The Interrelated Roles of TGF-β and IL-10 in the Regulation of Experimental Colitis

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In the present study, we define the relation between TGF-β and IL-10 in the regulation of the Th1-mediated inflammation occurring in trinitrobenzene sulfonic acid (TNBS)-colitis. In initial studies, we showed that the feeding of trinitrophenol-haptenated colonic protein to SJL/J mice induces CD4$^+$ regulatory T cells that transfer protection from induction of TNBS-colitis, and that such protection correlates with cells producing TGF-β, not IL-10. Further studies in which SJL/J mice were fed haptenated colonic protein, and then administered either anti-TGF-β or anti-IL-10 at the time of subsequent TNBS administration per rectum, showed that while both Abs abolished protection, anti-TGF-β administration prevented TGF-β secretion, but left IL-10 secretion intact; whereas anti-IL-10 administration prevented both TGF-β secretion and IL-10 secretion. Thus, it appeared that the protective effect of IL-10 was an indirect consequence of its effect on TGF-β secretion. To establish this point further, we conducted adoptive transfer studies and showed that anti-IL-10 administration had no effect on induction of TGF-β producing T cells in donor mice. However, it did inhibit their subsequent expansion in recipient mice, probably by regulating the magnitude of the Th1 T cell response which would otherwise inhibit the TGF-β response. Therefore, these studies suggest that TGF-β production is a primary mechanism of counter-regulation of Th1 T cell-mediated mucosal inflammation, and that IL-10 is necessary as a secondary factor that facilitates TGF-β production. ***The Journal of Immunology*, 2002, 168: 900–908.**

In recent years, it has become apparent that regulatory T cells play a key role in the immunopathogenesis of the inflammation occurring in experimental colitis (1, 2). In general, two such types of regulatory cells have been defined. The first, a TGF-β-producing T cell, was demonstrated in studies of trinitrobenzene sulfonic acid (TNBS)[3]-colitis, a Th1 T cell-mediated inflammation induced by intrarectal administration of the haptenating agent, TNBS. Here it was shown that the feeding of trinitrophenol (TNP)-haptenated colonic protein (HCP) prevented development of colitis and induced T cells in the lamina propria of the colon producing TGF-β. Furthermore, administration of anti-TGF-β to the fed mice abolished the protective effect of the feeding (3). TGF-β-producing T cells were also demonstrated in the SCID-transfer model of colitis, which is another Th1 T cell-mediated colitis, with studies showing that prevention of colitis in SCID mice given CD45RB^{high} T cells by cotransfer of CD45RB^{low} T cells is abolished by concomitant administration of anti-TGF-β (4). This observation has been supported by the recent observation that a CD25$^+$ T cell subpopulation in the CD45RB^{low} T cells is responsible for the prevention of colitis, and that such prevention is again abolished in mice by co-administration of anti-TGF-β (5).

A second type of regulatory T cell implicated in experimental colitis is a T cell-producing IL-10. The evidence supporting the existence of this regulatory T cell comes from studies showing that the ability of CD45RB^{low} cells to inhibit the development of colitis in the SCID transfer model (referred to above) is abrogated by the administration of Abs to the IL-10 receptor. In addition, protection from colitis is not observed when CD45RB^{low} T cells from IL-10-deficient mice are cotransferred with CD45RB^{high} T cells from wild-type mice (6). Finally, it has been shown that Ag-specific clones producing IL-10 (and perhaps TGF-β), namely Tr1 T cells, prevents colitis in the SCID transfer model when cotransferred with CD45RB^{high} T cells (7).

In the above studies, the neutralization of suppressive cytokines from regulatory T cells producing either TGF-β or IL-10 led to the onset of colitis. This suggests that TGF-β and IL-10 do not act independently in the prevention of colitis, but rather TGF-β and IL-10 regulatory functions are interrelated. To examine this possibility, we conducted a series of studies focusing on the conditions necessary for the induction and subsequent effector function of regulatory T cells arising in mice fed HCP, either in fed mice with TNBS-colitis, or in mice with TNBS-colitis to which the regulatory cells were transferred. We found that regulatory T cells preventing colitis must produce TGF-β to do so, but that such production requires the presence of IL-10 to down-regulate the level of Th1 cytokine production, which would otherwise inhibit TGF-β-producing cells.

Materials and Methods

Induction of colitis

Colitis studies were performed in specific pathogen-free, 5- to 6-wk-old male SJL/J mice obtained from the National Cancer Institute (National Institutes of Health, Bethesda, MD), and maintained in a specific pathogen-free animal facility at the National Institute of Allergy and Infectious Diseases (National Institutes of Health). Experiments were performed after 3 days of the arrival of the animals. Animals were treated in accordance with the National Institutes of Health guidelines. For induction of colitis, 2.5 mg

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Received for publication August 15, 2001. Accepted for publication November 8, 2001.

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[1] This study was supported in part by the research project, "Malattie infiammatorie croniche intestinali ed autoimmuni-componenti immunoregolatorie della mucosa nella patogenesi e prevenzione" art 12 del D.L.gs 502/1992.

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[3] Abbreviations used in this paper: TBNS, trinitrobenzene sulfonic acid; HCP, haptenated colonic protein; hpf, high-power field; LP, lamina propria; LPMC, LP mononuclear cells; TNP, trinitrophenol; EAE, experimental autoimmune encephalomyelitis.

0022-1767/02/$02.00

TNBS (pH 1.5–2.0; Sigma Aldrich, St. Louis, MO) in 50% ethanol was administered per rectum to lightly anesthetized mice through a 3.5 F catheter inserted into the rectum. The catheter tip was inserted 4 cm proximal to the anal verge, 100 ml of fluid (TNBS/ethanol) was slowly instilled into the colon, and the mouse was held in a vertical position for 30 s.

Histologic assessment of colitis

Tissues removed from mice at indicated times of death were fixed in 10% neutral buffered formalin solution (Sigma Aldrich), embedded in paraffin, cut into tissue sections, and stained with H&E. Stained sections were examined for evidence of colitis using the following criteria: the presence of lymphocyte infiltration, elongation, and/or distortion of crypts, frank ulceration, and thickening of the bowel wall. The degree of inflammation on microscopic cross-sections of the colon was graded semi-quantitatively from 0 to 4 (0: no evidence of inflammation; 1: low level of lymphocyte infiltration with infiltration seen in a $<$10% high-power field (hpf), no structural changes observed; 2: moderate lymphocyte infiltration with infiltration seen in 10–25% hpf, crypt elongation, bowel wall thickening which does not extend beyond mucosal layer, no evidence of ulceration; 3: high level of lymphocyte infiltration with infiltration seen in 25–50% hpf, high vascular density, thickening of bowel wall which extends beyond mucosal layer; 4: marked degree of lymphocyte infiltration with infiltration seen in $>$50% hpf, high vascular density, crypt elongation with distortion, transmural bowel wall-thickening with ulceration).

Generation of and feeding of HCP

HCP was generated as previously described (3). SJL/J mice were then fed 100 μg of HCP using an 18-gauge feeding needle every other day over an 8-day period.

Treatment of mice with anti-TGF-β or anti-IL-10 Abs

Anti-mouse TGF-β and anti-IL-10 Abs were obtained from ascites fluid generated in nude mice by hybridomas producing these Abs. For this purpose, nude mice were injected i.p. with hybridoma cell lines, and then maintained until ascites developed using standard procedures (8). Abs were purified from the ascites fluid using E-Z-SEP purification kits (Middlesex Sciences, Foxborough, MA). The hybridoma cell line 2G75A9 (neutralizing anti-TGF-$\beta_{1, 2, 3}$ was kindly donated by Dr. J. Leterio, Lab of Cancer Immunopathogenesis, National Cancer Institute, National Institutes of Health). The hybridoma cell lines (SXC-1 and SXC-2) producing neutralizing rat anti-mouse IL-10 Ab was a gift of Dr. B. Segal (Laboratory of Immunoregulation, National Institutes of Allergy and Infectious Diseases, National Institutes of Health). Mice were administered 1 mg of each Ab at indicated times.

Isolation and purification of lamina propria (LP) mononuclear cells and T cell subsets

LP lymphocytes were isolated from freshly obtained colonic specimens, as previously described (3). Enriched LP T cell and CD4$^+$ T cell populations were obtained from these lymphocyte populations by positive selection, using mouse T cell and CD4$^+$ T cell isolation columns (Isocell; Pierce, Rockford, IL). Information on the selecting Abs bound to the column and the use of the column is available from the manufacturer. The resulting cell population, when analyzed by flow cytometry (FACScan; BD Biosciences, Mountain View, CA) contained $>$90% LP T cells or LP CD4$^+$ T cells (RM4-4 (CD4 stain); BD PharMingen, San Diego, CA). In some experiments, purified LP CD4$^+$ T cells were subjected to a further negative selection isolation process. In brief, LP CD4$^+$ T cells were suspended at $1\times$ 10^6/ml in calcium-free PBS with 1% FCS (coating media), to which anti-CXCR3 Ab was added. Ab to CXCR3 was a gift from Dr. J. Farber (Laboratory of Clinical Investigation, National Institutes of Health), and prepared as follows. Rabbit anti-murine CXCR3 at a concentration of 0.3 mg/ml was incubated with 2 μl of ultra-pure H_2O and 1.2 μl of 1 M HEPES buffer for 30 min at room temperature. Purified LP CD4$^+$ T cells were then added to this preparation and incubated for an additional 40 min at 4°C, washed 3 times with cold $1\times$ PBS and resuspended in coating media at a concentration of $2\times$ 10^7 cells/ml. The Ab-coated cell population were then removed by incubation with immunomagnetic beads coated with sheep anti-rabbit IgG obtained from Dynal (New York, NY).

Adoptive transfer of LP CD4$^+$ T cells

LP cells were isolated from HCP-fed mice 5 days after completion of the last feeding, or from TNBS-induced colitis mice 4 or 5 days after intrarectal administration. Enriched CD4$^+$ T cells were obtained from these lymphocytes by positive selection using CD4$^+$ T cell isolation columns (Isocell; Pierce). Purified CD4$^+$ T cells (3.0×10^5) were then injected i.v. into the tail vein of normal SJL/J mice.

Cell culture of LP mononuclear cells (LPMC) and CD4$^+$ T cells

Cell culture of LPMC or CD4$^+$ T cell subset were performed in complete medium consisting of RPMI 1640 (Whittaker, Walkersville, MD) supplemented with 3 mM L-glutamine, 10 mM HEPES buffer, 10 mg/ml gentamicin (Whittaker), 100 U/ml each of penicillin and streptomycin (Whittaker), 0.05 mM 2-ME (Sigma Aldrich), and 10% FCS (Sigma Aldrich). Cultures of LPMC for evaluation of TGF-β production were performed in serum-free media supplemented with 1% nutridoma-SP (Roche Diagnostics, Indianapolis, IN).

Stimulation and measurement of cytokine production by LPMC

To measure the capacity of isolated LPMC to produce cytokines, the LPMC populations were cultured in complete medium (or serum-free media in the case of TGF-β) at 10^6 cells/ml in 24-well plates (Falcon; BD Biosciences) coated or uncoated with anti-CD3ϵ Ab (clone 145-2C11; BD PharMingen). Coating was accomplished by pre-exposure of individual wells to 10 μg/ml murine-anti-CD3ϵ Ab in carbonate buffer (pH 9.6) overnight at 4°C. Culture fluid for cell populations in coated wells also contained 1 μg/ml soluble CD28 Ab (clone 37.51; PharMingen). After 48 h of culture under these conditions (or 72 h for TGF-β), culture supernatants were removed and assayed for the presence of cytokines (IFN-γ, IL-10, and TGF-β) by ELISA. To measure IL-12 and TNF-α production, LPMC cells were preincubated for 18 h with 1000 U/ml recombinant murine IFN-γ (Genzyme; R&D Systems, Minneapolis, MN), followed by stimulation with 0.03% *Staphylococcus aureus*, Cowan's strain I (Calbiochem, La Jolla, CA). Culture supernatants were harvested after an additional 24 h.

ELISAs

Cytokine concentrations (except for TGF-β) were determined by commercially available specific ELISAs using paired murine cytokines per the manufacturer's recommendations (Endogen, Woburn, MA). TGF-β concentrations were determined using the commercially available TGF-β Quantikine kit (R&D Systems). Optical densities were measured on a Dynatech MR 5000 ELISA reader at a wavelength of 490 nm. Data was analyzed against the linear portion of the generated standard curve.

Statistical analysis

Assessment of statistical differences was determined by Student's *t* test and χ^2 test as indicated.

Results

Adoptive transfer of LP CD4$^+$ T cells from TNP-HCP-fed mice can down-regulate TNBS-colitis via the production of TGF-β

In previous studies, we showed that the Th1 T cell-mediated transmural colitis induced in SJL/J mice by the administration of TNBS per rectum can be suppressed by prior induction of "oral tolerance" to TNBS, which is accomplished by feeding TNBS-HCP (3). This protective effect of feeding HCP was accompanied by the induction of CD4$^+$ T cells producing TGF-β, and could be abrogated by the co-administration of anti-TGF-β Ab. In initial studies to further define this regulatory effect in the present study, we used an adoptive transfer model of TNBS-colitis, in which primed CD4$^+$ T cells obtained from mice with TNBS-colitis or LP CD4$^+$ T cells from HCP-fed mice were transferred to naive mice. Accordingly, we isolated LP CD4$^+$ T cells from mice with TNBS-colitis or LP CD4$^+$ T cells obtained from HCP-fed mice, and then administered these cells (i.v.) to naive recipient mice. The recipient mice were then administered TNBS per rectum at a dosage of TNBS known to cause colitis (2.5 mg) (see *Materials and Methods*). As shown in Fig. 1, we found that whereas mice administered TNBS per rectum which had been adoptively transferred LP CD4$^+$ T cells from TNBS-colitis mice alone experienced severe and persistent weight loss, mice that had been administered LP CD4$^+$ T cells from HCP-fed mice did not experience weight loss.

Similarly, as shown in Fig. 2, recipient mice that were administered LP CD4$^+$ T cells from TNBS-colitis mice showed evidence of severe inflammation, manifested by shortened, thickened, and

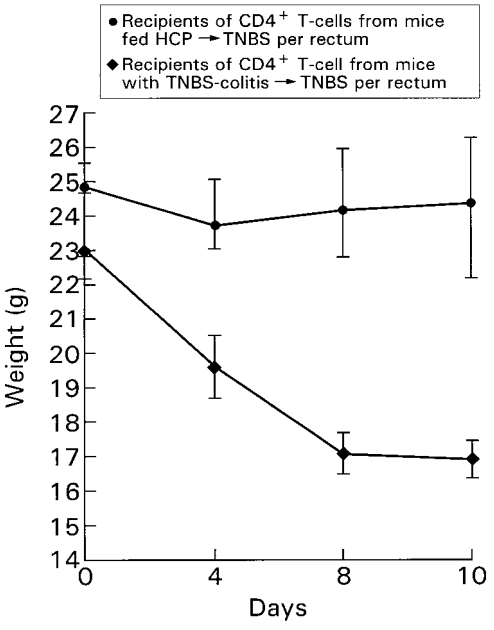

FIGURE 1. TNBS-induced colitis can be suppressed by the adoptive transfer of LP $CD4^+$ T cells from HCP-fed mice. Weight changes of recipients of LP $CD4^+$ T cells obtained from: mice fed HCP (HCP-fed $CD4^+$ T cells) (●), and mice with TNBS-colitis (TNBS $CD4^+$ T cells (◆). Recipient mice received TNBS per rectum 5 days after adoptive transfer of LP $CD4^+$ T cells (see *Materials and Methods*). Data shown are from one representative experiment of three similar experiments. Each point represents the average weight of five mice and bars SD

erythematous colons; whereas mice that were administered LP $CD4^+$ T cells from HCP-fed mice showed no evidence of macroscopic colonic inflammation.

In further studies to define the mechanism of the protective effect of LP $CD4^+$ T cells from HCP-fed mice, we extracted LP $CD4^+$ T cells from recipient mouse colons 5 days after TNBS challenge, and determined their capacity to secrete cytokines after

FIGURE 2. Macroscopic appearance of colons from recipients of LP $CD4^+$ T cells. Recipients of LP $CD4^+$ T cells obtained from mice fed HCP (*upper colon*), and mice with TNBS-colitis (*lower colon*). All mice received an intrarectal challenge of TNBS 5 days after adoptive transfer of LP $CD4^+$ T cells (see *Materials and Methods*) and all mice were sacrificed 5 days after TNBS challenge. As shown, there was severe colitis in recipients of LP $CD4^+$ T cells obtained from TNBS colitis mice (*lower colon*), whereas there was no significant macroscopic colitis seen in recipients of LP $CD4^+$ T cells obtained from HCP-fed mice (*upper colon*).

stimulation with anti-CD3/anti-CD28 Abs in vitro (see *Materials and Methods*). As shown in Fig. 3*A*, LP $CD4^+$ T cells isolated from the colons of mice that had been administered LP $CD4^+$ T cells from TNBS-colitis mice (LP $CD4^+$ effector cells) produced large amounts of IFN-γ, whereas $CD4^+$ T cells isolated from recipients that were administered LP $CD4^+$ T cells from HCP-fed mice (LP $CD4^+$ regulatory cells) produced strikingly lower amounts of IFN-γ ($p < 0.002$). In addition, the LP $CD4^+$ T cells isolated from recipients of LP $CD4^+$ regulatory cells produced greatly increased amounts of TGF-β, in contrast to $CD4^+$ T cells from recipients of LP $CD4^+$ effector cells, which produced low amounts of TGF-β ($p < 0.002$), as shown in Fig. 3*B*. Finally, we measured IL-10 production by the isolated LP $CD4^+$ T cells. In this case, we found no marked differences in the production of IL-10 by LP $CD4^+$ T cells from recipient mice who had received LP $CD4^+$ T cells from mice with colitis as compared with mice that were administered LP $CD4^+$ T cells from HCP-fed mice (308 vs 587 pg/ml, respectively). These findings were replicated and further explored in the subsequent studies described below.

Taken together, these data show that oral feeding of HCP to SJL/J mice leads to the induction of LP $CD4^+$ regulatory T cells that prevents the induction of TNBS-colitis. In addition, they show that the transferred cells from fed mice lead to a population of LP cells that produce TGF-β in the recipient colons. Finally, they suggest that increased production of TGF-β, rather than IL-10, may correlate with protection from colitis.

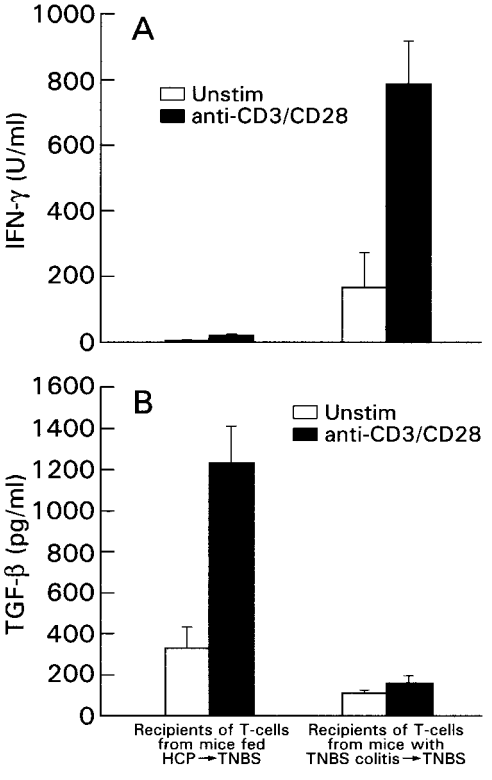

FIGURE 3. Cytokine production by LP $CD4^+$ T cells isolated from recipient mice colons after TNBS challenge per rectum. *A*, IFN-γ; *B*, TGF-β secretion from recipients of LP $CD4^+$ T cells obtained from mice fed HCP, or from mice with TNBS-colitis. All recipient mice were administered TNBS per rectum 5 days after adoptive transfer of LP $CD4^+$ T cells (see *Materials and Methods*). Data shown represent mean values obtained from three independent experiments. In each experiment, culture supernatants from cultures of pooled cells extracted from five mice per group were analyzed. Bars represent SEs. IFN-γ and TGF-β secretion, $p < 0.002$ TNBS vs HCP.

Treatment with either anti-TGF-β or anti-IL-10 mAb at the time of TNBS administration abolishes the protective effect of feeding HCP

To further explore the relative roles of TGF-β and IL-10 in the suppression of TNBS-colitis, we determined the effect of anti-TGF-β and anti-IL-10 Ab administration on the protective effect of feeding HCP. In these studies, mice were fed HCP every other day for 8 days. After an additional 5 days, the mice were administered TNBS per rectum. In addition, certain groups of mice also received anti-TGF-β or anti-IL-10 (1 mg/mouse) i.p. at the time of TNBS administration per rectum (see *Materials and Methods*).

As shown by the weight curves in Fig. 4, we found that whereas mice fed HCP alone did not develop colitis following administration of TNBS per rectum, mice fed HCP and administered anti-TGF-β or anti-IL-10 Ab (at the time of intrarectal TNBS administration) both developed colitis which did not differ from that of mice given TNBS per rectum which had not been fed HCP. However, as shown in the microscopic analysis of colonic tissue depicted in Fig. 5, while mice administered either anti-TGF-β or anti-IL-10 developed histologic evidence of colitis, those administered anti-TGF-β displayed a more severe inflammation than mice administered anti-IL-10. Thus, when colons from these mouse groups were subjected to histologic scoring (see *Materials and Methods*), a statistical difference in histologic scores was observed with a score of 3.6 + 0.3 for the anti-TGF-β treated mice, and a score of 2.7 + 0.4 for the anti-IL-10 treated mice ($p < 0.05$). This disparity was also evident in the mortality of the anti-TGF-β and anti-IL-10 treated mice at day 4 after TNBS administration. Mice administered anti-TGF-β displayed a high mortality rate (70%) as compared with mice given TNBS per rectum alone or in combination with anti-IL-10 Abs (18 and 22% respectively) ($p < 0.01$ mortality anti-TGF-β treated mice as compared with anti-IL-10 treated mice). Finally, recognizing that these differences in the effects of anti-IL-10 and anti-TGF-β could have been due to differences in the potencies of the Abs used, we also performed comparative studies in which the dose of anti-IL-10 was increased to 2 mg i.p. Again we noted that reversal of protection by anti-TGF-β was more complete, both in terms of microscopic appearance of the colon and mortality (data not shown). It should be noted that normal SJL mice which were administered anti-IL-10 and anti-TGF-β mAbs, and not given TNBS per rectum did not display evidence of weight loss or intestinal inflammation. In addition, these mice did not manifest any change in IFN-γ or TGF-β secretion as compared with normal SJL mice which were not administered these Abs (data not shown).

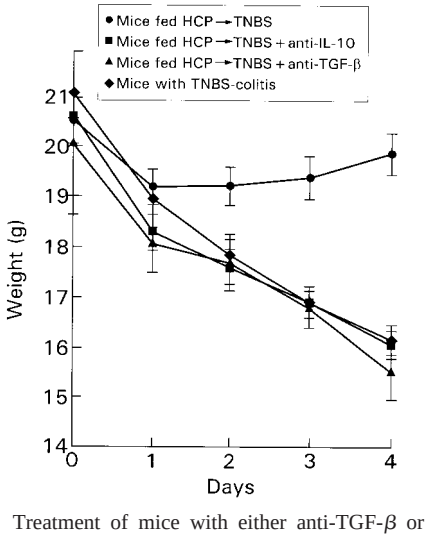

FIGURE 4. Treatment of mice with either anti-TGF-β or anti-IL-10 mAb at the time of TNBS administration abrogates the protective effect of feeding HCP on the development of TNBS-colitis. Weight changes of mice that were administered TNBS per rectum after HCP feeding (●), TNBS per rectum and anti-TGF-β IP after HCP feeding (▲), TNBS per rectum and anti-IL-10 IP after HCP feeding (■), or TNBS per rectum without any previous treatment (♦). Each point represents cumulative mean weight data from five different experiments. In each experiment, the various experimental groups consisted of at least five mice. Bars represent SE.

In further analysis of the various mouse groups in this phase of the study, we measured the capacity of mononuclear cells extracted from the LP of the mice to produce Th1 cytokines in response to anti-CD3/anti-CD28, or to produce IL-12 and TNF-α in response to SAC plus IFN-γ stimulation in vitro (see *Materials and Methods*). As shown in Fig. 6, *A* and *B*, LPMC from mice given TNBS per rectum (and who manifested TNBS-colitis) secreted large amounts of IL-12 and IFN-γ, whereas those from mice fed HCP produced only small amounts of these cytokines. However, cells from mice fed HCP that had been concomitantly treated with anti-TGF-β produced even greater amounts of Th1 cytokines than mice given TNBS per rectum alone. Finally, cells from mice fed HCP that had been concomitantly treated with anti-IL-10 also produced increased amounts of IL-12 and IFN-γ, although such production was lower than that of mice treated with anti-TGF-β or those given TNBS per rectum alone. It should also be noted that mice treated with anti-IL-10 produced substantial amounts of TNF-α (617 pg/ml) as compared with mice treated with anti-TGF-β (951 pg/ml), or administered TNBS alone (1086 pg/ml); perhaps explaining the fact that these mice displayed a weight loss equal to that of mice given TNBS per rectum alone or in conjunction with anti-TGF-β.

In parallel studies, we measured TGF-β and IL-10 production by LPMC extracted from the various mouse groups. As shown in Fig. 6*C*, LPMC from mice given TNBS per rectum that had been fed HCP and which did not have colitis produced large amounts of TGF-β, compared with LPMC from mice given TNBS alone ($p < 0.01$). In addition, LPMC from mice treated with either anti-TGF-β or anti-IL-10 and which also had colitis produced only small amounts of TGF-β which did not differ from those produced by mice given TNBS per rectum alone ($p > 0.05$). However, a somewhat different picture was obtained for IL-10 production (Fig. 6*D*). In this case, cells from mice treated with TNBS per rectum alone produced a moderate amount of IL-10, while cells from mice that had been fed HCP produced large amounts of IL-10 ($p < 0.01$). Furthermore, whereas cells from mice given TNBS per rectum and treated with anti-IL-10 produced low amounts of IL-10 ($p < 0.01$, HCP-fed and anti-IL-10 vs HCP-fed alone), cells from mice given TNBS per rectum and treated with anti-TGF-β produced high levels of IL-10, i.e., levels equal to those produced by cells of mice that had been fed HCP and not administered an Ab ($p > 0.05$). It should be noted that such IL-10 production most likely represents IL-10 produced by both T cells and APCs in the LPMC population, because anti-CD3 stimulation of T cells results in indirect stimulation of APCs via CD40L expression (9).

Taken together, these studies demonstrate that the effectiveness of feeding HCP and preventing a Th1 T cell-mediated inflammation correlates best with the presence of cells producing TGF-β. In addition, while administration of anti-IL-10 also prevents the protective effect of HCP prefeeding, such prevention correlates with the presence of decreased TGF-β production. Severe colitis following anti-TGF-β administration occurs despite high levels of IL-10 production. These results are compatible with the view that while counter-regulation by TGF-β occurs as a direct effect of suppression by this cytokine, counter-regulation by IL-10 occurs

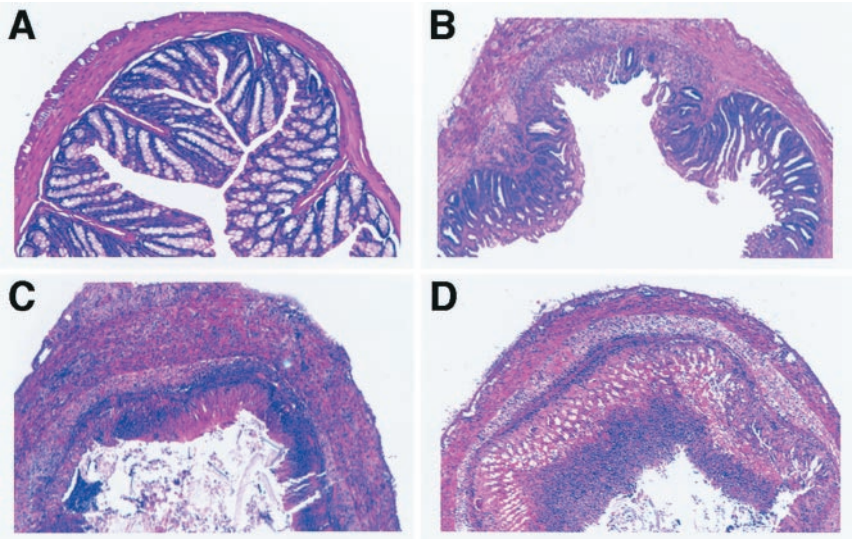

FIGURE 5. Histologic analysis of colons of the groups of SJL/J mice with TNBS-colitis, defined in legend to Fig. 4. Treatment with anti-TGF-β at the time of TNBS per rectum administration after HCP feeding induces a more severe colitis as compared with the colitis observed in mice treated with anti-IL-10. *A*, Photomicrograph (×100) of H&E-stained paraffin section of a representative colon from a mouse fed HCP and then administered TNBS per rectum; no significant inflammation is observed. *B*, Photomicrograph (×100) of H&E-stained paraffin section of a representative colon from a mouse fed HCP and then administered TNBS per rectum along with anti-IL-10 IP; moderate to severe inflammation is observed with thickening of bowel wall which extends beyond the mucosal layer, lymphocytic infiltrates in at least 50% of hpfs, distortion of crypts is present. *C*, Photomicrograph (×100) of H&E-stained paraffin section of a representative colon from a mouse fed HCP and then administered TNBS per rectum along with anti-TGF-β i.p.; severe transmural inflammation is present with significant bowel wall thickening, lymphocytic infiltrates in greater than 50% of hpfs, epithelial cell ulcerations and loss of goblet cells. *D*, Photomicrograph (×100) of H&E-stained paraffin section of a representative colon of a mouse with TNBS colitis. Transmural inflammation is present with bowel wall thickening, lymphocytic infiltrates in greater than 50% of hpfs; histologic score (see *Materials and Methods*) represents cumulative microscopic analysis of serial cross-section and longitudinal sections per mouse. In each experiment, each group consisted of at least five mice, with three experiments performed.

as an indirect effect on TGF-β production, and IL-10 has no significant suppressor effect in the absence of an effect through TGF-β production.

The effect of IL-10 on the induction of TGF-β-producing T cells

To further clarify the role of IL-10 in the counter-regulation of TNBS-colitis, we conducted a series of studies in which anti-IL-10 was given to mice either during the induction phase of TGF-β-producing T cells (i.e., at the time of HCP-feeding), or at the time these cells are stimulated to produce large (suppressive) amounts of TGF-β (i.e, at the "effector phase" of TGF-β producing cell activity).

In a first series of such studies addressing the induction of TGF-β-producing T cells, LP T cells were isolated from the colons of either HCP-fed mice (given HCP orally every other day for 8 days) or HCP-fed mice which were administered anti-IL-10 on days in between feedings (0.5 mg/dose/mouse each day during feedings for total 2 mg mAb/mouse). The LP cells from the animals treated were then pooled and adoptively transferred to naive SJL/J mice who were subsequently administered TNBS per rectum 5 days later. As shown in Fig. 7, the recipient of the cells from the mice fed HCP or fed HCP and administered anti-IL-10 did not display significant weight loss following administration of TNBS per rectum, whereas the mice given TNBS per rectum alone experienced considerable weight loss. This result was verified by an additional experiment in which the amount of anti-IL-10 mAb administered to mice during feedings was doubled to 4 mg/mouse. In this case as well, mice which were administered anti-IL-10 still did not manifest significant weight loss more than mice fed HCP alone ($p > 0.05$). However, it should be noted that in both experiments that all of the recipients of cells from mice fed HCP alone manifested no weight loss or macroscopic evidence of colitis, whereas the recipients of cells from mice fed HCP and administered anti-IL-10 displayed a mixed picture. Two-thirds developed no macroscopic evidence of colitis, whereas one-third displayed evidence of macroscopic colitis; despite the fact that all of the mice were recipients of aliquots of the same pooled cell population in any given experiment. We return to an explanation of this incomplete effect below.

Studies of cytokine production by LP T cells corroborated these macroscopic results. Thus, as shown in Fig. 8,*A* and *B*, the recipients of LP T cells from HCP-fed mice or the two-thirds of the recipients of LP T cells from HCP-fed and anti-IL-10-treated mice manifesting no colitis yielded LPMC cells that displayed minimal IL-12 and IFN-γ production, whereas LPMC cells from mice that were administered TNBS per rectum alone displayed high IL-12 and IFN-γ production. Similarly, as shown in Fig. 8*C*, recipients of LP $CD4^+$ T cells from HCP-fed mice, or the two-thirds of the recipients of LP T cells from HCP-fed and administered anti-IL-10 mice that did not manifest colitis yielded LPMC cells that exhibited high TGF-β production; whereas the cells from mice that were administered TNBS per rectum alone produced low TGF-β production. In contrast, the one-third of the recipients of LP T cells from HCP-fed and anti-IL-10-treated mice that manifests colitis yield LPMC cells, which displayed high IL-12 and IFN-γ production, and low TGF-β production.

The above data relating to the two-thirds of the recipient mice who were administered cells from HCP-fed mice and anti-IL-10 at the same time (i.e., during the induction of TGF-β-producing T cells, and were protected from colitis) strongly suggest that the negative effect of anti-IL-10 on protection from colitis observed in the studies above was not due to the fact that IL-10 is necessary during the induction of TGF-β producing T cells. On the contrary, they suggest that the latter induction is independent of IL-10. One

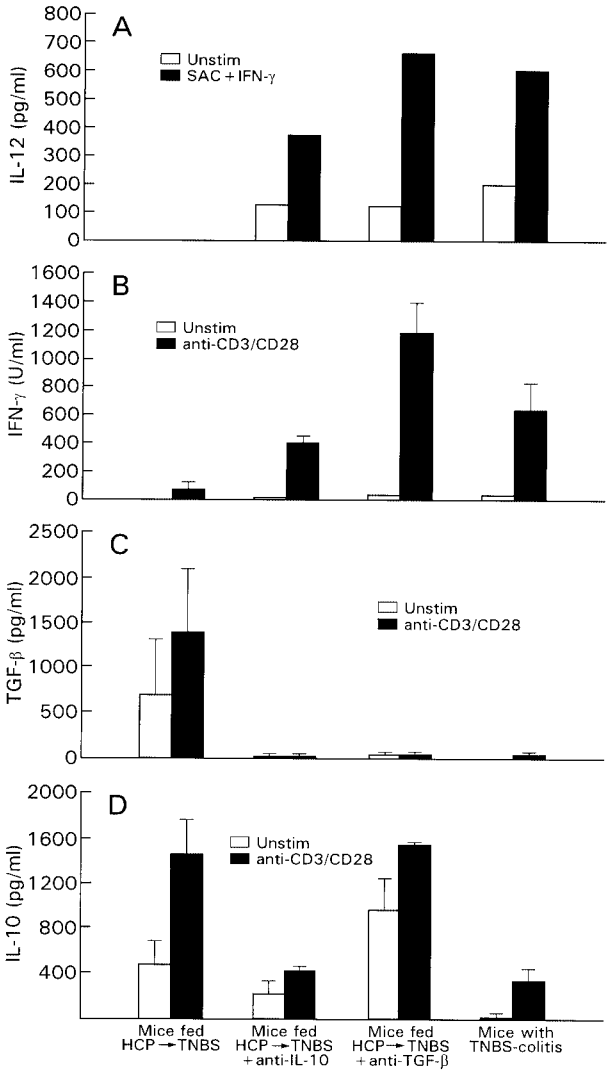

FIGURE 6. Cytokine secretion by LP MNCs isolated from colons of mice after HCP-feeding either untreated or treated with either IL-10 or anti-TGF-β. Groups identical to those in Fig. 4 were administered TNBS per rectum after HCP feeding, TNBS per rectum and anti-TGF-β IP after HCP feeding, TNBS per rectum and anti-IL-10 IP after HCP feeding, or TNBS per rectum without any previous treatment. Culture supernatants were assayed by specific ELISA for *A*, IL-12 secretion, *B*, IFN-γ secretion, $p < 0.01$ TNBS, HCP-fed and anti-IL-10, and HCP-fed and anti-TGF-β vs HCP-fed alone; *C*, TGF-β secretion, $p < 0.01$ TNBS, HCP-fed and anti-IL-10, and HCP-fed and anti-TGF-β vs HCP-fed alone; *D*, IL-10 secretion, $p < 0.01$ TNBS vs HCP, $p < 0.01$ HCP-fed and anti-TGF-β vs TNBS. Data of IFN-γ, TGF-β, and IL-10 represent mean values obtained from five independent experiments. In each experiment, culture supernatants from cultures of pooled cells extracted from five mice per group were analyzed. Bars represent SEs.

caveat to this conclusion relates to the finding that one-third of the recipient mice that were administered cells from HCP-fed mice, administered anti-IL-10 at the same time, and were not protected from colitis (despite the fact that they were recipient of the same pool of cells as the protected mice) in any given experiment. However, this can be explained by the fact that in feeding mice in the presence of anti-IL-10 (as in these studies), one is also inducing Th1 effector cells capable of mediating colitis, as well as regulatory T cells capable of suppressing colitis. Therefore, in some recipients, the transferred cells led to colitis rather than to protection. This possibility is strongly supported by the observation that when

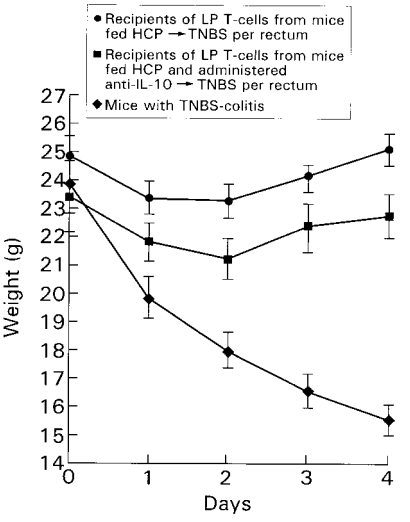

FIGURE 7. Treatment with anti-IL-10 during HCP feeding does not prevent the protective effect of transferred LP CD4$^+$ T cells on TNBS colitis development in recipients. Weight changes of mice administered TNBS who were recipients of LP CD4$^+$ T cells obtained from mice fed HCP (●), mice fed HCP and concomitantly administered anti-IL-10 (■), or mice administered TNBS alone (◆). Recipient mice were administered TNBS per rectum 4 days after adoptive transfer of LP CD4$^+$ T cells (see *Materials and Methods*). Each point represents mean weight values from groups of mice in three independent experiments. In each experiment, each group consisted of at least five mice. Bars represent SE.

the LP T cells from fed animals that were being adoptively transferred were placed into culture before transfer and stimulated with anti-CD3/anti-CD28, we observed that cells from mice that were fed HCP alone produced low amounts of IFN-γ (27 ± 24 U/ml) as compared with cells from mice fed HCP and administered anti-IL-10 (196 ± 31 U/ml). Perhaps more importantly, we observed that cells from mice that were fed HCP and administered anti-IL-10 still produced comparable amounts of TGF-β as compared to mice fed HCP alone (831 pg/ml vs 1122 pg/ml, respectively). Thus, whether or not the transferred cells suppressed the development of colitis, anti-IL-10 treatment did not adversely affect the induction of TGF-β producing regulatory cells.

In a related experiment to further explore this possibility, we subjected LP CD4$^+$ T cells obtained from HCP-fed mice administered anti-IL-10 to a negative selection process using an Ab to CXCR3 (a chemokine receptor found on mainly Th1 T cells (see *Materials and Methods*) (10–12). We found that in this case that >95% of recipient mice administered TNBS per rectum not only did not display significant weight loss, they also did not display evidence of macroscopic or microscopic colitis. These findings correlated with the fact that when LP CD4$^+$ T cells obtained from HCP-fed mice administered anti-IL-10 and subjected to anti-CXCR3 negative selection process were placed into culture before transfer and stimulated with anti-CD3/anti-CD28, the cells produced lower amounts of IFN-γ (76 U/ml), as compared with HCP-fed mice administered anti-IL-10 alone, yet still produced amounts of TGF-β secretion (996 pg/ml) that were comparable to that produced by LP CD4$^+$ T cells obtained from HCP-fed mice. Finally, cytokine secretion by LPMC cell populations obtained from recipient mice of these LP CD4$^+$ T cells after transfer and TNBS administration per rectum displayed no significant IFN-γ secretion (6 U/ml), but did display increased TGF-β secretion (1110 pg/ml). On this basis, we would conclude that protection from colitis in recipients of LP T cells from HCP fed mice also administered anti-IL-10 depends on the delicate balance between the rate of

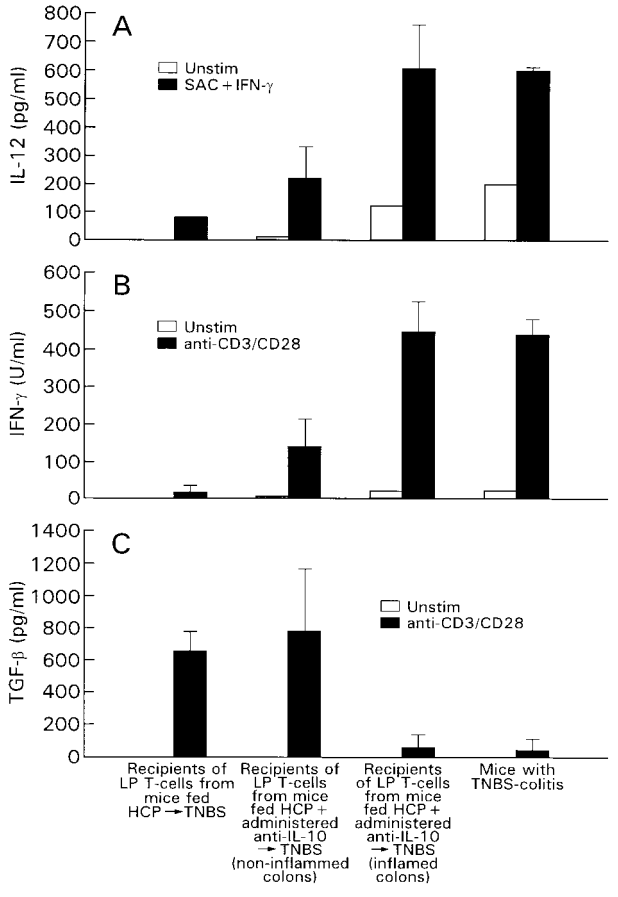

FIGURE 8. Cytokine secretion by cultured LP MNCs isolated from colons of mice administered TNBS per rectum who were recipients of LP $CD4^+$ T cells from mice fed HCP or mice fed HCP and concomitantly administered anti-IL-10. Culture supernatants were assayed for *A*, IL-12 secretion; *B*, IFN-γ secretion; and *C*, TGF-β secretion by specific ELISA. Each bar represents mean values from three independent experiments. In each experiment, cultures of pooled cells extracted from five mice per group were analyzed. Bars represent SE. Mouse recipients of cells of mice administered anti-IL-10 are broken into two groups, those with and without inflamed colons (see text).

expansion of TGF-β-producing suppressor cells, and the rate of expansion of Th1 T cells, which can oppose TGF-β-producing cell development (see further data below).

The effect of IL-10 on the effector phase of TGF-β-producing T cell activity

In a second type of adoptive transfer experiment designed to determine the relation of IL-10 to regulate TNBS-colitis, anti-IL-10 was administered to recipient mice rather than donor mice to determine its effect on the effector phase of suppressor T cell activity. In these studies, mice were fed HCP as in previous studies. Five days after completion of the feeding, LP $CD4^+$ T cells were harvested and adoptively transferred to recipient mice. Five days later, the recipient mice were administered TNBS per rectum alone or in combination with anti-IL-10 or anti-TGF-β.

As shown in Fig. 9, all of the recipient mice administered anti-IL-10 or anti-TGF-β manifested marked weight loss similar to mice given TNBS per rectum alone, whereas the recipient mice not given these Abs did not. However, as in prior studies, mice administered anti-IL-10 exhibited a colitis which was histologically milder than those given anti-TGF-β, i.e., they manifest less bowel wall thickening and lymphoid infiltration (data not shown). Similarly, as shown in Fig. 10,*A* and *B*, the weight loss in the anti-IL-10 and anti-TGF-β treated mice was accompanied by the presence of LP $CD4^+$ T cells that secreted large amounts of IFN-γ and small amounts of TGF-β as compared with mice not administered Abs, with the mice administered anti-TGF-β showing more marked differences than the mice administered anti-IL-10. These data thus show that whereas the IL-10 does not affect the induction of TGF-β producing $CD4^+$ T cells, it does affect the subsequent effector phase of these cells.

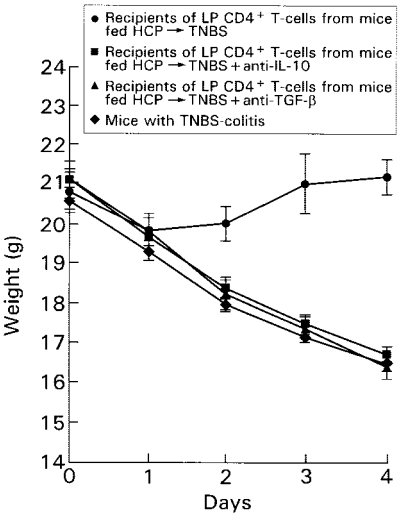

FIGURE 9. The protective effect of transferred $CD4^+$ T cells from mice fed HCP on the development of TNBS-colitis is abolished by treatment of recipients with anti-TGF-β or anti-IL-10. Weight changes of mouse recipients of LP $CD4^+$ T cells obtained from mice fed HCP and then administered TNBS (●), mice administered TNBS and anti-TGF-β (▲), mice administered TNBS and anti-IL-10 (■), or mice administered TNBS alone (◆). Recipient mice were administered TNBS per rectum 4 days after adoptive transfer of LP $CD4^+$ T cells. Each point represents cumulative mean weight data from two similar experiments. In each experiment the various experimental groups consisted of at least five mice. Bars represent SD.

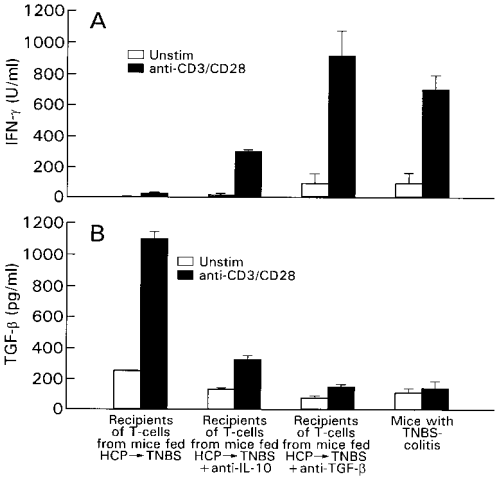

FIGURE 10. Cytokine secretion by LP MNCs isolated from colons of mouse recipients of LP $CD4^+$ T cells from mice fed HCP and administered either anti-IL-10 or anti-TGF-β. Recipient mice received TNBS per rectum 4 days after adoptive transfer of LP $CD4^+$ T cells. Culture supernatants were assayed for *A*, IFN-γ secretion; *B*, TGF-β secretion by specific ELISA. Data shown are from two similar experiments. In each experiment, cultures of pooled cells were extracted from five mice per group were analyzed. Bars represent SD.

Discussion

In this study, we show that protection of mice from the development of experimental mucosal inflammation (TNBS-colitis) by the pre-feeding of TNP-HCP requires the induction of T cells producing TGF-β. In addition, we define the role of IL-10 in such protection with studies showing that while TGF-β-producing regulatory T cells can be induced in the absence of IL-10, the latter cytokine is necessary for the production of TGF-β, because it down-regulates the ambient levels of Th1 cytokine, which would otherwise inhibit the expansion of TGF-β-producing T cells. Before we discuss these findings, it is important to mention that many of our studies were based on the in vivo administration of anti-IL-10 Abs to investigate IL-10 effects during Th1-mediated inflammation. This was justified by the fact that we used a combination of murine monoclonal IgM Abs (SXC-1 and SXC-2), that have been shown to neutralize 95–100% of the IL-10 responses in vitro (13, 14) and to efficiently block a variety of IL-10-mediated phenomena in vivo at the concentrations used here (15–17). In fact, in most of our studies, the use of these Abs did inhibit potential IL-10 suppressive effects at a dose of 1 mg/mouse. In the study in which they did not inhibit such effects even when given at a total dose of 2 mg/mouse (studies in which anti-IL-10 was given during HCP feedings before transfer of cells with regulatory activity), we verified the finding with an additional study in which the mice were given a total dose of 4 mg/mouse. In addition, in the study in which anti-IL-10 had an effect on the development of regulatory cells, albeit a somewhat less robust effect than did TGF-β, we again verified the result with an additional experiment in which the dose of anti-IL-10 was increased from 1 to 2 mg/mouse. Finally, it should be noted that in a previous study in which the in vivo inhibitory effect of the anti-IL-10 Ab was shown to be suboptimally effective, this Ab was a IgG monoclonal anti-IL-10 not used here (4).

The role of TGF-β as a key mediator of suppression of experimental mucosal inflammation has previously been shown both in the SCID transfer model of colitis and in the hapten-induced model studied here (TNBS-colitis) (3, 4). In the former case, it was shown that the protective effect of transferring mature $CD45RB^{low}$ T cells (along with naive $CD45RB^{high}$ T cells) can be reversed by the administration of anti-TGF-β. Similarly, in the latter case, it was shown that the protective effect of feeding hapten (in the form of TNP-HCP) is accompanied by the production of TGF-β by LP T cells, and that this protective effect is again reversed by the administration of anti-TGF-β, either during the period of feeding HCP or later when TNBS was administered per rectum (3). In the present study, this TGF-β effect was initially examined with adoptive transfer studies in which it was shown that $CD4^+$ T cells are the source of the TGF-β. In addition, these studies showed that while transferred $CD4^+$ T cells confer the ability to produce high amounts of TGF-β, it does not confer the ability to produce high amount of IL-10. They thereby presaged the conclusion that it is TGF-β rather than IL-10 that is primarily responsible for suppression of the experimental mucosal inflammation.

A more direct demonstration of TGF-β as the major protective element induced by feeding comes from additional studies in which mice fed HCP and later treated with TNBS per rectum to induce inflammation are concomitantly administered anti-TGF-β or anti-IL-10 mAbs at the time of TNBS administration. This type of experiment showed that while anti-TGF-β administration abolishes TGF-β production and protection, it leaves IL-10 production intact, thereby establishing that IL-10 does not itself act as protective cytokine in this context. This finding correlates with previous studies in which inflammation induced by TNBS per rectum is prevented or treated with a DNA plasmid-encoding TGF-β, which leads to TGF-β producing T cells that prevent inflammation even in the absence of IL-10 production (see further discussion below) (18).

In the same experiments in which protection was abolished by anti-TGF-β Abs, it was also abolished by administration of anti-IL-10 Abs. However, since anti-IL-10 administration was associated with inhibition of TGF-β secretion, and anti-TGF-β treatment was not associated with inhibition of IL-10 secretion, we assumed that the reversal of protection brought about by anti-IL-10 administration was due to an indirect effect on TGF-β secretion. To prove this point, we conducted two kinds of adoptive transfer studies in which anti-IL-10 was administered either during the period of induction of T cells producing TGF-β (i.e., at the time of HCP feeding) or after transfer of such cells to recipients. The former study provided evidence compatible with the conclusion that IL-10 had no effect on the induction of TGF-β producing T cells, in which two-thirds of recipient mice were subsequently protected from TNBS-colitis. The fact that not all mice were protected (even though they received cells from same pooled cell population as the protected mice) can be explained by the fact that mice administered anti-IL-10 during feeding were also developing Th1 T cells in the LP, albeit at a relatively low level. Thus, the transferred cells in some mice evoked a Th1 response which pre-empted the further expansion of T cells potentially able to produce TGF-β. This explanation is favored by the finding that the T cells being transferred, when stimulated in vitro by anti-CD3/anti-CD28, did in fact produce increased amounts of IFN-γ as compared with T cells from HCP-fed mice not administered anti-IL-10. In an additional study, we showed the depletion of such IFN-γ-producing cells from the transferred cell inoculum led to protection from colitis in almost all recipients, not just a large subset. Finally, this explanation is also consonant with data from the study of mice in which anti-IL-10 is administered to recipient mice of LP $CD4^+$ T cells from HCP-fed mice. In this case, the anti-IL-10 abolished protection, and at the same time shifted the response of the recipient mice to a large Th1 cytokine response and a small TGF-β response. Overall, the picture that emerges is that at least in the present context, while IL-10 is an important and indeed essential regulatory cytokine, its effects are indirect and are related to its capacity to facilitate the regulatory role of TGF-β.

Accepting the latter conclusion, the question arises as to the mechanism by which IL-10 facilitates TGF-β regulatory effects. As reported here, IL-10 is important for the expansion of induced TGF-β-producing regulatory cells and the secretion of TGF-β from those cells. Thus, one possibility is that IL-10 regulates the level of Th1 cytokine production, which if unchecked would inhibit expansion of TGF-β cells or TGF-β secretion. Several previous in vitro and in vivo studies support this possibility. Thus, in studies by Seder et al. (19) in which in vitro generation of TGF-β-producing cells from naive T cells were studied, it was shown that IL-12/IFN-γ inhibits TGF-β production, whereas IL-10 enhances such production via an indirect effect on IL-12 secretion. Similarly, in in vivo studies of oral tolerance in OVA-TCR transgenic mice by Marth et al. (20), it was shown that induction of TGF-β-producing cells by intermittent high dose feeding of OVA was greatly enhanced by coadministration of anti-IL-12. In a more recent study by these authors, it was shown that continuous feeding of OVA to OVA-TCR transgenic mice resulted in the cytokine response gradually shifting from IFN-γ-production to TGF-β and IL-10 production (21). Taken together, these previous data provide evidence that high level TGF-β production occurs within a cytokine environment in which Th1 cytokine production is relatively low, and that IL-10 supports TGF-β production by regulating Th1

cytokine production. A second possibility is that IL-10 is necessary for responsiveness to TGF-β regulatory effects. This view is supported by recent data from Cottrez et al. (22) who have recently reported that activated cells manifest reduced TGF-βR2 expression and this effect is reversed by IL-10. In addition, it is supported by studies showing that IFN-γ can induce intracellular production of SMAD-7 in fibroblasts and thus interfere with intracellular TGF-β signaling (23). Therefore, if IFN-γ is down-regulated by IL-10, TGF-β signaling is better maintained. Finally, it is important to note that these possibilities are not mutually exclusive, and that IL-10 may act through several different ways to support TGF-β regulation.

One study superficially in disagreement with the view that IL-10 acts through TGF-β as a regulatory cytokine is that of Powrie et al.(6), in which it was shown that transfer of $CD45RB^{low}$ T cells, normally a population that protects recipients from colitis, is ineffective in doing so if the $CD45RB^{low}$ T cells are derived from IL-10 deficient mice. However, it is reasonable to suggest that the T cells capable of producing IL-10 are necessary to control the level of the Th1 response and thus the level of TGF-β production. This latter interpretation, of course, is consonant with the observation in that the SCID-transfer model of colitis administration of anti-TGF-β also abolishes protection from colitis mediated by $CD45RB^{low}$ T cells, as mentioned above. Other studies seemingly at odds with the indirect role of IL-10 are those showing the existence of Tr1 T cells, i.e., T cells capable of producing high levels of IL-10 plus or minus relatively low levels TGF-β and shown in vivo to inhibit SCID-transfer colitis (7). It is possible that these cells have counter-regulatory effects by virtue of their capability to control Th1 responses as in the scenario defined for IL-10 above. Alternatively, it is possible that direct delivery of high amounts of IL-10 by these cells can down-regulate Th1 mediated inflammation in the absence of TGF-β. Evidence that this may be so comes from studies of Cua et al. (24), which show that IL-10 delivered directly into the CNS by recombinant adenovirus can ameliorate experimental autoimmune encephalomyelitis (EAE).

To conclude, the results reported in this study have an important bearing on the possible treatment of human disease characterized by Th1 T cell-mediated inflammation, such as Crohn's disease. First, they suggest that it may be difficult to stimulate an endogenous TGF-β response in the face of an active Th1 response, since the latter may down-regulate a nascent TGF-β response before it can gain the upper hand. TGF-β-centered therapy must instead come from cells programmed to produce TGF-β, such as those containing a TGF-β-encoding gene which is not subject to down-regulation, such as that recently described by Kitani et al. (18). Second, they show that the production of substantial amounts of IL-10 does not necessarily turn off the Th1 response, and is again more effective in the prevention of a Th1 response than in its reversal, as when Tr1 T cells are administered to prevent SCID-transfer colitis (7). This may arise from the fact that the amounts of IL-10 necessary to suppress IL-12 production after a full-blown Th1 T cell response has been established may be very difficult to achieve. Evidence for this comes from the study of mice that overproduce IL-10 because they bear an IL-10 transgene (9, 24). Here it was shown that increased IL-10 production by APCs does not affect the Th1 response induced by infection with intracellular pathogens (e.g., *Leishmania*), or by administration of myelin basic protein to induce EAE. Nevertheless, in these same studies IL-10 overproduction did prevent EAE development, most likely through its direct effect on macrophage function. As already mentioned, delivery of IL-10 directly to Th1 lesions by a recombinant adenovirus ameliorates inflammation (24). Thus, cells bearing IL-10-encoding plasmids that are not subject to down-regulation may also be a useful therapeutic option.

References

1. Strober, W., B. Kelsall, T. Marth, B. Ludviksson, R. Erhardt, and M. Neurath. 1997. Reciprocal IFN-γ and TGF-β responses regulate the occurrence of mucosa inflammation. *Immunol. Today 18:61.*
2. Groux, H., and F. Powrie. 1999. Regulatory T cells and inflammatory bowel disease. *Immunol. Today 20:442.*
3. Neurath, M. F., I. Fuss, B. L. Kelsall, D. H. Presky, W. Waegell, and W. Strober. 1996. Experimental granulomatous colitis in mice is abrogated by induction of TGF-β-mediated oral tolerance. *J. Exp. Med. 183:2605.*
4. Powrie, F., J. Carlino, M. W. Leach, S. Mauze, and R. L. Coffman. 1996. A critical role for transforming growth factor-β but not interleukin-4 in the suppression of T helper type 1-mediated colitis by $CD45RB^{low}$ $CD4^+$ T cells. *J. Exp. Med. 183:2669.*
5. Read, S., V. Malmstrom, and F. Powrie. 2000. Cytotoxic T Lymphocyte-associated antigen 4 plays an essential role in the function of $CD25^+CD4^+$ regulatory cells that control intestinal inflammation. *J. Exp. Med. 192:295.*
6. Asseman, C., S. Mauze, M. W. Leach, R. L. Coffman, and F. Powrie. 1999. An essential role for interleukin-10 in the function of regulatory T cells that inhibit intestinal inflammation. *J. Exp. Med. 190:995.*
7. Groux, H., A. O'Garra, M. Bigler, M. Rouleau, S. Antonenko, J. E. de Vries, and M. G. Roncarolo. 1997. A $CD4^+$ T-cell subset inhibits antigen-specific T-cell responses and prevents colitis. *Nature 389:737.*
8. Yokoyama, Y. M. 1991. Monoclonal antibody supernatant and ascites fluid production. In *Current Protocols of Immunology*. J. E. Coligan, A. M. Kruisbeek, D. H. Margulies, E. M. Shevach, and W. Strober, eds. Greene Publishing and Wiley Intersciences, New York, Ch. 2.6.4.
9. Groux, H., F. Cottrez, M. Rouleau, S. Mauze, S. Antonenko, S. Hurst, T. McNeil, M. Bigler, M. G. Roncarolo, and R. L. Coffman. 1999. A transgenic model to analyze the immunoregulatory role of IL-10 secreted by antigen-presenting cells. *J. Immunol. 162:1723.*
10. Syrbe, U., J. Siveke, and A. Hamann. 1999. Th1/Th2 subsets: distinct differences in homing and chemokine receptor expression? *Springer Semin. Immunopathol. 21:263.*
11. Agace, W. W., A. I. Roberts, L. Wu, C. Greineder, E. C. Eberts, and C. M. Parker. 2000. Human intestinal lamina propria and intraepithelial lymphocytes express receptors specific for chemokines induced by inflammation. *Eur. J. Immunol. 30:819.*
12. Ebert, L. M., and S. R. McColl. 2001. Coregulation of CXC chemokine receptor and CD4 expression on T lymphocytes during allogenic activation. *J. Immunol. 166:4870.*
13. Mosmann, T. R., J. H. Schumacher, D. F. Fiorentino, J. Leverah, K. W. Moore, and M. W. Bond. 1990. Isolation of monoclonal antibodies specific for IL-4, IL-5, IL-6, and a new Th2 specific cytokine (IL-10), cytokine synthesis inhibitory factor, by using a solid phase radioimmunoadsorbent assay. *J. Immunol. 145:2938.*
14. Ding, L., and E. M. Shevach. 1992. IL-10 inhibits mitogen-induced T cell proliferation by selectively inhibiting macrophage costimulatory function. *J. Immunol. 148:3133.*
15. Wagner, R. D., N. M. Maroushek, J. F. Brown, and C. J. Czuprynski. 1994. Treatment with anti-interleukin-10 monoclonal antibody enhances early resistance to but impairs complete clearence of *Listeria* monocytogenes infection in mice. *Infect. Immun. 62:2345.*
16. Segal, B. M., B. K. Dwyer, and E. M. Shevach. 1998. An interleukin (IL)-10/IL-12 immunoregulatory circuit controls susceptibility to autoimmune disease. *J. Exp. Med. 187:537.*
17. Li, W., F. Fu, L. Lu, S. K. Narula, J. J. Fung, A. W. Thomson, and S. Qian. Systemic administration of anti-IL-10 antibody prolongs organ allograft survival in normal and presensitized recipients. *Transplantation* 66:1587.
18. Kitani, A., I. J. Fuss, K. Nakamura, O. M. Schwartz, T. Usui, and W. Strober. 2000. Treatment of experimental (trinitrobenzene sulfonic acid) colitis by intranasal administration of transforming growth factor (TGF)-β1 plasmid: TGF-β1-mediated suppression of T helper cell type 1 response occurs by interleukin (IL)-10 induction and IL-12 receptor β2 chain downregulation. *J. Exp. Med. 192:41.*
19. Seder, R. A., T. Marth, M. C. Sieve, W. Strober, J. J. Letterio, A. B. Roberts, and B. Kelsall. 1998. Factors involved in the differentiation of TGF-β-producing cells from naive $CD4^+$ T cells: IL-4 and IFN-γ have opposing effects, while TGF-β positively regulates its own production. *J. Immunol. 160:5719.*
20. Marth, T., W. Strober, R. A. Seder, and B. L. Kelsall. 1997. Regulation of transforming growth factor-β production by interleukin-12. *Eur. J. Immunol. 27:1213.*
21. Marth, T., S. Ring, D. Schulte, N. Klensch, W. Strober, B. L. Kelsall, A. Stallmach, and M. Zeitz. 2000. Antigen-induced mucosal activation is followed by Th1 T cell suppression in continuously fed ovalbumin TCR-transgenic mice. *Eur. J. Immunol. 30:3478.*
22. Cottrez, F., and H. Groux. 2001. Regulation of TGF-β response during T cell activation is modulated by IL-10. *J. Immunol. 167:773.*
23. Ulloa, L., J. Doody, and J. Massague. 1999. Inhibition of transforming growth factor-β/SMAD signalling by the interferon-γ/STAT pathway. *Nature* 397:710.
24. Cua, D. J., H. Groux, D. R. Hinton, S. A. Stohlman, and R. L. Coffman. 1999. Transgenic interleukin-10 prevents induction of experimental autoimmune encephalomyelitis. *J. Exp. Med. 189:1005.*