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

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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 (T_{reg}) 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.

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Abbreviations used: DSS, dextran sulfate sodium; Ebi3, Epstein Bar virus–induced protein 3; Foxp3, forkhead box P3; IBD, inflammatory bowel disease; LP, lamina propria; LPDC, lamina propria DC; mLN, mesenteric LN; nTreg, natural regulatory T cell; Treg, regulatory T cell.

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,

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IL-27p28 was found to be associated specifically with human early onset inflammatory bowel disease (IBD; Imielinski 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 that *Il27ra* deficiency impairs the intestinal TH1 response, resulting both in ineffective worm expulsion and delayed onset of colitis (Villarino et al., 2008). Finally, *Ebi3*$^{-/-}$ mice were almost completely protected against colitis-induced by oxazolone, which induces a TH2-type immune pathology and involves invariant NK-T cells, but not at all protected when colitis was induced by 2,4,6-trinitrobenzenesulfonic acid, a TH1-promoting agent (Nieuwenhuis et al., 2002).

These 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. In an effort to reduce complexity, we decided to study the role of IL-27 in a T cell transfer model (Powrie et al., 1993), which allows for the specific assessment of T cell restricted *Il27ra* deficiency. This model is characterized by colitis and systemic wasting disease. Because colitis depends on IL-23 and TH17 cells (Ahern et al., 2008), and because IL-27 acts to suppress TH17 development (Batten et al., 2006; Stumhofer et al., 2006), we expected exacerbated colitis in recipients of *Il27ra*$^{-/-}$ CD45Rb^{hi} cells. In contrast, absence of IL-27Ra on transferred T cells resulted in diminished weight loss and reduced colonic inflammation.

RESULTS

Il27ra$^{-/-}$ CD4^{+}CD45Rb^{hi} 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^{+}CD45Rb^{hi} 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*$^{-/-}$ CD45Rb^{hi} T cells into CB17-SCID recipient mice resulted in partial protection against weight loss compared with transfer of WT CD45Rb^{hi} 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*$^{-/-}$ CD45Rb^{hi} cells also displayed a reduced amount of colonic shortening (Fig. 1 C) and had significantly reduced histological scores

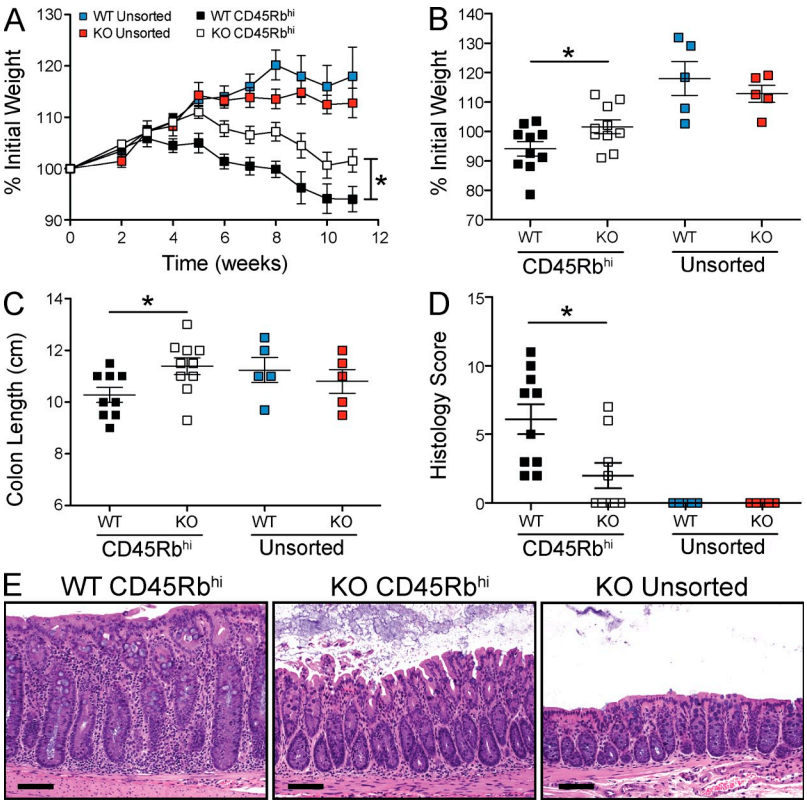

Figure 1. Decreased severity of CD45Rb^{hi} colitis in the absence of T cell–derived IL-27R. (A) Relative weight loss after transfer of CD4^{+}CD45Rb^{hi} or unsorted CD4^{+} cells from WT or *Il27ra*$^{-/-}$ (KO) mice into CB17-SCID recipients. (B) Weight loss relative to initial weight at 11 wk after transfer of CD4^{+}CD45Rb^{hi} 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 CD45Rb^{hi}, *Il27ra*$^{-/-}$ CD45Rb^{hi}, or *Il27ra*$^{-/-}$ unsorted CD4^{+} cells. Data are from two experiments representing four individual experiments. Bars, 100 µm. *, $P < 0.05$.

(Fig. 1, D and E). Therefore, the presence of *Il27ra* on T cells is required in this model for the development of both fulminant colitis and maximal weight loss.

IL-27Ra signaling limits conversion of naive T cells into Foxp3$^+$ regulatory T cells (T_{regs})

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 T_{regs} (nT_{regs}) 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 T_{reg} cells in the absence of *Il27ra* on peripheral blood T cells as early as 5 wk after transfer of CD45Rb^{hi} cells. Furthermore, when we sacrificed mice at the end of the study, we found that recipients of *Il27ra*$^{-/-}$ CD45Rb^{hi} 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$^+$CD45Rb^{hi} cells contained ~0.5% nT_{regs} (Fig. S2 B), it remained possible that the increased frequency of Foxp3$^+$ cells in *Il27ra*$^{-/-}$ CD45Rb^{hi} recipients resulted from preferential in vivo expansion of or enhanced in vivo suppressive capacity by *Il27ra*$^{-/-}$ nT_{regs}. 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$^+$CD45Rb^{hi} cells 7 wk before. nT_{regs} 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 nT_{regs} is not affected by the *Il27ra* genotype (Fig. S2, E and F).

IL-27 limits T_{reg} conversion in an OVA-dependent tolerization model in vivo

Inducible T_{regs} develop from naive 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 restrains T_{reg} conversion in vivo. To enable experiments that are not encumbered by nT_{reg} 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 nT_{regs} (Fig. 3 A), and hence represent an ideal system to study T_{reg} 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 T_{reg} conversion (Fig. 3 B). However, in this experimental system IL-27 also affected CD25 expression

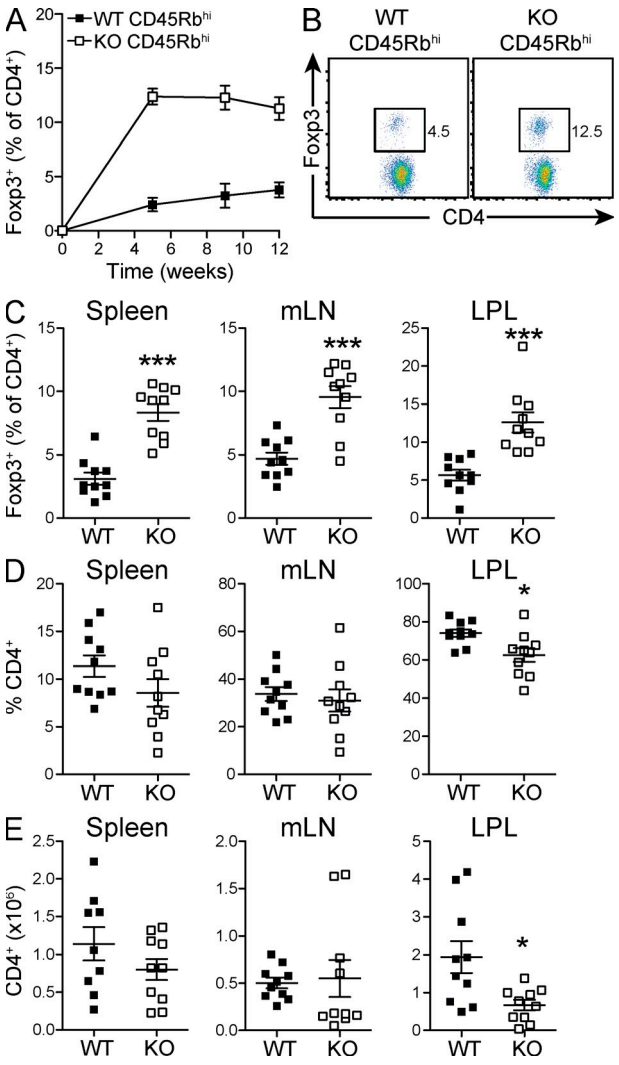

Figure 2. *Il27ra*$^{-/-}$ CD45Rb^{hi} cells preferentially assume a Foxp3$^+$ phenotype. (A) Time course of the percentage of Foxp3$^+$ cells relative to CD4$^+$ cells in peripheral blood of mice transferred with CD45Rb^{hi} cells from WT or *Il27ra*$^{-/-}$ donor mice. (B) Representative Foxp3 staining of splenocytes gated on CD4$^+$ cells at 12 wk after transfer of CD45Rb^{hi} cells. (C and D) Frequencies of Foxp3$^+$ CD4$^+$ T cells (C) and total CD4$^+$ cells (D) obtained from the spleen, mLN, and lamina propria at 12 wk after transfer. (E) Absolute number of CD4$^+$ T cells in spleen, mLN, and lamina propria at 12 wk after transfer. Data are representative of three individual experiments. *, $P < 0.05$; ***, $P < 0.001$.

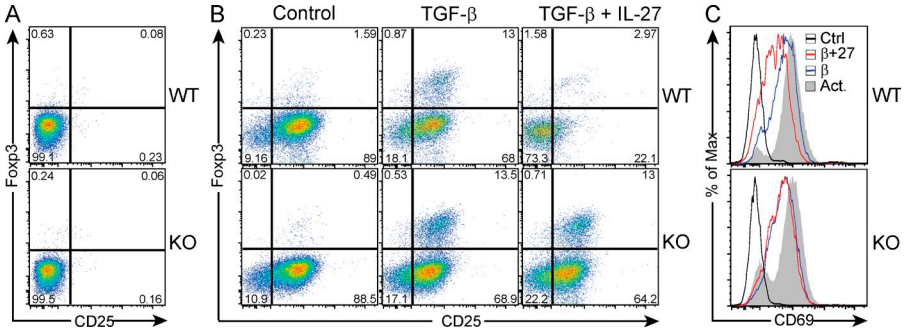

Figure 3. IL-27 inhibits the induction of OVA-specific T regulatory cells in vitro. (A) DO11.10^{+}Rag2$^{-/-}$ CD4^{+} T cells were isolated from WT or *Il27ra*$^{-/-}$ (KO) mice and confirmed to be Foxp3^{-}CD25^{-}. (B and C) DO11.10^{+}CD4^{+} cells from WT or *Il27ra*$^{-/-}$ mice were incubated with CD11c^{+}MHCII^{+} DCs isolated from the colonic lamina propria in the presence of $OVA_{323-339}$ and TGF-β, with or without IL-27, and stained for Foxp3 and CD25 (B) and CD69 (C). Ctrl, Control; β+27, TGF-β + IL-27; β, TGF-β; Act., Activated. Data are representative of two individual experiments.

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 T_{reg} development, indicating that IL-27 limits T_{reg} 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 T_{reg} conversion rather than expansion of nT_{regs}. Consistent with previous observations (Villarino et al., 2006), *Il27ra*$^{-/-}$ DO11.10 cells produced more IL-2 (Fig. 4 D),

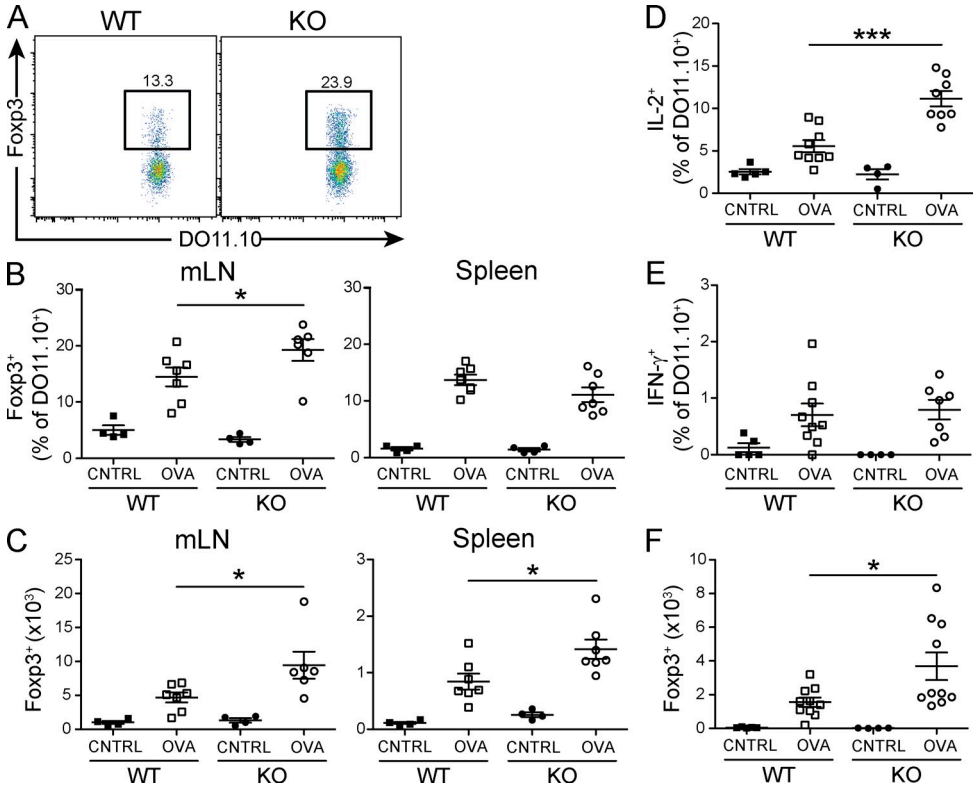

Figure 4. *Il27ra*$^{-/-}$ DO11.10^{+} T cells convert more readily to T regulatory cells in vivo. (A) Representative Foxp3 staining of WT or *Il27ra*$^{-/-}$ (KO) DO11.10^{+} cells after transfer to *balb/c* mice and oral administration of 1.5% OVA in water for 5 d. (B and C) Quantitative analysis of percentage (B) and absolute numbers (C) of Foxp3^{+} cells in the mLNs and spleens of mice receiving WT or *Il27ra*$^{-/-}$ DO11.10^{+} T cells and fed 1.5% OVA or control water. (D-E) Intracellular cytokine staining analysis of IL-2 (D) and IFN-γ (E) in the mLNs from OVA-fed or control mice. (F) Absolute numbers of Foxp3^{+} DO11.10^{+} cells in the mLN of control or OVA-treated mice that had received 3×10^{5} CD45Rb^{hi} cells 4 wk earlier. Data are from a single experiment representing two individual experiments. *, $P < 0.05$; ***, $P < 0.001$.

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 T_{reg} 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 T_{reg} conversion when we repeated this experiment under inflammatory conditions in mice that had received WT CD45Rb^{hi} 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*$^{-/-}$ CD45Rb^{hi} 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*$^{-/-}$ CD45Rb^{hi} 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 CD45Rb^{hi} 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*$^{-/-}$ CD45Rb^{hi} 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*$^{-/-}$ CD45Rb^{hi} 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$^{-/-}$ CD45Rb^{hi} 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$^{-/-}$ CD45Rb^{hi} 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 T_{reg} 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*$^{-/-}$ CD45Rb^{hi} 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

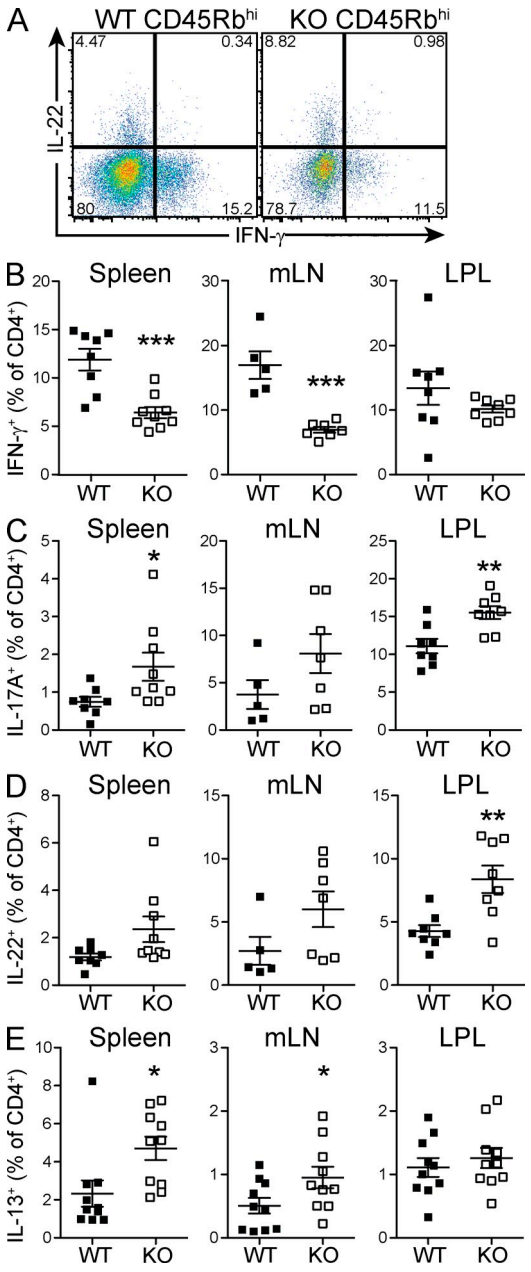

Figure 5. Reduced TH1 and enhanced TH17 polarization after transfer of *Il27ra*$^{-/-}$ CD45Rb^{hi} cells. (A) Representative IFN-γ and IL-22 intracellular cytokine staining of lamina propria isolates from CB17-SCID mice injected with WT or *Il27ra*$^{-/-}$ (KO) CD45Rb^{hi} CD4^{+} T cells stimulated with PMA, ionomycin, and brefeldin A. Samples are gated on CD4^{+} cells. (B–E) Quantitative analysis of intracellular IFN-γ (B), IL-17A (C), IL-22 (D), and IL-13 (E) staining in splenocytes, mLNs, and colonic lamina propria lymphocytes from CB17-SCID mice transferred with WT or *Il27ra*$^{-/-}$ CD45Rb^{hi} cells. Data are representative of three individual experiments. *, P < 0.05; **, P < 0.01; ***, P < 0.001.

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*$^{-/-}$ CD45Rb^{hi} 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*$^{-/-}$ CD45Rb^{hi} 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*$^{-/-}$ CD45Rb^{hi} 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*$^{-/-}$ CD45Rb^{hi} T cells is a consequence of increased T_{reg} 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 proliferate less because there are more Foxp3^{+} cells present, 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 T_{reg} conversion? Several of our data points favor the first of these two possibilities: First, IL-27 is known to antagonize TGF-β driven T_{reg} 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, T_{reg} conversion of IFN-γ$^{-/-}$ T cells was not enhanced, and they still caused significant wasting disease. Therefore, reduced T_{reg} 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 T_{reg} conversion.

We attempted to therapeutically rescue mice with preestablished transfer colitis by introducing *Il27ra*$^{-/-}$ CD45Rb^{hi} 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*$^{-/-}$ CD45Rb^{hi} 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 T_{reg} 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^{-}, IFN-γ^{+}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^{-} CD45Rb^{hi} graft is minimal (Uhlig et al., 2006). We found no *Il27ra*-dependent difference in IL-10 production by transferred CD45Rb^{hi} 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 T_{reg} differentiation has not been noted previously.

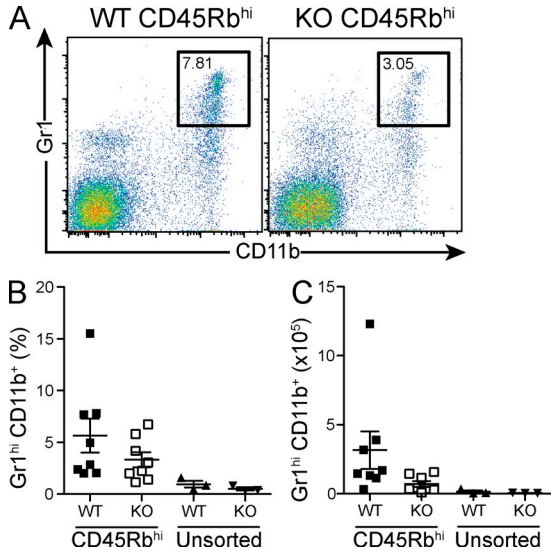

Figure 6. Neutrophil content in the lamina propria of WT and *Il27ra*$^{-/-}$ CD45Rb^{hi} recipient mice. (A) Representative flow cytometry of colonic lamina propria isolates 12 wk after transfer of CD45Rb^{hi} cells. Lamina propria leukocytes were surface stained with anti-Gr1 and anti-CD11b. Quantification of percentage (B) and absolute number (C) of Gr1^{hi}CD11b^{+} cells. Data are from one single experiment.

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 T_{reg} 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 T_{reg}-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 *balb/c* background for 12 generations. This strain was crossed further to the DO11.10^{+}Rag2$^{-/-}$ background (Taconic). CB17-SCID mice were purchased from Charles River. *balb/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 naive CD4^{+}CD45Rb^{hi} T cells. Naive CD4^{+}CD45Rb^{hi} cells were isolated from the spleens of female *Il27ra*$^{+/+}$ or *Il27ra*$^{-/-}$ 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 Biotec). After staining with Pacific blue–conjugated anti-CD4, PE-conjugated anti-CD45Rb, and PECy5-conjugated anti-CD44, CD4^{+}CD44^{lo}CD45Rb^{hi} naive T cells were purified (>98%) by cell sorting (FACSAria; BD). Female CB17-SCID mice were injected with 3×10^5 CD45Rb^{hi} cells or unsorted CD4^{+} cells i.v. Mice were monitored for weight loss and sacrificed by CO_2 asphyxiation 11–12 wk after initiation of the experiment. Blood was obtained by retroorbital bleeds 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–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 (5×10^4 cells/well) with LPDCs (5×10^3 cells/well) in the presence of $OVA_{323-339}$ (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 *Il27ra*$^{-/-}$ DO11.10^{+}Rag2$^{-/-}$ mice by negative MACS selection (Miltenyi Biotec). Female *balb/c* recipient mice (Taconic) were injected with 1.8×10^6 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, Peyer's 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 3×10^5 WT CD45Rb^{hi} cells to initiate colitis and, 4 wk later, were transferred with 2×10^6 CD4^{+}DO11.10^{+} cells from WT or *Il27ra*$^{-/-}$ mice. Mice were either given normal water or 1.5% OVA for 5 d, as indicated.

Online supplemental material. Fig. S1 shows that naive *Il27ra*$^{-/-}$ mice have unaltered levels of Foxp3^{+} T_{regs} and decreased levels of IFN-γ–producing CD4^{+} cells. Fig. S2 shows that *Il27ra*$^{-/-}$ natural T_{reg} have normal suppressive capacity. Fig. S3 shows that *Il27ra*$^{-/-}$ CD45Rb^{hi} cells fail to control previously established colitis. Fig. S4 shows cytokine expression during transfer colitis in recipients of WT or *Il27ra*$^{-/-}$ cells, assessed by qPCR. Fig. S5 details transfer colitis elicited by transfer of IFN-γ$^{-/-}$ CD45Rb^{hi} cells into rag2-deficient recipients. Onlen supplemental material is available at http://www.jem.org/cgi/content/full/jem.20100410/DC1.

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