IFNαR Signaling in Effector but Not Regulatory T Cells Is Required for Immune Dysregulation during Type I IFN–Dependent Inflammatory Disease

Shivani Srivastava, Lisa K. Koch and Daniel J. Campbell

J Immunol 2014; 193:2733-2742; Prepublised online 4 August 2014;
doi: 10.4049/jimmunol.1401039
http://www.jimmunol.org/content/193/6/2733

References

This article cites 43 articles, 23 of which you can access for free at:
http://www.jimmunol.org/content/193/6/2733.full#ref-list-1

Subscription

Information about subscribing to The Journal of Immunology is online at:
http://jimmunol.org/subscription

Permissions

Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts

Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts

The Journal of Immunology is published twice each month by
The American Association of Immunologists, Inc.,
1451 Rockville Pike, Suite 650, Rockville, MD 20852
Copyright © 2014 by The American Association of Immunologists, Inc. All rights reserved.
Print ISSN: 0022-1767 Online ISSN: 1550-6606.
IFNαR Signaling in Effector but Not Regulatory T Cells Is Required for Immune Dysregulation during Type I IFN–Dependent Inflammatory Disease

Shivani Srivastava,*† Lisa K. Koch,*‡ and Daniel J. Campbell*†

Type I IFNs are a family of proinflammatory cytokines that are essential for antiviral immunity but whose overexpression is associated with several autoimmune disorders. In this study, we asked how chronic IFN overexpression regulates the activity of different cell types and how this contributes to immune dysfunction during IFN-associated inflammatory diseases. We show that in mice that chronically overproduce type I IFNs owing to loss of the DNA exonuclease Trex1, inflammatory disease completely depends on IFNαR signaling in T cells. Although IFNs directly inhibited the proliferation and activation of Foxp3+ regulatory T cells, this was neither required nor sufficient for development of inflammatory disease. Rather, chronic IFN expression directly promoted the expansion and activation of effector T cells, and disease development was completely dependent on IFNαR signaling in these cells. Thus, chronic IFN expression can drive inflammatory disease via its direct effects on effector, but not regulatory, T cells.

dendritic cells is required for their maturation and their ability to present Ag and induce CD4+ and CD8+ T cell responses in certain tumor and vaccine models (17, 18). In addition, Tregs from healthy controls showed defective suppression in vitro in the presence of APCs from SLE patients, and this was linked to their production of IFN-α (19).

By contrast, during infection with vaccinia virus, IFNoR signaling is required primarily in NK cells, but not dendritic cells, for efficient viral clearance (20), whereas IFN signaling remained unclear. Using a well-established model of inflammatory response to IFN signaling in different cell types for disease development has completely dependent on IFN expansion of effector T (Teff) cells, and inflammatory disease was caused as follows. The intestinal epithelium was stripped, as previously described (22, 23), and the remaining intestinal pieces were washed three times in CMF solution (HBSS, Ca2+ and Mg2+ free, 100 mM HEPES (Sigma-Aldrich), 250 mM sodium bicarbonate (Fisher Scientific, Pittsburgh, PA), and 2% FBS (HyClone, Logan, UT)). The tissue was then placed into a flask containing CMF with 1 mM DTT and shaken for 20 min at 37°C. The IEL-containing supernatant was removed and transferred into 50-ml centrifuge tubes and pelleted by centrifugation. The cells were resuspended in 44% Percoll (Sigma-Aldrich) and layered onto a 67% Percoll cushion in a 15-ml polycarbonate centrifuge tube. The tubes were centrifuged (2800 rpm) for 20 min at room temperature. The IELs were removed from the 44/67% Percoll interface and washed with RPMI 1640. For LPL isolation, immediately following incubation with DTT, HBSS with 0.01 M EDTA was added to the intestinal tissue pieces and the tissue was incubated an additional 30 min on ice. Intestinal pieces were washed with RPMI 1640 and added to 50 ml RPMI 1640 plus 100 μl 0.5 M MgCl2, 100 μl 0.5 M CaCl2, and 150 μ/ml collagenase (Roche). Samples were stirred at 37°C for 1 h, and the released cells were then filtered through Nitex. Cells isolated from the LP were pelleted, resuspended in 44% RPMI/86% FBS, and layered onto a 44%/67% Percoll cushion in RPMI 1640, layered over 67% Percoll, and spun at 2800 rpm for 20 min. Lymphocytes were isolated from the interface and used for subsequent flow cytometry analyses.

**Histology and colitis scoring**

Colons were immersion fixed in 10% neutral buffered formalin, paraffin embedded, cut into 5-μm sections, and stained with H&E by the Benaroya Research Institute Histology Core. Sections were scored semiquantitatively from 0 to 4 for colitis severity in a blinded fashion (24). A grade of 0 was given when no changes were observed. Changes typically associated with other grades are as follows: grade 1, minimal scattered mucosal inflammatory cell infiltrates, with or without minimal epithelial hyperplasia; grade 2, mild scattered to diffuse inflammatory cell infiltrates, sometimes extending into the submucosa and associated with erosions, with minimal to mild epithelial hyperplasia and minimal to mild mucin depletion from goblet cells; grade 3, mild to moderate inflammatory cell infiltrates that were sometimes transmural, often associated with ulceration, with moderate epithelial hyperplasia and mucin depletion; grade 4, marked inflammatory cell infiltrates that were often transmural and associated with ulceration, with marked epithelial hyperplasia and mucin depletion; and grade 5, marked transmural inflammation with severe ulceration and loss of intestinal glands.

**Enumeration of lymphocytes**

Absolute numbers of lymphocytes in various tissues were determined using Polybead polystyrene nonfluorescent microspheres (15 μm, Polysciences). Briefly, 100 μl of the cell suspension to be counted was mixed with 100 μl of a fixed concentration (C0) of Polybeads (one drop of Polybeads per milliliter of PBS) in a FACS tube. Without washing, the samples were acquired on a FACSCalibur (BD) and were quantified using the appropriate gates. Beads and lymphocytes were identified by their distinct forward- and side-scatter characteristics. The ratio of lymphocyte gate events (nL) to bead gate events (nB) was determined and used to calculate the concentration (C) of the original cell suspension as follows: $C = \frac{n_L}{n_B} \cdot C_0$. Statistics

All data are presented as the mean values ± SEM. Statistical significance was determined by one-way ANOVA with a Tukey posttest, two-tailed unpaired t-test, or linear regression as indicated in the figure legends. Statistical significance was established at the levels of *p* < 0.05, **p** < 0.01, and ***p*** < 0.001.

**Results**

IFNoR signaling in T cells is required for immune dysfunction in Trex1−/− mice

To assess how dysregulated overproduction of type IFNs drives inflammatory disease, we used mice lacking Trex1, a 3′→5′
cytoplasmic DNA exonuclease that is ubiquitously expressed and functions to degrade endogenous retroelements and other cytoplasmic DNA (25, 26). Trex1 is a critical negative regulator of the IFN-stimulatory DNA response, and mutations in Trex1 cause an accumulation of endogenous cytoplasmic DNA that triggers type I IFN production, inflammation, and autoimmunity in mice and humans (25–28). Autoimmunity in Trex1−/− mice is entirely dependent on lymphocyte function and type I IFN expression, as Trex1−/−/Rag2−/− and Trex1−/−/Ifnar1−/− double-deficient animals are completely protected from disease development (26, 29).

Importantly, although Rag2−/−/Trex1−/− mice remain healthy, they display chronically elevated type I IFN production as early as 8 d after birth (29).

We used a well-described T cell transfer model of colitis to determine how type I IFN overproduction in Trex1−/− mice affects different cell types in vivo. In this model, adoptive transfer of CD4+Foxp3− CD45RBα naive T cells (TNaive) into Rag2−/− hosts induces inflammatory colitis and wasting disease that can be prevented by cotransfer of purified CD4+CD25+ Tregs (24, 30, 31). To determine how overproduction of type I IFNs affects immune regulation in this system, we sorted CD4+Foxp3GFP−CD45RBα naive T cells from CD45.1+Foxp3GFP mouse and CD4+CD25+ Tregs from CD45.2+ C57BL/6 [wild-type (WT)] mice and cotransferred them into either Rag2−/−/Trex1−/− or Rag2−/−/Trex1−/− recipients. As expected, Rag2−/−/Trex1−/− animals given WT TNaive cells and WT Tregs remained healthy, gained weight, and showed no new external signs of colitis development. By contrast, Rag2−/−/Trex1−/− recipients exhibited clinical signs of colitis, including hunching, diarrhea, and rectal inflammation, and gained significantly less weight than Rag2−/−/Trex1−/− recipients as early as 4 wk post transfer (Fig. 1A; not shown). Moreover, Rag2−/−/Trex1−/− recipients developed significant colonic inflammation, with higher numbers of IELs and LPLs in the colon, compared with Rag2−/−/Trex1−/− recipients (Fig. 1A). Consistent with this, histological analysis of colons showed leukocytic infiltrate in the LP, depletion of goblet cells, and moderate epithelial cell hyperplasia, as well as disrupted colonic architecture in Rag2−/−/Trex1−/− mice compared with Rag2−/−/Trex1−/− mice (Fig. 1B).

Altogether, these data clearly demonstrate that normal immunoregulation is impaired in Trex1-deficient recipients.

Trex1 deficiency results in the dysregulation of many cellular processes, such as the clearance of extranuclear DNA, activation of the IFN-stimulatory DNA response, and production of type I IFNs. To determine if overproduction of IFNs and its corresponding effects on T cells were responsible for the inflammatory disease that developed in Trex1-deficient mice, we cotransferred sorted CD45.1+ TNaive and CD45.2+ Tregs from Ifnar1−/− mice that lack the ability to signal through the type I IFNR into Rag2−/−/Trex1−/− or Rag2−/−/Trex1−/− mice. Importantly, Ifnar1−/− naive T cells are just as capable as WT naive T cells of inducing colitis in Rag-deficient mice (32). Of interest, neither Rag2−/−/Trex1−/− nor Rag2−/−/Trex1−/− recipients developed colitis, showing comparable weight gain, similar IEL and LPL numbers, and histologically normal colons (Fig. 1C, 1D). Thus, although IFNs are capable of acting on many different innate immune cell types in these recipient animals, their direct effects on the transferred T cells specifically were required for the impaired immune regulation observed during colitis induction in Trex1−/− mice.

**IFNs directly inhibit Tregs and promote Teff cell proliferation and activation**

In our colitis system, type I IFNs could be driving inflammatory disease in Trex1-deficient mice through their effects on Tregs, Teff cells, or both. By cotransferring different combinations of WT and Ifnar1−/− Treg and naive T cells into Rag2−/−/Trex1−/− mice, this system also allows us to determine how IFNs directly influence Teff cell and/or Treg activation and function and how this contributes to the development of inflammatory disease.

We have previously shown that IFNs have direct antiproliferative effects on Tregs during acute viral infection, and this was especially pronounced in the intestinal mucosa (8). Consistent with this finding, CD45.2+Ifnar1−/− Tregs transferred into Rag2−/−/Trex1−/− mice consistently showed enhanced proliferation in the IEL and LPL compartments of the colon, compared with transferred CD45.2+ WT Tregs, as measured by the percentage of cells expressing the cell cycle–associated nuclear Ag Ki-67 (Fig. 2A). This was true in the presence of either WT or Ifnar1−/− Teff cells, indicating that IFNs directly inhibit Treg proliferation in the mucosa. Moreover, Ifnar1−/− Tregs also displayed a more activated phenotype than did WT Tregs in the IEL and LPL compartments, with a greater proportion expressing the chemokine receptor CXCR3 and elevated expression of the activation marker CD44 (Fig. 2B, 2C). By contrast, Ifnar1−/− Tregs expressed lower levels of CD69 than did WT Tregs in the gut, consistent with the ability of type I IFNs to induce CD69 expression in T cells (33, 34). Thus, similar to what we observed during acute viral infection, chronic IFN expression in Trex1−/− mice appears to directly inhibit Treg proliferation and activation.

In contrast to their inhibitory effects on Tregs, IFNs can deliver prosurvival signals to Ag-activated CD4+ and CD8+ Teff cells, and are actually required for the expansion of virus-specific T cells during LCMV infection (11, 12). Consistent with this observation, CD45.1+Ifnar1−/− Teff cells transferred into Rag2−/−/Trex1−/− mice proliferated at significantly lower levels than did transferred WT cells in the IEL and, to a lesser degree, in the LPL compartments (Fig. 3A). Moreover, CD45.1+Ifnar1−/− Teff cells appeared significantly less activated than CD45.1+ WT Teff cells by several measures: although CXCR3 expression did not differ significantly between WT and Ifnar1−/− Teff cells, Ifnar1−/− Teff cells expressed significantly lower levels of the activation marker CD44 and produced significantly less IFN-γ upon restimulation with PMA and ionomycin (Fig. 3C, 3D). This decline in proliferation and activation among Ifnar1−/− Teff cells was evident regardless of the presence of either WT or Ifnar1−/− Tregs. No differences in the frequency of IL-17A− or IL-10−producing Teff cells were observed between WT and Ifnar1−/− Teff cells (data not shown). However, because our donor naive T cell and Treg populations were congenically marked, we were able to determine if any CD4+Foxp3GFP− naive T cells transferred into Rag2−/−/Trex1−/− recipients upregulated expression of Foxp3 and differentiated into peripheral Tregs (pTregs). Of note, the number of CD4+Foxp3+ Tregs arising from donor CD45.1+Ifnar1−/− naive T cells was significantly higher than those arising from CD45.1+ WT naive T cells, particularly in the IEL compartment (Fig. 3E).

Altogether, these data indicate that type I IFN signaling directly promotes Teff cell proliferation and proinflammatory activation, while inhibiting the generation of anti-inflammatory pTregs.

**Ifnar signaling in effector, but not regulatory, T cells is required for colitis development in Trex1−/− recipients**

Given that IFNs both inhibited Tregs and activated Teff cells, we next asked whether IFNs’ effects on Tregs and/or naive T cells was responsible for the loss of immunoregulation observed during colitis development in Trex1-deficient recipients. To test this, we assessed disease development in Rag2−/−/Trex1−/− mice given different combinations of WT and Ifnar1−/− Tregs and TNaive cells. Consistent with our previous findings, Rag2−/−/Trex1−/− mice receiving WT TNaive and WT Tregs developed severe colitis,
Ifnar1–/– Rag2–/– group. * significance was determined using the unpaired two-tailed Student t test. Data are representative of two independent experiments with three to four mice per group. *p < 0.05, **p < 0.0001.

whereas those receiving Ifnar1–/– TNaive and Ifnar1–/– Tregs were completely protected from disease, as measured by weight loss, lymphocytic infiltrates in the colon, and histological analysis of colon cross-sections (Fig. 4A, 4D, 4E).

We next asked whether IFNαR signaling in Tregs, specifically, was responsible for the loss of Treg suppressive function in Trex1–/– mice. Surprisingly, all Rag2–/– Trex1–/– mice developed colitis regardless of whether they received WT or Ifnar1–/– Tregs in combination with WT TNaive cells (Fig. 4B), indicating that loss of immunoregulation in Trex1-deficient mice does not depend on direct type I IFN signaling in Tregs. Transfer of WT TNaive cells with either WT or Ifnar1–/– Tregs induced weight loss with similar kinetics and magnitude, with no difference in the numbers of IELs or LPLs (Fig. 4B, 4D). Histological analysis of colon cross-sections displayed similar levels of epithelial cell hyperplasia and disruption of crypt architecture in Rag2–/– Trex1–/– mice that received WT or Ifnar1–/– Tregs. However, there was slightly more severe goblet cell depletion, leukocytic infiltrate, and ulceration in mice that received WT Tregs compared with those that received Ifnar1–/– Tregs, resulting in colitis scores that trended higher in mice that received WT Tregs, although this did not reach statistical significance. Thus, despite the ability of type I IFNs to directly inhibit Treg activation and function in vivo, WT and Ifnar1–/– Tregs do not differ significantly in their ability to suppress colon inflammation in Trex1-deficient mice.

Because direct inhibition of Tregs by type I IFNs was not necessary for the failed immunoregulation observed in Trex1–/– recipient mice, we next determined how type I IFN signaling in Teff cells contributes to disease development. For this, we transferred either WT or Ifnar1–/– TNaive cells, together with WT Tregs, into Rag2–/– Trex1–/– mice and monitored disease. Whereas recipients of WT naive T cells developed colitis, as ex-
In addition, recipients of wasting disease, showing no significant weight loss (Fig. 4C, 4D).

The effects of type I IFNs are incredibly complex and vary based on a number of contextual factors, such as the cell type acted on, its activation status, and the timing and extent of IFN expression. Although inflammatory disease in Trex1-deficient mice is known to depend on IFNαR signaling on hematopoietic cells (29), it has been unclear what cell type is the major mediator of inflammatory disease. IFNs are known to have proinflammatory effects on NK cells, macrophages, and dendritic cells, in addition to their effects on T cells, and the importance of each of each of these effects varies considerably in different inflammatory contexts. In this study, we found that IFNαR signaling in T cells, and not innate immune cells, was the major driver of disease in a model of chronic inflammatory bowel disease in Trex1-deficient mice. That is, in the presence of Ifnar1−/− Tregs and Ifnar1−/− T cells, IFN-responsive myeloid and NK cells were not sufficient to drive colitis in Trex1-deficient mice, as these mice were completely protected from disease. Although IFNs directly activated T effector cells and inhibited Tregs, only their effects on T effector cells were required for colitis development, we examined the absolute numbers of Tregs and T effector cells in Rag2−/−/Trex1−/− mice that received different combinations of WT and Ifnar1−/− naive T cells and Tregs. Consistent with their higher levels of proliferation (Fig. 3A), WT T effector cells were present at ~10-fold higher levels in the IEL and LPL compartments of the colon, compared with Ifnar1−/− T effector cells (Fig. 5A). Of interest, WT T effector cells were also increased in number in the mesenteric lymph node, suggesting that Ifnar1−/− T effector cells are impaired in their priming and expansion in lymphoid tissues. By contrast, despite their lower levels of proliferation and activation (Fig. 2), WT Tregs were not significantly reduced in number compared with Ifnar1−/− Tregs in either the mesenteric lymph node or the colonic IEL or LPL compartments (Fig. 5B). Accordingly, the ratio of Tregs to T effector cells in the IEL and LPL compartments of the colon was significantly lower in recipients of WT naive T cells than in recipients of Ifnar1−/− naive T cells, owing primarily to the numbers of T effector cells in these mice (Fig. 5C). The ratio of Treg to T effector cells correlated significantly with percent weight loss (Fig. 5D), with the lowest Treg/T effector cell ratios associated with the most severe weight loss, suggesting that the ability of type I IFNs to promote T effector cell population expansion is the primary factor contributing to disease development in recipients of WT cells.

## Discussion

The effects of type I IFNs are incredibly complex and vary based on a number of contextual factors, such as the cell type acted on, its activation status, and the timing and extent of IFN expression. Although inflammatory disease in Trex1-deficient mice is known to depend on IFNαR signaling on hematopoietic cells (29), it has been unclear what cell type is the major mediator of inflammatory disease. IFNs are known to have proinflammatory effects on NK cells, macrophages, and dendritic cells, in addition to their effects on T cells, and the importance of each of each of these effects varies considerably in different inflammatory contexts. In this study, we found that IFNαR signaling in T cells, and not innate immune cells, was the major driver of disease in a model of chronic inflammatory bowel disease in Trex1-deficient mice. That is, in the presence of Ifnar1−/− Tregs and Ifnar1−/− T effector cells, IFN-responsive myeloid and NK cells were not sufficient to drive colitis in Trex1-deficient mice, as these mice were completely protected from disease. Although IFNs directly activated T effector cells and inhibited Tregs, only their effects on T effector cells were required for the onset of inflammatory disease.

Previous studies have provided conflicting results regarding the impact of type I IFNs on Tregs and generally have not used ex-
Experimental systems that directly examined the effects of IFNs on Treg homeostasis and function (35–39). Recently, we showed that type I IFNs can directly inhibit Treg activation and proliferation both in vitro and in vivo during acute viral infection, and that this transient inhibition is necessary for the generation of optimal antiviral T cell responses (8). Similar to what we observed during acute viral infection, we demonstrated that type I IFNs directly promote Teff cell proliferation and activation in Trex1−/− mice. (A) Summary of Ki-67 expression by CD45.1+CD4+Foxp3− Teff cells in the IEL (left) and LPL (right) in the colons of Rag2−/−Trex1−/− mice receiving the indicated combinations of CD45.2+ WT or Ifnar1−/− (KO, knockout) Treg and CD45.1+ T naive cells. (D) Summary of IFN-γ expression by CD45.1+CD4+Foxp3− Teff cells in the small intestinal LP of Rag2−/−Trex1−/− mice receiving the indicated combinations of CD45.2+ WT or Ifnar1−/− KO Tregs and CD45.1+ T naive cells. IFN-γ expression was determined by intracellular cytokine staining after 5-h stimulation of small intestine LPLs (SI-LPL) with PMA and ionomycin. (E) Summary of absolute numbers of CD45.1+CD4+Foxp3gfp− pTregs in the IEL (left) and LPL (right) in the colons of Rag2−/−Trex1−/− mice receiving the indicated combinations of CD45.2+ WT or Ifnar1−/− KO Tregs and CD45.1+ T naive cells. Data are summarized from eight independent experiments with three to four mice per group. Statistical significance was determined using one-way ANOVA with the Tukey posttest. *p < 0.05, **p < 0.005, ***p < 0.0001.
FIGURE 4. IFNαR signaling in Teff cells, but not Tregs, is required for colitis development in Trex1−/− mice. (A–C) Top, Percent weight change in Rag2−/−Trex1−/− mice at various time points after cotransfer of WT Tregs + WT TNaive cells (■) or cotransfer of (A) Ifnar1−/− Tregs + Ifnar1−/− TNaive cells (○); (B) Ifnar1−/− Tregs + WT TNaive cells (□); or (C) WT Tregs + Ifnar1−/− TNaive cells (○). Bottom, Absolute number of IELs and LPLs in the colons of the indicated Rag2−/−Trex1−/− mice at time of sacrifice. Data are representative of two to three independent experiments with three to four mice per group. (D) Summary of the final percent weight change at time of sacrifice in Rag2−/−Trex1−/− mice receiving the indicated WT or Ifnar1−/− (KO, knockout) Tregs and TNaive cells. (E) Summary of colitis scores based on histological analysis of colon cross-sections from Rag2−/−Trex1−/− mice receiving the indicated WT or Ifnar1−/− KO Tregs and TNaive cells. (F) Representative H&E staining of cross-sections of intermediate to distal colon from Rag2−/−Trex1−/− recipients of the indicated Tregs and TNaive cells (original magnification ×10). (D and E) Data are (Figure legend continues)
LCMV infection, chronic IFN expression directly inhibited Treg proliferation in the gut, as Ifnar1−/− Tregs consistently proliferated more and exhibited a more activated phenotype than WT Tregs in Trex1-deficient mice. These results are in contrast to those of a recent study that demonstrated a direct role for IFN in the maintenance of Tregs in the mucosa during inflammatory colitis (40). This discrepancy may be due to differences in the mode of IFN induction in the different models used. Whereas Lee et al. (40) treated mice with pegylated IFN-α i.p. for several weeks, in Trex1−/− mice elevated expression of IFN-β initiates in nonhematopoietic cells during embryonic development (26, 29). Differences in the timing and extent of type I IFN expression, the IFN subtypes induced, or the sites of IFN production may all contribute to differences in the ways IFNs modulate Treg activity in these different systems. However, unlike what we observed during acute LCMV infection, the inhibition of Treg proliferation in Trex1-deficient mice by IFNs did not affect Treg numbers in the gut and was not required for the overall immune dysfunction observed in Trex1-deficient mice. Although during acute LCMV infection Ifnar1−/− Tregs were able to suppress antiviral T cell responses better than WT Tregs, in Trex1-deficient mice Ifnar1−/− Tregs were no better at suppressing Teff cell expansion or inflammatory colitis than were WT Tregs. This finding may be due to differences in the way IFNs inhibit Tregs directly in these two models: although IFNs inhibited both proliferation and accumulation of Tregs during LCMV infection, they only inhibited Treg proliferation without affecting their accumulation in the guts or lymphoid tissues of Trex1-deficient mice, suggesting differences in the survival of Tregs in these two models. Treg numbers may not have been dramatically affected by IFNs in Trex1-deficient mice owing to the lymphopenia present in Rag2−/− Trex1−/− mice. That is, the extensive lymphoproliferation of Tregs that occurred upon transfer into lymphopenic Rag2−/− Trex1−/− mice may have obscured any potential anti-proliferative or proapoptotic effects of IFNs on these cells. In addition, IL-2 is a critical factor for Treg survival that inhibits apoptosis via promotion of Bcl2 and Mcl1 activity and may promote Treg survival in Trex1-deficient mice despite the anti-proliferative effects of IFNs (41, 42). Consistent with this, as IL-2 signaling is known to promote expression of the high-affinity IL-2R chain CD25, Tregs transferred into Rag2−/− Trex1−/− mice had elevated levels of surface CD25 expression compared with those transferred into Rag2−/− Trex1+/+ mice, suggesting they received more IL-2 signaling in Trex1-deficient mice (data not shown). Finally, although Trex1-deficient mice receiving Ifnar1−/− naïve T cells with WT Tregs were protected significantly from colitis, there was still evidence of epithelial cell hyperplasia in their colons that was not present in the colons of mice receiving Ifnar1−/− naïve T cells with Ifnar1+/− Tregs. Likewise, Trex1-deficient mice receiving Ifnar1−/− Tregs with WT naïve T cells exhibited slightly, although not significantly, lower colitis scores and weight loss than did mice receiving WT Tregs with WT naïve T cells. Altogether, these data suggest that although Ifnar1−/− Tregs are unable to completely suppress inflammatory disease in Trex1-deficient mice, they may still protect from some aspects of inflammatory colitis better than WT Tregs.

Of note, despite the effects of IFNs on Tregs, immune dysfunction in Trex1-deficient recipient mice seemed primarily due to the direct effects of IFNs on Teff cells. WT effector cells were significantly more proinflammatory than Ifnar1−/− cells, proliferating at higher levels and producing more IFN-γ, which is a major cytokine involved in the pathogenesis of this colitis model (43). By contrast, we found no difference in IL-17A or IL-10 expression between WT and Ifnar1−/− Teff cells (data not shown), suggesting that IFNs specifically promote Th1 proinflammatory function. Of interest, transferred WT cells were also less likely to differentiate into pTregs than Ifnar1−/− cells. Thus, although the tolerogenic environment of the gut is known to support pTreg development, IFNs likely promote intestinal inflammation in this model by supporting Th1 differentiation at the expense of pTreg development. In addition to being more activated, accumulation of WT Teff cells in the guts of Trex1-deficient mice was significantly higher than that of Ifnar1−/− Teff cells. This may be due to defects in the priming and expansion of Ifnar1−/− T cells in secondary lymphoid tissues, as Ifnar1−/− Teff cells were also present at significantly lower numbers in the mesenteric lymph nodes. The number of Teff cells in the intestine correlated with disease severity, as measured by percent weight loss, suggesting that IFNs’ ability to promote Teff cell proliferation and accumulation is a major contributor to the severity of inflammatory disease that develops in Trex1-deficient mice.

The heightened proinflammatory function of WT Teff cells compared with Ifnar1−/− cells may also be due in part to their resistance to Treg-mediated suppression. The resistance of Teff cells to Treg-mediated suppression has been described both in patients and in mouse models of many autoimmune disorders, including type I diabetes, multiple sclerosis, and SLE. This resistance often occurs as a result of proinflammatory cytokine signaling in Teff cells. For example, Tregs are able to migrate to the CNS during experimental autoimmune encephalomyelitis, a mouse model of multiple sclerosis, but are unable to suppress Teff cells during active disease owing to the production of IL-6 and TNF-α, both of which have been implicated in driving Teff cell activation and resistance to suppression (44). A recent study also demonstrated that IL-1/MyD88 signaling in Teff cells is required to overcome suppression by Tregs (45). Thus, despite the presence of normal numbers of Tregs, factors in the inflammatory milieu may still circumvent Treg-mediated suppression and drive inflammatory disease. Activation of Teff cells by IFNs in Trex1-deficient mice, therefore, may render them resistant to suppression by Tregs. The longer duration of IFN signaling in Trex1-deficient mice compared with that during acute LCMV infection may also make Teff cells more resistant to suppression, even by Ifnar1−/− Tregs.

Although important for immunity to viral infections, type I IFNs are strongly linked to the development of certain autoimmune diseases, such as SLE, and there have been several reports of autoimmune and inflammatory diseases developing in patients receiving type I IFN therapeutically. Our work demonstrating that Teff cells are the major IFN-responsive cell type mediating inflammatory disease in Trex1-deficient mice has important implications for treatment of SLE and other type I IFN–associated autoimmune diseases. Targeted blockade of IFNαRs signaling in Teff cells, for example, may be an attractive therapy for IFN-associated autoimmune diseases, as it disrupts a central proinflammatory axis while avoiding off-target effects.
on other cell types. Moreover, considering that administration of type I IFNs is a commonly prescribed treatment for chronic infection, cancer, and even multiple sclerosis (46), it will be crucial to understand how type I IFNs regulate different cell types—both directly and indirectly—in these different contexts, to tailor the effectiveness of this treatment option.

FIGURE 5. Type I IFNs directly promote Teff cell expansion in Trex1−/− mice. (A and B) Summary of the absolute number of CD45.1+CD4+Foxp3− Teff cells (Teff) (A) and CD45.2+CD4+Foxp3+ Tregs (B) in the mesenteric lymph node (MLN) (right) and IEL (left) and LPL (middle) of the colons of Rag2−/−Trex1−/− mice receiving the indicated combinations of CD45.2+ WT or Ifnar1−/− (KO, knockout) Tregs and CD45.1+ TNaive cells. (C) Summary of the ratio of Tregs to Teff cells in the MLN (right) and IEL (left) and LPL (middle) of the colons of Rag2−/−Trex1−/− KO Tregs and CD45.1+ TNaive cells. (D) Correlation plot comparing the Treg/Teff ratio in the IEL of the colon with the percent weight change at time of sacrifice in Rag2−/−Trex1−/− mice receiving the indicated combinations of WT or Ifnar1−/− KO Tregs and TNaive cells. Data are summarized from eight independent experiments with three to four mice per group. Statistical significance was determined using one-way ANOVA with the Tukey posttest (A–C) or the linear regression slope test (D). *p < 0.05, **p < 0.005, ***p < 0.0001.
Acknowledgments

We thank K. Arumugamathan for assistance in flow cytometry and cell sorting, Pamela Johnson and Mary Beauchamp for assistance with histology, and Sylvia McCoy for administrative assistance.

Disclosures

The authors have no financial conflicts of interest.

References