Interleukin 25 regulates type 2 cytokine-dependent immunity and limits chronic inflammation in the gastrointestinal tract

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The cytokine interleukin (IL) 25 has been implicated in the initiation of type 2 immunity by driving the expression of type 2 cytokines such as IL-5 and IL-13, although its role in the regulation of immunity and infection-induced inflammation is unknown. Here, we identify a dual function for IL-25: first, in promoting type 2 cytokine–dependent immunity to gastrointestinal helminth infection and, second, in limiting proinflammatory cytokine production and chronic intestinal inflammation. Treatment of genetically susceptible mice with exogenous IL-25 promoted type 2 cytokine responses and immunity to Trichuris. IL-25 was constitutively expressed by CD4+ and CD8+ T cells in the gut of mouse strains that are resistant to Trichuris, and IL-25–deficient mice on a genetically resistant background failed to develop a type 2 immune response or eradicate infection. Furthermore, chronically infected IL-25−/− mice developed severe infection–induced intestinal inflammation associated with heightened expression of interferon-γ and IL-17, identifying a role for IL-25 in limiting pathologic inflammation at mucosal sites. Therefore, IL-25 is not only a critical mediator of type 2 immunity, but is also required for the regulation of inflammation in the gastrointestinal tract.

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C57BL/6 mice) is associated with the hallmarks of type 2 immunity: outgrowth of antigen-specific CD4+ TTH2 cells that produce IL-4 and IL-13, mastocytosis, IgG1 and IgE production, goblet cell hyperplasia, and production of RELMβ, which is a novel goblet cell-specific protein associated with immunity to helminth parasites (14–18). In contrast, CD4+ TH1 cells that produce IFN-γ promote parasite persistence, resulting in chronic infection (e.g., in AKR mice). Most susceptible strains of mice that harbor persistent parasites exhibit moderate infection-induced pathology, whereas strains deficient in IL-10 or NFκB1 develop severe intestinal inflammation that can lead to death (19, 20). Thus, infection with Trichuris provides a tractable system to study immunity and inflammation in the GI tract. Here, we provide evidence that IL-25 is a critical cytokine in both promoting type 2 cytokine-dependent resistance to Trichuris infection and in inhibiting destructive intestinal inflammation.

RESULTS AND DISCUSSION
To test whether IL-25 could induce a protective type 2 response and confer immunity to Trichuris in a normally susceptible strain, infected AKR mice were treated with recombinant IL-25 (rIL-25). Although control-treated mice exhibited a polarized Trichuris-specific IFN-γ response in the absence of detectable IL-4 (Fig. 1 A), administration of rIL-25 resulted in the induction of an antigen-specific IL-4 response and extinguished IFN-γ production (Fig. 1 A). In addition, rIL-25–treated AKR mice exhibited the hallmarks of a protective TH2 cell response, including reduced levels of Trichuris-specific IgG2a (Fig. S1, available at http://www.jem.org/cgi/content/full/jem.20051496/DC1), an increase in the numbers of goblet cells in the cecum (Fig. 1 B) and increased expression of RELMβ (Fig. 1 C). Consistent with the development of a Trichuris-specific type 2 response, rIL-25 treatment conferred resistance to infection, as untreated AKR mice had a high parasite burden, whereas treated AKR mice achieved sterile cure (Fig. 1 D). IL-25–induced immunity was lymphocyte dependent, as infected SCID mice treated with rIL-25 did not exhibit goblet cell responses (Fig. 1 B) and were unable to eradicate infection (Fig. 1 D). Thus, exogenous IL-25 can promote protective lymphocyte-dependent type 2 responses and act as an effective therapeutic treatment to enhance type 2 immunity in the GI tract.

We next tested whether early differences in expression of IL-25 was a predictor of infection outcome in genetically resistant versus susceptible strains. Although no infection-induced increases in IL-25 mRNA were observed after infection with Trichuris (unpublished data), we found that resistant BALB/c mice expressed constitutively higher levels of IL-25 mRNA in the cecum but not the mesenteric lymph node (mLN) compared with susceptible AKR mice, whereas both strains expressed equivalent but low levels of IL-25 mRNA in the mLN (Fig. 2 A). Furthermore, IL-25 receptor (IL-25R) mRNA expression by BALB/c mice was elevated in both compartments (Fig. 2 A). To determine the cellular source of IL-25, we examined the expression of a lacZ reporter construct inserted into the IL-25 locus. This reporter system allows for the amplification of the endogenous IL-25 response by using the fluorescent β-galactosidase substrate, FDG. Upon examination of the mLN and cecal patch (CP)
IL-25 is expressed constitutively in the cecal patch of mice resistant to *Trichuris*. (A) Increased expression of IL-25 and IL-25R mRNA in the cecum of resistant BALB/c (B/c) mice. IL-25 and IL-25R mRNA levels were determined by real-time PCR from the cecum or mLN of naive BALB/c or AKR mice. (B) CD4+ and CD8+ T cells in the cecal patch express lacZ/IL-25. Cells from the mLN or cecal patch of IL-25−/− mice were stained with FDG and CD4 or CD8. Numbers represent frequency of FDG+ CD4+ or CD8+ T cells. Data are representative of three to four independent experiments with three to four mice per group.

Endogenous IL-25 is required for resistance to *Trichuris*. (A) IL-25 is required for optimal production of Th2 cytokines after infection. IL-4, IL-13, and IFN-γ expression in the mLN and cecum in WT and IL-25−/− mice at day 18 after infection as measured by real-time PCR. (B) Defective goblet cell responses in IL-25−/− mice after infection with *Trichuris*. Cecal sections from day 18 infected WT or IL-25−/− mice were stained for goblet cells. Bar, 50 μm. (C) IL-25−/− mice fail to up-regulate RELMβ after infection. Protein isolated from fecal pellets on various days after infection was analyzed by SDS-PAGE and immunoblotted for RELMβ. (D) IL-25 is required for resistance to infection with *Trichuris*. Worm burdens in WT, IL-25−/− and RAG−/− mice at day 30 after infection. Data are presented as mean ± SEM and are representative of four independent experiments with three to four mice per group. N, naive; I, infected.
observed a critical role for this cytokine in limiting chronic helminth infection. IL-25 may function to actively inhibit expression of type 1 cytokines after infection, in addition to promoting type 2 responses. These data show that the requirement for IL-25 in the development of TH2 cells and type 2 responses in vivo is critical. IL-25 has been shown to activate NF-κB in mice, suggesting it may be a critical component in infections but display moderate infection-induced inflammation characterized by minimal crypt hyperplasia.

Although the cytokines that drive TH1 cell differentiation and type 1 inflammation are well characterized, the factors that promote the development of type 2 responses in vivo remain elusive. Based on higher levels of IL-25 in genetically resistant mouse strains, coupled with the results demonstrating that exogenous IL-25 can drive expression of TH2 cytokines, whereas type 2 immunity is severely impaired in IL-25−/− mice, we hypothesized that this cytokine may be the critical factor required for the development of TH2 cells and type 2 responses in vivo. To test whether IL-25 expression was essential for immunity to Trichuris, infected IL-25−/− mice were treated with a combination of anti–IL-12 and anti–IFN-γ monoclonal antibodies. Previous studies have shown that in vivo depletion of type 1 cytokines can promote type 2 cytokine-dependent resistance to Trichuris in normally susceptible hosts.

Blockade of type 1 cytokines in infected IL-25−/− mice was associated with elevated production of Trichuris-specific IL-4, IL-5, and IL-13 in the draining mLN (Fig. 4 A), enhanced serum IgE responses (Fig. S4 A, available at http://www.jem.org/cgi/content/full/jem.20051496/DC1), and a recovery of intestinal goblet cell responses (Fig. 4 B). Furthermore, anti–IL-12/anti–IFN-γ–treated IL-25−/− mice exhibited reduced Trichuris-specific IFN-γ production (Fig. 4 A) and lower serum IgG2a levels (Fig. S4 B). Although control-treated IL-25−/− mice displayed high worm burdens, inhibition of type 1 responses resulted in successful expulsion of Trichuris in IL-25−/− mice (Fig. 4 C). Contrary to the hypothesis that IL-25 is essential for type 2 responses, these results show that expression of IL-4, IL-5, and IL-13 was recovered in the absence of IL-25 if endogenous type 1 cytokines were blocked. Therefore, these data show that the requirement for IL-25 in the development of type 2 immunity is conditional and suggest that, in addition to promoting type 2 responses, IL-25 may function to actively inhibit expression of type 1 cytokines after helminth infection.

In support of an antiinflammatory function of IL-25, we observed a critical role for this cytokine in limiting chronic infection-induced intestinal inflammation. Genetically susceptible strains of mice, such as AKR, as well as mice deficient in IL-4Rα signaling, develop persistent chronic intestinal goblet cell responses. Furthermore, anti–IL-12 and anti–IFN-γ treatment recovers goblet cell responses in infected IL-25−/− mice. Goblet cell responses in the cecum of infected WT and control or IL-25−/− mice were determined by cytometric bead array (CBA; IL-4, IL-5, and IFNγ) or ELISA (IL-13). Anti–IL-12/anti–IFN-γ treatment recovered goblet cell responses in infected IL-25−/− mice. Goblet cell responses in the cecum of infected WT and control or IL-25−/− mice were determined at day 20 after infection. Bar, 50 μm.

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Figure 4. Type 2 inflammation is IL-25 independent when endogenous type 1 responses are inhibited. Infected IL-25−/− mice were treated with anti–IL-12 and anti–IFN-γ every 4 d beginning on day 4 and 20. (A) Blockade of type 1 cytokines in infected IL-25−/− mice recovers TH2 cytokine responses. Antigen-specific IL-4, IL-5, IL-13, and IFN-γ responses by restimulated mLN cells from control (lg) or αIL-12/αIFN-γ–treated (α12/γ) IL-25−/− mice determined by cytometric bead array (CBA; IL-4, IL-5, and IFNγ) or ELISA (IL-13). (B) Anti–IL-12/anti–IFN-γ treatment recovers goblet cell responses in infected IL-25−/− mice. Goblet cell responses in the cecum of infected WT and control or αIL-12/αIFN-γ–treated IL-25−/− mice on day 20 after infection. Bar, 50 μm. (C) IL-25 is dispensable for resistance to infection with Trichuris when type 1 responses are neutralized. Worm burdens in infected WT and control or αIL-12/αIFN-γ–treated IL-25−/− mice were determined at day 20 after infection. Results are presented as mean ± SEM and represent two independent experiments with three to four mice per group. *, P < 0.01.
IL-25 is required to limit infection-induced intestinal inflammation. WT and IL-25/− mice were infected with Trichuris and allowed to progress to the chronic phase of infection (day 30 after infection). (A) IL-25 is required to inhibit infection-induced pathology. Cecal sections from naive or infected (day 30) AKR, B6 WT, or IL-25/− mice were stained with hematoxylin and eosin. Bar, 50 μm. (B and C) IL-25 limits expression of proinflammatory cytokines in the draining mLN and gut after infection with Trichuris. (B) Increased Trichuris-specific IFN-γ and IL-17 produced by mLN cells from WT or IL-25/− mice at day 18 after infection as measured by CBA (IFN-γ) or ELISA (IL-17). White bars, medium; black bars, Trichuris antigen. (C) Expression of mRNA for IFN-γ and IL-17 in the mLN and cecum of WT and IL-25/− mice at day 30 after infection. (D) IL-10 expression in the mLN is intact in infected IL-25/− mice. mLN cells from WT or IL-25/− mice at day 18 after infection were restimulated with (black bars) or without (white bars) Trichuris antigen and IL-10 levels were determined by CBA. IL-10 mRNA levels in the mLN at day 30 after infection were determined by real-time PCR. Results are presented as mean ± SEM and represent three independent experiments with three to four mice per group. ND, not detected.
BglII site of pbgal-Basic (CLONTECH Laboratories, Inc.). A 1,049-bp 3′ region of homology was generated by PCR using a genomic subclone (C6), incorporating unique SpeI and NotI restriction sites, and cloned into pBluescriptNeo-lex. This plasmid was digested with Sall and the lex-Neo-lex-3′ region of homology was ligated into the unique Sall site of the 5′ arm-pbgal-Basic, yielding the final targeting vector. The Nhel linearized targeting vector was electroporated into C57BL/6 ES cells and Neo-resistant clones were analyzed for HR using a PCR-based screening strategy followed by Southern blot confirmation with 5′ and 3′ probes. A confirmed HR clone was electroporated with a Cre recombinase expression containing plasmid and several Neo-sensitive clones were screened using Southern blot to identify the Neo-flipped HR. IL-25–LacZ ES line. This line was injected into C57BL/6 blastocysts and IL-25–LacZ–knockin (Kl)−/− progeny were obtained by intercrossing IL-25–LacZ–Kl+/− animals. A PCR-based genotyping strategy was developed to track the IL-25 WT and Kl alleles as follows: IL-25 targeted allele, sense 5′-GCTGACTCTCAACATTCATCTTCC-3′, antisense 5′-CTGCTGCTTCAGTAGGCTTG-3′; wild-type IL-25 allele, sense 5′-CTACAGACACGCTCCACCATGOMACC-3′, antisense 5′-CTGCTGCTTCAGTAGGCTTG-3′.

C57BL/6j, AKR, SCID (CBySnNJ CB17-Pkd−/−j), and RAG−/− (B6.129S7-Rag1tm1Jow/J) mice were obtained from The Jackson Laboratory. Animals were maintained in a specific-pathogen-free environment and tested negative for pathogens in routine screening. All experiments were performed following the guidelines of the University of Pennsylvania Institutional Animal Care and Use Committee. Trichuris muris was maintained in genetically susceptible animals. Isolation of Trichuris excretory-secretory antigen and eggs was performed as described previously (14). Mice were infected on day 0 with 150–200 embryonated eggs, and parasite burdens were assessed on various days after infection.

Cytokines and monoclonal antibodies. Monoclonal antibodies against IL-12 (C17.8) and IFN-γ (XMG-6) were purified from ascites by ammonium sulfate precipitation and extensively dialyzed against PBS. Mice were treated i.p. with 500 μg of each antibody every 4 d between days 4 and 20 after infection. IL-12 was synthesized and purified as described previously (11). Mice were treated i.p. with 10 μg of rIL-12 every other day between days 8 and 18 after infection.

mRNA analysis. mRNA expression levels were analyzed using real-time PCR. Total RNA from tissue was isolated, cDNA was prepared, and 10–25 ng of cDNA was used to analyze gene expression in either the SYBR green real-time PCR assay or using predesigned TaqMan probe sets for IL-25, IL-25R, IL-4, IL-13, IL-10, IFN-γ, ubiquitin, and β-actin (Applied Biosystems). Reactions were run on the GeneAmp 5700 Sequence Detection System (Applied Biosystems), and expression was normalized to the housekeeping genes ubiquitin or β-actin.

Cell culture and cytokine and lacZ analysis. At necropsy, the mesenteric LN was 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 × 10−3 M 2-ME. Cells were plated in medium alone or in the presence of Trichuris muris ES Ag (50 μg/ml). Cell-free supernatants were harvested after 48 h, and analyzed for cytokine secretion by sandwich ELISA or cytometric bead array (mouse Th1/Th2 cytokine CBA; BD Biosciences). For determination of lacZ expression, the fluorocent β-galactosidase substrate fluorescein di-β-D-galactopyranoside (FDG; Invitrogen) was added to cells isolated from the mLNs or CP of naive or infected IL-25−/− mice that express lacZ in place of β-galactosidase (50 μg/ml). After 20 min on ice, cells were surface stained with fluorescein-labeled antibodies against CD4, CD8, CCR3, CD11b, CD11c, CD19, FcεRI, and NK1.1 (eBioscience) and analyzed by flow cytometry on a FACS Calibur using CELLQuest Pro software (BD Biosciences).

Serum immunoglobulin analysis. Serum was analyzed by ELISA for Trichuris-specific IgG1 and IgG2a as described previously (19). Total serum IgE was analyzed with an OptElA IgE ELISA kit following the manufacturer’s recommendations (BD Biosciences).

Goblet cell, RELMβ, and mucosal mast cell responses. Segments of cecum were removed and fixed in 4% paraformaldehyde. For detection of intestinal goblet cells, 5-μm paraffin-embedded sections were cut and stained with Alcian blue-periodic acid Schiff's reagent or with hematoxylin and eosin. Isolation of proteins from stool samples was performed as described previously (15). Equal amounts of protein were analyzed by SDS-PAGE and immunoblotted for RELMβ with a polyclonal rabbit α-murine RELMβ antibody. Intestinal mast cells were identified by immunohistochemistry using rat anti-mouse mast cell protease-1 (mMCP-1) antibody as described previously (30). Concentrations of mMCP-1 in serum were quantitated as described previously (30).

Statistics. Results represent the mean ± SEM unless otherwise stated. Statistical significance was determined by Student’s t test.

Online supplemental material. Trichuris-specific serum IgG2a response in AKR mice after infection with Trichuris with or without exogenous rIL-25 is shown in Fig. S1. Fig. S2 depicts the serum immunoglobulin responses (IgG1, IgG2a, and IgE) of wild-type and IL-25−/− mice after infection with Trichuris. Fig. S3 shows the local and systemic mucosal mast cell responses in wild-type and IL-25−/− mice after infection with Trichuris. Fig. S4 shows that blocking IL-12 and IFN-γ in IL-25−/− mice after infection with Trichuris results in decreased serum IgG2a and increased IgG1 and IgE responses. Online supplemental material is available at http://www.jem.org/cgi/content/full/jem.20051496/DC1.

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