IL-27 promotes T cell–dependent colitis through multiple mechanisms

Jennifer H. Cox,1 Noelyn M. Kljavin,1 Nandhini Ramamoorthi,1 Lauri Diehl,2 Marcel Batten,3 and Nico Ghilardi1

1Department of Molecular Biology, 2Department of Pathology, Genentech Inc., South San Francisco, CA 94080
3Garvan Institute of Medical Research, Darlinghurst, Sydney, NSW 2010, Australia

Interleukin-27 (IL-27) is a cytokine known to have both proinflammatory and immunoregulatory functions. The latter appear to dominate in vivo, where IL-27 suppresses TH17 responses and promotes the differentiation of Tr1 cells expressing interferon-γ and IL-10 and lacking forkhead box P3 (Foxp3). Accordingly, IL-27 receptor α (IL27ra)–deficient mice suffer from exacerbated immune pathology when infected with various parasites or challenged with autoantigens. Because the role of IL-27 in human and experimental mouse colitis is controversial, we studied the consequences of Il27ra deletion in the mouse T cell transfer model of colitis and unexpectedly discovered a proinflammatory role of IL-27. Absence of Il27ra on transferred T cells resulted in diminished weight loss and reduced colonic inflammation. A greater fraction of transferred T cells assumed a Foxp3+ phenotype in the absence of Il27ra, suggesting that IL-27 functions to restrain regulatory T cell (Treg) development. Indeed, IL-27 suppressed Foxp3 induction in vitro and in an ovalbumin–dependent tolerization model in vivo. Furthermore, effector cell proliferation and IFN-γ production were reduced in the absence of Il27ra. Collectively, we describe a proinflammatory role of IL-27 in T cell–dependent intestinal inflammation and provide a rationale for targeting this cytokine in pathological situations that result from a breakdown in peripheral immune tolerance.

© 2011 Cox et al. This article is distributed under the terms of an Attribution–Noncommercial–Share Alike–No Mirror Sites license for the first six months after the publication date (see http://www.rupress.org/terms). After six months it is available under a Creative Commons License (Attribution–Noncommercial–Share Alike 3.0 Unported license, as described at http://creativecommons.org/licenses/by-nc-sa/3.0/).

IL-27 is a heterodimeric cytokine formed by association of the subunit proteins IL-27p28 and Epstein Bar virus–induced protein 3 (Ebi3; Pflanz et al., 2002). It is predominantly expressed by myeloid cells and signals through a heterodimeric receptor that consists of IL-27Ra (WSX-1, TCCR) and gp130 (Pflanz et al., 2004) and is expressed throughout the immune system. Most of the studies on IL-27 have focused on T cells, where receptor ligation results in activation of the TH1 transcription factors T-bet and STAT1, as well as subsequent up-regulation of the IL-12Rβ2 chain. Despite this apparent TH1-inducing signaling profile, mice deficient in Ebi3 (Ebi3−/−) or Il27ra (Il27ra−/−) do not display major defects in the ability to mount TH1 responses, even though TH1 responses are somewhat delayed in a limited number of infectious scenarios (Batten and Ghilardi, 2007). Instead, these mice exhibit exacerbated inflammation in response to a wide variety of immune challenges, including pathogens that elicit TH1 and TH2 responses and inflammatory models of disease that rely on TH2 and TH17 activity (Batten and Ghilardi, 2007; Kastelein et al., 2007). Several possible mechanisms for this immunomodulatory activity have been identified: IL-27 is known to antagonize TH17 development (Batten et al., 2006; Stumhofer et al., 2006), induce IL-10 production (Awasthi et al., 2007; Fitzgerald et al., 2007; Stumhofer et al., 2007; Batten et al., 2008), and suppress IL-6–induced T cell proliferation (Batten et al., 2006). Nevertheless, IL-27 plays a proinflammatory role in some situations. For example, Il27ra−/− mice are protected from proteoglycan-induced arthritis (Cao et al., 2008) and deletion of the Il27ra gene in the MRL/lpr model of lupus results in lower TH1 cytokine production, diminished anti-dsDNA antibodies, and enhanced survival (Shimizu et al., 2005).

Colitis occurs when tolerance to microbial antigens is broken, resulting in mucosal inflammation. In a recent genome-wide association study,
IL-27p28 was found to be associated specifically with human early onset inflammatory bowel disease (IBD; Imlerlinski et al., 2009). Consistent with a proposed immunoregulatory function of IL-27, the risk allele was found to result in lower expression of IL-27 by donor-derived lymphoblastoid cell lines. However, two other studies found transcripts for IL-27p28 (Schmidt et al., 2005) and Ebi3 (Omata et al., 2001) to be overexpressed in biopsy samples from IBD patients, which would be consistent with either a proinflammatory or an ineffective protective role of IL-27 in IBD. Thus, the pathophysiological relevance of IL-27 in human IBD remains unresolved.

Similar controversy exists in regard to the role of IL-27 in mouse models of colitis. Two groups have studied Il27ra−/− mice in the context of dextran sulfate sodium (DSS)–induced colitis, which is an epithelial damage model, and have come to diametrically opposed conclusions. Troy et al. (2009) studied a high-dose, acute inflammation model (5–10% DSS in drinking water) and found that Il27ra−/− mice are more susceptible, whereas Honda et al. (2005) used much lower concentrations of DSS (0.5% DSS in drinking water) and found Il27ra−/− mice to be protected. Interestingly, the former study identified both adaptive and innate immune cells as targets for IL-27, because the difference in DSS–induced disease severity persisted when the Il27ra−/− allele was crossed onto a Rag-deficient background (Troy et al., 2009). Another group studied the role of IL-27 in a model of helminth-induced IBD and found IL-27 in human IBD remains unresolved. Thus, the pathophysiological relevance of IL-27 in human IBD remains unresolved.

To determine whether IL-27 responsiveness of T cells was required for the establishment of the colitis phenotype, we used the CD4+CD45Rbhi transfer model of colitis (Powrie et al., 1993). First, we crossed the Il27ra−/− allele (Chen et al., 2000) into the balb/c background for 12 generations. As previously described in C57BL/6 mice, Il27ra deficiency causes no overt abnormalities in the balb/c background (unpublished data). However, to our surprise, transfer of FACS-purified balb/c Il27ra−/− CD45Rbhi T cells into CB17-SCID recipient mice resulted in partial protection against weight loss compared with transfer of WT CD45Rbhi T cells (Fig. 1, A and B), suggesting that IL-27 plays a proinflammatory role in this model. Upon analysis of the colons at the end of the study, we found that recipients of Il27ra−/− CD45Rbhi cells also displayed a reduced amount of colonic shortening (Fig. 1 C) and had significantly reduced histological scores. The discrepant results suggest that IL-27 may have distinct effects on various cell compartments, and may promote or ameliorate colitis depending on the specific circumstances.

**RESULTS**

Il27ra−/− CD4+CD45Rbhi T cells fail to induce fulminant colitis

To determine whether IL-27 responsiveness of T cells was required for the establishment of the colitis phenotype, we used the CD4+CD45Rbhi transfer model of colitis (Powrie et al., 1993). First, we crossed the Il27ra−/− allele (Chen et al., 2000) into the balb/c background for 12 generations. As previously described in C57BL/6 mice, Il27ra deficiency causes no overt abnormalities in the balb/c background (unpublished data). However, to our surprise, transfer of FACS-purified balb/c Il27ra−/− CD45Rbhi T cells into CB17-SCID recipient mice resulted in partial protection against weight loss compared with transfer of WT CD45Rbhi T cells (Fig. 1, A and B), suggesting that IL-27 plays a proinflammatory role in this model. Upon analysis of the colons at the end of the study, we found that recipients of Il27ra−/− CD45Rbhi cells also displayed a reduced amount of colonic shortening (Fig. 1 C) and had significantly reduced histological scores.

**Figure 1. Decreased severity of CD45Rbhi colitis in the absence of T cell–derived IL-27.** (A) Relative weight loss after transfer of CD4+CD45Rbhi or unsorted CD4+ cells from WT or Il27ra−/− (KO) mice into CB17-SCID recipients. (B) Weight loss relative to initial weight at 11 wk after transfer of CD4+CD45Rbhi or unsorted CD4+ cells from WT or KO mice. (C) Colon length measurements at 11 wk after transfer. (D) Histological scoring of colitis severity (E) Representative hematoxylin and eosin staining of colons from mice transferred with WT CD45Rbhi, Il27ra−/− CD45Rbhi, or Il27ra−/− unsorted CD4+ cells. Data are from two experiments representing four individual experiments. Bars, 100 µm. *, P < 0.05.
To enable experiments that are not encumbered by nTreg contamination, we bred the Il27ra−/− allele into a DO11.10+ and Rag2−/− background. Such mice contain a pristine population of naive, OVA-specific T cells that is completely devoid of nTreg (Fig. 3 A), and hence represent an ideal system to study Treg conversion. To validate this system, we first cultured DO11.10+Rag2−/− T cells in the presence of lamina propria DCs (LPDCs), OVA peptide, and TGF-β. Indeed, lamina propria DCs allowed for Foxp3+ conversion, and IL-27 suppressed Treg conversion (Fig. 3 B). However, in this experimental system IL-27 also affected CD25 expression (Fig. 1, D and E). Therefore, the presence of Il27ra on T cells is required in this model for the development of both fulminating colitis and maximal weight loss.

II-27Ra signaling limits conversion of naïve T cells into Foxp3+ regulatory T cells (Treg)

As expected, control animals transferred with total CD4+ cells from either genotype suffered from neither weight loss nor colitis, and this is because of the presence of natural Treg (nTreg) in the cell graft (Fig. 1). We therefore investigated whether a difference in the presence of forkhead box P3-positive (Foxp3+) cells might account for the observed phenotype. As shown in Fig. 2 A, we could detect elevated Treg cells in the absence of Il27ra on peripheral blood T cells as early as 5 wk after transfer of CD45Rbhi cells. Furthermore, when we sacrificed mice at the end of the study, we found that recipients of Il27ra−/− CD45Rbhi cells contained approximately two to three times the normal proportion of Foxp3+ T cells in blood, spleen, mesenteric LN (mLN), and lamina propria (Fig. 2, A–C). In agreement with enhanced suppressive activity, we also noted that Il27ra−/− recipients contained fewer total CD4+ T cells, especially in the lamina propria (Fig. 2, D and E).

Previous studies and experiments done here revealed no reduction in the frequency of Foxp3+ cells in naive Il27ra−/− mice (Fig. S1 A; Batten et al., 2006). Furthermore, the suppressive capacity of these cells in vitro is unaffected by the absence of Il27ra (Fig. S2 A; Batten et al., 2006). However, because FACS-sorted WT and Il27ra−/− CD4+CD45Rbhi cells contained ~0.5% nTreg (Fig. S2 B), it remained possible that the increased frequency of Foxp3+ cells in Il27ra−/− CD45Rbhi recipients resulted from preferential in vivo expansion of or enhanced in vivo suppressive capacity by Il27ra−/− nTreg. To address this concern, we transferred purified CD4+CD25+ cells from WT or Il27ra−/− mice into Rag2-deficient C57BL/6 mice that had been transferred with WT CD4+CD45Rbhi cells 7 wk before. nTreg from either genotype were fully capable of rescuing their hosts from systemic wasting disease (Fig. S2, C and D). Furthermore, we observed similar frequencies of Foxp3+ cells of both genotypes in the blood, spleen, and mLNs of the rescued recipient animals, suggesting that the in vivo expansion rate of nTreg is not affected by the Il27ra genotype (Fig. S2, E and F).

IL-27 limits Treg conversion in an OVA-dependent tolerization model in vivo

Inducible Treg develop from naïve CD4+ T cells upon stimulation in the presence of TGF-β. It has been demonstrated in the context of transfer colitis that this type of conversion occurs in vivo in a small fraction of the transferred cells (Sun et al., 2007); however, the resulting number of Foxp3+ cells is insufficient to afford the host full protection, and colitis ensues. Prior studies have suggested that IL-27 can suppress the TGF-β-driven induction of Foxp3+ cells in vitro (Neufert et al., 2007; Huber et al., 2008); therefore, we investigated whether IL-27 normally restraints Treg conversion in vivo.

To enable experiments that are not encumbered by nTreg contamination, we bred the Il27ra−/− allele into a DO11.10+ and Rag2−/− background. Such mice contain a pristine population of naïve, OVA-specific T cells that is completely devoid of nTreg (Fig. 3 A), and hence represent an ideal system to study Treg conversion. To validate this system, we first cultured DO11.10+Rag2−/− T cells in the presence of lamina propria DCs (LPDCs), OVA peptide, and TGF-β. Indeed, lamina propria DCs allowed for Foxp3+ conversion, and IL-27 suppressed Treg conversion (Fig. 3 B). However, in this experimental system IL-27 also affected CD25 expression (Fig. 1, D and E). Therefore, the presence of Il27ra on T cells is required in this model for the development of both fulminating colitis and maximal weight loss.

II-27Ra signaling limits conversion of naïve T cells into Foxp3+ regulatory T cells (Treg)

As expected, control animals transferred with total CD4+ cells from either genotype suffered from neither weight loss nor colitis, and this is because of the presence of natural Treg (nTreg) in the cell graft (Fig. 1). We therefore investigated whether a difference in the presence of forkhead box P3-positive (Foxp3+) cells might account for the observed phenotype. As shown in Fig. 2 A, we could detect elevated Treg cells in the absence of Il27ra on peripheral blood T cells as early as 5 wk after transfer of CD45Rbhi cells. Furthermore, when we sacrificed mice at the end of the study, we found that recipients of Il27ra−/− CD45Rbhi cells contained approximately two to three times the normal proportion of Foxp3+ T cells in blood, spleen, mesenteric LN (mLN), and lamina propria (Fig. 2, A–C). In agreement with enhanced suppressive activity, we also noted that Il27ra−/− recipients contained fewer total CD4+ T cells, especially in the lamina propria (Fig. 2, D and E).

Previous studies and experiments done here revealed no reduction in the frequency of Foxp3+ cells in naïve Il27ra−/− mice (Fig. S1 A; Batten et al., 2006). Furthermore, the suppressive capacity of these cells in vitro is unaffected by the absence of Il27ra (Fig. S2 A; Batten et al., 2006). However, because FACS-sorted WT and Il27ra−/− CD4+CD45Rbhi cells contained ~0.5% nTreg (Fig. S2 B), it remained possible that the increased frequency of Foxp3+ cells in Il27ra−/− CD45Rbhi recipients resulted from preferential in vivo expansion of or enhanced in vivo suppressive capacity by Il27ra−/− nTreg. To address this concern, we transferred purified CD4+CD25+ cells from WT or Il27ra−/− mice into Rag2-deficient C57BL/6 mice that had been transferred with WT CD4+CD45Rbhi cells 7 wk before. nTreg from either genotype were fully capable of rescuing their hosts from systemic wasting disease (Fig. S2, C and D). Furthermore, we observed similar frequencies of Foxp3+ cells of both genotypes in the blood, spleen, and mLNs of the rescued recipient animals, suggesting that the in vivo expansion rate of nTreg is not affected by the Il27ra genotype (Fig. S2, E and F).

IL-27 limits Treg conversion in an OVA-dependent tolerization model in vivo

Inducible Treg develop from naïve CD4+ T cells upon stimulation in the presence of TGF-β. It has been demonstrated in the context of transfer colitis that this type of conversion occurs in vivo in a small fraction of the transferred cells (Sun et al., 2007); however, the resulting number of Foxp3+ cells is insufficient to afford the host full protection, and colitis ensues. Prior studies have suggested that IL-27 can suppress the TGF-β-driven induction of Foxp3+ cells in vitro (Neufert et al., 2007; Huber et al., 2008); therefore, we investigated whether IL-27 normally restraints Treg conversion in vivo.
IL-27 promotes transfer colitis | Cox et al.

observed that Il27ra deficiency significantly augmented peripheral Treg development, indicating that IL-27 limits Treg conversion even in a noninflammatory environment. This effect was further accentuated when we measured absolute numbers of Foxp3+ DO11.10+Rag2−/− cells (Fig. 4 C). Because only naive Foxp3− cells were transferred into recipients, this experiment also conclusively proves that IL-27 signaling limits Treg conversion rather than expansion of nTregs. Consistent with previous observations (Villarino et al., 2006), Il27ra−/− DO11.10 cells produced more IL-2 (Fig. 4 D), and generally suppressed T cell activation as measured by CD69 surface expression (Fig. 3 C). IL-27 acted directly on T cells to down-regulate Foxp3, CD25, and CD69, because it had no effect when Il27ra−/− T cells were used in the experiment.

Next, we transferred purified DO11.10+Rag2−/− T cells into naive balb/c recipients and exposed them to OVA in the drinking water. Exposure to antigen led to a significant increase in Foxp3+ cells in the spleens and mLNs (Fig. 4, A–C). Consistent with our data obtained from the colitis model, we observed that Il27ra deficiency significantly augmented peripheral Treg development, indicating that IL-27 limits Treg conversion even in a noninflammatory environment. This effect was further accentuated when we measured absolute numbers of Foxp3+ DO11.10+Rag2−/− cells (Fig. 4 C). Because only naive Foxp3− cells were transferred into recipients, this experiment also conclusively proves that IL-27 signaling limits Treg conversion rather than expansion of nTregs. Consistent with previous observations (Villarino et al., 2006), Il27ra−/− DO11.10 cells produced more IL-2 (Fig. 4 D),
whereas IFN-γ production was minimal irrespective of Il27ra expression in the noninflammatory environment of unchallenged balb/c mice (Fig. 4 E). However, increased production of IL-2 is not responsible for enhanced Treg conversion because IL-2 does not override the suppressive effect of IL-27 on Foxp3 induction (Neufert et al., 2007 and unpublished data), which has been shown in vitro to be a direct, STAT3-mediated effect of IL-27 on T cells (Huber et al., 2008). Importantly, we still observed enhanced Treg conversion when we repeated this experiment under inflammatory conditions in mice that had received WT CD45Rbhi cells 4 wk earlier (Fig. 4 F), suggesting that the inflammatory milieu by itself is insufficient to restrict Foxp3 expression in the absence of IL-27 signaling. However, introduction of Il27ra−/− CD45Rbhi cells into mice with preestablished colitis failed to demonstrate a therapeutic effect (Fig. S3).

Il27ra−/− effector T cells have a different cytokine secretion profile compared with their WT counterparts

Despite the increased conversion rate of Il27ra−/− CD45Rbhi cells, the majority of the transferred cells remained Foxp3−. We therefore also examined cytokine production in the colon by RT-PCR and in restimulated lymphocytes isolated from the spleen, the mLN, and the colonic lamina propria of CD45Rbhi recipient animals by intracellular staining. Although IL-12, IL-23, IL-2, IL-6, and IL-27 were all found to be induced in colitic mice, no statistically significant changes were noted between WT and Il27ra−/− CD45Rbhi recipients (Fig. S4). In accordance with prior observations (Chen et al., 2000; Artis et al., 2004; Batten et al., 2006; Stumhofer et al., 2006), T cells isolated from Il27ra−/− CD45Rbhi recipients produced significantly less IFN-γ as assessed by intracellular staining (Fig. 5, A and B), mimicking decreased production of IFN-γ in naive mice (Fig. S1 B). The overall decrease of CD4+ cells (Fig. 2 E) further accentuates the decrease in absolute numbers of IFN-γ–producing cells (not depicted). Therefore, the question of whether diminished IFN-γ production is partially responsible for the protective effect of Il27ra deficiency merits consideration. Using IFN-γ neutralizing antibodies, others have previously reported a pathogenic role for IFN-γ in this model (Powrie et al., 1994). In our hands, IFN-γ−/− CD45Rbhi cells elicited less severe colitis, but also caused highly aggressive wasting disease requiring early termination of the experiment (Fig. S5, A–C). Consistent with findings obtained by others (Wang et al., 2006), IFN-γ−/− CD45Rbhi cells had a diminished propensity to become Foxp3+ and expanded more aggressively (Fig. S5, D and E). Thus, IFN-γ deficiency phenocopies IL-27Ra deficiency in terms of reduced colitis, but not in terms of the improved wasting disease, increased Treg conversion, or reduced expansion of effector cells. Therefore, a mere reduction in IFN-γ production cannot fully explain the Il27ra−/− phenotype.

Conversely, and consistent with prior studies (Artis et al., 2004; Batten et al., 2006; Stumhofer et al., 2006; Yang et al., 2008), we observed mild elevations in TH17 (IL-17A and IL-22) and TH2 (IL-5 and IL-13) cytokines produced by Il27ra−/− CD45Rbhi cells (Fig. 5, C–E and not depicted). However, these changes were effectively neutralized in absolute terms by the lower total number of CD4+ cells (Fig. 2 E and not depicted). We did not observe increased neutrophil...
infiltration, which one would expect as a consequence of IL-17 overexpression (Fig. 6). Therefore, the minor relative changes in IL-17 and IL-22 production by Il27ra−/− CD45Rbhi cells are unlikely to contribute significantly to disease protection, although IL-17 (Izcue et al., 2008; Leppkes et al., 2009; O’Connor et al., 2009) has been shown to either be neutral or exert protective effects in the context of this model, and IL-22 is protective (Zenewicz et al., 2008).

**DISCUSSION**

We demonstrate conclusively that Il27ra−/− CD45Rbhi T cells are less colitogenic than their WT counterparts in the murine transfer colitis model. Our analysis of the T cell graft after 12 wk of incubation in lymphopenic hosts revealed that a much greater proportion of IL27ra−/− CD45Rbhi T cells assumed a Foxp3+ phenotype, whereas the remaining Foxp3− cells proliferated less and produced much less of the TH1 cytokine IFN-γ. Although there were mild relative increases in IL27ra−/− effector cells producing TH2 and TH17 cytokines, the lower abundance of IL27ra−/− effector cells effectively neutralized this difference, which is consistent with the absence of an increase in neutrophil infiltration into the lamina propria. Therefore, the reduced colitis and weight loss in recipients of IL27ra−/− CD45Rbhi T cells is a consequence of increased Treg conversion, lower IFN-γ production, decreased effector cell proliferation, or a combination of these effects.

Together, the changes in T cell phenotype and abundance form a chicken and egg conundrum: do effector cells or is there an increase in Foxp3+ cells because effector cells fail to proliferate and cause inflammation efficiently in the absence of IL-27Ra, which in turn de-represses Treg conversion? Several of our data points favor the first of these two possibilities: First, IL-27 is known to antagonize TGF-β driven Treg conversion in vitro (Fig. 3; Neufert et al., 2007; Huber et al., 2008), and thus conversion is expected to be de-restrained in IL27ra−/− T cells in vivo. Second, we observed an increase in absolute numbers, not just relative percentages, of IL27ra−/− Foxp3+ T cells in our OVA-dependent tolerization model (Fig. 4). This difference persisted even in the context of established colitis (Fig. 4 F), and thus represents a cell intrinsic effect that is independent of the inflammation status of the environment into which the cells are transferred. The OVA oral tolerance model is a much shorter term experiment than the 12-wk colitis study, and is therefore more amenable to mechanistic interpretation. And lastly, although a reduction in IFN-γ production could potentially explain the reduction in colitis, Treg conversion of IFN-γ−/− T cells was not enhanced, and they still caused significant wasting disease. Therefore, reduced Treg conversion is not secondary to reduced production of IFN-γ in the absence of IL-27Ra. In summary, these observations provide strong suggestive evidence that the primary effect of IL-27 in this context is to restrain Treg conversion.

We attempted to therapeutically rescue mice with preestablished transfer colitis by introducing Il27ra−/− CD45Rbhi cells, but this experiment was not successful (Fig. S3). Although a trend toward increased Foxp3 expression was still noticed 6 wk after transfer of the Il27ra−/− CD45Rbhi cells into the inflammatory environment, and although the transferred cells clearly produced less IFN-γ (Fig. S3, F and G), we did not detect any changes in weight loss or colon length. This result suggests that although de-repression of Treg conversion through loss of IL-27Ra at the onset of inflammation significantly affects the ultimate outcome, it is not robust enough to overcome preestablished inflammation.

Earlier studies by our group and others showed that IL-27 can induce IL-10 production in T cells and lead to the development of Foxp3+ IL-10+ Tr1 cells (Awasthi et al., 2007; Fitzgerald et al., 2007; Stumhofer et al., 2007; Batten et al., 2008). Because Tr1 cells have potent immunoregulatory effects (Anderson et al., 2007; Jankovic et al., 2007; Trinchieri, 2007), it has been postulated that this is a mechanism by which IL-27 exerts immune suppression in infectious and autoimmune disease. These earlier observations are at odds with the proinflammatory role of IL-27 described here. However, in transfer colitis, IL-10 production by cells originating from the Foxp3+ CD45Rbhi graft is minimal (Uhlig et al., 2006). We found no Il27ra−/−-dependent difference in IL-10 production by transferred CD45Rbhi cells, and IL-10 production in general was minimal in this model (unpublished data). Thus, the Foxp3-suppressing effects of IL-27 are not in contradiction with its Tr1-inducing effects; the dichotomy merely reflects the differences between the physiological contexts in which IL-27 stimulation occurs and may explain why the essential function of IL-27 in the regulation of Treg differentiation has not been noted previously.
It is perhaps appropriate to point out that the majority of in vivo effects assigned to IL-27 have been inferred from the analysis of Il27ra−/− mice, and only some have been confirmed by studies of Il27p28−/− or Ebi3−/− mice. This study is no different in that regard. To date, IL-27 is the only confirmed ligand for IL-27Ra. However, IL-35 has been described as an IL-27–related heterodimer with potent immunoregulatory effects (Collison et al., 2007, 2010). IL-35 consists of IL-12p35 and Ebi3, and although its receptor has not been identified, IL-27Ra remains a candidate. Another recently described IL-27–related heterodimer consists of IL-27p28 and cytokine-like factor and appears to bind to IL-27Ra, but conclusive proof that IL-27Ra is required for signaling is currently not available (Crabé et al., 2009). Thus, it remains possible that IL-27Ra has ligands other than IL-27, which might further contribute to the apparent complexity and dichotomous nature of IL-27 biology in vivo.

In summary, we demonstrate that IL-27 exerts proinflammatory effects in the T cell transfer colitis model. Our experiments demonstrate conclusively that IL-27 acts to suppress induced Treg development in vivo, and thus reveal a hitherto unrecognized proinflammatory mechanism. Collectively, our results suggest that targeting of IL-27 in situations where pathology results from a breakdown in Treg-mediated tolerance may result in significant therapeutic benefit.

**MATERIALS AND METHODS**

**Mice.** All mice were maintained under pathogen-free conditions, and experiments were approved by the Institutional Animal Care and Use Committee of Genentech, Inc. The Il27ra−/− allele (Chen et al., 2000) was backcrossed onto the b6 background for 12 generations. This strain was crossed further to the DO11.10+Rag2−/− background (Taconic). CB17-SCID mice were purchased from Charles River. b6/c and Rag2−/−, C57BL6 mice were obtained from Taconic.

**Cytokines.** Unless otherwise indicated, all cytokines were purchased from R&D Systems and all antibodies used in flow cytometry or culture experiments were purchased from BD. Anti–IL-22 (clone 3F11, isotype mouse IgG2a; Genentech) was directly conjugated to Alexa Fluor 647 (Invitrogen).

**Induction of colitis with naïve CD4+CD45Rbhi T cells.** Naïve CD4+CD45Rbhi cells were isolated from the spleens of female Il27ra−/− or Il27a−/− mice by FACS sorting. In brief, single-cell suspensions were depleted of red blood cells by hypotonic lysis and CD4+ cells were purified by MACS–positive L3T4 selection (Miltenyi Biotech). After staining with Pacific blue–conjugated anti–CD4, PE-conjugated anti–CD45Rb, and PE/Cy5-conjugated anti–CD8, CD4+CD45Rbhi naïve T cells were purified (>98%) by cell sorting (FACSARia; BD). Female CB17-SCID mice were injected with 3 × 105 CD45Rbhi cells or unsorted CD4+ cells i.v. Mice were monitored for weight loss and sacrificed by CO2 asphyxiation 11–12 wk after initiation of the experiment. Blood was obtained by retroorbital bleed under isoflurane anesthesia at the indicated time points.

**Assessment of intestinal inflammation.** At the time of sacrifice, mouse colons were removed and flushed, and the length was measured from rectum to cecum. Tissues were immediately fixed in 10% buffered formalin, and 4–5-µm paraffin–embedded sections were stained with hematoxylin and eosin. Colitis severity was scored in the proximal colon, medial colon, distal colon, and rectum on a scale of 0–5, with 0 and 5 representing a normal colon and severe colitis, respectively. The scores of four anatomical regions were totaled for each mouse to yield a total histological score.

**Flow cytometry.** Single-cell suspensions were obtained from spleens and mLNs. Lamina propria leukocytes were isolated as described previously (Zheng et al., 2008). Cell counts were measured by ViaCount analysis on the Guava PCA-96 (Millipore). Anti–CD4 and anti–CD25 were used for surface staining of lymphocytes. For intracellular cytokine staining, single-cell suspensions from spleens, mLNs, and lamina propria were restimulated for 4 h in RPMI containing 10% FBS with 50 ng/ml of phorbol 12-myristate 13-acetate and 500 ng/ml of ionomycin in the presence of 5 µg/ml of brefeldin A (Sigma-Aldrich). Cells were then fixed in 1% paraformaldehyde in PBS, permeabilized with 0.5% saponin in flow cytometry buffer (0.5% BSA in PBS), and stained intracellularly with PE-conjugated anti–IFN-γ, PECy7-conjugated anti–mouse IL-17, PECy7-conjugated anti–mouse IFN-γ, Alexa Fluor 647–conjugated anti–mouse IL-22 (Genentech), and PE-conjugated anti–mouse IL-13. Alternatively, Foxp3 staining was performed according to manufacturer’s protocol (eBioscience).

**In vitro conversion of DO11.10+ T cells.** Lamina propria DCs were purified after collagenase treatment by sorting of CD11c+MHCII+ cells. DO11.10+CD4+ cells were MACS purified and incubated (5 × 104 cells/well) with LPDCs (5 × 104 cells/well) in the presence of OVA323-339 (0.3 µM), TGF-β (3 ng/ml), and IL-27 (20 ng/ml) as indicated. Cells were stimulated for 5 d in round-bottom 96-well plates, and intracellular Foxp3 was analyzed as described in the previous paragraph.

**In vivo conversion of DO11.10+ T cells.** CD4+ T lymphocytes were enriched (~85% purity) from the spleens and mLNs of female Il27ra−/− or Il27a−/− DO11.10+Rag2−/− mice by negative MACS selection (Miltenyi Biotech). Female b6/c recipient mice (Taconic) were injected with 1.8 × 106 cells i.v. on day 0. On day 1, mice were given 1.5% OVA (Grade V; Sigma-Aldrich) dissolved in drinking water for 5 d, as previously described (Sun et al., 2007). OVA water was replaced every 48 h, and control mice received normal drinking water. On day 6, mice were sacrificed and spleens, mLNs, Peyers patches, and lamina propria lymphocytes were assessed for Foxp3 expression. Cells were surface stained with PE-conjugated anti–KJ1-26 for the DO11.10 TCR, and then stained for intracellular IL-2, IFN-γ, or Foxp3. In some experiments, mice were injected with 3 × 104 WT CD45Rbhi cells to initiate colitis and, 4 wk later, were transferred with 2 × 106 CD4+DO11.10+ cells from WT or Il27a−/− mice. Mice were either given normal water or 1.5% OVA for 5 d, as indicated.

**Online supplemental material.** Fig. S1 shows that naïve Il27ra−/− mice have unaltered levels of Foxp3+ Treg and decreased levels of IFN-γ–producing CD4+ cells. Fig. S2 shows that Il27a−/− natural Treg have normal suppressive capacity. Fig. S3 shows that Il27a−/− CD45Rbhi cells fail to control previously established colitis. Fig. S4 shows cytokine expression during transfer colitis in recipients of WT or Il27a−/− cells; assessed by qPCR. Fig. S5 details transfer colitis elicited by transfer of IFN-γ−/− CD45Rbhi cells into rag2−/− deficient recipients. Online supplemental material is available at http://jem.org/cgi/content/full/jem.20100410/DC1.

We would like to thank Jeong Kim for critical discussion of the manuscript and William Forrest for assistance with statistical analysis. We also thank Jim Cupp and members of the Genentech flow cytometry group for contributions in cell sorting, the Genentech histology laboratory for specimen processing and staining, and Ben Torres for animal husbandry. Nico Ghilardi, Nandhini Ramamoorthi, Noelyn M. Kljavin, Jennifer H. Cox, and Lauri Diehl are full-time employees at Genentech, a member of the Roche Group. Marcel Batten is supported by a fellowship from The Cancer Institute of New South Wales.

The authors declare no additional conflicting financial interests.

Submitted: 1 March 2010
Accepted: 12 November 2010
REFERENCES

Ahern, P.P., A. Iacue, K.J. Maloy, and F. Powrie. 2008. The interleukin-23 axis in intestinal inflammation. *Immunol. Rev.* 226:147–159. doi:10.1111/j.1600-065X.2008.00705.x

Anderson, C.F., M. Oukka, V.J. Kuchroo, and D. Sacks. 2007. CD4+CD25+Foxp3+ Th1 cells are the source of IL-10–mediated immune suppression in chronic cutaneous leishmaniasis. *J. Exp. Med.* 204:285–297. doi:10.1084/jem.20061886

Artis, D., A. Villanorno, M. Silverman, W. He, E.M. Thornton, S. Mu, S. Summer, T.M. Covey, E. Huang, H. Yoshida, et al. 2004. The IL-27 receptor (WSX-1) is an inhibitor of innate and adaptive elements of type 2 immunity. *J. Immunol.* 173:5626–5634

Awasthi, A., Y. Carrier, J.P. Peron, E. Bettelli, M. Kamanaka, R.A. Flavell, V.K. Kuchroo, M. Oukka, and H.L. Weiner. 2007. A dominant function for interleukin 27 in generating interleukin 10-producing anti-inflammatory T cells. *Nat. Immunol.* 8:1380–1389. doi:10.1038/ni1541

Batten, M., and N. Ghilardi. 2007. The biology and therapeutic potential of interleukin 27. *J. Mol. Med.* 85:661–672. doi:10.1007/s00109-007-0164-7

Batten, M., J. Li, S. Yi, N.M. Kljavin, S. Lucas, J. Lee, F.J. de Sauvage, and N. Ghilardi. 2009. Interleukin 27 limits autoimmune encephalomyelitis by suppressing the development of interleukin 17-producing T cells. *Nat. Immunol.* 7:929–936. doi:10.1038/ni1375

Batten, M., N.M. Kljavin, J. Li, M.J. Walter, F.J. de Sauvage, and N. Ghilardi. 2008. Cutting edge: IL-27 is a potent inducer of IL-10 but not Foxp3+ in murine T cells. *J. Immunol.* 180:2752–2756.

Cao, Y., P.D. Doodes, T.T. Glant, and A. Finnegan. 2008. IL-27 induces a Th1 immune response and susceptibility to experimental arthritis. *J. Immunol.* 180:922–930

Chen, Q., N. Ghilardi, H. Wang, T. Baker, M.H. Xie, A. Gurney, I.S. Grewal, and F.J. de Sauvage. 2000. Development of Th1-type immune response and susceptibility to experimental arthritis. *Cell.* 102:151–163. doi:10.1016/S0092-8674(00)00067-9

Collison, L.W., V. Chaturvedi, A.L. Henderson, P.R. Giacomin, C. Guy, J. Molnar, J. Cui, L. Aksamit, J. Moss, et al. 2009. IL-27 limits autoimmune encephalomyelitis by suppressing the development of interleukin 17-producing T cells. *Nat. Immunol.* 10:603–609. doi:10.1038/ni1376

Imielinski, M., R.N. Baldassano, A. Griffiths, R.K. Russell, V. Annese, M. Katan, J. Heilmann, F. Powrie, and F. Altieri. 2009. Common variants at five new loci associated with early-onset inflammatory bowel disease. *Nat. Genet.* 41:1335–1340. doi:10.1038/ng.489

Iacue, A., S. Hue, S. Buonocore, C.V. Arancibia-Circacio, P.P. Ahern, Y. Iwakura, K.J. Maloy, and F. Powrie. 2008. Interleukin-23 restraints regulatory T cell activity to drive T cell-dependent colitis. *Immunity.* 29:559–570. doi:10.1016/j.immuni.2008.02.012

Jankovic, D., M.C. Kulberg, C.G. Feng, B.S. Goldszmid, C.M. Collazo, M. Wilson, T.A. Wynn, M. Kamanaka, R.A. Flavell, and A. Sher. 2007. Conventional T-betFoxp3+ Th1 cells are the major source of host-protective regulatory IL-10 during intracellular protozoan infection. *J. Exp. Med.* 204:273–283. doi:10.1084/jem.20062175

Kastelein, R.A., C.A. Hunter, and D.J. Cua. 2007. Discovery and biology of IL-23 and IL-27: related but functionally distinct regulators of inflammation. *Annu. Rev. Immunol.* 25:221–242. doi:10.1146/annurev.immunol.22.012703.104758

Leppkes, M., C. Becker, I.I. Ivanov, S. Hirih, S. Wirtz, C. Neufert, S. Pouly, A.J. Murphy, D.M. Valenzuela, G.D. Yancopoulos, et al. 2009. RORgamma-expressing Th17 cells induce murine chronic intestinal inflammation via redundant effects of IL-17A and IL-17F. *Gastroenterology.* 136:257–267. doi:10.1053/j.gastro.2008.10.018

Neufert, C., C. Becker, S. Wirtz, M.C. Fantini, B. Weigmann, R.R. Galle, and M.F. Neurath. 2007. IL-27 controls the development of inducible regulatory T cells and Th17 cells via differential effects on STAT1. *Eur. J. Immunol.* 37:1809–1816. doi:10.1002/eji.200636896

Nieuwenhuis, E.E., M.F. Neurath, N. Corazza, H. Iijima, J. Trgovcich, S. Wirtz, J. Glickman, D. Bailey, M. Yoshida, P.R. Galle, et al. 2002. Disruption of T helper 2 immune responses in Epstein-Barr virus-induced gene 3–deficient mice. *Proc. Natl. Acad. Sci. USA.* 99:16951–16956. doi:10.1073/pnas.025648899

O’Connor, W. Jr., M. Kamanaka, C.J. Booth, T. Town, S. Nakae, Y. Iwakura, J.K. Kolls, and R.A. Flavell. 2009. A protective function for interleukin 17A in T cell-mediated intestinal inflammation. *Nat. Immunol.* 10:603–609. doi:10.1038/ni1376

Omata, F., M. Birkenbach, S. Matsuizaki, A.D. Christ, and R.S. Blumberg. 2001. The expression of IL-12 p40 and its homologue, Epstein-Barr virus-induced gene 3, in inflammatory bowel disease. *Inflamm. Bowel Dis.* 7:215–220. doi:10.1038/sj.ibd.200108006-00006

Pflanz, S., J.C. Timans, J. Cheung, R. Rosales, H. Kanzler, J. Gilbert, L. Hibbert, T. Churakova, M. Travis, E. Vaisberg, et al. 2002. IL-27, a heterodimeric cytokine composed of EB13 and p28 protein, induces proliferation of naive CD4+ T cells. *Immunology.* 169:779–790. doi:10.1111/j.1600-065X.2005.00468.x

Pflanz, S., L. Hibbert, J. Mattson, R. Rosales, E. Vaisberg, J.F. Bazan, J.P. McGlathery, N. de Waal Malefyt, and R.A. Kastelein. 2004. WSX-1 and glycoprotein 130 constitute a signal-transducing receptor for IL-27. *J. Immunol.* 172:2228–2231.

Powrie, F.M., W.R. Leach, S. Maurice, L.B. Caddle, and R.L. Coffman. 1993. Phenotypically distinct subsets of CD4+ T cells induce or protect from chronic intestinal inflammation in C. B-17 scid mice. *Int. Immunol.* 5:1461–1471. doi:10.1093/intimm/5.11.1461

Powrie, F.M., W.R. Leach, S. Maurice, S. Menon, L.B. Caddle, and R.L. Coffman. 1994. Inhibition of Th1 responses prevents inflammatory bowel disease in scid mice reconstituted with CD45RBhi CD4+ T cells. *Immunology.* 10:553–562. doi:10.1046/j.1600-065X.2000.00049.x

Rubtsov, A.M., K. Miyazaki, R.N. Baldassano, J. Heilmann, K. Kiyono, Y. Iwakura, R. Matsumoto, K. Kiyosawa, and T. Shimazaki. 2003. Membranous glomerulonephritis development with Th2-type immune deviations in MRL/lpr mice deficient for IL-27 receptor (WSX-1). *J. Immunol.* 175:7185–7192.

Stunshofer, J.S., A. Laurence, E.H. Wilson, E. Huang, C.M. Tato, L.M. Johnson, A.V. Villanorno, Q. Huang, A. Yoshuma, D. Sehy, et al. 2006. Interleukin 27 negatively regulates the development of interleukin 17-producing T helper cells during chronic inflammation of the central nervous system. *Nat. Immunol.* 7:937–945. doi:10.1038/ni1376

Stunshofer, J.S., J.S. Silver, A. Laurence, P.M. Porrett, T.H. Harris, L.A. Turka, M. Ernst, C.J. Saris, J.J. O’Shea, and C.A. Hunter. 2007.
Interleukins 27 and 6 induce STAT3-mediated T cell production of interleukin 10. Nat. Immunol. 8:1363–1371. doi:10.1038/ni1537
Sun, C.M., J.A. Hall, B.B. Blank, N. Bouladoux, M. Oukka, J.R. Mora, and Y. Belkaid. 2007. Small intestine lamina propria dendritic cells promote de novo generation of Foxp3 T reg cells via retinoic acid. J. Exp. Med. 204:1775–1785. doi:10.1084/jem.20070602
Trinchieri, G. 2007. Interleukin-10 production by effector T cells: Th1 cells show self control. J. Exp. Med. 204:239–243. doi:10.1084/jem.20070104
Troy, A.E., Zaph, Y. Du, B.C. Taylor, K.J. Guild, C.A. Hunter, C.J. Saris, and D. Artis. 2009. IL-27 regulates homeostasis of the intestinal CD4+ effector T cell pool and limits intestinal inflammation in a murine model of colitis. J. Immunol. 183:2037–2044. doi:10.4049/jimmunol.0802918
Uhlig, H.H., J. Coombes, C. Mottet, A. Izcue, C. Thompson, A. Fanger, A. Tannapfel, J.D. Fontenot, F. Ramsdell, and F. Powrie. 2006. Characterization of Foxp3+CD4+CD25+ and IL-10-secreting CD4+CD25+ T cells during cure of colitis. J. Immunol. 177:5852–5860.
Villarino, A.V., J.S. Stumhofer, C.J. Saris, R.A. Kastelein, F.J. de Sauvage, and C.A. Hunter. 2006. IL-27 limits IL-2 production during Th1 differentiation. J. Immunol. 176:237–247.
Villarino, A.V., D. Artis, J.S. Bezbradica, O. Miller, C.J. Saris, S. Joyce, and C.A. Hunter. 2008. IL-27R deficiency delays the onset of colitis and protects from helminth-induced pathology in a model of chronic IBD. Int. Immunol. 20:739–752. doi:10.1093/intimm/dxn032
Wang, Z., J. Hong, W. Sun, G. Xu, N. Li, X. Chen, A. Liu, L. Xu, B. Sun, and J.Z. Zhang. 2006. Role of IFN-gamma in induction of Foxp3 and conversion of CD4+ CD25- T cells to CD4+ Tregs. J. Clin. Invest. 116:2434–2441.
Yang, J., M. Yang, T.M. Htut, X. Ouyang, A. Hanidu, X. Li, R. Sellati, H. Jiang, S. Zhang, H. Li, et al. 2008. Epstein-Barr virus-induced gene 3 negatively regulates IL-17, IL-22 and RORgamma t. Eur. J. Immunol. 38:1204–1214. doi:10.1002/eji.200838145
Zenzewicz, L.A., G.D. Yancopoulos, D.M. Valenzuela, A.J. Murphy, S. Stevens, and R.A. Flavell. 2008. Innate and adaptive interleukin-22 protects mice from inflammatory bowel disease. Immunity. 29:947–957. doi:10.1016/j.immuni.2008.11.003
Zheng, Y., P.A. Valdez, D.M. Danilenko, Y. Hu, S.M. Sa, Q. Gong, A.R. Abbas, Z. Modrusan, N. Ghilardi, F.J. de Sauvage, and W. Ouyang. 2008. Interleukin-22 mediates early host defense against attaching and effacing bacterial pathogens. Nat. Med. 14:282–289. doi:10.1038/nm1720