IL-27 Is a Key Regulator of IL-10 and IL-17 Production by Human CD4+ T Cells

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Although the physiologic pathways that control regulatory T cells (Foxp3-expressing regulatory T cells, IL-10-secreting Tr1 cells) and Th17 cells in rodents have been defined, the factors that control these differentiation pathways in humans are not well understood. In this study, we show that IL-27 promotes the differentiation of IL-10-secreting Tr1 cells while inhibiting Th17 generation and molecules associated with Th17 function. Furthermore, IL-27 inhibits IL-17-polarizing cytokines on dendritic cells, which in turn decrease IL-17 secretion from T cells. Our results demonstrate that IL-27 plays a key role in human T cells by promoting IL-10-secreting Tr1 cells and inhibiting Th17 cells and thus provides a dual regulatory mechanism to control autoimmune and tissue inflammation. The Journal of Immunology, 2009, 183: 2435–2443.
of inducing IL-10 from murine T cells and act as a negative feedback mechanism against proinflammatory immune responses (32–35). However, the role of IL-27 in the human immune system is not well understood. In this study, we show that IL-27 is a key regulator of IL-10 and IL-17 production by human CD4+ T cells.

Materials and Methods

**Media and reagents**

RPMI 1640 was supplemented (complete RPMI with 10% heat-inactivated FCS, 1 mM nonessential amino acids, 45 μg/ml penicillin and streptomycin, and 2 mM L-glutamine (all from Life Technologies)). Recombinant human IL-27, IL-2, IL-10, IL-23, and IL-27 receptor Abs were obtained from R&D Systems. mAbs specific for HLA-DR, CD80, CD86, CD83, CD40, and CCR7 were purchased from BD Biosciences. Isotype control Ab mouse IgG1 and mouse IgG2a were also from BD Biosciences. TLR ligands LPS (Escherichia coli) and peptidoglycan (PGN) (Staphylococcus aureus) were obtained from InvivoGen.

**Human T cell stimulation**

Peripheral blood was obtained after informed consent from healthy subjects. PBMCs were isolated from heparinized venous blood by Ficoll-Hypaque density gradient centrifugation (Amersham Pharmacia Biotech). Total, naïve, and memory CD4+ T cells were prepared using magnetic beads by negative selection (Miltenyi Biotec). The CD4+ T cells were cultured at a concentration of 1.5 × 10^6 cells/ml in 24-well plates coated with anti-CD3 and anti-CD28 mAb (1.0 μg/ml) in the presence or absence of 100 ng/ml of recombinant human IL-27. For polarization experiments, naïve CD4+ T cells were seeded at a density of 1.5 × 10^6 cells/ml in 24-well plates coated with anti-CD3 and anti-CD28 (1 μg/ml). In some cases, IL-1β (50 ng/ml), IL-6 (50 ng/ml), IL-21 (25 ng/ml), IL-23 (25 ng/ml), TGF-β1 (2 ng/ml), and IL-27 (100 ng/ml) were added at day 0 and were maintained throughout the experiment. Cell-free culture supernatants were collected on day 5 for ELISA.

**Primary and secondary cell stimulation**

In vitro primary stimulation was conducted in 96-well culture plates coated with mAbs to CD3 (1.0 mg/ml), CD28 (1.0 mg/ml), and IL-27 (100 ng/ml). The wells were washed and purified CD4+ T cells (2.0–3.0 × 10^5 cells/well) were added in 200 μl of culture medium. After 3 days of primary stimulation, cells were washed and expanded for 3 days in medium supplemented with 100 U/ml human IL-2. At day 6, cells were counted and subjected to secondary stimulation under conditions similar to those of the primary activation. The experiment had each activation condition in triplicate. For the bystander T cell suppression assay, naive CD4+ T cells were seeded at a density of 2 × 10^5 cells/well in a 96-well plate coated with mAbs to CD3 (1.0 μg/ml) and CD28 (1.0 μg/ml). Cell-free supernatants from the CD3/28/IL-27- or CD3/28-activated cell populations were transferred to the above culture with or without neutralizing Ab to IL-10 and proliferation was measured at day 3. In some culture conditions, anti-IL-27 Ab was added at a concentration of 20 μg/ml.

**Generation of monocyte-derived dendritic cells (DCs)**

Human PBMCs were isolated from buffy coats by centrifugation through a Ficoll-Paque Plus (Amersham Pharmacia Biotech) density gradient. Cells were enriched for monocytes (CD14+ cells) by using a monocyte enrichment kit (Miltenyi Biotec). Monocytes were resuspended at 2 × 10^6 cells/ml in DC medium (RPMI 1640 plus Glutamax (Invitrogen), 5% FBS, 100 U/ml penicillin, and 100 μg/ml streptomycin) containing IL-4 (50 ng/ml) and GM-CSF (100 ng/ml; BD Biosciences) and cultured for 6 days with replacement of half of the medium and addition of fresh cytokines every alternate day. Nonadherent DCs were harvested by centrifugation and suspended in DC medium without antibiotics. The resulting cells were determined to be >95% CD11c+ by flow cytometry.

**DC-T cell coculture assay**

Total and naïve CD45RA+ Th cells were enriched by immunomagnetic negative selection. They were then cocultured with DCs that had been activated alone with LPS (0.5 μg/ml), PGN (10 μg/ml), or combined with IL-27 (100 ng/ml) for 12 h. The DCs were washed three times before they were cultured with T cells. Cocultures were performed with a DC:T cell ratio of 1:3 in U-bottom 96-well plates. The culture supernatants were assayed for IL-17 levels by ELISA.

**Flow cytometric analysis**

Cells were resuspended in PBS containing 1% BSA (Sigma-Aldrich) and 0.1% sodium azide (Sigma-Aldrich) and incubated with FITC- or PE-conjugated Abs or isotype control Abs at the recommended dilutions for 30 min on ice.

**Cytokine measurement**

Supernatants from DCs, DC-T cell cocultures, and IL-27-treated T cells were harvested at the indicated time points and stored at −80°C. Cytokines were quantified by the ELISA using OptEIA kits (BD Pharmingen) or by cytokine bead array (inflammation kit; BD Pharmingen) according to the manufacturer’s instructions.

**Real-time quantitative RT-PCR**

Total RNA was extracted with the RNaseasy Mini Kit (Qiagen) according to the manufacturer’s instructions. First-strand cDNA synthesis was performed for each RNA sample using TaqMan reverse transcription reagents (Applied Biosystems). Transcripts were quantified by real-time quantitative PCR on an Applied Biosystems 7500 Sequence Detector with Applied Biosystems predesigned TaqMan Gene Expression Assays and reagents according to the manufacturer’s instructions. The following probes were used (identified by Applied Biosystems assay identification number): TBX21, Hs00203436_m1; IL-17A, Hs99999082_m1; IL-22, Hs00209294_m1; CCL20, Hs00171125_m1; GATA-3, Hs00231122_m1; FOXP3, Hs00203958_m1; retinoic orphan nuclear receptor (RORC), which encodes the human ortholog of mouse RORγt, Hs01076112_m1; IL-17F, Hs00369400_m1; and CCR6, Hs01711212_m1. For each sample, mRNA abundance was normalized to the amount of the housekeeping gene GAPDH.

**Results**

**IL-27 induces IL-10 production from human CD4+ T cells**

We stimulated ex vivo total CD4+ T cells with plate-bound anti-CD3 and anti-CD28 in the presence or absence of recombinant human IL-27 and assessed their cytokine profile. Stimulation in the presence of IL-27 induced large amounts of IL-10 with a moderate increase in IFN-γ (Fig. 1, A and B). By contrast, IL-27 stimulation inhibited anti-CD3 and anti-CD28 induced IL-17 without affecting TGF-β production (Fig. 1, C and D). Neither stimulatory condition induced IL-4. The transcription factors T-bet, GATA-3, RORC, and Foxp3 are required for the generation of Th1, Th2, Th17, and Treg cells, respectively. We found that cells stimulated with anti-CD3 and anti-CD28 induced expression of mRNA encoding Foxp3, GATA-3, T-bet, and RORC. Addition of IL-27 to the above culture condition greatly reduced the expression of GATA-3 and RORC, whereas the expression of T-bet and Foxp3 transcripts were not affected (Fig. 1, E–H). These observations suggest that IL-10 production induced by IL-27 did not depend on GATA-3, a transcription factor required for the generation of Th2 cells (36) and that IL-27-stimulated T cells have a cytokine profile that is distinct from that induced by stimulation with Abs to CD3 and CD28 but similar to that of Tr1 cells.

To determine whether the IL-10-producing cells were derived from the naive or memory T cell population, CD4+ T cells were separated into CD45RA- and CD45RO- T cells and were then stimulated with plate-bound anti-CD3 and anti-CD28 in the presence or absence IL-27 (Fig. 2A). Naive CD4+ T cells stimulated with IL-27 responded more robustly in that they produced IL-10 in greater folds (Fig. 2A). Neither naive nor memory CD4+ T cells produced IL-4. However, both cell populations secreted moderate quantities of IFN-γ in response to IL-27 stimulation (Fig. 2B). Consistent with IL-10 secretion, naive CD4+ T cells expressed more IL-27 receptor on their surface (Fig. 2C). In addition, we found that IL-27 did not affect the proliferation of either naive or
FIGURE 1. IL-27 stimulation induces IL-10 production from human peripheral blood CD4+ T cells. ELISA of total CD4+ T cells stimulated with plate-bound anti-CD3 and anti-CD28 in the presence or absence of recombinant human IL-27 (100 ng/ml). A and B, IL-27 stimulation increased anti-CD3- and anti-CD28-induced IL-10 and IFN-γ production. C and D, IL-27 inhibited anti-CD3- and anti-CD28-induced IL-17 without affecting TGF-β production. E–H, Quantitative PCR of the expression of mRNA encoding T-bet, GATA-3, RORC, and Foxp3 in total CD4+ T cells stimulated with plate-bound anti-CD3 and anti-CD28 in the presence or absence of IL-27 (100 ng/ml), presented relative to the expression of mRNA encoding GAPDH. Data are from 11 randomly selected healthy donors. Horizontal bars indicate the median.

The above data suggest that primary activation of naive CD4+ T cells with IL-27 induces an IL-10-producing T cell phenotype. To determine whether these cells are then committed to maintain this phenotype, we analyzed the properties of these CD4+ T cells on secondary stimulation. Purified naive CD4+ T cells were initially stimulated for 3 days and subsequently expanded for 4 days in medium supplemented with rIL-2. These cells were then subjected to secondary stimulation and analyzed for IL-10 production. CD4+ T cells, first activated with CD3/CD28/IL-27, produced large amounts of IL-10 on secondary stimulation (Fig. 2D). By contrast, CD4+ T cells initially activated without IL-27 produced small amounts of IL-10 on secondary stimulation with IL-27 (Fig. 2E). Therefore, primary stimulation through IL-27 is required for IL-10 production in restimulated cells. Of note, secondary stimulation with CD3 or CD3/CD28 alone became sufficient for the induction of IL-10 synthesis from the cells received IL-27 primary stimulation, suggesting these cells are differentiated and committed to the IL-10-producing phenotype (Fig. 2D). In addition, restimulated cells did not produce IL-4 but synthesized small amounts of IL-2 (data not shown).

It has been evident from the previous studies that the cytokine production profile of Tr1 cells was their key trait. Tr1 cells, upon activation via the TCR, produce high amounts of IL-10 but are distinct from Th2 cells since they do not produce IL-4 and produce very low levels of IL-2 (5–7). It has been shown that human Tr1 cells also produce IFN-γ in addition to IL-10 (5, 7). Consistent with ELISA data, intracellular cytokine analysis revealed that IL-27 stimulation of naive CD4+ T cells lead to increased coexpression of IL-10 and IFN-γ (Fig. 2F). The addition of IL-2 further enhanced the expression of IL-10 in these cells. It has been shown that IL-10 secretion by human Tr1 cells requires IL-2 (7). Because Tr1 cells are characterized by release of IL-10 without concurrent production of IL-2, we tested the role of IL-2 in IL-27-induced IL-10 secretion. CD4+ T cells activated with either CD3/CD28/IL-27 or CD3/IL-27 in the presence of IL-2 produced large amounts of IL-10 (Fig. 2G). The addition of a neutralizing mAb to IL-2 abrogated IL-10 production, suggesting that IL-10 production is dependent on IL-2 in IL-27-activated T cells (Fig. 2H).

IL-27 inhibits IL-17 production and molecules associated with its effector functions from CD4+ T cells

Although the factors that promote and/or control murine Th17 differentiation have been extensively studied, there has been much less information on the regulation of this cytokine in human T cells. It has been shown that IL-1β, IL-6, and IL-23 are important in promoting IL-17 secretion from human CD4+ T cells (21, 22). The nuclear receptor RORC acts as a key transcription factor in

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4 The online version of this article contains supplemental material.
FIGURE 2. IL-27 stimulation induces Tr1 like cells. A, Purity of naive and memory CD4⁺ T cells. Flow cytometry of naive CD4⁺ T cells after purification and staining with anti-CD4 PE, anti-CD45RA allophycocyanin, and anti-CD45RO FITC. B, Analysis of the cytokine expression of naive and memory CD4⁺ T cells stimulated with anti-CD3 and anti-CD28 in the presence or absence of recombinant human IL-27 (100 ng/ml). C, Naive CD4⁺ T cells expressed more IL-27 receptor on their surface compared with memory CD4⁺ T cells as measured by FACS. D and E, Analysis of the cytokine expression of secondary stimulated T cells. CD4⁺ T cells, first activated with CD3/CD28/IL-27, produced large amounts of IL-10 on secondary stimulation. Cells were stimulated for 3 days with anti-CD3 and anti-CD28 in the presence or absence of IL-27 and 3 days after primary stimulation cells were expanded and subjected to secondary stimulation. IL-10 secretion was measured at day 3. F, IL-27 induces the generation of IFN-γ and IL-10⁺ CD4⁺ T cells. Flow cytometry of naive CD4⁺ T cells activated with anti-CD3 and anti-CD28 in the presence or absence of IL-27 stained for intracellular IL-10 and IFN-γ. G and H, IL-27-induced IL-10 production is IL-2 dependent. CD4⁺ T cells were incubated with the plate-bound anti-CD3 and anti-CD28 in the presence or absence of IL-27 and IL-10 secretion was measured at day 3. rIL-2 (100 U/ml) or neutralizing Ab to IL-2 (20 μg/ml) was added as indicated. I, IL-27-stimulated T cells inhibit the proliferation of bystander CD4⁺ T cells in an IL-10-dependent manner. Naive CD4⁺ T cells were incubated with the plate-bound anti-CD3 and anti-CD28 in the presence or absence of IL-27 for 3 days. Cell-free culture supernatants were transferred to freshly purified CD4⁺ T cells cultured with plate-bound anti-CD3 alone or with anti-CD3 and CD28. Neutralizing anti-IL-10 Ab (20 μg/ml) was added to the indicated condition and proliferation was measured at day 5. Data represent one of four (A–C) experiments or one of three (D and E) or one of two (F–I) experiments involving nine randomly selected donors, and the error bars represent the mean ± SD.
this lineage commitment process. However, the factors that control IL-17 production from human T cells are not known. Therefore, we examined the effect of IL-27 on IL-17 secretion from CD4⁺ T cells. In agreement with published studies (37, 38), induction of IL-17 from naive T cells was not observed in response to CD3 alone or CD3/CD28 stimulation (data not shown). However, total CD4⁺ T cells produced IL-17 in response to CD3/CD28 stimulation and was inhibited by the addition of IL-27 (Fig. 3A). Furthermore, IL-27 induced inhibition of IL-17 expression was associated with substantial reduction of RORC (Fig. 3B, 3D). It has been reported that IL-17A, IL-22, and IL-17F are mainly produced by Th17 cells (21–25). IL-23 is involved in the generation and maintenance of Th17 cells and constitutive expression of the IL-23 receptor on Th17 cells has been described (39). Thus, we asked whether IL-27 modulates molecules associated with function and maintenance of Th17 cells. We found that IL-27 inhibited CD3/CD28-induced IL-17F and IL-22 expression from total CD4⁺ T cells (Fig. 3, C and D). The inhibitory effect of IL-27 was also well pronounced on IL-23 receptor expression as determined by both real-time PCR and flow cytometry (Fig. 3E). Human IL-17-producing cells also express the chemokine receptor CCR6 and its ligand CCL20 (40). We found that the addition of IL-27 inhibited the expression of both CCR6 and CCL20 (Fig. 3, F and G). We next tested whether the inhibition of IL-17 production by IL-27 was dependent on IL-10. We found that the addition of neutralizing IL-10 Ab did not reverse IL-27-mediated inhibition of IL-17. However, addition of neutralizing IFN-γ Ab completely reversed the IL-27-mediated suppression of IL-17 (Fig. 3H). Furthermore, IL-27-induced inhibition of IL-17 expression was associated with a substantial reduction of RORC, and neutralizing IFN-γ Ab restored the IL-27-mediated suppression of RORC (Fig. 3H). The addition of recombinant IFN-γ led to significant inhibition of IL-17 production from CD3/CD28-stimulated total CD4⁺ T cells. Furthermore, IFN-γ-induced inhibition of IL-17 expression was associated with a substantial reduction in RORC expression (Fig. 3I).

Next, we tested whether IL-27 is capable of inhibiting IL-17 secretion under Th17-polarizing conditions. We stimulated naive CD4⁺ T cells with IL-17-polarizing cytokines IL-1β and IL-23 with or without IL-27. In agreement with previous studies, IL-17 was produced under this condition; moreover, the addition of IL-27 suppressed IL-17 secretion. We found that addition of IL-27 inhibited IL-17 production from naive T cells (21–25). We next tested whether IL-27 is capable of inhibiting IL-17 secretion under Th17-polarizing conditions. We stimulated naive CD4⁺ T cells with IL-17-polarizing cytokines IL-1β and IL-23 with or without IL-27. In agreement with previous studies, IL-17 was produced under this condition; moreover, the addition of IL-27 suppressed IL-17 secretion. We found that addition of IL-27 inhibited IL-17 production from naive T cells (21–25).
culture conditions, demonstrating a wide effect of IL-27 on IL-10 expression (Fig. 4E). Furthermore, IL-27 showed an inhibitory effect on memory cell IL-17 production and molecules associated with its effector function and maintenance of the Th17 phenotype (supplemental Fig. 5). Collectively, these data indicated that IL-27 inhibits IL-17 and molecules associated with function and maintenance of Th17 cells.

**IL-27 inhibits IL-17-polarizing cytokines from DCs**

It is well established that the development of Th cell subsets is orchestrated by cytokines produced by DCs that differentially sense archetypical structures defining different classes of pathogens. For example, DC-secreted IL-12 has been shown to favor Th1 development while DC-secreted IL-10 is known to inhibit these cytokines (41, 42). In addition, TGF-β secretion by DCs can modulate Foxp3+ Treg cell development (43). Likewise, DC-secreted IL-1β and IL-6 appear to synergize with IL-23 in the induction of IL-17 by human CD4+ T cells. It has been shown that human DCs primed by TLR agonists produce enhanced amounts of IL-1β, IL-6, and IL-23 (21). Therefore, we tested whether IL-27 modulates IL-17-polarizing cytokine secretion by DCs. For this, we stimulated DCs with TLR ligands in the presence or absence of IL-27, and analyzed IL-17 production. ELISA of IL-17 secretion by Th17-polarized cells. Naive CD4+ T cells were activated with plate-bound anti-CD3 and anti-CD28 in the presence of IL-27 or with or without IL-27. Data represent one of three randomly selected donors.

**Discussion**

In this study, we show that in humans IL-27 induces the generation of T cells that secrete large amounts of IL-10 and suppress T cell proliferation in an IL-10-dependent manner. On the contrary, IL-27 inhibited IL-17 secretion and the molecules associated with function and maintenance of the Th17 phenotype. Control of self-reactive T lymphocytes by Tr1 cells has been proposed to maintain tolerance and prevention of autoimmunity (4, 5). These cells suppress immune responses through either direct cell-cell interactions or the release of inhibitory cytokine IL-10. The differentiation of

**FIGURE 4.** IL-27 inhibits IL-17 production from Th17-polarized cells. Naive CD4+ T cells were activated with plate-bound anti-CD3 and anti-CD28 in the presence of Th17-polarizing cytokines IL-1β (50 ng/ml) and IL-23 (50 ng/ml) with or without IL-27 (100 ng/ml). A, ELISA of IL-17 in cell-free culture supernatants. B, Real-time quantitative RT-PCR of transcript expression of RORC at 24 h after activation. C, IL-27 inhibits TGF-β in combination with other proinflammatory cytokine-induced IL-17 secretion. ELISA of IL-17 secretion by Th17-polarized cells by TGF-β in combination with IL-1β (50 ng/ml), IL-6 (50 ng/ml), IL-21 (12.5 ng/ml), and IL-23 (50 ng/ml) with or without IL-27. D, IL-27 inhibits RORC expression as determined by real-time quantitative RT-PCR. E, IL-27 induces IL-10 secretion from CD4+ T cells stimulated with TGF-β in combination with other proinflammatory cytokines. ELISA of IL-10 secretion from T cells stimulated with TGF-β in combination with IL-1β (50 ng/ml), IL-6 (50 ng/ml), IL-21 (25.0 ng/ml), and IL-23 (50 ng/ml) with or without IL-27. Data represent one of three independent experiments with cells from three randomly selected donors.
CD4+ T cells into Tr1 cells is poorly defined, in part because of difficulties in inducing and culturing such cells. Tr1 cells produce large amounts of IL-10 and moderate amounts of IFN-γ but no IL-4 and are able to suppress in vitro T cell responses (5). In agreement with these features, our results suggest that IL-27-induced CD4+ T cells produced large amounts of IL-10 and moderate amounts of IFN-γ but no IL-4 and are able to suppress in vitro T cell responses. Tr1 cells are characterized by the release of IL-10 without concomitant production of IL-2 (7). Our results suggest that in humans activation of CD4+ T cells with IL-27 in the presence of IL-2 enhanced the production of IL-10, whereas neutralizing anti-IL-2 Ab abrogated the effect, suggesting that IL-10 production is dependent on IL-2 in IL-27-activated T cells.

IL-17 has been shown to have a pathogenic role in autoimmunity. IL-17 expression has been detected in the target tissue during the progression of various human autoimmune diseases such as multiple sclerosis, rheumatoid arthritis, psoriasis, and Crohn's disease (9). Consistent with these observations, IL-17-deficient mice or mice treated with an IL-17 receptor antagonist are resistant to the development of adjuvant-induced arthritis (44, 45). Similarly, IL-17-deficient animals develop experimental autoimmune encephalomyelitis with delayed onset and reduced severity (46). Given the pathogenic relevance of IL-17, it is important to understand how IL-17 is controlled in human T cells.

Despite a great deal of information on the regulation of IL-17 in the mouse, until recently there has been a paucity of information on the differentiation pathways that lead to IL-17 generation in humans. It has been recently shown that the cytokines IL-1β, IL-6, and IL-23 are capable of driving IL-17 secretion from human CD4+ T cells (21, 22). Most recently, TGF-β in combination with other proinflammatory cytokines has been shown to induce Th17 differentiation from cord blood-derived naive CD4+ T cells (23–25). However, the factors that negatively regulate IL-17 production in humans are not known. In this study, we show that IL-27 is capable of inhibiting IL-17 production both from total and memory CD4+ T cells. In addition, IL-27 exerted its suppressive role on IL-17 production even under strong Th17-polarizing conditions. Also, IL-27 showed a broad inhibitory effect on the Th17 subset-associated effector cytokines such as IL-17F and IL-22. It has been proposed that IL-23 is required to shape a stable Th17 population in the secondary lymphoid tissue and to maintain a pathogenic Th17 population at the site of inflammation in animals (47). Furthermore, constitutive expression of IL-23R on IL-17-producing T cells was observed in humans (27). We found that anti-CD3/CD28 stimulation induced IL-23R on total and memory CD4+ T cells. The addition of IL-27 markedly inhibited the expression of IL-23R on these cells, suggesting that IL-27 can inhibit expansion of the IL-17-producing cells. Th17 cells in humans have been reported to
express chemokine receptor CCR6 and its ligand CCL20, indicating that Th17 cells might regulate their own recruitment to inflamed tissues in an autocrine manner. We found that addition of IL-27 inhibited the expression of both CCR6 and CCL20, suggesting a role for IL-27 in limiting the molecule associated with Th17 recruitment to the site of inflammation. Thus, our results suggest that IL-27 inhibits not only IL-17 production from T cells but also molecules associated with function and maintenance of the Th17 phenotype.

The differentiation of CD4+ T cells into effector population is profoundly influenced by cytokine produced by DCs. DC-secreted IL-1β and IL-6 appear to synergize with IL-23 in the induction of IL-17 by human CD4+ T cells. It has been shown that human DCs primed by TLR agonists produce enhanced amounts of IL-1β, IL-6, and IL-23 and that culturing these DCs with T cells induced IL-17 production from T cells (21). Our results suggest an additional mechanism for IL-27 in suppressing IL-17 production from T cells. IL-27 inhibited TLR ligand induced IL-17-polarizing cytokines IL-1β, IL-6, and IL-23. Moreover, culturing IL-27-pretreated DCs with T cells showed reduced IL-17 secretion, suggesting that IL-27 is capable of inhibiting IL-17-inducing cytokines from DCs and thereby inhibiting IL-17 production from T cells.

Our results demonstrate that IL-27 plays a key role in human T cells by promoting a specific subset of Tregs (IL-10 secreting) and inhibiting Th17 cells and thus provides a dual regulatory mechanism to control autoimmunity and tissue inflammation.

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Disclosures

The authors have no financial conflict of interest.

References


Supplementary Figure: 1

A

Naive CD4+ T cell

B

Memory CD4+ T cell

Thymidine Incorporation

Thymidine incorporation

Med CD3
CD3/IL-27
CD3/IFN-γ
CD3/28/IL-27
Supplementary figure: 2

A

![Graph A](image)

B

![Graph B](image)

Culture supernatants added from cells stimulated with:
- Medium
- CD3/28
- CD3/28/IL-27
- CD3/28/IL-27/anti-IL-10
- CD3/28/IL-27/anti-IL-27

Thymidine Incorporation


y-axis: 0, 2500, 5000, 7500, 10000, 12500
Supplementary Figure: 4

A

B

P = 0.0013

P = 0.0011

C

D

P = 0.0037

P = 0.0042

E

P = 0.0104

Med

Th17

Th17/IL-27

IL-17F (Rel. expression)

IL-22 (Rel. expression)

IL-23R (Rel. expression)

CCR6 (Rel. expression)

CCL20 (Rel. expression)
Supplementary figure: 5

A

B

C

D

E

F

G

IL-17 (pg/ml)

RORC (Rel. expression)

IL-17F (Rel. expression)

IL-22 (Rel. expression)

IL-23R (Rel. expression)

CCR6 (Rel. expression)

CCL20 (Rel. expression)
Supplementary figure: 6
Figure Legends

Supplementary Figure 1. Effect of IL-27 on naïve and memory CD4$^+$ T cell proliferation. IL-27 do not inhibit either naïve or memory CD4$^+$ T cell proliferation. Naïve and memory CD4$^+$ T cells (2x10$^5$ cells per well in a 96 well plate) were stimulated with plate bound anti-CD3 and CD28 with or without recombinant human IL-27 (100 ng/ml) and proliferation was measured at day 3. Data are representative of two separate experiments with 3 donors.

Supplementary Figure 2. Direct effect of IL-27 on CD4$^+$ T cell proliferation. (A) IL-27 does not inhibit naïve CD4$^+$ T cell proliferation. Naïve CD4$^+$ T cells were stimulated with plate bound anti-CD3 and CD28 with or without recombinant human IL-27 (100 ng/ml) and proliferation was measured at day 3. (B) IL-27 in the culture did not affect bystander T cell proliferation. Naïve CD4$^+$ T cells were seeded at a density of 2x10$^5$ cells per well in a 96 well plate coated with monoclonal antibodies to CD3 (1.0 $\mu$g/ml), CD28 (1.0 $\mu$g/ml). Cell free supernatants from the CD3/28/IL-27 or CD3/28 activated cell populations were transferred to the above culture with or without neutralizing antibody to IL-10 and proliferation was measured at day 3. In some culture condition anti-IL-27 antibody was added at a concentration of 20 $\mu$g/ml. Data are representative of two separate experiments.

Supplementary Figure 3. IL-27 in combination with TGF-β induces IL-10 production from CD4$^+$ T cells (A and B) Analysis of IL-10 and IFN-γ expression of naïve CD4$^+$ T cells stimulated with IL-27 (100 ng/ml) in the presence or absence of recombinant human TGF-β (20 ng/ml). (C) Quantitative PCR of the expression of mRNA encoding FOXP3 in CD4$^+$ T cells stimulated with IL-27 (100 ng/ml) in the presence or absence of
recombinant human TGF-β (20 ng/ml). Data are from 7 different healthy donors. Horizontal bars indicate the median. Data represent one of three independent experiments.

Supplementary Figure 4. IL-27 inhibits molecules associated Th17 effector molecules in total CD4⁺ T cells. (A-E) Quantitative PCR of the expression of mRNA encoding IL-17F, IL-22, IL-23R, CCR6 and CCL20 from total CD4⁺ T cells stimulated with plate bound anti-CD3 and anti-CD28 in the presence or absence of IL-27 (100 ng/ml). Data are representative of three separate experiments.

Supplementary Figure 5. IL-27 inhibits IL-17 production from memory CD4⁺ T cells. (A) Memory CD4⁺ T cells were stimulated with anti-CD3 and anti-CD28 (1 μg/ml) in the presence or absence of IL-27 (100 ng/ml) for 4 days and cell free culture supernatants was assayed for IL-17A by ELISA. (B) IL-27 inhibits RORC expression in memory CD4⁺ T cells. (C-G) IL-27 inhibits molecules associated Th17 effector molecules in total CD4⁺ T cells. Quantitative PCR of the expression of mRNA encoding IL-17F, IL-22, IL-23R, CCR6 and CCL20 from memory CD4⁺ T cells stimulated with plate bound anti-CD3 and anti-CD28 in the presence or absence of IL-27 (100 ng/ml). Data are representative of three separate experiments with 3 donors.

Supplementary Figure 6. IL-27 prestimulation of DCs inhibits IL-17 production from memory CD4⁺ T cells. (A) and (B) show naïve and memory CD4⁺ T cells were cultured with a 1: 3 ratio of DCs prestimulated with LPS or PGN alone or in the presence of IL-27 (100 ng/ml). After 5 days of coculture the cell free culture supernatants were tested for IL-17 by ELISA. Data are representative of three independent experiments.