Stimulatory and Inhibitory Effects of Interleukin (IL)-4 and IL-13 on the Production of Cytokines by Human Peripheral Blood Mononuclear Cells: Priming for IL-12 and Tumor Necrosis Factor α Production

By Annalisa D'Andrea, Xiaojing Ma, Miguel Aste-Amezaga, Carla Paganin, and Giorgio Trinchieri

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

Summary

The production of cytokines in monocytes/macrophages is regulated by several different cytokines that have activating or inhibitory effects. Interleukin (IL)-10, IL-4, IL-13, and transforming growth factor (TGF)-β are usually considered to be the most important macrophage-deactivating factors, with inhibitory effects on cytokine production. Unlike IL-10 and TGF-β, which appear to act as downmodulators of many phagocytic cell functions, the mode of action of IL-4 and IL-13 is more complex. Addition of IL-4 and IL-13 to peripheral blood mononuclear cell (PBMC) cultures inhibited production of IL-12, tumor necrosis factor (TNF)-α, IL-10, and IL-10 induced by lipopolysaccharide (LPS) or Staphylococcus aureus added simultaneously with the cytokines. However, pretreatment of PBMC with IL-4 or IL-13 for >20 h enhanced the production of IL-12 and TNF-α in response to LPS or S. aureus several fold in these cells; this IL-4–induced priming for the two cytokines was inhibited by anti-IL-4 neutralizing antibodies. IL-4 priming also enhanced the accumulation of IL-12 and TNF-α mRNA induced by LPS and S. aureus. The enhanced accumulation of transcripts for the IL-12 p35 and p40 chains by IL-4 priming was reflected in enhanced secretion of both the IL-12 free p40 chain and the p70 heterodimer. These results suggest an unexpected complexity in the regulatory role of IL-4 and IL-13 in immune responses.

The ability of monocytes/macrophages to act as APCs for T cells and as accessory cells for activation of NK cells is mediated in part by the expression of costimulatory molecules, either present at the cell surface (e.g., B7 and cell adhesion molecules) or secreted (e.g., IL-12, TNF-α, and IL-1). IL-12 is a heterodimeric cytokine produced by several cell types with APC function. IL-12 induces production of IFN-γ from T and NK cells, proliferation of activated T and NK cells, and enhancement of the cytotoxic activity of lymphocytes (1). Upon infection with bacteria or intracellular parasites, monocytes/macrophages produce IL-12 (2), which cooperates with TNF-α and IL-1 as well as with B7 stimulation of the CD28 receptor on T cells in inducing production of IFN-γ and lymphocyte proliferation (3–5). In cooperation with other costimulatory molecules, IL-12 induces development of Th1 cells during an immune response to antigen (6, 7) and is required for optimal proliferation and IFN-γ production by differentiated Th1 cells (4, 8). The light chain (p35) and the heavy chain (p40) of IL-12 are encoded by two distinct genes (9, 10); although only the covalently linked p70 heterodimer has known biological activity, the p40 heavy chain is always secreted in large excess over the heterodimer (2, 11).

The expression or production of costimulatory molecules in monocytes/macrophages is regulated by several cytokines, which have either activating or deactivating effects on various functions of these cells. IFN-γ and GM-CSF are among the stimulatory cytokines, enhancing IL-12 production and other functions of monocytes/macrophages (12, 13), whereas IL-10, IL-4, and TGF-β are usually considered as the most important deactivating factors (14). Through their effect on accessory cell functions, this latter group of cytokines has an inhibitory effect on many responses of T and NK cells; however, they can also directly affect lymphocyte functions, for example, IL-10 inhibits production of IL-2 by T cells, IL-4 inhibits the response to IL-2, and TGF-β inhibits the response to both IL-2 and IL-12 in T and NK cells (15–17, Scharton-Kerston, T., G. Trinchieri, and P. Scott, unpublished results). IL-13 is a recently described cytokine that shares one chain (IL-2Rα) of its receptor with the IL-4 and IL-2 receptors and mediates similar functions to those mediated by IL-4 on monocytes/macrophages and B cells, but not on T or NK cells (18–20).

Unlike IL-10 and TGF-β, which appear to downmodulate many phagocytic cell functions, the mode of action of IL-4 and IL-13 is more complex. On monocytes/macrophages, IL-4
inhibits the expression of IFN-γ-induced genes and the differentiation of these cells, whereas IFN-γ induces their differentiation. IL-4 or IL-13 treatment of monocytes induces the formation of long cytoplasmic protrusions that tightly adhere to substrates, giving them a dendritic appearance (21). Addition of IL-4 to long-term monocyte cultures grown in the presence of GM-CSF blocks the differentiation to macrophages and the expression of macrophage markers such as CD14 or Fc receptors, while inducing cells that resemble dendritic/Langerhans cells with respect to antigenic phenotype (e.g., expression of CD11c) and potent APC functions (22, 23). Short-term treatment of monocytes with IL-4 or IL-13 enhances the expression of the surface antigens class II MHC, CD11b, CD11c, CD18, and CD23, but downregulates the expression of CD16, CD32, and CD64 (14, 24-30). Furthermore, IL-4 and IL-13 inhibit production of IL-1α and -β, IL-6, IL-8, IL-10, IL-12 p35 and p40, GM-CSF, M-CSF, G-CSF, IFN-α, and TNF-α, but enhance production of the IL-1 receptor antagonist (IL-1RA)1 by monocytes (14, 30-35). However, in one study, IL-4 was shown to induce production of G-CSF and M-CSF from human monocytes (36). IL-4 was also shown to inhibit cytokine production, including TNF-α, by mouse peritoneal macrophages, especially if these cells were pretreated with IL-4 before stimulation. However, IL-4 has also been reported to enhance or induce TNF-α production in LPS-treated or Leishmania-infected peritoneal macrophages (37, 38). These contrasting results may reflect the use of different cell populations and experimental conditions (39), but possibly also species differences, as suggested by the observation that pretreatment with IL-4 inhibits the stimulus-dependent respiratory burst in human monocytes but enhances it in murine macrophages (40).

In this study, we show that pretreatment of PBMC with IL-4 or IL-13 for >20 h enhances several fold their ability to produce IL-12 and TNF-α in response to Staphylococcus aureus or LPS. This priming effect was reflected in a similar increase in stimulus-dependent accumulation of cytokine mRNA.

Materials and Methods

Cytokines and Reagents. CHO cell-derived human rIL-12 was a gift from Dr. S. Wolf (Genetics Institute, Boston, MA); rIL-1β (3.8 × 10^7 U/ml) was provided by the Division of Cancer Treatment (National Cancer Institute, Bethesda, MD); rIL-2 (10^6 U/μg) by Dr. T. Taguchi (Osaka University, Osaka, Japan); CHO cell-derived rIL-10 (1.5 × 10^8 U/μg) and rIL-13 by Dr. A. O’Garra (DNAX Research Institute, Palo Alto, CA), and rTNF-α (5 × 10^7 U/μg) by Dr. H. M. Shepard (Genentech, South San Francisco, CA). The following reagents were purchased from commercial sources: rFGF-β1 (R&D Systems, Inc., Minneapolis, MN); rIL-4 (2 × 10^8 U/μg, Genzyme Corp., Cambridge, MA); PHA-M (Wellcome Diagnostics, Dartford, UK); 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 and Cytokine Assays. Neutralizing anti-IL-10 mAb 19F1 and anti-IL-4 mAb 25D2 were kindly provided by Dr. A. O’Garra and anti-IL-12 p57 and IL-206 were kindly provided by Dr. M. A. Cousin (Roussel-Uclaf, Romainville, France) (41). RIAs for human IFN-γ and human TNF-α were performed as described (42, 43) with mAbs B133.1/B133.5 and B154.9/B154.7, respectively. IL-12 p40 was measured in cell-free supernatants by RIA as described (2) by use of the mAb pair C11.7/CB8.6. RIA for IL-1β was performed with mAbs F18.609/F18.206 (44). ELISA for human IL-10 was purchased from Biosource International (Camarillo, CA).

Quantitation of Biologically Active IL-12 Heterodimer by Antibody Capture Bioassay. The biological activity of IL-12 was determined by use of the capture bioassay previously described (3). Briefly, the anti-IL-12 p40 antibody C11.5 (2) was absorbed (15 μg/ml, 100 μl/well, in 0.1 M carbonate buffer, pH 9.5) for ≥24 h at 4°C to bacterial 96-well plates (Flow Laboratories, Inc., Rockville, MD). Plates were washed three times with PBS, and PBS/FCS 5% was added (200 μl, 1 h, 37°C). After three washes, dilutions of rIL-12 standard or supernatant fluids to be tested were added (100 μl/well, for 3 h, at room temperature), plates were again washed (five times) and PHA blasts (5 × 10^5/well) together with 100 U/ml of rIL-2 were added to the plates. After an 18-h incubation (37°C, 5% CO2), supernatants were collected and tested for IFN-γ production by RIA. The assay detects >1 pg/ml of biologically active IL-12, is specific for IL-12, and at least in the concentration range used in these experiments, is not affected by the presence of an excess of free p40 chains.

Cell Lines and PBMC Preparation. Peripheral blood obtained from healthy donors was anticoagulated with heparin. PBMC were separated on Ficoll-Hypaque (Lymphoprep; Nyegaard Co., Oslo, Norway) density gradient. When monocytes were used, they were obtained after adherence of the PBMC to plastic flasks by scraping the flasks with a rubber policeman after carefully rinsing out nonadherent cells with three washes with PBS. PHA blasts (>98% activated T cells) were obtained after 3-d culture of PBL in the presence of 1% PHA-M (44, 45). The human EBV-transformed B lymphoblastoid cell line RPMI-8866 and the monocytic leukemia cell line THP-1 were grown in RPMI-1640 medium (Flow Laboratories, Inc.) supplemented with 10% (20% for THP-1) heat-inactivated FCS (Irvine Scientific, Santa Ana, CA). All tissue culture media and supplements were endotoxin-free. Cultures were performed in 96-well round-bottomed plates (Flow Laboratories, Inc.) with 10^6 cells/well. Cytokines to be tested were added at the onset of culture and kept in culture for 20 h before addition of the stimuli; supernatants were harvested after 18 h; IL-12 p40, TNF-α, IL-1β, or IL-10 release was determined by use of specific RIAs, and IL-12 p70, by antibody capture bioassay. In some experiments, neutralizing anti-IL-4 mAb (25D2), anti-IL-10 mAb (19F1), at a concentration of 10 μg/ml each, were added to PBMC at different times during the culture assays before harvesting the supernatants for the specific RIAs.

Northern Blot Hybridization. Northern blots were performed as described (46). Briefly, total RNA was extracted from induced and uninduced PBMC by the guanidine isothiocyanate method. Equal amounts of RNA (15 μg/lane) were fractionated in a 1% agarose-formaldehyde gel. Gene expression was detected by sequential hybridization of nylon membranes (Schleicher & Schuell, Inc., Keene, NH) with 32P-cDNA probes for IL-12 p40, TNF-α, IL-1β, and glyceraldehyde phosphate dehydrogenase (GAPD). Filters were exposed to film (X-Omat AR; Eastman Kodak Co., Rochester,
Results

Effect of Concomitant Treatment with IL-4, IL10, and TGF-β1 on LPS- and S. aureus-Induced Production of IL12 and Other Cytokines by PBMC. As previously reported (14, 30), stimulation of human PBMC with LPS or S. aureus in the presence of IL-4 or IL-13 inhibited the production of TNF-α, IL-1β, and IL-10 (results not shown). Production of the free IL-12 p40 chain or the biologically active IL-12 p70 heterodimer by LPS-stimulated PBMC was also inhibited by both IL-4 and IL-10 (results not shown). Production of the free IL-12 p40 chain or the biologically active IL-12 p70 heterodimer by LPS-stimulated PBMC was also inhibited by both IL-4 and IL-10 present during stimulation profoundly inhibited expression of both the IL-12 p40 and TNF-α transcripts. These results extend the previous report that IL-4 and IL-13 inhibit the accumulation of IL-12 transcripts induced by LPS in the presence of IL-10 (30). The accumulation of IL-1β transcripts was only minimally decreased by IL-4, in contrast with the IL-4-mediated inhibition of IL-1β secretion (30 and Fig. 1). Unlike IL-4 inhibition of LPS-induced IL-12 production, the inhibition by IL-4 or IL-13 of IL-12 p40 or p70 production induced by S. aureus stimulation was often less consistent and, in most experiments, minimal or absent (Table 1). TGF-β1 and IL-10 effectively inhibited IL-12 production induced by either LPS or S. aureus (Table 1).

PBMC Priming for IL-12 and TNF-α Production by Pretreatment with IL-4 or IL-13. Unlike the inhibitory effects exerted by IL-4 and IL-13 when present during stimulation, pretreatment of PBMC for 20 h with IL-4 or IL-13 induced a three- to fourfold enhancement in their ability to produce IL-12 p40 and p70 in response to either LPS or S. aureus, and an approximately twofold increase in the production of TNF-α, whereas IL-1β and IL-10 production in response to LPS was inhibited (Tables 1 and 2). The inhibitory effect of TGF-β1 or IL-10 on IL-12 p40 and p70 production induced by either S. aureus or LPS was observed when PBMC were either stimulated in the presence of TGF-β1 or IL-10 or were pretreated for 20 h with the two cytokines (Table 1).

Neutralizing anti-IL-4 antibodies suppressed the ability of IL-4 to prime PBMC for IL-12 p40 production in response to S. aureus (Fig. 2), indicating that this priming effect was mediated by IL-4 and not by contaminants in the preparation. Similar results were obtained with enriched monococyte preparations (adherent PBMC) instead of total PBMC (Table 3 and results not shown). Pretreatment of the macrophage-like cell line THP-1 with IL-4 also increased the production of IL-12 p40 in response to LPS and, to a lesser extent, S. aureus; however, IL-4 neither enhanced nor inhibited the constitutive or TPA-induced production of IL-12 p40 from the EBV-transformed B lymphoblastoid cell line RPMI-8866 (Table 3).

Northern blot analysis (Fig. 3) showed that, like the effect of IL-4 on cytokine secretion, IL-4 pretreatment enhanced severalfold the accumulation of IL-12 p40 and TNF-α mRNA induced by either LPS or S. aureus. Unlike IL-4, pretreatment with TGF-β1 inhibited accumulation of the transcripts for the two cytokines. Accumulation of the IL-12 p35 mRNA, much less abundant than p40 mRNA, was analyzed by the RNase protection method. IL-12 p35 transcripts were induced by LPS or S. aureus with a kinetics similar to that of IL-12 p40 mRNA (Fig. 3 and not shown), and this induced accumulation was also enhanced severalfold by pretreatment of the PBMC with IL-4.

Inhibition of IL-10 Production Does Not Play a Major Role in the Priming Effect of IL-4 and IL-13 on IL-12 Production. Because monocytes produce IL-10, a potent inhibitor of IL-12 production (3) (Fig. 4), and because IL-10 production is inhibited by IL-4 (30) (Table 2), we tested the possibility that the priming effect of IL-4 and IL-13 was mediated by their inhibition of IL-10 production. However, in the present experimental conditions, IL-4 and IL-13 induced only a partial inhibition of LPS-induced IL-10 production and had no demonstrable effect on S. aureus–induced production (Table 2).
Table 1. Effect of Simultaneous Addition or Pretreatment with IL-4 and IL-13 on the LPS- and S. aureus-induced Production of IL-12 by PBMC

| Time of preincubation | Cytokine | Stimulus | IL-12 p40 *pg/ml (n = 14) | IL-12 p70 *pg/ml (n = 3) |
|-----------------------|----------|----------|--------------------------|--------------------------|
| 0                     | None     | Medium   | 161 ± 79                 | <8                       |
| 0                     | IL-13    | Medium   | 152 ± 68                 | <8                       |
| 20                    | IL-13    | S. aureus| 61,214 ± 12,828          | 893 ± 370                |
| 0                     | IL-13    | S. aureus| 18,515 ± 4,410           | 366 ± 320                |
| 20                    | IL-4     | S. aureus| 48,031 ± 7,487           | 869 ± 434                |
| 0                     | IL-4     | S. aureus| 13,402 ± 4,165           | 60 ± 48                  |
| None                  | Medium   |          | 79 ± 25                  | <8                       |
| 0                     | TGF-β    | Medium   | 120 ± 64                 | <8                       |
| 0                     | IL-10    | Medium   | 25 ± 13                  | <8                       |
| None                  | S. aureus|          | 10,340 ± 2,222           | 239 ± 204                |
| 20                    | TGF-β    | S. aureus| 1,213 ± 435              | 18 ± 15                  |
| 0                     | TGF-β    | S. aureus| 4,690 ± 1,256            | 89 ± 52                  |
| 20                    | IL-10    | S. aureus| 1,719 ± 456              | 27 ± 17                  |
| 0                     | IL-10    | S. aureus| 398 ± 115                | <8                       |
| None                  | LPS      |          | 387 ± 174                | 157 ± 140                |
| 20                    | TGF-β    | LPS      | 324 ± 97                 | <8                       |
| 0                     | TGF-β    | LPS      | 105 ± 5                  | <8                       |
| 20                    | IL-10    | LPS      | 180 ± 78                 | 8 ± 6                    |
| 0                     | IL-10    | LPS      | 37 ± 10                  | 17 ± 12                  |

* IL-4 (10 ng/ml) or IL-13 (10 ng/ml) was added to PBMC cultures 20 h before or at the time of stimulation with S. aureus (1:10,000 wt/vol) or with LPS (1 μg/ml). Cell-free supernatant fluids were collected after 18 h of stimulation; IL-12 p40 was determined by RIA and IL-12 p70 by antibody-capture bioassay. Results are mean ± SE of n experiments.

However, anti-IL-10 antibodies enhanced production of IL-12 p40 from both untreated and IL-4- or IL-13–primed PBMC (Fig. 4). Thus, although endogenous IL-10 has an inhibitory effect on IL-12 p40 production, inhibition of IL-10 production is unlikely to be an important mechanism in the IL-4/IL-13–mediated priming in light of the at least additive effect of IL-4/IL-13 priming with anti-IL-10. However, addition of exogenous IL-10 almost completely inhibited IL-12 p40 production by either untreated or IL-4/IL-13–primed PBMC (Fig. 4).

Kinetics and Dose Dependence of the Priming Effect of IL-4 and IL-13 on IL-12 p40 Production. The maximum priming effect (20-h pretreatment) of both IL-4 and IL-13 for production of IL-12 p40 from human PBMC was observed at concentrations of 1–10 ng/ml. No significant increase in IL-12 p40 production was observed with any doses of IL-4 or IL-13 between 1 pg/ml and 10 ng/ml added during the 18-h stimulation, and an inhibitory effect was observed with the highest doses of IL-4 and IL-13 present during the 18-h-stimulation with LPS, but not consistently with S. aureus (Fig. 5). An optimal priming effect was observed when PBMC were pretreated with IL-4 or IL-13 for at least 20–24 h (Fig. 5). In longer-term cultures, the IL-4–mediated enhancement of
Table 2. Effect of 20-h Pretreatment with IL-4 or IL-13 on Cytokine Production by PBMC Stimulated with LPS or S. aureus

| Cytokine* | Stimulus  | TNF-α (n = 16) | IL-1β (n = 6) | IL-10 (n = 4) |
|-----------|-----------|----------------|--------------|--------------|
| None      | Medium    | 13 ± 4         | 1,322 ± 1,321 | 25 ± 3       |
| IL-4      | Medium    | 27 ± 7         | 54 ± 54      | 23 ± 4       |
| IL-13     | Medium    | 19 ± 6         | 181 ± 126    | 22 ± 2       |
| None      | S. aureus | 1,679 ± 260    | 19,105 ± 3,601 | 103 ± 73    |
| IL-4      | S. aureus | 2,628 ± 314    | 12,935 ± 2,855 | 177 ± 77    |
| IL-13     | S. aureus | 2,636 ± 31     | 21,834 ± 3,104 | 129 ± 67    |
| None      | LPS       | 236 ± 42       | 14,108 ± 9,307 | 80 ± 30     |
| IL-4      | LPS       | 512 ± 89       | 5,682 ± 3,805 | 41 ± 11     |
| IL-13     | LPS       | 604 ± 99       | 7,130 ± 4,473 | 43 ± 14     |

* IL-4 (10 μg/ml) or IL-13 (10 μg/ml) was added to PBMC cultures. After a 20-h incubation, cultures were stimulated with S. aureus (1:10,000 wt/vol) or LPS (1 μg/ml). Cell-free supernatant fluid was collected after an 18-h stimulation, and TNF-α, IL-1β, and IL-10 were quantitated by immunoassays. Results are mean ± SE of n experiments.

Discussion

The data presented here show that treatment of PBMC with IL-4 or IL-13 for ≥20 h primes them for production of IL-12 and TNF-α in response to S. aureus and LPS, whereas their ability to produce IL-1β or IL-10 in response to LPS is impaired. These data contrast with the well-described ability of IL-4 and IL-13, at least when added at the beginning or a few hours before stimulation of monocytes/macrophages, to inhibit production of a large number of cytokines, with the sole exception of IL-1RA (14, 30). However, the ability of IL-4 in some experimental conditions to act as costimulus for production of G-CSF and M-CSF from human monocytes (36) and TNF-α from mouse peritoneal macrophages (37, 38) has been reported. Thus, the regulation of activa-

Figure 2. Specificity of IL-4 in the enhancement of IL-12 p40 production by PBMC. Human PBMC were precultured in medium alone or medium supplemented with rIL-4 (10 ng/ml) and/or neutralizing anti-IL-4 (10 μg/ml) for 20 h (solid bar) before adding S. aureus (1:10,000 wt/vol); alternatively, rIL-4 and/or anti-IL-4 were added at the same time as the S. aureus (open bar). After an 18-h incubation in the presence of S. aureus, cell-free supernatant fluids were collected and tested for the presence of IL-12 p40 by RIA. Results are mean ± SE (n = 11).

Table 3. Effect of IL-4 on IL-12 p40 Production by Cell Lines

| Cells* | Stimulus | Medium (0 h) | IL-4 (−20 h) |
|--------|----------|--------------|--------------|
|        |          | IL-12 p40 (pg/ml) |              |
| Monocytes | − | 52 | 64 | 152 |
| S. aureus | 2,074 | 74 | 20,409 |
| LPS | 562 | 242 | 2,307 |
| THP-1 | − | 51 | 27 | 57 |
| S. aureus | 104 | 85 | 288 |
| LPS | 2,215 | 1,987 | 6,734 |
| RPMI-8866 | − | 3,941 | 3,783 | 4,534 |
| S. aureus | 3,986 | 3,983 | 4,132 |
| TPA | 7,953 | 8,125 | 7,967 |

* The macrophagic cell line THP-1 and the EBV-transformed B lymphoblastoid cell line RPMI-8866 were stimulated for 18 h with S. aureus (1:10,000 wt/vol), LPS (1 μg/ml), or TPA (1.6 x 10⁻⁷ M) in medium only, in the presence (0 h) of IL-4 (10 ng/ml), or after a 20-h pretreatment with IL-4. After an 18-h stimulation, cell-free supernatant fluids were collected and IL-12 p40 was measured by RIA. A representative experiment is shown for each cell line and for a preparation of adherent PBMC (>95% monocytes).
Figure 3. Effect of IL-4 and rTGF-β on the induction of IL-12 p35, IL-12 p40, and TNF-α mRNA accumulation in human PBMC treated with LPS (1 μg/ml) or *S. aureus* (1:10,000 wt/vol). Cells were preincubated for 20 h at 37°C in medium alone or medium supplemented with 10 ng/ml rIL-4 or rTGF-β before LPS or *S. aureus* stimulation (18 h). For IL-12 p40 and TNF-α, 15 μg total RNA was loaded per lane. Filters were sequentially hybridized with 32p-labeled IL-12 p40, TNF-α, and GAPD cDNA probes. The bottom panel shows the autoradiography of the Northern blot of one of two experiments with similar results. For IL-12 p35, total RNA (20 μg) in solution was hybridized with 3P-riboprobe transcribed from IL-12 p35 cDNA, and mRNA accumulation was analyzed by RNase protection. Both Northern blot hybridization and RNase protection were quantitated by use of a phosphorimager. Results in the bar plots are given as percentage of maximum accumulation after normalizing to the GAPD values. Open and solid bars indicate the results of two experiments.

Figure 4. Role of IL-10 in IL-12 p40 production by PBMC after pretreatment with rIL-4 or rIL-13. Human PBMC were preincubated for 20 h in medium alone (open bars) or in medium supplemented with 10 ng/ml of rIL-4 (solid bars) or rIL-13 (striped bars) in the presence or absence of either IL-10 (50 U/ml) or anti-IL-10 mAb (ascites 1:100), followed by addition of *S. aureus* (1:10,000 wt/vol) or LPS (1 μg/ml). IL-12 p40 production was determined after an 18-h incubation by use of an RIA. Results are presented as mean ± SE (n = 3).

IL-4 and IL-13 Prime PBMC for IL-12 and TNF-α Production

The inhibition of IL-12 and TNF-α production and, partially, IL-1β production by IL-4 is reflected by a decrease in accumulation of their mRNA in LPS-stimulated cells. Previous studies (31) have indicated that this effect of IL-4 may be mediated by both transcriptional and posttranscriptional mechanisms. Unlike the inhibitory effect of IL-4 and IL-13 when present together with the cytokine-inducing stimulus, preincubation of PBMC with IL-4 and IL-13 for >20 h primed the cells for production of IL-12 and TNF-α in response to both *S. aureus* and LPS, resulting in a three- to fourfold increase of both mRNA accumulation and protein secretion. In the absence of a cytokine-inducing stimulus such as *S. aureus* or LPS, IL-4 did not have a direct stimulatory effect on the mRNA accumulation or production of either IL-12 or TNF-α. The priming effect of IL-4 and IL-13 was demonstrable on the induction of both the p35 and p40 genes. However, the ratio between the transcripts was not changed by priming, and, like unprimed cells, the IL-4- or IL-13-primed PBMC secreted the IL-12 p40 chain in large excess over the biologically active IL-12 p70 heterodimer. These effects of IL-4 and IL-13 contrast with those of IFN-γ, which directly stimulate expression of the p35 gene and induce...
because identical results were obtained with recombinant IL-4 phils (46a).

priming for IL-12 p70 production more effectively than for IL-12 p40 production in both monocytes and neutrophils (46a).

The ability of IL-4 to prime PBMC for cytokine production was not due to contaminants present in the preparation, because identical results were obtained with recombinant IL-4 from three different sources (not shown), and the effect was completely blocked by neutralizing anti-IL-4 antibodies. The nearly identical results obtained with IL-4 and IL-13, two cytokines that share many biological actions, confirms the specificity of the priming effect. Because IL-4 and IL-13 inhibit phagocytic cell production of IL-10, a powerful inhibitor of IL-12 and TNF-α production, it was possible that the priming effect of IL-4 and IL-13 on the production of these two cytokines was due to inhibition of IL-10 production. However, our data do not support this hypothesis because (a) IL-4 and IL-13 prime PBMC for production of IL-12 and TNF-α in response to S. aureus, whereas they have only limited ability to inhibit IL-10 production in response to this stimulus; and (b) neutralizing anti-IL-10 antibodies enhance production of IL-12 in response to either LPS and S. aureus by abolishing the inhibitory effect of endogenous IL-10, and this effect is synergistic with IL-4 or IL-13 priming, indicating that the ability of IL-4- or IL-13-primed PBMC to produce IL-12 is still inhibited by endogenous IL-10. Thus, although the inhibition of IL-10 may play a minor role in the mechanisms of IL-4 or IL-13 priming, especially in the case of LPS stimulation, the major mechanism of the priming appears to be independent of inhibition of IL-10 production.

The ability of IL-4 and IL-13 to prime PBMC for IL-12 production is particularly surprising because IL-12 and IL-4 are known to play an antagonistic role in inducing development of Th1 and Th2 immune response, respectively (47). The equilibrium between IL-12 and IL-4 early during an immune response most likely determines whether the response is Th1 or Th2 (48). However, it is likely that several modulatory mechanisms and both positive and negative feedback mechanisms intervene in the fine regulation of the Th1/Th2 dichotomy. IL-4 acts not only by downmodulating costimulatory signals expressed or secreted by accessory cells, but also by preventing the responsiveness of T cells to IL-2 (49). The priming effect of IL-4 and IL-13 on IL-12 production by PBMC is almost completely suppressed by IL-10, another cytokine prevalently produced by Th2 cells, suggesting that the priming effect of IL-4 on IL-12 production is not effective when a Th2 response predominates. Even in the presence of high concentrations of IL-12 during an immune response, factors such as TGF-β may suppress Th1 cell differentiation in response to IL-12 and favor Th2 cell differentiation (Scharton-Kersten, T., L. C. C. Afonso, M. Wysocka, G. Trinchieri, and P. Scott, manuscript submitted for publication). Other exceptions to the rule that IL-4 and IL-13 favor Th2 and Th1 differentiation, respectively, include the findings that IL-12 enhanced IL-4 production by IL-4-primed human cord blood CD4+ cells (50), and that IL-12 in vivo induced IL-10 production in the mouse (51).

The ability of IL-4 and IL-13 to prime monocytes for IL-12 and TNF-α production is likely reflected in enhanced accessory cell activity of these cells. We observed that IL-4- and IL-13-primed PBMC have an enhanced ability to act as APC for antigen-induced IFN-γ production by most human tetanus toxoid-specific CD4+ clones (results not shown). IL-12 production from APC plays an important role in antigen-induced IFN-γ production and, to some extent, in proliferation of CD4+ clones (4). Thus, it is likely that the enhanced production of IL-12 is important for the enhanced APC activity of IL-4- and IL-13-primed cells, although other effects of IL-4, in particular the enhanced expression of MHC class II and other costimulatory factors such as TNF-α, may play a role (52, 53). The exact mechanism of the enhanced APC activity of IL-4- and IL-13-primed PBMC remains to be characterized. We have also observed that IL-4 and IL-13 treatment

![Image](https://example.com/image.png)

Figure 5. Effect of IL-4 and IL-13 on IL-12 p40 production. (A) Human PBMC were preincubated (for 20 h at 37°C) in the absence (O-O) or presence (O-○) of increasing concentrations of human rIL-4 or rIL-13 followed by the addition of LPS (1 μg/ml) or S. aureus (1:10,000 wt/vol). After an 18-h incubation at 37°C, cell-free supernatant fluids were collected and tested for the presence of IL-12 p40. Results are mean ± SE (n = 4). (B) Human PBMC were preincubated with human rIL-4 or rIL-13 (10 ng/ml) for the time periods indicated, after which S. aureus (1:10,000) was added and the cells were cultured for an additional 18 h. Supernatant fluids were collected and tested for the presence of IL-12 p40. Results (▲▲) are mean ± SE (n = 3). Dashed lines in all panels represent the amount of IL-12 produced in the absence of treatment with either IL-4 or IL-13.
of monocytes enhances their ability to mediate TNF-α-dependent cytotoxicity of the WEHI-164 cell line (results not shown), contrasting with the ability of IL-4 to inhibit antibody-dependent cell-mediated cytotoxicity, due to inhibition of expression of Fe receptor on monocytes/macrophages (14, 37). Not only does short-term treatment of PBMC with IL-4 or IL-13 enhance their APC function and ability to support antigen-dependent IFN-γ production, but long-term cultures of monocytes in the presence of GM-CSF and IL-4 give rise to cells with dendritic-like phenotype, which are very powerful

of APC (22, 23) and extremely effective producers of IL-12 (Kubin, M., and G. Trinchieri, unpublished results). These data suggest that IL-4 and IL-13, primary products of Th2 cells, might play a role in the differentiation and activation of APC, which, in the absence of other regulatory mechanisms, produce large amounts of IL-12 and may favor Th1 cell development. The role of this activity of IL-4 or IL-13 in the complex regulatory interactions of the immune response remains to be clarified.

We thank Ms. Marion Kaplan for typing the manuscript.

This work was supported in part by National Institutes of Health grants CA-10815, CA-20833, CA-32898, and AI-34412. C. Paganin is a recipient of a fellowship for the Istituto Superiore di Sanità (Rome, Italy) for the study of AIDS.

Address correspondence to Dr. Giorgio Trinchieri, The Wistar Institute, 3601 Spruce Street, Philadelphia, PA 19104. A. D’Andrea’s present address is DNAX Research Institute of Molecular and Cellular Biology, 901 California Avenue, Palo Alto, CA 94304-1104.

Received for publication 19 August 1994 and in revised form 30 September 1994.

References
1. Kobayashi, M., L. Fitz, M. Ryan, R.M. Hewick, S.C. Clark, S. Chan, R. Loudon, F. Sherman, B. Perussia, and G. Trinchieri. 1989. Identification and purification of natural killer cell stimulatory factor (NKSF), a cytokine with multiple biologic effects on human lymphocytes. J. Exp. Med. 170:827-845.
2. D'Andrea, A., M. Rengaraju, N.M. Valiante, J. Chehimi, M. Kubin, M. Aste, S.H. Chan, M. Kobayashi, D. Young, E. Nickbarg, et al. 1992. Production of natural killer cell stimulatory factor (interleukin 12) by peripheral blood mononuclear cells. J. Exp. Med. 176:1387-1398.
3. D'Andrea, A., M. Aste-Amezaga, N.M. Valiante, X. Ma, M. Kubin, and G. Trinchieri. 1993. Interleukin 10 (IL-10) inhibits human lymphocyte interferon-γ production by suppressing natural killer cell stimulatory factor/IL-12 synthesis in accessory cells. J. Exp. Med. 178:1041-1048.
4. Murphy, E.E., G. Terres, S.E. Macatonia, C.S. Hsieh, J. Mattson, L. Lanier, M. Wysocka, G. Trinchieri, K. Murphy, and A. O’Garra. 1994. B7 and interleukin 12 cooperate for proliferation and interferon γ production by mouse T helper clones that are unresponsive to B7 costimulation. J. Exp. Med. 180:223-231.
5. Kubin, M., M. Kamoun, and G. Trinchieri. 1994. Interleukin 12 synergizes with B7/CD28 interaction in inducing efficient proliferation and cytokine production of human T cells. J. Exp. Med. 180:211-222.
6. Manetti, R., P. Parronchi, M.G. Giudizi, M.P. Piccinni, E. Maggi, G. Trinchieri, and S. Romagnani. 1993. Natural killer cell stimulatory factor (interleukin 12 [IL-12]) induces T helper type 1 (Th1)-specific immune responses and inhibits the development of IL-4 producing Th cells. J. Exp. Med. 177:1199-1204.
7. Hsieh, C., S.E. Macatonia, C.S. Tripp, S.F. Wolf, A. O’Garra, and K.M. Murphy. 1993. Listeria-induced Th1 development in αβ-TCR transgenic CD4+ T cells occurs through macrophage production of IL-12. Science (Wash. DC). 260:547-549.
8. Germann, T., M.K. Gately, D.S. Schoenhaut, M. Lohoff, F. Mattner, S. Fischer, S. Jin, E. Schmitt, and E. Rüde. 1993. Interleukin-12/T cell stimulating factor, a cytokine with multiple effects on T helper type 1 (Th1) but not on Th2 cells. Eur. J. Immunol. 23:1762-1770.
9. Wolf, S.F., P.A. Temple, M. Kobayashi, D. Young, M. Dicig, L. Lowe, R. Dzialo, L. Fitz, C. Ferenz, R.M. Hewick, et al. 1991 Cloning of cDNA for natural killer cell stimulatory factor, a heterodimeric cytokine with multiple biologic effects on T and natural killer cells. J. Immunol. 146:3074-3081.
10. Gubler, U., A.O. Chua, D.S. Schoenhaut, C.M. Dwyer, W. McComas, R. Motyka, N. Nabavi, A.G. Wolitzky, P.M. Quinn, P.C. Familleit, et al. 1991. Coexpression of two distinct genes is required to generate secreted bioactive cytotoxic lymphocyte maturation factor. Proc. Natl. Acad. Sci. USA. 88:4143-4147.
11. Stern, A.S., F.J. Podlaski, J.D. Hulmes, Y.E. Pan, P.M. Quinn, A.G. Wolitzky, P.C. Famileit, et al. 1990. Purification to homogeneity and partial characterization of cytotoxic lymphocyte maturation factor. J. Immunol. 146:3074-3081.
12. Stern, A.S., F.J. Podlaski, J.D. Hulmes, Y.E. Pan, P.M. Quinn, A.G. Wolitzky, P.C. Famileit, et al. 1990. Purification to homogeneity and partial characterization of cytotoxic lymphocyte maturation factor from human B-lymphoblastoid cells. Proc. Natl. Acad. Sci. USA. 87:6808-6812.
13. Nathan, C.F., H.W. Murray, M.E. Wiebe, and B.Y. Rubin. 1983. Identification of interferon-γ as the lymphokine that activates human macrophage oxidative metabolism and antimicrobial activity. J. Exp. Med. 158:670-689.
14. Kubin, M., J.M. Chow, and G. Trinchieri. 1993. Differential regulation of interleukin-12 (IL-12), tumor necrosis factor-α,
and IL-1β production in human myeloid leukemia cell lines and peripheral blood mononuclear cells. Blood. 83:1847–1855.
14. Bogdan, C., and C. Nathan. 1993. Modulation of macrophage function by transforming growth factor β, interleukin-4, and interleukin-10. Ann. NY Acad. Sci. 685:713–739.
15. Yaga, K., H. Mostowski, and G. Tosato. 1993. Human interleukin-10 can directly inhibit T-cell growth. Blood. 81:2964–2971.
16. de Waal Malefyt, R., H. Yssel, and J.E. de Vries. 1993. Direct effects of IL-10 on subsets of human CD4+ T cell clones and resting T cells. Specific inhibition of IL-2 production and proliferation. J. Immunol. 150:4754–4765.
17. Hsu, D., K.W. Moore, and H. Spits. 1992. Differential effects of IL-4 and IL-10 on IL-2-induced IFN-γ synthesis and lymphokine-activated killer activity. Int. Immunol. 4:563–569.
18. Minty, A., P. Chalon, J. Deroce, X. Dumont, J. Guilleminot, M. Kaghad, C. Labit, P. Leplatois, P. liaison, B. Miloux, et al. 1993. Interleukin-13 is a new human lymphokine regulating inflammatory and immune responses. Nature (Lond.). 362:248–250.
19. McKenzie, A.N.J., J.A. Culppepper, R. de Waal Malefyt, F. Brière, J. Punnonen, G. Aversa, A. Sato, W. Dang, B.G. Cocks, S. Menon, et al. 1993. Interleukin-13, a T-cell-derived cytokine that regulates human monocyte and B-cell function. Proc. Natl. Acad. Sci. USA. 90:3735–3739.
20. Zuwara, G., and J.E. de Vries. 1994. Interleukin-13, an interleukin-4–like cytokine that acts on monocytes and B cells, but not on T cells. Immunol. Today. 15:19–26.
21. de Waal Malefyt, R., C.G. Figdor, and J.E. de Vries. 1993. Effects of interleukin-4 on monocyte functions: comparison to interleukin-13. Res. Immunol. 144:629–633.
22. Sallusto, F., and A. Lanzavecchia. 1994. Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin 4 and downregulated by tumor necrosis factor α. J. Exp. Med. 179:1109–1118.
23. Peters, J.H., J. Ruppert, R.K. Giesler, H.M. Najjar, and H. Xu. 1991. Differentiation of human monocytes into CD14 negative accessory cells: do dendritic cells derive from the monocytic lineage? Pathobiology. 59:122–126.
24. Vercelli, D., H.H. Jabara, B.W. Lee, N. Woodland, R.S. Geha, and D.Y.M. Leung. 1988. Human recombinant interleukin 4 induces FeR2/CD23 on normal human monocytes. J. Exp. Med. 167:1406–1416.
25. Te Velde, A.A., F. Rousset, C. Peronne, J.E. de Vries, and C.G. Figdor. 1990. IFN-α and IFN-γ have different regulatory effects on IL-4 induced membrane expression of FeR1 and release of soluble FeR1b by human monocytes. J. Immunol. 144:3052–3059.
26. Te Velde, A.A., J.P.G. Klomp, B.A. Yard, J.E. de Vries, and C.G. Figdor. 1988. Modulation of phenotypic and functional properties of human peripheral blood monocytes by IL-4. J. Immunol. 140:1548–1554.
27. Becker, S., and E.G. Daniel. 1990. Antagonistic and additive effects of IL-4 and IFNγ on human monocytes and macrophages: effects on Fe receptors, HLA-D antigens, and superoxide production. Cell. Immunol. 129:351–362.
28. Littman, B.H., F.E. Dastyan, P.L. Carlson, and K.M. Sanders. 1989. Regulation of monocyte/macrophage C2 production and HLA-DR expression by IL-4 (BSF-1) and IFNγ. J. Immunol. 142:520–525.
29. Gerrard, T.L., D.R. Dyer, and H.S. Mostowski. 1990. IL-4 and granulocyte-macrophage colony stimulating factor selectively increase HLA-DR and HLA-DP antigens, but not HLA-DQ antigens on human monocytes. J. Immunol. 144:4670–4674.
30. de Waal Malefyt, R., C.G. Figdor, R. Huijbers, S. Molan-Peterson, B. Bennett, J. Culppepper, W. Dang, G. Zuwara, and J.E. de Vries. 1993. Effects of IL-13 on phenotype, cytokine production, and cytotoxic function of human monocytes. J. Immunol. 151:6370–6381.
31. Donnelly, R.P., M.J. Fenton, J.D. Kaufman, and T.L. Gerrard. 1991. IL-1 expression in human monocytes is transcriptionally and posttranscriptionally regulated by IL-4. J. Immunol. 146:3431–3436.
32. Hart, P.H., G.F. Vitti, D.R. Burgess, G.A. Whitty, D.S. Piccoli, and J.A. Hamilton. 1989. Potential antiinflammatory effects of interleukin 4: suppression of human monocyte tumor necrosis factor α, interleukin 1 and prostaglandin E2. Proc. Natl. Acad. Sci. USA. 86:3803–3807.
33. Essner, R., K. Rhoades, W.H. McBride, D.L. Morton, and J.S. Economou. 1989. IL-4 down-regulates IL-1 and TNF gene expression in human monocytes. J. Immunol. 142:3857–3861.
34. Te Velde, A.A., R.J.F. Huijbers, J.E. de Vries, and C.G. Figdor. 1990. Interleukin 4 inhibits secretion of IL-1α, tumor necrosis factor α, and IL-6 by human monocytes. Blood. 76:1392–1397.
35. Hamilton, J.A., G.A. Whitty, A.K.M. Royston, J. Cebo, and J.E. Layton. 1992. Interleukin 4 suppresses granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor levels in stimulated human monocytes. Immunology. 76:566–571.
36. Wieser, M., R. Bonifet, W. Oster, A. Lindeman, R. Mertelsmann, and F. Herrmann. 1989. Interleukin-4 induces secretion of CSF for granulocytes and CSF for macrophages by peripheral blood monocytes. Blood. 73:1105–1108.
37. Suk, K., S.D. Somers, and K.L. Erickson. 1993. Regulation of murine macrophage function by IL-4: IL-4 and IFN-γ differentially regulate macrophage tumoricidal activation. Immunology. 80:617–624.
38. Stenger, S., W. Solbach, M. Rollinghoff, and C. Bogdan. 1991. Cytokine interactions in experimental cutaneous leishmaniasis. II. Endogenous tumor necrosis factor-alpha production by macrophages is induced by the synergistic action of interferon (IFN)-γ and interleukin (IL)-4 and accounts for the antiparasitic effect mediated by IFN-γ and IL-4. Eur. J. Immunol. 21:1669–1675.
39. Molina, I.J., and B.T. Huber. 1991. Regulation of macrophage activation markers by IL-4 and IFN-γ is subpopulation-specific. Cell. Immunol. 134:241–248.
40. Phillips, W.A., M. Croatto, and J.A. Hamilton. 1992. The effect of IL-4 on the macrophage respiratory burst is species dependent. Biochem. Biophys. Res. Commun. 182:727–732.
41. Fontaine, S., C. Danais, D. Lando, and M.A. Cousin. 1989. Monoclonal antibodies to human interleukin-1 beta and their use in a sensitive two-site enzyme linked immunosorbent assay. Lymphokine Res. 8:129–139.
42. Murphy, M., R. Loudon, M. Kobayashi, and G. Trinchieri. 1986. γ interferon and lymphotoxin, released by activated T cells, synergize to inhibit granulocyte/monocyte colony formation. J. Exp. Med. 164:263–279.
43. Cuturi, M.C., M. Murphy, M.P. Costa-Giomi, R. Weinmann, B. Perussia, and G. Trinchieri. 1987. Independent regulation of tumor necrosis factor and lymphotoxin production by human peripheral blood lymphocytes. J. Exp. Med. 165:1581–1594.
44. Chan, S.H., B. Perussia, J.W. Gupta, M. Kobayashi, M. Popstil, H.A. Young, S.F. Wolf, D. Young, S.C. Clark, and G. Trinchieri.
chieri. 1991. Induction of interferon γ production by natural killer cell stimulatory factor: characterization of the responder cells and synergy with other inducers. J. Exp Med. 173:869–879.

45. Chan, S.H., M. Kobayashi, D. Santoli, B. Perussia, and G. Trinchieri. 1992. Mechanisms of IFN-γ induction by natural killer cell stimulatory factor (NKSF/IL-12): role of transcription and mRNA stability in the synergistic interaction between NKSF and IL-2. J. Immunol. 148:92–98.

46. Cassatella, M.A., L. Hartman, B. Perussia, and G. Trinchieri. 1989. Tumor necrosis factor and immune interferon synergistically induce cytochrome b-245 heavy chain gene expression and NADPH oxidase in human leukemic myeloid cells. J. Clin. Invest. 83:1570–1579.

46a. Cassatella, M.A., L. Meda, S. Gasperini, A. D’Andrea, X. Ma, and G. Trinchieri. 1994. Interleukin-12 production by human polymorphonuclear leukocytes. Eur. J. Immunol. In press.

47. Trinchieri, G. 1993. Interleukin-12 and its role in the generation of Th1 cells. Immunol. Today. 14:335–338.

48. Trinchieri, G. 1995. Interleukin-12: a pro-inflammatory cytokine with immunoregulatory functions which bridge innate resistance and antigen-specific adaptive immunity. Annu. Rev. Immunol. 13:251–276.

49. Nagler, A., L.L. Lanier, and J.H. Phillips. 1988. The effects of IL-4 on human natural killer cells. A potent regulator of IL-2 activation and proliferation. J. Immunol. 141:2349–2351.

50. Wu, C.Y., C.E. Demeneur, M. Gately, F. Podlaski, H. Yssel, M. Kiniwa, and G. Delespesse. 1994. In vitro maturation of human neonatal CD4 T lymphocytes. I. Induction of IL-4-producing cells after long-term culture in the presence of IL-4 plus either IL-2 or IL-12. J. Immunol. 152:1141–1153.

51. Finkelman, F.D., K.B. Madden, A.W. Cheever, I.M. Katona, S.C. Morris, M.K. Gately, B.R. Hubbard, W.C. Gause, and J.F. Urban, Jr. 1994. Effects of interleukin 12 on immune responses and host protection in mice infected with intestinal nematode parasites. J. Exp. Med. 179:1563–1572.

52. Zlotnik, A., M. Fischer, N. Roehm, and D. Zipori. 1987. Evidence for effects of interleukin 4 (B cell stimulatory factor 1) on macrophages: enhancement of antigen presenting ability of bone marrow-derived macrophages. J. Immunol. 138:4275–4279.

53. Aiello, F.B., D.L. Longo, R. Overton, L. Takacs, and S.K. Durum. 1990. A role for cytokines in antigen presentation: IL-1 and IL-4 induce accessory functions of antigen-presenting cells. J. Immunol. 144:2572–2581.