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The IL-27 Receptor (WSX-1) Is an Inhibitor of Innate and Adaptive Elements of Type 2 Immunity

David Artis,* Alejandro Villarino,* Michael Silverman,† Weimian He,‡ Elizabeth M. Thornton,¶ Sharon Mu,* Shamin Summer,* Todd M. Covey,* Elaine Huang,* Hiroki Yoshida,† Gary Koretzky,† Michael Goldschmidt,* Gary D. Wu,§ Fred de Sauvage,∥ Hugh R. P. Miller,¶ Christiaan J. M. Saris,* Phillip Scott,* and Christopher A. Hunter*}

Although previous studies have investigated the role of IL-27/WSX-1 interactions in the regulation of Th1 responses, little is known about their role in regulating Th2-type responses. Studies presented in this work identify a direct role for IL-27/WSX-1 interactions in the negative regulation of type 2 responses independent of effects on type 1 cytokines. WSX-1−/− mice infected with the gastrointestinal helminth Trichuris muris displayed accelerated expulsion of parasites and the development of exaggerated goblet cell hyperplasia and mastocytosis in the gut due to increased production of Th2 cytokines. Enhanced mast cell activity in the absence of WSX-1 was consistent with the ability of wild-type mast cells to express this receptor. In addition, IL-27 directly suppressed CD4⁺ T cell proliferation and Th2 cytokine production. Together, these studies identify a novel role for IL-27/WSX-1 in limiting innate and adaptive components of type 2 immunity at mucosal sites. The Journal of Immunology, 2004, 173: 5626–5634.

The differentiation and expansion of naive CD4⁺ T cells into effector cell populations producing either Th1 (IFN-γ) or Th2 cytokines (IL-4, IL-5, IL-9, and IL-13) are influenced, in large part, by the cytokine environment in which T cell priming occurs (1). IL-12 is a critical cytokine that promotes the development of Th1-type responses, and recent studies have identified new cytokines that are closely related to IL-12 in both sequence and structure. One example is IL-27, a heterodimeric cytokine composed of EBI3, an IL-12p40-like soluble receptor, and IL-27p28, an IL-12p35-related helical protein. Like IL-12, IL-27 is produced by accessory cells following exposure to inflammatory stimuli (2). The receptor complex for IL-27 includes WSX-1, a class I cytokine receptor with homology to the IL-12R (3–5), and gp130, the common receptor chain used by several cytokines (6–8). In addition to the structural homology between IL-12/IL-12R and IL-27/IL-27R, there are also functional similarities. IL-27, like IL-12, can enhance production of IFN-γ by naive Th cells and NK cells (2). Moreover, while the IL-12R plays a critical role in the expansion of Th1 cells (9), it has been reported that IL-27/WSX-1-deficient T cells express lower levels of T-bet and IFN-γ (4, 5, 10). Furthermore, we recently demonstrated that in a nonpolarized cytokine environment that develops following exposure to Leishmania major infection, WSX-1 is critical in promoting the development of Th1 cells (11). As a consequence of these studies, a consensus has emerged that IL-27/WSX-1 is an important pathway for the generation of optimal type 1 responses (1, 12–14).

The complex biology of IL-27/WSX-1 interactions was illustrated by recent studies that demonstrated that this receptor can also deliver critical inhibitory signals to limit the magnitude and duration of type 1-mediated inflammatory responses. For instance, WSX-1−/− mice infected with the intracellular pathogen Toxoplasma gondii generated a robust type 1 response, but failed to regulate the intensity of effector T cell responses. Thus, hyperactive CD4⁺ T cells led to a lethal inflammatory disease during acute toxoplasmosis (15). Similarly, WSX-1 is a negative regulator of Th cell-dependent inflammation in an experimental model of hepatitis (16) and following infection with Trypanosoma cruzi (17). Although these studies have collectively demonstrated that IL-27/WSX-1 signaling can either promote or inhibit type 1 responses depending on the cytokine environment, there is little known about the role of WSX-1 in regulating type 2-mediated immunity. To address this question, we used infection with the intestinal-dwelling helminth pathogen Trichuris muris. This natural parasite of mice provides a well-characterized model with which to investigate the regulation of type 2 cytokine-dependent immunity. Chronic infection with T. muris is promoted by type 1 cytokines (IL-12, IL-18, and IFN-γ), whereas expulsion of parasites requires production of CD4⁺ T cell-dependent type 2 cytokines, including IL-4 and IL-13 (18–22). In common with other intestinal helminths, expulsion of T. muris is associated with goblet cell hyperplasia and mastocytosis in the intestinal epithelium and infection outcome provides a phenotypic and functional readout of type 2 immunity at the mucosa (23, 24). Following infection with T. muris, WSX-1−/− mice exhibited accelerated expulsion of larval...
parasites. Although Trichuris-specific IFN-γ responses were normal in WSX-1−/− mice, T cells isolated from these mice produced significantly higher levels of Th2 cytokines. In addition, infected WSX-1−/− mice exhibited increased goblet and mast cell activity, both innate components of type 2 immunity in the gut. Because mast cells were found to express WSX-1 and exhibited enhanced responses in the absence of this receptor, it is likely that IL-27 signaling regulates mast cell function. In vitro assays also demonstrated that IL-27/WSX-1 interactions directly inhibited CD4+ Th2 cell functions. Taken together, these studies identify a novel IFN-γ-independent role for IL-27/WSX-1 in limiting innate and adaptive components of mucosal type 2 responses, and suggest that IL-27/WSX-1 interactions may be a target for the treatment of inflammatory conditions associated with aberrant type 2 cytokine production.

Materials and Methods

Animals

Mice deficient in WSX-1 were generated, as previously described (5), and provided by C. Saris (Angen, Thousand Oaks, CA). WSX-1−/− mice were bred and maintained as homozygotes in a specific pathogen-free environment at the University of Pennsylvania. Mice deficient in IFN-γ (25) were purchased from The Jackson Laboratory (Bar Harbor, ME). Age- and sex-matched C57BL/6 mice (The Jackson Laboratory) were used as controls. In all experiments, mice were infected at 5–8 wk of age, and experimental groups contained three to five animals. All experiments were conducted following the guidelines of the University of Pennsylvania Institutional Animal Care and Use Committee.

T. muris infection and Ag

T. muris was maintained in genetically susceptible or immunocompromised animals. Between days 35 and 42 postinfection, adult worms were isolated and cultured in RPMI 1640 containing 500 U/ml penicillin and 500 μg/ml streptomycin for 24 h. T. muris excretory-secretory Ag was isolated at 8 and 24 h, dialyzed, and sterile filtered, and protein concentrations were determined by Bradford assay. Ag preparations were then used in lymphocyte restimulations (50 μg/ml). Deposited eggs were collected after 24 h of culture, washed three times in sterile water, incubated at room temperature for 6 wk, and stored at 4°C. Mice were infected on day 0 with 150–200 embryonated eggs, and parasite burdens were assessed on various days postinfection.

Detection of IL-27 and WSX-1 mRNA

IL-27 and WSX-1 levels were determined by RT-PCR. mRNA was isolated from whole mesenteric lymph node (LN)3 cell suspensions using TRIzol (Invitrogen Life Technologies, Carlsbad, CA) and reversed transcribed using Superscript II (Invitrogen Life Technologies), and PCR was performed (34 cycles: 95°C, 30 s/60°C, 30 s/72°C, 1 min) to detect IL-27p28 (primers: 5′-CTGTTCAACGTCGTTTCTTG, 5′-CTCCAGGGAGTAAAGAGCT), Eβ3 (primers: 5′-CCAGATGCGACTGCATGCTTCT-3′, 5′-CTGTTAGGCCTGAGCTGAC), and WSX-1 (primers: 5′-CAAGAAGGCTCCGTTGCTG, 5′-TTAGGGCCGATCCACACAT). Levels of β-actin transcripts were equalized to allow a semiquantitative comparison between samples.

In vivo depletions

Neutralizing anti-IL-12 mAb (C17.8) (2 mg per dose), anti-IFN-γ mAb (XMG.6) (2 mg per dose), and anti-IL-4 mAb (11B11) (4 mg per dose) were administered i.p. on days 0, 4, 8, and 12 postinfection. All Abs were generated from ascites grown by Harlan Bioscience (Madison, WI). Control mice received equivalent amounts of purified rat IgG (Sigma-Aldrich, St. Louis, MO).

Analysis of T. muris-specific cytokine responses

Mesenteric LN cell suspensions were prepared and resuspended in RPMI 1640 supplemented with 10% heat-inactivated FBS, 100 U/ml penicillin, 100 μg/ml streptomycin, nonessential amino acids, and 2-ME. Cells were plated at 4 × 106 cells/well and cultured alone or in the presence of T. muris Ag (50 μg/ml) for 72 h. Levels of IL-4, IL-5, IL-13, and IFN-γ were assayed by either sandwich ELISA or intracellular cytokine staining (see below). For detection of intracellular IL-12 IFN-γ after 14 days of infection, cells were also stimulated with anti-CD3 and anti-CD28 (both 1 μg/ml; BD Pharmingen, San Diego, CA) in the presence of rIL-4 (50 ng/ml; eBioscience) and anti-IL-12 mAb (C17.8: 10 μg/ml). After 48 h, cells were pulsed with PMA (50 ng/ml; Sigma-Aldrich), ionomycin (500 ng/ml; Sigma-Aldrich), and brefeldin A (10 μg/ml; Sigma-Aldrich) for 3–5 h, and stained for intracellular IFN-γ (eBioscience, San Diego, CA) in combination with surface CD4 (eBioscience). Cells were acquired on a FACSCalibur cytometer, and analyzed using CellQuest software (BD Biosciences, San Jose, CA).

In vitro differentiation assays

Splenocytes were isolated from naïve animals, depleted of CD8+ T cells and NK1.1+ cells by magnetic bead separation (BD Pharmingen), labeled with CFSE (Molecular Probes, Eugene, OR; 5 μg/ml), and cultured for 3–4 days. Cells were stimulated with soluble anti-CD3 mAb (0.1 μg/ml), soluble anti-CD28 mAb (0.5 μg/ml), and rIL-2 (10 IU/ml; Chiron, Emeryville, CA) under Th2-polarizing conditions (rIL-4 (eBioscience; 50 ng/ml), anti-IL-12 mAb (10 μg/ml), and anti-IL-10 (10 μg/ml)). In some studies, murine rIL-27 (200 ng/ml; Genentech, South San Francisco, CA) was added to cultures in the presence of rIL-4 (eBioscience; 50 ng/ml), anti-IL-12 (10 μg/ml), and anti-IFN-γ mAb (10 μg/ml). For detection of intracellular IL-4, cells were pulsed as above, stained with fluorochrome-labeled anti-CD4 and anti-IL-4 mAb (eBioscience), and analyzed by flow cytometry. For secondary stimulations, cells were harvested at day 4, washed, and restimulated with anti-CD3 for 48 h under neutral conditions. Secreted levels of IL-4, IL-5, and IL-13 were determined by ELISA.

Analysis of goblet cell and resistin-like molecule (RELM)β responses

Segments of midcecum were removed, washed in sterile PBS, and fixed for 24 h in 10% neutral buffered formalin. Tissues were processed routinely and paraffin embedded using standard histological techniques. For detection of intestinal goblet cells, 5-μm sections were cut and stained with H&E or Alcian blue-periodic acid Schiff. Enumeration of intestinal goblet cell hyperplasia was conducted by counting numbers of goblet cells per 100 crypt units. The anti-murine RELMβ Ab, its use in immunoblotting and immunohistochemistry, as well as the conditions used to isolate stool proteins have been described previously (26).

Analysis of mast cell responses

Cecal tissue was fixed in 4% paraformaldehyde, and numbers of intestinal mast cells per 100 cecal crypts were enumerated by immunohistochemistry using rat anti-mouse mast cell protease-1 (mMCP-1) Ab (RF6.1), as previously described (27). Concentrations of mMCP-1 in serum were quantified using the rat monoclonal RF 6.1-based ELISA with modifications (27).

For analysis of WSX-1 expression, primary mouse mast cells were generated ex vivo from C57BL/6 bone marrow. Whole bone marrow was plated in mast cell growth medium (IMDM, 15% fetal clone II serum supplemented with 100 μg/ml penicillin; 500 μg/ml streptomycin; 1% penicillin/streptomycin/glutamine (Invitrogen Life Technologies), 5 ng/ml murine IL-3, and 80 ng/ml rat stem cell factor), and nonadherent cells were transferred to new plates at 5 × 103 cells/ml and supplemented with 50% fresh medium twice weekly. Differentiation was monitored by surface staining for c-Kit. Homogeneous c-Kit+ cultures were typically obtained after 5–6 wk of culture. For immunoprecipitation, 9 × 106 mast cells or purified splenic CD4+ T cells were lysed on ice in buffer containing 1% Nonidet P-40, 0.25% deoxycholate, 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, and 1% (v/v) protease inhibitors (Sigma-Aldrich). Cell lysates were cleared and preabsorbed with a 1:1 mix of protein A-Sepharose and protein G-Sepharose. Lysates were then incubated with rabbit anti-WSX-1 peptide antiserum (5), followed by addition of protein A/G-Sepharose for 30 min at 4°C. Washed beads were resuspended in sample buffer and subjected to SDS-PAGE, transferred to a polyvinylidene difluoride membrane, incubated with rabbit anti-WSX-1 peptide antiserum plus HRP-conjugated protein A, and visualized using ECL (Amersham Biosciences, Arlington Heights, IL), according to the manufacturer’s instructions.

Passive cutaneous anaphylaxis

Mice were sensitized with 25 ng of anti-DNP IgE in 25 μl of PBS by intradermal injection into the base of the dorsal aspect of the right ear. As
a control, 25 μl of PBS was injected into base of the dorsal aspect of the left ear. Twenty-four hours later, mice were challenged with 100 μg of DNP-human serum albumin (HSA) in 1% Evans blue (Sigma-Aldrich) by i.v. retro-orbital injection. Thirty minutes after challenge, mice were euthanized, ears were collected and incubated at 55°C for 48 h in formamide, and OD (610 nm) was determined to quantify Evans blue extravasation as a measure of mast cell-dependent anaphylaxis. For histological analysis, ear tissue was fixed in 10% formalin, paraffin-embedded sections were stained with toluidine blue, and number of mast cells in 10 high power fields (>100) per mouse was determined.

**Statistical analysis**

Significant differences (p < 0.05) between experimental groups were determined by Student’s t test.

**Results**

*Infection with T. muris leads to increased IL-27 mRNA expression*

Previous studies have shown that infection with the protozoan pathogen *T. gondii* or LPS stimulation of macrophages and DC leads to the up-regulation of IL-27 mRNA (2, 15). However, little is known about expression of IL-27/WSX-1 during type 2 immunity. Therefore, C57BL/6 mice, a genetically resistant strain, were challenged with *T. muris*, and the levels of mRNA for WSX-1, EBI3, and IL-27p28 in the draining mesenteric LN were assayed by RT-PCR. Although expression of WSX-1 and EBI3 was constitutive, there was a marked increase in the levels of IL-27p28 mRNA at days 7 and 14 postinfection (Fig. 1, A and B). These data demonstrate that IL-27 is induced following *T. muris* infection, and suggest this cytokine may play a role in the regulation of the immune response to helmint parasites.

WSX-1<sup>−/−</sup> mice exhibit accelerated expulsion of *T. muris* mediated by enhanced production of Th2 cytokines

To assess the role of IL-27/WSX-1 interactions in immunity to *T. muris*, wild-type (WT) and WSX-1<sup>−/−</sup> mice were infected, and the parasite burden was measured at various times postinfection. Larval development and establishment of infection were comparable in WT and WSX-1<sup>−/−</sup> mice at day 11 postinfection (WT, 121 ± 34; WSX-1<sup>−/−</sup>, 108 ± 17). However, by day 14 postinfection, WT mice retained high numbers of *T. muris*, whereas WSX-1<sup>−/−</sup> mice had expelled larval parasites (Fig. 1C). At 21 days postinfection, WT mice also expelled their parasites, consistent with previously published studies (28). To determine whether accelerated resistance to *T. muris* in WSX-1<sup>−/−</sup> mice was associated with increased Th2-type cytokine responses, mesenteric LN cells were isolated from WT and WSX-1<sup>−/−</sup> mice at day 14 postinfection, and parasite-specific cytokine production was analyzed. Consistent with previous studies, levels of *Trichurus*-specific cytokine production were undetectable or low in naive WT and WSX-1<sup>−/−</sup> mice (data not shown). However, lymphocytes from infected WSX-1<sup>−/−</sup> mice secreted significantly higher levels of IL-4, IL-5, and IL-13 (Fig. 1, D–F) (<p>0.05) compared with those from WT mice. Furthermore, flow cytometric analysis demonstrated that although the number of Ag-reactive cells was low, there was a higher frequency of IL-4<sup>+</sup> CD4<sup>+</sup> T cells in Ag-restimulated WSX-1<sup>−/−</sup>-lymphocyte cultures at day 14 postinfection (data not shown).

To determine whether enhanced resistance to *T. muris* observed in WSX-1<sup>−/−</sup> mice was dependent on increased production of Th2 cytokines, infected WSX-1<sup>−/−</sup> mice were treated with anti-IL-4 mAb, and infection and immunological outcome were determined after 14 days. Blockade of IL-4 significantly reduced the production of *T. muris*-specific IL-4 and IL-13 following restimulation of mesenteric LN cells (Table I) (<p>0.05). Furthermore, significantly higher numbers of larval *T. muris* were detected in anti-IL-4 mAb-treated WSX-1<sup>−/−</sup> mice (Table I) (<p>0.05), demonstrating that in the absence of WSX-1, elevated production of Th2 cytokines mediates enhanced resistance to infection.

**Enhanced goblet cell responses in the absence of WSX-1**

The protective Th2-type responses required for resistance to *T. muris* are associated with intestinal goblet cell hyperplasia, an innate component of type 2 immunity at mucosal sites (22). In uninfected WT and WSX-1<sup>−/−</sup> mice, numbers of intestinal goblet cells were similar (Fig. 2, A and B). By day 14 postinfection, WSX-1<sup>−/−</sup> mice exhibited significantly elevated intestinal goblet cell hyperplasia and increased mucin production compared with infected WT animals (Fig. 2, C and D; enumerated in Fig. 2E) (<p>0.05). Furthermore, when the expression of RELMβ, a novel goblet cell-specific protein that is induced by Th2 cytokines (26), was examined, Western blot analysis revealed that secreted levels of RELMβ in fecal pellets were markedly enhanced in *T. muris*-infected WSX-1<sup>−/−</sup> mice (Fig. 2F). In accord with this finding, immunohistochemical analysis demonstrated a higher frequency of RELMβ<sup>+</sup> goblet cells in infected WSX-1<sup>−/−</sup> mice when compared with WT cohorts (Fig. 2, G–J). Together, these studies demonstrate that in the absence of WSX-1, goblet cell responses are enhanced following intestinal helmint infection.

**Table I. IL-4 is required for helmint resistance in WSX-1<sup>−/−</sup> mice**

<table>
<thead>
<tr>
<th>IL-4 (U/ml)</th>
<th>7.0 ± 0.9</th>
<th>2.2 ± 0.6&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-13 (pg/ml)</td>
<td>303.9 ± 43.0</td>
<td>151.7 ± 88.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Worm burden</td>
<td>0</td>
<td>77 ± 10&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>WSX-1<sup>−/−</sup> mice were infected with *T. muris* and treated with control Ab or anti-IL-4 mAb. After 14 days of infection, worm burdens were determined by microscopy and Ag-specific cytokine responses were assayed by ELISA (*p < 0.05, n = 4).
WSX-1 is a negative regulator of mast cell responses

Intestinal mastocytosis is also a hallmark of type 2 cytokine-dependent innate immunity in the gastrointestinal tract. To analyze intestinal mastocytosis, cecal sections were immunohistochemically stained with an Ab specific for mMCP-1. mMCP-1 is a granule chymase that is predominantly expressed by intestinal mouse mast cells, and serum levels of this protein provide an indication of mast cell degranulation in the gut (27). Although no intestinal mastocytosis nor serum mMCP-1 was detected in naive WT or WSX-1 mice, it was unclear whether this was a significant decrease in mast cell reactivity to this infection.

Although enhanced mast cell responses were observed in T. muris-infected WSX-1 mice, it was unclear whether this was a significant decrease in mast cell reactivity to this infection.
consequence of enhanced production of type 2 cytokines or whether WSX-1 directly regulates mast cell activity. To investigate this issue, expression of WSX-1 on murine bone marrow-derived mast cells was determined. Immunoprecipitation revealed that both CD4⁺ T cells and mast cells expressed WSX-1 (Fig. 3C), consistent with a recent study demonstrating that human mast cells express this receptor (8). To test whether WSX-1 directly regulates mast cell activity, an in vivo model of mast cell-dependent passive cutaneous anaphylaxis was used (29, 30). In these studies, WT and WSX-1⁻/⁻ mice were primed with intradermal injections of anti-DNP-IgE. Twenty-four hours later, mice were challenged with cognate Ag (DNP-HSA), and extravasation of Evans blue dye was used as indication of mast cell-dependent vascular permeability. Although there was no difference in the numbers of mast cells in the tissue of naive WT and WSX-1⁻/⁻ mice (WT, 5.02 ± 0.95; WSX-1⁻/⁻, 5.00 ± 0.96; mean number of mast cells (±SD) per 10 high power fields, n = 5), WSX-1⁻/⁻ mice exhibited enhanced mast cell responses following Ag challenge. Therefore, administration of DNP-HSA led to the extravasation of Evans blue in WT mice, but these levels were significantly increased in the absence of WSX-1 (Fig. 3D) (p < 0.05). These studies provide the first indication of a direct inhibitory role for WSX-1 signaling in the regulation of mast cell responses in vivo.

Enhanced production of Th2 cytokines and rapid expulsion of T. muris in WSX-1⁻/⁻ mice are independent of a defect in IFN-γ production

The studies presented above demonstrate that WSX-1⁻/⁻ mice develop enhanced resistance to T. muris, but these findings do not address the mechanisms that underlie the elevated Th2 responses. Because IL-27/WSX-1 can promote the production of IFN-γ under nonpolarizing conditions (4, 5, 10, 11), it was possible that a primary defect in IFN-γ production could lead to unopposed Th2 differentiation in T. muris-infected WSX-1⁻/⁻ mice. However, at 14 days postinfection, T. muris Ag-stimulated mesenteric LN cells produced equivalent levels of IFN-γ in WT and WSX-1⁻/⁻ T cell cultures (Fig. 4A). Moreover, polyclonal stimulation of mesenteric LN cells from these animals revealed that there was no intrinsic defect in the ability of WSX-1⁻/⁻ CD4⁺ T cells to produce IFN-γ when compared with WT controls (Fig. 4B). These findings demonstrated that early IFN-γ production is intact in WSX-1⁻/⁻ mice infected with T. muris, and are consistent with recent reports that have identified WSX-1-independent Th1 responses in vivo (11, 15, 17).

Nevertheless, to directly address whether reduced Th1 responses could contribute to accelerated type 2-mediated immunity observed in infected WSX-1⁻/⁻ mice, WT and WSX-1⁻/⁻ animals were treated with anti-IL-12 plus anti-IFN-γ mAb before and following T. muris infection. At day 14 postinfection, analysis of parasite burden and cytokine responses demonstrated that administration of anti-IL-12/anti-IFN-γ to WT mice did not result in accelerated expulsion of parasites as is the case in control-treated WSX-1⁻/⁻ mice (Fig. 5A). However, small increases in the production of Th2 cytokines observed in WT and WSX-1⁻/⁻ mice (Fig. 5, B and C) indicated the presence of functional Th1 response in both experimental groups. Critically, the levels of Th2 cytokines produced by anti-IL-12/anti-IFN-γ-treated WT mice were still significantly lower than those observed in WSX-1⁻/⁻ mice (Fig. 5, B

**FIGURE 5.** Enhanced type 2 responses in T. muris-infected WSX-1⁻/⁻ mice are independent of a defect in IFN-γ production. WT and WSX-1⁻/⁻ mice were infected with T. muris and treated with control or neutralizing anti-IL-12 and anti-IFN-γ mAb (2 mg/dose) on days 0, 4, 8, and 12 postinfection. After 14 days of infection, parasite burdens were assessed (A), and levels of IL-4 (B) and IL-13 (C) were secreted by T. muris Ag-stimulated mesenteric LN cells determined by ELISA. Histological sections of midcecum were stained for detection of intestinal goblet cells, and the number of goblet cells per 100 crypt units was enumerated under light microscopy (D). Representative sections stained for goblet cells from infected WT (F), WT + anti-IL-12/anti-IFN-γ (G), or WSX-1⁻/⁻ mice (H) are shown. Levels of secreted RELMβ in fecal pellets were determined by Western blotting (E). Values represent mean ± SEM, and are representative of two to three independent experiments. * Significant difference between anti-IL-12 and anti-IFN-γ mAb-treated WT and anti-IL-12 and anti-IFN-γ mAb-treated WSX-1⁻/⁻ mice (p < 0.05).
Inhibition of Th2 cell responses by IL-27/WSX-1 in vitro

To determine whether IL-27/WSX-1 interactions can directly regulate Th2 cell function, naive WT and WSX-1−/− CD4+ T cells were stimulated in vitro under Th2-polarizing conditions, and CD4+ T cell proliferation and cytokine production were assayed. Polyclonal stimulation was associated with increased levels of IL-27 mRNA in these cultures and sustained expression of WSX-1 mRNA in WT cells (data not shown), confirming the presence of functional IL-27/WSX-1 signaling in these cultures. After 3 days of primary stimulation, there were equivalent frequencies of WT and WSX-1−/− CD4+ T cells producing IL-4 (Fig. 6, A and B). However, upon secondary stimulation of these cells, production of IL-5 (Fig. 6C) and IL-13 (Fig. 6D), robust markers of Th2 cell differentiation, was significantly higher in WSX-1−/− T cell cultures (p < 0.05), while IL-4 was undetectable in WT or WSX-1−/− cultures. Associated with increased production of Th2 cytokines, WSX-1−/− CD4+ T cells exhibited enhanced proliferative responses. After 3 days of primary stimulation, there were increased numbers of WSX-1−/− CD4+ T cells present in the first to the fourth proliferative generations (Fig. 6, E and F). By day 4 poststimulation, the enhanced proliferative responses of WSX-1−/− CD4+ T cells over WT controls were more pronounced, with a marked increase in the percentage of WSX-1−/− T cells observed in the fifth and sixth generations (Fig. 6, F and H). Furthermore, the CD4+ T cell responder frequency, an indicator of the percentage of cells that have responded to antigenic stimulation by infected WSX-1−/− mice. Together, these studies suggest that the enhanced type 2 responses observed in the WSX-1−/− mice infected with *T. muris* are not a secondary consequence of intrinsic defects in IFN-γ production; rather, they are due to a direct inhibitory role for WSX-1 in limiting the magnitude and kinetics of type 2 immunity.
undergoing at least one proliferative cycle, was higher in WSX-1−/− compared with WT cultures (WT, 34%; WSX-1−/−, 46%). Differences in CD4+ T cell proliferation and Th2 cytokine production were not a reflection of increased numbers of precultivated T cells in naive WSX-1−/− cultures, as the frequencies of naive (CD62Lhigh, CD25low) vs activated (CD62Llow, CD25high) CD4+ T cells were similar in the mesenteric LN and spleen of WT and WSX-1−/− mice (data not shown).

The above studies demonstrated that the absence of WSX-1 can lead to enhanced CD4+ T cell proliferation and elevated production of Th2 cytokines. Consistent with these data, addition of rIL-27 to WT T cell cultures led to a 30% reduction in the frequency of CD4+ T cells producing IL-4 (Fig. 7A). Critically, the inhibition of Th2 responses, although not as dramatic as that observed in vivo, was not associated with reduced T cell proliferation (Fig. 7B), suggesting that in addition to shared functions, IL-27 and WSX-1 may have independent roles in the regulation of T cell proliferation vs the ability to express effector cytokines. Taken together, these in vitro studies demonstrate that IL-27/WSX-1 interactions can directly inhibit CD4+ Th2 effector cell proliferation and function, and provide a likely basis for the enhanced Th2 responses and accelerated resistance observed in T. muris-infected WSX-1−/− mice.

Discussion

The data presented in this work provide the first demonstration of a direct role for IL-27/WSX-1 interactions in negatively regulating innate and adaptive type 2 responses. We and others have previously demonstrated that in a nonpolarized cytokine milieu, IL-27/WSX-1 signaling can promote IFN-γ production and type 1-mediated immunity (4, 5, 11, 15, 31). For instance, following infection with L. major, which is characterized by the development of a mixed Th1/Th2 cytokine response, WSX-1 expression is required to promote optimal IFN-γ production, most likely via STAT1-mediated induction of IFN-γ (10). However, blockade of IL-4 extinguishes the requirement for WSX-1 in promoting Th1 cell differentiation and immunity to L. major, demonstrating that the requirement for WSX-1 in type 1 immunity is restricted to a nonpolarized cytokine environment (11). Consistent with this proposed pathway, previous studies have shown that STAT1-dependent Th1 cell development is regulated by the presence of IL-4 (32). Given this, it was possible that enhanced type 2 responses and resistance to T. muris in WSX-1−/− mice were the result of a defect in Th1 responses. Indeed, the initial finding that WSX-1−/− mice displayed accelerated expulsion of T. muris was consistent with this model. However, three key observations establish that the enhanced type 2 responses in WSX-1−/− mice are not a secondary consequence of defective IFN-γ production, but a function of enhanced type 2 responses in the gastrointestinal tract. First, infected WSX-1−/− mice did not have an early defect in IFN-γ production following Trichuris-specific and polyclonal restimulation of T cells. Second, blockade of IL-4 inhibited type 2 responses and early worm expulsion in WSX-1−/− mice, demonstrating that unregulated type 2 responses elicit accelerated resistance to infection in the absence of WSX-1. Finally, blockade of type 1 responses in infected WT mice did not recapitulate the enhanced type 2 responses and accelerated expulsion of T. muris observed in WSX-1-deficient animals. Together with in vitro studies that demonstrated that WSX-1−/− CD4+ T cells exhibited enhanced proliferation, increased production of Th2 cytokines, and that exogenous IL-27 could down-regulate the production of IL-4 by WT CD4+ T cells, these results identify a novel role for IL-27/WSX-1 as a direct inhibitor of Th2 cell responses. Therefore, unlike nonpolarized cytokine environments in which WSX-1 can promote Th1 cell development, these studies identify a new role for IL-27/WSX-1 in limiting type 2-mediated inflammatory responses.

In a recent study using a low dose inoculum of T. muris, it was reported that while WT B6 mice developed Th1 responses and were susceptible to chronic trichuriasis, mice deficient in WSX-1 exhibited reduced IFN-γ responses and were more resistant to infection (33). These results are consistent with cytokine environment-dependent functions for IL-27/WSX-1 interactions, i.e., a low dose infection may generate a nonpolarized cytokine environment in which WSX-1 is important for promoting optimal Th1 cell differentiation. In contrast, higher doses of infection used in the present study promote polarized type 2 cytokine responses in which the inhibitory functions of IL-27/WSX-1 dominate. Alternatively, the failure to establish chronic infections in WSX-1−/− mice in a low dose model may be due to the development of rapid and enhanced type 2 responses that precipitate early worm expulsion, similar to the outcome observed in the present study. Consistent with this hypothesis, WSX-1−/− mice exhibit enhanced IL-4 and IL-13 responses compared with WT mice following low or high dose challenge (33). Reduced IFN-γ production in the low dose model may therefore be a secondary consequence of enhanced production of type 2 cytokines. The recent in vitro study by Lucas et al. (34), demonstrating that IL-27 can directly inhibit transcription of GATA3, a critical transcription factor for expression of Th2 cytokines, provides a likely molecular mechanism underlying some of the biological functions of WSX-1 described in this work.

In combination with previous studies demonstrating that following induction of hepatitis or exposure to T. gondii or T. cruzi, two intracellular pathogens that elicit polarized type 1 cytokine responses, WSX-1 is a critical negative regulator of CD4+ Th1 cell functions (15–17), the data presented in this work establish that the IL-27/WSX-1 interaction can deliver inhibitory signals to limit type 1- and type 2-mediated responses in vivo. A possible mechanism underlying the ability of WSX-1 to suppress T cell function is provided by the observation that in multiple systems WSX-1−/− T cells are hyperproliferative. The ability of naive T cells to acquire an effector phenotype requires passage through the cell cycle (35), and therefore, the increased proliferation of WSX-1−/− T cells is likely to contribute to their ability to produce accelerated type 1 and type 2 inflammatory responses in vivo. The enhanced proliferation of WSX-1−/− T cells may be a function of improved cell survival, accelerated cell cycle progression, or increased sensitivity to growth factors such as IL-2. Furthermore, the recognition that IL-27 can activate STAT1 and STAT3 (10, 15, 36), two transcription factors that are associated with the prevention of immune hyperactivity (37–39), suggests that a defect in these signaling pathways could lead to increased T cell proliferation and cytokine production in WSX-1−/− T cells. Whether WSX-1-mediated STAT activation directly inhibits Th2 cell function or requires downstream trans factors is unknown. One pathway that may provide a mechanism for the inhibitory effects of STAT proteins is their ability to induce expression of suppressor of cytokine signaling (SOCS) proteins that limit JAK/STAT-mediated signaling. Relevant to the studies presented in this work, SOCS-1, SOCS3, and SOCS5 are known to regulate the magnitude of type 2 cytokine production (40–43), and may provide a link between WSX-1 signaling and negative regulation of type 2 responses in the gastrointestinal tract.

To date, most studies on IL-27/WSX-1 have focused on the role of this cytokine/receptor interaction in the regulation of T cell functions. The findings reported in this work demonstrate that murine WT mast cells also express high levels of WSX-1 and that
mast cell responses in WSX-1−/− mice are enhanced during infection with *T. muris* as well as in a model of passive cutaneous anaphylaxis. Indeed, the results presented in this work provide the first in vivo evidence that IL-27/WSX-1 interactions directly regulate mast cell function. While the present studies were in progress, Kastelein and colleagues (8) reported that macrophages, dendritic cells, and mast cells express the IL-27R, and that stimulation of primary human mast cells with IL-27 activates STAT3. Therefore, it is now clear that lymphoid and nonlymphoid cell types express a functional IL-27R (i.e., WSX-1 and gp130), and that the functions of this dimeric receptor are not restricted to the regulation of T cell activity. The recognition that gp130, a receptor chain that has been linked to the suppression of inflammation (44, 45), forms part of the IL-27R complex supports an inhibitory role for IL-27. Thus, IL-27/IL-27R interactions may provide a general mechanism to down-regulate immune activity in multiple hematopoietic cell types.

The data presented in this work also highlight the paradoxical nature of IL-27/WSX-1 signaling. Specifically, we and others have found that IL-27 can enhance T cell proliferation, whereas WSX-1−/− T cells are hyperproliferative (2, 5, 15). Similarly, the in vitro Th2 cell differentiation assays used in this study reveal that naive WT and WSX-1−/− CD4+ T cells generate a similar frequency of IL-4 producers, while exogenous IL-27 can suppress the number of IL-4+ CD4+ T cells. These contradictory findings indicate that the biology of IL-27 and its receptor is more complex than current models suggest. This complexity is further illustrated by reports that EBI3, a component of IL-27, is required for the development of invariant NKT cells, whereas WSX-1 is not (17, 31) (our unpublished observations). Together, these findings suggest that the different IL-27R components mediate different functional activities (i.e., proliferation vs. acquisition of effector functions) or that additional ligands for WSX-1 are involved in the differential regulation of these activities. For example, previous studies have reported that EBI3 and IL-12p35 can dimerize, but no function has been ascribed to this potential cytokine (46). It will be critical to understand how IL-27, or related cytokines, interact with WSX-1 and gp130 to differentially regulate multiple immune cell types in the context of type 2 immunity. Nonetheless, the identification of IL-27, or related cytokines, interacting with WSX-1 and their cognate receptors. *Immunity* 19:159.


