Suppression of TNF-α and IL-1 Signaling Identifies a Mechanism of Homeostatic Regulation of Macrophages by IL-27

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Suppression of TNF-α and IL-1 Signaling Identifies a Mechanism of Homeostatic Regulation of Macrophages by IL-27

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IL-27 is a pleiotropic cytokine with both activating and inhibitory functions on innate and acquired immunity. IL-27 is expressed at sites of inflammation in cytokine-driven autoimmune/inflammatory diseases, such as rheumatoid arthritis, psoriasis, inflammatory bowel disease, and sarcoidosis. However, its role in modulating disease pathogenesis is still unknown. In this study, we found that IL-27 production is induced by TNF-α in human macrophages (Mφ) and investigated the effects of IL-27 on the responses of primary human Mφ to the endogenous inflammatory cytokines TNF-α and IL-1. In striking contrast to IL-27–mediated augmentation of TLR-induced cytokine production, we found that IL-27 suppressed Mφ responses to TNF-α and IL-1β, thus identifying an anti-inflammatory function of IL-27. IL-27 blocked the proximal steps of TNF-α signaling by downregulating cell-surface expression of the signaling receptors p55 and p75. The mechanism of inhibition of IL-1 signaling was downregulation of the ligand-binding IL-1RII concomitant with increased expression of the receptor antagonist IL-1Ra and the decoy receptor IL-1RIII. These findings provide a mechanism for suppressive effects of IL-27 on innate immune cells and suggest that IL-27 regulates inflammation by limiting activation of Mφ by inflammatory cytokines while preserving initial steps in host defense by augmenting responses to microbial products. The Journal of Immunology, 2010, 185: 7047–7056.

Interleukin-27 is a heterodimeric cytokine composed of EBI3 and p28 subunits that share similarity with the p40 and p35 subunits of IL-12 (1). The IL-27R is a heterodimer composed of a WSX-1 subunit, which confers ligand specificity, and the gp130 signaling subunit, which is also used by the IL-6 family of cytokines (2). WSX-1 bears a STAT1-binding site, and gp130 has four STAT3-docking sites, two of which can also recruit STAT1. In lymphocytes, IL-27 activates STAT1, STAT3, STAT5, and low amounts of STAT4 (3). In myeloid cells, IL-27–induced phosphorylation of STAT1 and STAT3 has been observed (2). Our group has found that in human monocytes (Mo), IL-27 induces sustained activation of STAT1, resulting in potent induction of inflammatory STAT1 target genes and augmentation of TLR responses. In contrast, STAT3-mediated gene induction and suppressive functions were not readily apparent in IL-27–stimulated primary human Mo (4).

The major cellular source of IL-27 is APC: Mo, macrophages (Mφ), and dendritic cells (DCs) (1). In the setting of infections, pathogen recognition by TLRs triggers the synthesis of IL-27. Downstream of TLRs, MyD88-dependent activation of NF-κB and TRIF-dependent activation of IFN regulatory factor (IRF) 3 drive the induction of EBI3 and p28 subunits of IL-27 (5). Type I and II IFNs are also involved in the induction of p28 by activating several members of the IRF family including IRF1, IRF3, IRF7, and IRF9 (6–9). Recently, it has been reported that type I IFNs trigger production of IL-27 by downregulating the expression of the intracellular isoform of osteopontin in murine DCs (10).

In humans, IL-27 is expressed at sites of chronic sterile inflammation, such as the pannus (inflammatory synovial tissue) of rheumatoid arthritis (RA) (11), psoriatic skin lesions (12), inflamed intestine in Crohn’s disease, and sarcoid granulomas (13). IL-27 is a pleiotropic cytokine, and its role in these diseases remains enigmatic and elusive (14–17). Initially, IL-27 was discovered as a cytokine that promotes the early stages of Th1 differentiation by inducing expression of IL-12Rβ2 and rendering T cells responsive to the Th1-promoting effects of IL-12 (3, 18, 19). More recently, the preponderance of evidence suggests that the dominant in vivo function of IL-27 is immunoregulatory by suppressing Th1, Th2, and Th17 cells (5). IL-27 also has both activating and suppressive effects on innate immune cells (16). Our group has described STAT1-mediated proinflammatory effects of IL-27 in human Mo including: 1) induction of chemokines, such as CXCL9 and CXCL10, that are well-known for recruiting inflammatory cells; and 2) augmented production of proinflammatory cytokines in response to TLR ligands (4). In contrast, our laboratory and others (20, 21) observed that IL-27 has tissue-protective capacities by inhibiting osteoclastogenesis. Other reports suggest that IL-27 can suppress inflammatory cytokine production in Mφ and DCs (22, 23), although these suppressive effects in murine cells appeared modest relative to IL-10, the potent Mφ-deactivating cytokine that strongly activates STAT3 (24). Overall, evidence from animal models supports the concept that IL-27, depending on the context, can be either pro- or anti-inflammatory (25). Along these lines, in adjuvant-induced
arthritis, proteoglycan-induced arthritis, and animal models of diabetes mellitus, IL-27 is pathogenic (26–28), whereas in experimental autoimmune encephalomyelitis and in collagen-induced arthritis, IL-27 is protective and may represent a potential treatment (11, 29).

In our current study, we wished to investigate the potential role of IL-27 in the process of chronic sterile inflammation. We first observed that TNF-α, a key player in the synovial inflammation of RA, skin inflammation of psoriasis, and intestinal inflammation of inflammatory bowel disease, triggers the production of large amounts of IL-27 by human Mφ (synovial fluid Mφ of patients with RA or Mφ generated from peripheral blood CD14+ Mo). In striking contrast to augmentation of inflammatory TLR responses (4), we found that IL-27 suppressed responses of human Mφ to endogenous inflammatory factors TNF-α and IL-1β, thus identifying a potent anti-inflammatory function of IL-27. In contrast to IL-10, which suppresses both TLR and TNF-α responses by STAT3-mediated suppression of downstream cytokine gene transcription (24, 30), IL-27 worked by suppressing proximal TNF-α and IL-1 signaling. IL-27 suppressed TNF-α-mediated proinflammatory functions by downregulating cell-surface expression of the signaling receptors TNFRSF1A (p55) and TNFRSF1B (p75). IL-27 inhibited IL-1β-induced signaling in human Mφ by downregulating the expression of the signaling receptor IL-1RI, inducing expression of the receptor antagonist IL-1Ra, and by upregulating the expression of the decoy receptor IL-1RⅢ. These results identify a mechanism by which IL-27 suppresses Mφ activation by endogenous inflammatory factors and provide insights about the basis of context-dependent activating versus suppressive functions of IL-27 in innate immune and chronic inflammatory responses.

**Materials and Methods**

**Cell culture**

PBMCs from whole blood of healthy volunteers and mononuclear cells from synovial fluids of 13 patients with RA (fulfilling the American College of Rheumatology criteria [31]) were isolated by density gradient centrifugation using Ficoll (Invitrogen Life Technologies, Carlsbad, CA). CD14+ cells were purified from fresh PBMCs and synovial fluid-derived mononuclear cells using anti-CD14 magnetic beads (Miltenyi Biotec, Auburn, CA) as recommended by the manufacturer. Purity of CD14+ cells was >97% as verified by FACS. Cells were cultured in RPMI 1640 medium (Invitrogen Life Technologies) supplemented with 10% FBS (Hyclone, Logan, UT) and 100 U/ml penicillin and streptomycin in the presence or absence of 10 ng/ml human M-CSF (hM-CSF) (PeproTech, Rocky Hill, NJ). The following human cytokines were used to stimulate cells as indicated: human (h)IL-27 (3–100 ng/ml; R&D Systems, Minneapolis, MN), hTNF-α (10 ng/ml; PeproTech), hIL-1β (10 ng/ml; R&D Systems), and hIL-10 (100 ng/ml; R&D Systems). In some experiments, etanercept (10 μg/ml; Amgen, Thousand Oaks, CA) was used to block the effects of TNF-α, hIgG1 (10 μg/ml; R&D Systems) was used as an isotype control. The following TLR ligands were used to stimulate cells as indicated: PamCysSer(Lys)4 (EMC Microcollections, Tübingen, Germany), control. The following TLR ligands were used to stimulate cells as indicated: human (h)IL-27 (3–100 ng/ml; R&D Systems), and hIL-10 (100 ng/ml; R&D Systems) were approved by the Hospital for Special Surgery Institutional Review Board.

**Immunoblotting, ELISA, and FACS**

Whole-cell extracts were prepared by lysis of cells in buffer containing 20 mM HEPEs (pH7), 300 mM NaCl, 10 mM KCl, 1 mM MgCl2, 0.1% Triton X-100, 0.5 mM DTT, 20% glycerol, and 1× proteinase inhibitor mixture (Roche, Basel, Switzerland). A total of 5 or 10 μg whole-cell lysates were fractioned on polyacrylamide gels using SDS-PAGE, transferred to polyvinylidene fluoride membranes (Millipore, Bedford, MA), and incubated with specific Abs. ECL was used for detection. Abs to ERK, p-ERK, p-p38, p-IκBα, IκBα, and cleaved IL-1β were purchased from Cell Signaling Technology (Beverly, MA), and the Abs to p38 and c-Fos were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). For sandwich ELISA, paired capture and detection Abs to IL-27 (R&D Systems), IL-8 (R&D Systems), IL-6 (BD Pharmingen, San Diego, CA), and IL-1Ra (R&D Systems) were used according to the instructions of the manufacturer. For flow cytometry, goat PE-conjugated Ab to human IL-1RⅠ, goat PE-conjugated control Ab, mouse PE-conjugated Ab to human TNFRSF1A (p55), mouse PE-conjugated control Ab, mouse fluorescein-conjugated Ab to human TNFRSF1B (p75), and mouse fluorescein-conjugated control Ab (R&D Systems) were used according to the instructions of the manufacturer. FCRs on cell surface were blocked by human FcR Blocking Reagent (Miltenyi Biotec).

**Real-time quantitative RT-PCR**

Total RNA was extracted using a RNeasy mini kit (Qiagen, Valencia, CA), and 1 μg total RNA was reverse transcribed using a First Strand cDNA Synthesis kit (Fermentas, Glen Burnie, MD). Quantitative PCR (qPCR) was performed using iQ SYBR Green Supermix and iCycler iQ thermal cycler (Bio-Rad, Hercules, CA) following the manufacturer’s protocols, and triplicate reactions were run for each sample. The oligonucleotide primers used were: hGAPDH: 5′-ATCAAGAAGGTGGTGAAGCA-3′ and 5′-GTCGCTTTGGAGATGGCTTCT-3′; hIL-1Ra: 5′-TTTCTGCAACAGTGGAAAGC-3′ and 5′-TGGAGACACCCGTTGGTTCG-3′; hIL-6: 5′-TAATGGGCACTCTTCTTCTTCT-3′ and 5′-TGTCCTTAAGCCCTATCTTT-3′; hTNFRSF1A: 5′-CCCCCGCTGACTGTCGCTCCACATTTT-3′ and 5′-GGGGCTGTCCTCCACCTACTGTA-3′; hTNFRSF1B: 5′-TGAGCTTCCGGACCTGAACTA-3′ and 5′-AGGCGCCAGCTGACAACCTG-3′; hIL-1RⅠ: 5′-AAGCTGCTGCTGCTGCTGCT-3′ and 5′-CAGATGCGATTCTTCTTCTCTC-3′; hIL-1RⅡ: 5′-GGGGGAAATGATCACAGGAATGTG-3′ and 5′-CCCCATGGAAGGCACAGTACACAA-3′; hIL-1Ra: 5′-GCGTCAGGATGTTAGAG-3′ and 5′-GAGATGTTGCGTCCATTGT-3′; hIL-1AcP: 5′-CTCTCCGGGGCCACATGAC-3′ and 5′-GACCACCGCTGGACCTTTTCCTTCT-3′.

**Results**

Human Mφ derived from synovial fluid of patients with RA and from peripheral blood of healthy donors produce IL-27 in response to TNF-α

Recently, it has been reported that IL-27 is expressed in the pannus of patients with RA (11), but the stimulus for IL-27 production during synovial inflammation has not yet been identified. TLR-mediated stimulation of APCs is a well-characterized inducer of IL-27 (5–9). Although TLR ligands have been implicated in synovial inflammation (32), we wished to investigate whether endogenous inflammatory factors, such as TNF-α, which has a more established role in RA pathogenesis and is abundant in the inflamed synovium (33), can trigger production of IL-27. In this context, we isolated Mφ (CD14+ cells) from synovial fluid of 13 patients with active RA and cultured them overnight (12 h) in the presence or absence of TNF-α (10 ng/ml). Interestingly, we observed that TNF-α stimulation of patients’ Mφ led to a significant production of IL-27 protein (n = 13; p < 0.001, paired Student t test) (Fig. 1A). It is of note that the amount of the TNF-α-induced IL-27 was substantial; the mean concentration of IL-27 in culture supernatants was 0.5 ng/ml (ranging from 0.18–1.11 ng/ml), and induction of IL-27 by TNF-α was observed in all 13 independent experiments (Fig. 1A). When TNF-α function was blocked using the soluble TNFR etanercept, TNF-α–induced production of IL-27 by patients’ Mφ was abrogated (n = 5; p < 0.05, paired Student t test) (Fig. 1B), supporting the conclusion that, in our experimental system, TNF-α triggered the production of IL-27 by RA patient-derived synovial Mφ.

We next wished to explore whether TNF-α stimulation induces IL-27 production by human Mφ and Mφ obtained from peripheral blood of healthy individuals. CD14+ cells from PBMCs of healthy
donors cultured for 2 d with M-CSF to differentiate them into Mφ-like cells were stimulated overnight with TNF-α. In all eight independent experiments, there was a robust (0.26–1.85 ng/ml) and significant (p = 0.001, paired Student t test) induction of IL-27 protein post stimulation of cells with 10 ng/ml TNF-α (Fig. 1C) that was prevented by etanercept (Fig. 1D). Freshly isolated peripheral blood Mo produced, as expected, high amounts of IL-27 in response to overnight stimulation with 10 ng/ml LPS (Fig. 1E). TNF-α stimulation of Mo induced a robust production of IL-8 (Fig. 1F), but surprisingly failed to induce IL-27 production even when TNF-α was used at the high concentration of 50 ng/ml (n = 5; p > 0.05, paired Student t test) (Fig. 1E). Overall, these results indicate that human Mφ (derived from synovial fluid of RA patients or peripheral blood of healthy donors), but not Mo, produce substantial amounts of IL-27 in response to TNF-α stimulation; the amounts of TNF-α-induced IL-27 in Mφ were comparable to those induced in Mo by LPS.

IL-27 suppresses TNF-α responses by downregulating the expression of the signaling receptors p55 and p75 and by inhibiting proximal TNF-α-induced signaling in human Mφ

We have recently reported that IL-27 enhances responses of human Mo/Mφ to TLR ligands (4). Cells exposed to IL-27 produce significantly higher amounts of protein and mRNA of the proinflammatory cytokines IL-6, IL-1β, and TNF-α in response to various TLR ligands including Pam3CysSer(Lys)4 (TLR2 ligand), LPS (TLR4 ligand), and CL097 (TLR7/8 ligand) (Fig. 2A, 2B) (4). In our current study, we wished to explore whether IL-27 also modulates responsiveness of human Mφ to mediators that drive synovial inflammation including TNF-α and IL-1β. CD14+ cells, derived from healthy donors’ PBMCs, were cultured for 2 d with M-CSF in the presence or absence of IL-27 and were then stimulated with TNF-α. As expected, M-CSF–generated Mφ responded to TNF-α stimulation with a robust induction of IL-8 mRNA (Fig. 2C, black bar). Surprisingly, in cells exposed to 100...
ng/ml IL-27, the induction of IL-8 mRNA following TNF-α stimulation was attenuated (Fig. 2C, gray bar; \( n = 5; p < 0.0001 \), paired Student \( t \) test). A significant suppressive effect of IL-27 on TNF-α-mediated induction of IL-8 and IL-1β mRNA was also observed in the absence of M-CSF in our experimental system (Fig. 2D; \( n = 3; p = 0.001 \) for the attenuation of IL-8 mRNA expression and \( p = 0.014 \) for the suppression of IL-1β mRNA expression, paired Student \( t \) test). The above-mentioned suppressive effects of IL-27 on human MΦ were dose dependent. Whereas 3 ng/ml IL-27 was only moderately suppressive, pre-exposure of cells to higher doses of IL-27 (10, 30, and 100 ng/ml) significantly suppressed the mRNA expression of the TNF-α target IL-8 (Fig. 3A; \( n = 4; p < 0.01 \)) in a dose-dependent manner. Interestingly, the inhibitory effects of 100 ng/ml IL-27 on the TNF-α-mediated expression of IL-8 mRNA were significant even when the cells were pre-exposed to IL-27 for only 1 h (Fig. 3B, first gray bar on the left; \( n = 3; p < 0.05 \), paired Student \( t \) test). The potency of inhibition increased with increased duration of IL-27 exposure, with an average inhibition of 46.1% after 1 h (Fig. 3B) increasing to 91.7% after 48 h of IL-27 pretreatment (Fig. 3B, last gray bar on the right; \( n = 3; p < 0.01 \), paired Student \( t \) test). To further address the potency of the anti-inflammatory effects of IL-27 on human MΦ at the earlier time points, a head-to-head comparison of IL-27 with the powerful anti-inflammatory cytokine IL-10 was performed. Interestingly, at both time points tested (3 h and 24 h pretreatment with 100 ng/ml IL-27 or 100 ng/ml IL-10), IL-27 displayed a suppressive effect comparable to that of IL-10 on the TNF-α-mediated induction of IL-8 mRNA expression in human MΦ (Fig. 3C; \( n = 3; \) no statistically significant difference was found by paired Student \( t \) test).

The induction of TNF-α target genes, including induction of IL-8, is dependent upon TNF-α-induced activation of NF-κB and MAPK pathways (34), and thus we investigated whether IL-27 inhibited TNF-α signaling. In agreement with the literature, we found that stimulation of human MΦ with TNF-α (10 ng/ml) induced rapid phosphorylation of IκBα (Fig. 4A, upper panel, lanes 2–4) and rapid phosphorylation of ERK and p38 MAPKs (Fig. 4B, first and third panel, lanes 2–4). Notably, we observed strong inhibition of TNF-α-induced IκBα, ERK, and p38 phosphorylation by IL-27 (Fig. 4A, 4B, lanes 6–8). In summary, these results demonstrate that IL-27 attenuates responses of human MΦ to TNF-α by inhibiting TNF-α-induced signaling.

We next investigated the effects of IL-27 on the expression of the two signaling receptors for TNF-α. In the presence of IL-27 (100 ng/ml) for 48 h, we observed a significant downregulation of the p55 receptor on the cell surface of human MΦ, measured by FACS (Fig. 5A; \( n = 5; \) average percentage of inhibition, 50; \( p = 0.001 \), paired Student \( t \) test). Interestingly, exposure of MΦ to IL-27 for 1, 3, 6, 24, and 48 h had no effect on the expression level of p55

**FIGURE 2.** IL-27 primes human MΦ for enhanced TLR responses and suppresses responses of human MΦ to TNF-α. A and B, CD14+ cells isolated from healthy donors’ peripheral blood were cultured with M-CSF (10 ng/ml) in the presence or absence of IL-27 (100 ng/ml) for 24 h and then were stimulated with various TLR ligands including Pam3CysSer(Lys)3 (TLR2 ligand), LPS (TLR4 ligand), and CL097 (TLR7/8 ligand). For A, ELISA was used to measure production of IL-6 protein in culture supernatants following 6 h stimulation with Pam3CysSer(Lys)3 (10 ng/ml), LPS (1 ng/ml), or CL097 (1 μg/ml). For B, qPCR was used to measure the mRNA expression of IL-6 and IL-1β mRNA following 3 h stimulation with Pam3CysSer(Lys)3 (10 ng/ml), and results are depicted as expression relative to GAPDH. For A and B, paired Student \( t \) test was used for statistical analysis. CD14+ cells isolated from healthy donor PBMCs were cultured for 2 d with M-CSF (10 ng/ml) (C) or without M-CSF (D) in the presence or absence of IL-27 (100 ng/ml) and then were stimulated with TNF-α (10 ng/ml) for 3 h. Induction of IL-8 and IL-1β mRNA was measured by qPCR, and the results of the independent experiments were pooled (\( n = 5 \) for C and \( n = 3 \) for D). The mean levels of TNF-α-induced IL-8 mRNA were 238% relative to GAPDH mRNA levels (C) and 240% of GAPDH (D); IL-1β mRNA was induced to 22% of GAPDH levels (D). For comparison of pooled data from different donors, the induction of IL-8 and IL-1β mRNA in response to TNF-α in control cells (cells cultured without IL-27 pretreatment) was set as 100. Induction of IL-8 and IL-1β mRNA in cells pretreated with IL-27 is depicted as percentage relative to induction in control cells. For statistical analysis, paired Student \( t \) test was used. 

![Graph of IL-27 INHIBITS RESPONSES OF MACROPHAGES TO TNF-α AND IL-1](http://www.jimmunol.org/)

*Figure 2. IL-27 primes human MΦ for enhanced TLR responses and suppresses responses of human MΦ to TNF-α. (A) IL-6 and (B) IL-1β production by unstimulated (Control) or IL-27-stimulated (IL-27) human MΦ following 6 h stimulation with Pam3CysSer(Lys)3 (10 ng/ml), LPS (1 ng/ml), or CL097 (1 μg/ml). (C) qPCR analysis of IL-8 mRNA expression. (D) qPCR analysis of IL-1β mRNA expression.*

*Processed by guest on January 14, 2018*
of TNFRs may contribute to suppression of downstream signal that IL-27–mediated downregulation of cell-surface expression

**FIGURE 3.** IL-27 suppresses responses of human MΦ to TNF-α in a dose- and time-dependent manner, and these suppressive effects of IL-27 are comparable to the effects of IL-10. CD14+ cells isolated from healthy donor PBMCs were cultured for 48 h with M-CSF (10 ng/ml) in the presence or absence of IL-27 (A–C) or IL-10 (C) and then were stimulated with TNF-α (10 ng/ml) for 3 h. Induction of IL-8 mRNA was measured by qPCR and the results of the independent experiments were pooled. A, Cells were pretreated for 48 h with different doses of IL-27 (3–100 ng/ml) and then were stimulated with TNF-α. B, Cells were pretreated with 100 ng/ml IL-27 for 1, 3, 6, 24, or 48 h and then were stimulated with TNF-α. The induction of IL-8 mRNA in response to TNF-α in control cells (cells cultured without IL-27 pretreatment) was set as 100. Induction of IL-8 mRNA in cells pretreated with IL-27 is depicted as percentage relative to induction in control cells (A, B). C, Cells were pretreated for 3 or 24 h with IL-27 (100 ng/ml) or IL-10 (100 ng/ml) and then stimulated with TNF-α. Results are depicted as percentage of inhibition relative to control cells (cells stimulated with TNF-α without IL-27 or IL-10 pretreatment). For statistical analysis, paired Student t test was used. *p < 0.01, **p < 0.001, ***p < 0.0001.

mRNA (Fig. 5B). Similarly, cell-surface expression of the second receptor for TNF-α, p75, was also significantly reduced by IL-27 (Fig. 5C; n = 5; average percentage of inhibition, 29.5%; p = 0.019, paired Student t test), whereas the mRNA expression of p75 was not inhibited (Fig. 5D).

**FIGURE 4.** IL-27 blocks TNF-α–induced activation of signaling pathways in human MΦ. CD14+ cells isolated from healthy donor PBMCs were cultured for 2 d with M-CSF (10 ng/ml) in the presence or absence of IL-27 (100 ng/ml) and then were stimulated with TNF-α (10 ng/ml) for 5, 10, and 15 min. Immunoblotting was used to measure Ser32 phosphorylation of IκBα (A), Thr202/Tyr204 phosphorylation of Erk1/Erk2, and Thr180/Tyr182 phosphorylation of p38 (B). Representative results of at least three independent experiments are shown.

IL-27 abrogates responses of human MΦ to IL-1β

IL-1β is an important inflammatory cytokine that utilizes MyD88-dependent signaling pathways similar to TLR-induced MyD88-dependent pathways (35). We then investigated whether IL-27 enhances (similar to LPS and other TLR ligands) or suppresses (similar to TNF-α) responses of human MΦ to IL-1β. In agreement with the literature, human MΦ stimulated with IL-1β produced mature IL-1β protein (cleaved form with molecular mass of 17 kDa) (Fig. 6A, upper panel, lanes 3 and 4). In the presence of IL-27, production of the cleaved form of IL-1β protein in response to IL-1β stimulation was strongly attenuated (Fig. 6A, upper panel, lanes 6–8). We also used qPCR to measure expression of IL-1β target genes. IL-27 significantly suppressed the expression of IL-1β, IL-6, and IL-8 mRNA in response to IL-1β stimulation (data not shown and Fig. 6B, C; n = 3; p < 0.05, paired Student t test). Interestingly, in the absence of M-CSF in our system, the IL-1β–mediated induction of IL-1β, IL-8, and IL-6 mRNA was almost completely inhibited (average inhibition >97%) by pre-exposure of cells to 100 ng/ml IL-27 (Fig. 6D; n = 3; p < 0.001, paired Student t test).

We next investigated whether IL-27 suppresses IL-1β responses by inhibiting IL-1β–induced signaling. After stimulating human MΦ with 10 ng/ml IL-1β, we observed the expected activation of the classical NF-κB pathway as manifested by rapid degradation of the IκBα protein (Fig. 7A, upper panel, lanes 1–4). We also observed rapid phosphorylation of ERK and p38 (Fig. 7B, first and third panel, lanes 1–4), indicating activation of MAPK pathways. IL-1β–mediated degradation of IκBα and phosphorylation of ERK and p38 were prevented in the presence of IL-27 (Fig. 7A, 7B, lanes 5–8), suggesting that IL-27 suppresses the effects of IL-1β on human MΦ by blocking IL-1β–induced signaling.

IL-27 downregulates the expression of the signaling receptor IL-1RI and induces IL-1Ra and the decoy receptor IL-1RII

IL-1β first binds to the IL-1RI receptor subunit on the surface of target cells, and subsequently, IL-1 accessory protein (IL-1AcP)
is recruited, thus forming a trimolecular signaling complex that activates NF-κB and MAPK signaling pathways (35). Because IL-1β has potent proinflammatory and tissue-destructive effects, its function is tightly regulated by the decoy receptor IL-1RII that binds and sequesters IL-1β but does not elicit any signal and by IL-1Ra that competes with IL-1β for binding with IL-1RI (35). In this context, we wished to investigate whether the inhibitory effect of IL-27 on IL-1β signaling and function was due to an effect on

**FIGURE 5.** IL-27 downregulates the cell-surface protein levels of the TNF-α signaling receptors p55 and p75 on human Mφ. CD14+ cells isolated from healthy donors’ peripheral blood were cultured with M-CSF (10 ng/ml) in the presence or absence of IL-27 (100 ng/ml) for 48 h. **A,** Expression level of p55 protein on the cell surface was measured by FACS. **B,** qPCR was used to measure the mRNA expression of p55, and results are depicted relative to GAPDH. **C,** Expression level of p75 protein on the cell surface was measured by FACS. Panel labels same as defined in **A.** **D,** qPCR was used to measure the mRNA expression of p75, and results are depicted relative to GAPDH. For **A** and **C,** representative results of five independent experiments are shown. For **B** and **D,** pooled results of three independent experiments are shown. Control, control Mφ stained for p55; IL-27, IL-27–treated Mφ stained for p55; Control Isotype, control Mφ stained with isotype control; IL-27 Isotype, IL-27–treated Mφ stained with isotype control.

**FIGURE 6.** IL-27 suppresses responses of human Mφ to IL-1β. CD14+ cells isolated from healthy donor PBMCs were cultured for 1 d with M-CSF (10 ng/ml) (**A–C**) or without M-CSF (**D**) in the presence or absence of IL-27 (100 ng/ml) and then were stimulated with IL-1β (10 ng/ml) for 1, 3, or 6 h. For **A,** immunoblotting was used to measure production of the cleaved mature form of IL-1β protein, and representative results of three independent experiments are shown. For **B–D,** qPCR was used to measure induction of IL-1β, IL-6, and IL-8 mRNA, and the results of three independent experiments were pooled. In **B,** the mean levels of IL-1β–induced IL-1β mRNA relative to GAPDH mRNA levels were 670%, 144%, and 176% at the 1 h, 3 h, and 6 h time points, respectively. In **C,** the mean levels of IL-1β–induced IL-6 mRNA were 1.0% of GAPDH. In **D,** the mean levels of IL-1β–induced mRNA relative to GAPDH were 510% (IL-1β), 470% (IL-8), and 4.5% (IL-6). For comparison of pooled data from different donors, induction of IL-1β, IL-6, and IL-8 mRNA in cells pretreated with IL-27 was depicted as percentage relative to induction in control cells. For statistical analysis, paired Student t test was used.
the above-mentioned molecules. Interestingly, we observed that in human CD14+ cells purified from PBMCs of healthy individuals, IL-27 significantly suppressed the mRNA expression of the signaling receptor IL-1RI (Fig. 8A; n = 9; p < 0.01, paired Student t test). This suppressive effect on IL-1RI mRNA expression was readily observed within 3 h following stimulation of cells with IL-27 and was sustained for at least 48 h (Fig. 8B). A similarly significant suppressive effect of IL-27 on the IL-1RI mRNA expression was observed in our system in the absence of M-CSF (average inhibition of IL-1RI mRNA, 77.5%; n = 5; p < 0.001, paired Student t test, data not shown). In agreement with our observations at the mRNA level, the expression of IL-1RI protein on the cell surface was also significantly reduced by IL-27 in all four donors tested (Fig. 8C, average percentage of inhibition, 69.2). IL-1RI expression has recently been shown to depend on basal expression of the transcription factor c-Fos (36). IL-27 downregulated the expression of c-Fos protein in human Mφ (Fig. 8D), suggesting that IL-27 suppresses IL-1RI expression by targeting its transcriptional regulator c-Fos.

In contrast to the suppression of IL-1RI expression, IL-27 significantly increased the production of the receptor antagonist IL-1Ra by human Mφ (Fig. 9A). Interestingly, the induction of IL-1Ra protein by IL-27 was more robust in the absence of M-CSF in our system. In supernatants collected from 24-h cultures, the average amount of the IL-27–induced IL-1Ra protein measured by ELISA was 4.87 ng/ml and 1.64 ng/ml, in the absence and presence of M-CSF, respectively (Fig. 9A). The decoy receptor IL-1RII was also induced in human Mφ by IL-27 in the presence of M-CSF (Fig. 9B; n = 7; p < 0.01, paired Student t test). Finally, the expression of IL-1AcP was not modulated by IL-27 (Fig. 9C). Overall, IL-27 coordinately suppressed expression of an activating IL-1R component while

![FIGURE 7.](image)

**FIGURE 7.** IL-27 inhibits IL-1β–induced activation of NF-κB and MAPK signaling pathways in human Mφ. CD14+ cells isolated from healthy donors’ PBMCs were cultured for 1 d with M-CSF (10 ng/ml) in the presence or absence of IL-27 (100 ng/ml) and then were stimulated for 5, 10, and 15 min with IL-1β (10 ng/ml). Immunoblotting was used to measure total IκBα (A), Thr202/Tyr204 phosphorylation of Erk1/Erk2, and Thr180/Tyr182 phosphorylation of p38 (B). Representative results of at least three independent experiments are shown.

![FIGURE 8.](image)

**FIGURE 8.** IL-27 downregulates IL-1RI and suppresses the expression of c-Fos. CD14+ cells isolated from healthy donors’ peripheral blood were cultured with M-CSF (10 ng/ml) in the presence or absence of IL-27 (100 ng/ml) for 24 h (A, C, D) or for 3, 24, and 48 h (B). A and B, qPCR was used to measure the mRNA expression of IL-1RI, and results are depicted relative to GAPDH. Paired Student t test was used for statistical analysis. For B, representative results of three independent experiments are shown. C, Expression level of IL-1RI protein on the cell surface was measured by FACS. Left panel shows representative results of four independent experiments, and right panel depicts the percentage of inhibition of IL-1RI expression by IL-27 in four donors that were tested. D, Immunoblotting was used to measure the expression of the transcription factor c-Fos (representative results of at least five independent experiments are shown). Control Stained, control Mφ stained for IL-1RI; Control Isotype, control Mφ stained with isotype control; IL-27 Stained, IL-27–treated Mφ stained for IL-1RI; IL-27 Isotype, IL-27–treated Mφ stained with isotype control.
inducing inhibitors of the IL-1R and thereby suppressed human Mφ responses to IL-1β.

Discussion
IL-27 is a highly pleiotropic cytokine that can promote or suppress immune and inflammatory responses depending on context (15–17, 25). One of the challenges in understanding IL-27 function has been to elucidate molecular mechanisms that underlie its activating versus suppressive functions. In this study, we have identified a new suppressive function of IL-27, namely, inhibition of Mφ responses to the key endogenous inflammatory cytokines TNF-α and IL-1β. IL-27 suppressed Mφ responses to TNF-α and IL-1β by blocking proximal steps in signaling by TNFRs and IL-1Rs. IL-27 downregulated the protein expression of the signaling receptors p55 and p75 on the surface of human Mφ. The mechanism of inhibition of IL-1 signaling was downregulation of the ligand-binding IL-1RII concomitant with increased expression of the IL-1Ra protein and the decoy receptor IL-1RII. These findings provide a mechanism for suppressive effects of IL-27 on innate immune cells and suggest that IL-27 regulates innate immune and inflammatory responses by attenuating Mφ activation in response to endogenous inflammatory cytokines.

Our findings that IL-27 inhibits TNF and IL-1 responses stand in striking contrast to IL-27–mediated augmentation of TLR-induced inflammatory cytokine production in human Mφ (Fig. 2A, 2B) (4). This dual regulation suggests that IL-27 has a different function in the context of an infection due to TLR stimulation than its function during chronic sterile inflammation that is driven primarily by endogenous cytokines, such as TNF-α and IL-1. In the first case, IL-27 augments innate immunity to eliminate pathogens by increasing responses of Mφ to pathogen-associated molecular patterns (TLR ligands) (4). In the latter case, IL-27 functions as a homeostatic cytokine by suppressing responses of Mφ to TNF-α and IL-1β. A similar pleiotropic and context-dependent function of IL-27 has been described in adaptive immunity (5, 14–17). In the early phase of the adaptive immune response, IL-27 promotes Th1 polarization of naive lymphocytes to promote a robust response to weak infectious stimuli (37). When an immune response is established, the function of IL-27 transitions to immunosuppressive aiming to restrict inflammation by inhibiting polarization of T cells (5).

IL-27 is expressed at sites of TNF-α–driven inflammation, such as the RA synovium (11), the psoriatic skin (12), and the intestine of patients with Crohn’s disease (13). Our finding that TNF-α induces production of IL-27 by synovial fluid Mφ supports the hypothesis that TNF-α stimulation of Mφ in the joint microenvironment may contribute to the observed expression IL-27 in the pannus of RA patients. It is not yet clear whether IL-27 expressed in TNF-α–driven diseases is predominantly pathogenic or represents a homeostatic attempt to control inflammation and restrain tissue destruction. In a recent report, we observed that IL-27 is tissue protective by inhibiting osteoclastogenesis (20). Our current study further supports a homeostatic function of IL-27 in cytokine-driven inflammation by suppressing Mφ activation. Another group of investigators that described the expression of IL-27 in psoriatic skin lesions also observed anti-inflammatory effects on human keratinocytes (12). In addition, several studies indicate that IL-27 suppresses Th1 and Th17 cells (10, 29, 38–41), two subsets of T cells that have been implicated in the pathogenesis of RA (42), psoriasis, and Crohn’s disease (43, 44). These results are consistent with reports of suppressive properties of IL-27 in several mouse models of autoimmune and inflammatory diseases (10, 11, 22, 29, 38, 40, 41, 45–51). However, the contribution of IL-27–mediated suppression of Mφ function, as described in this study, to suppressing inflammation in vivo is difficult to assess in animal models, as murine Mφ are minimally responsive to IL-27 (4). The alternative approach of ex vivo analysis of human arthritic Mφ suggests that the in vivo functions of IL-27 are regulated by the synovial microenvironment (20), and definitive resolution of this question would require a clinical trial of IL-27 in human diseases with concomitant measurement of Mφ function and cytokine production.

The anti-inflammatory effects of IL-27 on human Mφ are more circumscribed than the effects of the prototypic Mφ-deactivating cytokine IL-10. IL-10 potently blocks inflammatory cytokine production in response to multiple Mφ-activating stimuli, including TNF, IL-1, and multiple TLRs (24). The broad suppressive activity of IL-10 is explained by its targeting of inflammatory cytokine gene transcription; inhibition of transcription appears to occur via STAT3-mediated induction of as yet unknown transcriptional repressors (30). In contrast, IL-27 exhibits receptor-specific inhibitory effects that are explained by selective targeting of IL-1 and TNFRs and proximal signaling components, whereas TLR-induced cytokine responses are actually augmented...
(4). Consistent with this, we found that although STAT3 is activated by IL-27, IL-27 is a weak inducer of STAT3 target genes relative to IL-10 (4). This difference in downstream gene expression and mechanism of inhibition between IL-27 and IL-10 suggests that, in contrast to IL-10, IL-27-mediated suppressive effects may not be predominantly STAT3 dependent. Indeed, IL-27 inhibits IL-1 responses at least in part by downregulating Foxp3 expression and thus expression of IL-1R1. As Fox is also downregulated by IFNs acting via STAT1 (52, 53), it is possible that STAT1 contributes to the homeostatic functions of IL-27 that we have described.

TLR ligands, TNF-α, and IL-1β are potent activators of Mφ, inducing production of proinflammatory mediators that are crucial for an effective immune response to pathogens. Activators of Mφ also trigger homeostatic mechanisms that restrain inflammation and limit associated tissue damage. One key homeostatic mechanism that TLR ligands activate is the production of IL-10 (54), a potent anti-inflammatory cytokine that limits inflammatory cytokine production and controls the duration and intensity of immune responses (24). Interestingly, TNF-α does not induce IL-10 production in Mφ (55), but according to our findings, induces the synthesis of IL-27 that suppresses responses of Mφ to TNF-α and IL-1β. In this context, our observations suggest that TNF-α-stimulated production of IL-27 may represent a selective homeostatic mechanism that limits activation of Mφ by inflammatory cytokines, but preserves responses to microbial products that are sensed by TLRs.

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Disclosures
The authors have no financial conflicts of interest.

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