neutrophil scores (minimum – maximum) per colon section.

Preparations of tissue homogenates. To determine mast cell infiltration into the tissue, whole colon homogenates were made. After the mice were killed, the colon was excised carefully and opened longitudinally. Feces were removed by gently washing in saline. The colon was placed in ice-cold PBS enriched with protease inhibitors (Complete Mini) in flat-bottom tubes. The tissue was dispersed on ice for 10 s according the rotor-stator principle (Ystral). Homogenates were centrifuged (14,000 rpm, 4°C, 10 min), and the supernatant was frozen until further use to assess mMCP-1 and TNF-α levels by ELISA.

Mast cell activation and infiltration in vivo. To monitor mast cell activation in time, blood samples of DNFB- and vehicle-sensitized mice were taken 30 min, 24 h, and/or 72 h after the intrarectal DNS challenge. Blood samples were collected via heart puncture, and 4% EDTA was added (10% vol/vol) to obtain plasma. After centrifugation, the plasma was stored at ~70°C until use. Levels of mMCP-1 in plasma were measured as described previously using a commercially available ELISA assay (21). Results are expressed as nanograms of mMCP-1 per milliliter of plasma.

In addition, to determine mast cell infiltration in the colon, mMCP-1 levels were measured in the supernatant of colon homogenates with the commercially available ELISA kit. Samples were used undiluted. Samples were diluted 10-fold for total protein measurements (BCA protein assay). Results are expressed as nanograms of mMCP-1 per milligram of total protein.

TNF-α levels in vivo. To determine TNF-α levels in vivo, TNF-α was measured in the supernatant of colon homogenates 72 h after the DNS challenge with a commercially available ELISA kit. Samples were used undiluted. Total protein content of the supernatant of colon homogenates was quantified with a commercial available detection kit (BCA protein assay). Samples were diluted 10-fold for total protein measurements. Results are expressed as picograms of TNF-α per milligrams of total protein.

Materials. DNFB, olive oil, the AEC chromogen staining kit, normal goat serum, rat control IgG, DAB, hematoxylin, and eosin were all purchased from Sigma Chemical (St. Louis, MO). DNS was purchased from Eastman Kodak (Rochester, NY). Tween 20 was purchased from Janssen Pharmaceutica (Beerse, Belgium). Sodium pentobarbital was purchased from Sanofi (Maassluis, The Netherlands). Anti-TNF MAb was purified from the XT22 hybridoma. Dexamethasone was purchased from Intervet (Boxmeer, The Netherlands). The rat Ig anti-mMCP-1 antibody was a kind gift of Dr. H. R. Miller, Royal (Dick) School of Veterinary Studies (University of Edinburgh), and the biotin-conjugated goat anti-rat Ig specific polyclonal antibody was purchased by Pharmingen (Aalt, Belgium). The BCA protein assay was purchased from Pierce (Elten-Leur, The Netherlands).
To investigate the percentages of total scored stool (score 0, diarrhea/watery stool (solid bars). Results are expressed as cumulative 20B

RESULTS

Fig. 1. Anti-TNF-α/H9251 treatment profoundly inhibits diarrhea found in dinitrofluorobenzene (DNFB)-sensitized and dinitrobenzene sulfonic acid (DNS)-challenged mice. A and B: stool consistency 72 h after the challenge of vehicle- and DNFB-sensitized mice treated with anti-TNF-α MAb (A) or dexamethasone (B). 0, normal well-formed pellets; 1, loose stool/easy to smear (shaded bars); 2, diarrhea/watery stool (solid bars). Results are expressed as cumulative percentages of total scored stool (score 0 not shown); n = 9–10 mice/group. *P < 0.05 compared with vehicle-sensitized mice subjected to the same treatment; †P < 0.05 compared with DNFB-sensitized mice treated with control compounds (Kruskal-Wallis test followed by a Dunn’s multiple-comparison test).

Netherlands). Complete mini-protease inhibitors were from Roche Diagnostics (Almere, The Netherlands). The mMCP-1 ELISA was from Moreaun Scientific (Midlothian, UK). Maxisorp surface 96-well plates were purchased from Nunc Immunoplate (Roskilde, Denmark). The TNF-α ELISA kit was purchased from Biosource (Nivelles, Belgium). Maxisorp surface 96-well plates were purchased from Nunc Immunoplate.

Statistics. Stool consistency data were analyzed from raw scoring data using a distribution-free Kruskal-Wallis test followed by a Dunn’s multiple-comparison test. Tissue damage scores, mast cells, and colonic patch numbers are all expressed as medians (ranges) and were also analyzed with the use of a distribution-free Kruskal-Wallis test followed by a Dunn’s multiple-comparison test. The following data were analyzed by two-way ANOVA and a Bonferroni multiple-comparison test: mMCP-1 content in plasma and colon tissue and TNF-α levels in colon tissue. In the figures, group means ± SE are given. P < 0.05 was considered to be significant. All data manipulations and statistical analysis were conducted with the use of Graphpad Prism (version 3.0, San Diego, CA).

RESULTS

Table 1. Effects of neutralizing anti-TNF MAb or corticosteroids by dexamethasone treatment in vehicle- and DNFB-sensitized mice 72 h after challenge

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Numbers of Colonic Patches</th>
<th>Tissue Damage Scores</th>
<th>Numbers of Mast Cells</th>
<th>Neutrophil Infiltration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control MAb</td>
<td>4 (3–5)</td>
<td>0 (0–1)</td>
<td>11 (5–20)</td>
<td>0 (0–1)</td>
</tr>
<tr>
<td>Vehicle</td>
<td>4 (5–8)*</td>
<td>2 (1–3)*</td>
<td>62 (32–99)*</td>
<td>1 (0–2)*</td>
</tr>
<tr>
<td>Anti-TNF-α MAb</td>
<td>4 (3–5)</td>
<td>0 (0–1)</td>
<td>11 (1–27)</td>
<td>0 (0–1)</td>
</tr>
<tr>
<td>Vehicle</td>
<td>5 (4–5)+</td>
<td>0 (2–2)†</td>
<td>58 (27–87)*</td>
<td>0 (0–1)+†</td>
</tr>
<tr>
<td>Vehicle</td>
<td>3.5 (3–6)</td>
<td>0 (0–1)</td>
<td>10 (6–2)†</td>
<td>0 (0–1)†</td>
</tr>
<tr>
<td>Vehicle</td>
<td>6.5 (5–8)+</td>
<td>2 (1–4)*</td>
<td>142 (58–222)*</td>
<td>1 (0–2)*†</td>
</tr>
<tr>
<td>Vehicle</td>
<td>2 (0–5)</td>
<td>0 (0–1)</td>
<td>7.5 (2–20)</td>
<td>0 (0–0)</td>
</tr>
<tr>
<td>Vehicle</td>
<td>3 (1–5)+</td>
<td>0 (0–1)†</td>
<td>32 (8–55)+</td>
<td>0 (1–1)+†</td>
</tr>
</tbody>
</table>

Colonic patches were counted at the mucosal side of the colon of vehicle- or dinitrofluorobenzene (DNFB)-sensitized mice, and results are expressed as median numbers of colonic per colon (minimum-maximum); n = 7–9 mice. Colon tissue was scored for cellular infiltration and tissue damage after hematoxylin-eosin staining. Per mouse, three sections of colon tissue were examined at three different longitudinal depths leaving at least 100 μm in between. A score was given to the total appearance of the colon, and results are expressed as median tissue damage scores (minimum-maximum); n = 3 mice. Mast cells were counted after mouse mast cell protease (mMCP)-1 staining. Per mouse, three different longitudinal sections were counted and averaged, and results are expressed as median average numbers of mMCP-1-positive cells per colon section (minimum-maximum); n = 3 mice. Neutrophil infiltration was scored after peroxidase staining. Per mouse, three different longitudinal sections were examined. A score was given per longitudinal section, and results are expressed as median neutrophil infiltration per colon section (minimum-maximum); n = 3 mice. *P < 0.05 compared with vehicle-sensitized mice subjected to the same treatment protocol; †P < 0.05 compared with DNFB-sensitized mice treated with control compound.

Treatment with anti-TNF-α Ab and corticosteroids can abolish DNFB-induced colonic hypersensitivity. To investigate the role of TNF-α in colonic hypersensitivity, which has been previously shown to be associated with diarrhea, cellular infiltration, and mast cell infiltration and activation (31), treatment with anti-TNF-α Ab was conducted in the present experiments. Administration of the corticosteroid dexamethasone in colonic hypersensitivity was used as a reference treatment.

The formation of diarrhea is indicative of a disturbed intestinal homeostasis and can be caused by inflammation or infection and is therefore an important feature of IBD. Figure 1 demonstrates that DNFB sensitization followed by an intrarectal challenge with DNS led to significantly deteriorated stool consistency in mice treated with control compounds 72 h after the challenge. However, treatment with both anti-TNF-α Ab (Fig. 1A) and dexamethasone (Fig. 1B) could completely abolish diarrhea formation in DNFB-sensitized mice 72 h after the challenge.

Colonic patches are small lymphoid follicles that appear on the mucosal site of the colon and are easily visible. An increase in visible colonic patches is indicative of hypertrophy of these structures (10, 31). Seventy-two hours after the challenge, the numbers of colonic patches were significantly increased in DNFB-sensitized mice treated with the control Ab as well as PBS compared with vehicle-sensitized mice subjected to the same treatment protocol (Table 1). Treatment with anti-TNF-α Ab and dexamethasone could significantly decrease the quantity of visible colonic patches to a number not significantly different from vehicle-sensitized mice exposed to the same treatment protocol (Table 1), indicating that cellular recruit-
ment to and/or proliferation of these lymphoid structures was affected by this treatment.

This was confirmed by the observation that tissue damage and cellular infiltration, combined in the total damage score, could significantly be reduced by treatment with anti-TNF-α Ab and dexamethasone in DNFB-sensitized mice 72 h after the challenge (Table 1). Figure 2 shows representative pictures of colon tissue of vehicle-treated (A and B) and DNFB-sensitized mice (C and D) treated with either control Ab (A and C) or anti-TNF-α Ab (B and D). Infiltration of inflammatory cells could be observed in control Ab-treated DNFB-sensitized mice 72 h after the challenge (Fig. 2C). This cellular infiltration could significantly be attenuated by treatment with the anti-TNF-α Ab (Fig. 2D and Table 1). Dexamethasone treatment also led to significant inhibition of cellular infiltration into the colon (Fig. 2, E–H, and Table 1). Infiltration of inflammatory cells in PBS-treated DNFB-sensitized mice 72 h after the challenge (Fig. 2G) could also be completely abolished by

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**Fig. 2.** Anti-TNF-α and dexamethasone treatment inhibits cellular infiltration and tissue damage found in DNFB-sensitized and DNS-challenged mice. Representative samples of hematoxylin-eosin-stained colon tissue of vehicle-sensitized animals treated with control Ab (A), anti-TNF-α MAb (B), PBS (E), or dexamethasone (F) show normal intact colon structure. Cellular infiltration in the colon of DNFB-sensitized mice 72 h after the challenge treated with control MAb (C) or PBS (G) could almost completely be abolished by treatment with anti-TNF-α MAb (D) or dexamethasone (H).
treatment with dexamethasone (Fig. 2H). The colonic lining of vehicle-sensitized mice was not affected by treatment with either PBS or dexamethasone (Fig. 2, E and F).

Furthermore, treatment with anti-TNF-α Ab and dexamethasone could significantly reduce the infiltration of neutrophils into the colon of DNFB-sensitized mice 72 h after the challenge compared with control-treated DNFB-sensitized mice (Table 1).

DNFB-induced mast cell infiltration and activation could be inhibited by dexamethasone but not by anti-TNF-α Ab treatment. Mucosal mast cells contain mMCP-1 in the granules, and this protein was visualized by specific immunohistochemical staining. The numbers of mMCP-1-positive cells were counted, and the results are presented in Table 1. As Table 1 demonstrates, the numbers of mast cells present in the colon were not affected by anti-TNF-α Ab treatment in DNFB-sensitized mice 72 h after the challenge. This is in agreement with the total mMCP-1 levels in the supernatant of colon homogenates 72 h after the challenge (Fig. 3A). However, treatment of DNFB-sensitized mice with dexamethasone did result in significantly decreased levels of mMCP-1 in the colon 72 h after the challenge compared with PBS-treated DNFB-sensitized mice (Fig. 3B). Indeed, dexamethasone treatment also significantly decreased total mMCP-1 levels in the colon of vehicle-sensitized mice 72 h after the challenge (Fig. 3B). Immunohistochemical staining also demonstrated significantly reduced mMCP-1-positive cells (Table 1).

After activation and degranulation of the mast cell, mMCP-1 is secreted into the bloodstream (17). Increased mMCP-1 levels in plasma are indicative of mast cell activation. Both 30 min and 72 h after the challenge, mMCP-1 levels in plasma were significantly increased in DNFB-sensitized mice compared with vehicle-sensitized mice (Fig. 4). Treatment with anti-TNF-α Ab did not affect mMCP-1 levels in plasma of vehicle- and DNFB-sensitized mice after the challenge at both time points (Fig. 4A).

In DNFB-sensitized mice treated with dexamethasone, no significant differences in mMCP-1 plasma levels 30 min after the challenge could be observed compared with PBS-treated DNFB-sensitized mice (Fig. 4B). Seventy-two hours after the challenge, dexamethasone treatment did cause significantly decreased mMCP-1 levels in both vehicle- and DNFB-sensitized mice compared with PBS-treated mice subjected to the same sensitization protocol (Fig. 4B).
Anti-TNF-α Ab treatment did not influence total TNF-α levels in the colon. As described previously, TNF-α in the colon has been demonstrated to be increased and mainly derived from mast cells in our murine colitis model (31). TNF-α measured in the supernatant of colon homogenates is indicative of the total TNF-α level present in the colon. Figure 5 demonstrates that total TNF-α levels were significantly enhanced in DNFB-sensitized mice subjected to treatment with the control Ab and could not be affected by treatment with anti-TNF-α Ab 72 h after the challenge. Even though the total TNF-α levels in DNFB-sensitized mice compared with vehicle-sensitized mice both treated with PBS barely reached statistical significance \((P = 0.06; \text{Fig. } 5B)\), dexamethasone treatment resulted in significantly decreased total TNF-α levels in the colon of DNFB-sensitized mice 72 h after the challenge (Fig. 5B).

**DISCUSSION**

TNF-α is a proinflammatory cytokine known to be of importance in IBD. Patients suffering from IBD show increased levels of TNF-α in the circulation, intestinal tissue at the site of inflammation, and stool (7, 26, 27). Moreover, current therapeutic strategies in IBD by neutralization of TNF-α show beneficial effects (32). Intestinal mast cells are suggested to be an important source for TNF-α in both Crohn’s disease and ulcerative colitis (6, 22). In the present study, we proposed a role for TNF-α in a murine colitis model associated with mast cell infiltration and activation (31). To further validate this model for translational medicine from animal models to the human situation, treatment with neutralizing anti-TNF-α MAb was conducted. Our data presented here indicate that treatment with anti-TNF-α could ameliorate the severity of the disease by reducing the development of diarrhea and infiltration of inflammatory cells. This occurred to the same extent as treatment with the corticosteroid dexamethasone.

TNF-α has shown to be an important mediator of diarrhea in human IBD patients and rodent experimental colitis (4, 12, 28). Mast cells can rapidly release prestored TNF-α upon activation (37). Counteracting the effect of TNF-α by the use of specific MAb contributes to improved stool consistency after the induction of colitis.

Mast cell-derived TNF-α is known to function as a chemoattractant of leukocytes to the site of inflammation in hypersensitivity reactions (5). The mechanism of TNF-α to recruit inflammatory cells to the site of inflammation can be subscribed to its ability to upregulate the expression of cellular adhesion molecules like ICAM-1, VCAM-1, and P- and E-selectin (15), and increased expression of cellular adhesion molecules can also be found in experimental and human IBD (8, 9). Blockade of TNF-α has been shown to inhibit cellular adhesion molecule expression in endothelial cells and therefore suppresses the migration of leukocytes (18, 33). This corresponds with our observation showing that treatment with anti-TNF-α MAb led to significantly reduced cellular and neutrophil infiltration into the colon and colonic damage. Furthermore, the reduced numbers of colonic patches observed in mice receiving anti-TNF-α treatment suggest that TNF-α is also involved in the enlargement of these lymphoid structures. This corresponds with the finding of McLachlan et al. (25), who demonstrated that peripheral mast cells could induce the hypertrophy of draining lymph nodes via the rapid secretion of TNF-α.

The total level of mMCP-1 in the colon is indicative of the total number of mast cells present in the colon. Treatment with anti-TNF-α MAb does not result in reduced infiltration and proliferation of mast cells to the colon as determined by total mMCP-1 levels as well as by immunohistochemical staining of mMCP-1 in the colon. Mast cell growth and proliferation is described to depend mainly on stem cell factor but also on several other factors including IL-3, IL-4, IL-9, and IL-10 but not TNF-α (13, 24). Treatment with anti-TNF-α MAb will therefore not affect the increase of mast cell number provoked by the induction of colitis. Moreover, the effect of anti-TNF-α treatment takes place downstream from mast cell activation because mMCP-1 levels could still be detected in plasma both 30 min and 72 h after the challenge. This is supported by the observation showing that the increased TNF-α levels in the colon during colitis after treatment with anti-TNF-α were unaffected. In conclusion, it can be stated that total mast cell numbers and TNF-α levels in the colon are not influenced by treatment with anti-TNF-α MAb and that the beneficial effect of this treatment is exerted downstream of mast cell activation.

As a reference treatment, the effect of the corticosteroid dexamethasone was studied to determine the therapeutic efficacy of anti-TNF-α in the DNFB-induced colitis model. Corticosteroids, like dexamethasone and prednisone, are fre-
quenty used as treatments in IBD. They show beneficial immunosuppressive effects by inhibition of the production of proinflammatory cytokines and by reducing the upregulation of adhesion molecules (2, 30). The data obtained in the present study demonstrate that treatment with dexamethasone in this colitis model also resulted in improved stool consistency, decreased cellular infiltration, and reduced numbers of colonic patches.

Furthermore, Soda et al. (35) have demonstrated that dexamethasone can destroy mast cells without degranulation and therefore abolish the induction of inflammatory responses. In addition, it was also shown that dexamethasone suppresses antigen-induced Fce receptor I-mediated mast cell activation (1). This is in agreement with our observations showing that dexamethasone was capable of reducing the total number of mast cells and inhibiting mast cell activation both in vehicle- and DNFB-sensitized mice. This finding, together with the reduced TNF-α levels after dexamethasone treatment, supports the proposal that TNF-α originates from mast cells in this colitis model. Furthermore, this strengthens the results obtained after treatment with anti-TNF-α MAb and sustains the suggestion that the beneficial effect of anti-TNF-α treatment occurs posterior from mast cell activation.

Because of the passage of a wide variety of pathogens (i.e., luminal microflora, food allergens, and parasitic nematodes), the gastrointestinal tract is constantly immunoinvasive. The immunosuppressive observations in vehicle-sensitized mice treated with dexamethasone indicate that dexamethasone can affect the constitutively activated state of the gastrointestinal tract.

Taken together, we demonstrated in this study that TNF-α is important for the development of mast cell-associated colitis in mice. Further validation of our recently described chemically induced immunological model for IBD shows that this model is useful to study translational medicine from animal models to the human situation.

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REFERENCES


