Antibodies to Interleukin 12 Abrogate Established Experimental Colitis in Mice

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Summary

In this study, we describe a novel murine model of chronic intestinal inflammation induced by the hapten reagent 2,4,6-trinitrobenzene sulfonic acid (TNBS). Rectal application of low doses of TNBS in BALB/c and SJL/j mice resulted in a chronic transmural colitis with severe diarrhea, weight loss, and rectal prolapse, an illness that mimics some characteristics of Crohn's disease in humans. The colon of TNBS-treated mice on day 7 was marked by infiltration of CD4+ T cells; furthermore, in situ polymerase chain reaction studies revealed high levels of interferon (IFN)-γ mRNA in diseased colons. Isolated lamina propria (LP) CD4+ T cells from TNBS-treated mice stimulated with anti-CD3 and anti-CD28 antibodies exhibited a Th1 pattern of cytokine secretion: a 20-50-fold increase in IL-2 and IFN-γ levels and a 5-fold decrease in IL-4 levels as compared with those of stimulated LP CD4+ T cells from control BALB/c mice. Administration of monoclonal anti–IL-12 antibodies to the TNBS-treated mice both early (at 5 d) and late (at 20 d) after induction of colitis led to a striking improvement in both the clinical and histopathological aspects of the disease and frequently abrogated the established colitis completely. Furthermore, LP CD4+ T cells isolated from anti–IL-12–treated mice failed to secrete IFN-γ upon in vitro stimulation. In summary, the data demonstrate the pivotal role of IL-12 and IFN-γ in a TNBS-induced murine model of chronic intestinal inflammation. Furthermore, they suggest the potential utility of anti–IL-12 antibodies in patients with Crohn's disease.

Inflammatory bowel disease (IBD), encompassing Crohn's disease (CD) and ulcerative colitis, are idiopathic chronic diseases occurring with increasing frequency in Western populations (1, 2). Recently, various animal models of chronic intestinal inflammation have been established, which will likely provide new insights into the pathogenesis of IBD (reviewed in 3). These include rats carrying transgenes of HLA-B27 and β2-microglobulin (4) and mice in which the genes for IL-2 (5), IL-10 (6), and the α or β chain of the TCR (7) have been inactivated by homologous recombination. In addition, a colitis model has been recently established by the adoptive transfer of normal CD45RBhi T cells from BALB/c mice to C.B.-17 scid mice (8). In this case, the transferred T cells manifest a Th1 cytokine response associated with granulomatous inflammation that can be abrogated by systemic treatment with rIL-10, but not with rIL-4 (8).

IL-12 is a recently characterized cytokine with unique structure and pleiotropic effects (9–12). It consists of two disulfide-linked subunits, p40 and p35, that form functionally active p40/p35 heterodimers or inhibitory p40 homodimers. IL-12 is produced mainly by macrophages/microcytes and can be efficiently induced by intracellular parasites, bacteria, and bacterial products. Functional studies showed that IL-12 enhances cytolytic activity of NK cells and macrophages, and that it induces, in synergism with the B7/CD28 interaction, cytokine production and proliferation of activated NK cells and T cells (13). Furthermore, IL-12 plays a pivotal role in Th1 T cell differentiation, and it induces naive T cells to produce IFN-γ. As a result of this ability to drive T cell responses to the Th1 phenotype, IL-12 has been shown to be an effective treatment of established parasitic infections in mice (14, 15) that elicit a Th2 T cell response. In addition, antibodies to IL-12 have been shown to prevent experimental autoimmune encephalitis, a disease mediated by Th1 T cells (16). In the present study, we describe a novel murine model of chronic intestinal inflammation by a single application of the hapten reagent 2,4,6-trinitrobenzene sulfonic acid (TNBS). Furthermore, we demonstrate that successful treatment of established colitis can be achieved by systemic administration of antibodies to IL-12. Since the lamina propria (LP) T cell responses induced by TNBS show similarity to those observed in human CD, the data suggest the potential therapeutic use of anti–IL-12 antibodies in this disease.
Materials and Methods

**Induction of Colitis.** Specific pathogen-free 2-4 mo-old female BALB/c or SJL/J mice were obtained from the National Cancer Institute (Bethesda, MD) and maintained in the Building 10A Animal Facility at the National Institutes of Health. To induce colitis, the mice were lightly anesthetized with metofane (methoxyflurane; Pitman-Moore, Mundelein, IL). A 3.5F catheter was then carefully inserted into the colon such that the tip was 4 cm proximal to the anus. To induce colitis, 0.5 mg of the hapten reagent TNBS (Sigma Chemical Co., St. Louis, MO) in 50% ethanol (to break the intestinal epithelial barrier) was slowly administered into the lumen of the colon via the catheter fitted onto a 1-ml syringe. In control experiments, mice received 50% ethanol alone using the same technique described above. The total injection volume was 100 µl in both groups allowing TNBS sensitivity; 1, very low level; and 2, low level of leukocytic infiltration; 3, moderate level (goblet cells, high vascular density, thickening of the colon wall); 4, transmural infiltrations, loss of appendix. Animals were then kept in a vertical position for 30 s and returned to their cages.

**Grading of Histologic Changes.** Tissues were removed at indicated time points and embedded in paraffin. Paraffin sections were made and stained with hematoxylin and eosin. The degree of inflammation on microscopic cross-sections of the colon was graded semiquantitatively from 0 to 4 (0, no signs of inflammation; 1, very low level; and 2, low level of leukocytic infiltration; 3, high level of leukocytic infiltration, high vascular density, thickening of the colon wall; 4, transmural infiltrations, loss of goblet cells, high vascular density, thickening of the colon wall). Grading was done in a blinded fashion by the same pathologist.

**Morphometric Assessment of Colon Wall Thickness.** Three or more animals from each treatment group were randomly selected at the indicated time points (see Results), and colon samples were removed and embedded in paraffin. Thickness of the colon wall was determined on cross-sections by measuring the distance from the serosal surface to the luminal surface at 2-mm intervals along the entire length of each section through a calibrated eyepiece using a Vanox S1 microscope (Olympus Corp., Lake Success, NY).

**Immunohistochemistry.** Samples were put into OCT compound on dry ice, and 7-µm cryosections were cut according to standard procedures. Sections were then air dried and fixed in cold acetone for 2 min at room temperature. Next, samples were rehydrated in PBS for 15 min, blocked with 5% FCS in PBS for 20 min, and incubated with FITC-conjugated rat anti-mouse CD4 antibody (1:100 dilution; obtained from Pharmingen, San Diego, CA) for 45 min in a dark humid chamber. Sections were then washed for an additional 15 min in PBS. Finally, sections were mounted and analyzed with a fluorescence microscope at an excitation wavelength of 490 nm.

**Quantification of CD4+ T lymphocytes.** T lymphocytes were performed on cryostat sections for at least three specimens from each time point and each treatment group by examining 10 randomly selected high power fields (HPFs). Under our experimental conditions (magnification of 400), one HPF represented 0.25 mm2.

**Cell Isolation and Purification of LP CD4+ T Cells.** LP lymphocytes were isolated from freshly obtained colonic specimens using a modification of the technique described by van der Heijden and Stok (17). After removal of the Peyer's patches, the colon was washed in Ca/Mg-free HBSS, cut into 0.5-cm pieces and incubated twice in HBSS containing EDTA (0.37 mg/ml) and DTT (0.145 mg/ml) at 37°C for 15 min. Next, the tissue was digested further in RPMI containing collagenase D (400 U/ml) and DNase I (0.1 mg/ml) (Boehringer Mannheim Biochemicals, Indianapolis, IN) in a shaking incubator at 37°C. LP cells were then layered on a 40-100% Percoll gradient (Pharmacia, Uppsala, Sweden), and lymphocyte-enriched populations were isolated from the cells at the 40-100% interface. Enriched CD4+ T cell populations were obtained by negative selection using mouse CD4+ T cell isolation columns (Isocell; Pierce Chemical Co., Rockford, IL). The resultant cells when analyzed by flow cytometry (FACScan®, Becton Dickinson & Co., Mountain View, CA) contained >85% CD4+ cells.

**Cell Culture of LP CD4+ T Cells.** Cell cultures of LP CD4+ T cells were performed in complete medium consisting of RPMI 1640 (Whitaker Bioproducts, Walkersville, MD) supplemented with 3 mM t-glutamine, 10 mM Hepes buffer, 10 mg/ml gentamycin (Whittaker), 100 U/ml each of penicillin and streptomycin (Whittaker), 0.05 mM 2-ME (Sigma Chemical Co.), and 10% FCS.

**Reagents and mAbs.** Unconjugated and biotinylated monoclonal rat anti–mouse IL-2 (clones JES6-1A12/JES6-SH4), IL-4 (BVD4-1D11/BVD6-24G2), IL-10 (JES5-2A5/SXC-1), and IFN-γ (R4-6A2/XMG1.2) antibodies and mouse rIL-2 (specific activity = 2.5 × 106 BRMP U/mg), IL-4 (105 U/mg by CTLL-2 assay), IL-10 (5 × 105 U/mg), and IFN-γ (105 U/mg) were purchased from Pharmingen and Genzyme Corp., (Cambridge, MA), respectively. Purified hamster anti–mouse CD3ε (clone 145-2C11) and hamster anti–mouse CD28 (clone 37.51) antibodies were obtained from Pharmingen.

**Cytokine Assays.** To measure cytokine production, 24-well plates (Costar Corp., Cambridge, MA) were coated with 10 µg/ml murine anti-CD3ε antibody in carbonate buffer (pH 9.6) overnight at 4°C. 105 LP CD4+ T cells were then cultured in 1 ml of complete medium in precoated or uncoated wells, and 1 µg/ml soluble anti-CD28 antibody was added to the anti-CD3ε-coated wells. Culture supernatants were removed after 48 h and assayed for cytokine concentration. Cytokine concentrations were determined by specific ELISA per the manufacturer's recommendation (PharMingen) using Immulon 4 96-well microtiter plates (Dynatech Laboratories Inc., Chantilly, VA). ODs were measured on a Dynatech MR 5000 ELISA reader at a wavelength of 490 nm.

**Isolation of Spleen CD4+ T Cells.** Spleens were aseptically removed and subsequently digested with collagenase (400 U/ml) and DNase 1 (0.1 mg/ml) at 37°C for 15 min. After filtration straining, the resulting splenocyte suspension was depleted of RBC by hypotonic lysis with ACK lysing buffer (B & B Scott, W. Warwick, RI). Cells collected from the 70–90% interface. Enriched CD4+ T cell populations were obtained by negative selection using mouse CD4+ T cell isolation columns as described above. As assessed by FACS® analysis, the resulting cell population contained >85% CD4+ cells.

**Cell Culture of Spleen CD4+ T Cells.** 105 spleen CD4+ T cells were cultured in 1 ml of complete medium (see above). Culture supernatants were removed after 48 h and assayed for cytokine concentration as described above.

**In Situ Reverse Transcriptase PCR (RT-PCR).** In situ RT-PCR for IFN-γ mRNA expression was performed as previously described (18). Cryosections were placed on charged glass slides that were cut to fit into 0.5-ml Eppendorf tubes. Samples were then fixed in 10% formaldehyde overnight at 4°C. Washed three times in PBS and four times in autoclaved dH2O for 5 min. Next, sections were permeabilized with 2 mg/ml trypsinogen (Sigma) in 0.01 N HCl for 15 min at 25°C followed by neutralization with buffer A (0.1 M Tris HCl, pH 7.5, 0.1 M NaCl). For DNA degradation, the sections were then incubated in RQ1 RNase-free DNase (8 U/100 ml; obtained from Promega Corp., Madison, WI).
son, WI) in buffer B containing 40 mM Tris HCl, pH 7.9, 10 mM NaCl, 6 mM MgCl$_2$, and 0.1 mM CaCl$_2$ at 37°C for 12 min and at 50°C for 10 min. Next, sections were incubated for 60 min at 50°C in a Perkin Elmer thermocycler in 100 μl of the following reaction mixture: 10 mM Tris HC1, 50 mM KCl, 1.5 mM MgCl$_2$, 25 μM dATP, 25 μM dTTP, 25 μM dCTP, 25 μM dGTP (Pharmacia Fine Chemicals, Piscataway, NJ), 100 nM of either IFN-γ primer (sense: 5'-GACAATCGGCGATGATCCGGGACAAC-3'; and antisense primer: 5'-TCTCTGAGGCTG-GATTCCGGCAACA-3' [19]), 10 mM DTT, 75 U RNasin, 400 U M-MLV reverse transcriptase (GIBCO BRL, Gaithersburg, MD). Slides were washed five times each in sodium citrate buffer (3 M NaCl, 0.3 M Na$_3$ citrate, pH 7.0, 2X SSC), 1X SSC, and twice in dH$_2$O.

Histologic analysis during the first days after induction of colitis showed infiltrations of neutrophil granulocytes into the colon. On day 7, a transmural inflammation affecting the entire colon (but sparing the small bowel) was found. The colitis was mainly characterized by lymphocytic infiltrates that were associated with thickening of the colon wall, ulcerations, loss of goblet cells, and the presence of granulomas (Fig. 3, a–b).

Immunohistochemical staining showed that on day 7, CD4+ T cells were increased in colons of TNBS-treated mice compared to control mice (Fig. 3, c–d). The differences in inflammatory activity were further confirmed by histologic grading of the colon sections (Table 1). As assessed by morphometric analysis of colon wall thickness and number of CD4+ T lymphocytes (Table 2), disease in-

![Figure 1](https://example.com/figure1.png)

**Figure 1.** Intrarectal administration of TNBS induces severe diarrhea and wasting disease (a). Rectal prolapse of a BALB/c mouse 7 d after administration of TNBS in 50% ethanol. (b) Weight changes of normal BALB/c mice, control mice treated with 50% ethanol alone, and BALB/c mice were treated with TNBS in 50% ethanol over a 12-d period. Weight data from one representative experiment is shown. Each point represents average weight data pooled from five mice. Standard errors are indicated. —, Ethanol group; —•—, normal BALB/c mice; —Θ—, TNBS-colitis group.
Intensity usually peaked between 2 and 4 wk after administration of TNBS. At later stages of the disease, there was a reduction in the number of granulocytes, but intramural lymphoid aggregates persisted and beginning fibrosis was found (Fig. 3 e). These histological signs of inflammation were still detected up to 2 mo after TNBS treatment, but were absent in ethanol-treated mice.

Histologically, the spleens of TNBS-treated mice showed an increase in the size of the red pulp and the periaorteriol lymphoid sheaths on day 7 when compared with spleens from control mice (Fig. 4, a–b). FACS® analysis of spleen lymphocytes revealed a twofold increase in the percentage of CD4+ and CD8+ T cells in TNBS-treated mice compared with control ethanol-treated mice and normal BALB/c mice, along with a reduction in B220+ B cells (data not shown).

Figure 2. Macrophage changes of colon and spleen in TNBS-treated mice. Photographs of dissected large intestine and spleen of a normal BALB/c mouse (top), a control BALB/c mouse treated with 50% ethanol (second row), and two BALB/c mice treated with TNBS in 50% ethanol (bottom row) 7 d after the initial rectal administration. The colons of the TNBS-treated mice are severely inflamed, hyperemic, and they contain less feces due to massive diarrhea.

Figure 3. Histologic analysis of the colon from BALB/c mice with TNBS-induced colitis and control mice. (a) Photomicrograph of hematoxylin and eosin-stained paraffin section of colon from a TNBS-treated BALB/c mouse on day 7. Loss of goblet cells and lymphocytic infiltrations are present. (b) Photomicrograph of hematoxylin and eosin-stained section of colon from a TNBS-treated mouse on day 7 showing a granuloma. (c) Detection of CD4+ cells in the colon of a BALB/c mouse treated with TNBS on day 7 by immunofluorescence. FITC-positive cells were seen in the subepithelial area and the lamina propria (X100). (d) Immunostaining with FITC-labeled anti-mouse CD4 antibodies in the colon of a control ethanol-treated BALB/c mouse on day 7. Only few positive cells were detected (X100). (e) Photomicrograph of hematoxylin and eosin-stained section of colon from a TNBS-treated mouse after 7 wk showing chronic inflammation with intramural lymphoid aggregates and beginning fibrosis.
Table 1. Histologic Grading of Colon Sections from Control BALB/c Mice and from Mice with TNBS-induced Colitis Treated with Anti-IL-12, Control Rat IgG, or with No Additional Reagent

| Severe colitis | Mild colitis | No signs of colitis |
|----------------|--------------|--------------------|
| 4              | 2            | 0                  |
| 3              | 1            | 1                  |
| 2              | 1            | 0                  |

Colon specimens were taken at indicated time points after administration of TNBS or ethanol, and the magnitude of inflammatory changes in the colon was analyzed on hematoxylin and eosin-stained cross-colon sections. Data were pooled from three independent experiments in each group. Ethanol group day 7; TNBS group day 7; anti-IL-12 group day 12; rat IgG group day 12.

Figures 4 and 5. Histologic analysis of the spleen from mice with TNBS-induced colitis and control mice. Photomicrographs of hematoxylin and eosin-stained sections (×100) from spleens of a control ethanol-treated BALB/c mouse (a) and a TNBS-treated BALB/c mouse (b) on day 7. The spleen of the TNBS-treated mouse reveals hypervascularisation and strikingly increased red pulp and periarteriolar lymphoid sheet areas.

Stimulated LP CD4+ T Lymphocytes of TNBS-treated Mice Secrete Th1 Cytokines. To examine cytokine production by infiltrating LP CD4+ T cells in the colon of TNBS-treated mice, we purified this population from colonic tissue specimens 7 d after the induction of colitis and compared their cytokine pattern with that of LP CD4+ T cells obtained from colonic tissue specimens of control ethanol-treated mice. Cells were cultured for 2 d and culture supernatants were analyzed for concentration of Th1 (IL-2, IFN-γ) and Th2 (IL-4, IL-10) cytokines by specific ELISA. As shown in Fig. 5 a, a 10-fold increase in the spontaneous IFN-γ production of LP CD4+ T cells was found in TNBS-treated mice. Furthermore, LP CD4+ T cells from TNBS-treated mice stimulated with anti-CD3 and anti-CD28 produced 20-50-fold higher levels of IL-2 and IFN-γ than LP CD4+ T cells from control mice (Fig. 5, a-b). Similarly, an increase in the spontaneous (4.5 vs 1.8 U) and induced (46 vs 16.8 U after stimulation with anti-CD3 and anti-CD28) IFN-γ production by spleen CD4+ T cells was found in the TNBS-treated animals compared with the ethanol control group at this time point.

In contrast to the above finding, secretion of IL-4 by unstimulated LP CD4+ T cells from TNBS-treated mice was identical compared to LP CD4+ T cells from ethanol-treated control mice, and in stimulated LP CD4+ T cells from TNBS-treated mice, the average secretion of IL-4 was reduced about fivefold compared with ethanol-treated control mice (Fig. 5 d). Finally, the secretion of IL-10 by unstimulated LP CD4+ T cells was similar in TNBS- and ethanol-treated mice (Fig. 5 d).

In Situ PCR Studies Show Elevated IFN-γ mRNA Expression in the Colon of TNBS-treated BALB/c Mice. To determine if the observed increase in IFN-γ production was also observed at the mRNA level, we evaluated the mRNA expression of IFN-γ in the colon of TNBS-treated mice by in situ PCR studies. As shown in Fig. 6 a, control ethanol-treated animals did not show significant expression of IFN-γ mRNA on day 7. In the TNBS-treated animals, however, we found a dramatic upregulation of IFN-γ mRNA expression at the same time point (Fig. 6 b). High staining intensity was seen particularly in the subepithelial areas.
Figure 6. Increased expression of IFN-γ mRNA in the colon of BALB/c mice with TNBS-induced colitis. (a) In situ PCR staining for IFN-γ mRNA expression in the colon of an ethanol-treated mouse. Only very low staining intensity is found using antisense-primed cDNA as template for the PCR reaction. The luminal site (L) of the colon is indicated (×50). (b) In situ PCR staining for IFN-γ mRNA expression in the colon of a TNBS-treated mouse. High staining intensity is seen in the LP. The luminal site of the colon is indicated. No staining was found with the antidiogoxigenin antibody using sense-primed cDNA as template for the PCR reaction (data not shown) (×100).

Figure 5. Cytokine production of stimulated and unstimulated LP CD4+ T cells in TNBS-induced colitis. LP CD4+ T cells were isolated from TNBS- and control ethanol-treated mice on day 7, cultured for 2 d in the presence or absence of anti-CD3 and anti-CD28 (see Materials and Methods) and culture supernatants were analyzed for concentration of IFN-γ (a), IL-2 (b), IL-4 (c), and IL-10 (d). Data represent three independent experiments done in triplicate. Standard errors are indicated. Ethanol group; TNBS-colitis group.

Early Administration of Antibodies to IL-12 Represses Colitis and Abolishes Wasting Disease in TNBS-treated Mice. Since the previous data suggested the presence of activated Th1 cells in TNBS-induced colitis, we sought to determine if antibodies to IL-12 might influence disease activity. We therefore treated mice 5 and 9 d after induction of the colitis systemically with anti-IL-12 or control rat IgG (see Materials and Methods). When mice were treated with anti-IL-12, a striking improvement of the wasting disease became apparent. Anti-IL-12-treated mice became more active and lost their ruffled coat appearance when com-
Figure 7. Anti-IL-12 antibodies abrogate colitis present 5 d after administration of TNBS. (a) Weight changes of BALB/c mice with TNBS-induced colitis after early administration (day 5) of anti-IL-12 antibodies or rat control IgG. After initial reduction of the body weight in both TNBS-treated groups, the mice treated with anti-IL-12 showed increase in the average body weight after day 5, whereas mice treated with rat control IgG continuously lost body weight. Each point represents data from five mice. The standard errors are indicated. — , Anti-IL-12 group; ——, rat IgG control group. (b) Gross appearance of the colon from two TNBS-treated mice given anti-IL-12 antibodies (top row) and two TNBS-treated mice given rat IgG (bottom row) at day 12 after initial administration of TNBS. There was a reduction in inflammatory activity in the anti-IL-12-treated mice. One representative experiment out of three is shown.

pared with untreated mice or mice given control rat IgG (data not shown); in addition, as depicted in Fig. 7 a, mice that had received anti-IL-12 usually obtained their initial body weight, whereas control IgG-treated mice continued to lose weight. Finally, gross inspection of the colon on day 12 revealed reduction in inflammatory activity in animals administered anti-IL-12 (Fig. 7 b).

Histologic studies showed significantly less inflammatory cells in the colons of anti-IL-12–treated mice (Fig. 8 a–b). In most cases, anti-IL-12 treatment completely abrogated the TNBS-induced inflammation and restored a normal histologic appearance of the colon. This was confirmed by histologic grading of colon sections: pooled data from three independent experiments showed significant reduction in inflammatory activity after anti-IL-12 treatment (Table 1).

IFN-γ Production by Stimulated LP CD4+ T Cells Is Reduced in TNBS-treated Mice Given Anti-IL-12. Next, we analyzed IFN-γ production by LP CD4+ T cells in anti-IL-12–treated animals. As shown in Fig. 9 a, we found an abrogation of high level IFN-γ production in TNBS-treated mice that had received anti-IL-12 compared with rat IgG-treated mice suggesting that the anti-IL-12 treatment might act by influencing the Th1-like response of local CD4+ T cells. In addition, Elispot assays for IFN-γ secretion by LP CD4+ T cells showed a dramatic reduction in the average number of Elispots in the anti-IL-12–treated group compared to the rat control IgG-treated group (Fig. 9 b–c). The size of the Elispots, however, was similar in both groups, indicating that the reduction in IFN-γ secretion by LP CD4+ T cells from anti-IL-12–treated mice is mainly due to a reduction in the number of IFN-γ-secreting cells.

Late Administration of Antibodies to IL-12 Abolishes Wasting Disease in Mice with TNBS-induced Colitis. Finally, we wanted to determine if anti-IL-12 treatment would be effective during later phases of the disease when colitis was fully established. We thus started administration of anti-IL-12 or control rat IgG on day 20 and repeated such treatment on days 24 and 28. As shown in Fig. 10 a, a striking increase in the average weight of mice was found after anti-IL-12 treatment but not after rat control IgG treatment. Furthermore, when LP CD4+ T cells from such mice were stimulated with anti-CD3 and anti-CD28, we found a reduction of IFN-γ secretion in those mice given anti-IL-12, but not those given rat control IgG (Fig. 10 b).
Figure 9. Analysis of IFN-γ production in BALB/c mice with TNBS-induced colitis given anti-IL-12 or rat control IgG. (a) Cytokine production of LP CD4+ T cells from TNBS-treated animals on day 12. There was an abrogation of high-level IFN-γ secretion in the anti-IL-12-treated animals, 1, Rat IgG control group; 2, anti-IL-12 group. (b) Elispot assay for IFN-γ secretion. LP CD4+ T lymphocytes from TNBS-treated mice given rat control IgG were isolated and analyzed as specified in Materials and Methods. A high number of Elispots per high power field was seen. (c) Elispot assay for IFN-γ secretion using LP CD4+ T cells from anti-IL-12-treated mice. There was a striking reduction in the average number of Elispots per high power field in the mice treated with anti-IL-12 antibodies compared with those given control IgG.

Discussion

In the present study, we describe a novel murine model of chronic intestinal inflammation induced by a single rectal administration of the hapten reagent TNBS. Furthermore, we demonstrate that the inflammation is associated with a Th1 T cell response and can be abrogated by systemic treatment with antibodies against IL-12, even after inflammation is well established.

The immune responses to hapten determinants such as TNBSs are believed to depend on the haptenization of autologous proteins and presentation of MHC class II-fitting peptides to CD4+ T cells by antigen presenting cells, ultimately leading to specific CD4+ T cell recognition, T cell expansion, and T cell cytokine responses (24). While hap-
In summary, the data demonstrate the pivotal role of IL-12 and IFN-γ in a murine Th1 model of chronic intestinal inflammation induced by the hapten reagent TNBS. The fact that this inflammation is abrogated by anti–IL-12 treatment, together with its similarity to CD, suggests that anti–IL-12 antibodies have potential therapeutic utility in patients with this disease. This hypothesis is supported by the recent finding (unpublished data) that there is a striking increase in IL-12 p35/p40 (p70) heterodimer expression, as assessed by immunohistochemistry, in the colon of patients with CD compared to normal colon.

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