IL-27 Suppresses Th2 Cell Development and Th2 Cytokines Production from Polarized Th2 Cells: A Novel Therapeutic Way for Th2-Mediated Allergic Inflammation

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IL-27 Suppresses Th2 Cell Development and Th2 Cytokines Production from Polarized Th2 Cells: A Novel Therapeutic Way for Th2-Mediated Allergic Inflammation\(^1\)

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IL-27 up-regulates Th1 but down-regulates Th2 responses. However, its molecular mechanism and regulatory effects on polarized Th2 cells remain unclear. In this study, we have revealed that IL-27 inhibits Th2 cell development as well as Th2 cytokines production from already polarized Th2 cells by down-regulation of GATA-3 and up-regulation of T-bet expression simultaneously. In vivo daily IL-27 treatment for 1 wk after Leishmania major infection protects BALB/c mice from footpad swelling by diminishing parasite burden via reciprocal regulation of Th1 and Th2 responses. Furthermore, IL-27 stimulation causes marked reduction in the capacity of host mouse to mount a Th2 response against Strongyloides venezuelensis infection. Thus, IL-27-treated mice failed to develop intestinal mastocytosis after S. venezuelensis infection and exhibited a marked delay in parasite expulsion. Finally, intranasal administration of IL-27 inhibits OVA-induced airway hyperresponsiveness and inflammation in OVA-sensitized animals. Thus, IL-27 could provide us with a novel therapeutic way for treating Th2-associated diseases such as bronchial asthma. The Journal of Immunology, 2007, 179: 4415–4423.

The differentiation of naive CD4\(^+\) T cells into Th1 or Th2 cells is a critical process in the development of adaptive immune responses (1). Th1 cells mediate cellular immunity by production of IFN-\(\gamma\), which is critical for eradication of intracellular pathogens. In contrast, Th2 cells produce IL-4, IL-5, and IL-13, which in combination induce humoral immunity, allergic inflammation, and promote host resistance, particularly to intestinal nematodes (2, 3). Although many factors influence the developmental process of CD4\(^+\) T cells into Th1 or Th2 cells, cytokines have emerged as key determinants of the outcome (1). IL-12, a heterodimer of the p40 and p35 subunits, induces IFN-\(\gamma\)-producing Th1 cells through activation of Stat4 (4, 5). IFN-\(\gamma\) signal, transduced by Stat1, activates a downstream transcription factor, T-bet, that enhances the expression of genes specific to Th1 cells (6). Then, IL-18 acts on IL-18R-expressing Th1 cells to induce GATA-3 activation (9, 10), promoting the expression of GATA-3, the transcription factor essential for both IL-4 production and Th2 cell polarization (11).

Recently, a novel member of the IL-12 family, IL-27, has been identified (12). IL-27, a heterodimeric cytokine produced by activated APCs, is composed of IL-12 p40-related protein, EBV-induced gene 3 (EBI3),\(^3\) and newly discovered IL-12 p35-related protein, p28 (12, 13). IL-27R consists of IL-27Ra chain (WSX-1/TCCR), homologous to IL-12R\(\beta\)2, and gp130, a common subunit of the IL-6R family (14, 15). IL-27 induces naive CD4\(^+\) T cells to proliferate and to express T-bet and subsequently exhibit IL-12R\(\beta\)2 (16). Thus, IL-27 renders naive CD4\(^+\) T cells to the Th1-polarizing effects of IL-12 (12, 16–18).

Previous studies of IL-27Ra-deficient (IL-27Ra\(^{-/-}\)) mice revealed that they are susceptible to infection with Leishmania major and Listeria monocytogenes due to impaired Th1 response (14, 15, 19). However, subsequent studies have demonstrated the regulatory effects of IL-27 on progressive CD4\(^+\) T cell-dependent inflammatory responses. Indeed, IL-27Ra\(^{-/-}\) mice infected with Toxoplasma gondii or Trypanosoma cruzi develop lethal inflammatory responses, illustrating its regulatory effects (20). In contrast, IL-27Ra\(^{-/-}\) mice are resistant to infection with Trichuris muris, which is a well-characterized helminth expelled by Th2-mediated immune responses, suggesting the inhibitory effect of IL-27 on Th2 cytokine production (21). IL-27Ra\(^{-/-}\) mice also exhibited enhanced asthmatic phenotypes, including airway hyperresponsiveness (AHR), eosinophilic infiltration, and mucus overproduction in response to Ag challenge (22). Furthermore, IL-27 directly inhibits GATA-3 expression through the Stat1-dependent pathway (23). Therefore, IL-27 may play a critical role in suppression of Th2 responses. Very recent studies have noticed another important function of IL-27, that IL-27 inhibits development of IL-17-producing helper T (Th-17) cells (24, 25). Therefore, IL-27 is a critical cytokine possibly involved in regulation of human immunological diseases induced by Th1, Th2, or Th-17 cells (13, 26).

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\(^{3}\)Abbreviations used in this paper: EBI3, EBV-induced gene 3; AHR, airway hyperresponsiveness; Tg, transgenic; L3, third-stage larvae; mMCP-1, mouse mast cell protease 1; Th-17, IL-17-producing helper T.

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Flow cytometry

For preparation of CD4⁺CD26L⁺ resting T cells, splenic CD4⁺ T cells from DO11.10 mice were purified by MicroBeads (anti-mouse CD4; clone RM4-5; Miltenyi Biotech). The enriched CD4⁺ T cells were first treated with 10 µg/ml anti-CD4-PE and FITC-anti-CD26L for 30 min at 4°C, followed by treatment with PE-anti-CD27 and PE-anti-CD62L for 30 min at 4°C in staining buffer (PBS/1% FCS). Stained samples were separated into CD4⁺ CD26L⁺ T cells by FACSAria (BD Biosciences). Purity of sorted cells was >98.5% after reanalysis. For intracellular cytokine staining, polarized Th2 cells (1 × 10⁶/ml) were restimulated with 50 ng/ml PMA plus 0.5 µM monomycin for 4 h with a pulse of 1 µM brefeldin A (BD Biosciences) during the final 2 h to inhibit cytokine secretion. Cells were stained with CyChrome-anti-CD4 and followed by fixation with 4% (v/v) paraformaldehyde in PBS and permeabilization of cell membrane with ice-cold PBS containing 1% FCS plus 0.1% saponin. Resultant cells were further stained with 0.5 µg of PE-anti-mouse IL-4 plus 0.5 µg of FITC-anti-mouse IFN-γ or isotype-matched control Abs (BD Biosciences) and analyzed for their proportion of cytoplasmic IL-4⁺ or IFN-γ⁺ cells by FACS-Calibur (BD Biosciences).

Materials and Methods

Mice

Specific pathogen-free female BALB/c or C57BL/6 mice, 8 wk of age, were purchased from The Jackson Laboratory. Mice transgenic for αβ TCR recognizing OVA₃₃₃₃–₃₄₃₃ (DO11.10; BALB/c genetic background) were provided by D. Loh (Washington University, St. Louis, MO). IL-27 Tg mice were generated as described below. The cDNA for the pLG1-SAP vector that contains the liver-specific human serum amyloid P component promoter and the rabbit α-globin gene (28). IL-27 Tg lines were generated by pronuclear microinjection of the cDNA into fertilized eggs obtained from C57BL/6 mice. All mice were bred under specific pathogen-free conditions at the animal facilities of the Hyogo College of Medicine (Nishinomiya, Japan) and were used at 8–12 wk of age.

Reagents

Recombinant mouse IL-12 and IL-18 were purchased from Genetics Institute (Cambridge, MA) and MBL, respectively. Recombinase mouse IL-23 and IL-27 were prepared in our laboratory as described before (29, 30). Endotoxin level was <8 pg/ml of rIL-27 or rIL-23 as determined by the Limulus amebocyte lysate method (Wako Pure Chemical). Recombinase mouse IL-4 was purified as described before (8). Purified Abs (anti-mouse CD28 (37.51), anti-mouse CD23 (2C11), anti-mouse IL-4 (11B11), anti-mouse IL-12p40 (C17.8), linker, in p3XFLAG-CMV-9 (Sigma-Aldrich) vector (27), was amplified using standard PCR methods and inserted into the pLG1-SAP vector that contains the liver-specific human serum amyloid P component promoter and the rabbit β-globin gene (28). IL-27 Tg mice were generated as described below. The cDNA for the pLG1-SAP vector that contains the liver-specific human serum amyloid P component promoter and the rabbit β-globin gene (28). IL-27 Tg lines were generated by pronuclear microinjection of the cDNA into fertilized eggs obtained from C57BL/6 mice. All mice were bred under specific pathogen-free conditions at the animal facilities of the Hyogo College of Medicine (Nishinomiya, Japan) and were used at 8–12 wk of age.

In this study, we investigated the molecular mechanism and regulatory effects of IL-27 on Th2 cells. We showed that mice treated with IL-27 or IL-27- transgenic (Tg) mice are resistant to L. major infection but simultaneously develop a disability to promptly expel gastrointestinal nematode Strongyloides venezuelensis infection. Finally, we have shown that intranasal administration of a mixture of IL-27 and OVA into OVA-immunized animals can inhibit OVA-induced allergic airway inflammation, suggesting its therapeutic usage for the treatment of Th2 cell-mediated allergic diseases.

S. venezuelensis infection

S. venezuelensis has been maintained as described in our previous report (33). Animals were s.c. inoculated with 5000 S. venezuelensis L3 to initiate a complete infection. The degree of infection of individual mice was monitored by counting the number of eggs excreted daily (eggs per gram of feces).

Generation and analysis of OVA-specific bronchial asthma

BALB/c mice were immunized i.p. with 50 µg of OVA complexed with aluminum potassium sulfate on day 1 and challenged intranasally with 50 µg of OVA in 50 µl of PBS on days 7–9. Control mice were immunized with OVA and exposed to PBS alone. Mice were analyzed as described below at 24 h after the final exposure to PBS or OVA. For measurement of AHR to β-methacholine inhalation in mice, we used Palamos-I (MIPS) hardware and software as described in our previous reports (34, 35). Bronchial lavage was performed with three aliquots of 1.0 ml of PBS/mouse. Total cell counts were performed. Cytosin preparations of bronchial lavage fluid were stained with Diff-Quik (Baxter Healthcare), and differentials were performed based on morphology and staining characteristics. Histological analysis of lungs was performed as described previously (34, 35). For detection of IL-13 production, lungs were removed at 24 h after the final exposure to PBS or OVA and homogenized and centrifuged as described in our previous report (34). The obtained supernatants were tested for IL-13 contents using ELISA.

In vivo treatment

For host defense experiments for L. major or S. venezuelensis infection, infected mice were daily i.p. injected with PBS, IL-27 (1 µg/day), or a combination of IL-12 (10 ng/day) and IL-18 (1 µg/day) for 7 days. For experiments of airway inflammation, OVA-immunized mice were intranasally challenged with 50 µg of OVA with or without IL-27 (0.2, 1, 5 µg) in 50 µl of PBS on days 7–9.

ELISA

ELISA kits for IL-4, IL-5, IL-13, and IFN-γ (R&D Systems) were used. Serum levels of mouse mast cell protease-1 (mMCP-1) and IgE were measured by ELISA as described in our previous reports (36). Serum level of FLAG-tagged IL-27 protein was determined by sandwich ELISA using anti-FLAG (M2; Sigma-Aldrich) as capture Ab and biotin-anti-IL-27p28 (R&D Systems) as detection Ab. rIL-27 prepared as a FLAG-tagged single chain protein by flexibly linking EBI3 to p28 as described before (30) was used as a standard.

In vitro cytokines production

Polarized Th2 cells in vitro were recultured at 1 × 10⁷/0.2 ml per well with 100 µl IL-2, 1 µM OVA33-343, and 1 × 10⁷/0.2 ml of APCs (irradiated T cell-depleted BALB/c splenocytes) in the presence of IL-27 (0–100 µg/ml). Proliferation or meseenteric lymph nodes cells from mice infected with L. major or S. venezuelensis third-stage larvae (L3) were cultured at 2 × 10⁷/0.2 ml per well under stimulation with immobilized anti-CD3 and anti-CD28 (each 5 µg/ml) for 48 h and tested for IFN-γ, IL-4, IL-5, and IL-13 production using ELISA.
FIGURE 1. IL-27 inhibits Th2 cell polarization. Naive splenic CD4+ CD62L− T cells (10^5/ml) from DO11.10 mice were cultured with 100 pM IL-2, 1 μM OVA_{323-339} and 2 × 10^4/ml irradiated BALB/c splenic dendritic cells in Th2 condition (1000 U/ml IL-4 plus 20 μg/ml anti-IL-12p40/anti-IFN-γ) or Th1 condition (10 ng/ml IL-12 and 10 μg/ml anti-IL-4) in 24-well plates in a total 1-ml volume for 7 days. Twenty micrograms of IL-18, IL-23, and IL-27 per milliliter, either by itself or in various concentrations (A and B) or various concentrations of IL-27 (0–100 ng/ml; C and D) were added from the beginning of the culture. After initial priming, cells were washed and recultured with 100 pM IL-2, 1 μM OVA_{323-339} plus irradiated T cell-depleted BALB/c splenocytes for 48 h for induction of cytokine secretion (A and C), or recultured with PMA (50 ng/ml) plus ionomycin (500 ng/ml) for 4 h and analyzed by FACs for cytosolic IL-4 and IFN-γ (D). Supernatants were harvested and tested for IL-4, IL-5, IL-13, and IFN-γ production by ELISA (A and C). Results are the percentage of IL-4+ or IFN-γ+ cells gated on CD4+ T cells (D). After 5 days of initial priming and washing cells, nuclei or total cell lysates were prepared and subjected to Western blotting using anti-GATA-3 or anti-T-bet, respectively (B). Results are representative of three independent experiments.

Western blotting

Protein levels of GATA-3 and T-bet were determined using Western blot techniques. To detect GATA-3 expression, cells were washed once with PRS and resuspended in buffer A (10 mM HEPES (pH 7.8), 10 mM KCl, 0.1 mM EDTA, 1 mM DTT, 2 μg/ml aprotinin, 0.5 mM PMSF, and 0.5% Nonidet P-40). Nuclei were pelleted, and the cytoplasmic proteins were carefully removed. The nuclei were then resuspended in buffer C (50 mM HEPES (pH 7.8), 420 mM KCl, 0.1 mM EDTA, 5 mM MgCl2, 10% glycerol, 1 mM DTT, 2 μg/ml aprotinin, and 0.5 mM PMSF) as described elsewhere (37). After vortexing and stirring for 30 min at 4°C, the samples were centrifuged, and the nuclear proteins in the supernatant were transferred to a fresh vial. Nuclear extracts were resolved by SDS-PAGE and transferred to polyvinylidene difluoride membranes. Blots were probed with murine mAbs against GATA-3 (40). Primers used for HPRT RT-PCR were described previously (40).

RT-PCR

Total RNA was extracted by using a guanidine thiocyanate procedure. cDNA was prepared using an oligo(dT) primer and SuperScript RT (Invitrogen Life Technologies), and RT-PCR was performed using TaqDNA polymerase as described elsewhere (39). Cycle conditions were 94°C for 40 s, 60°C for 20 s, and 72°C for 40 s. The following primers were used to detect FLAG-tagged IL-27: sense primer, 5′-GATCCTGAGAACTTCAGGCTC-3′; antisense primer and 5′-GATCCTGAGAACTTCAGGCTC-3′; antisense primer and 5′-GTA ACCAGGTAGCTTGATCATCGTACATCC-3′. Primers used for HPRT were described previously (40).

Statistics

Statistical comparisons between experimental groups were determined by the paired Student t test. All analysis was performed by GraphPad Instat Software. A value of p < 0.05 was considered to be significantly different.

Results

IL-27 inhibits Th2 cell differentiation

We first examined the capacity of IL-27 to inhibit Th2 cell differentiation in vitro. We established Th2 cells by culturing naive CD4+ T cells obtained from OVA-specific TCR Tg BALB/c (DO11.10) mice with OVA peptide, IL-4, and anti-IL-12 plus anti-IFN-γ for 1 wk (35). The resultant Th2 cells challenged with OVA produced significant amounts of IL-4, IL-5, and IL-13 but not...
FIGURE 2. IL-27 suppresses cytokine production from polarized Th2 cells. After initial priming of Th2 conditions as indicated in Fig. 1, cells were washed and recultured with 100 pM IL-2, 1 μM OVA323–339 plus irradiated T cell-depleted BALB/c splenocytes in the presence of IL-27 (0–100 ng/ml) for 48 h for induction of cytokine secretion (A) and expression of GATA-3 and T-bet (B). Results are geometric means + SEM. Results are representative of three independent experiments. C. Polarized Th2 cells were stimulated with IL-27 (10 ng/ml) or IFN-γ (20 ng/ml) for 10 and 30 min. Total cell lysates were then prepared and subjected to Western blotting with Abs specific for indicated phospho-regulated Stat proteins. To control for equal protein loading, blots were stripped and reprobed with Abs specific for the indicated total Stat proteins. Experiments were repeated twice with similar results. D. Polarized Th2 cells were recultured with 100 pM IL-2, 1 μM OVA323–339, plus irradiated T cell-depleted BALB/c splenocytes with IL-27 (10 ng/ml) or IFN-γ (20 ng/ml) for 48 h for induction of cytokine secretion. Results are geometric means + SEM. Results are representative of two independent experiments. *, p < 0.05 vs corresponding value for Th2 cells recultured with OVA323–339 alone.

IFN-γ (Fig. 1, A and C). Addition of IL-27 directly and dose-dependently inhibited Th2 cell differentiation and conversely induced Th1 cell differentiation even in the absence of IFN-γ and IL-12 (Fig. 1, A and C). We also examined the mechanism by which IL-27 inhibits Th2 development but simultaneously induces Th1 development and found that IL-27 suppressed GATA-3 expression but induced T-bet expression (Fig. 1B). Since IL-12 and IL-18 synergistically inhibit Th2 responses (7), we simultaneously examined the potential of IL-18 to enhance the action of IL-27 and found that IL-18 has no such function (Fig. 1, A and B). Another member of the IL-12 family, IL-23 (26, 41), failed to replace IL-27 found that IL-18 has no such function (Fig. 1, A and B). We also examined the effects of IL-27 on Th2 polarization by FACs analysis. We found that 57 and 0.56% of CD4+ T cells cultured under Th2-inducing condition were positive for cytoplasmic IL-4 and IFN-γ respectively. Addition of IL-27 dose-dependently decreased the proportion of cytoplasmic IL-4-positive cells (17.5%) but increased the proportion of cytoplasmic IFN-γ-positive cells (to 6.73); Fig. 1D).

IL-27 inhibits Th2 cytokines production from already polarized Th2 cells

It is important to examine the inhibitory effect of IL-27 on the function of already differentiated Th2 cells. Polarized Th2 cells produce Th2 cytokines (Fig. 2A). We found that IL-27 dose-dependently inhibited IL-5 and IL-13 but not IL-4 production from polarized Th2 cells (Fig. 2A). Surprisingly, IL-27 diminished GATA-3 expression and conversely induced T-bet expression even in highly polarized Th2 cells (Fig. 2B). As reported previously (16–18, 23, 30, 38, 42), both IL-27 and IFN-γ activated Stat1 (Fig. 2C). However, only IL-27 stimulation strongly activated Stat3 (Fig. 2C). Furthermore, IL-27 stimulation but not IFN-γ stimulation suppressed IL-5 and IL-13 production from polarized Th2 cells (Fig. 2D). Further studies are definitely needed to understand the role of Stat1 and Stat3 in regulation of Th2 cytokine production.

Administration of IL-27 protects BALB/c mice from leishmaniasis

BALB/c mice infected with L. major developed progressive disease, as assessed by footpad swelling, while C57BL/6 mice are highly resistant to L. major (Fig. 3A). The resistance and susceptibility of inbred strains of mice to infection are intimately associated with the capacity to produce IFN-γ and IL-4, respectively. Susceptible BALB/c mice develop a Th2 response and resistant C57BL/6 mice develop a Th1 response following L. major infection (43, 44). As reported elsewhere, popliteal lymph nodes cells from L. major–infected BALB/c mice strongly produced IL-4 and little IFN-γ, while those from L. major-infected C57BL/6 mice strongly produced IFN-γ and little IL-4 (Fig. 3B). Consistent with our previous report (32), administration of a mixture of IL-12 (10 ng/day) and IL-18 (1 μg/day) for the first week of infection protected BALB/c mice from footpad swelling (Fig. 3A) and diminished parasite burden (Fig. 3C) by down-regulation and up-regulation of Th2 and Th1 responses, respectively (Fig. 3B), whereas administration of IL-18 alone did not show such protective effects (data not shown) (32). Next, to address the inhibitory effect of IL-27 on the generation of Th2 cells in vivo, we daily administered IL-27 (1 μg/day) into L. major-infected BALB/c mice for 1 wk. This administration partially but significantly protected mice from footpad swelling (Fig. 3A) and considerably diminished parasite burden (Fig. 3C). In addition to 1-wk treatment with IL-27, we administered IL-27 for 2 wk into L. major-infected BALB/c mice and found that both group showed similar footpad swelling. Importantly, IL-27-treated L. major-infected BALB/c mice all survived at 12-16 wk after infection, whereas control PBS-treated L. major-infected BALB/c mice died of disseminated infection.
around these periods (data not shown). We simultaneously measured the capacity of popliteal lymph nodes cells to produce IL-4 and IFN-γ upon stimulation with plate-bound anti-CD3 plus anti-CD28 in vitro. Lymph nodes cells from \( \text{L. major} \)-infected and \( \text{IL-27} \)-administered \( \text{BALB/c} \) mice diminished IL-4 production but increased IFN-γ production (Fig. 3B). These results taken together indicated that administration of \( \text{IL-27} \) into \( \text{L. major} \)-infected \( \text{BALB/c} \) mice protected them from progressive leishmaniasis by direct inhibition of Th2 response and simultaneous induction Th1 response. We suspect that \( \text{IL-27} \)-stimulated T cells come to express IL-12Rβ2 and develop into Th1 cells under the stimulation of endogenous IL-12 in vivo.

**Exogenous IL-27 in wild-type or endogenous IL-27 in IL-27 Tg mice inhibit S. venezuelensis -induced Th2 responses**

Mice infected with L3 of the gastrointestinal nematodes \( \text{S. venezuelensis} \) increased serum IgE levels, number of intestinal mucosal mast cells, and serum mMCP-1 through primary Th2 responses (33, 45). The expulsion of parasites is tightly associated with the level of intestinal mastocytosis, which correlates well with the serum mMCP-1 level (33, 46). Infected mice completed parasite expulsion within 2 wk. Because IL-27 reciprocally regulates Th1 and Th2 responses (Figs. 1–3), we tested whether in vivo administration of IL-27 into mice inoculated with \( \text{S. venezuelensis} \) L3 inhibits Th2-driven IgE and intestinal mastocytosis. This treatment considerably inhibited IgE and mMCP-1 levels and parasite expulsion (Fig. 4, A–C). Furthermore, this IL-27 administration markedly increased Th1 cells but diminished Th2 cells by reciprocal regulation of Th1 and Th2 responses in \( \text{S. venezuelensis} \) L3-infected \( \text{C57BL/6} \) mice (Fig. 4D).

To substantiate further this conclusion, we generated two IL-27 Tg mouse lines, 1 and 6, as described in Materials and Methods. Circulating FLAG-tagged IL-27 in each Tg mice was determined by ELISA and further analyzed by Western blot analysis using anti-FLAG Ab (Fig. 5, A and B). Expression of FLAG-tagged IL-27 mRNA in liver was detected by RT-PCR analysis (Fig. 5C). Liver-selective expression of FLAG-tagged IL-27 was also determined by RT-PCR analysis using RNA obtained from various tissues (Fig. 5D). The mean concentrations of FLAG-tagged IL-27 in sera of IL-27 Tg1 and Tg6 mice at 6–8 wk of age were 0.962 ± 0.543 ng/ml (\( n = 17 \)) and 0.569 ± 0.338 ng/ml (\( n = 19 \)), respectively. Although the IL-27 Tg mice appeared normal at birth, approximately one-half of IL-27 Tg1 mice and 10% of IL-27 Tg6 mice died by 10 mo of age. More shortened survival rate in IL-27 Tg1 mice than that in IL-27 Tg6 mice could be due to more increased expression of FLAG-tagged IL-27 (Fig. 5, A–C). IL-27 Tg

**FIGURE 3.** IL-27 protects \( \text{BALB/c} \) mice from leishmaniasis. A, Mice were s.c. inoculated with stationary-phase promastigotes on the first day of experiment. \( \text{BALB/c} \) mice (five per group) were daily i.p. injected with PBS, IL-12 (10 ng/day) plus IL-18 (1 μg/day) or IL-27 (1 μg/day) during the first 7 days after infection. Weekly footpad measurements represent the average footpad swelling ± SEM. *\( p < 0.05 \); †\( p < 0.01 \) vs corresponding value for infected mice treated with PBS. B, Draining popliteal lymph node cells from each group of mice (five per group) were harvested at 7 wk after infection. Cell suspensions were cultured at 2 × 10^7/ml per well under stimulation with immobilized anti-CD3 and anti-CD28 (each 5 μg/ml). After 48 h of culture, supernatants were harvested and tested for IFN-γ and IL-4 production by ELISA. C, At 7 wk after infection, parasite burdens in 1 × 10^7 cells of the popliteal lymph node draining the site of infection were determined. Statistical differences between samples were determined using Student’s \( t \) test. Results are representative of three independent experiments.

**FIGURE 4.** IL-27 inhibits \( \text{S. venezuelensis} \)-induced Th2 responses. \( \text{C57BL/6} \) mice were inoculated with 5000 \( \text{S. venezuelensis} \) L3 on the first day of experiment. Mice (five per group) were daily i.p. injected with PBS or IL-27 (1 μg/day) during the first 7 days after infection. Serum levels of IgE (A) and mMCP-1 (B) and the number of eggs per gram of feces (C) at day 10 after infection were measured. D, Draining mesenteric lymph node cells from each group of mice were harvested at day 11 after infection. Cell suspensions were cultured at 1 × 10^7/ml per well under stimulation with immobilized anti-CD3 and anti-CD28 (each 5 μg/ml). After 48 h of culture, supernatants were harvested and tested for IFN-γ and IL-4 production by ELISA. Results are geometric means ± SEM of five animals per group and are representative of three independent experiments. Statistical differences between samples were determined using Student’s \( t \) test.
mice could live at least by 6 mo of age. Histological analysis at 8–12 wk of age revealed extramedullary hemopoiesis in the spleens of IL-27 Tg mice compared with wild-type mice. However, their livers had no obvious morphological abnormalities. Hematological examination of peripheral blood revealed no significant difference in the number of leukocytes, erythrocytes, and platelets. Furthermore, IL-27 Tg mice had no immunological defects and percentages of CD4+ and CD8+ T cells in their thymus and secondary lymphoid organs were comparable to those in wild-type mice. Finally, both T cells and B cells were not activated and secondary lymphoid organs were comparable to those in wild-type mice. We inoculated wild-type C57BL/6 mice and C57BL/6 backcrossed with IL-27 Tg mice for these experiments. Inoculated wild-type mice (five per group) inoculated with 5000 S. venezuelensis L3 had increased serum levels of mMCP-1 (B) and IgE (C) from C57BL/6 wild-type (WT) and IL-27 Tg mice (five per group) inoculated with 5000 S. venezuelensis L3. D, Draining mesenteric lymph node cells from each group of mice (five per group) were harvested at day 11 after infection. Cell suspensions were cultured at 2 × 10^6/0.2 ml per well under stimulation with immobilized anti-CD3 and anti-CD28 (each 5 μg/ml). After 48 h of culture, supernatants were harvested and tested for IFN-γ and IL-4 contents using ELISA. nd: Not detected. Results are geometric means ± SEM of five animals per group and are representative of three independent experiments.

**Intranasal administration of IL-27 suppresses allergic airway inflammation**

Because IL-27 has a striking capacity to inhibit Th2 cells to produce IL-5 and IL-13 (Fig. 2A), we examined its therapeutic potential on allergic diseases. For this purpose, we established an experimental allergic asthma model. BALB/c mice, immunized with OVA 1 wk before and challenged with intranasal OVA administration for 3 days developed AHR in response to β-methacholine exposure (Fig. 7A). Furthermore, they showed peribronchial accumulation with eosinophils and neutrophils (Fig. 7B) and goblet cell metaplasia in their airways (Fig. 7C). Daily i.p. injections of IL-27 (1 μg/day) for the first 7 days following immunization with OVA modestly diminished AHR to that of β-methacholine (data not shown). However, intranasal administration of IL-27 at the time of OVA challenge dose-dependently diminished AHR (Fig. 7A), airway eosinophilic inflammation (Fig. 7B), and goblet cell metaplasia (Fig. 7C). Among the Th2-related cytokines, IL-13 is thought to be most bronchogenic (34, 47). Thus, we nasally administered various doses of IL-27.
formalin, cut into 3-
via the right ventricle with 10 ml of PBS, then fixed in 10% buffered  
buffers of cells.  
evaluation of cytospin preparations. Data are expressed as absolute num-

Inflammatory cell composition of bronchoalveolar lavage from each group  
exposed to PBS alone. The allergic phenotype was assessed 24 h after the  
final exposure to Ag.  

FIGURE 7. IL-27 protects against allergic airway inflammation.  
BALB/c mice (five mice per group) were immunized i.p. with 50 μg of  
OVA with aluminum potassium sulfate (alum) on day 1 and challenged  
intranasally with 50 μg of OVA with or without IL-27 (0.2, 1, 5 μg) in 50  
μl of PBS on days 7–9. Control mice were immunized with OVA and  
exposed to PBS alone. The allergic phenotype was assessed 24 h after the  
final exposure to Ag. A. AHR in response to increased concentrations of  
hailed β-methacholine was measured in a whole-body plethysmograph. B.  
Inflammatory cell composition of bronchoalveolar lavage from each group  
of mice. Cell differential percentages were determined by light microscopic  
evaluation of cytoospin preparations. Data are expressed as absolute num-
bers of cells. C. Lungs were prepared for histology by perfusing the animal  
via the right ventricle with 10 ml of PBS, then fixed in 10% buffered formalin, cut into 3-μm sections, and stained with predigested periodic acid-Schiff. Original magnification, ×100. D. Lungs were homogenized and centrifuged as described in Materials and Methods. The obtained supernatants were tested for IL-13 contents by ELISA. In. Intranasal. Results are geometric means ± SEM of five animals per group and are representa-
tive of three independent experiments. *, p < 0.05 vs corresponding value for OVA-sensitized and OVA-administered mice.  

found that IL-27 dose-dependently diminished the IL-13 level in the lung (Fig. 7D). These results clearly indicated that in-

hibits IL-13 production from OVA-stimulated Th2-polarized cells, providing promising a therapeutic way for the treatment  
of allergic asthma.  

Discussion  
Yoshida and his colleagues (22) reported that IL-27Rα−/− mice immunized with OVA exhibited enhanced asthmatic phenotypes in  
response to OVA challenge. In addition, Hunter and colleagues (21) reported that IL-27Rα−/− mice infected with gastrointestinal  
nematode T. muris displayed increased Th2 cell-dependent intesti-
nal goblet cell hyperplasia, mastocytosis, and Th2 cytokine pro-
duction. Therefore, IL-27 seems to inhibit Th2 responses in vivo.  
Furthermore, IL-27 inhibits CD4+ T cell expression of GATA-3 by activation of Stat1 (23). However, the direct evidence and mo-

lecular mechanism by which IL-27 exerts an inhibitory effect on  
Th2 responses remains unclear. To address these issues, we ex-
amined the capacity of rIL-27 to inhibit Th2 polarization in vitro and  
found that IL-27 inhibits Th2 cell development, even in the presence of anti-IFN-γ Ab and anti-IL-12 Ab (Fig. 1A). Further-
more, IL-27 induced Th1 cell differentiation under Th2-inducing  
conditions (Fig. 1, C and D). Thus, IL-27 can directly inhibit Th2  
cell development and conversely induce them to develop into Th1  
cells that produce IFN-γ. In line with this, in vivo treatment with  
IL-27 for the first week after L. major infection significantly di-
minished footpad swelling and parasite burden by induction of Th1  
cell development and inhibition of Th2 cell development simulta-

neously (Fig. 3).  

It is well known that expulsion of some types of helminthes de-
pends on the action of activated mast cells (45, 46, 48). Recently,  
we reported that administration of a mixture of IL-2 and IL-18  
duces intestinal mastocytosis by induction of mast cell growth  
factors such as IL-3 and IL-9 and that such IL-2 and IL-18-pre-
treated mice rapidly expel implanted adult worms of S. venezuel-
enesis (33). However, as we reported here, IL-27-treated S. ven-
zuelensis-infected C57BL/6 mice failed to do so and sustained  
infection. They exhibited significantly reduced levels of IgE and  
mMCP-1 in their sera and decreased Th2 response but increased  
Th1 response simultaneously (Fig. 4). IL-27 Tg mice, that we have  
newly established, could not mount the Th2 response in response  
to infection with S. venezuelensis. Accumulated IL-27 in IL-27 Tg  
mice strongly induces Th1 cells before infection with S. venezuel-
enesis. Thus, infected IL-27 Tg mice could not increase mMCP-1 and IgE levels in their sera (Fig. 6, B and C), resulting in  
sustained infection (Fig. 6A). The molecular mechanism by which  
IL-27 inhibits Th2 cell development could be explained by  
down-regulation of GATA-3 and simultaneous up-regulation of  
T-bet (Fig. 1B).  

The most remarkable inhibitory effect of IL-27 on Th2 cells  
presented here is direct suppression of IL-5 and IL-13 production  
from already polarized Th2 cells (Fig. 2A). It is well known that  
IFN-γ counteracts Th2 development (49). However, IFN-γ failed to  
suppress IL-5 and IL-13 production from polarized Th2 cells  
(Fig. 2D), even though IFN-γ could induce Stat1 activation in  
polarized Th2 cells (Fig. 2C). Furthermore, it was recently dem-

onstrated that IL-27 more efficiently inhibits Th-17 cell develop-
ment than IFN-γ (24). These results taken together indicate the  
importance of IL-27 for the treatment of diseases induced by Th2  
cells or Th-17 cells.  

It is well known that IFN-γ increases the expression of T-bet  
through Stat1 activation (50). However, IL-27 induced Stat1 acti-
vation and T-bet expression in the absence of IFN-γ and IL-12  
(Fig. 1). These results suggest that IL-27 directly induces T-bet via  
Stat1 activation independently of IFN-γ and IL-12. Usui et al. (51)
have revealed that administration of retroviral T-bet into established Th2 clones inhibits IL-5 production without affecting IL-4 production by down-regulation of GATA-3 expression. Furthermore, Zhu et al. (52) have shown that deletion of GATA-3 in established Th2 cells completely abolished their capacity to produce IL-5 and IL-13 but not IL-4. Functional GATA-3 binding sites were found in both il5 and il13 promoters but not in il4 promoter (53, 54), suggesting the involvement of GATA-3 as an essential transcription factor in directly activating il5 and il13 promoters. Thus, IL-27 can inhibit production of IL-5 and IL-13 by down-regulation of GATA-3 and up-regulation of T-bet through Stat1 activation (Fig. 2, B and C), substantiating further these previous reports. Moreover, IL-27 can activate both Stat1 and Stat3 in polarized Th2 cells, while IFN-γ only activates Stat1 (Fig. 2C). Further studies are necessary to elucidate the precise molecular mechanisms by which IL-27 inhibits the Th2 differentiation and Th2 cytokine production from polarized Th2 cells through activation of both Stat1 and Stat3.

In vivo treatment with high doses of IL-12 alone or low doses of IL-12 plus IL-18 prevents the development of Th2 cells (55), which results in down-regulation of allergic inflammation including bronchial asthma (49). In this report, we showed that IL-27 significantly suppressed IL-5 and IL-13 production from already differentiated Th2 cells by down-regulating GATA-3 and up-regulation of T-bet simultaneously (Fig. 2, A and B). According to this result, intranasal administration of IL-27 with OVA into OVA-sensitized animals dose-dependently diminished AHR to β-methacholine exposure, airway eosinophilic inflammation, and goblet cell metaplasia with mucus overproduction by suppressing IL-13 production (Fig. 7). IL-13 is suggested to play a critical role in induction of AHR, airway eosinophilia, and mucus oversecretion. Indeed, our group and others (34, 47) have reported that blockade of IL-13 markedly inhibits allergen-induced AHR, eosinophilic inflammation, and goblet cell metaplasia in animal models. We have elucidated that IL-27 treatment has no apparent adverse effects such as inflammatory bowel disease and liver injury with elevated serum glutamic-oxaloacetic transaminase and alanine aminotransferase activities, which are often seen with high doses of IL-12 treatment (27, 56). These results taken together strongly suggest that intranasal IL-27 administration might become an important therapeutic approach for the establishment of the treatment for bronchial asthma.

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References


