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Blocking CD27-CD70 costimulatory pathway suppresses experimental colitis

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Abstract

The pathogenesis of human inflammatory bowel disease (IBD) and most experimental models of IBD is dependent on the activation and expansion of $CD4^+$ T cells via interaction with mucosal antigen-presenting cells. The costimulatory receptor CD70 is transiently expressed on the surface of conventional dendritic cells, but is constitutively expressed by a unique antigen presenting cell (APC) population in the intestinal lamina propria. We used two experimental IBD models to evaluate whether interfering the interaction between CD70 and its T cell ligand CD27 would affect the development of colitis. Adoptive transfer of naive CD27-deficient $CD45RB^{high}$ $CD4^+$ T cells into Rag-$1^{-/-}$ mice resulted in significantly less disease than when wild type $CD45RB^{high}CD4^+$ T cells were used. Moreover, a monoclonal anti-CD70 antibody prevented the disease caused by the transfer of wild type $CD45RB^{high}$ $CD4^+$ T cells into Rag-$1^{-/-}$ mice and the same antibody also ameliorated an established disease. The colitis associated pro-inflammatory cytokines IL-6, TNF-α and IFN-γ were significantly reduced after anti-CD70 antibody treatment, suggesting an overall reduction in inflammation due to blockade of pathogenic T cell expansion. Anti-CD70 antibody treatment also suppressed TNBS-induced colitis in SJL/J mice. Since anti-CD70 antibody treatment suppressed multiple proinflammatory cytokines, this may be a more potent therapeutic approach for IBD than blockade of individual cytokines.

Introduction

Inflammatory bowel disease (IBD) is a chronic inflammatory disorder of the gastrointestinal tract that occurs in immunocompetent individuals and is characterized by an aberrant mucosal T cell-mediated inflammation (1, 2). Despite intensive study of IBD pathogenesis, the initiating antigens and the mechanisms that sustain the inflammatory process remain incompletely understood (3, 4). Interaction of the T cell expressed tumor necrosis factor receptor (TNFR) family of costimulatory molecules with their respective TNF-related ligands found predominantly on antigen presenting cells (APC) play a critical role during T

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cell activation and differentiation (5–8). The role of many TNF family members in IBD has been well studied in experimental models and TNF antibody is also being used to treat human IBD (9, 10). Many recent studies have also shown that interaction of the co-stimulatory molecule CD27 with its ligand CD70 plays a key role in the expansion and survival of antigen activated T cells (11–13). However, the role of this pathway in IBD has not been studied.

In both mice and humans, CD27 is constitutively expressed on naïve and memory T cells as well as on subsets of activated B cells, NK cells and hematopoietic progenitor cells (11). In contrast, the expression of its ligand CD70 is tightly regulated (14). CD70 is absent on quiescent T, B and dendritic cells but can be induced transiently on T cells after activation and on dendritic cells after stimulation with anti-CD40 or lipopolysaccharide (LPS) (15, 16). Interaction of CD27 with CD70 appears to be important for an effective T cell response in vivo because CD27-deficient mice generate lower numbers of effector CD4 and CD8 T cells in response to a viral infection compared to wild type mice (17). Similarly, administration of recombinant soluble CD70 protein (sCD70) during antigen stimulation enormously enhances the T cell response *in vivo* (18). However, unchecked expression of CD70 predisposes to immunopathology. Aberrant expression in CD70 transgenic mice results in massive activation of T cells responding to self-antigens, attended with depletion of naïve T cell pool that eventually leads to immunosuppression (19). sCD70 treatment also abrogates the requirement for adjuvants and prevents the tolerance induction observed with administration of antigen alone (18). Persistent CD70 expression also characterizes the human rheumatoid arthritis and systemic lupus erythematosis (20, 21). Thus, controlled expression of CD70 appears to be crucial for proper T cell activation and to prevent pathogenesis. By corollary, the CD27-CD70 costimulatory pathway may also provide an important target to prevent T cell-mediated immunopathology. Indeed, the beneficial effect of blocking this pathway with anti-CD70 antibody has been shown in animal models of cardiac allograft rejection and experimental autoimmune encephalomyelitis (22, 23).

We have previously reported that a novel type of antigen presenting cells in the mouse intestinal lamina propria constitutively express CD70 and critically contributes to the mucosal T cell expansion in response to an oral infection (24). In the present study, we analyze the role of CD27-CD70 interaction in IBD using two murine experimental models of colitis. Our results suggest that CD27-CD70 interaction is critical to sustain T cell-mediated intestinal inflammation and blocking this pathway may provide a potential tool for therapeutic intervention in IBD.

Material and methods

Mice

C57BL/6, Rag-1$^{-/-}$ and SJL/J mice were purchased from the Jackson Laboratory (Bar Harbor, ME). CD27-deficient mice on C57BL/6 background has been described (17). All mice were maintained in the specific pathogen–free animal facility at the Immune Disease Institute (IDI) and were used when they were 4–6 weeks of age. All animal experiments had been approved by the Institutional Review Board of IDI.

Antibody Treatment

In the T cell transfer model, wild type $CD4^{+}CD45RB^{high}$ T cell-transferred Rag-$1^{-/-}$ mice were injected i.p with 500 μg of anti-CD70 antibody (clone 3B9) (14) or control hamster IgG (Jackson Immunoresearch Laboratories, Inc) twice a week starting from the day of adoptive transfer or 3 or 5 wk after transfer. In the TNBS model, the antibodies were administered on the day of TNBS injection and repeated 2 and 4 days later. In the innate colitis model, hamster IgG or anti-CD70 antibodies were injected 4h before injecting anti-CD40 and repeated on days 2 and 4.

Flow cytometry and cell sorting

FITC-, PE- or PerCP- conjugated antibodies to mouse CD4, CD45RB, CD8, CD27 and CD70 were from BD PharMingen. Immunostaining and flow cytometric analysis were done as described earlier (24) using FACScan flow cytometer. Cell sorting was done using a FACSAria Sorter (Becton Dickinson, San Jose, CA)

T cell transfer colitis

Naive $CD4^{+}CD45RB^{high}$ T cells were isolated from the spleens of naïve C57BL/6 wild type mice or CD27-deficient mice by cell sorting. After confirming that the isolated cells were >95% pure, 5×10^{5} sorted cells were suspended in 200 μl of sterile PBS and injected *i.p* into Rag-$1^{-/-}$ recipient mice of same age and sex as the donor mice. The recipient Rag-$1^{-/-}$ mice were weighed initially and then weekly after cell transfer. All mice were sacrificed when signs of diarrhea, hunching and wasting disease appeared in the control hamster IgG treated mice. Mice were monitored for colitis as described previously (25). Briefly, DAI was compiled as sum of four parameters: hunching and wasting were scored 0 or 1, stool consistency 0–3 and colon thickening 0–3 with higher scores representing more severe colitis. Histological colitis scores were obtained using tissue samples from the proximal, middle and distal colon. The histology scores were assigned in a blinded manner by our participating pathologist, Dr. A. K. Bhan. The sections were scored for the presence of crypt abscesses (0–1), the degree of mucosal thickness (0–3), and the degree of inflammatory infiltrate (0–3). The maximum score for DAI was 8 and for histological index it was 7.

TNBS Colitis

Colitis was induced in SJL/J mice as described by Neurath et al (26). Mice were anesthetized with *i.p* injection of ketamine/xylazine and 0.5 mg of TNBS (Sigma, St. Louis, MO) in 25% ethanol (150 μl) administered intrarectally through a catheter inserted 4 cm deep from the anus. Animals were then kept in a vertical position for 30 seconds and returned to their cages. Control animals received 150 μl of 25% ethanol. All mice were weighed prior to disease induction and every day thereafter until sacrifice on day 7. On day 7, DAI and histology scores were measured as described earlier except that the following criteria were used for histological scoring: ulceration (0–2+); inflammatory infiltrate (0–3+); edema (0–2+); crypt abscess (0–1+); goblet cell depletion (0–1+); and mucosal thickening (0–3+) with the maximum histological colitis score of 12.

Innate Immune colitis

All the age and sex-matched Rag-$1^{-/-}$ mice were injected *i.p* with 200 μgs of an agonistic CD40 monoclonal antibody (FGK45) (27) or with Isotype control (Rat IgG2a). All the mice were weighed daily and monitored for colitis as described in the T cell transfer model.

Isolation of lamina propria mononuclear cells

The lamina propria lymphocytes were isolated from whole colons as described earlier (24). Briefly, the excised colons were cut longitudinally, washed thoroughly and cut into ~5mm pieces. The pieces were transferred to a 50 ml tube containing 10 ml of CMF-FBS-EDTA solution (CMF-HEPES solution with 10% FBS, 5 mM EDTA and 100 μg/ml gentamycin). The tubes were shaken in a magnetic stirrer for 20 min at room temperature, the supernatant collected and the EDTA treatment repeated 2 more times. Finally, the colonic tissue was washed in tissue culture media (to remove residual EDTA) and digested with 300 U/ml of collagenase (type VIII; Sigma) at 37°C for 1h with shaking. The supernatants were passed through a 70 μm nylon wool strainer and lamina propria lymphocytes were harvested by Percoll gradient centrifugation.

Cytokine analysis

Lymphocytes from the mesenteric lymph node (MLN) of T cell-transferred mice were isolated and 2×10^5/ml cells were stimulated with plate-bound anti-CD3ε antibody (10 μg/mL, 145-2C11; eBioscience, Inc, San Diego, CA) in triplicates in 96 well plates. After 48 h of culture, supernatants were harvested and stored at −20°C for cytokine analysis. For colon explant cultures, small pieces of the colonic tissue samples (~5mm of mid-colon weighing 100 mgs each) were washed extensively and cultured in 500 μl of tissue culture media in 24 well plates at 37°C overnight. The culture media were centrifuged and the supernatants stored for analysis. Cytokine levels were quantitated using the cytometric bead array (CBA kit, BD Biosciences) according to the manufacturer's instructions and data analysis was performed using the BD CBA software.

Statistical Analysis—Non-parametric data were analyzed using two tailed Mann–Whitney U test (DAI and histological data). Levels of cytokine are presented as the mean ±SEM. These data were analyzed by the Student's *t* test. $p \leq 0.05$ was considered statistically significant and the statistical analysis was done with Graph Pad Prism 4.00 (Graph Pad, San Diego, CA).

Results

Blockade of CD70 or absence of CD27 prevents the induction of colitis in the CD45RB^{high} CD4$^+$ T cell ⇒ Rag-$1^{-/-}$ transfer model

Adoptive transfer of *wt* CD4$^+$CD45RB^{high} naïve T cells into SCID or Rag-$1^{-/-}$ mice leads to weight loss, diarrhea and severe colitis in 6–8 wk (28). Hence, we used this model to test the importance of CD27-CD70 costimulatory pathway in the mucosal T cell-mediated pathology. We transferred sorted CD45RB^{high}CD4$^+$ T cells (5×10^5 cells/mouse) isolated from either wild type or CD27-deficient mice into Rag-$1^{-/-}$ mice. The wild type CD45RB^{high}CD4$^+$ T cell-transferred recipient mice were either treated with a control

hamster IgG or anti-CD70 antibody (500μg/mouse by *i.p.* injection twice weekly starting at the time of T cell transfer). The mice were monitored for weight loss and clinical symptoms of colitis over time. Eight weeks after transfer, when the recipient mice treated with the control hamster IgG developed a moderate to severe colitis, all mice were scored for disease activity, the mice sacrificed and their colonic tissues histologically examined. Rag-$1^{-/-}$ recipient mice transferred with wild type $CD45RB^{high}CD4^{+}$ T cells and treated with hamster IgG showed a progressive weight loss and developed clinical disease by 8 weeks. In contrast, wild type $CD45RB^{high}CD4^{+}$ T cell-transferred recipient mice treated with anti-CD70 antibody as well as Rag-1−/− mice transferred with CD27-deficient T cells, showed significantly less weight loss and disease activity (Fig. 1a,b). Histological examination of colonic sections in Hamster IgG treated mice showed a transmural inflammation with mononuclear cell infiltration in the lamina propria and prominent epithelial hyperplasia with loss of goblet cells. These features were much less evident in the anti-CD70 antibody treated mice as well as in mice transferred with CD27-deficient T cells (Fig. 1c,d). Moreover, a quantitative evaluation of the $CD4^{+}$ T cell infiltrates, measured by flow cytometric analysis of isolated lamina propria mononuclear cells revealed a significantly reduced CD4 T cell numbers in anti-CD70 antibody treated mice compared to hamster IgG treated mice (0.8 +/− 0.08 X 10^6 and 0.15 +/− 0.6 X 10^6 +/− respectively, for control and anti-CD70 antibody treated mice, measured at 8 weeks after transfer, n=5, $p<.05$). Thus, CD27-CD70 costimulation appears to be important for sustaining T-cell mediated intestinal inflammation.

We also analyzed the cytokine production by colonic tissue and mesenteric lymph nodes (MLN) in Rag-$1^{-/-}$ recipient mice 8 weeks after T cell transfer. For analysis of colonic samples, equivalent sized colonic tissue was incubated with tissue culture media overnight and supernatants collected for the cytokine assay. MLN cells (1×10^5/ml) were stimulated with anti-αCD3 (10 μg/ml) and culture supernatants collected after 48h of culture. IFN-γ, TNF-α and IL-6 levels were tested by cytometric bead array (CBA). Compared to the wild type T cell-transferred Rag-$1^{-/-}$ recipient mice treated with hamster IgG which showed highly elevated levels of these cytokines, cytokine levels were significantly reduced in the colon and MLN of both CD27-deficient T cell-transferred mice and anti-CD70 antibody treated wild type T cell-transferred mice (Fig. 2a,b). Collectively our results suggest that CD27-CD70 costimulatory pathway plays an important role in the development of colitis and blockade of this pathway suppresses the pathogenic T cell expansion attended by reduced production of inflammatory cytokines and intestinal inflammation.

Anti-CD70 antibody therapy ameliorates an established colitis

Our preceding results show that blocking CD27-CD70 costimulation prevents the development of colitis. However, it was not clear whether this costimulatory pathway is only involved during the priming phase of the response or is important throughout the course of the disease. Only in the latter case would anti-CD70 antibody could have a therapeutic potential. Although for $CD8^{+}$ T cells, CD27-CD70 interactions appear to regulate expansion at the site of priming as well as expansion and survival at effector sites, this is much less clear for $CD4^{+}$ T cells. Therefore, we tested the effect of anti-CD70 antibody treatment started after initiation of the disease in the Rag-transfer model.

In the preventive studies described earlier, weight loss in the hamster IgG treated mice started at approximately 3 wk and clinical symptoms started to appear by 5 wk. Thus, we compared the anti-CD70 antibody treatment started from the day of transfer to that initiated 3 wk or 5 wk after the transfer of wild type $CD45RB^{high}CD4^{+}$T cells into Rag-$1^{-/-}$ recipients. Mice received 500 μg of hamster IgG or anti-CD70 antibody twice a week starting on the day of transfer or 3 wk or 5 wk after cell transfer. The mice were observed for weight loss and mortality till 12 wk. The hamster IgG-treated mice showed progressive weight loss, developed clinical symptoms and 90% of the animals died by 12 wk. In contrast, animals treated with anti-CD70 antibody starting on the day of transfer or after 3 wk of transfer did not show significant weight loss and over 90% of animals survived for the 12 wk period of observation (Fig 3a,b). The wild type T cell-transferred Rag-$1^{-/-}$ recipient mice that had started losing weight 5 wk after transfer, started to regain body weight after initiation of anti-CD70 antibody treatment and 70% of animals survived during the 12 wk period of observation (Fig 3a,b). Clinical and histopathological examination in the surviving mice showed that compared to hamster IgG-treated mice shown in Fig.1 (colonic sections were not taken from Hamster IgG treated mice in this experiment because most of the animals died during the extended observation period) much less inflammation was seen in the colons of anti-CD70 antibody treated mice (Fig. 3c,d,e). Thus, CD70 blockade appears to be capable of reversing an established colitis.

Anti-CD70 antibody treatment also inhibits TNBS-induced colitis

To further confirm the importance of CD27-CD70 pathway in the intestinal T cell response, we also tested the ability of anti-CD70 antibody to reduce inflammation in the TNBS model of colitis. Intra-rectal administration of trinitrobenzene sulphonic acid (TNBS) in certain strains of mice results in a Th1 T cell-mediated transmural infiltrative colitis (29). Thus, groups of SJL/J mice were injected intra-rectally with 0.5mg of TNBS in 25% ethanol or 25% ethanol alone for non-specific toxicity control. TNBS administered mice were *i.p* injected with control hamster IgG or anti-CD70 antibody on days 0, 2 and 4. By day 7, the hamster IgG-treated mice had lost weight and showed clinical symptoms, whereas the anti-CD70 antibody treated and ethanol alone-injected mice did not lose weight or develop clinical symptoms (Fig. 4a,b). Correspondingly, the colonic histopathology was also significantly reduced after anti-CD70 antibody treatment when compared to hamster IgG treated mice, which showed depletion of goblet cells, hemorrhagic necrosis, ulceration and transmural infiltration of mononuclear cells (Fig. 4c,d). These results confirm that anti-CD70 antibody blockade reduces the T cell-mediated immunopathology, irrespective of the method of induction of colitis.

Anti-CD70 antibody treatment does not inhibit non-T cell mediated colitis

Although the effect of CD27-CD70 interaction on T cells is well studied, whether this interaction can also affect the CD70 expressing APC function remains largely unknown. Thus, a possibility existed that anti-CD70 antibody treatment could affect innate immunity by interfering with cytokine production by CD70 expressing macrophage/dendritic cells. Thus, we also tested anti-CD70 antibody in an innate immune model of colitis. Administration of an agonistic CD40 antibody to the T and B-cell deficient Rag-$1^{-/-}$ mice induces a rapid Th-1 cytokine-dependant systemic disease as well as an IL-23-dependant

non-T cell mediated colitis with myeloid cell infiltration of the colonic mucosa (27). Thus, we tested the effect of anti-CD70 antibody treatment in this model. Rag-1$^{-/-}$ mice were injected with 200 μg of anti-CD40 or an isotype control antibody once to induce the disease. The anti-CD40 injected mice were treated with control hamster IgG or anti-CD70 antibody on days 0, 2 and 4 after anti-CD40 injection and the mice were monitored for weight loss and disease development. Anti-CD70 antibody treatment failed to reduce either the systemic disease (rapid weight loss, spleen weight and cellularity) (Fig. 5a,b,c) or the intestinal disease (DAI, histological score (Fig. 5d,e). In a separate experiment we confirmed that CD70 is indeed induced on the splenic dendritic cells after treatment with anti-CD40 mAb treatment (supplementary Fig. S1). Taken together, these results suggest that CD70 blockade does not interfere with non-T cell mediated colitis.

Discussion

Our results suggest that CD27-CD70 costimulatory pathway plays an important role in the development of colitis and blockade of this pathway suppresses the pathogenic T cell accumulation in the gut mucosa attended by reduced intestinal inflammation and proinflammatory cytokine production.

Although the role of CD27-CD70 interaction in the differentiation and survival of CD8 T cells has been well studied, the role of this costimulatory pathway for CD4 T cells is less analyzed (30). Our results suggest that CD27-CD70 interaction is important for CD4 T cell activation in the intestinal mucosa. This is also consistent with studies showing the importance of this costimulatory pathway for the T cell response in the respiratory mucosa following influenza infection (31, 32). Indeed, although both CD28 and CD27 were required for priming a response to influenza virus in the lymph nodes, CD27 was more critical for the accumulation of virus specific T cells in the lungs (27). Thus, CD27-CD70 pathway may be particularly important in determining the expansion/survival of T cells at tissue sites of inflammation.

CD27-CD70 interaction appears to be particularly important to initiate a Th-1 pattern of differentiation for CD4 T cells with increased potential for IFN-γ and IL-2 production (30, 33). We also observed that the cytokines were significantly reduced after CD70 blockade. Considering that anti-CD70 antibody treatment failed to reduce inflammation in the innate model of colitis, the reduction in proinflammatory cytokines observed after anti-CD70 antibody treatment in the Rag-1$^{-/-}$ transfer model of colitis appears to be a consequence, rather than the cause for the reduction of T cell numbers.

It is noteworthy that the improvement in clinical and histological features of colitis was much more pronounced in the anti-CD70 antibody treated mice compared to CD27-deficient T cell transferred Rag-1$^{-/-}$ recipient mice. In addition to T cells, CD27 is also expressed by subsets of NK cells and CD27^{high} NK cells have lower activation threshold and secrete more cytokines than CD27^{low} NK cells (34). Deliberate stimulation through this receptor is also known to augment inflammatory cytokine production by NK cells (35). Moreover, the NK cell numbers are significantly increased in the immunodeficient Rag-1$^{-/-}$ mice compared to wild type mice (36). Because the CD27-CD70 pathway is only blocked on T cells after

transfer of CD27-deficient T cells, whereas this pathway is also blocked in NK cells after anti-CD70 antibody treatment, it is possible that combined inhibition of T and NK cells after antibody treatment gave better protection compared to knocking out CD27 on T cells alone. However, it has been reported that NK cells can play a regulatory role in IBD since the colitis was augmented when IL-10-deficient or even sufficient CD4 T cells were transferred into NK cell-depleted Rag-$1^{-/-}$ mice (37). Thus alternatively, compensatory mechanisms in CD27-deficient T cells or the presence of unidentified additional ligand(s) for CD70 may possibly account for the differences that we observed .

The fact that anti-CD70 antibody treatment could reverse the pathology even when administered after the disease has already become established suggests that continued CD27-CD70 interaction may be necessary to maintain the T cell-mediated inflammation and pathology of chronic colitis. These results are also consistent with earlier studies in the CD70 Tg mice. In CD70 Tg mice, both CD4 and CD8 T cells (responding to environmental or autoantigens) get profoundly activated (38) and sustained interaction of CD27-CD70 is necessary for this because treatment of 4 wk old CD70 Tg mice with blocking CD70 antibody effectively reverses the phenotype (19). Although costimulation is generally thought to be important during APC-T cell interactions in the early stage of an immune response, APCs are also present at effector tissue sites and express costimulatory molecules. However, their role during chronic T cell activation is not well understood. Our results suggest that tissue-specific APCs may be necessary to maintain a sustained inflammation in the gut mucosa. Because CD70 is only transiently expressed by conventional dendritic cells, these cells are unlikely to be involved in the sustained activation of mucosal T cells during chronic colitis. On the other hand, we have previously reported that a novel type of antigen presenting cell present exclusively in the intestinal lamina propria constitutively expresses CD70 (24). The CD70 expressing APC is also present in the Rag-$1^{-/-}$ mice (supplementary Fig. S2), as well as in SJL/J mice (not shown). Although treatment with anti-human CD70 antibody is known to activate ADCC-mediated depletion of CD70 expressing tumor cells (39), antibody mediated depletion of lamina propria effector T cells is unlikely to be the cause for protection against IBD because i) we did not observe significant CD70 expression by lamina propria T cells by flow cytometric examination either in wild type mice or in our IBD models ((24) and data not shown). The fact that protection against IBD was also seen when CD27-deficient T cells were transferred to Rag-$1^{-/-}$ mice without antibody treatment also supports this hypothesis. Moreover, the anti-mouse antibody used in this (clone 3B9) appears to be non-depleting antibody in that the CD70+ APC were still present after antibody treatment (supplementary Fig. S3). Thus, it is likely that blockade of CD70 expressed on the CD70+ APC is the cause of protection induced by anti-CD70 antibody treatment in this study.

In summary, our results show that the CD27-CD70 costimulatory pathway is important in sustaining the T cell-mediated inflammation in IBD and blockade of this pathway may provide a tool for therapeutic intervention. The fact that continuous interaction of CD27/CD70 is necessary for the gut mucosal T cell expansion/survival is particularly important from a therapeutic viewpoint since it would enable treatment after the disease is diagnosed. Since CD70 blockade directly leads to reduction in T cell numbers and thus reduce

inflammation, this may provide a superior therapeutic approach for IBD than suppressing individual cytokines.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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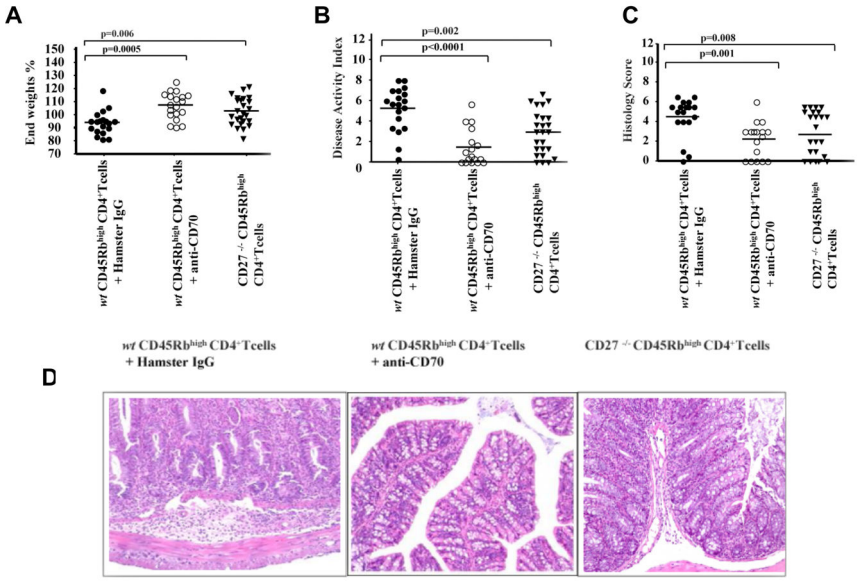

Fig 1. CD27-CD70 interaction is required for the development of colitis in the T cell transfer model

Rag-1$^{-/-}$ mice were transferred with CD4^{+}CD45RB^{hi} CD4 T cells from wild type or CD27-deficient mice and the wild type T cell transferred mice treated with control hamster IgG or anti-CD70 antibody. Induction of colitis was assessed 8 wk after transfer. (a) Weight loss (b) Disease activity index (DAI) and (c) Histology score. The data shown is pooled from 3 independent experiments with a total of 17–25 mice in each group. Each symbol represents an individual mouse and the horizontal line represents the median value. (d) One representative histology slide from each of *wt* CD45RB^{hi} CD4 T cells transferred mice treated with hamster IgG (histology score=7) or anti-CD70 antibody (histology score=1) and CD27-deficient CD45RB^{hi} CD4 T cells transferred mice (histology score=2) is shown (magnification, 10X). Colon sections from unmanipulated wt C57/BL-6 and Rag-1$^{-/-}$ mice is shown in supplementary Fig. S4.

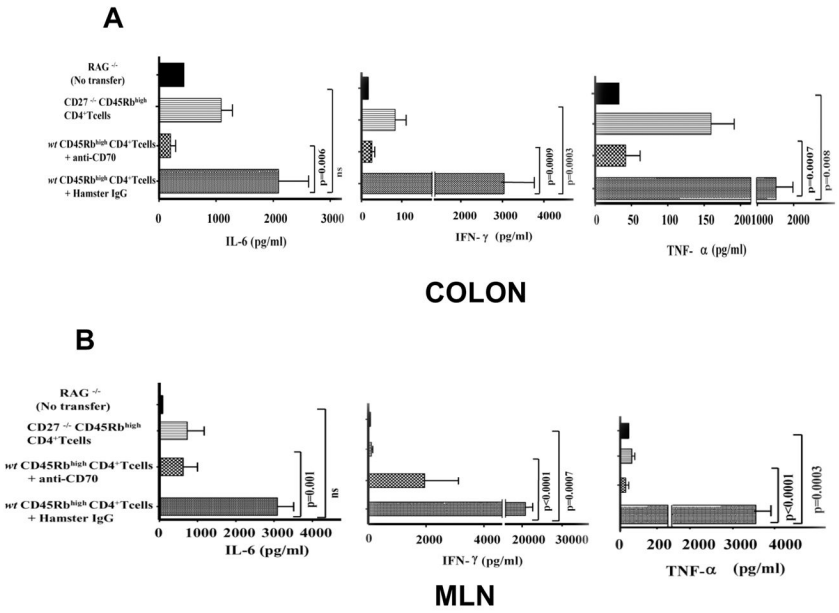

Fig 2. Decreased cytokine production in the absence of CD27-CD70 interaction

Cytokine concentration in mice in Fig.1 was measured in (a) colon explant cultures or (b) MLN T cell cultures using a cytometric bead array (CBA kit) (n= 5–18 mice per group). The bar graphs represent Mean values ±SD.

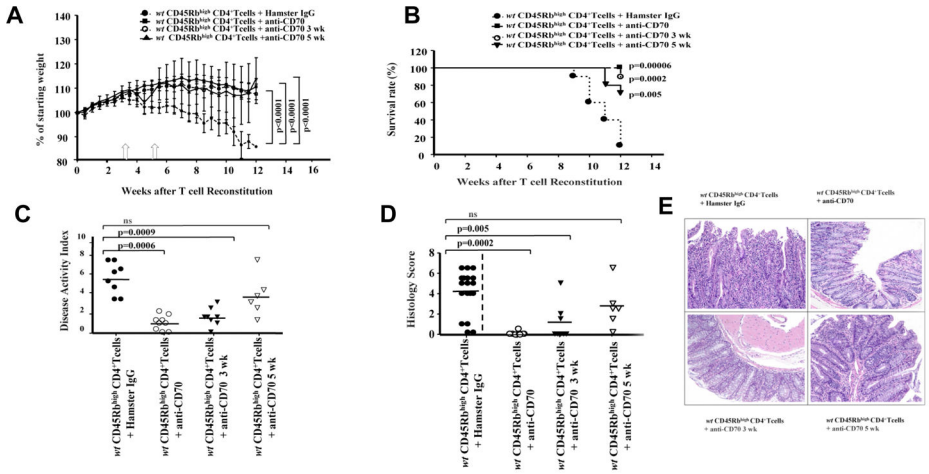

Fig 3. Anti-CD70 antibody therapy ameliorates an established colitis

Wild type CD45RB^{hi} CD4 T cell-transferred Rag-1$^{-/-}$ mice were treated with a control hamster IgG or anti-CD70 antibody starting from the day of transfer or 3 wk or 5 wk after T cell transfer. Severity of colitis was assessed by (a) weight loss as a percentage of the starting weight (b) survival rate percentage (c) disease activity index and (d) histology score (n = 5–7 mice per group). Data represent the Mean values ±SD *p< 0.05 (e) One representative histology slide from hamster IgG control and each treatment group (anti-CD70 antibody treatment initiated on day 0, 3 wk and 5 wk after transfer) is shown (magnification, 10X).

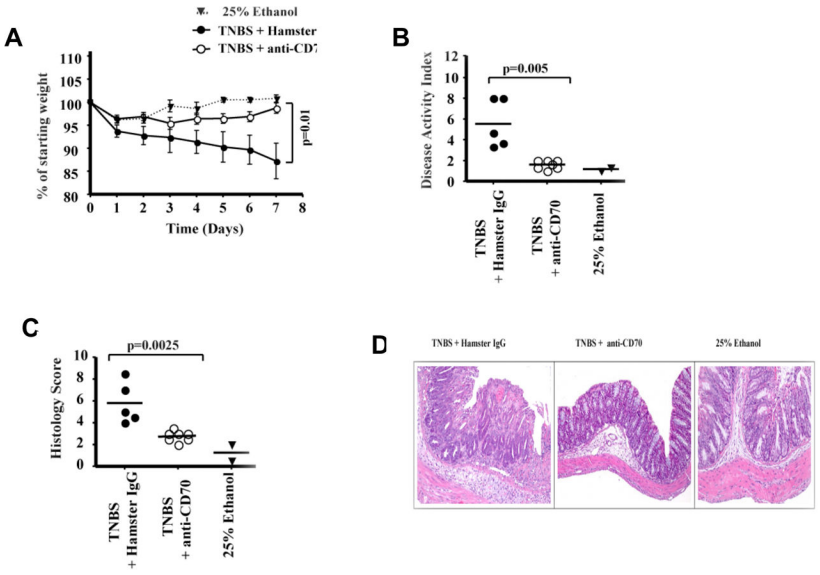

Fig 4. Anti-CD70 antibody treatment prevents TNBS-induced colitis

SJL/J mice were injected intrarectally with 25% ethanol alone or with TNBS in 25% ethanol and treated with control hamster IgG or anti-CD70. Induction of colitis was assessed by (a) weight loss as a percentage of the starting weight (b) DAI and (c) histology score. (d) One representative histology slide from each treatment group of mice is shown (magnification, 10X).

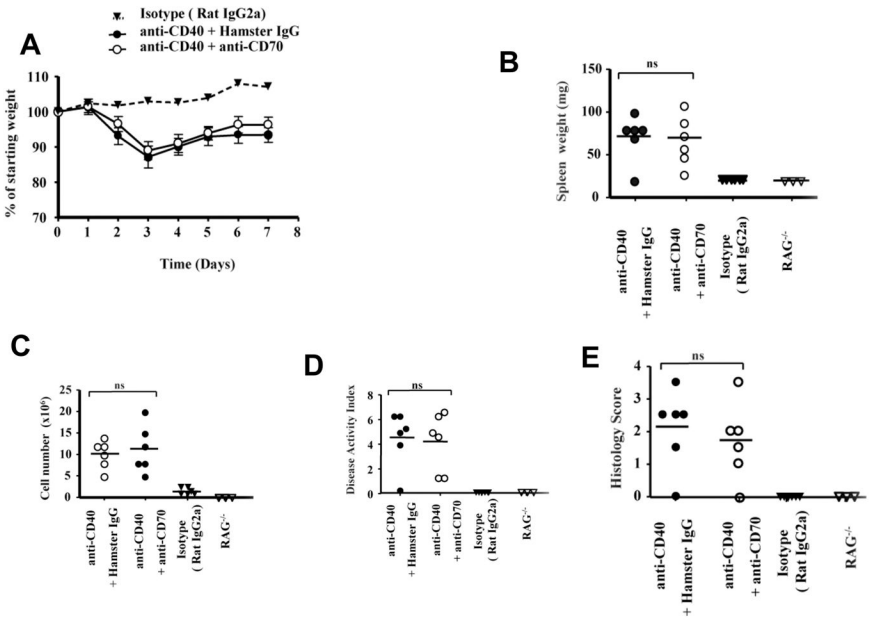

Fig 5. Anti-CD70 antibody treatment does not inhibit a non-T cell-mediated colitis

Rag-1$^{-/-}$ mice were injected with 200 μg anti-CD40 and treated with control hamster IgG or anti-CD70 antibody. Disease induction was assessed by (a) weight loss (b) splenic weight (c) spleen total cell number (d) DAI and (e) histological score. Data represent the Mean ±SD (n= 3 for isotype control (no anti-CD40) and n = 6 for anti-CD40 injected group.