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Inhibition of IL-12 Production in Human Monocyte-Derived Macrophages by TNFα

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IL-12 is a pivotal cytokine that links the innate and adaptive immune responses. TNF-α also plays a key role in orchestrating inflammation and immunity. The reciprocal influence of these two inflammatory mediators on each other may have significant impact on the cytokine balance that shapes the type and extent of immune responses. To investigate the relationship between TNF-α and IL-12 production, we analyzed the effects of exposure of human monocyte-derived macrophages to TNF-α on LPS- or Staphylococcus aureus-induced IL-12 production in the presence or absence of IFN-γ. TNF-α is a potent inhibitor of IL-12 p40 and p70 secretion from human macrophages induced by LPS or S. aureus. IL-10 is not responsible for the TNF-α-mediated inhibition of IL-12. TNF-α selectively inhibits IL-12 p40 steady-state mRNA, but not those of IL-12 p35, IL-1α, IL-1β, or IL-6. Nuclear run-on analysis identified this specific inhibitory effect at the transcriptional level for IL-12 p40 without down-regulation of the IL-12 p35 gene. The major transcriptional factors identified to be involved in the regulation of IL-12 p40 gene expression by LPS and IFN-γ, i.e., c-Rel, NF-κB p50 and p65, IFN regulatory factor-1, and ets-2, were not affected by TNF-α when examined by nuclear translocation and DNA binding. These data demonstrate a selective negative regulation on IL-12 by TNF-α, identifying a direct negative feedback mechanism for inflammation-induced suppression of IL-12 gene expression. The Journal of Immunology, 2000, 164: 1722–1729.

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prompt resolution of C. parvum-injected wild-type mice with lower IL-12 production suggested TNF-α may play a role in limiting the extent and duration of murine inflammatory responses. The role of TNF-α in human proinflammatory diseases and the pivotal importance of IL-12 in innate and adaptive cell-mediated responses underscores the importance of understanding TNF-α mechanisms of action in inhibiting pathogen-induced IL-12 production by macrophages. We show here that in human monocyte-derived macrophages (MDM),3 TNF-α selectively inhibits IL-12 p40 transcription, but not p35, identifying a novel regulatory mechanism of action for TNF-α-induced suppression of IL-12 production.

Materials and Methods

Cytokines and reagents

Human TNF-α was obtained from Genentech (San Francisco, CA); murine TNF-α and anti-murine TNF-α (XT22) were purchased from Genzyme (Cambridge, MA); human IFN-γ was obtained from Endogen (Woburn, MA); fixed Staphylococcus aureus Cowan strain I (binding capacity to human IgG: 2.0 ± 0.1 mg/ml suspension) and actinomycin D were obtained from Calbiochem-Behring (La Jolla, CA); LPS and polyvinyl B were obtained from Sigma (St. Louis, MO).

Preparation of MDM

Human PBMC were isolated from healthy donors by the procedure of Ficoll/Hypaque gradient centrifugation (Pharmacia, Uppsala, Sweden). Mononuclear cells were incubated for an hour in polystyrene tissue culture flasks ( Falcon, Becton Dickinson, Franklin Lakes, NJ). Adherent cells, typically ≥94% CD14+ by FACS analysis, were cultured in 10% pooled AB+ human serum (Sigma, St. Louis, MO) for 20 h before harvesting for experimental treatments. In some experiments, human monocytes were prepared by countercurrent elutriation. No difference was observed between elutriated and adherence-purified monocytes with regard to responses analyzed in this study.

Abs and cytokine assays

All Abs used for supershift and Western blot experiments were purchased from Santa Cruz Biotechnology (Santa Cruz, CA); RIA for human TNF-α were performed using mAb pairs B154.9/B154.7 (15). Human IL-12 p70 and p40 were measured in cell-free supernatants by RIA as described in (16) using the mAb pairs 12H4/5C8.6 and 11.79/5C8.6, respectively. The neutralizing anti-IL-10 mAb 10F1 was kindly provided by Dr. K. Moore of DNAX (Palo Alto, CA).

RNAse protection assay

RNAse protection assays were performed using the PharMingen Multiprobe system hCK-2 (PharMingen, San Diego, CA) following the manufacturer’s instructions.

Nuclear run-on

Isolation of nuclei and in vitro transcription in the presence of [32P]UTP (3000Ci/mmole, DuPont, Wilmington, DE) were performed essentially as described (17, 18). Nuclear RNA was isolated after DNase I (Boehringer Mannheim, Indianapolis, IN) and proteinase K (Boehringer Mannheim) treatment followed by three phenol/chloroform/isoamyl alcohol extractions and ethanol precipitation. Unincorporated [32P]UTP was removed using Sephadex G-50 columns (Boehringer Mannheim). RNA was partially degraded by treatment with 0.2 N NaOH for 10 min at 4°C. 32P-labeled nuclear RNA was hybridized for 2 days at 60°C to prehybridized nylon filters (Schleicher & Schuell, Keene, NH), on which 250 ng of denatured PCR-amplified cDNAs corresponding to the coding regions of the IL-12 p40 and p35, TNF-α, and β-actin genes had been immobilized using a slot-blot apparatus (Hoeffer Scientific, San Francisco, CA). After hybridization, filters were washed at room temperature with 2× SSC, and single-stranded RNA was digested with the same solution containing 10 µg/ml RNase A at 37°C for 15 min. Filters were then washed twice in 2× SSC, 0.1% SDS for 15 min at 50°C and once in 0.1× SSC, 0.1% SDS for 30 min at 50°C. The extent of hybridization was quantified using the ImageQuant software on a PhosphorImager 445SSi (Molecular Dynamics, Sunnyvale, CA).

Nuclear extract preparation

Nuclear proteins were isolated as described (19).

EMSA

End-labeled DNA probes (50,000 cpm/sample) were mixed with 1–2 µg of crude nuclear extracts and incubated at room temperature for 20–30 min in the presence of 1 µg of poly(dId-c) (Boehringer Mannheim) in a volume of 10 µl of 0.5× dialysis (D) buffer (10 mM HEPES, pH 7.9, 10% glycerol, 50 mM KCl, 0.1 mM EDTA, 0.25 mM DTT, 0.25 mM PMSF, 10 µg/ml leupeptin, 10 µg/ml pepstatin A). The mix was then fractionated through a 4% polyacrylamide gel in buffer containing 6.7 mM Tris-HCl, pH 7.5, 3.3 mM sodium acetate, 0.1 mM EDTA for 1 h at 200 V. The gel was dried and exposed in a PhosphorImager storage screen (Molecular Dynamics) and scanned. Supershift experiments were conducted by preincubating the nuclear extract with 1–2 µg of affinity-purified polyclonal Abs for 30 min at 4°C before the probe was added.

Western blot

Reducing SDS-PAGE (15%) was performed according to Laemmli (20) with 6–8 µg of nuclear proteins in each sample. The gel was then electroblotted in Tris-glycine buffer containing 40% methanol onto a nitrocellulose membrane (Trans-blot; Bio-Rad, Hercules, CA). After blocking the membrane with TBST buffer (10 mM Tris, pH 8.0, 150 mM NaCl, 0.05% Tween 20) containing 5% milk powder for 1 h at room temperature, primary Abs (rabbit anti-mouse IgG) were added at 1:1000 dilution (1 µg/ml) in TBST containing 1% milk powder for 1 h at room temperature. The membrane was washed three times for 7 min each with TBST, and incubated with secondary Ab (anti-rabbit or anti-goat IgG conjugated to HRP; Amersham, Arlington Heights, IL) at 1:5–10,000 dilution for 1 h at room temperature. After washing three times in TBST for 5 min each, and once in TBS for 5 min, the membrane was drained briefly and subjected to the enhanced chemiluminescence detection procedure (Amersham).

Results

TNF-α is a potent and specific inhibitor of IL-12 p40 and p70 secretion from human macrophages

MDM were pretreated with TNF-α (20 ng/ml) for 20 h followed by IFN-γ (100 ng/ml) for 16 h before being stimulated with S. aureus (1:10,000) for 24 h. Cell-free supernatants were assayed by RIA for hIL-12 p40 and p70 secretion. Fig. 1A shows that preexposure of MDM to TNF-α inhibited the production of IL-12 p40 and p70 induced by IFN-γ and S. aureus in a dose-dependent manner. TNF-α also inhibited IFN-γ and LPS-stimulated IL-12 p40 and p70 production similarly to S. aureus (data not shown). In all experiments performed, regulation of IL-12 p70 secretion by TNF-α is also observed if measuring the secretion of IL-12 p40 chain. To determine the specificity of the inhibitory effect of TNF-α, MDM were incubated with an anti-TNF-α Ab B154.9 for 24 h or an isotype control Ab (C20.8) before IFN-γ treatment. All cultures were kept in the presence of 10 µg/ml of polyvinyl B to eliminate possible contaminating endotoxin in our Ab preparations, which may complicate the interpretation of the data (21). Importantly, polyvinyl B did not restrict MDM IL-12 secretion following S. aureus stimulation. Fig. 1B shows that addition of anti-TNF-α Ab B154.9 completely abolished the inhibitory effect of the exogenous TNF-α on IFN-γ/S. aureus-induced IL-12 production. The selectivity of the suppressive effect of TNF-α on IL-12 production was tested in parallel against proinflammatory cytokine IL-6 as shown in Fig. 1C. Although IL-6 has been reported to inhibit IL-12 secretion (22), our data showed a selective effect by TNF-α in suppressing IL-12 p40 secretion. Finally, TNF-α exposure of MDM had a positive effect on viability as previously reported (23), ruling out a decrease of IL-12 p70 and p40 secretion due to an increase in programmed cell death. Taken together, these results show that TNF-α is a potent and specific inhibitor of IL-12 production in macrophages.

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3 Abbreviations used in this paper: MDM, monocyte-derived macrophages; IRF-1, IFN regulatory factor 1; NOS, NO synthase.
Kinetics of TNF-α-mediated inhibition of IL-12 production

To determine the temporal characteristics of the TNF-α action, MDM were exposed to TNF-α at different times during the stimulation procedure, i.e., TNF-α was added a day before IFN-γ priming, at the time of IFN-γ priming, or at the time of stimulation with S. aureus. In addition, a parallel culture treated equally was washed at 20 h following TNF-α treatment to determine the temporal requirement for the presence or absence of TNF-α to exert its inhibitory effect. Fig. 2a shows that the IFN-γ/S. aureus-stimulated IL-12 p70 production was inhibited strongly when TNF-α was added before or at the time of IFN-γ treatment (samples 3 and 4). However, the inhibition was less pronounced when TNF-α was added together with IFN-γ. No inhibition was observed when TNF-α was added at the same time as S. aureus (sample 5). Washing the cells after an overnight exposure to TNF-α resulted in higher induction of IL-12 p70 production, but failed to reverse the inhibition (Fig. 2, a and b, samples 2 and 3), suggesting that a constant presence of TNF-α is not required for its inhibitory effect.

TNF-α pretreatment interferes with the signaling of both IFN-γ and S. aureus

To determine whether TNF-α inhibitory effects extended to S. aureus-induced or only IFN-γ-enhanced IL-12 production as observed in the murine system (13), MDM stimulation and IL-12 secretion was measured in the presence of neutralizing Ab to eliminate the endogenously produced IFN-γ in PBMC cultures with 10 μg/ml polymixin B. As expected, Fig. 3 shows that S. aureus-induced IL-12 p40 secretion by PBMC was strongly inhibited by

FIGURE 1. Inhibition of IL-12 p40 and p70 production by TNF-α in MDM. Human MDM at 1 × 10⁶/ml in 96-well plates were pretreated with human TNF-α and were used at decreasing concentrations (A) or at 20 ng/ml (B and C) for 20 h with or without anti-TNF-α Ab B154.9 (10 μg/ml), then primed with hIFN-γ (100 ng/ml) for 16 h before S. aureus stimulation (1:10,000) for another 24 h. IL-12 p40 and p70 protein secretion into the supernatant fluid was measured by RIA in quadruplicates. A, Inhibition of IL-12 p40 and p70 production by TNF-α. Data (mean ± SEM) are from two donors. B, Prevention of TNF-α-mediated inhibition of IL-12 p40 production by neutralizing anti-TNF-α Abs in the presence of 10 μg/ml polymixin B. Data (mean ± SEM) are from four donors.

FIGURE 2. Kinetics of TNF-α-mediated inhibition of IL-12 p70 production. Human MDM were seeded and treated with TNF-α (20 ng/ml), IFN-γ (100 ng/ml), and S. aureus (1:10,000) at the indicated times postisolation, in the presence of polymixin B (10 μg/ml). A, No wash. B, After the 20 h exposure to TNF-α, all wells were washed and fresh media replenished. After 24 h S. aureus stimulation, all wells were harvested, and cell-free supernatants were measured for IL-12 p70 secretion. Data (mean ± SEM) are from two donors.

FIGURE 3. TNF-α inhibits IL-12 p40 production induced by S. aureus alone or by IFN-γ and S. aureus. Human PBMCs were seeded at 10⁶/ml in 96-well plates and treated with TNF-α (20 ng/ml) in the presence or absence of an anti-IFN-γ mAb or an isotype-matching Ab for 24 h followed by S. aureus (1:10,000) stimulation, in the presence of polymixin B (10 μg/ml). After 24 h S. aureus stimulation, cell-free supernatant fluids were harvested and measured for IL-12 p40 secretion. Data (mean ± SEM) are from four donors.
TNF-α (sample 2 vs 3). Anti-IFN-γ treatment resulted in a significant loss of *S. aureus*-induced IL-12 p40 production (sample 4), indicating that the endogenous IFN-γ contributed to the production of IL-12 p40. However, the IFN-γ-independent IL-12 p40 production was significantly inhibited by TNF-α treatment. These results show that TNF-α inhibits *S. aureus*-induced IL-12 p40 production as well as the IFN-γ-enhanced production shown in Fig. 1A.

**The inhibitory effect of TNF-α on IL-12 production is not mediated by IL-10**

IL-10 is one of the most potent inhibitors of macrophage activation including the suppression of IL-12 production (24). IL-10 exerts its repressive effect on IL-12 production by inhibiting directly the transcription of both the IL-12 p40 and the p35 genes (24). We tested whether TNF-α-induced inhibition of IL-12 production in human MDM was mediated by IL-10 because TNF-α induces IL-10 expression (25). MDM cultured in media containing 10 μg/ml polymixin B were stimulated with *S. aureus* alone or with IFN-γ and *S. aureus* after the pretreatment with TNF-α, in the absence or presence of the neutralizing anti-IL-10 Ab (12G8), or an irrelevant isotype control Ab (C20.8). Fig. 4 shows that the production of IL-12 p40 was stimulated with *S. aureus* or IFN-γ priming plus *S. aureus*. Anti-IL-10 treatment substantially enhanced this production, while TNF-α pretreatment greatly inhibited it in the absence or presence of anti-IL-10. These results show that TNF-α inhibits IL-12 p40 production in human MDM independently of IL-10.

**TNF-α-mediated inhibition is selective for IL-12 p40 gene expression**

RNase protection assay using the Pharmingen multiprobe system was performed on RNA samples derived from human MDM exposed to TNF-α before IFN-γ/LPS stimulation. Fig. 5 shows that IFN-γ priming before LPS stimulation significantly enhanced the accumulation of mRNA for IL-12 p35, IL-12 p40, IL-10, IL-1α, IL-1β, IL-1Ra, and IL-6. TNF-α alone induced IL-10, IL-1β, IL-1Ra, and IL-6 mRNA expression. Pretreatment with TNF-α strongly inhibited IL-12 p40 mRNA induced by either LPS alone or IFN-γ plus LPS while having only a modest and statistically nonsignificant effect on the p35 message and an enhancing effect on IL-1Ra. These results indicate that the inhibitory effect of TNF-α is selective for IL-12 p40 steady-state mRNA.

**TNF-α inhibits IL-12 p40 gene expression at the transcriptional level**

To determine the molecular pathway through which TNF-α affects IL-12 gene expression, nuclear run-on assays were performed with isolated nuclei derived from human MDM exposed to TNF-α before IFN-γ/LPS stimulation. Fig. 6 shows that TNF-α strongly inhibited the IFN-γ/LPS-stimulated IL-12 p40 transcription, and to a lesser degree that of the TNF-α gene itself. Interestingly, transcription of the IL-12 p35 gene was not affected by TNF-α priming. The stability of the IFN-γ/LPS-induced IL-12 p35 and p40 mRNA was assessed following the blockade of de novo transcription with actinomycin D. TNF-α did not affect the half-life of the
NF-κB and IRF-1. Nuclear extracts were prepared as described in Fig. 6. Six micrograms of nuclear proteins for each condition was applied to a 15% SDS-PAGE under reducing conditions. After electrophoresis, the gel was electrophoretically blotted to a nitrocellulose membrane and hybridized to Abs of interests, visualized using the enhanced chemiluminescence detection reagents (Amersham). One of two independent experiments with highly similar results is shown.

![FIGURE 8.](Image)

**Discussion**

Because of the pivotal role of IL-12 as a proinflammatory and an immunoregulatory agent in immune responses against infectious agents and cancer, elucidation of the mechanisms of positive and negative regulation of IL-12 production in human myelomonocytic cells is of critical importance. The last 4 years have seen a rapid accumulation of studies identifying mediators of IL-12 inhibition as potential mechanisms of antinflammatory or immunosuppressive responses. Since the early description of IL-10 and TGF-β as inhibitors of IL-12 production, numerous other agents have been implicated such as prostaglandin E2 (29), thalidomide (30), 1,25-dihydroxyvitamin D3 (31), phosphodiesterase (32), β2 agonists (33), glucocorticoids (34), intracellular cAMP (35), histamine (36), endotoxin tolerance (21), Fc receptor/complement receptor (CD46 and CD11b) and their respective ligands or microorganisms.
that either mimic or carry them (e.g., measles virus) (37, 38), cholera toxin (39), and type I IFNs (40). However, the mechanisms by which these agents suppress IL-12 production remain largely unexplored.

In this report, we observed that TNF-α is a potent and selective inhibitor of IL-12 p40 and p70 production from human MDM (Fig. 1). This inhibition is observed when MDM were pre-exposed to TNF-α before, but not at, the time of stimulation with LPS or S. aureus (Fig. 2). The fact that the inhibitory effects of TNF-α on macrophages take a considerable preincubation period (~16 h) to attain suggests that TNF-α may be inducing de novo protein syntheses required for its inhibitory activities. Interestingly, inhibition of IL-12 can result after transient exposure to TNF-α, i.e., the contact between and its receptors needs not to be continuous. In addition, the sustained inhibitory effect of TNF-α of S. aureus-induced IL-12 in the presence of neutralizing Ab against endogenously produced IFN-γ suggests that TNF-α mechanism of action is independent of IFN-γ signaling (Fig. 3). The TNF-α-mediated inhibition is also highly selective against IL-12 p40 gene expression because it does not affect IL-12 p35 or other inflammatory cytokines such as IL-1α, IL-1β, and IL-6 expressed in macrophages in response to inflammatory stimuli (Fig. 5).

TNF-α has been shown to induce the production of IL-10 by resting and LPS-stimulated macrophages in a potential mechanism of negative feedback regulation of the immune response (25). However, the TNF-α action in the regulation of human IL-12 production is independent of IL-10, as we showed that the inhibition of IL-12 by TNF-α was unabated in the presence of an effective neutralizing IL-10 Ab (Fig. 4). Interestingly, our observation of an IL-10 independence of the TNF-α action in human MDM is different from the report by Hodge-Dufour et al. (13) on TNF-α pretreatment of thioglycolate-elicited murine macrophages resulting in suppression of IL-12 p40 and p70 production following LPS or low m.w. hyaluronic stimulation. The latter observations from comparing IL-10 null and wild-type mice indicated that the inhibition of IL-12 by TNF-α can be mediated by both IL-10-dependent and IL-10-independent mechanisms, although the IL-10-dependent mechanism appears dominant and the other redundant and compensatory in the absence of the endogenous IL-10 production.

In contrast to the mechanism of IL-10-mediated suppression of both IL-12 p40 and p35 gene transcription, nuclear run-on analysis indicated that TNF-α induced transcriptional inhibition of the IL-12 p40 gene only, not of the p35 gene (Fig. 6). Moreover, mRNA stability analysis in MDM after TNF-α treatment showed that the half-life of IFN-γ/LPS-induced IL-12 p40 mRNA was not affected by TNF-α treatment (data not shown). The latter shows the mechanism of inhibition of IL-12 p40 by TNF-α is different to that described for other macrophage-derived mRNAs inhibited by TNF-α (41). Taken together, our data strongly suggest a selective inhibition by TNF-α of IL-12 p40 gene transcription induced by LPS/S. aureus alone or the combination of IFN-γ and LPS/S. aureus.

The binding of LPS/IFN-γ-induced NFκB to the κB “half site” at −107/−117 of the human IL-12 p40 promoter was not inhibited by the pretreatment with TNF-α (Fig. 7). Nor did this treatment alter the composition of the NFκB complex, e.g., from the transcriptionally activating p65/p50 heterodimer to an inhibitory homodimer such as p50/p50 (42). However, we cannot rule out the possibility that there might be a difference in the transactivating potential of the p65/p50 heterodimer, which may be adversely affected by TNF-α, as has been shown in the case of LPS induction of NFκB in THP-1 cells (43). Another site of critical importance for the human IL-12 p40 transcription in macrophages is the cts element located at −206 to −211 of the p40 promoter. Cotransfection experiments with various combinations of expression vectors for NFκB p65, p50, c-Rel, and ets-2 demonstrated that ets-2 and c-Rel synergistically activate the transfected p40 promoter in the IL-12 p40-expressing cell lines (RPMI 8866 and RAW264.7), and nonexpressing cells such as Bjab (EBV− B cell line) and Jurkat (T cell line), strongly suggesting that c-Rel and ets-2 functionally cooperate as the major transcription factors necessary and sufficient to direct the cell type-specific expression of the p40 gene (27). Analysis of several known components of the FI complex, including c-Rel, IRF-1, and ets-2, by Western blot indicated that the nuclear translocation of these three transcription factors induced by IFN-γ and LPS is not affected by pretreatment with TNF-α (Fig. 8). The nuclear translocation of IRF-1 in the presence of TNF-α is consistent with an inhibitory mechanism by TNF-α of IL-12 production following IFN-γ/LPS/S. aureus stimulation that is independent of upstream IFN-γ signaling events. Overall, the lack of evidence for a defect in the known IL-12 p40 transcription machinery induced by LPS/S. aureus or IFN-γ/LPS/S. aureus in the presence of TNF-α pretreatment indicate that the molecular target(s) of TNF-α involved in the suppression of IL-12 p40 transcription may be novel. Interestingly, this novel regulation of transcription appears to be specific to the IL-12 p40 gene without affecting any of the upstream pathways.

Another candidate mediator of the inhibition of IL-12 by TNF-α in the presence of IFN-γ is NO (44). Several reports demonstrated that inducible nitric oxide synthase (NOS2)-deficient mice developed enhanced Th1 cell responses following infections and antigenic stimulation, producing more IFN-γ and less IL-4 compared with similarly treated wild-type mice (45, 46). The same group subsequently showed that peritoneal macrophages from NOS2-deficient mice infected with Leishmania major in vivo or stimulated with IFN-γ or LPS in vitro produced significantly higher levels of IL-12 than those from heterozygous and wild-type mice. IL-12 production from the macrophage cell line J774 activated with LPS or LPS plus IFN-γ could be markedly enhanced by the NOS2 inhibitor l-N<sup>ω</sup>-monomethyl arginine (l-NMMA) and profoundly inhibited by the NO-generating compound S-nitroso-N-acetyl-penicillamine (47). Interestingly, the effect of NO on IL-12 p40 production is also transcriptional and is activation dependent, as is the effect of TNF-α on IL-12 p40 expression shown in our study. However, another group recently reported the opposite observation that at day 1 of L. major infection of mice, genetic deletion or functional inactivation of NOS2 abolished IFN-γ and NK cell response with a correlative down-modulation of IL-12 p40 mRNA expression in the lymph node of infected mice. In contrast, peritoneal macrophages from both NOS2<sup>−/−</sup> and NOS2<sup>+/−</sup> mice produced similar amounts of IL-12 p70 in response to IFN-γ plus LPS within 24 h of stimulation (48). The reasons underlying the differences in the data generated by the two groups are presently unclear.

In our own experiment where we used a neutralizing mAb to eliminate endogenous and exogenous IFN-γ, hence the generation of NO, TNF-α was still able to inhibit S. aureus-induced IL-12 p40 production. In addition, our preliminary data (not shown) indicated that the use of l-N<sup>ω</sup>-monomethyl arginine in IFN-γ/LPS-treated human MDM failed to reverse TNF-α-inhibited inhibition of IL-12 p40 production.

TNF-α was originally defined on the basis of its ability to induce hemorrhagic necrosis of transplanted mouse tumors and by its selective cytotoxicity for transformed cells (49). Since then, TNF-α has been found to play a key role in orchestrating a wide spectrum of complex events involved in inflammation and immunity. Recent studies (14, 50, 51) in TNF-α- or TNFR p55-deficient mice subjected to various pathological challenges suggest that TNF-α has an essential homeostatic role in limiting the extent and duration of
an inflammatory process in vivo (14). For example, TNF-α-deficient mice showed little initial response and a late but vigorous and disorganized inflammatory response to heat-killed C. parvum leading to death, in contrast to the prompt response (granuloma formation and hepatosplenomegaly) and subsequent resolution phase in C. parvum-injected wild-type mice (14). The ability of TNF-α to inhibit IL-12 production by human macrophages demonstrated here provides direct evidence that in addition to the cascade of inflammatory and anti-inflammatory cytokines induced during inflammation, TNF-α signaling can selectively inhibit IL-12 gene expression upon macrophage activation as part of the scheme of cytokine feedback and self-limiting modulations. This may benefit the host in controlling inflammatory responses as exemplified in murine models above while at the same time providing a potential immunosuppression mechanism to intracellular pathogens whose infection is associated with chronic TNF-α expression. Indeed, ongoing experiments are directed at assessing the role of TNF-α in inducing a decreased IL-12 response in HIV-1 infection where sustained immune activation and expression of TNF-α is associated with a decrease in IL-12 and cell-mediated responses. It remains to be tested whether this mechanism can provide a novel target for reversing immune defense suppression during chronic inflammation.

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References
vation is required for lipopolysaccharide induction of interleukin-1β and NFκB activation, but not NFκB nuclear translocation. J. Biol. Chem. 271:18306.


