IL-27 Activates Human Monocytes via STAT1 and Suppresses IL-10 Production but the Inflammatory Functions of IL-27 Are Abrogated by TLRs and p38

George D. Kalliolias and Lionel B. Ivashkiv

*J Immunol* 2008; 180:6325-6333; doi: 10.4049/jimmunol.180.9.6325

http://www.jimmunol.org/content/180/9/6325
IL-27 Activates Human Monocytes via STAT1 and Suppresses IL-10 Production but the Inflammatory Functions of IL-27 Are Abrogated by TLRs and p38

George D. Kalliolas* and Lionel B. Ivashkiv2‡

IL-27 is a member of the IL-12 family of cytokines that activates the Jak-STAT signaling pathway in a context-dependent manner and has pleiotropic effects on acquired immunity. IL-27 has the capacity to promote early stages of Th1 generation, but recent evidence has suggested a predominant suppressive effect on Th1, Th2, and Th17 differentiation. Although modest suppressive effects of IL-27 on myeloid lineage cells have been observed, there is limited knowledge about the role of IL-27 in the regulation of innate immunity. In this study we report that although in resting murine macrophages IL-27 had minimal if any effects, in resting human monocytes IL-27 had profound proinflammatory functions. IL-27 activated a STAT1-dominant pattern of signaling in human monocytes with the consequent activation of STAT1-dependent inflammatory target genes. IL-27 primed monocytes for augmented responses to TLR stimulation in a STAT1-dependent manner, altered IL-10 signaling, and attenuated IL-10-induced gene expression. Strikingly, IL-27 strongly suppressed TLR-induced IL-10 production in human monocytes. However, the proinflammatory effects of IL-27 on human monocytes were rapidly abrogated by LPS via a p38-mediated mechanism that inhibited IL-27 signaling. Our findings identify a predominantly proinflammatory function for IL-27 in human monocytes and suggest a mechanism by which the activating effects of IL-27 on innate immunity are attenuated as an immune response proceeds and IL-27 transitions to predominantly suppressive effects on acquired immunity. *The Journal of Immunology, 2008, 180: 6325–6333.

IL-27 is a heterodimeric cytokine comprised of EB13 (EBV-induced protein 3) and p28 subunits that share similarity with the p40 and p35 subunits of IL-12, respectively (12). The IL-27 receptor is a heterodimer composed of a WSX-1 subunit (also termed TCCR for T cell cytokine receptor), which confers ligand specificity, and the gp130 signaling subunit, which is also used by the IL-6 family of cytokines and activates the Jak-STAT signal transduction pathway (13). IL-27 activates STATs in a context-dependent manner, depending on cell type and activation state. In resting lymphocytes IL-27 activates STAT1, STAT3, STAT5, and low amounts of STAT4 (14, 15), whereas activation of STAT1 is decreased in fully activated relative to resting CD4+ T cells (16). STAT1 plays a key role in mediating the suppressive effects of IL-27 on Th17 (17–19) and Th2 differentiation (15), whereas both STAT1 and STAT3 are required for IL-27-induced IL-10 production in T cells (10, 11). In myeloid cells, IL-27-induced phosphorylation of STAT1 and STAT3 has been observed (8, 13, 20–22), but activation of these STATs has not been linked to IL-27 function in the myeloid lineage. STAT1 and STAT3 have many antagonistic functions in myeloid cells. For example, STAT1 mediates inflammatory, proapoptotic and antiproliferative effects, while STAT3 mediates anti-inflammatory, antiapoptotic, and proliferative effects (22, 23).

© 2008 by The American Association of Immunologists, Inc. 0022-1767/08/S2.00

www.jimmunol.org
priming macrophages (Mφs)3 with IFN-γ, increase the proinflammatory properties of cytokines and can even confer activating functions to IL-10 (24, 26, 35). IL-27 activates both STAT1 and STAT3 in myeloid cells and thus has the potential to induce both proinflammatory and anti-inflammatory effects.

There is limited knowledge about the role of IL-27 in the regulation of innate immunity. An early study showed that IL-27 can induce proinflammatory cytokines in human monocyes and mast cells (13). Follow-up studies in murine systems have suggested a moderately suppressive function of IL-27 on activated myeloid cells. IL-27 inhibited the function of murine mast cells (36), partially suppressed the production of proinflammatory cytokines by activated murine Mφs (8), and attenuated the Ag-presenting capacity and Th1-promoting function of activated murine dendritic cells in vitro (21). We investigated the function and signaling of IL-27 in monocytes and Mφs. We found that resting murine Mφs are minimally responsive to IL-27, but human monocyes are strongly activated by IL-27 in a STAT1-dominant manner. IL-27 primed human monocyes to produce augmented inflammatory responses to LRR ligation, strongly suppressed TLR-induced IL-10 production, and altered IL-10 function by increasing IL-10-induced STAT1 activation and attenuating IL-10-induced gene expression. IL-27 signaling to STAT1 and downstream gene activation were rapidly abrogated by TLR signaling via a p38-dependent mechanism. These results identify STAT1-dependent inflammatory functions for IL-27 in human monocyes and suggest a mechanism by which the inflammatory properties of IL-27 are extinguished as the immune response proceeds and IL-27 assumes a more regulatory role.

Materials and Methods

Cell culture

Bone marrow-derived Mφs, resident peritoneal Mφs, and splenocytes were obtained as described (25) from C57BL/6j mice (The Jackson Laboratory). Cells were cultured in DMEM supplemented with 20% FBS (HyClone), 100 U/ml penicillin, and streptomycin in the presence or absence of 10 ng/ml murine M-CSF (PeproTech). The following murine (m) cytokines were used to stimulate cells as indicated: mIFN-γ (100 U/ml) (PeproTech), mL-27 (100 ng/ml), and mL-6 (50 ng/ml) (R&D Systems). PBMCs were obtained from whole blood from healthy volunteers by density gradient centrifugation using Ficoll (Invitrogen). CD14+ monocyes were purified from fresh PBMCs using anti-CD14 magnetic beads (Miltenyi Biotec) as recommended by the manufacturer. Purity of monocyes was >97% as verified by FACS. Cells were cultured in RPMI 1640 medium (Invitrogen) supplemented with 10% FBS (HyClone), 100 U/ml penicillin, and streptomycin in the presence or absence of 10 ng/ml human (h) M-CSF (hM-CSF) (PeproTech). The following human cytokines were used to stimulate cells as indicated: hIFN-γ (100 U/ml) (Roche), hL-27 (100 ng/ml) (PeproTech), hL-6 (100 ng/ml) (R&D Systems), and hL-6 (50 ng/ml) (R&D Systems), and soluble IL-6R (60 ng/ml). Monocytes were stimulated with (S)-2-[1,3-bis(palmitoyloxy)propyl]-N-palmitoyl-(R)-Cys-(S)-Ser-(S)-Lys-OH, 3HC (Pam,Cys; ECM Microcollections), LPS (Sigma), CL097 (InvivoGen), and zymosan (Molecular Probes) as indicated in the figure legends. In some experiments cells were pretreated with cycloheximide (A.G. Scientific), actinomycin D (Sigma), the p38 inhibitor SB203580, or the MEK inhibitor U0126 (Calbiochem). The experiments using human and murine monocyes were approved by the Hospital for Special Surgery Institutional Review Board and Institutional Animal Care and Use Committee (New York, NY), respectively.

Immunoblotting and ELISA

Whole cell extracts were obtained and protein levels were quantitated using the Bradford assay (Bio-Rad). For immunoblotting, 5 μg of whole cell lysates were fractionated on 7.5% polyacrylamide gels using SDS-PAGE, transferred to polysyndetide fluoride membranes (Millipore), and incubated with specific Abs. ECL was used for detection. Abs for STAT1 and STAT3 were purchased from BD Transduction Laboratories. Phosphorylation-specific (tyrosine 701) STAT1 Ab and phosphorylation-specific (tyrosine 705) STAT3 Ab were obtained from Cell Signaling Technology. For ELISA, paired TNF-α, IL-6, and IL-10 capture and detection Abs were purchased from R&D Systems and used in a sandwich ELISA according to the instructions of the manufacturer.

Real-time quantitative RT-PCR

For real-time PCR, total RNA was extracted using an RNasy mini kit (Qiagen) and 1 μg of total RNA was reverse transcribed using a first strand cDNA synthesis kit (Fermentas). Quantitative PCR was performed using iQ SYBR Green Supermix and iCycler iQ thermal cycle (Bio-Rad) following the manufacturer’s protocols. Triplicate reactions were run for each sample and expression of a tested gene was normalized relative to levels of GAPDH. The oligonucleotide primers used are as follows: mgAPD, 5′-ATCAAGAAGGTGTTGAAGCA′-3′ (forward) and 5′-AGACAACCTG TGCTCCTAGT-3′ (reverse); murine suppressor of cytokine signaling (SOCS) 1, 5′-CATCCCTCTTTAACCCGTAC-3′ (forward) and 5′-TGGA AGGGGAAGAACCTACG-3′ (reverse); mSOCS1, 5′-ATTGCCCTAAT CACTTTAT-3′ (forward) and 5′-ACTGCGATTGTGTGATTT-3′ (reverse); murine interferon (IRF-1), 5′-CACCACAGAGG CATAGCAC-3′ (forward) and 5′-AGACCTTTCTTTGGAATAGG-3′ (reverse); mouse inducible protein-10 (IP-10), 5′-ATTCTTAAAGGCT GGTCTGA-3′ (forward) and 5′-CACCTCCACATACTTCAAGT-3′ (reverse); hGAPDH, 5′-ATCAAGAAGGTGTTGAAGCA′-3′ (forward) and 5′-GTGCGTGTTGAATGACAGGA-3′ (reverse); hSOCS1, 5′-TGGTGA CACGTTAATGAT-3′ (forward) and 5′-AGAGTAGAGAG GTGGCAGT-3′ (reverse); hSOCS3, 5′-CCACCTCCAGATCTGTGC GGAAG-3′ (forward) and 5′-CATAGGACGTCAGCCTGGCTG GAC-3′ (reverse); hCXCL10, 5′-ATTGCTGTCTTCTTCTTCTC-3′ (forward) and 5′-TCTACACCTCTTTTCTTATTGTAG-3′ (reverse); human complement receptor 1 (CR1), 5′-GGATCCTCTCTGCGCCGGT GTG-3′ (forward) and 5′-CCAGGCGCATTGACATGCAAG-3′ (reverse); human decidual protein-induced progesterone (DEPP), 5′-GGCC GCCCTGCTGTCC-3′ (forward) and 5′-CCCTCGGCTGTGCTT TTCTGT-3′ (reverse); hCD163, 5′-CCAGTCTAAACAACCTGTCT-3′ (forward) and 5′-CACCTCCTTGATGCGACCACA-3′ (reverse); human IL-15 hydroyx prostaglandin dehydrogenase (PDGH), 5′-GGTCGAGTGA TAATGGAAGAAAAC-3′ (forward) and 5′-CAATAACGCGCCGCT GTGT-3′ (reverse); hCXCL9, 5′-ATCGACCAACCAAACAGGACT-3′ and 5′-TGCGGCTGTGCTTTGG-3′ (reverse); hCCL5, 5′-TGCGGCTCTTCTTCTCTC-3′ (forward) and 5′-TATGCA GTGCCACCGAAAG-3′ (reverse); hIRF-1, 5′-GCACGAGGCGAAAAT GCA-3′ (forward) and 5′-GGAGGCTCCTTCTCCATCTCA-3′ (reverse); hTNF-α, 5′-AATGGCTTTGCTTCCAGTAGC-3′ (forward) and 5′-AG AGCTCAGAATGGATAAAGCTGGA-3′ (reverse); hIL-6, 5′-TGATGGCCATT CTTCTCT-3′ (forward) and 5′-TGTCCTCATTGGCGCTTATCCTT-3′ (reverse); hIL-10, 5′-TTATCCTGTCTGTGGGCTGTTG-3′ (forward) and 5′- GTCCTGGGAATAGGTTAAGG-3′ (reverse).

Results

Nonactivated murine Mφs are minimally responsive to IL-27 IL-27 strongly suppresses murine T cells via STAT1 and STAT3 (12, 16), and IL-27-induced STAT3 activation, along with moderately suppressive effects on activation-induced cytokine production, has been described in murine myeloid cells (8). We wished to investigate the effects of IL-27 on resting Mφs not exposed to activation stimuli. We analyzed IL-27-induced activation of STAT tyrosine phosphorylation and downstream gene expression. Stimulation of murine splenocytes, which are comprised predominantly of lymphocytes, with IL-27 rapidly induced tyrosine phosphorylation of STAT1 and STAT3 as expected (Fig. 1A). Surprisingly, tyrosine phosphorylation of STAT1 and STAT3 was not observed after the stimulation of bone marrow-derived Mφs (BMDMs) with the same preparation of IL-27 in parallel wells in the same experiment (Fig. 1B), and similar results were obtained using resident peritoneal Mφs (data not shown). In contrast, IFN-γ and IL-6, used

3 Abbreviations used in this paper: Mφ, macrophage; BMDM, bone marrow-derived macrophage; CR1, complement receptor 1; DEPP, disease-specific protein-induced proges
teron; h (prefix), human; IRF-1, IFN regulatory factor 1; m (prefix), murine; Pam,Cys,Ser(Lys), (S)-[2-(3-hydroxipalmitoyloxy)]-N-palmitoyl-(R)-Cys-(S)-Ser-(S)-Lys-OH, 3HC (Pam,Cys; ECM Microcollections); LPS (Sigma), CL097 (InvivoGen), and zymosan (Molecular Probes) as indicated in the figure legends. In some experiments cells were pretreated with cycloheximide (A.G. Scientific), actinomycin D (Sigma), the p38 inhibitor SB203580, or the MEK inhibitor U0126 (Calbiochem). The experiments using human and murine monocyes were approved by the Hospital for Special Surgery Institutional Review Board and Institutional Animal Care and Use Committee (New York, NY), respectively.
mRNA expression was measured using real-time PCR and normalized relative to GAPDH expression. Representative results of at least 15 independent experiments are shown; small SDs are not readily apparent because of large inductions. Thus, we tested the effects of IL-27 stimulation on the expression of the STAT1-target genes SOCS1, IRF1, and IP-10 and the STAT3 target gene SOCS3. IL-27 stimulation had minimal, if any, effect on expression of these STAT target genes, in contrast to the robust activation of STAT1 target genes by IFN-γ and SOCS3 by IL-6 (Fig. 1C). Overall, these results indicate that resting mBMDMs (and peritoneal Mφs) are minimally responsive to IL-27, which is consistent with low or undetectable expression of the IL-27 receptor component WSX-1 in these cells (Ref. 3 and C. Hunter, unpublished observations).

**FIGURE 1.** Nonactivated murine Mφs are minimally responsive to IL-27. A. Splenocytes obtained from C57BL/6J mice were stimulated with mIFN-γ (100 U/ml) for 20 min or mIL-27 (100 ng/ml) for 10, 20, and 30 min, and STAT1 and STAT3 tyrosine phosphorylation (pY) was measured by immunoblotting. B. BMDMs obtained from C57BL/6J mice were stimulated with mIFN-γ (100 U/ml) or IL-6 (50 ng/ml) for 20 min or mIL-27 (100 ng/ml) for 10, 20, and 30 min, and STAT1 and STAT3 tyrosine phosphorylation (pY) was measured by immunoblotting. C. BMDMs were cultured for 3 h with mIFN-γ (300 U/ml), IL-6 (50 ng/ml), or mIL-27 (100 ng/ml) and mRNA was measured using real-time PCR and normalized relative to mGAPDH expression. Results are depicted as expression in cytokine-stimulated monocytes relative to unstimulated monocytes (set as 1) and the means ± SD of triplicate determinants in a representative experiment are shown; small SDs are not readily apparent because of large inductions.

**FIGURE 2.** IL-27 activates STAT1 and STAT1 target genes in human monocytes. A and B. Freshly isolated human monocytes were stimulated with IFN-γ (100 U/ml), IL-10 (100 ng/ml), or IL-27 (100 ng/ml) for 15 min and STAT1 (A) or STAT3 (B) tyrosine phosphorylation (pY) was measured by immunoblotting. Representative results of at least three independent experiments are shown. C. Monocytes were cultured for 3 h with IFN-γ (100 U/ml) or IL-27 (100 ng/ml) and mRNA expression was measured using real-time PCR and normalized relative to GAPDH expression. Representative results of at least 15 independent experiments are shown. D. Monocytes were cultured in the presence or absence of cycloheximide (CHX; 15 μg/ml) for 30 min, then stimulated with IL-27 (100 ng/ml) for 3 h, and mRNA was measured using real-time PCR. E. Monocytes were cultured for 3 h with IL-10 (100 ng/ml) or IL-27 (100 ng/ml) and mRNA was measured using real-time PCR. For D and E, representative results from at least three independent experiments are shown. Results are depicted as expression in cytokine-stimulated monocytes relative to unstimulated monocytes (set as 1) and the means ± SD of triplicate determinants in a representative experiment are shown.

IL-27 activates predominantly STAT1-mediated responses in human monocytes and Mφs

We next tested whether human monocytes and Mφs were responsive to IL-27. IL-27 induced tyrosine phosphorylation of both STAT1 and STAT3 in freshly isolated human monocytes (Fig. 2, A and B). IL-27 appeared to activate moderate levels of both of these STATs relative to the strong activation of STAT1 by IFN-γ and the strong activation of STAT3 by IL-10 (Fig. 2, A and B). Thus, at the time point tested (20 min after cytokine stimulation), IL-27 appeared to activate a balanced signal mediated by both STAT1 and STAT3 (Fig. 2, A and B). These results suggest that IL-27 activates STAT1 and STAT3 weakly in murine Mφs, below the limits of detection by the immunoblotting of whole cell lysates. Activation of STAT target genes is a very sensitive measure of STAT activation, and robust gene activation can be observed downstream of minimal or undetectable signals (24). Thus, we tested the effects of IL-27 stimulation on the expression of the STAT1-target genes SOCS1, IRF1, and IP-10 and the STAT3 target gene SOCS3. IL-27 stimulation had minimal, if any, effect on expression of these STAT target genes, in contrast to the robust activation of STAT1 target genes by IFN-γ and SOCS3 by IL-6 (Fig. 1C). Overall, these results indicate that resting mBMDMs (and peritoneal Mφs) are minimally responsive to IL-27, which is consistent with low or undetectable expression of the IL-27 receptor component WSX-1 in these cells (Ref. 3 and C. Hunter, unpublished observations).

**FIGURE 2.** IL-27 activates STAT1 and STAT1 target genes in human monocytes. A and B. Freshly isolated human monocytes were stimulated with IFN-γ (100 U/ml), IL-10 (100 ng/ml), or IL-27 (100 ng/ml) for 15 min and STAT1 (A) or STAT3 (B) tyrosine phosphorylation (pY) was measured by immunoblotting. Representative results of at least three independent experiments are shown. C. Monocytes were cultured for 3 h with IFN-γ (100 U/ml) or IL-27 (100 ng/ml) and mRNA expression was measured using real-time PCR and normalized relative to GAPDH expression. Representative results of at least 15 independent experiments are shown. D. Monocytes were cultured in the presence or absence of cycloheximide (CHX; 15 μg/ml) for 30 min, then stimulated with IL-27 (100 ng/ml) for 3 h, and mRNA was measured using real-time PCR. E. Monocytes were cultured for 3 h with IL-10 (100 ng/ml) or IL-27 (100 ng/ml) and mRNA was measured using real-time PCR. For D and E, representative results from at least three independent experiments are shown. Results are depicted as expression in cytokine-stimulated monocytes relative to unstimulated monocytes (set as 1) and the means ± SD of triplicate determinants in a representative experiment are shown.
proinflammatory effects of IL-27 on human monocytes

We wished to investigate the functional consequences of IL-27 signaling on inflammatory cytokine production by monocytes. IFN-γ enhances TLR-induced inflammatory cytokine production, whereas IL-10 suppresses cytokine production by a STAT3-dependent mechanism (31, 32). We tested whether IL-27 would enhance or suppress TLR-induced cytokine production in primary human monocytes. We cultured human monocytes in the presence or absence of IL-27, and then stimulated cells with TLR ligands and measured cytokine production using ELISA. As expected, stimulation of monocytes with Pam3Cys (TLR2 ligand) induced the production of the proinflammatory cytokines TNF-α and IL-6 (Fig. 4A, open bars). Cells that had been pretreated with IL-27 produced substantially higher levels of TNF-α and IL-6 in response to Pam3Cys (Fig. 4A, gray bars). In parallel, IL-27 pretreatment augmented TLR2-induced accumulation of TNF-α and IL-6 mRNA (Fig. 4B), and similar results were obtained when IL-1β and IL-12 p40 mRNA were measured (data not shown). IL-27 also augmented inflammatory cytokine production in response to the TLR4 ligand LPS (Fig. 4, C and D) and the TLR7/8 ligand CL097 (data not shown).

The priming effects of IL-27 on TLR-induced inflammatory cytokine production are similar to those exerted by IFN-γ, a strong activator of STAT1 (31). Therefore, we wished to test whether augmented cytokine production was dependent on STAT1. We approached this question by using human monocytic THP-1 cells that had been stably transduced with lentiviral vectors encoding short hairpin RNA (shRNA) that effectively silences STAT1 expression (or control shRNA) as previously described (27). Consistent with our observations using primary monocytes, THP-1 cells transduced with control shRNA produced increased levels of TLR2-induced IL-6 after priming with IL-27 (Fig. 4E). In contrast, this priming effect on TLR2-induced IL-6 production was abolished in cells in which STAT1 expression was silenced (Fig. 4E). These results show that IL-27 augments TLR-induced inflammatory cytokine production by a STAT1-dependent mechanism.

IL-27 modulates IL-10 function and suppresses IL-10 production

Effective priming and activation of monocytes involves overcoming suppression by regulatory factors such as IL-10 and...
TGF-β (39). As IL-10 is a key deactivator of monocytes (22, 32), we investigated the effects of IL-27 on IL-10 signaling and function. The suppressive effects of IL-10 are mediated by STAT3, and we tested the effects of IL-27 on IL-10-induced STAT activation. IL-10 induced tyrosine phosphorylation of STAT3 as expected, and this induction was not affected by IL-27 (Fig. 5A). As predicted based on the mRNA results (Fig. 2D), IL-27 strongly increased STAT1 protein expression (Fig. 5A). Concomitant with increased STAT1 expression, IL-10 induced increased levels of STAT1 tyrosine phosphorylation in monocytes that had been primed with IL-27 (Fig. 5A). Because STAT1 can suppress the expression of STAT3 target genes (27), we tested the effects of IL-27 on IL-10-induced gene expression. Interestingly, IL-27 attenuated the induction of SOCS3, PGDH, DEPP, CD163, and CR1 genes by IL-10 (Fig. 5B), suggesting that IL-27 may attenuate IL-10 function, including suppressive functions mediated by SOCS3 and PGDH (38, 40).

**FIGURE 4.** IL-27 primes monocytes for enhanced TLR responses by a STAT1-dependent mechanism. A–D, Monocytes were cultured with M-CSF (10 ng/ml) in the presence or absence of IL-27 (100 ng/ml) for 24 h and stimulated for the indicated times with Pam3Cys (Pam; 10 ng/ml) (A and B) or LPS (10 ng/ml) (C and D). TNF-α and IL-6 protein were measured in culture supernatants using ELISA (A and C) and mRNA was measured using real time PCR (B and D). Representative results of at least five independent experiments are shown. For B and D, results are depicted as expression in cytokine-stimulated monocytes relative to unstimulated monocytes (set as 1) and the means ± SD of triplicate determinants in a representative experiment are shown. E, THP-1 monocytic cells transduced with lentiviral vectors encoding control or STAT1-specific shRNA interference cells (STAT1 Low) were cultured in the presence or absence of IL-27 (200 ng/ml) for 24 h, stimulated with Pam3Cys, and IL-6 in culture supernatants was measured using ELISA. Representative results of at least three independent experiments are shown.

**FIGURE 5.** IL-27 modulates IL-10 signaling and function. Monocytes were cultured with M-CSF (10 ng/ml) in the presence or absence of IL-27 (30 or 100 ng/ml) for 24 h. A, Control and IL-27-primed cells were stimulated with or without IL-10 (20 ng/ml) for 20 min and cell lysates were analyzed by immunoblotting. PY, Tyrosine phosphorylation. B, Control and IL-27-primed cells were stimulated with IL-10 (20 ng/ml) for 3 h and SOCS3, PGDH, DEPP, CD163, and CR1 mRNA were measured using real-time PCR and normalized relative to GAPDH expression. C, Control and IL-27-primed cells were cultured for 3 h in the presence or absence of IL-10 (20 ng/ml) and then stimulated for 6 h with Pam3Cys (Pam). Production of TNF-α and IL-6 protein was measured in the culture supernatants using ELISA. Representative results from at least three independent experiments are shown. For B, results are depicted as expression in cytokine-stimulated monocytes relative to unstimulated monocytes (set as 1), and the means ± SD of triplicate determinants in a representative experiment are shown.
and D induction of IL-10 protein and mRNA (Fig. 6, 5). Monocytes with IL-27 resulted in a striking inhibition of Pam3Cys induced TLR-induced IL-10 production, which serves an important role in cytokine production, we next addressed whether IL-27 regulates its ability to suppress TLR-induced cytokine production in unstimulated monocytes (set as 1) and the means ± SD of triplicate determinants in a representative experiment are shown.

For B and D, results are depicted as expression in cytokine-stimulated monocytes relative to unstimulated monocytes (set as 1) and the means ± SD of triplicate determinants in a representative experiment are shown.

We then tested whether IL-27 could attenuate a key suppressive function of IL-10, namely IL-10-mediated down-regulation of inflammatory cytokine production. As expected, IL-10 suppressed Pam3Cys-induced production of TNF-α and IL-6 (Fig. 5C). In accord with our previous results, IL-27 augmented TLR-induced TNF-α and IL-6 production (Fig. 5C). This augmented cytokine production was still effectively suppressed by IL-10. However, even though IL-10 still maintained suppressive effects on cytokine production in IL-27-primed monocytes, monocytes that were treated with both IL-27 and IL-10 produced relatively higher levels of TNF-α and IL-6 (Fig. 5C, light gray bars), comparable to or higher than cytokine levels produced by control monocytes stimulated with TLR ligands in the absence of IL-10 (Fig. 5C, open bars). Similar results were obtained when monocytes were stimulated with LPS (data not shown). These results make two points: 1) when the total monocyte output of TNF-α and IL-6 are considered, the proinflammatory effects of IL-27 dominate over the suppressive effects of IL-10; and 2) despite these quantitative differences, IL-10 retains its ability to suppress TLR-induced cytokine production in the presence of IL-27.

Because IL-27 did not abrogate the suppressive effects of IL-10 on cytokine production, we next addressed whether IL-27 regulated TLR-induced IL-10 production, which serves an important feedback inhibitory and homeostatic function (32). Treatment of monocytes with IL-27 resulted in a striking inhibition of Pam3Cys induction of IL-10 protein and mRNA (Fig. 6, A and B). IL-27 had similar inhibitory effects on the induction of IL-10 by LPS (Fig. 6, C and D) and by zymosan (Fig. 6E), in which case IL-10 production is mainly dependent on dectin-1, which signals by tyrosine kinase pathways (41). Thus, in striking contrast to T cells where IL-27 induces IL-10 production (9–11), IL-27 broadly suppresses IL-10 production in human monocytes. Collectively, the results indicate that disruption of IL-10-mediated feedback inhibition and suppression is a component of the activating function of IL-27 on human monocytes.

Activation of monocytes by IL-27 is abrogated by TLRs via a p38-dependent mechanism

The activating effects of IL-27 on fresh human monocytes that we have described stand in stark contrast to reports that document a predominantly suppressive function of IL-27 on acquired immunity at later stages of immune responses (6–8, 12, 16–19, 36). We reasoned that IL-27 may prime the first steps of innate immune responses, similar to early activation of naive T cells (2–4, 16, 42–44), but that these priming functions may be blocked as the immune response evolves and IL-27 function shifts to a suppressive mode. We tested this notion by investigating whether activated monocytes became refractory to the effects of IL-27. Strikingly, activation of primary human monocytes with LPS strongly inhibited IL-27-induced STAT1 activation over a sustained time period (Fig. 7A). Concomitant with the inhibition of signaling, LPS inhibited IL-27-induced activation of the STAT1 target genes IRF1, STAT1, CXCL10, and CXCL9 (Fig. 7B); the time frame of these experiments is short, such that the LPS-mediated induction of CXCL9 and CXCL10 that occurs indirectly via autocrine IFN-β is not readily detectable. The IL-27 receptor is comprised of gp130 and WSX-1 subunits (13), and we and others have shown that gp130-mediated signaling is strongly inhibited by TLRs via a rapidly induced mechanism that is dependent on p38 and independent of de novo protein synthesis (45–51). Interestingly, inhibition of IL-27 signaling was induced rapidly, within 30 min of TLR stimulation (Fig. 7A), and was independent of de novo RNA or protein synthesis (Fig. 7C). In addition, LPS-induced suppression was reversed when the p38 inhibitor SB203580 was used (Fig. 7D) and, thus, was dependent on the activation of p38. As LPS inhibits IL-6 and IL-27 signaling under the same conditions (Fig. 7E) by a similar p38-dependent but RNA and protein synthesis-independent mechanism (45–51), these results suggest that TLRs inhibit IL-27 signaling in part by targeting the gp130 receptor subunit. This raises the possibility that although IL-27 and IFN-γ share STAT1-activating properties, the functions of these cytokines may be differentially regulated by TLRs. This notion was supported by the
finding that, in contrast to the inhibition of IL-27 responses, IFN-γ-induced activation of STAT1 and downstream expression of STAT1 target genes were preserved in LPS-activated monocytes (Fig. 7, E and F). Collectively, the results show that IL-27 function and production. These findings suggest an activating function of IL-27 on human innate immunity that contrasts with its suppressive effects on acquired immunity. However, this activating function of IL-27 is transient, as IL-27 signaling in Mψs provides a mechanism by which IL-27 transitions from early activating effects on innate immunity to switch to predominantly suppressive effects on acquired immunity.

The activation of human monocytes by IL-27 has similarities but also significant differences from activation by IFN-γ, the prototypic Mψ-activating cytokine. Many of these similar actions can be explained on the basis of STAT1 activation, which leads to expression of canonical inflammatory STAT1 target genes such as chemokines (27) and to suppression of IL-10 production in myeloid cells in vitro (39) and in vivo (52). Interestingly, STAT1 appears to have the opposite effect on IL-10 production in T cells, where IL-27 induces IL-10 production by a STAT1-dependent (and also STAT3-dependent) mechanism (10, 11). We have also
shown for the first time that priming for increased TLR-induced inflammatory cytokine production is dependent on STAT1; it is likely that priming by IFN-γ depends on STAT1, similar to priming by IL-27 (Fig. 4). Interesting differences between IL-27 and IFN-γ include cellular source, regulation of production, regulation of cellular responses to these cytokines, and different effects on acquired immunity. IL-27 is produced mainly by APCs in response to microbial products. IFN-γ is also produced by NK and NKT cells that participate in innate immunity; however, Ag-specific T cells are a major source of IFN-γ, and thus IFN-γ production can be finely regulated in an Ag-specific manner. In contrast, Mφ responses to IL-27 are more tightly regulated than responses to IFN-γ, as TLR stimulation inhibited IL-27 but not IFN-γ signaling. Thus it appears that IL-27 and IFN-γ have similar functions on human monocytes, but the expression of and signaling by these cytokines are differentially regulated to tune Mφ responses to be appropriate for the environment.

IL-27 uses gp130 that is also used by IL-6 family cytokines; gp130 activates both STAT3 and STAT1 with a tendency to activate a STAT3-dominant response (53, 54). In contrast to IL-6 that activated STAT1 transiently in monocytes, IL-27 activated sustained tyrosine phosphorylation of STAT1 that correlated with a much stronger IL-27 induction of STAT1 target gene expression (Fig. 3). The duration of STAT activation has been associated with the magnitude of target gene expression (28, 37, 38), and the sustained pattern of STAT1 activation by IL-27 is likely conferred by WSX-1, because the activation of STATs by gp130 was transient in monocytes (Fig. 3) and WSX-1 has been shown to activate STAT1 in other systems (13). Interestingly, our group and others have shown that gp130 signaling is highly regulated and is targeted for inhibition by multiple mechanisms, including SOCS3 and Src homology domain 2-containing phosphatase (SHP-2), that bind to tyrosine 759 in the gp130 cytoplasmic domain and by a rapidly acting p38-dependent pathway (45–51, 55, 56) that likely induces receptor internalization (Ref. 57 and G. Kalliolias, unpublished observations) (Fig. 3A). Thus, gp130 functions not just as a signal transducer but as a target for inhibition that confers suppression on signaling by receptor subunits such as WSX-1, with which it associates. In contrast, activation of STAT1 by IFN-γ is resistant to inhibition by TLR-induced pathways. The association of WSX-1 with gp130 thus provides a mechanism by which STAT1 can be strongly activated but also becomes subject to inhibition by factors that activate p38.

Our results suggest differences between human monocytes that are strongly activated by IL-27 and murine BMDMs and peritoneal Mφs that have minimal responsiveness in our hands (Fig. 1), consistent with low WSX-1 expression in these Mφ populations (Ref. 3 and C. Hunter, unpublished observations). IL-27 function is context dependent and it is possible that IL-27 activates STAT1 in murine myeloid populations more directly comparable (but difficult to access experimentally) to the human monocytes studied here. Other laboratories have actually detected modest direct suppressive effects of IL-27, accompanied by STAT3 activation, in activated murine Mφs and dendritic cells cultured under different conditions than those used in our study (8, 21). It is not yet clear whether these differences between the activation of human monocytes and the modest suppression of murine myeloid cells reflect differences related to cell differentiation and activation state or represent a qualitative species difference, possibly related to differential relative activation of STAT3 vs STAT1 by murine and human IL-27 receptors. Alternatively, these differences may be related to differences in WSX-1 expression, which is regulated by cytokines (58, 59) in different monocyte/Mφ populations, or to differential targeting and modulation of gp130 signaling. To date we have been unsuccessful in detecting activating responses to IL-27 in several murine Mφ populations while finding that human cells maintain IL-27 responsiveness during differentiation into Mφs during culture with M-CSF (G. Kalliolias, unpublished observations). Future work comparing the expression and signaling of human vs murine WSX-1 will help resolve these issues.

Our results suggest a temporal regulation of monocyte/Mφ responses to IL-27 during the course of an immune response. In this model, during the early phases of innate immune responses circulating monocytes or recent mononuclear emigrants into inflammatory sites are strongly activated by systemic or paracrine IL-27, respectively. If monocytes encounter microbes or their products at sites of infection, they will start to produce IL-27 but at the same time become refractory to its effects. Thus the amplifying effects and potential toxicity of autocrine IL-27 are avoided, and IL-27 can act in a paracrine manner, eventually to suppress lymphocyte responses. This model postulates that the pleiotropic biological effects of IL-27 are in part mediated by induced changes in IL-27 signaling in monocytes. This mechanism of regulation is similar to the reprogramming of the pattern of STAT activation that occurs in T cells during their activation (16) and has been suggested to alter T cell responses to IL-27 in a context-dependent manner. Overall, our observations extend our understanding of the bidirectional and context-dependent role of IL-27 in the orchestration of immune responses.

Acknowledgments
We thank Jong Dae Ji and Kyung-Hyun Park-Min for critical reading of the manuscript. We thank Simi Ahmed and Zhimei Du for some of the IFN-γ experiments.

Disclosures
The authors have no financial conflict of interest.

References


