TSLP regulates intestinal immunity and inflammation in mouse models of helminth infection and colitis

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Intestinal epithelial cells (IECs) produce thymic stromal lymphopoietin (TSLP); however, the in vivo influence of TSLP–TSLP receptor (TSLPR) interactions on immunity and inflammation in the intestine remains unclear. We show that TSLP–TSLPR interactions are critical for immunity to the intestinal pathogen *Trichuris*. Monoclonal antibody–mediated neutralization of TSLP or deletion of the TSLPR in normally resistant mice resulted in defective expression of Th2 cytokines and persistent infection. Susceptibility was accompanied by elevated expression of interleukin (IL) 12/23p40, interferon (IFN) γ, and IL-17A, and development of severe intestinal inflammation. Critically, neutralization of IFN-γ in *Trichuris*-infected TSLPR$^{-/-}$ mice restored Th2 cytokine responses and resulted in worm expulsion, providing the first demonstration of TSLPR-independent pathways for Th2 cytokine production. Additionally, TSLPR$^{-/-}$ mice displayed elevated production of IL-12/23p40 and IFN-γ, and developed heightened intestinal inflammation upon exposure to dextran sodium sulfate, demonstrating a previously unrecognized immunoregulatory role for TSLP in a mouse model of inflammatory bowel disease.

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Abbreviations used: BMDC, bone marrow–derived DC; DSS, dextran sodium sulfate; ES, excretory–secretory; H&E, hematoxylin and eosin; IBD, inflammatory bowel disease; IEC, intestinal epithelial cell; IEC-Sup, supernatant(s) from the IEC line CMT-93; IF, immunofluorescent; GI, gastrointestinal; MLN, mesenteric LN; PAS, periodic acid-Schiff; TSLP, thymic stromal lymphopoietin.

Intestinal epithelial cells (IECs) are a critical cell population that maintains intestinal immune homeostasis through both barrier function and the ability to actively modulate intestinal immune responses (1–3). One IEC-derived cytokine with immunomodulatory properties is thymic stromal lymphopoietin (TSLP) (4). TSLP is a four-helix bundle cytokine that is expressed both in humans and mice. Despite poor sequence homology, human and mouse TSLP exhibit similar biological functions (4). Expression of TSLP is regulated by NF-κB and can be induced by exposure to viral, bacterial, and parasitic pathogens, inflammatory cytokines, and the Th2 cell–associated cytokines IL-4 and IL-13 (3, 5–8). TSLP binds to its high affinity receptor, a heterodimer composed of a unique TSLPRα chain and the IL-7Rα chain, that is expressed on hematopoietic cell lineages, including B cells, T cells, mast cells, and DCs (4, 5, 9–12).

In vitro studies demonstrated that TSLP-conditioned human DCs can promote Th2 cell responses (11, 13–15). Mechanistically, TSLP treatment of DCs induces Th2 cell differentiation by inhibiting IL-12 production while simultaneously inducing OX40L expression (14–16). The in vivo functions of TSLP have been most extensively studied in the skin and the lung (11, 13, 17, 18). Transgenic overexpression of TSLP in cutaneous or pulmonary epithelial cells results in the onset of Th2 cytokine–mediated inflammation resembling atopic dermatitis or asthma, respectively (17, 18). Based on these studies, it has been proposed that TSLP is both necessary and sufficient for the initiation of Th2 cytokine–driven inflammation (4, 19, 20). We recently showed that TSLP responsiveness is an important component of early immunity to the intestinal nematode pathogen *Trichuris* (2). However, the mechanisms and absolute requirements for TSLP–TSLPR interactions in the regulation of intestinal immunity and inflammation in vivo remain undefined.

In this study, we identify constitutive TSLP expression in IECs throughout the lower gastro-

The Rockefeller University Press $30.00
J. Exp. Med. Vol. 206 No. 3 655-667
www.jem.org/cgi/doi/10.1084/jem.20081499

intestinal (GI) tract, with the highest level of expression in the proximal large intestine. When challenged with *Trichuris*, genetically resistant WT mice in which TSLP was neutralized or TSLPR$^{-/-}$ mice failed to express protective Th2 cell–associated cytokines and maintained persistent parasites beyond day 34 after infection. Disruption of the TSLP–TSLPR pathway additionally resulted in increased expression of IL-12/23p40, IFN-γ, and IL-17A, and the development of severe infection-induced inflammation. Recombinant TSLP inhibited production of IL-12/23p40 in DCs in vitro, and DCs isolated from infected TSLPR$^{-/-}$ mice exhibited dysregulated production of IL-12/23p40 ex vivo. Significantly, blockade of IFN-γ in *Trichuris*-infected TSLPR$^{-/-}$ mice restored expression of Th2 cytokines and host protective immunity. Additionally, TSLPR$^{-/-}$ mice exhibited elevated expression of proinflammatory cytokines and early onset of intestinal inflammation in a mouse model of inflammatory bowel disease (IBD). Collectively, these data suggest that within the intestinal microenvironment, one function of TSLP–TSLPR interactions may be to limit proinflammatory cytokine production and inflammation.

RESULTS

Constitutive expression of TSLP in IECs

The GI tract is composed of region-specific microenvironments defined by the presence of distinct accessory cell and lymphocyte populations, and varied populations of commensal bacteria (21–23). The large intestine differs from the small intestine in immunoregulatory pressures, as it carries a higher level of commensal bacteria and is a common site for intestinal inflammatory diseases like ulcerative colitis (24). We sought to examine whether basal expression of TSLP differed in these distinct microenvironments. There was low expression of TSLP in the small intestine of naive mice (Fig. 1 A). TSLP mRNA expression was also detected throughout the large intestine, with the highest expression levels in the proximal large intestine (Fig. 1 A). TSLP protein expression was examined by immunofluorescent (IF) staining of cryosections of the cecum and large intestine. In the cecum, TSLP was found predominately in mature enterocytes located at the crypt table, whereas in the proximal large intestine TSLP was expressed throughout the length of the crypt (Fig. 1 B). The variable expression of TSLP throughout the length of the GI tract suggests differential regulation depending on the microenvironment. As TSLP expression can be induced through exposure to TLR ligands (3, 5, 6, 15) and commensal bacteria have been demonstrated to influence cytokine production in the intestine (25, 26), it is possible that alterations in the abundance or composition of communal bacterial populations in the distinct regions in the GI tract may influence TSLP mRNA expression. Not withstanding this, the high levels of basal expression and the extensive distribution pattern of TSLP in the crypts of the proximal large intestine suggest a possible regulatory function for IEC-derived TSLP in immune homeostasis within the large intestine.

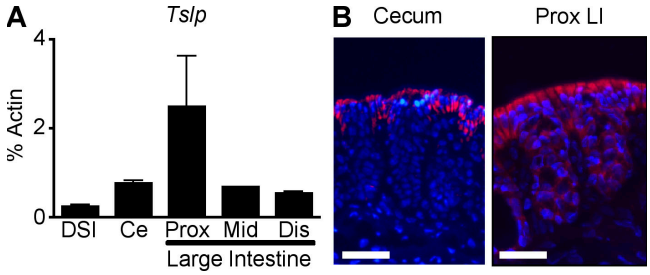

Figure 1. TSLP is basally expressed in the large intestine. (A) RNA was isolated from sections of the intestine and expression of TSLP was analyzed by RT-PCR. Values represent the percentage of actin expression in each sample. Results represent means ± SEM. Ce, cecum; dis, distal; DSI, distal small intestine; prox, proximal. (B) IF staining of TSLP (red) in intestinal cryosections, costained with DAPI (blue). Prox LI, proximal large intestine. Data represent two individual experiments with two to three mice per group. Bars, 50 µm.

In vivo neutralization of TSLP in WT mice results in susceptibility to *Trichuris*

Challenge of genetically resistant mice with the intestinal nematode pathogen *Trichuris* results in the dynamic up-regulation of TSLP mRNA in the large intestine early during infection (8). Further, we recently demonstrated that in the absence of the TSLP receptor, genetically resistant mice exhibit an early defect in the clearance of *Trichuris* (2). However, TSLP has been shown to influence lymphocyte development both in vivo and in vitro (10, 27, 28), and previous work has established that alterations in the circulating levels of TSLP can influence the frequency and composition of B cell populations in vivo (9). To investigate whether the impaired early immunity to infection in the TSLPR$^{-/-}$ mice was caused by alterations in immune cell development or a definitive requirement for TSLP in protective immunity, we neutralized endogenous TSLP in genetically resistant animals during *Trichuris* infection using a neutralizing anti-TSLP mAb. Although mesenteric LN (MLN) cells isolated from control-treated WT mice at day 21 after infection produced IL-4 and IL-13 after antigen-specific restimulation, MLN cells isolated from anti-TSLP mAb–treated mice exhibited significantly reduced expression of these cytokines (Fig. 2 A). Consistent with a defect in Th2 cell differentiation in vivo, the frequency of IL-13$^+$ CD4$^+$ T cells was lower in MLNs isolated from anti-TSLP mAb–treated mice than in control-treated mice (Fig. 2 B). Expression of Th2 cytokines in the intestine leads to physiological changes in the intestinal epithelium, including increased cell turnover, goblet cell hyperplasia, and the elevated expression of goblet cell–associated genes that are correlated with worm expulsion (29–35). Histological examination of ceca isolated from infected WT animals revealed goblet cell hyperplasia and increased mucin staining, consistent with the presence of Th2 cytokines (Fig. 2 C). In contrast, mucin staining of cecal tissue sections from anti-TSLP mAb–treated mice failed to show detectable goblet cell responses (Fig. 2 C). Expression of the goblet cell–specific proteins RELMβ and GOB5 were also

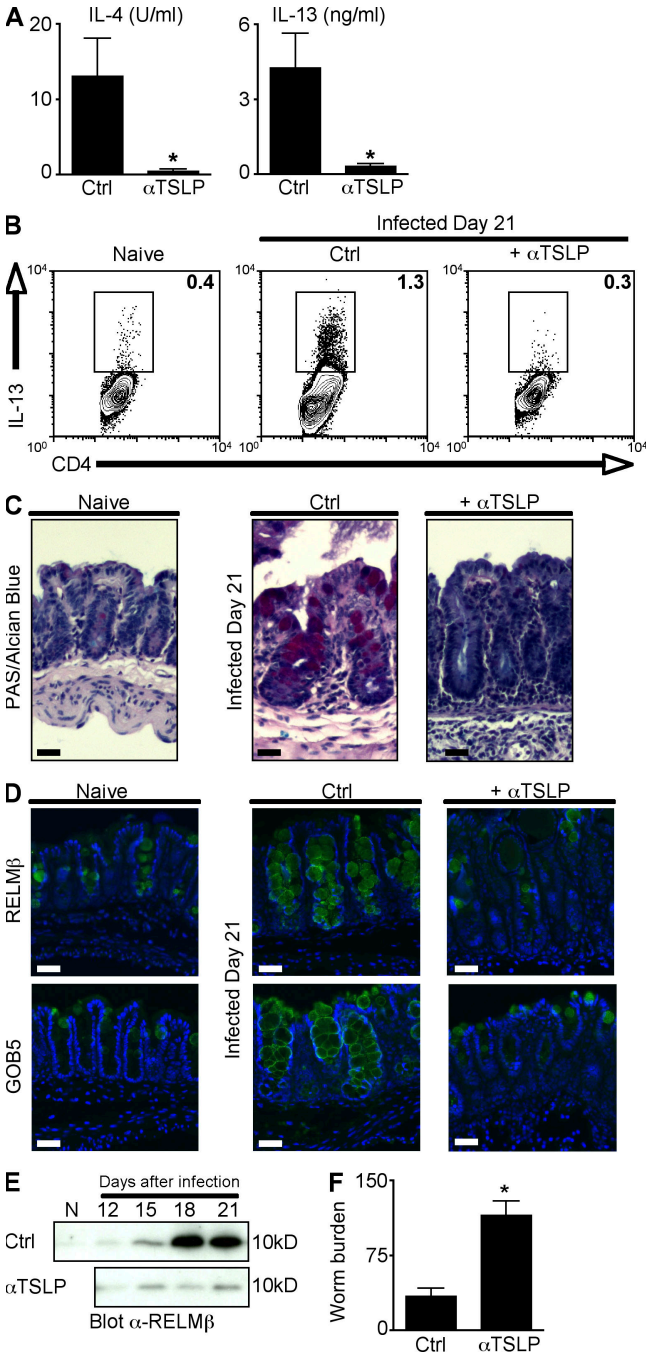

Figure 2. In vivo neutralization of TSLP in WT mice results in susceptibility to *Trichuris*. (A) Antigen-specific cytokine production from restimulated MLN cells was determined by ELISA. (B) Frequencies of $CD4^+$ $IL\text{-}13^+$ T cells in the MLNs after 72 h of antigen restimulation (percentages are shown). Plots are gated on $CD4^+$ T cells. (C–E) Anti-TSLP–treated mice fail to induce goblet cell responses after infection. (C) Paraffin sections of cecal tissue were stained with PAS/Alcian blue for goblet cell visualization. (D) Paraffin sections of cecal tissue were IF stained for RELMβ and GOB5 (green) and costained with DAPI (blue). (E) Protein isolated from fecal pellets on various days after infection was analyzed by SDS-PAGE and immunoblotted for RELMβ. N, naive, uninfected control. (F) Worm burdens were assessed at day 21 after infection. Results represent means ± SEM. Data represent three individual experiments with three to six mice per group. *, $P \leq 0.05$. Bars, 50 µm.

decreased in the anti-TSLP mAb–treated mice (Fig. 2 D). Further, RELMβ secretion, as determined by protein analysis of fecal samples, was also defective in infected mice treated with anti-TSLP mAb (Fig. 2 E). Consistent with these defective Th2 cytokine responses, anti-TSLP mAb–treated mice failed to exhibit worm expulsion at day 21 after infection (Fig. 2 F). These results identify that optimal expression of TSLP is critical for the development of pathogen-specific Th2 cytokine responses and early immunity to *Trichuris*.

TSLP–TSLPR interactions are critical for immunity to *Trichuris*

Deletion of TSLPR in normally resistant mice resulted in increased IFN-γ production and elevated worm burdens at day 21 after infection (2). In genetically resistant strains of mice, $CD4^+$ Th2 cell–mediated expulsion of *Trichuris* occurs between days 18–21, whereas genetically susceptible mice develop persistent infection and retain parasites for the lifetime of the host (36). However, impaired early worm expulsion is not always indicative of a failed host protective response. For example, *Trichuris*-infected mice deficient in inducible T cell co-stimulator fail to expel worms at day 18 after infection but are able to successfully mount a protective Th2 cytokine–mediated response and clear infection by day 34 after infection (32). Thus TSLP–TSLPR interactions could be required for optimal early Th2 cytokine responses but not for subsequent Th2 cell–mediated clearance of infection.

To determine whether blockade of the TSLP–TSLPR pathway resulted in persistent chronic infection or simply resulted in delayed worm expulsion, $TSLPR^{+/+}$ and $TSLPR^{-/-}$ mice were infected with *Trichuris*, and the kinetics of worm expulsion were monitored over 34 d. MLN cells isolated from infected $TSLPR^{-/-}$ mice failed to produce detectable levels of Th2 cytokines (Fig. 3 A). Histological examination of cecal sections also revealed defective Th2 cytokine–dependent goblet cell hyperplasia in infected $TSLPR^{-/-}$ mice in comparison to infected $TSLPR^{+/+}$ mice (Fig. 3 B). Further, production of goblet cell–derived RELMβ and GOB5 was decreased in infected $TSLPR^{-/-}$ mice compared with infected $TSLPR^{+/+}$ mice (Fig. 3 C). Luminal secretion of RELMβ was also decreased and unsustained in infected $TSLPR^{-/-}$ mice (Fig. 3 D). The defective Th2 cytokine responses functionally altered the outcome of infection, as $TSLPR^{-/-}$ mice failed to mediate worm expulsion even at a late time point and remained heavily infected beyond day 34 after infection (Fig. 3 E). Consistent with this, chronic infection was also observed in WT mice treated with anti-TSLP mAb, as they exhibited high worm burdens at day 34 after infection (Fig. S1, available at http://www.jem.org/cgi/content/full/jem.20081499/DC1). Collectively, neutralization of TSLP and deletion of the TSLPR identify a critical role for TSLP–TSLPR interactions in the

development of a host protective Th2 cytokine–dependent immune response to *Trichuris*.

Proinflammatory cytokine production in the gut-associated lymphoid tissues is increased in the absence of TSLP

The defective production of Th2 cytokines and susceptibility to *Trichuris* after the disruption of the TSLP–TSLPR pathway could be the result of impaired responsiveness to infection or dysregulation of Th cell responses. Histological examination of cecal sections taken at day 34 after infection revealed immune-mediated alterations in both WT and TSLPR$^{-/-}$ mice (Fig. 4 A). Cecal sections from WT mice exhibited minimal to mild submucosal edema, mixed inflammatory cell infiltrate, and mild crypt hyperplasia indicative of a recent infection. In contrast, TSLPR$^{-/-}$ mice exhibited severe infection-induced inflammation characterized by severe submucosal edema and transmural inflammation with lymphocytic infiltrate in the muscularis, and mixed lymphocytic and neutrophilic infiltrate in the submucosa and lamina propria (Fig. 4 A). Additionally, IECs in the TSLPR$^{-/-}$ mice appeared activated, and numerous mitotic figures were observed (Fig. 4 B). TSLPR$^{-/-}$ mice also exhibited foci of inflammation with disruption of crypt architecture (Fig. 4 C). The severe infection-induced inflammation exhibited in the TSLPR$^{-/-}$ mice contrasts with the mild to moderate inflammation seen in genetically susceptible AKR mice that also exhibit chronic infection (37–39). Similar pathology to the infected TSLPR$^{-/-}$ mice was also observed in infected anti-TSLP mAb–treated WT mice (Fig. S2, available at http://www.jem.org/cgi/content/full/jem.20081499/DC1).

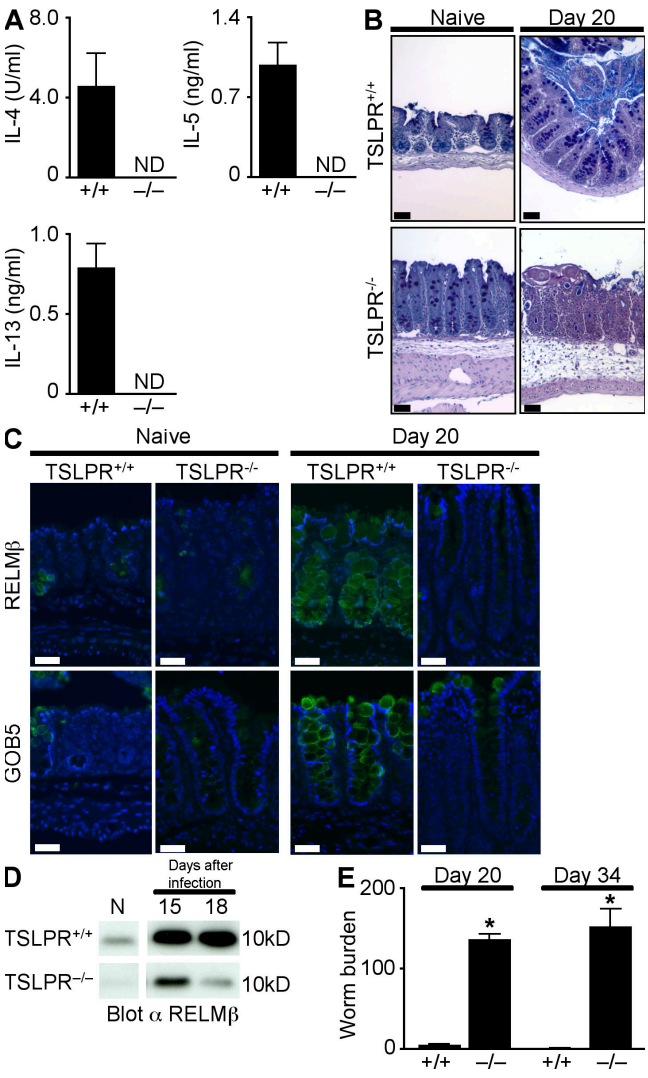

Figure 3. TSLP responsiveness is required for immunity to *Trichuris*. (A) TSLPR$^{-/-}$ mice are unable to induce Th2 cytokine production. Antigen-specific cytokine production from restimulated MLN cells was determined by ELISA. (B–D) TSLPR$^{-/-}$ mice have decreased goblet cell responses. ND, not detected. (B) Cecal sections from TSLPR$^{+/+}$ or TSLPR$^{-/-}$ mice were taken at day 20 after infection and stained with PAS/Alcian blue. (C) Paraffin sections of cecal tissue were IF stained for RELMβ and GOB5 (green) and costained with DAPI (blue). (D) Protein isolated from fecal pellets on various days after infection was analyzed by SDS-PAGE and immunoblotted for RELMβ. N, naive, uninfected control. (E) Worm burdens in TSLPR$^{+/+}$ and TSLPR$^{-/-}$ mice were assessed at days 20 and 34 after infection. Results represent means ± SEM. Data represent three individual experiments with three to four mice per group. *, $P \leq 0.05$. Bars, 50 µm.

The presence of severe intestinal inflammation in the TSLPR$^{-/-}$ mice suggested that susceptibility to *Trichuris* was not solely caused by a lack of Th2 responsiveness but involved a more general dysregulation of infection-induced innate and adaptive immune responses. Consistent with this hypothesis, there was a significant increase in the frequency of CD4$^+$ IFN-γ$^+$ T cells isolated from the MLNs of TSLPR$^{-/-}$ mice at days 21 and 34 after infection compared with WT mice (Fig. 4 D; and Fig. S3, available at http://www.jem.org/cgi/content/full/jem.20081499/DC1). Restimulated MLN cells isolated from infected TSLPR$^{-/-}$ mice exhibited significantly elevated production of IFN-γ and IL-17A in comparison to infected WT mice after either antigen-specific or polyclonal stimulation (Fig. 4, E and F). Elevated levels of IFN-γ and IL-17A were also observed in the *Trichuris*-infected WT anti-TSLP mAb–treated mice at the same time points (Fig. S4). The heightened expression of IFN-γ and IL-17A and the severe intestinal inflammation exhibited upon disruption of the TSLP–TSLPR pathway demonstrates that susceptibility to infection was not solely caused by the impairment of Th2 cytokine responses but also by the development of an unregulated proinflammatory cytokine response.

TSLP regulates DC production of IL-12/23p40 in vivo and in vitro

The development of an inappropriate pathogen-specific CD4$^+$ Th1 cell response upon the disruption of TSLP–TSLPR interactions suggests an alteration in signals that influence Th cell differentiation. Previous in vitro studies demonstrated that TSLP could inhibit IL-12 secretion from human DCs (13–15). Given the critical importance of IL-12 in the promotion of Th1 cell differentiation, we next examined whether there was a dysregulation of DC-derived IL-12/23p40 early in infection. Analysis of CD11c$^+$ CD11b$^+$ DCs isolated from

MLNs of infected TSLPR$^{-/-}$ mice showed increased production of the proinflammatory cytokines TNF-α and IL-12/23p40 at day 10 after infection (Fig. 5, A and B). Additionally, levels of IL-12/23p40 mRNA were increased in the large intestine of TSLPR$^{-/-}$ mice at day 12 after infection (Fig. 5 C). Although we have previously shown dysregulation of DC-derived IL-12/23p40 under homeostatic conditions (2), these data demonstrate an in vivo role for TSLP in the regulation of infection-induced IL-12/23p40 production from intestinal DCs.

To examine the ability of IEC-derived TSLP to modulate DC function, an in vitro conditioning system was used. Bone marrow–derived DCs (BMDCs) were cultured overnight in the presence of supernatants from the IEC line CMT-93 (IEC-Sup), which is known to express TSLP (2). Control and conditioned BMDCs were subsequently stimulated with LPS for 8 h. Exposure to IEC-Sup did not impair the ability of DCs to respond to LPS, as IEC-Sup–conditioned BMDCs exhibited normal up-regulation of surface expression of MHC class II and the co-stimulatory

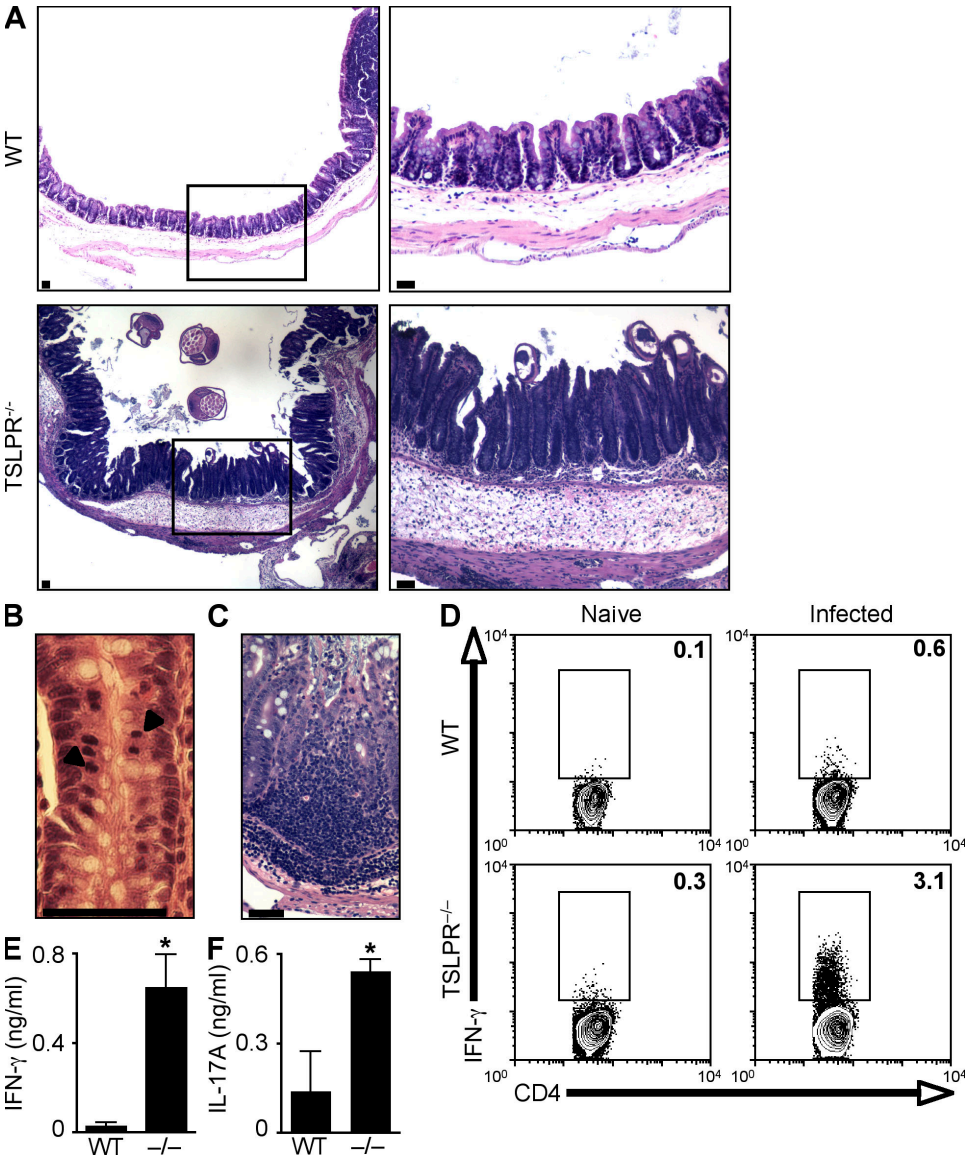

Figure 4. TSLP–TSLR interactions limit proinflammatory cytokine production and infection-induced inflammation. (A–C) TSLPR$^{-/-}$ mice have increased infection-induced inflammation. (A) Paraffin-embedded cecal sections from day 34 after infection were stained with H&E. (B) Epithelial cells in TSLPR$^{-/-}$ mice exhibit numerous mitotic figures (arrowheads). (C) TSLPR$^{-/-}$ mice exhibit foci of inflammation with loss of crypt architecture. (D–F) TSLPR$^{-/-}$ mice have increased proinflammatory cytokine production at day 20 after infection. (D) Frequencies of CD4$^+$ IFN-γ$^+$ T cells in the MLNs at day 20 after infection (percentages are shown). (E) Antigen-specific IFN-γ production from restimulated MLNs was determined by ELISA. (F) Polyclonal IL-17A production from restimulated MLNs was determined by ELISA. Results represent means ± SEM. Data represent two to three individual experiments with three to four mice per group. *, $P \leq 0.05$. Bars, 50 µm.

molecules CD80 and CD86 as compared with unconditioned BMDCs (Fig. 5 D). Further, conditioning with IEC-Sup did not alter or inhibit LPS-induced production of TNF-α (Fig. 5, E and G). However, IEC-Sup–conditioned DCs exhibited reduced production of IL-12/23p40 (Fig. 5, F and H). As IEC-Sup contains multiple IEC-derived factors, we sought to determine the contribution of TSLP in the regulation of DC responses using rTSLP. Similar to the results with IEC-Sup, exposure of BMDCs to rTSLP did not alter their surface marker expression or production of TNF-α after LPS stimulation but did result in decreased IL-12/23p40 production in comparison to control-treated BMDCs (Fig. 5, I–K). These findings demonstrate the ability of TSLP to selectively regulate DC production of IL-12/23p40. Consistent with their inhibition of IL-12/23p40, both IEC-Sup– or rTSLP-conditioned BMDCs induced fewer $CD4^+$ T cells to produce IFN-γ in antigen-specific co-culture assays (Fig. 5 L). These results indicate that TSLP is important in the regulation of DC-derived IL-12/23p40 both in vitro and in vivo.

TSLP is not required to initiate Th2 cytokine responses in the intestine

Previous studies in both skin and lung models of Th2 cytokine–mediated allergic inflammation implicated TSLP as both necessary and sufficient to drive the development of Th2 cytokine–dependent inflammation, suggesting that TSLP may be a "master switch" for the development of Th2 cytokine

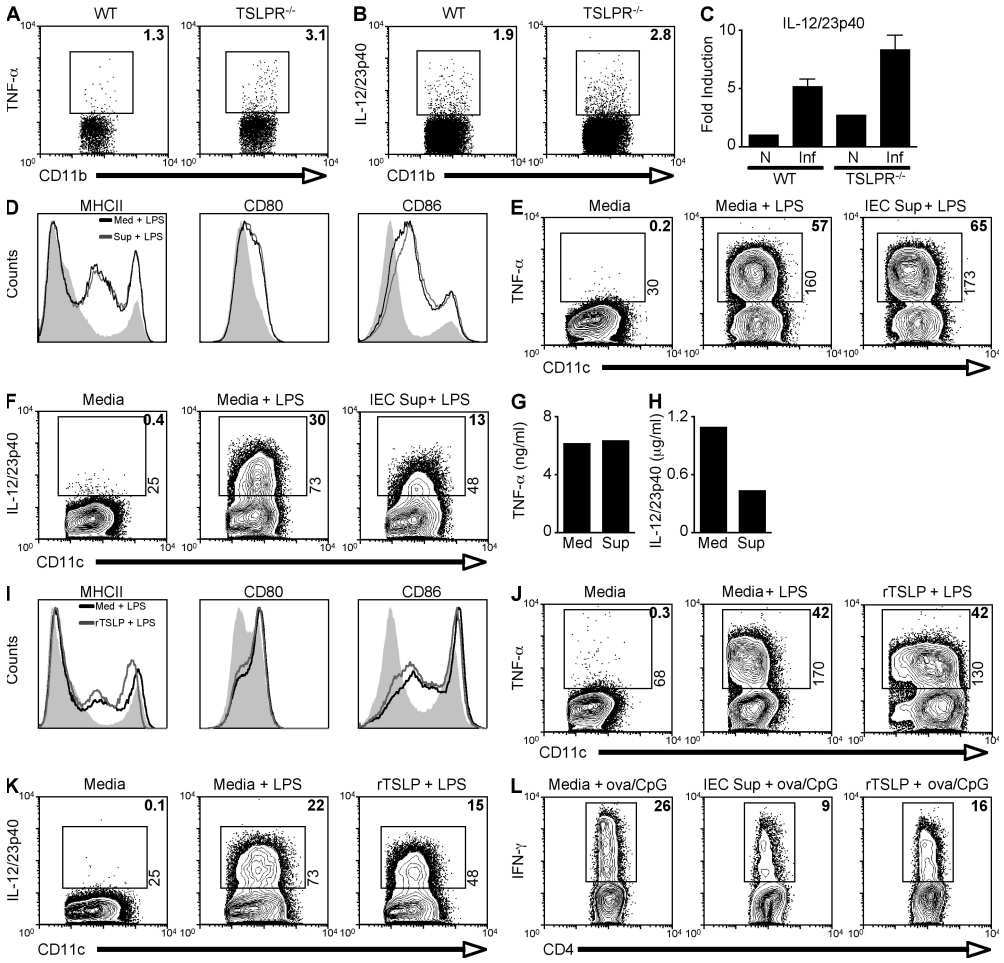

Figure 5. TSLP inhibits DC production of IL-12/23p40 in vivo and in vitro. (A and B) $TSLPR^{-/-}$ mice have increased DC-derived proinflammatory cytokine production at day 10 after infection. Cells were isolated from MLNs and restimulated directly ex vivo with *Trichuris* ES antigen. Cells are gated on $CD11c^+$ $CD11b^+$ populations (A, TNF-α; B, IL-12/23p40). (C) IL-12/23p40 mRNA expression in the colon at day 12 after infection. Results represent means ± SEM. N, naive, uninfected control. Inf, infected day 12. (D–F) IEC-Sup specifically inhibit LPS-induced IL-12/23p40 production. (D) LPS-induced up-regulation of MHC class II, CD80, and CD86. (E and F) BMDC cytokine production was measured using intracellular cytokine staining (E, TNF-α; F, IL-12/23p40). (G and H) Cytokine secretion was assayed by ELISA of the supernatants (G, TNF-α; H, IL-12/23p40). Med, media + LPS; Sup, IEC-Sup + LPS. (I–K) rTSLP specifically inhibits LPS-induced IL-12/23p40 production. (I) LPS-induced up-regulation of MHC class II, CD80, and CD86. (J and K) BMDC cytokine production as measured using intracellular cytokine staining (J, TNF-α; K, IL-12/23p40). (L) IEC and rTSLP conditioning of BMDCs decreased $CD4^+$ T cell production of IFN-γ upon co-culture. Percentages are shown in A, B, E, F, and J–L. Data in A–C represent two individual experiments with three mice per group. Data in D–K represent three to five individual experiments, and data in L represent two individual experiments. Shaded histograms in D and I indicate control, unstimulated BMDCs.

responses (17–19). The impaired immunity to *Trichuris* exhibited after disruption of the TSLP–TSLPR pathway supports this model. However, anti-TSLP mAb treatment or deletion of TSLPR resulted in the exaggerated expression of IL-12/23p40 in ex vivo–isolated DCs and heightened $CD4^+$ T cell–derived IFN-γ production. To test whether TSLP–TSLPR interactions were absolutely required or dispensable for *Trichuris*-induced Th2 cytokine responses, *Trichuris*-infected $TSLPR^{-/-}$ mice were treated with neutralizing mAbs against IFN-γ throughout the course of infection. If TSLP–TSLPR interactions were necessary for Th2 cytokine responses, anti–IFN-γ–treated $TSLPR^{-/-}$ mice would remain susceptible to *Trichuris*.

After neutralization of IFN-γ in infected $TSLPR^{-/-}$ mice, there was a marked reduction in the frequency of IFN-γ^+ $CD4^+$ T cells in the MLNs at day 21 after infection compared with control-treated $TSLPR^{-/-}$ mice, consistent with the role of IFN-γ in promoting Th1 responses (Fig. 6 A). Coincident with decreased levels of IFN-γ, there was an increase in levels of IL-4, IL-5, and IL-13 secreted from antigen-restimulated MLN cells from anti–IFN-γ–treated $TSLPR^{-/-}$ mice compared with the control-treated $TSLPR^{-/-}$ mice (Fig. 6 B). Critically, the levels of IL-4, IL-5, and IL-13 in the anti–IFN-γ–treated $TSLPR^{-/-}$ mice were comparable to those seen in resistant $TSLPR^{+/+}$ mice (Fig. 6 B). Blockade of IFN-γ did not affect *Trichuris*-specific IgG1 serum antibody levels (not depicted) but did inhibit the development of a *Trichuris*-specific IgG2a serum antibody response, as serum levels of IgG2a in anti–IFN-γ–treated $TSLPR^{-/-}$ mice were comparable to levels in $TSLPR^{+/+}$ mice (Fig. 6 C). Anti–IFN-γ treatment also augmented levels of total serum IgE (Fig. S5, available at http://www.jem.org/cgi/content/full/jem.20081499/DC1). Goblet cell responses were restored as the expression of both GOB5 and RELMβ was increased in anti–IFN-γ–treated $TSLPR^{-/-}$ mice (Fig. 6, D and E). Further, histological examination revealed that the severe crypt elongation, edema, and inflammatory infiltrate present in the *Trichuris*-infected $TSLPR^{-/-}$ mice were absent after anti–IFN-γ treatment (Fig. 6 F). Critically, anti–IFN-γ treatment also recovered immunity in the $TSLPR^{-/-}$ mice. Although control-treated $TSLPR^{-/-}$ mice exhibited persistent infection, anti–IFN-γ–treated mice expelled their worms by day 34 after infection (Fig. 6 G). These results demonstrate that in the absence of IFN-γ in an in vivo system, TSLP-independent mechanisms exist that allow for the generation of a protective Th2 cytokine response in the intestine.

$TSLPR^{-/-}$ mice exhibit exaggerated expression of proinflammatory cytokines and severe intestinal inflammation in dextran sodium sulfate (DSS)–induced colitis

As TSLP was not strictly required to generate a protective Th2 cytokine response in the intestine, we hypothesized that TSLP may limit proinflammatory cytokine production and inflammation. To assess whether the ability of TSLP to influence intestinal immune responses extended beyond helminth infection, we used the DSS model of chemical-induced colitis. Oral administration of DSS is toxic to IECs and induces IEC damage, barrier leakage, and exposure of subepithelial immune cells to commensal bacteria. Barrier breakdown results in immune cell recruitment, increased expression of proinflammatory cytokines, and the development of focal ulcerations in the large intestine (40).

WT and $TSLPR^{-/-}$ mice were exposed to 5% DSS in their drinking water for 4 days, and disease was assessed daily. DSS-treated $TSLPR^{-/-}$ mice exhibited rapid weight loss in comparison to treated WT mice, displaying weight loss as early as day 1 after treatment (Fig. 7 A). Increased weight loss in the $TSLPR^{-/-}$ mice correlated with early disease onset and increased disease severity as measured by fecal consistency, rectal bleeding, general appearance, and weight loss (Fig. 7 B). At day 4 after DSS treatment, sections from the mid-colon of DSS-treated WT mice revealed mild thickening of the muscularis and a minimal presence of inflammatory cells. In contrast, tissue sections from DSS-treated $TSLPR^{-/-}$ mice exhibited severe thickening of the muscularis, a marked mucosal inflammatory cell infiltrate, and large numbers of focal lesions characterized by epithelial cell sloughing, increased inflammatory cell infiltrate, and complete loss of crypt architecture (Fig. 7 C). Consistent with the increased severity of disease score and inflammation, direct ex vivo organ culture of colonic segments displayed increased production of IL-12/23p40 in the DSS-treated $TSLPR^{-/-}$ mice (WT, 0.18 ± 0.05 ng/ml/mg of tissue; $TSLPR^{-/-}$, 0.33 ± 0.08 ng/ml/mg of tissue). Ex vivo analysis of MLN cells from day 4 after DSS treatment demonstrated an increased number of IFN-γ^+ $CD4^+$ T cells in DSS-treated $TSLPR^{-/-}$ mice compared with WT DSS-treated mice (Fig. 7 D). In addition, polyclonal restimulations of MLN cells harvested at day 4 after DSS–treated $TSLPR^{-/-}$ mice revealed increased levels of IFN-γ in comparison to DSS-treated WT mice (Fig. 7 E). DSS-treated $TSLPR^{-/-}$ mice also displayed exacerbated colonic shortening, thickening, and enteric bleeding at day 6 after treatment compared with DSS-treated WT mice (Fig. 7, F and G). Collectively, these results demonstrate a previously unrecognized role for TSLP–TSLPR interactions in the regulation of intestinal inflammation in a mouse model of IBD.

DISCUSSION

This study demonstrates a key role for TSLP–TSLPR interactions in regulating intestinal immunity and inflammation. We show for the first time that TSLP is constitutively expressed throughout the length of the GI tract at both the mRNA and protein level under homeostatic conditions. The TSLP–TSLPR pathway within the intestine is critical for host protective immunity, as disruption of TSLP–TSLPR interactions in vivo led to susceptibility to *Trichuris* infection. After infection, anti-TSLP mAb–treated WT mice and $TSLPR^{-/-}$ mice remained chronically infected, had elevated expression of IFN-γ and IL-17A, and exhibited severe infection-induced inflammation. Disruption of the TSLP–TSLPR pathway also resulted in dysregulated DC production of infection-induced IL-12/23p40. However, unlike models of

inflammation in the skin or the lung, we found that TSLP was not required for pathogen-specific Th2 cytokine responses in the intestine. Infected TSLPR$^{-/-}$ mice treated with neutralizing anti–IFN-γ antibodies recovered pathogen-specific Th2 cytokine production and immunity, thus identifying TSLP-independent pathways for the initiation of intestinal Th2 cytokine responses. Additionally, we demonstrate that TSLP–TSLPR interactions limit IL-12/23p40 and IFN-γ production in the DSS model of chemical-induced intestinal inflammation. Consistent with the elevated proinflammatory cytokine production, DSS-treated TSLPR$^{-/-}$ mice displayed increased intestinal inflammation compared

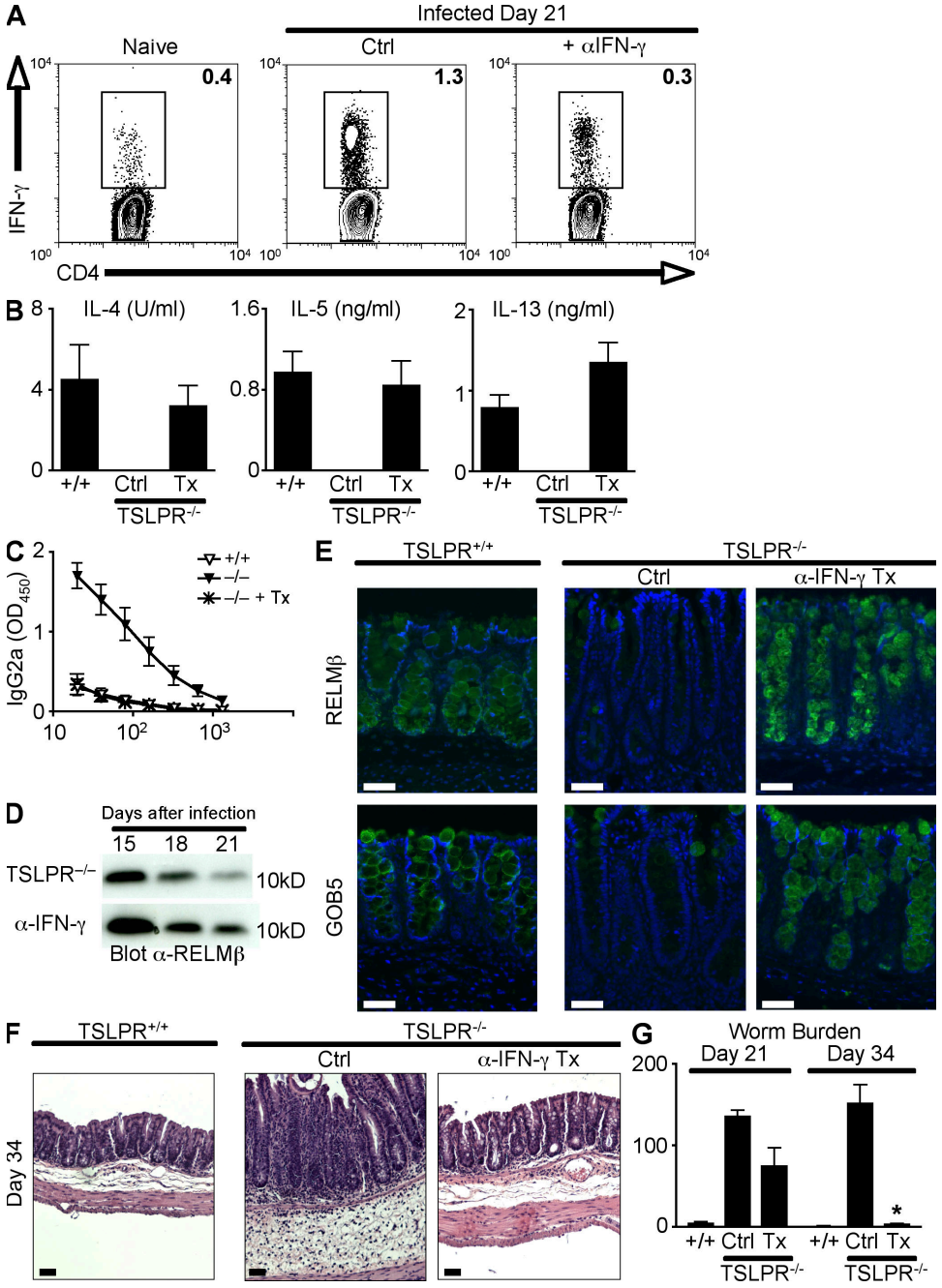

Figure 6. TSLP is not required for the initiation of an intestinal Th2 cytokine response to *Trichuris*. (A) Frequencies of CD4$^+$ IFN-γ$^+$ T cells isolated from the MLNs and stimulated directly ex vivo (percentages are shown). (B) Antigen-specific cytokine production from restimulated MLN cells was determined by ELISA. (C) Antigen-specific serum IgG2a levels. (D and E) Anti–IFN-γ treatment (Tx) restored goblet cell function in TSLPR$^{-/-}$ mice. (D) Protein isolated from fecal pellets on various days after infection was analyzed by SDS-PAGE and immunoblotted for RELMβ. (E) Paraffin sections of cecal tissue were IF stained for RELMβ and GOB5 (green) and costained with DAPI (blue). (F) Day 34 cecal sections were stained with H&E. (G) Worm burdens were assessed at days 21 and 34 after infection. Results represent means ± SEM. Data are representative of two individual experiments with three to four mice per group. *, $P \leq 0.05$. Bars, 50 µm.

with WT animals. Collectively these results indicate that in addition to promoting Th2 cytokine responses, a function of TSLP in the intestinal microenvironment may be to either directly or indirectly inhibit proinflammatory cytokine production and help prevent the development of severe intestinal inflammation.

The putative link between TSLP and the promotion of Th2 cell development has been well developed in both in vitro and in vivo studies (4, 19, 20). Initial in vitro studies found that human monocyte-derived DCs treated with TSLP induced $CD4^+$ T cells to produce IL-4 upon co-culture (11, 13, 15), and that TSLP-induced up-regulation of OX40L on DCs promoted $CD4^+$ Th2 cell differentiation (14). Recent work also demonstrated the ability of TSLP to directly influence $CD4^+$ T cells: in vitro treatment of purified $CD4^+$ T cells with TSLP induced T cell IL-4 production and Th2 cell differentiation (41, 42). Further, in the presence of TNF-α or IL-1, TSLP has been shown to induce IL-5 and IL-13 from mast cells (5). Supporting these in vitro studies, the transgenic overexpression of TSLP by epithelial cells in the skin or the lung induces Th2 cytokine–mediated inflammation in vivo (17, 18). Thus, through its ability to influence both innate cell populations as well as $CD4^+$ T cells, TSLP could contribute both directly and indirectly to Th2 cell differentiation in vivo. Directly, TSLP has been shown to promote *Il-4* gene transcription in $CD4^+$ T cells (42). Although the exact mechanisms remain unknown, it is possible that TSLP-induced STAT5 activation plays a role in promoting *Il-4* gene transcription independently of IL-4, STAT6, or GATA-3 (43–45). Once initiated, IL-4 may act in an autocrine fashion on $CD4^+$ T cell to activate STAT6 and up-regulate GATA-3 expression, promoting and stabilizing Th2 cell differentiation (46, 47). Indirectly, TSLP could promote Th2 cell differentiation by inducing innate cell production of IL-13, which could signal through STAT6 to promote proximal events (including GATA-3 up-regulation) in Th2 cell differentiation (46, 47). Neither of these possibilities is mutually exclusive, however, and it is likely that TSLP acts through multiple pathways to promote Th2 cell differentiation and Th2 cytokine production.

Although these data strongly support a role for TSLP in directly influencing and promoting Th2 cytokine responses, our finding that TSLP was not required for the initiation of pathogen-specific Th2 cytokine responses identifies TSLP-independent pathways for the generation of intestinal Th2 cytokine responses. In the absence of TSLP it is possible that a combination of other Th2-promoting factors present in the intestine are able to compensate and either act alone or

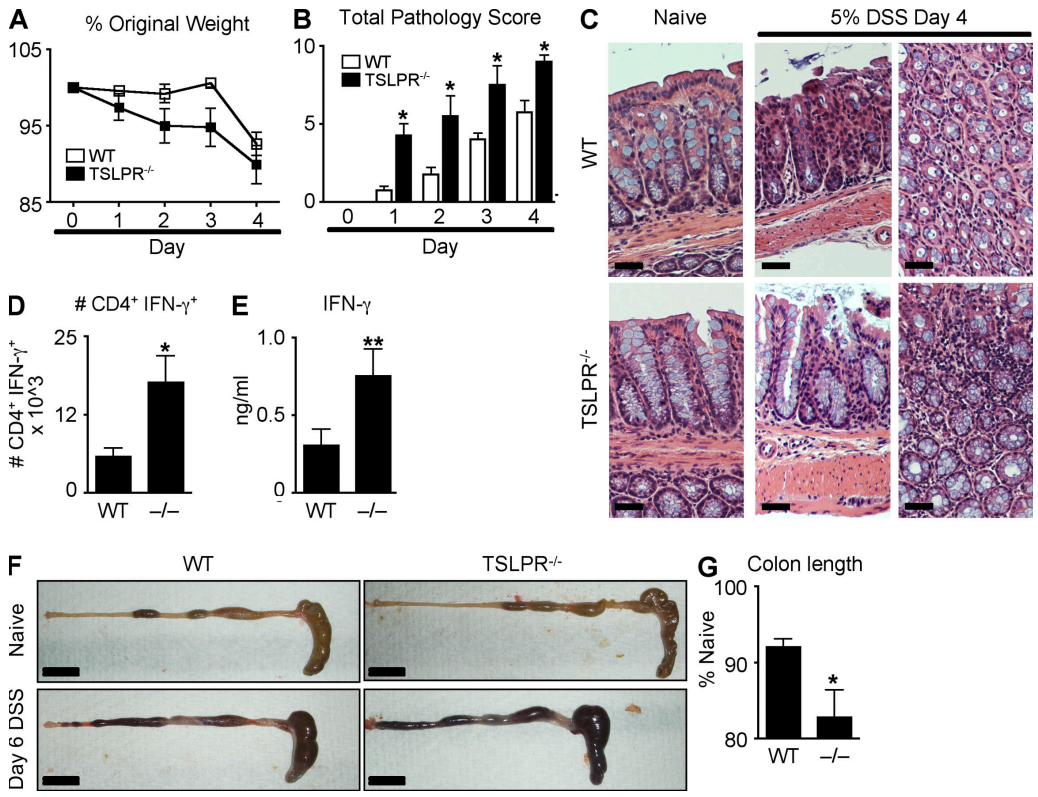

Figure 7. TSLP–TSLPR interactions limit proinflammatory cytokine production and inflammation in DSS colitis. (A) Weight loss. (B) Total pathology score for WT and $TSLPR^{-/-}$ mice on 5% DSS. (C) Paraffin-embedded cecal sections from day 4 after DSS administration were stained with H&E. Bars, 50 µm. (D) Total number of $CD4^+$ IFN-γ^+ T cells isolated from the MLNs at day 4 after DSS. (E) Cytokine production from anti-CD3/anti-CD28–restimulated MLN cells was determined by ELISA. (F) Colon of day 6 DSS-treated WT and $TSLPR^{-/-}$ mice, Bars, 1 cm. (G) Colonic shortening. Data are presented as the percent length of naive. Results represent means ± SEM. Data are representative of three individual experiments with four to five mice per group. *, $P \leq 0.05$; **, $P \leq 0.08$.

in concert to create a microenvironment permissive for Th2 cell differentiation. Candidates include cytokines such as IL-25 and IL-33, as they have each been demonstrated to promote Th2 cytokine responses (48, 49). Further, both IL-25 and IL-33 have been shown to play important roles in immunity to *Trichuris* infection (8, 39, 50). The Notch–Notch ligand pathway may also play a role in the generation of TSLP-independent Th2 cytokine responses in the intestine. Previous work has demonstrated that the Notch–Notch ligand interactions are important in mediating immunity to *Trichuris* (51), and Notch may act either directly in the promotion of Th2 cell differentiation (52–56) or through its influence on epithelial cell function (57). Thus, TSLP may be another factor that, although not itself essential for the development of Th2 cytokine responses in the intestine, is critical in combination with several other factors to create a Th2-permissive microenvironment.

The elevated levels of proinflammatory cytokines and severe infection-induced inflammation displayed in both infected TSLPR$^{-/-}$ mice and anti-TSLP mAb–treated WT mice suggests that TSLP may be playing a role in limiting proinflammatory cytokine production. This is further supported by the elevated expression of IL-12/23p40 and IFN-γ, and the severe inflammation exhibited in the DSS-treated TSLPR$^{-/-}$ mice. Although these data appear contradictory to the established function of TSLP in directly promoting Th2 cytokine responses, the ability of TSLP to modulate proinflammatory cytokine production has been previously indicated. In vitro studies showed that TSLP-treated DCs exhibit reduced production of IL-12/23p40 upon TLR ligation, and the ability of DC-derived OX40L to drive Th2 cell differentiation is critically dependent on the absence of IL-12 (2, 11, 14, 15). In addition, transgenic overexpression of TSLP in the skin decreased the frequency of CD4$^+$ IFN-γ$^+$ T cells in transgenic mice in comparison to normal littermate controls, and TSLPR$^{-/-}$ mice exhibited significantly increased IL-12 mRNA levels in the lungs compared with WT animals after OVA-induced allergic inflammation (13, 17). These findings are consistent with a role for TSLP in negatively regulating proinflammatory cytokine production in DC populations in peripheral sites. Thus, the exaggerated Th2 cytokine–mediated diseases in mice with transgenic overexpression of TSLP may be a result of simultaneous increases in Th2 cytokine responses and decreased expression of proinflammatory cytokines, including IL-12 and IFN-γ, that would counterregulate developing Th2 cytokine responses. TSLP is known to promote expression of GATA-3 and IL-4, pathways that would inhibit expression of IFN-γ (46, 58), but molecular mechanisms underlying its direct antiinflammatory functions remain to be defined. For instance, although TSLP has been demonstrated to inhibit DC production of IL-12/23p40 (2, 11, 14, 15), the signaling pathways induced by TSLP in DCs that regulate gene expression are unknown at present. TSLP may also act directly on CD4$^+$ T cells to limit Th1 and Th17 cell differentiation. For example, TSLP is known to activate STAT5 (27, 59), and STAT5a activation in CD4$^+$ T cells has been shown to inhibit IL-12–induced STAT4 activation through the induction of suppressor of cytokine signaling 3 (60). Further studies are required delineate the molecular mechanisms through which TSLP creates a Th2-permissive microenvironment in both the intestine and in other peripheral tissues.

Beyond a direct influence on balancing production of pro- and antiinflammatory cytokine production, TSLP may also regulate recruitment and/or retention of immune cells at the site of infection or inflammation. For example, TSLP treatment of human mDCs induces expression of the chemokines CCL17 and CCL22, known ligands for CCR4, a chemokine receptor found on effector Th2 cells (11, 19). Thus, after exposure to *Trichuris*, IEC-derived TSLP could be acting on intestinal DCs to simultaneously create conditions permissive to Th2 cell differentiation and to promote Th2 effector cell recruitment or retention in the intestine.

As there is a strong correlation of increased TSLP expression and disease in human patients with Th2 cytokine–mediated diseases such as atopic dermatitis and asthma, the TSLP–TSLPR pathway has emerged as a novel therapeutic target for allergic inflammatory disorders (11, 61). Supporting this, targeted blockade of the downstream OX40-OX40L pathway has been shown to successfully inhibit TSLP-induced and antigen-induced inflammation in mouse and nonhuman primate models of asthma and atopic dermatitis (62). However, the results of the present studies demonstrate that disruption of the TSLP–TSLPR pathway may lead to elevated production of proinflammatory cytokines and a predisposition to intestinal inflammation. As such, these findings suggest that manipulation of the TSLP–TSLPR pathway in the treatment of allergic diseases will require careful management. Notwithstanding this, the ability of TSLP to inhibit IL-12/23p40 and IFN-γ production suggests that TSLP-based biologics may offer a novel therapeutic modality in the treatment of IBD and other chronic inflammatory disorders. Consistent with this, TSLP expression was decreased in intestinal biopsies from patients with Crohn's disease, suggesting that increased TSLP expression may provide therapeutic benefits (15). Thus, the TSLP–TSLPR pathway may be a novel therapeutic target for a variety of inflammatory conditions associated with the overproduction of proinflammatory cytokines.

MATERIALS AND METHODS

Animals, parasites, antigens, and infections. WT C57BL/6J and OT-II transgenic mice were obtained from the Jackson Laboratory. TSLPR$^{+/+}$ and TSLPR$^{-/-}$ mice were obtained from J. Ihle (St. Jude's Children's Hospital, Nashville TN). Mice were bred and maintained in a specific-pathogen free environment at the University of Pennsylvania. Animal protocols were approved by the University of Pennsylvania Institutional Animal Care and Use Committee (IACUC), and all experiments were performed according to the guidelines of the University of Pennsylvania IACUC. *Trichuris muris* was maintained in genetically susceptible animals (63, 64); isolation of *Trichuris* excretory–secretory (ES) antigen and eggs was performed as previously described (65). Mice were infected on day 0 with 150–200 embryonated eggs, and parasite burdens were assessed at various time points after infection. Serum was analyzed by ELISA for *Trichuris*-specific IgG2a, as previously described (66).

Cytokines and mAbs. Neutralizing mAb against IFN-γ (XMG-6) was purified from ascites by ammonium sulfate precipitation and dialyzed against PBS. Mice were injected i.p. with 1 mg of antibody at the time of

infection and every 5 days after infection. Neutralizing mAb against mouse TSLP (M702) was obtained from Amgen. Mice were injected i.p. with 1 mg of antibody at the time of infection and every 5 days after infection. rTSLP (final concentration of 10 or 100 ng/ml in in vitro cultures) was obtained from R&D Systems.

Isolation of cells. At necropsy, MLNs were harvested and single-cell suspensions were prepared in DMEM supplemented with 10% heat-inactivated FBS, 2 mM glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, 25 mM Hepes, and 5×10^{-5} M 2-ME. A portion of cells was analyzed ex vivo for cytokine production by stimulating with 10 μg/ml brefeldin A, 50 ng/ml PMA, and 750 ng/ml ionomycin for 4–5 h. For DC experiments, MLN cells were restimulated in the presence of 50 μg/ml of *T. muris* ES antigen and 10 μg/ml brefeldin A. Cells were harvested and stained with fluorochrome-conjugated antibodies against CD4, IFN-γ, IL-13, CD11c, CD8α, CD11b, TNF-α, and IL-12/23p40. Cells were analyzed by flow cytometry on a FACSCalibur using CellQuest Pro software (BD); further analysis was performed using FlowJo software (Tree Star, Inc.). MLN cells were restimulated by plating cells in medium alone, in the presence of 50 μg/ml of *T. muris* ES antigen, or with 1 μg/ml each of soluble anti-CD3 and anti-CD28 antibodies. After 72 h in culture, cells were stimulated with PMA and ionomycin in the presence of brefeldin A for 4–5 h. Cell-free supernatants were harvested and analyzed for cytokine secretion by sandwich ELISA (eBioscience). The cells were harvested and analyzed for cytokine production as described for the ex vivo analysis.

Tissue staining and RELMβ responses. At necropsy, cecal sections were removed and fixed in 4% paraformaldehyde or snap frozen in OCT medium (Tissue Tek; Sakura Inc.). 5-μm paraffin-embedded sections were cut and stained with hematoxylin and eosin (H&E) or periodic acid-Schiff (PAS)/Acian blue, or by IF for RELMβ and GOB5 for visualization of goblet cell responses. IF staining was performed as previously described (2). IF for TSLP was performed on cryosections of the large intestine and cecum using a biotinylated monoclonal anti-TSLP antibody (eBioscience). Fecal protein isolation was performed as previously described (29). Samples were equalized by protein content and analyzed by SDS-PAGE, and then immunoblotted for RELMβ using a polyclonal rabbit anti–murine RELMβ antibody (PeproTech).

BMDCs and BMDC/T cell co-culture. BMDCs were derived as follows. Bone marrow from WT C57BL/6 mice was plated in 6-well cell-culture plates in RPMI 1640 supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin, 5×10^{-5} M 2-ME, 10% FBS, 2 mM L-glutamine, and 20 ng/ml GM-CSF (PeproTech). Media was changed at days 3, 6, and 8; cells were harvested at day 9. BMDCs were plated out at 4×10^6 cells/ml and mixed 1:1 with either media alone, IEC-Sup, or rTSLP (final concentration of 10 or 100 ng/ml; R&D Systems) for 16–18 h. Cells were stimulated with 10 ng/ml LPS. At various time points, cell-free supernatants were harvested for analysis by ELISA, and cells were harvested either for analysis by flow cytometry or RT-PCR for mRNA expression. For BMDC/T cell co-culture, BMDCs were harvested and conditioned with IEC-Sup or rTSLP, as described. They were then pulsed with 0.5 mg/ml ovalbumin (Worthington) and 1 μg/ml CpG (Coley Pharmaceutical Group) for 16–18 h. Purified OT-II $CD4^+$ T cells were cultured with the BMDCs for 4 d. Cells were stimulated with PMA, ionomycin, and brefeldin A for the final 4 h and analyzed by flow cytometry.

RNA isolation and real-time PCR. RNA was isolated from the intestinal tissues of mice using a TRIzol extraction and from BMDCs using RNEasy spin columns (QIAGEN). Tissues were first disrupted in a tissue homogenizer (TissueLyzer; QIAGEN). cDNA was synthesized from the isolated RNA using Superscript Reverse Transcriptase (Invitrogen). Quantitative real-time PCR was performed on cDNA samples using commercial primer sets (QIAGEN) and SYBR green chemistry. All reactions were run on an ABI 7500 Fast Real-Time PCR System (Applied Biosystems). Samples are normalized to naive controls unless otherwise stated in the figures.

DSS-induced intestinal inflammation. DSS (MP Biomedicals) was added to drinking water at 5% weight/volume for 4 days. Mice were monitored for weight loss, rectal bleeding, and general appearance (piloerection and lethargy). Pathology was scored as follows: (a) weight loss (no change = 0; <5% = 1; 6–10% = 2; 11–20% = 3; >20% = 4), (b) feces (normal = 0; pasty, semi-formed = 2; liquid, sticky, or unable to defecate after 5 min = 4), (c) blood (no blood = 0; visible blood in rectum = 1; visible blood on fur = 2), and (d) general appearance (normal = 0; piloerection = 1; lethargy and piloerection = 2; motionless, sickly = 4).

Statistics. Results represent means ± SEM unless otherwise stated. Statistical significance was determined by the Student's *t* test or the Wilcoxon Mann-Whitney test for nonparametric samples (*, $P \leq 0.05$; **, $P \leq 0.08$).

Online supplemental material. Fig. S1 shows chronic *Trichuris* infection in WT mice treated with anti-TSLP mAb at day 34 after infection. Severe infection-induced inflammation in anti-TSLP mAb–treated mice is displayed in Fig. S2. Increased frequencies of $CD4^+$ IFN-γ^+ cells in the MLNs at day 34 after infection in $TSLPR^{-/-}$ mice are displayed in Fig. S3. Fig. S4 shows the elevated levels of IFN-γ and IL-17A in *Trichuris*-infected WT mice treated with anti-TSLP mAb. Fig. S5 depicts the increased levels of total serum IgE seen in *Trichuris*-infected $TSLPR^{-/-}$ mice treated with anti–IFN-γ mAb. Online supplemental material is available at http://www.jem.org/cgi/content/full/jem.20081499/DC1.

We would like to thank P. Giacomin and M. Nair for critical reading of this manuscript, and T. Kirn for assistance with histological analysis.

This work was supported by grants from the National Institutes of Health (AI61570 and AI74878 to D. Artis, F32-AI72943 to A.E. Troy, and T32-CA09140-30 to B.C. Taylor), the Burroughs Wellcome Fund (Investigator in Pathogenesis of Infectious Disease Award to D. Artis), the Crohn's and Colitis Foundation of America (William and Shelby Model Family Foundation Research Award to D. Artis), and pilot grants from the University of Pennsylvania (Center for Infectious Diseases and University Research Fund to D. Artis). C. Zaph is funded by the Irvington Institute Fellowship Program of the Cancer Research Institute.

M.R. Comeau is employed by Amgen and supported by Amgen Inc. The authors declare no other competing financial interests.

Submitted: 11 July 2008
Accepted: 11 February 2009

REFERENCES

1. Nenci, A., C. Becker, A. Wullaert, R. Gareus, G. van Loo, S. Danese, M. Huth, A. Nikolaev, C. Neufert, B. Madison, et al. 2007. Epithelial NEMO links innate immunity to chronic intestinal inflammation. *Nature*. 446:557–561.
2. Zaph, C., A.E. Troy, B.C. Taylor, L.D. Berman-Booty, K.J. Guild, Y. Du, E.A. Yost, A.D. Gruber, M.J. May, F.R. Greten, et al. 2007. Epithelial-cell-intrinsic IKK-beta expression regulates intestinal immune homeostasis. *Nature*. 446:552–556.
3. Zeuthen, L.H., L.N. Fink, and H. Frokiaer. 2008. Epithelial cells prime the immune response to an array of gut-derived commensals towards a tolerogenic phenotype through distinct actions of thymic stromal lymphopoietin and transforming growth factor-beta. *Immunology*. 123:197–208.
4. Liu, Y.J., V. Soumelis, N. Watanabe, T. Ito, Y.H. Wang, W. Malefyt Rde, M. Omori, B. Zhou, and S.F. Ziegler. 2007. TSLP: an epithelial cell cytokine that regulates T cell differentiation by conditioning dendritic cell maturation. *Annu. Rev. Immunol.* 25:193–219.
5. Allakhverdi, Z., M.R. Comeau, H.K. Jessup, B.R. Yoon, A. Brewer, S. Chartier, N. Paquette, S.F. Ziegler, M. Sarfati, and G. Delespesse. 2007. Thymic stromal lymphopoietin is released by human epithelial cells in response to microbes, trauma, or inflammation and potently activates mast cells. *J. Exp. Med.* 204:253–258.
6. Kato, A., S. Favoreto Jr., P.C. Avila, and R.P. Schleimer. 2007. TLR3- and Th2 cytokine-dependent production of thymic stromal lymphopoietin in human airway epithelial cells. *J. Immunol.* 179:1080–1087.
7. Lee, H.C., and S.F. Ziegler. 2007. Inducible expression of the proallergic cytokine thymic stromal lymphopoietin in airway epithelial cells is controlled by NFkappaB. *Proc. Natl. Acad. Sci. USA*. 104:914–919.

8. Humphreys, N.E., D. Xu, M.R. Hepworth, F.Y. Liew, and R.K. Grencis. 2008. IL-33, a potent inducer of adaptive immunity to intestinal nematodes. *J. Immunol.* 180:2443–2449.
9. Astrakhan, A., M. Omori, T. Nguyen, S. Becker-Herman, M. Iseki, T. Aye, K. Hudkins, J. Dooley, A. Farr, C.E. Alpers, et al. 2007. Local increase in thymic stromal lymphopoietin induces systemic alterations in B cell development. *Nat. Immunol.* 8:522–531.
10. Chappaz, S., L. Flueck, A.G. Farr, A.G. Rolink, and D. Finke. 2007. Increased TSLP availability restores T- and B-cell compartments in adult IL-7 deficient mice. *Blood.* 110:3862–3870.
11. Soumelis, V., P.A. Reche, H. Kanzler, W. Yuan, G. Edward, B. Homey, M. Gilliet, S. Ho, S. Antonenko, A. Lauerma, et al. 2002. Human epithelial cells trigger dendritic cell mediated allergic inflammation by producing TSLP. *Nat. Immunol.* 3:673–680.
12. Sokol, C.L., G.M. Barton, A.G. Farr, and R. Medzhitov. 2008. A mechanism for the initiation of allergen-induced T helper type 2 responses. *Nat. Immunol.* 9:310–318.
13. Al-Shami, A., R. Spolski, J. Kelly, A. Keane-Myers, and W.J. Leonard. 2005. A role for TSLP in the development of inflammation in an asthma model. *J. Exp. Med.* 202:829–839.
14. Ito, T., Y.H. Wang, O. Duramad, T. Hori, G.J. Delespesse, N. Watanabe, F.X. Qin, Z. Yao, W. Cao, and Y.J. Liu. 2005. TSLP-activated dendritic cells induce an inflammatory T helper type 2 cell response through OX40 ligand. *J. Exp. Med.* 202:1213–1223.
15. Rimoldi, M., M. Chieppa, V. Salucci, F. Avogadri, A. Sonzogni, G.M. Sampietro, A. Nespoli, G. Viale, P. Allavena, and M. Rescigno. 2005. Intestinal immune homeostasis is regulated by the crosstalk between epithelial cells and dendritic cells. *Nat. Immunol.* 6:507–514.
16. Liu, Y.J. 2007. Thymic stromal lymphopoietin and OX40 ligand pathway in the initiation of dendritic cell-mediated allergic inflammation. *J. Allergy Clin. Immunol.* 120:238–244.
17. Yoo, J., M. Omori, D. Gyarmati, B. Zhou, T. Aye, A. Brewer, M.R. Comeau, D.J. Campbell, and S.F. Ziegler. 2005. Spontaneous atopic dermatitis in mice expressing an inducible thymic stromal lymphopoietin transgene specifically in the skin. *J. Exp. Med.* 202:541–549.
18. Zhou, B., M.R. Comeau, T. De Smedt, H.D. Liggitt, M.E. Dahl, D.B. Lewis, D. Gyarmati, T. Aye, D.J. Campbell, and S.F. Ziegler. 2005. Thymic stromal lymphopoietin as a key initiator of allergic airway inflammation in mice. *Nat. Immunol.* 6:1047–1053.
19. Liu, Y.J. 2006. Thymic stromal lymphopoietin: master switch for allergic inflammation. *J. Exp. Med.* 203:269–273.
20. Ziegler, S.F., and Y.J. Liu. 2006. Thymic stromal lymphopoietin in normal and pathogenic T cell development and function. *Nat. Immunol.* 7:709–714.
21. Macdonald, T.T., and G. Monteleone. 2005. Immunity, inflammation, and allergy in the gut. *Science.* 307:1920–1925.
22. Mowat, A.M. 2003. Anatomical basis of tolerance and immunity to intestinal antigens. *Nat. Rev. Immunol.* 3:331–341.
23. Artis, D. 2008. Epithelial-cell recognition of commensal bacteria and maintenance of immune homeostasis in the gut. *Nat. Rev. Immunol.* 8:411–420.
24. Tlaskalova-Hogenova, H., R. Stepankova, T. Hudcovic, L. Tuckova, B. Cukrowska, R. Lodinova-Zadnikova, H. Kozakova, P. Rossmann, J. Bartova, D. Sokol, et al. 2004. Commensal bacteria (normal microflora), mucosal immunity and chronic inflammatory and autoimmune diseases. *Immunol. Lett.* 93:97–108.
25. Zaph, C., Y. Du, S.A. Saenz, M.G. Nair, J.G. Perrigoue, B.C. Taylor, A.E. Troy, D.E. Kobuley, R.A. Kastelein, D.J. Cua, et al. 2008. Commensal-dependent expression of IL-25 regulates the IL-23–IL-17 axis in the intestine. *J. Exp. Med.* 205:2191–2198.
26. Ivanov, I.I., L. Frutos Rde, N. Manel, K. Yoshinaga, D.B. Rifkin, R.B. Sartor, B.B. Finlay, and D.R. Littman. 2008. Specific microbiota direct the differentiation of IL-17-producing T-helper cells in the mucosa of the small intestine. *Cell Host Microbe.* 4:337–349.
27. Levin, S.D., R.M. Koelling, S.L. Friend, D.E. Isaksen, S.F. Ziegler, R.M. Perlmutter, and A.G. Farr. 1999. Thymic stromal lymphopoietin: a cytokine that promotes the development of IgM+ B cells in vitro and signals via a novel mechanism. *J. Immunol.* 162:677–683.
28. Sims, J.E., D.E. Williams, P.J. Morrissey, K. Garka, D. Foxworthe, V. Price, S.L. Friend, A. Farr, M.A. Bedell, N.A. Jenkins, et al. 2000. Molecular cloning and biological characterization of a novel murine lymphoid growth factor. *J. Exp. Med.* 192:671–680.
29. Artis, D., M.L. Wang, S.A. Keilbaugh, W. He, M. Brenes, G.P. Swain, P.A. Knight, D.D. Donaldson, M.A. Lazar, H.R. Miller, et al. 2004. RELMbeta/FIZZ2 is a goblet cell-specific immune-effector molecule in the gastrointestinal tract. *Proc. Natl. Acad. Sci. USA.* 101:13596–13600.
30. Theodoropoulos, G., S.J. Hicks, A.P. Corfield, B.G. Miller, and S.D. Carrington. 2001. The role of mucins in host-parasite interactions: Part II–helminth parasites. *Trends Parasitol.* 17:130–135.
31. Anthony, R.M., L.I. Rutitzky, J.F. Urban Jr., M.J. Stadecker, and W.C. Gause. 2007. Protective immune mechanisms in helminth infection. *Nat. Rev. Immunol.* 7:975–987.
32. Wilson, E.H., C. Zaph, M. Mohrs, A. Welcher, J. Siu, D. Artis, and C.A. Hunter. 2006. B7RP-1-ICOS interactions are required for optimal infection-induced expansion of CD4+ Th1 and Th2 responses. *J. Immunol.* 177:2365–2372.
33. Zaph, C., K.A. Rook, M. Goldschmidt, M. Mohrs, P. Scott, and D. Artis. 2006. Persistence and function of central and effector memory CD4+ T cells following infection with a gastrointestinal helminth. *J. Immunol.* 177:511–518.
34. Artis, D. 2006. New weapons in the war on worms: identification of putative mechanisms of immune-mediated expulsion of gastrointestinal nematodes. *Int. J. Parasitol.* 36:723–733.
35. Nair, M.G., K.J. Guild, and D. Artis. 2006. Novel effector molecules in type 2 inflammation: lessons drawn from helminth infection and allergy. *J. Immunol.* 177:1393–1399.
36. Else, K.J., F.D. Finkelman, C.R. Maliszewski, and R.K. Grencis. 1994. Cytokine-mediated regulation of chronic intestinal helminth infection. *J. Exp. Med.* 179:347–351.
37. Artis, D., N.E. Humphreys, A.J. Bancroft, N.J. Rothwell, C.S. Potten, and R.K. Grencis. 1999. Tumor necrosis factor α is a critical component of interleukin 13–mediated protective T helper cell type 2 responses during helminth infection. *J. Exp. Med.* 190:953–962.
38. Bancroft, A.J., D. Artis, D.D. Donaldson, J.P. Sypek, and R.K. Grencis. 2000. Gastrointestinal nematode expulsion in IL-4 knockout mice is IL-13 dependent. *Eur. J. Immunol.* 30:2083–2091.
39. Owyang, A.M., C. Zaph, E.H. Wilson, K.J. Guild, T. McClanahan, H.R. Miller, D.J. Cua, M. Goldschmidt, C.A. Hunter, R.A. Kastelein, and D. Artis. 2006. Interleukin 25 regulates type 2 cytokine-dependent immunity and limits chronic inflammation in the gastrointestinal tract. *J. Exp. Med.* 203:843–849.
40. Byrne, F.R., and J.L. Viney. 2006. Mouse models of inflammatory bowel disease. *Curr. Opin. Drug Discov. Devel.* 9:207–217.
41. Rochman, I., N. Watanabe, K. Arima, Y.J. Liu, and W.J. Leonard. 2007. Cutting edge: direct action of thymic stromal lymphopoietin on activated human CD4+ T cells. *J. Immunol.* 178:6720–6724.
42. Omori, M., and S. Ziegler. 2007. Induction of IL-4 expression in CD4(+) T cells by thymic stromal lymphopoietin. *J. Immunol.* 178:1396–1404.
43. Takatori, H., H. Nakajima, K. Hirose, S. Kagami, T. Tamachi, A. Suto, K. Suzuki, Y. Saito, and I. Iwamoto. 2005. Indispensable role of Stat5a in Stat6-independent Th2 cell differentiation and allergic airway inflammation. *J. Immunol.* 174:3734–3740.
44. Zhu, J., H. Yamane, J. Cote-Sierra, L. Guo, and W.E. Paul. 2006. GATA-3 promotes Th2 responses through three different mechanisms: induction of Th2 cytokine production, selective growth of Th2 cells and inhibition of Th1 cell-specific factors. *Cell Res.* 16:3–10.
45. Zhu, J., J. Cote-Sierra, L. Guo, and W.E. Paul. 2003. Stat5 activation plays a critical role in Th2 differentiation. *Immunity.* 19:739–748.
46. Murphy, K.M., and S.L. Reiner. 2002. The lineage decisions of helper T cells. *Nat. Rev. Immunol.* 2:933–944.
47. Mowen, K.A., and L.H. Glimcher. 2004. Signaling pathways in Th2 development. *Immunol. Rev.* 202:203–222.
48. Fort, M.M., J. Cheung, D. Yen, J. Li, S.M. Zurawski, S. Lo, S. Menon, T. Clifford, B. Hunte, R. Lesley, et al. 2001. IL-25 induces IL-4, IL-5, and IL-13 and Th2-associated pathologies in vivo. *Immunity.* 15:985–995.
49. Schmitz, J., A. Owyang, E. Oldham, Y. Song, E. Murphy, T.K. McClanahan, G. Zurawski, M. Moshrefi, J. Qin, X. Li, et al. 2005. IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-

related protein ST2 and induces T helper type 2-associated cytokines. *Immunity*. 23:479–490.
50. Saenz, S.A., B.C. Taylor, and D. Artis. 2008. Welcome to the neighborhood: epithelial cell-derived cytokines license innate and adaptive immune responses at mucosal sites. *Immunol. Rev.* 226:172–190.
51. Tu, L., T.C. Fang, D. Artis, O. Shestova, S.E. Pross, I. Maillard, and W.S. Pear. 2005. Notch signaling is an important regulator of type 2 immunity. *J. Exp. Med.* 202:1037–1042.
52. Ansel, K.M., I. Djuretic, B. Tanasa, and A. Rao. 2006. Regulation of Th2 differentiation and Il4 locus accessibility. *Annu. Rev. Immunol.* 24:607–656.
53. Kubo, M. 2007. Notch: filling a hole in T helper 2 cell differentiation. *Immunity*. 27:3–5.
54. Perrigoue, J.G., F.A. Marshall, and D. Artis. 2008. On the hunt for helminths: innate immune cells in the recognition and response to helminth parasites. *Cell. Microbiol.* 10:1757–1764.
55. Amsen, D., A. Antov, D. Jankovic, A. Sher, F. Radtke, A. Souabni, M. Busslinger, B. McCright, T. Gridley, and R.A. Flavell. 2007. Direct regulation of Gata3 expression determines the T helper differentiation potential of Notch. *Immunity*. 27:89–99.
56. Fang, T.C., Y. Yashiro-Ohtani, C. Del Bianco, D.M. Knoblock, S.C. Blacklow, and W.S. Pear. 2007. Notch directly regulates Gata3 expression during T helper 2 cell differentiation. *Immunity*. 27:100–110.
57. Demehri, S., Z. Liu, J. Lee, M.H. Lin, S.D. Crosby, C.J. Roberts, P.W. Grigsby, J.H. Miner, A.G. Farr, and R. Kopan. 2008. Notch-deficient skin induces a lethal systemic B-lymphoproliferative disorder by secreting TSLP, a sentinel for epidermal integrity. *PLoS Biol.* 6:e123.
58. Szabo, S.J., B.M. Sullivan, S.L. Peng, and L.H. Glimcher. 2003. Molecular mechanisms regulating Th1 immune responses. *Annu. Rev. Immunol.* 21:713–758.
59. Isaksen, D.E., H. Baumann, P.A. Trobridge, A.G. Farr, S.D. Levin, and S.F. Ziegler. 1999. Requirement for stat5 in thymic stromal lymphopoietin-mediated signal transduction. *J. Immunol.* 163:5971–5977.
60. Takatori, H., H. Nakajima, S. Kagami, K. Hirose, A. Suto, K. Suzuki, M. Kubo, A. Yoshimura, Y. Saito, and I. Iwamoto. 2005. Stat5a inhibits IL-12-induced Th1 cell differentiation through the induction of suppressor of cytokine signaling 3 expression. *J. Immunol.* 174:4105–4112.
61. Ying, S., B. O'Connor, J. Ratoff, Q. Meng, K. Mallett, D. Cousins, D. Robinson, G. Zhang, J. Zhao, T.H. Lee, and C. Corrigan. 2005. Thymic stromal lymphopoietin expression is increased in asthmatic airways and correlates with expression of Th2-attracting chemokines and disease severity. *J. Immunol.* 174:8183–8190.
62. Seshasayee, D., W.P. Lee, M. Zhou, J. Shu, E. Suto, J. Zhang, L. Diehl, C.D. Austin, Y.G. Meng, M. Tan, et al. 2007. In vivo blockade of OX40 ligand inhibits thymic stromal lymphopoietin driven atopic inflammation. *J. Clin. Invest.* 117:3868–3878.
63. Wakelin, D. 1967. Acquired immunity to *Trichuris muris* in the albino laboratory mouse. *Parasitology*. 57:515–524.
64. Wakelin, D. 1969. The development of the early larval stages of *Trichuris muris* in the albino laboratory mouse. *J. Helminthol.* 43:427–436.
65. Artis, D., A. Villarino, 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.
66. Artis, D., S. Shapira, N. Mason, K.M. Speirs, M. Goldschmidt, J. Caamano, H.C. Liou, C.A. Hunter, and P. Scott. 2002. Differential requirement for NF-kappa B family members in control of helminth infection and intestinal inflammation. *J. Immunol.* 169:4481–4487.