Interleukin 10 (IL-10) Inhibits Human Lymphocyte Interferon \( \gamma \)-Production by Suppressing Natural Killer Cell Stimulatory Factor/IL-12 Synthesis in Accessory Cells

By Annalisa D’Andrea, Miguel Aste-Amezaga, Nicholas M. Valiante, Xiaojing Ma, Marek Kubin, and Giorgio Trinchieri

From The Wistar Institute of Anatomy and Biology, Philadelphia, Pennsylvania 19104

Summary
Natural killer cell stimulatory factor or interleukin 12 (NKSF/IL-12) is a heterodimeric cytokine produced by monocytes/macrophages, B cells, and possibly other accessory cell types primarily in response to bacteria or bacterial products. NKSF/IL-12 mediates pleiomorphic biological activity on T and NK cells and, alone or in synergy with other inducers, is a powerful stimulator of interferon \( \gamma \) (IFN-\( \gamma \)) production. IL-10 is a potent inhibitor of monocyte-macrophage activation, that inhibits production of tumor necrosis factor \( \alpha \) (TNF-\( \alpha \)), IL-1 and also IFN-\( \gamma \) from lymphocytes acting at the level of accessory cells. Because TNF-\( \alpha \) and IL-1 are not efficient inducers of IFN-\( \gamma \), the mechanism by which IL-10 inhibits IFN-\( \gamma \) production is not clear. In this paper, we show that IL-10 is a potent inhibitor of NKSF/IL-12 production from human peripheral blood mononuclear cells activated with \textit{Staphylococcus aureus} or lipopolysaccharide (LPS). Both the production of the free NKSF/IL-12 p40 chain and the biologically active p70 heterodimer are blocked by IL-10. NKSF/IL-12 p40 chain mRNA accumulation is strongly induced by \textit{S. aureus} or LPS and downregulated by IL-10, whereas the p35 mRNA is constitutively expressed and only minimally regulated by \textit{S. aureus}, LPS, or IL-10. Although IL-10 is able to block the production of NKSF/IL-12, a powerful inducer of IFN-\( \gamma \) both in vitro and in vivo, the mechanism of inhibition of IFN-\( \gamma \) by IL-10 cannot be explained only on the basis of inhibition of NKSF/IL-12 because IL-10 can partially inhibit IFN-\( \gamma \) production induced by NKSF/IL-12, and also, the IFN-\( \gamma \) production in response to various stimuli in the presence of neutralizing antibodies to NKSF/IL-12. Our findings that antibodies against NKSF/IL-12, TNF-\( \alpha \), or IL-1\( \beta \) can significantly inhibit IFN-\( \gamma \) production in response to various stimuli and that NKSF/IL-12 and IL-1\( \beta \) can overcome the IL-10-mediated inhibition of IFN-\( \gamma \), suggest that IL-10 inhibition of IFN-\( \gamma \) production is primarily due to its blocking production from accessory cells of the IFN-\( \gamma \)-inducer NKSF/IL-12, as well as the costimulating molecule IL-1\( \beta \).

1 Abbreviations used in this paper: NKSF, natural killer cell stimulatory factor; TPA, tetradecanoylphorbol acetate.

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type 1 (Th-1) response and to inhibit the differentiation of IL-4-producing Th-2 cells, both in the response of human PBLs to allergens or bacterial antigens (12), and in the response of anti-OVA TCR transgenic mice to OVA (13). Importantly, in both systems (12, 13), anti-IL-12 Abs prevented Th-1 differentiation, suggesting that IL-12 is a necessary factor for Th-1 cell differentiation.

IL-10 is a product of various cell types including monocytes and B and T cells, that, like IL-12, is associated with differentiation of Th-2 cells (14, 15). IL-10 inhibits lymphocyte cytokine production, particularly IFN-γ, by T and NK cells, and also inhibits proliferation of T cells, acting primarily at the level of monocyte/macrophage accessory cells (16–18).

IL-10 is a potent inhibitor of monocyte/macrophage functions, including oxidative burst, nitric oxide production, cytotoxicity, and production of cytokines, such as TNF-α and IL-1 (19–24). TNF-α has been shown to be an important cofactor for the induction of IFN-γ production by murine T and NK cells, and the ability of IL-10 to prevent TNF production by monocyte/macrophages has been proposed to be one of the mechanisms by which IL-10 inhibits IFN-γ production (25). However, TNF-α by itself is unable to induce IFN-γ production, and cells other than monocytes and macrophages, including T and NK cells, also produce TNF-α, making it unlikely that inhibition of TNF-α production by IL-10 may fully account for its effect on IFN-γ production (25).

In this paper, we demonstrate that IL-10 efficiently inhibits, in addition to TNF-α and IL-1β, IL-12 production by human PBMC. Abs against IL-12, TNF-α, and IL-1β and, more effectively, their combinations, inhibit IFN-γ production from human PBMC in response to several different inducers. IL-12 and IL-1β, but not TNF-α, are able to partially restore IFN-γ production in the presence of IL-10. These results suggest that IL-10 inhibits IFN-γ production by preventing production by accessory cells of the IFN-γ–inducing cytokine NKSF/IL-12 and, in part, the costimulatory cytokines IL-1β and possibly TNF-α.

Materials and Methods

Cytokines and Reagents. CHO cell-activated rNKSF/IL-12 (5 × 10⁸ U/mg) was a gift from Dr. S. Wolf (Genetics Institute, Boston, MA); rIL-1β (3.8 × 10⁷ U/ml) was provided by the Division of Cancer Treatment (National Cancer Institute, Bethesda, MD); rIL-2 (10⁴ U/mg) by Dr. H. M. Shepard (Genentech, South San Francisco, CA). The following reagents were purchased from commercial sources: PHA-M (Wellcome Diagnostics, Dartford, England); fixed S. aureus Cowan strain 1 (Pansorbin; Calbiochem-Behring Corp., La Jolla, CA); LPS (from Escherichia coli, serotype 0127:B8; Sigma Chemical Co., St. Louis, MO); and 12-O-tetradecanoylphorbol 13-acetate (TPA; Sigma Chemical Co.).

Antibodies. mAbs B154.2 (anti-TNF-α, IgG1) and C8.6.2 (anti-NKSF/IL-12 p40, IgG1) were produced and characterized in our laboratory as previously described (4, 26). Neutralizing anti-IL-10 mAb 19F1 was kindly provided by Dr. K. Moore and anti-IL-1β F18.609 and F18.206 were kindly provided by Dr. M. A. Cousin (Roussel-Uclaf, Romainville, France) (27).

Cytokine Assays. RIA for human IFN-γ and human TNF-α were performed as previously described (26, 28) using mAbs B133.1/B133.5 and B154.9/B154.7, respectively. NKSF/IL-12 p40 was measured in cell-free supernatants by RIA as described (4) using the mAb pairs C11.79/C8.6. RIA for IL-1β was performed using F18.609/F18.206 mAbs (27).

Quantitation of Biologically Active NKSF/IL-12 Heterodimer by an Ab Capture Bioassay. The biological activity of NKSF/IL-12 was determined using a modification of the capture bioassay previously described (29). Briefly, the anti-NKSF/IL-12 p40 Ab C11.5 (4) was absorbed (15 μg/ml, 100 μl/well, in 0.1 M carbonate buffer, pH 9.5) for 24 h or longer at 4°C to bacteriological 96-well plates (Flow Laboratories, McLean, VA). The plates were washed three times with PBS and PBS/FCS 5% was added to plates (200 μl, 1 h, 37°C). After three washes, dilutions of rNKSF/IL-12 standard or supernatant fluids to be tested were added (100 μl/well, 3 h, room temperature), the plates were again washed (five times) and PBMC (5 × 10⁴/well) together with 100 U/ml or rIL-2 were added to the plates. After an 18-h incubation (37°C, 5% CO₂), the supernatants were collected and tested for IFN-γ production by RIA. The assay detects ≥ 1 pg/ml of biologically active NKSF/IL-12, specific for NKSF/IL-12, and at least in the concentration range used in the present experiments, is not affected by the presence of an excess of p40 chains.

Preparation of Human PBMC. Peripheral blood obtained from healthy donors was anticoagulated with heparin. PBMC were separated on Ficoll-Hypaque (Lymphoprep; Nyegard and Co., Oslo, Norway) density gradient, and PBL were obtained after adherence of PBMC to plastic flasks (>98%) and transduced and uninduced PBMC by the gnanidine isothiocyanate method. Equal amounts of RNA (15 μg/lane) were fractionated and transcribed using 32p-UTP (NEN; DuPont Co.) and the instructions of the manufacturer (Invitrogen Co., San Diego, CA). The vectors were linearized with appropriate restriction enzymes already described (32). Briefly, total RNA was extracted from induced and uninduced PBMC by the guanidine isothiocyanate method. Equal amounts of RNA (15 μg/lane) were fractionated and transcribed using 32p-UTP (NEN; DuPont Co.). The filters were then exposed to X-Omat AR film (Eastman Kodak Co., Rochester, NY) between double intensifying screens (DuPont Co., Wilmington, DE).

Northern Blot Hybridization. Northern blots were performed as already described (32). Briefly, total RNA was extracted from induced and uninduced PBMC by the guanidine isothiocyanate method. Equal amounts of RNA (15 μg/lane) were fractionated and transcribed using 32p-UTP (NEN; DuPont Co.). The filters were then exposed to X-Omat AR film (Eastman Kodak Co., Rochester, NY) between double intensifying screens (DuPont Co., Wilmington, DE).

RNAse Protection Assay. The entire coding regions of p40- and p35-NKSF/IL-12 were amplified by PCR and subcloned into the pCRT™1000 pCR™II vectors, respectively, according to the suggestions of the manufacturer (Invitrogen Co., San Diego, CA). The vectors were linearized with appropriate restriction enzymes and transcribed using 32p-UTP (NEN; DuPont Co.) and the riboprobe kit (Promega Biotec, Madison, WI) into complementary RNA (antisense) riboprobes containing regions of 266 and
cells (4). At concentrations inhibitory for IL-12 production, antibody-capture biological assay based on the ability of IL-

PBMC always produce an excess of the free 1>40 chain over

the 1070 heterodimer (4), we tested whether

the 1040 chain either in free form or associated with the p35

monocyte-depleted, nonadherent (<0.5% monocytes) popu-

lations, whereas LPS or S. aureus

are powerful inducers of NKSF/IL-12 production (Fig. 1). Murine rIL-10, inactive

on human cells, did not inhibit NKSF/IL-12 production at

concentrations up to 100 U/ml (data not shown). When

IL-10 also inhibited LPS- and

TNF-ot and

IL-1/~ production, confirming and extending the results of

other investigators (21).

In endotoxin-free conditions, insignificant levels of NKSF/IL-12 p40 are spontane-

ously released by human PBMC in vitro (data not shown), whereas LPS or S. aureus are powerful inducers of NKSF/IL-

12 production (Fig. 1). Human rIL-10 induced a dose-
dependent inhibition of NKSF/IL-12 production in response
to either inducer which was almost complete at IL-10 concen-
trations of 10–30 U/ml (Fig. 1). Murine rIL-10, inactive on human cells, did not inhibit NKSF/IL-12 production when

PBMC were separated into adherent (>95% monocytes) and

monocyte-depleted, nonadherent (<0.5% monocytes) popula-

tions, IL-10 inhibited IL-12 production in both prepara-
tions (data not shown). In the nonadherent cell preparation, IL-

12 is produced in part by B cells, although the major nonad-

herent producer cells are HLA-DR + , non-B, nonmonocytic

cells (4). At concentrations inhibitory for IL-12 production, IL-

10 also inhibited LPS- and S. aureus-induced TNF-ot and

IL-1$/ production, confirming and extending the results of

other investigators (21).

The RIA used to measure NKSF/IL-12 in Fig. 1 detects the p40 chain either in free form or associated with the p35

chain in the biologically active p70 heterodimer (4). Because

PBMC always produce an excess of the free p40 chain over

the p70 heterodimer (4), we tested whether IL-10 also in-

hibits the production of the p70 heterodimer using an antibody-capture biological assay based on the ability of IL-

12 captured by anti-IL-12 p40 coated on plastic to induce IFN-7 production from PBL in the presence of IL-2 (29).

As shown in Fig. 2, IL-10 was found to inhibit production of both the free p40 chain and the p70 heterodimer.

We analyzed by Northern blotting the ability of IL-10 to

inhibit S. aureus- (Fig. 3A) and LPS-induced (data not shown) accumulation of NKSF/IL-12 p40 and TNF-ot mRNA. S. aureus induced maximal accumulation of p40 mRNA at 3–5 h, whereas accumulation of TNF-ot mRNA was max-

imal at 1–3 h. At all time points analyzed, 50 U/ml IL-10 almost completely blocked S. aureus and LPS-induced accumu-

lation of both p40 and TNF-ot mRNA. Because p35 mRNA is at very low abundance, RNase protection assay was used for its detection (Fig. 3B). Unlike p40 mRNA, that was strongly induced by SAC stimulation and inhibited by IL-

10, p35 mRNA accumulation was only minimally stimu-

lated by SAC at 4–6 h and also minimally downregulated by IL-10. These results confirm previous data (4) that p35 expression is constitutive in PBMC and only minimally regulated.

Role of NKSF/IL-12, TNF-ot, and IL-1$ Production by Ac-

cessory Cells in the Induction of IFN-7. As shown in Fig. 4,

IL-10 efficiently suppressed IFN-7 production by resting

PBMC in response to IL-2, PHA, S. aureus, and, to a lesser extent, NKSF/IL-12. Abs to NKSF/IL-12 also significantly

inhibited IFN-7 production in the same experimental condi-
tions. However, the ability of IL-10 to still inhibit IFN-7

production in the presence of anti-NKSF/IL-12 Abs (Fig. 4),
Figure 3. Effect of rIL-10 on the *S. aureus* induced accumulation of IL-12 p40, p35, and TNF-α mRNA in human PBMC. Total RNA was extracted from PBMC incubated for the indicated times in tissue culture medium or in the presence of *S. aureus* (1:10,000 wt/vol) with or without 50 U/ml human rIL-10. (A) Cells stimulated in the presence of IL-10 were pretreated for 4 h with the same concentrations of IL-10. The RNA (15 μg/lane) was then hybridized in a Northern blotting with 32P-labeled IL-12 p40, TNF-α, or β-actin cDNA. Representative experiment out of four with similar results. (B) Total RNA from the RPMI-8866 cell line, constitutively expressing high levels of NKSF/IL-12 (1) and from PBMC incubated for 4 h in medium, IL-10 50 U/ml, *S. aureus* (1:10,000 wt/vol), or *S. aureus* and IL-10, was hybridized with 32P-labeled probes transcribed from NKSF/IL-12 p40 and p35 cDNA. 20 μg RNA were used for hybridization with the p35 probe and 5 μg with the p40 probe. Equivalency of the different RNA preparations was determined by optical density and by hybridization with a β-actin probe in a Northern blotting (data not shown). mRNA accumulation was quantitated by RNase protection and autoradiograms showing the protected RNA fragment (240 bp for p35 and 266 bp for p40) are presented. Representative experiment out of three with similar results. All the experiments shown in this figure were performed in endotoxin-free conditions.

suggested that IL-10-mediated inhibition of IFN-γ production was not solely due to suppression of NKSF/IL-12 production. We therefore investigated the role in IFN-γ production of two other cytokines inhibited by IL-10, IL-1β, and TNF-α. Neutralizing Abs against NKSF/IL-12, TNF-α, and IL-1β at least partially inhibited IFN-γ production individually (Fig. 5). IL-2, IL-12, and TPA-induced IFN-γ production also on purified preparations of cultured NK and T cells, whereas *S. aureus*, which presumably acts indirectly by stimulating accessory cells to produce NKSF/IL-12 (4), was unable to induce IFN-γ by the purified cell preparations. The IFN-γ production by purified activated NK and T cells in response to IL-2, IL-12, and TPA was not inhibited by IL-10, unlike the significant inhibition observed in the same conditions with resting PBMC (Fig. 6). The inability of IL-10 to suppress IFN-γ in the purified activated cell preparations was likely due to the absence of accessory cells which are required for IFN-γ production by resting but not by activated lymphocytes (5). Addition of cultured monocyte-derived macrophages to the purified NK and T cells in most experiments reconstituted the ability of the cells to produce IFN-γ in response to *S. aureus* and enhanced the production in response to IL-2 or IL-12. The IFN-γ production in response to *S. aureus* or the enhanced production of IFN-γ in response to IL-2 or IL-12 in the presence of macrophages was inhibited by IL-10 (data not shown). Purified NK and T cell preparations were unable to produce NKSF/IL-12 (4) and anti-NKSF/IL-12 Abs failed to inhibit IL-2 or TPA-induced IFN-γ production in purified NK cells (Fig. 7) and T cells (data not shown), unlike what was observed with PBMC (Fig. 4). The enhanced IFN-γ production in response to *S. aureus* obtained by adding monocyte-derived macrophages to NK or T cells, was, however, inhibited by anti-NKSF/IL-12 Abs. Anti-TNF-α Abs, unlike anti-NKSF/IL-12, significantly inhibited IFN-γ production in response to IL-2, NKSF/IL-12, and TPA in purified NK (Fig. 7) and T cells (data not shown), indicating that TNF-α, known to be produced by NK and T cells in response to these stimuli (6, 26, 31), participates in IFN-γ production not only by resting, but also by activated NK and T cells.

NKSF/IL-12 and IL-1β Reconstitute IFN-γ Production in the Presence of IL-10. The observation that neutralizing Abs against NKSF/IL-12, TNF-α, and IL-1β inhibited IFN-γ production by PBMC in response to various stimuli, prompted us to test the ability of these three cytokines to stimulate IFN-γ production and to prevent IL-10 inhibition. To de-

![Figure 4](image-url)
crease the constitutive production of these cytokines, their effect was tested in endotoxin-free conditions. In these conditions, IFN-γ production was found to be consistently lower than that observed when nonendotoxin tested lots of PBS

Figure 5. Effect of neutralizing Abs anti-NKSF/IL-12, anti-TNF-α, and anti-IL-1β on IFN-γ production by human PBMC in response to various inducers. Human PBMC were incubated (5 × 10^6/ml, 18 h, 37°C) with various IFN-γ inducers (IL-2, 100 U/ml; IL-12, 1 ng/ml; S. aureus, 1:10,000 vol/vol; PHA, 1%; anti-CD3 and mAb OKT3, and the indicated combinations of anti-NKSF/IL-12 (C8.6, 10 μg/ml) anti-TNF-α (B154.2, 10 μg/ml) or anti-IL-1β (F18.609, 10 μg/ml) Abs. The cell-free supernatant fluid was then assayed for IFN-γ content by RIA. Results are mean ± SE, n = 4.

Discussion

IL-10 was shown to inhibit macrophage activation and production of cytokines such as TNF-α and IL-1 (19-24). IL-10 can also inhibit T cell lymphokine production and proliferation by suppressing the function of accessory and APCs (16). IL-10 inhibits the accessory function of macrophages, but not of other APCs such as dendritic or B cells, by suppressing both secretion of costimulatory cytokines and FCS were used, suggesting that activation of accessory cells by contaminant endotoxin has an enhancing role on IFN-γ production by PBMC. To obtain consistently measurable levels of IFN-γ in these experiments, 100 U/ml of rIL-2 were added. In these conditions, NKSF/IL-12, but not TNF-α or IL-1β induced IFN-γ production (Fig. 8). IL-1β but not TNF-α (tested in a range of concentrations from 1 to 30 U/ml), slightly enhanced IFN-γ production induced by NKSF/IL-12 and IL-2. IL-10 (10 U/ml) inhibited IFN-γ production induced by NKSF/IL-12 and IL-2, and this inhibition was largely reversed by the addition of 10 ng/ml IL-1β, whereas addition of TNF-α had no effect (Fig. 8).

Figure 6. Effect of rIL-10 on IFN-γ production by purified preparations of activated NK cells (A), T cells (B), and resting PBMC (C), in response to various stimuli. Purified NK cells from 8-d cocultures of PBL stimulated with irradiated RPMI-8866 cells, purified T cells from 5-d PHA-stimulated cultures of PBMC, and total resting PBMC were incubated (18 h, 5 × 10^6 cells/ml, 37°C) in medium or in the presence of S. aureus (1:10,000 wt/vol), rIL-2 (100 U/ml), rNKSF/IL-12 (1 ng/ml), or TPA (10^-8 M), and in the presence (during the incubation and a 4-h pretreatment) or absence of 50 U/ml human rIL-10. Cell-free supernatant fluids were then collected and assayed for IFN-γ content by RIA. Results are expressed as mean ± SE, n = 7 for NK and T cells, n = 4 for PBMC.

Figure 7. Anti-TNF-α, but not anti-NKSF/IL-12 Abs inhibit IFN-γ production by purified cultured NK cells in response to various inducers. NK cells were purified from 6-d cocultures of PBMC with irradiated RPMI-8866 B cells and stimulated for 18 h with rIL-2 (100 U/ml), rNKSF/IL-12 (1 ng/ml), or TPA (10^-8 M), in the presence or absence of anti-NKSF/IL-12 (C8.6, 10 μg/ml) or anti-TNF-α (B154.2, 10 μg/ml) Abs. Cell-free supernatant fluids were collected and assayed for IFN-γ content by RIA. Results are expressed as mean ± SE, n = 5.
Concentrations of rNKSF/IL-12 and in the absence (Δ) or in the presence of containing 10 U/ml human rlbl0 (including a 4-h pretreatment with IL-rlbl0 (γ,). Cell-free supernatant fluids were collected and assayed for 37~ 100 U/ml rIL-2) in endotoxin-free conditions at the indicated con-

Expression of costimulatory molecules (e.g., B7) on the macrophage surface (33). The ability of IL-10 to inhibit IFN-γ production by T and NK cells is not easily explained by its inhibition of cytokines such as TNF-α and IL-1, which have costimulatory or enhancing effects on IFN-γ production, but which by themselves are not able to induce IFN-γ production (25).

We (1, 4, 5, 11) have reported that NKSF/IL-12, a product of monocytes/macrophages and other accessory cells, is a powerful inducer of IFN-γ production by NK and T cells, acting alone or in synergy with IL-2 or other IFN-γ inducing stimuli. NKSF/IL-12 has a major role in IFN-γ production by human PBL in vitro. When IFN-γ production is induced by stimuli able to induce monocytes to produce NKSF/IL-12, such as S. aureus or other bacteria or bacterial products, anti-NKSF/IL-12 Abs almost completely abolish IFN-γ production (4). However, a significant inhibition of IFN-γ production by anti-NKSF/IL-12 Abs is also observed when IFN-γ inducers are used such as IL-2 or PHA, that are not able to significantly induce NKSF/IL-12 production. These results, therefore, suggest that NKSF/IL-12, constitutively produced or possibly induced by contaminant endotoxin, plays a major role in IFN-γ production. Similarly, in the murine system, it was shown that NKSF/IL-12 is required for Toxoplasma-induced IFN-γ production in vitro (34) and we (Wysocka, M., and G. Trinchieri, unpublished observations) have observed that anti-NKSF/IL-12 mAbs block LPS-induced IFN-γ production in vivo in mice. Because of this central role of NKSF/IL-12 in IFN-γ production, we investigated the effect of IL-10 on the ability of monocytes to produce NKSF/IL-12. Similarly to what was previously reported for TNF-α, IL-1, and other monokines (21-23), IL-10 very efficiently suppressed in PBMC IL-12 p40 mRNA accumulation and secretion of IL-12 p40 free chain and p70 hetero-
dimers induced by LPS or S. aureus. Production of NKSF/IL-12 from both monocytes and nonadherent mononuclear cells was suppressed by IL-10. Because B cells apparently are responsible for only a fraction of NKSF/IL-12 production by nonadherent mononuclear cells and the nature of the NKSF/IL-12 producer HLA-DR nonadherent cells remains to be determined (4), it is not possible to conclude yet on which cells, in addition to monocytes, IL-10 is active in suppressing NKSF/IL-12 production.

Although NKSF/IL-12 plays a major role both in vivo and in vitro in the induction of IFN-γ production in response to various stimuli, IL-10-mediated inhibition of IFN-γ cannot be explained only by inhibition of NKSF/IL-12 production by accessory cells, because of the ability of IL-10 to at least partially suppress IFN-γ production by PBMC in response to NKSF/IL-12. We therefore analyzed the possibility that inhibition of other cytokines, such as TNF-α and IL-1β, play a role in the effect of IL-10 on IFN-γ production. Although TNF-α and IL-1β, unlike NKSF/IL-12, did not directly stimulate IFN-γ production, neutralizing Abs against these cytokines significantly suppressed IFN-γ production, suggesting that they are required costimulatory factors for IFN-γ production. Only IL-1β and not TNF-α was shown to be able to prevent the ability of IL-10 to inhibit IFN-γ production in response to NKSF/IL-12. We interpret these data to indicate that IL-10 inhibits IFN-γ production in PBMC by suppressing both NKSF/IL-12 and IL-1 production by monocytes and other accessory cells. Although TNF-α is also an important cofactor for IFN-γ production and IL-10 inhibits its production by monocytes, it appears not to play a primary role in the effect of IL-10 on IFN-γ production, probably because upon induction with IL-2, NKSF/IL-12 or other stimuli, TNF-α is produced not only by monocytes but also by NK and T cells, not affected by IL-10. This conclusion is supported by the observation that IFN-γ production by purified preparations of NK and T cells is partially inhibited by anti-TNF-α Abs, but is unaffected by IL-10 or by anti-NKSF/IL-12 Abs. Another possible mechanism of action of IL-10, not investigated in this study, could be mediated by its effect on costimulatory molecules on the membrane of accessory cells (33). For example, we have observed that costimulation of T cells with anti-CD28 Abs or B7 antigen on accessory cells synergizes with NKSF/IL-12 in inducing IFN-γ production (Kubin, M., and G. Trinchieri, unpublished observations).

The ability of IL-10 to inhibit NKSF/IL-12 production has particular interest in light of the opposite effects of these two cytokines on Th-1/Th-2 cell differentiation (12, 13). Because NKSF/IL-12 may be an obligatory factor for efficient Th-1 responses (12, 13), IL-10 production by Th-2 cells or by monocytes may represent a feedback mechanism preventing differentiation of Th-1 clones and favoring the maintenance...
of a Th-2 response. The understanding of the reciprocal regulation of these cytokines in vitro and in vivo will shed light on the regulation of cellular and humoral immune responses and will possibly provide means of therapeutic intervention for modulating them.

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Address correspondence to Dr. Giorgio Trinchieri, The Wistar Institute, 3601 Spruce Street, Philadelphia, PA 19104.

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