IL-27R deficiency delays the onset of colitis and protects from helminth-induced pathology in a model of chronic IBD

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Abstract

Members of the IL-6/IL-12 cytokine family play central roles in Crohn’s disease. The present findings demonstrate that IL-27, a close relative of IL-12 and IL-23, can promote the onset of colitis in mice. We report that, compared with IL-10-deficient animals, which succumb to chronic intestinal disease at 3-6 months of age, mice lacking both IL-10 and the IL-27R (IL-27R/WSX-1) exhibit delayed pathology and prolonged survival (>1 year). Moreover, unlike highly susceptible IL-10-deficient counterparts, they were able to clear infection with Trichuris muris, a colon-dwelling nematode. In both models of intestinal inflammation, improved clinical outcome was associated with reduced inflammation and profound attenuation of Th1 responses and, consistent with these in vivo findings, we confirmed that during in vitro differentiation, IL-27 directly promotes CD4⁺ T cell IFN-γ production through effects on Tbet, a key Th1 transcription factor. We also found that its ability to suppress Th2 responses, which was clearly evident in helminth-infected IL-10−/−IL-27R−/− mice, was largely Tbet independent. Taken together, these studies demonstrate that, in the absence of IL-10, IL-27 can promote Th1-type and suppress Th2-type intestinal inflammation but, ultimately, is not required for the development of inflammatory bowel disease.

Introduction

Inflammary bowel disease (IBD) is a constellation of intestinal pathologies that share a common etiology, inappropriate responses to commensal gut flora, but are distinguished by unique histological and immunological features. The most common of these disorders is Crohn’s disease (CD), which is traditionally thought to derive from excessive type I (Th1) inflammation (1, 2). Consistent with that notion, a large portion of lymphocytes in affected tissues produce IFN-γ, the prototype Th1 cytokine, and, in several mouse models of CD, intestinal pathology is dependent on Th1-associated cytokines (IL-12) and transcription factors (STAT4 and Tbet) but, conspicuously, not often on IFN-γ itself (3–6). The latter point has raised the possibility that CD is not simply a Th1 disease and, recently, convincing evidence has emerged to argue that it is mediated by IL-23, a cytokine closely related to IL-12 and known to be pathogenic in models of autoimmunity like collagen-induced arthritis and experimental autoimmune encephalomyelitis (EAE) (7, 8). IL-23 can influence a range of immune cells, including macrophages and dendritic cells (DCs), but is best known for effects on Th17 cells, a subset of αβ T CD4⁺ cells that secrete the pro-inflammatory cytokines IL-17 (IL-17A/IL-17F), IL-21 and IL-22 (8–13). Although not required for de novo Th17 differentiation, which occurs when naive precursors are activated in the presence of transforming growth factor-β (TGF-β) and IL-6 (14–16), IL-23 appears to promote the survival and or expansion of Th17 cells, thereby sustaining autoimmune inflammation. Direct evidence for the pathogenic nature of IL-23 and Th17...
IL-27 promotes the onset of colitis

responses during intestinal disease has come recently, with four studies demonstrating that ablation of IL-27 renders mice resistant to spontaneous and induced forms of colitis and another linking heritable resistance in humans to a point mutation in the gene encoding for the IL-23R (17–21). In light of those findings, and the fact that IL-23 shares a common subunit with IL-12 (IL-12p40), it has also been proposed that the success of anti-IL-12p40 antibody therapy in treating CD may be more the consequence of its effects on IL-23 than on IL-12 (17–19, 22–24).

Due to the emergence of IL-6, IL-12 and IL-23 as causative agents, there is now considerable interest in determining if other members of the IL-6/IL-12 family also play a role in IBD. One cytokine that is particularly relevant in this context is IL-27, which was identified through sequence and structural homology to IL-6 and IL-12 (25–27). IL-27 is heterodimeric, composed of a helical subunit (IL-27p28) bound to a soluble, receptor-like molecule [Epstein–Barr-induced gene 3 (EBI3)] (28). Its receptor complex also has two parts: one unique, termed IL-27R or WSX-1, and GP130, which is shared by a range of cytokines, including LIF, IL-6, IL-11 and Oncostatin M (29). Because GP130 is expressed by most mammalian cells, IL-27 responsiveness is determined by the availability of IL-27R, which is largely restricted to immune cells of the lymphoid (T cells, B cells, NK cells and NKT cells) and myeloid (macrophages, DCs and mast cells) lineages (25–27). To date, most studies have focused on the ability of IL-27 to influence T cell responses and, based primarily on in vitro data, initial reports concluded that it is essential for Th1 differentiation (28, 30, 31), a notion bolstered by the finding that it can induce expression of IFN-γ, Tbet and IL-12RB2 (32–35). However, IL-27R-deficient (IL-27R−/−) mice display only a mild, transient susceptibility to classic Th1-type pathogens, like Leishmania major and Listeria monocytogenes, suggesting that IL-27 is not strictly required for cellular immunity (30, 31, 36).

Furthermore, when challenged with Toxoplasma gondii, a potent stimulus for IL-12-dependent Th1 responses, IL-27R−/− mice rapidly clear parasites but develop a lethal inflammatory disease characterized by severe hepatic pathology and a gross accumulation of IFN-γ-producing T cells in the lymphoid organs (37). Thus, beyond its Th1-inducing capacity, a major non-redundant function for IL-27 is to limit potentially harmful pathogen-induced Th1 responses. That regulatory capacity, which is manifested in numerous models of infection, including Chagas’ disease and tuberculosis (25–27, 38), has also been extended to other effector T cell lineages, namely Tc1 and Tc17. Support for this latter point comes from in vivo studies demonstrating that both Tc2 and Tc17 responses are exaggerated in IL-27R−/− mice and from in vitro data showing that IL-27 can directly inhibit the production of Th2- and Th17-type cytokines and transcription factors (34, 39–44).

While the bulk of in vitro evidence suggests that IL-27 is suppressive in the context of pathogen-induced or autoimmune inflammation, its role in IBD is difficult to predict. There are studies showing that IL-27 is ‘overproduced’ in patients with CD and ulcerative colitis (UC) (45, 46) but whether it is protective or pathogenic remains uncertain. In terms of chemically induced IBD, one report states that IL-27R is required for DSS-induced colitis while another concludes that EBI3, a component of IL-27, is completely dispensable for TNBS-induced colitis (47, 48). It is also known that EBI3 deficiency protects mice from oxazolone-induced colitis, a more Th12-driven model of UC (48). To address this uncertainty, we performed a longitudinal (>1 year) comparison of intestinal pathology between IL-10-deficient mice, which have long been used as a model for spontaneous IBD (49), and mice lacking both IL-10 and the IL-27R [(double knock-out or IL-10−/− IL-27R−/− (dKO)]. In the absence of IL-27R, IL-10-deficient mice exhibited prolonged survival associated with a profound defect in IFN-γ production and only slight decreases in Th2- and Th17-type cytokines. The dKOs also became resistant to infection with Trichuris muris, an intestinal helminth that induced severe intestinal pathology in IL-10-deficient animals. Again, Th1-type cytokines were dramatically reduced following helmint infection but, in this case, there was evidence that exaggerated Th2 responses may have contributed to the phenotype of the dKOs. Taken together, these studies demonstrate that IL-27 is an important regulator of intestinal T cell responses and suggest that it promotes the onset of IBD through effects on both Th1 and Th2 responses.

Methods

Animals

Mice deficient in IL-27R/WSX-1 (C57B/6 background) were generated as described (30) and provided by C. Saris (Amgen, Thousand Oaks, CA, USA). IL-10-deficient mice and wild-type (WT) (C57BL/6) controls were purchased from Jackson Laboratories (Bar Harbor, ME, USA). IL-10−/− and IL-27R−/− mice were crossed over two generations to generate homozygous double mutants (IL-10−/− IL-27R−/−). PCR genotyping for IL-10 and IL-27R was performed according to the protocols supplied by Jackson Laboratories and Amgen, respectively. STAT4 and Tbet-deficient mice, along with WT controls (Balb/c), were purchased from Jackson Laboratories. All animals were housed in a specific-pathogen free environment at the University of Pennsylvania. Experiments were carried out following the guidelines of the University of Pennsylvania Institutional Animal Care and Use Committee.

Histology

At the indicated time points, colonic sections were isolated, flushed with PBS, fixed in 10% buffered formalin, embedded in paraffin, sectioned and then stained with hematoxylin and eosin. A portion of the cecum was fixed in neutral buffered formaldehyde, processed and stained with periodic acid and Schiff’s reagent followed by Alcian blue to visualize goblet cells. All microscopy images were taken at ×20 magnification.

Infections

Trichuris muris was maintained as described (39). Briefly, adult worms were isolated from susceptible hosts and cultured in vitro. Media containing secretory antigens were collected, dialyzed and sterilized for use in re-stimulation studies (see below). Deposited eggs were washed,
incubated and used for oral infections. Age- and sex-matched cohorts (female: 4–6 weeks) were challenged with 150–200 embryonated eggs and parasite burdens were assessed by microscopy. Toxoplasma gondii was maintained in mice (Swiss Webster and CBA/CaJ; Jackson Laboratories) and tissue cysts were prepared as described (37). Age- and sex-matched cohorts (female: 4–6 weeks) were challenged with 10 cysts by oral injection.

Ex vivo analysis
For all ex vivo assays, mesenteric lymph nodes (MLNs) were dissected from age- and sex-matched cohorts, dissociated into a single-cell suspension, washed and counted. For semi-quantitative real-time PCR, 3–5 × 10^6 lymphocytes were put directly into 1 ml of Trizol reagent (Invitrogen, Carlsbad, CA, USA). mRNA was isolated by standard methods and converted to cDNA using SuperScript III reverse transcriptase (400–500 ng RNA per reaction; Invitrogen). Amplification of cytokine transcripts was performed with SYBR green PCR master mix (20–50 ng cDNA per reaction; Applied Biosystems, Foster City, CA, USA) using an iQ5 Real-Time PCR Thermal Cycler (Bio-Rad, Hercules, CA, USA). Primer sequences and relevant information are provided in Supplementary Table 1 (available at International Immunology Online). Each primer set yields a unique product as measured by melting curve analysis and has been validated for specificity using in vitro culture systems [bone marrow-derived DCs for IL-6, IL-12p35/p40, IL-23p19 and IL-27p28/EBI3; purified CD4 T cells for IFN-γ; tumor necrosis factor-α (TNF-α), IL-4, IL-13, IL-17A, IL-17F and IL-22 and whole spleen for TGF-β and amphiregulin]. Reactions were performed in duplicate, C_t values normalized to β-actin levels and fold induction (n > 1) or reduction (n < 1) of each gene calculated (ΔΔC_t) with respect to a pool of two to three age- and sex-matched WT controls (n = 1).

For ex vivo flow cytometry, 1–2 × 10^6 lymphocytes were washed and stained with αCD4, αCD8 and αNK1.1 antibodies (eBioscience, San Diego, CA, USA). NKt and invariant NKT (iNKT) cells were measured as described (50). In brief, thymus, liver and spleen were dissected from sex- and age-matched cohorts (female: 4–8 weeks) and homogenized into a single-cell suspension. Cells (1 × 10^7) were then washed and stained with the following antibodies: αHSA, αB220, αNK1.1, αCD1d, αCD3ε, αCD4 and αCD8 (PharMingen, San Diego, CA, USA). αGalCer-loaded CD1d tetramers were generated and used as described (51).

For re-stimulation studies, 24-well tissue culture plates (Sigma/Costar, St Louis, MO, USA) were coated with αCD3ε mAb (2 μg ml⁻¹ in PBS for 2 h at 37°C; eBioscience). Lymphocytes (2–4 × 10^6) were allocated per well and, 12–16 h later (overnight), brefeldin A (BFA) was added (10 μg ml⁻¹; Sigma). After 2–3 h, cells were collected, washed, fixed (4% PFA; Sigma), permeabilized (0.25% saponin; Sigma) and stained for intracellular IFN-γ (eBioscience). Culture supernatants were also collected prior to addition of BFA and IFN-γ. IL-4 and IL-13 measured by ELISA. For the T. muris studies, lymphocytes were re-stimulated with either αCD3ε mAb or secreted worm antigen (50 μg ml⁻¹). For all experiments, lymphocytes were cultured in supplemented tissue culture media (RPMI-1640 with 10% FCS, 1% sodium pyruvate, 1% non-essential amino acids, 0.1% β-mercaptoethanol, 100 U ml⁻¹ penicillin and 100 μg ml⁻¹ streptomycin; Gibco/Invitrogen). Four-color flow cytometry was performed on a FACS caliber instrument and analyzed using CellQuest Pro Software (Becton Dickinson; Franklin Lakes, NJ, USA). Logarithmic scales were used for all dot plots; 25 000–50 000 events per plot for ex vivo studies and 5000–10 000 events per plot for in vitro experiments.

In vitro analysis
Spleens were isolated from WT, IL-27R−/− and Tbet−/− mice, dissociated into a single-cell suspension, depleted of erythrocytes using 0.86% (w/v) ammonium chloride (Sigma) and depleted of CD8+ and NK1.1+ cells by magnetic bead separation (Polysciences Inc., Niles, IL, USA). Cells were stimulated with soluble αCD3 and αCD28 antibodies (1 μg ml⁻¹ each; eBioscience) and cultured in 48-well plates for 48–72 h (2 × 10^5 ml⁻¹ in 0.5 ml media per well; Costar). IL-27 was provided by C. Saris (Armen) and used at 100 ng ml⁻¹. For detection of intracellular IFN-γ, cells were stimulated for 72 h, pulsed with phorbol 12-myristate 13-acetate ([PMA] 50 ng ml⁻¹; Sigma) and ionomycin (500 ng ml⁻¹; Sigma), treated with BFA and then stained as above (4 h PMA/Iono with BFA for the last 2 h). For qPCR and ELISA, cells or supernatants were collected after 48 h (no PMA/Iono/BFA). mRNA levels were measured as in the previous section (200–400 ng RNA and 20–40 ng cDNA per reaction).

Statistics
For figures 2, 3, 5, 6 and 7, statistical differences between experimental groups were determined by paired Student’s t-test. A star above the lower of two values (brackets) represents significant differences (P < 0.05) or, when this symbol is not used, actual P values are given.

Results
Delayed onset of colitis in mice lacking the IL-27R
To assess whether IL-27 plays a role in the development and/or progression of IBD, IL-27R-deficient mice were bred with IL-10-deficient mice. The resulting F1 generation (IL-10−/−/IL-27R−/−) was then intercrossed, thereby generating all possible genotypes, including homozygous double mutants. These animals were born at the predicted Mendelian ratios and, at weaning age, were indistinguishable from WT, IL-10−/− and IL-27R−/− littermates. Consistent with previous accounts (3, 19, 49), IL-10−/− mice began to show obvious signs of intestinal disease (weight loss and prolapsed rectum) after ~3 months and none survived beyond 8 months (Fig. 1C and data not shown). Colon sections from moribund animals revealed, as expected, severe crypt elongation and hyperplasia, mononuclear cell infiltrates and widespread disruption of the epithelial barrier (Fig. 1A). In contrast, age-matched (4 months) IL-10−/−/IL-27R−/− mice displayed only mild crypt elongation when compared with unaffected WT and IL-27R−/− counterparts (Fig. 1A). The dKO mice continued to thrive for several months but, between 9 and 18 months of age, clinical symptoms began to appear.
In each case, disease progression was rapid, often going from healthy to completely dehabilitated in <1 month. Colonic pathology in these mice was not only similar to that of 4-month-old IL-10−/− cohorts in some respects, such as the pronounced crypt elongation, but also distinct in others, as with the large, organized aggregates of mononuclear cells within the crypts and lamina propria (Supplementary Figure 1, available at International Immunology Online). Thus, while deletion of the IL-27R significantly delayed the onset of disease, it did not completely protect IL-10-deficient mice from lethal colitis (Fig. 1C).

To determine the immunological basis for improved survival in IL-10−/−IL-27R−/− mice, we performed a PCR-based survey of pro-inflammatory cytokine production in 4-month-old cohorts. This time point was chosen because it coincided with the divergence between IL-10−/− mice and dKOs, with the former exhibiting obvious clinical symptoms and the latter appearing largely healthy. To first gain a broad overview of this difference, mRNA was extracted from colon-draining MLNs and cytokine transcripts measured using semi-quantitative real-time PCR (Supplementary Table 1, available at International Immunology Online). As shown previously (19), these studies revealed that intestinal pathology in IL-10−/− mice is attendant to enhanced expression of numerous Th1- and Th17-associated factors, including IL-6, IL-12p35, IL-23p19, IL-17A, IL-17F, IL-22 and IFN-γ (Fig. 2). There was considerable heterogeneity in the IL-10-deficient cohorts, reflecting the fact that disease is not completely synchronous, but it should also be noted that individuals with the most dramatic cytokine profiles tended to display the most severe pathology (data not shown). Additionally, and of particular relevance for the current work, levels of IL-27p28 were substantially increased which, coupled to an abundance of EBI3 transcripts, suggest that IL-27 could play a role in the development of colitis in these animals (Fig. 2).

Consistent with their improved clinical outcome, expression of pro-inflammatory cytokines was decreased in IL-10−/−IL-27R−/− mice relative to IL-10−/− counterparts. This effect was unambiguous for Th1-type factors IL-12p35 and IFN-γ but less so for Th17-type factors, with IL-6, IL-23p19, IL-17A, IL-17F and IL-22 remaining elevated in the dKOs (Fig. 2). Expression of Tn12-type cytokines IL-4 and IL-13 was comparable in all groups but IL-10−/− and IL-10−/−IL-27R−/− mice both exhibited increased expression of amphiregulin (Fig. 2), a myeloid cell-derived cytokine recently implicated in mucosal Tn2 responses (52). Thus, while deletion of the IL-27R led to a marked reduction in classic Th1-type factors, it had less effect on other pro-inflammatory mediators.

To determine whether the diminished Tn1 responses noted in IL-10−/−IL-27R−/− mice were due to a requirement for IL-27 in the development and/or recruitment of IFN-producing lineages, we performed ex vivo flow cytometry to determine the composition of the MLNs. These studies revealed that, in younger animals (4–6 weeks), the percentages of CD4+, CD8+ and NK1.1+ cells were similar for all the strains tested (Supplementary Figure 2, available at International Immunology Online and data not shown). Additionally, despite one report showing that EBI3-deficient mice are

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**Fig. 1.** Delayed onset of colitis in the absence of IL-27R. (A and B) Shown are representative images of hematoxylin and eosin-stained colons from 4-month-old cohort (A) and 10-month-old cohort (B) of WT, IL-10−/−, IL-27R−/− and IL-10−/−IL-27R−/− mice. Microscopy data are representative of three separate experiments (n = 2–4 mice per group; ×20 magnification; at least three sections/slides inspected per individual). (C) Survival of littermate cohorts was monitored. Three experiments were pooled for Kaplan–Meier curves (n = 8–12 mice per group).
resistant to oxazolone-induced colitis due to a lack of iNKT cells (48), our analysis revealed no such defect for either IL-27R−/− or IL-10−/− IL-27R−/− mice. Compared withagematched WT counterparts, percentages and total numbers of iNKT cells were similar in the thymus, spleen and liver of IL-27R-deficient strains (Supplementary Figure 3, available at International Immunology Online). Taken together, these data indicate that the phenotype of the dKOs is not explained by an inherent deficiency in NK cells, NKT cells or T cells.

Fig. 2. Cytokine profiles in IL-10 and IL-10 × IL-27R-deficient mice. Semi-quantitative real-time PCR was used to measure cytokine mRNA in MLNs of 4-month old cohorts. Data are pooled from two separate experiments (n = 2–4 mice per group). Each plot element represents an individual animal and has been grouped by symbol/color according to genotype. Expression is represented as the fold induction (x > 1) or reduction (x < 1) relative to a pool of two to three WT controls (x = 1). Direct comparisons between experimental groups were subjected to a paired t-test and only those with P values < 0.08 are shown (brackets). Primer/product information is provided in Supplementary Table 1 (available at International Immunology Online).
Concurrent to the onset of clinical symptoms, there was a significant increase in the percentage of CD8\(^+\) T cells, and to a lesser extent CD4\(^+\) T cells, within the MLNs of IL-10\(^-/-\) mice (Supplementary Figure 2, available at International Immunology Online). Given the many published reports demonstrating a predominance of T\(_h1\) responses in these animals, we reasoned that those were likely to be IFN-\(\gamma\) producing cells that may be lacking in IL-10\(^-/-\) IL-27R\(^-/-\) counterparts. To test that hypothesis, lymphocytes from 4-month-old cohorts were re-stimulated ex vivo and intracellular cytokine production measured by flow cytometry. As anticipated, these studies revealed an obvious accumulation of IFN-\(\gamma\)+ CD4\(^+\) and CD8\(^+\) cells in IL-10\(^-/-\) mice and little evidence of an active T\(_h1\) response in the dKOs (Fig. 3). When measured by ELISA, the supernatants of IL-10\(^-/-\) cultures also contained three to four times more IFN-\(\gamma\) than those of dKOs (Fig. 3). Thus, in the context of IL-10 deficiency, the ability of IL-27 to promote T\(_h1\) and Tc1 differentiation appears to promote the onset of IBD.

IL-27R deficiency protects IL-10\(^-/-\) mice from helminth-induced colitis

Aside from its role in maintaining intestinal homeostasis, IL-10 is a critical regulator of helminth-induced mucosal immune responses (53, 54). That capacity is well illustrated when IL-10\(^-/-\) mice are infected with T. muris, a murine homolog to human Trichurid pathogens (55, 56). Unlike resistant WT animals, which clear these colon-dwelling worms through efficient induction of protective T\(_h2\) responses, IL-10-deficient animals sustain high parasite burdens and quickly succumb to a lethal T\(_h1\)-dominated intestinal disease (57). Given the results of our colitis studies, we reasoned that IL-27R deficiency might also protect IL-10\(^-/-\) mice from this helminth-induced pathology and, to test that hypothesis, cohorts of younger animals were challenged with T. muris. Predictably, IL-10\(^-/-\) mice could not clear these parasites and, after 25 days, exhibited severe intestinal pathology characterized by crypt ablation, disruption of the mucosal barrier and pronounced mononuclear cell infiltrates (Fig. 4). In contrast, infected dKOs did not harbor worms and were histologically similar to resistant WT and IL-27R\(^-/-\) mice, with all three strains exhibiting the pronounced goblet cell hyperplasia that is typical of productive anti-helminth responses (Fig. 4). Consistent with previous reports, ex vivo PCR analysis confirmed that the susceptibility of IL-10\(^-/-\) mice was associated with an abundance of pro-inflammatory (IL-6 and TNF-\(\alpha\)) and T\(_h1\)-type factors (IFN-\(\gamma\) and IL-12p35) coupled to a relative lack of protective T\(_h2\)-type factors (IL-4 and IL-13) (Fig. 5). Infected IL-10\(^-/-\) mice also exhibited high levels of T\(_h17\)-type factors (IL-17A, IL-17F, IL-22 and IL-23p19), amphiregulin and IL-27 (p28/EBI3). IL-10\(^-/-\) IL-27R\(^-/-\) mice had a more ‘resistant’ cytokine profile, with T\(_h2\)-type factors dominating over T\(_h1\)-type factors and expression of TNF-\(\alpha\), IL-6, IL-27 and amphiregulin returning to WT levels (Fig. 5).

Fig. 3. Defective T\(_h1\) responses in IL-10 × IL-27R-deficient mice. (A) MLNs were collected from 4-month-old cohorts and re-stimulated with plate-bound anti-CD3 mAb. After 16 h, cells were treated with BFA and stained for surface CD4/CD8 and intracellular IFN-\(\gamma\). Percentages represent the fraction of CD4\(^+\) or CD8\(^+\) events (x-axis) that are also positive for IFN-\(\gamma\) (y-axis). Data are representative of three separate experiments (\(n = 2-3\) mice per group). (B) Lymphocytes were stimulated as in (A), supernatants collected (no BFA) and IFN-\(\gamma\) measured by ELISA. Shown are pooled data (\(n = 2-4\) mice per group) from one of three similar experiments. Standard deviations are represented by error bars and significant differences (\(P < 0.05\)) denoted by stars.
T17-type factors were also modestly reduced but remained substantially higher than in WT and IL-27R−/− controls (Fig. 5).

To confirm the findings of our PCR-based survey, lymphocytes from infected cohorts were re-stimulated with soluble T. muris worm antigen and cytokine production measured by ELISA. Supernatants from T. muris−challenged cohorts of WT, IL-10−/−, IL-27R−/− and IL-10−/−IL-27R−/− mice. Microscopy data are representative of two separate experiments (n = 3–4 mice per group; ×20 magnification; at least three sections/slides inspected per individual). Note the cross-sectioned Trichurid worm in the upper right panel of Fig. 5(C).

As before, we assessed helminth-induced T1,1 responses by stimulating lymphocytes from infected cohorts with anti-TCR mAbs and then measuring IFN-γ production. Again, there was a pronounced accumulation of IFN-γ* CD4+ and CD8+ cells in IL-10−/− mice. These percentages were greatly reduced in the dKOs, though it bears noting that, when compared with WT controls, the number of IFN-γ* producing CD8+ cells was still significantly elevated (Fig. 6C and D). Nevertheless, taken together, the data presented in this section demonstrate that, unlike highly susceptible IL-10-deficient animals, IL-10−/−IL-27R−/− mice are able to generate the protective T1,2 responses necessary to control infection with T. muris.

IL-27 promotes T1,1 responses by Tbet-dependent mechanisms

Based on our in vivo studies, which highlighted the ability of IL-27 to promote intestinal T1,1 responses, we performed a series of in vitro experiments to investigate the molecular mechanisms for that effect. It has been established that, via STAT1, IL-27 promotes expression of Tbet, the key transcription factor in driving Th1 differentiation (32–35). Our studies confirm those reports by demonstrating that IL-27 can promote the expression of Tbet mRNA when WT CD4+ T cells are cultured under non-polarizing conditions (Fig. 7D). That activity was strictly dependent on the interaction between IL-27 and IL-27R as it was not apparent when IL-27R−/− cells were used (Fig. 7D). As expected, IL-27 also enhanced the production of IFN-γ by WT cells, here prompting a significant rise in IFN-γ mRNA, secreted IFN-γ protein and in the percentage of IFN-γ* CD4+ cells (Fig. 7A and C and
In contrast, it did not augment the percentage of IFN-γ+ cells or the abundance of IFN-γ in Tbet-deficient cultures (Fig. 7B and C). These data are consistent with previous reports demonstrating that IL-27 promotes Th1 responses through largely Tbet-dependent mechanisms (58) and, since these transcription factors are known to promote experimental colitis (5), they also imply that the IL-27/Tbet axis may promote Th1-type intestinal inflammation.

**Fig. 5.** Cytokine profiles of IL-10 and IL-10 × IL-27R-deficient mice following *Trichuris muris* infection. Semi-quantitative real-time PCR was used to measure cytokine mRNA in the MLNs of uninfected and *T. muris*-challenged cohorts. Data are pooled from two separate experiments (*n* = 3–4 mice per group). Each plot element represents an individual animal and has been grouped by symbol/color according to genotype. Expression is represented as the fold induction (*x* > 1) or reduction (*x* < 1) relative to a pool of two to three WT controls (*x* = 1). Direct comparisons between experimental groups (brackets) were subjected to a paired *t*-test and *P* values are given. Primer/product information is provided in Supplementary Table 1 (available at *International Immunology* Online).
Aside from its role in Th1 responses, IL-27 can inhibit Th2 responses (39, 40, 44, 59). To determine whether this latter property is also Tbet dependent, we cultured WT, IL-27R−/−/− and Tbet−/− CD4+ T cells with and without IL-27 and used real-time PCR to measure Th2-type factors. As shown previously (34, 39), IL-27 strongly inhibited expression of IL-4 and IL-13.
IL-4, IL-13 and GATA-3 in WT but not IL-27R−/− CD4+ T cells (Fig. 7E–G and data not shown). Tbet is known to suppress T1,2 responses and, accordingly, IL-4, IL-13 and GATA-3 were higher in Tbet−/− cells than in WT controls. However, IL-27 was still able to inhibit all three transcripts in the absence of Tbet, thus demonstrating that it can suppress T1,2 responses through largely Tbet-independent mechanisms (Fig. 7E–G).

**Discussion**

The preceding studies reveal that, in contrast to highly susceptible IL-10-deficient counterparts, IL-10−/−/IL-27−/− mice are resistant to spontaneous and helminth-induced forms of colitis. This improved outcome was associated with greatly diminished T1,1 responses suggesting that, despite a growing literature on the anti-inflammatory properties of IL-27, its pro-inflammatory activities may be relevant for IBD. Consistent with the ability of IL-27 to promote T1,1 responses, production of IFN-γ, the signature T1,1 cytokine, was dramatically reduced in dKOs; with the CD4+ and CD8+ T cell compartments being most affected. These findings suggest a causative link between the T1,1-inducing capacity of IL-27 and the onset of intestinal disease in IL-10−/− mice, an idea bolstered by previous work demonstrating that, similar to the dKO phenotype, neutralization of IFN-γ delays colitis in these animals (3). Additionally, since IL-27 promotes T1,1 responses through largely Tbet-dependent mechanisms (Fig. 7), and Tbet is required for the development of experimental colitis (5), the data presented here also support the following statement: IL-27 is critical for the induction of Tbet-dependent T1,1 responses during the onset phase of chronic intestinal inflammation but, over time, the requirement for its T1,1-inducing capacity can be overcome, presumably once the abundance of other pro-T1,1 cytokines, like IL-12, becomes so great as to make the contribution of IL-27 redundant. This dichotomy, which has been raised previously by us and others (37, 38, 58, 60, 61), is well supported by the delayed T1,1 phenotype that is characteristic of EB13−/− and IL-27R−/− mice following infection with L. major and by numerous studies showing that IL-27R−/− mice exhibit no defect in the generation of T1,1 responses following infection with strongly T1,1-polarizing pathogens like Trypanosoma cruzi, Mycobacterium tuberculosis or T. gondii (36–38, 60–62). It is also well illustrated by the data presented here; we find that, despite the delayed T1,1 phenotype of IL-10−/−/IL-27R−/− mice, IL-27R is completely dispensable for the generation of intestinal T1,1 responses following infection with T. gondii, a parasite known to rapidly induce systemic levels of IL-12 (Supplementary Figure 4, available at International Immunology Online). In fact, when compared with WT controls, oral infection in IL-27R−/− mice leads to greatly exaggerated T1,1 responses, thereby demonstrating the paradoxical, yet essential, anti-inflammatory properties of IL-27/IL-27R (Supplementary Figure 4, available at International Immunology Online).

Following infection with T. muris, IL-10−/−/IL-27R−/− mice displayed a marked increase in IL-13 production that likely contributed to their ability to clear the parasites. As discussed above, these animals also had diminished T1,1
Although T<sub>r1</sub> and T<sub>r17</sub> responses can be pathogenic, they also have essential pro-inflammatory functions that are acutely relevant in the intestine, where there is perpetual risk of infection and/or tissue injury. Various mechanisms have evolved to encourage these beneficial aspects while protecting against unwanted effects, one of which is IL-10, a cytokine that is constitutively produced in the intestine and known to curb both pathogen and commensal-induced inflammation (53, 54, 72, 73). Demonstrating its immunoregulatory properties, IL-10-deficient mice develop severe colitis and, as such, have become an accepted model for human CD. In that capacity, they have facilitated a number of discoveries but, as with all models, they also have caveats that should be considered. Most notably, IL-10-deficient mice disregard the fact that CD develops in an IL-10-sufficient environment and, since IL-10 has been deleted, any effects that a given factor might have on IL-10 itself are obscured. That limitation is particularly relevant here since recent work indicates that IL-27 can promote T cell IL-10 production and that this capacity is critical for limiting inflammation in the CNS (74–76). Thus, while it is clear that IL-27 promotes colitis in IL-10<sup>−/−</sup> mice, it may also have additional, likely anti-inflammatory, functions that are important in an IL-10-sufficient context.

From the preceding studies, we concluded that the delayed colitis in IL-10<sup>−/−</sup>IL-27R<sup>−/−</sup> mice is due, in large part, to a lack of IL-27-dependent T<sub>r1</sub> responses. There is solid evidence to support this statement but several other interpretations can be made. For instance, the phenotype of the dkOs could be due to an improved capacity to defend against colitis-induced sepsis. A recent study has shown that neutralization of IL-27 ameliorates pathology in a mouse model of cecal puncture and it is reasonable to assume that IL-10<sup>−/−</sup>IL-27R<sup>−/−</sup> mice would be similarly protected (77). Given that intestinal T<sub>r2</sub> responses are enhanced in the absence of IL-27, the phenotype of the dkOs could also be due to a more T<sub>r2</sub>-skewed intestinal environment that may temper the development of pathogenic T<sub>r1</sub>1-type inflammation. On that note, it is tempting to speculate that the disease that eventually develops in these animals, which is histologically distinct from that of moribund IL-10-deficient mice (Supplementary Figure 1, available at International Immunology Online), is also immunologically distinct, perhaps manifesting a more T<sub>r2</sub>-like UC. Whatever the case, it is clear that IL-27 does have profound impact in this model of IBD and, as such, the present findings provide a valuable insight toward the human disorder.
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**Supplementary data**

Supplementary Table 1 and Figures 1–4 are available at International Immunology Online.

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**Abbreviations**

BFA brefeldin A  
CD: Crohn's disease  
CNS central nervous system  
DC dendritic cell  
dKO double knock-out or IL-10−/−IL-27R−/−  
EAE experimental autoimmune encephalomyelitis  
EBI3 Epstein–Barr-induced gene 3  
IBD inflammatory bowel disease  
INKT invariant NKT  
lono ionomycin  
MLN mesenteric lymph node  
PMA phorbol 12-myristate 13-acetate  
TGF-β transforming growth factor-β  
TNF-α tumor necrosis factor-α  
UC ulcerative colitis  
WT wild type

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