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Cutting Edge: Suppression of GM-CSF Expression in Murine and Human T Cells by IL-27

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GM-CSF is a potent proinflammatory cytokine that plays a pathogenic role in the CNS inflammatory disease experimental autoimmune encephalomyelitis. As IL-27 alleviates experimental autoimmune encephalomyelitis, we hypothesized that IL-27 suppresses GM-CSF expression by T cells. We found that IL-27 suppressed GM-CSF expression in CD4+ and CD8+ T cells in splenocyte and purified T cell cultures. IL-27 suppressed GM-CSF in Th1, but not Th17, cells. IL-27 also suppressed GM-CSF expression by human T cells in nonpolarized and Th1- but not Th17-polarized PBMC cultures. In vivo, IL-27p28 deficiency resulted in increased GM-CSF expression by CNS-infiltrating T cells during Toxoplasma gondii infection. Although in vitro suppression of GM-CSF by IL-27 was independent of IL-2 suppression, IL-10 upregulation, or SOCS3 signaling, we observed that IL-27-driven suppression of GM-CSF was STAT1 dependent. Our findings demonstrate that IL-27 is a robust negative regulator of GM-CSF expression in T cells, which likely inhibits T cell pathogenicity in CNS inflammation. The Journal of Immunology, 2012, 189: 2079–2083.

Granulocyte-macrophage CSF is a proinflammatory hematopoietic growth factor produced by many cell types, including T cells (1). GM-CSF is important in many cellular processes such as dendritic cell activation, granulocyte survival, and enhancement of macrophage and microglial function (2–4).

Previous studies in experimental autoimmune encephalomyelitis (EAE) have demonstrated an essential pathogenic role for T cell–derived GM-CSF in CNS inflammation (4–8). Studies using Toxoplasma gondii infection in mice, which can also cause CNS inflammation, reported a detrimental role for GM-CSF by increasing parasitic burden in peritoneal macrophages (9). Taken together, these studies suggest that therapeutic targeting of GM-CSF may be beneficial in CNS inflammatory disease. However, regulation of GM-CSF expression, particularly by T cells, is not well understood and is a critical gap in our knowledge of GM-CSF biology.

IL-27 is a heterodimeric member of the IL-12 cytokine family (10) that is produced mainly by activated APCs (10). IL-27 was initially shown to promote Th1 differentiation (11), as well as to inhibit the differentiation of naive CD4+ T cells to other helper subsets (11, 12). However, reports have shown an insensitivity of committed Th17 cells to IL-27 in vitro (12, 13). IL-27 also influences production of a range of cytokines, including IL-2 and IL-10 (14–17). In addition, IL-27 suppresses EAE, in part via Th17 cell inhibition and IL-10 upregulation (12, 18, 19). Moreover, IL-27R–deficient mice exhibit enhanced CNS inflammation when chronically infected with T. gondii, further demonstrating that IL-27 regulates CNS inflammation (20).

Given the suppressive effect of IL-27 on CNS inflammation (16, 18–20) and the pathogenic role of GM-CSF in CNS inflammation, we hypothesized that IL-27 negatively regulates GM-CSF. In this article, we show that IL-27 suppressed GM-CSF production by CD4+ and CD8+ T cells under nonpolarizing (NP) and Th1 conditions, but not Th17 conditions, in both murine and human cultures. This suppression was mediated by JAK2/Tyk2 and STAT1 signaling. IL-27p28–deficient mice exhibited elevated proportions of GM-CSF–producing T cells during T. gondii infection in vivo. Taken together, these findings indicate that IL-27 is a key negative regulator of GM-CSF expression by T cells, which likely regulates inflammation in health and disease.

Materials and Methods

Mice

C57BL/6 mice were obtained from Charles River Laboratories. 2D2 mice were a kind gift from Prof. S. Anderton (University of Edinburgh, Edin- burgh, U.K.). stat3−/− mice were purchased from Taconic. Il10−/− mice were a kind gift from Mouse Genetics Cologne Foundation and were provided by

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Abbreviations used in this article: EAE, experimental autoimmune encephalomyelitis; JI-1, Jak inhibitor 1; NP, nonpolarized/nonpolarizing; WT, wild-type.

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Prof. Anne O’Garra (Medical Research Council National Institute for Medical Research, London, U.K.), il27p28Δ/Δ mice were obtained from Lexicon Pharmaceuticals (The Woodlands, TX) (21). SOCS3 boxed (sox3Δ/fl) mice (The Jackson Laboratory) were crossed with CD44cre mice (European Mouse Mutant Archive) to generate mice that lacked SOCS3 in CD4+ cells only (CD44creSox3Δ/fl). All animal maintenance and experiments were in compliance with the UK Home Office or in accordance with the federal and institutional guidelines of the University of Pennsylvania School of Veterinary Medicine, and approved by the Queen’s University Ethical Review Committee or the University of Pennsylvania Institutional Animal Care and Use Committee.

**Cell culture and T cell purification**

Murine splenocytes were cultured in complete RPMI 1640 or X-VIVO 15 (Lonza). Cells were stimulated with soluble anti-CD3 and anti-CD28 (1 μg/ml each) Abs at 2 × 10^6 cells per milliliter. For purified T cell cultures, cells were purified by magnetic microbead separation (Stemcell) and cultivated as above at 10^6 cells per milliliter. For Th17 cell culture, cells were cultured in IMDM or X-VIVO 15 and activated as above. When indicated, Jak inhibitor 1 (1-10 μM) (Calbiochem) was added to cultures.

Human PBMCs from healthy volunteers were purified by Ficoll density gradient centrifugation. PBMCs or purified CD4+ cells (Miltenyi Biotec) were cultured in RPMI 1640 or IMDM and activated as above at 2 × 10^6 cells/ml. All activation Abs were obtained from eBioscience.

**T cell polarization and cytokines**

For experiments performed under NP conditions, no exogenous cytokines were added to the cultures. When indicated, the following were used for Th1 polarization, IL-12 (10 ng/ml; eBioscience); for Th17 polarization, TGF-β (2 ng/ml; R&D Systems) or IL-23 (50 ng/ml; eBioscience), IL-6 (200 U/ml; R&D Systems), IL-1β (10–20 ng/ml; Peprotech), and anti–IFN-γ Ab (10 μg/ml clone XMG1.2; BioXcell). For human Th17 cell polarization, the following were used: IL-23 (10 ng/ml), anti–IFN-γ Ab (10 μg/ml), and anti–IL-4 Ab (5 μg/ml); for GM-CSFΔ/Δ T cell polarization, anti–IFN-γ Ab and anti–IL-12 Ab (10 μg/ml clone 11B11; BioXcell) were used. For reactivated murine Th17 cells, IL-23 (10 ng/ml; eBioscience) was added to cultures. When indicated, IL-27 (10–20 ng/ml; eBioscience) was used. Where stated, daily IL-2 (100 U/ml; R&D Systems) or anti–IFN-γ Ab was added to cultures.

**Toxoplasma gondii infection**

The ME49 strain of T. gondii was maintained and prepared as described (22). For infections, wild-type (WT) and il27p28Δ/Δ mice were administered 20 tissue cysts i.p. and treated with sulfadiazine (200 mg/l; Sigma-Aldrich) in drinking water from day 4 for 2 wk. Mice were sacrificed 3–4 wk post infection, and GM-CSF expression by CD4+ T cells from spleen and CNS was assessed by flow cytometry.

**Flow cytometry**

Cells were stimulated with PMA (50 ng/ml), ionomycin (500 ng/ml), and brefeldin A (1 μg per 10^6 cells) for up to 5 h before staining. Cells were surface stained, fixed, permeabilized, and stained intracellularly with fluorescently conjugated Abs (all from eBioscience).

**Real-time PCR**

Extracted mRNA was converted to cDNA (Applied Biosystems). PCR amplification (Quantitect SYBR Green; Qiagen) was carried out using primers for the csf2 gene (7). Values were normalized to β-actin and compared with controls (unstimulated splenocytes).

**Cytokine quantification**

GM-CSF protein was quantified by ELISA (R&D Systems).

**Statistics**

Data were tested for statistical significance using unpaired, two-tailed, Student t tests for parametric data and Mann-Whitney tests for nonparametric data.

**Results and Discussion**

**IL-27 suppresses GM-CSF expression in activated murine T cells**

To test the hypothesis that IL-27 negatively regulates GM-CSF expression by activated T cells, splenocytes were activated in the presence or absence (±) of exogenous IL-27. Over 96 h, IL-27 suppressed GM-CSF protein expression (Fig. 1A) in both CD4+ and CD8+ T cells (Fig. 1B). To determine whether IL-27 acted directly on T cells to suppress GM-CSF, purified CD4+ or CD8+ T cells were activated ± IL-27. IL-27 consistently suppressed GM-CSF expression in purified cultures (Fig. 1C). These studies demonstrate that IL-27 can directly suppress GM-CSF expression by T cells.

**IL-27 suppresses GM-CSF expression in Th1- but not Th17-polarizing conditions**

Given the importance of GM-CSF in the pathogenicity of Th1 cells in EAE (8), we examined whether IL-27 suppressed GM-CSF expression by Th1 cells. GM-CSF production was diminished under Th1-polarizing conditions (exogenous IL-12 added), consistent with recent findings (7). However, addition of IL-27 further suppressed GM-CSF expression (Fig. 2A, 2B, 2C).
Supplemental Fig. 1A), demonstrating the synergistic actions of IL-27 and IL-12 in regulating GM-CSF production.

As GM-CSF also contributes to the pathogenicity of Th17 cells (8), we examined the influence of IL-27 on Th17 cell-derived GM-CSF. Because IL-27 directly suppresses Th17 differentiation (12), we activated splenocytes in Th17-polarizing stimuli for 24 h before adding IL-27. Of interest, we did not observe GM-CSF suppression by IL-27 in this system (Fig. 2C, Supplemental Fig. 1B). To study more thoroughly if IL-27 could modulate Th17 cell-derived GM-CSF, we reactivated differentiated Th17 cells under the influence of IL-23 ± IL-27 (Fig. 2C). Again, we did not observe suppression of GM-CSF by IL-27. This finding is perhaps not surprising, considering published data demonstrating that differentiated Th17 cells lack responsiveness to IL-27 in vitro (12, 13). However, flow cytometric analysis of GM-CSF expression in these cultures yielded positive GM-CSF staining in only one of six repeated experiments (Supplemental Fig. 2A), which parallels lower GM-CSF detection in these culture supernatants (Fig. 2C, Supplemental Fig. 1B). Taken together, these data show that IL-27 suppresses GM-CSF production by Th1, but not Th17, cells in vitro.

As Th17 polarization was initiated before the addition of IL-27, Th17-polarizing stimuli may have rendered these cells unresponsive to IL-27. It is also possible that Th17-polarizing stimuli suppressed GM-CSF to such an extent that the addition of IL-27 had no additive suppressive effect. This finding may potentially be due to the presence of TGF-β, which is known to inhibit GM-CSF (8).

To address this possibility, we polarized Th17 cells in the absence of exogenous TGF-β (as described in Ref. 23). Although IL-23–polarized Th17 cells produced GM-CSF at levels equivalent to those of NP T cells (Supplemental Fig. 1B), IL-27 still did not suppress GM-CSF in these Th17 cultures (Fig. 2D, Supplemental Fig. 1B).

Recently Codarri et al. (7) showed that neutralization of IFN-γ and IL-12 promotes the development of highly encephalitogenic GM-CSFhigh T cells. To test whether IL-27 also inhibits GM-CSF expression in this GM-CSFhigh population, splenocytes were activated in the presence of neutralizing anti–IFN-γ and anti–IL-12 ± IL-27. Compared with NP conditions, the proportion of CD4+ T cells expressing GM-CSF was enhanced, and this was reduced by IL-27, as was GM-CSF secretion (Fig. 2E, Supplemental Fig. 2B). These data suggest that IL-27 could limit the encephalitogenicity of GM-CSFhigh T cells.

To reflect in vivo T cell activation more accurately, splenocytes from 2D2 transgenic TCR mice were activated in vitro with MOG35–55 Ag under NP and Th1 conditions ± IL-27. In this model, IL-27 suppressed GM-CSF production (Fig. 2F), demonstrating that GM-CSF expression by T cells in response to Ag can be suppressed by IL-27.

To determine if IL-27 regulated T cell-derived GM-CSF during CNS inflammation in vivo, WT and il27p28−/− mice were infected with T. gondii. Significantly higher proportions of GM-CSF–producing effector T cells were observed in spleens of il27p28−/− animals compared with WT animals during acute infection (Fig. 2G). IL-27p28 deficiency also resulted in higher proportions of GM-CSF–producing effector T cells in the CNS during chronic infection (Fig. 2H). These data demonstrate that IL-27 regulates GM-CSF expression by T cells in vivo, which may contribute to enhanced inflammation observed in IL-27R−/− mice infected with T. gondii (20).

Suppression of GM-CSF expression by IL-27 is independent of IL-10, IL-2, IFN-γ, and SOCS3 signaling but dependent on Jak2/Tyk2 activity and STAT1 signaling.

We next addressed the mechanism of GM-CSF suppression by IL-27. IL-10 is a potent inhibitor of GM-CSF (24), and as IL-27 has been demonstrated to induce IL-10 in T cells (15–17), we examined whether suppression of GM-CSF by IL-27 was dependent on IL-10. IL-27 suppressed GM-CSF expression in il10−/− splenocytes (Fig. 3A, Supplemental Fig. 2C), demonstrating that IL-10 is not required for GM-CSF suppression by IL-27.

IL-27 inhibits IL-2 expression by T cells (14). As IL-2 is an important T cell growth factor, IL-27 could suppress T cell-derived GM-CSF by depriving T cells of IL-2. However, daily
addition of exogenous IL-2 did not abrogate GM-CSF suppression by IL-27 (Fig. 3B, Supplemental Fig. 2D), showing that IL-2 inhibition does not mediate this phenomenon.

As IFN-γ can inhibit GM-CSF expression (7) and is induced by IL-27 (22), we studied whether IFN-γ mediated suppression of GM-CSF by IL-27. Addition of anti–IFN-γ did not prevent suppression of GM-CSF by IL-27 (Fig. 3C, Supplemental Fig. 2E), suggesting that IFN-γ does not mediate such suppression.

IL-27 induces SOCS3 (14), which regulates a range of cytokine signaling pathways (25) and thus could inhibit an inducer of GM-CSF. We examined the suppressive effect of IL-27 on GM-CSF expression in splenocytes from mice with SOCS3 deficiency restricted to CD4+ inducer of GM-CSF. We examined the suppressive effect of IL-27 on GM-CSF expression in splenocytes from mice with SOCS3 deficiency restricted to CD4+ cells (CD4−/cre/Socs3−/−). IL-27 suppressed GM-CSF expression in these cells (Fig. 3D, Supplemental Fig. 2F), demonstrating that this mechanism is SOCS3 independent.

As suppression of GM-CSF was independent of several known regulatory mechanisms of IL-27 (Fig. 3A, 3B, 3D), we hypothesized that IL-27 directly suppresses early GM-CSF gene transcription. We observed suppression of cf-2 expression by IL-27 as early as 3 h following T cell activation by real-time PCR (Fig. 3E). Such rapid suppression suggests that IL-27 directly inhibits GM-CSF gene transcription, potentially via antagonizing downstream TCR signaling targets that induce GM-CSF.

To determine the signaling pathway by which IL-27 mediates suppression of GM-CSF, we examined Jak/STAT signaling using JI-1 (Fig. 3F). At concentrations of JI-1 that selectively inhibited Jak2 and Tyk2 (1 nM), suppression of GM-CSF was lost (Fig. 3F, Supplemental Fig. 2G), demonstrating that suppression of GM-CSF by IL-27 is mediated by Jak2/Tyk2 activity.

STAT1 can be directly phosphorylated by Jak2 and mediates several anti-inflammatory properties of IL-27 (16, 17, 19). We therefore examined whether suppression of GM-CSF by IL-27 was dependent on STAT1. In cultures of activated stat1−/− splenocytes, IL-27 did not suppress GM-CSF expression (Fig. 3G), demonstrating that STAT1 signaling is required for IL-27–mediated suppression of GM-CSF. Of interest, suppression of GM-CSF by IL-12 was also lost in stat1−/− mice (data not shown), suggesting that both cytokines may suppress GM-CSF via similar mechanisms.

IL-27 suppresses GM-CSF expression in human T cells

To translate findings from murine to human systems, we investigated whether IL-27 suppressed GM-CSF expression by human T cells. PBMCs from healthy donors were activated under NP, Th1, and Th17 conditions ± IL-27. Robust GM-CSF expression was observed in this system (Fig. 4A, 4B). Even in such potent GM-CSF–producing cultures, IL-27 suppressed GM-CSF in NP and Th1-polarized conditions (Fig. 4A, 4B), consistent with murine observations (Figs. 1, 2). Of interest, IL-12 did not suppress GM-CSF in human T cells, the opposite of that observed in our murine model (Figs. 2, 4A, Supplemental Fig. 1A, 1C), suggesting differential kinetics of GM-CSF regulation by IL-12 in murine and human systems. However, IL-27 consistently suppressed both proportions of GM-CSF+ cells and secreted GM-CSF in supernatants of both murine and human cultures (Figs. 1, 4, Supplemental Fig. 1C), indicating that IL-27 is a conserved negative regulator of GM-CSF.

IL-27 did not suppress GM-CSF production in human Th17 cultures, as measured by ELISA (Fig. 4A). Despite confirmation of IL-27 bioactivity through inhibition of Th17 polarization (Fig. 4B), the percentage of Th17 cells (based on total IL-17+ cells) producing GM-CSF was unaffected (68% in control cells compared with 71% in IL-27–treated cells; Supplemental Fig. 1F). This finding further demonstrates that IL-27 does not suppress GM-CSF production in committed Th17 cells. IL-27 also directly suppressed GM-CSF by purified NP and Th1-polarized CD4+ T cells (Fig. 4C, Supplemental Fig. 1D, 1G), but not in Th17-polarized purified CD4+ T cell cultures (based on the percentage of total IL-17+ cells that coexpressed GM-CSF; Supplemental Fig. 1E, 1H).

These data show that IL-27 directly regulates GM-CSF expression by human Th1, but not Th17, cells.

In this study, we report that IL-27 suppresses GM-CSF in activated murine and human T cells; however, importantly, GM-CSF was never completely abolished. This finding suggests that IL-27 selectively inhibits only some of the signals that induce GM-CSF expression. Understanding these mechanisms would be important in the development of novel GM-CSF–directed therapies, such that selective antagonism of the GM-CSF system could be appropriated to maximize preservation of normal immune function but limit GM-CSF–associated immunopathogenesis.

Given the importance of GM-CSF in APC function during inflammation, inhibition by IL-27 may suppress inflammation by muting APC activation, further limiting T cell activation. Indeed, T cell–derived GM-CSF contributes to EAE pathogenesis by potentiating APC functions in the CNS, particularly in microglia, which augments recruitment and activation of T cells and other myeloid cells (5–7). This activity serves to propagate the local inflammatory response that drives tissue destruction. Thus, strategies that inhibit the pathogenic signature of effector T cells, such as high GM-CSF expression (7, 8), inhibit establishment and/or propagation of local inflammatory responses. In this regard, GM-CSF suppression by IL-27 may be an endogenous anti-inflammatory mechanism and may offer the potential for therapeutic exploitation in inflammatory disease.

Disclosures

The authors have no financial conflicts of interest.

References


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Supplementary Figure 1. A. ELISA of Th1 polarised murine splenocytes +/- IL-27 over 72hrs. GM-CSF was undetectable at the 24h timepoint of IL-27-treated cultures and has been marked as 0 on the graph. (n=4). B. ELISA of murine Non Polarised (NP) and Th17 polarised splenocytes polarised via TGF-β, IL-6, IL-18 and anti-IFN-γ (TGF-β) or IL-23, IL-6, IL-1β and anti-IFN-γ (IL-23) for up to 96hrs. In IL-27 treated cultures, IL-27 was added after 24hrs of culture activation and polarisation, (n=4). Statistical analysis; NP Control vs NP + IL-27 – 48hr = ***, 72hr = *, 96hr = *; Th17 (TGF-β) Control vs Th17 (TGF-β) + IL-27 – all timepoints = NS ; Th17 (IL-23) Control vs Th17 (IL-23) + IL-27 – all timepoints = NS. C. ELISA of human NP PBMCs +/- IL-27 and Th1 (IL-12) polarised human PBMCs over 120hrs. Results are pooled from 5 donors. Statistical analysis; Control vs IL-27 – 24hr = *, 48hr = **, 72hr = **, 96hr = NS, 120hr = *** ; Control vs IL-12 – 24 up to 96hr = NS, 120hr = **. D. Flow cytometry of activated human purified CD4+ T cells polarised under Th1 conditions (+ IL-12) +/- IL-27 for 24hrs. Image is representative of 5 donors. E. Flow cytometry of activated human purified CD4+ T cells polarised under Th17 conditions (+ IL-23 and neutralising anti-IFN-γ and anti-IL-4 antibodies) +/- IL-27 for 24hrs. Image is representative of 5 donors. F. Percentage of IL-17+CD4+ T cells that also express GM-CSF in total PBMC cultures activated +/- IL-27 for 120hrs, (n=5). G. Percentage of IFN-γ+CD4+ T cells that also express GM-CSF in activated purified Th1 polarised CD4+ cell cultures +/- IL-27 for 24hrs, (n=5). H. Percentage of IL-17+CD4+ T cells that also express GM-CSF in activated purified Th7 polarised CD4+ cell cultures +/- IL-27 for 24hrs, (n=5).
Supplementary Figure 2. Splenocytes were activated with anti-CD3/anti-CD28 antibodies and cells were stained for flow cytometric analysis. Data shown are gated on CD4+ T cells. A. WT splenocytes were polarised towards Th17 cells (1st Activation) for 96hrs. For IL-27 treated cultures, IL-27 was added 24hrs after Th17 polarisation stimuli were added to the culture. After the 1st activation, cells were then reactivated in the presence of IL-23 (2nd activation) +/- IL-27 for 48hrs. B. WT splenocytes were polarised towards GM-CSF high T cells +/- IL-27 for 72hrs. C. IL-10 +/- splenocytes were activated +/- IL-27 for 72hrs. D. WT splenocytes were activated in the presence of IL-2 +/- IL-27 for 72hrs. E. WT splenocytes were activated in the presence of neutralising anti-IFN-γ antibodies +/- IL-27 for 72hrs. F. Splenocytes from Socs3 low x CD4 ccs mice were activated +/- IL-27 for 72hrs. G. WT splenocytes were activated under increasing concentrations of Jak Inhibitor 1 +/- IL-27 for 72hrs. Data are representative of at least two and up to four independent experiments.