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J Immunol 1998; 160:5861-5868;
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Exogenous and Endogenous IL-10 Regulate IFN-α Production by Peripheral Blood Mononuclear Cells in Response to Viral Stimulation

Faribouz Payvandi,*‡ Sheela Amrute,* and Patricia Fitzgerald-Bocarsly2,†

IL-10 is an important regulator of the production of proinflammatory cytokines. Its effect on IFN-α production, however, has not been reported. In this study, PBMC from healthy donors were stimulated with virus in the presence of IL-10. Human IL-10 (hIL-10) caused reductions in both the frequency of IFN-α-producing cells (IPC) and bulk IFN in response to herpes simplex virus type-1 (HSV-1), Sendai virus, Newcastle disease virus, and vesicular stomatitis virus. The inhibitory effect occurred when IL-10 was added 2 or 4 h before, or 2 h poststimulation with HSV or Sendai virus, but not when added 4 h postinduction. Unlike IL-10, IL-4 did not affect the IFN-α response to HSV. However, when PBMC were induced with Sendai virus, IFN-α production was also reduced by IL-4. IL-10 treatment of PBMC resulted in strong reductions in the steady state levels of both HSV- and Sendai virus-induced IFN-α, α2, and αβ mRNA as determined by RT-PCR. IFN-α production to Sendai virus occurs predominantly by monocytes, whereas most enveloped viruses stimulate low frequency “natural IFN-producing cells (NIPC),” which are thought to be dendritic cells. Peripheral blood dendritic cells were found to express the IL-10 receptor, suggesting that IL-10 may directly act on the dendritic IPC. Addition of monoclonal anti-IL-10 to PBMC resulted in a significant increase in both the frequency of IPC and the amount of secreted IFN-α in response to HSV but not Sendai virus. We conclude that human IL-10 can serve as both an endogenous and exogenous regulator of IFN-α production.

IFN-α belongs to the type 1 family of IFNs, which are all derived from the same ancestral gene and exert their effects via a common cell surface receptor (1). IFN-α can be induced by a number of agents including foreign cells, virus-infected cells, tumor cells, bacterial cells, free virions, viral envelopes, and synthetic double stranded RNA (2). Several laboratories, including our own, have been studying the cells responsible for the secretion of IFN-α in response to a number of different viruses (3). These investigations have demonstrated two major IFN-α-producing cell populations: monocytes and a second population of cells that have been called “natural IFN-producing cells” (NIPC).3 The NIPC, which are negative for lineage-specific cell surface markers and share the phenotype of immature DCs, have been shown to be the primary cells among PBMC that produce IFN-α in response to a number of both enveloped DNA and RNA viruses (4–6). Of these, herpes simplex virus type-1 (HSV-1) and Sendai virus have been the best studied and induce predominantly NIPC and monocytes to produce IFN-α, respectively. Cederblad et al. have reported that the cytokines GM-CSF and IL-3 are able to up-regulate IFN-α secretion from both monocytes and NIPC in response to HSV (7). Although there have been several studies demonstrating the down-regulation of IFN-α in various diseases (8–10), there is very little information about the factors involved in the reduction of IFN-α production in the patient populations.

IL-10, a pleiotropic cytokine, is a natural immunosuppressant that shifts the body’s immune response away from an inflammatory response (11–13). It is produced by human CD4+ T cells and clones, monocytes, macrophages, keratinocytes, activated B cells, and B lymphoma lines and plays a major role in T helper cell regulation, serving to down-regulate Th1 responses (14, 15). The production of IL-10 by macrophages can be inhibited by IL-10 itself, which suggests an autocrine effect of IL-10 on monocyte function (16). In vitro, IL-10 has been shown to inhibit the production of several proinflammatory cytokines including IL-1β, IL-6, IL-8, TNF-α, GM-CSF, and G-CSF, as well as to inhibit monocyte MHC class II expression (17, 18). IL-10 also has been shown to synergize with other cytokines, including IL-4 and TGF-β, to inhibit macrophage cytotoxic activity (19). In addition to its inhibitory effects on monocyte function, IL-10 has been shown to exert a wide array of immuno-stimulatory effects on B cells and T cells (13). Clerici et al. have shown significant increases in the levels of IL-10 produced by PBMC from HIV infected individuals in whom T helper cell functions were severely compromised (20). It has been suggested that sustained production of IL-10 in various organs serves to protect patients with septic peritonitis, and IL-10 knock-out animals demonstrate chronic enterocolitis, indicating a failure to regulate response to bacterial gut pathogens (21). In general, the production of IL-10 is correlated with expression of Th2-like responses induced by infectious agents (22).

To determine the effects of IL-10 on IFN-α production, we stimulated PBMC with different viruses including HSV, Sendai virus, vesicular stomatitis virus (VSV), and Newcastle disease virus

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0022-1767/98/$02.00
(NDV) in the presence or absence of exogenous hIL-10. The frequency of IPC and the amount of secreted IFN-α were measured by ELISPOT and IFN bioassay, respectively. The influence of endogenously produced IL-10 on IFN-α secretion by PBMC stimulated with HSV and Sendai virus was also investigated. We observed that hIL-10 inhibited both the frequency of IPC and bulk IFN-α as well as the steady state levels of IFN-α mRNA. Furthermore, blocking of endogenous IL-10 enhanced both the frequency of IPC and the amount of secreted IFN-α produced in response to HSV but not Sendai virus, suggesting a role for endogenous IL-10 in regulation of IFN-α synthesis in certain viral infections.

Materials and Methods

Cell lines

GM-0459A (Human Genetic Mutant Cell Repository, Camden, NJ), a human fibroblast cell line trisomic for chromosome 21, was grown in DMEM (JRH, St.Lenexa, KS) supplemented with 15% FCS (Gemini Bio-Products, Inc., Calabasas, CA), 2 mM L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin (DMEM-15% FCS). The cells were passaged twice weekly and used up to passage 20. Vero cells (originally obtained from the American Type Culture Collection (ATCC, Manassas, VA) were grown in DMEM-10% FCS.

Cytokines and Abs

Recombinant IL-4 was purchased from R&D Systems (Minneapolis, MN). Recombinant hIL-10 and anti-hIL-10 mAb (JES3) were kindly supplied by Dr. G. Marchalonis (Scribing-Plough, Northfield, NJ). Rat IgG (isotype control for JES3) was purchased from Sigma (St. Louis, MO). Fluorescent-conjugated mouse anti-human CD14 and its isotype control were obtained from DAKO (Carpinteria, CA).

Viruses

HSV-1 strain 2931 was grown and titred on Vero cells as previously described (23). VSV, originally obtained from Dr. Nicholas Ponzio of New Jersey Medical School, was grown on Vero cells, whereas Sendai virus, strain Sendai/Cantell, was obtained from ATCC and grown in 10-day-old embryonated chicken eggs. NDV, strain VR-107, was also grown in embryonated chicken eggs. Sendai virus and NDV were titred in a hemagglutination assay using chicken RBC, and titers were expressed as hemagglutination units (HAU)/ml. All viruses were stored at −70°C.

Cell preparation

PBMC were prepared by Ficoll-hypaque (Lymphoprep; Accurate Chemical and Scientific, Westbury, NY) density sedimentation of heparinized peripheral blood obtained with informed consent from healthy volunteers. The cells were washed twice in HBSS with Ca2+ /Mg2+ (Mediatech, Herndon, VA) and resuspended in RPMI 1640 (JRH Biosciences) supplemented with 10% FCS, penicillin, streptomycin, L-glutamine, and 25 mM HEPES buffer (RPMI-10%). Cells were enumerated using a coulter Model ZBI electronic particle counter (Coulter Electronics, Hialeah, FL). For the enrichment of CD14+ and CD14- populations, magnetic cell separation was used. PBMC were incubated with mouse-anti-human CD14 Ab for 1 h at 4°C, washed, and resuspended in magnetic beads conjugated with sheep anti-mouse IgG and incubated at 4°C for another hour followed by separation by the MACS magnet (Miltenyi Biotec, Sunnyvale, CA). Both CD14+ and CD14- were harvested, and the purity of the populations was assessed by flow cytometry.

Analysis of IL-10 receptor expression on DCs

The expression of IL-10 receptor was determined for whole PBMC using an IL-10 Fluorochrome Kit (R&D Systems) according to the manufacturer’s protocol. PBMC (1 × 10⁶) were first stained with Tricolor-labeled anti- HLA-DR (Caltag, Burlingame, CA) and PE-conjugated anti-CD3, CD14, CD19 (DAKO) and CD16 and CD20 (both from Becton Dickinson, Sunnyvale, CA). The cells were then washed and separated into two groups to which were added the biotin-conjugated IL-10 or the biotin-labeled control protein. After incubation, avidin-FITC was added, followed by washing. Groups containing appropriate isotype controls were also used for each fluorochrome. All tubes were then washed and fixed in 1% paraformalde- hyde for flow analysis. Analysis was conducted on a FACS Caliber (Becton Dickinson). The PBMC were first analyzed for forward and side scatter, and the cells within the lymphocyte and monocyte gate were acquired. These were then analyzed for the tricolor and PE markers, and cells that were HLA-DR+ but negative for the PE-conjugated Abs were further analyzed for FITC-IL-10 binding.

ELISPOT assays

The ELISPOT assay for determination of frequency of IPC was performed as previously described (9). PBMC in RPMI-10% FCS were incubated at 1 × 10⁶ cells/ml with viruses at concentrations as described in the experiments for 6 to 8 h at 37°C in a 5% CO₂ incubator. Microtiter plates (96-well) with nitrocellulose membrane bottoms, type HATF (Millitter HA plates, Millipore, Bedford, MA), were coated with ammonium sulfate- precipitated bovine anti-human IFN-α antiseraum (Batch No. AS/4693, kindly provided by Wellcome Research Foundation, Beckenham, U.K.) and then fixed with 0.05% glutaraldehyde. Stimulated or mock-stimulated PBMC were washed and then added in serial threefold dilutions to the Millitter plates and to standard 96-well flat-bottom plates (Corning, Acton, MA) for bulk IFN generation. Cells in the Millitter plates were incubated at 37°C for 10 to 12 h to allow production and capture of IFN-α and were developed using a murine anti-human IFN-α mAb that cross-reacts with different subtypes of IFN-α (293, kindly provided by Drs. Brita Cederblad and Gunnar Alm, Upplasa, Sweden), followed by horseradish peroxidase- conjugated goat anti-mouse IgG (Jackson ImmunoResearch Laboratory, West Grove, PA) and the horseradish peroxidase substrate 3,3′ diaminobenzidine tetrahydrochloride (Sigma). The resulting brown spots were counted under a dissecting microscope, and the frequencies of IPC were calculated and expressed as mean IFN 10⁶/96 PBMC. The SDs for replicate wells were typically less than or equal to 10% of the mean values.

IFN bioassays

IFN bioassays were performed on samples harvested from the standard mirocytes plates described above. Supernatants were stored frozen at −20°C before quantification of IFN activity. IFN was quantified using a cytopathic effect (CPE) reduction assay as previously described (24) using GM 04592A as the indicator cell line and VSV as the challenge virus. Each IFN assay contained cell and virus controls, and the international leukocyte IFN (IFN-α) reference standard G-023-901-527, obtained from the National Institute of Allergy and Infectious Diseases. The lowest detectable IFN concentration was between 3 and 10 IU/ml of supernatant.

RNA isolation and RT-PCR analysis

Total RNA was prepared from virally stimulated PBMC using RNAzol B according to the manufacturer’s protocol (TEL-Test, Friendswood, TX). Reverse transcription and PCR were performed using the Perkin-Elmer Cetus Gene Amp RNA PCR kit (Perkin-Elmer Cetus, Norwalk, CT) using 1 μg total RNA from 10⁶ PBMC. All primers were synthesized by Operon Technologies (Alameda, CA): IFN-α1 sense primer, 5′-CAATATCTCTATTGGTCCTCCG-3′; IFN-α1 antisense primer, 5′-AGAGATTGCGTGGAGGACTTCTCTG-3′ (25); IFN-α2 sense primer, 5′-ATCCAGCAGACTCTCATACTG-3′; IFN-α2 antisense primer, 5′-AGAGAAAAAGTCTCAGATGT-3′; IFN-β sense primer, 5′-GATTTACTATGAGCCGCTTGG-3′; IFN-β antisense primer, 5′-CTTCCGAGTTAATGTCAGATCC-3′ (25); β-actin sense primer, 5′-GTTGGGGGCCCGACGCACTAC-3′; and β-actin antisense primer, 5′-GTCCCTTAAATGTAGCAGACGTTC-3′ (16).

For IFN-α1 and IFN-β PCR, the products were denatured at 94°C for 45 s, annealed at 6°C for 45 s, and extended at 72°C for 2 min. For IFN-α2 PCR, the products were held at 95°C for 2 min, annealed at 55°C for 1 min, extended at 72°C for 1 min, and denatured at 95°C for 1 min. β-actin PCR was performed using both sets of conditions. A total of 26 cycles of amplification were performed using the Gene Amp PCR system 9600 (Perkin-Elmer). After the PCR amplification reaction, 20 μl of each reaction was loaded on 2% agarose gels in TBE buffer, and PCR products were quantitated using a Fluorimag SI (Molecular Dynamics, Sunnyvale, CA).

Results

IL-10 down-regulates IFN-α production and frequency of NIPC in response to viral stimulation

PBMC from healthy individuals were stimulated for 6 h with either HSV at a multiplicity of infection (MOI) of 1 or Sendai virus at 16 HAU/ml in the presence or absence of different concentrations of hIL-10. The treated cells were placed into Millititer plates or flat-bottom plates for an additional 12 h for ELISPOT and bulk IFN assays, respectively. IL-10 inhibited both the frequency of IPC as well as total IFN produced in response to both HSV and Sendai virus in a dose-dependent manner (Fig. 1). In addition to decreasing the frequency of detected IPC, IL-10-treated PBMC also
tended to yield fainter spots in the ELISPOT assay, which is interpreted to indicate that, on a per cell basis, the IL-10 decreases IFN-\(\alpha\) production (data not shown). The inhibition was maximal at 10 ng/ml IL-10, and increasing amounts of IL-10 did not further enhance the inhibition. Therefore, a concentration of 10 ng/ml IL-10 was chosen for additional experiments. Statistical analysis using nine individual donors indicated that IL-10 treatment resulted in 2.3- and 2-fold reductions in frequencies of IPC responding to HSV and Sendai virus, respectively, as well as 4.6- and 3.3-fold reductions in bulk IFN production, all of which were highly significant (data not shown.) Moreover, inclusion of monoclonal anti-IL-10 Ab (10 \(\mu\)g/ml) totally abrogated the inhibitory effects of exogenously added IL-10 (Fig. 2), confirming the specificity of the inhibitory effects of IL-10 on IFN-\(\alpha\) production.

We have previously determined that, in addition to HSV, other viruses containing both RNA and DNA genomes are able to induce a low frequency IFN-\(\alpha\) response from PBMC (26). We therefore tested the effects of IL-10 on the frequency of NIPC and IFN-\(\alpha\) secreted by PBMC that were stimulated with either NDV or VSV, two RNA viruses. IL-10 inhibited the frequency of NIPC 65% and 55% and bulk IFN-\(\alpha\) 6.5-fold and 3-fold from PBMC stimulated with NDV or VSV, respectively (Fig. 3).

**IL-10 inhibits the level of IFN-\(\alpha\) when added up to 2 h postinduction with HSV or Sendai virus**

To further define the parameters of IL-10 inhibition of IFN-\(\alpha\) production, IL-10 (10 ng/ml) was added to PBMC culture at different times before and after the start of stimulation by either HSV or Sendai virus. As shown in Figure 4, hIL-10 inhibited the frequency of NIPC and the amount of IFN-\(\alpha\) secreted by PBMC stimulated with either HSV or Sendai virus when added either 2 h before or 2 h after addition of viruses. However, when IL-10 was added 4 h after addition of viruses, no inhibition was observed. These results indicate that IL-10 acts during early phases of IFN-\(\alpha\) induction.

**FIGURE 1.** Dose-dependent effects of hIL-10 on the IFN-\(\alpha\) production by PBMC stimulated with HSV or Sendai virus. PBMC from healthy donors were stimulated with either HSV at an MOI of 1 (A and B) or Sendai virus at 16 HAU/ml (C and D) at a final concentration of 1 \(\times\) 10\(^6\) cell/ml in the presence or absence of hIL-10 (from 0.1 ng/ml to 100 ng/ml). Data are expressed as number of responding IPC/10\(^6\) PBMC (A and C) as measured by ELISPOT assay and bulk IFN-\(\alpha\) produced as measured by bioassay (B and D) and is representative of four separate experiments.

**FIGURE 2.** Effect of Ab specific for IL-10 on IFN-\(\alpha\) production by PBMC stimulated with HSV or Sendai virus. PBMC from healthy donors were either unstimulated (control) or stimulated with either HSV at an MOI of 1 (A) or Sendai virus at 16 HAU/ml (B) at a final concentration of 1 \(\times\) 10\(^6\) cells/ml in the presence or absence of hIL-10 (10 ng/ml) or hIL-10 plus anti-IL-10 (10 \(\mu\)g/ml). The frequency of IFN-\(\alpha\)-producing cells and the amount of secreted IFN-\(\alpha\) were determined as described above. Data are representative of three separate experiments.
**IL-10 and IL-4 differentially affect HSV- and Sendai virus-induced IFN-α production**

In addition to IL-10, other cytokines including IL-4 have been shown to be important antiinflammatory agents that suppress proinflammatory activities of macrophages by down-regulating the production of TNF-α, GM-CSF, G-CSF, IL-6, and IL-8 (19, 27–29). We therefore investigated the effect of IL-4 on production of IFN-α by PBMC stimulated with virus. IL-10 but not IL-4 significantly lowered the level of secreted IFN-α and the frequency of NIPC responding to HSV (Table I). Different concentrations of IL-4 (from 0.1 to 10 ng/ml) or up to a 4-h preincubation with this cytokine did not lower the level of IFN-α secreted by PBMC stimulated with HSV (data not shown).

In contrast, IL-10 and IL-4 were each able to reduce the frequency of IFN-α-producing cells and bulk IFN-α secreted by PBMC stimulated with Sendai virus. The data for inhibition of Sendai virus-induced but not HSV-induced IFN-α by IL-4 is in agreement with published data by Gobl et al. (30). No effects on HSV- or Sendai virus-induced IFN-α were observed using TGF-β or IL-13 (data not shown).

**IL-10 inhibits IFN-α1, -α2, and -β at the mRNA level**

Several reports have demonstrated that IL-10 inhibition of cytokine production is accompanied by decreased steady-state levels of mRNA (31, 32). IFN-α1, -α2, and -β mRNA are known to be strongly expressed by NIPC and monocytes in response to viral stimulation (33). We therefore investigated the effects of IL-10 on the mRNA levels for IFN-α1, -α2, and -β in PBMC stimulated by either HSV or Sendai virus. Total RNA was prepared as described in Materials and Methods, reverse transcribed into cDNA, and subsequently amplified with cytokine-specific primers and run on 2% agarose gels with ethidium bromide.

PBMC stimulated with either HSV or Sendai virus resulted in strong IFN-α and -β mRNA expression whereas the addition of IL-10 with virus inhibited these mRNA levels (Fig. 5). The levels of inhibition of IFN-α-specific mRNA correlated well with inhibition of both frequency of IPC and secretion of IFN-α by PBMC stimulated with either HSV and Sendai virus.
to demonstrate the elevation of TNF levels in serum of mice pre-treated with anti-IL-10. Clerici et al. (20) have found significant increases in the levels of IL-10 production by PBMC from HIV+ individuals in whom Th cell functions were severely compromised. In an in vitro study, they demonstrated that neutralization of IL-10 reversed the defect in Ag-specific Th function from HIV-infected individuals. To evaluate the role of endogenous IL-10 in the regulation of IFN-α production, PBMC were stimulated with HSV and Sendai virus in the presence or absence of anti-IL-10, and the frequency of NIPC and bulk IFN generation were determined. Anti-IL-10 at a concentration of 20 μg/ml significantly increased the frequency of NIPC and the amount of IFN-α production by HSV stimulated PBMC whereas isotype control Ab had no effect (Fig. 6A). Anti-IL-10, however, did not have a significant effect on IFN-α production by PBMC induced with Sendai virus (Fig. 6B).

**IL-10 inhibits IFN-α production by both NIPC and monocytes**

Human monocytes are important producers of several cytokines, including IL-1α, IL-6, IL-8, TNF-α, and GM-CSF. Several laboratories, including ours, have shown that, in addition to NIPC, monocytes are the second major population in peripheral blood that produce IFN-α (3, 5). IL-10 has been show to inhibit the production of IL-1α, IL-6, and IL-8 by LPS-activated human monocytes. To determine the effects of IL-10 on the production of IFN-α by highly purified monocytes (CD14+ vs CD14- cells), we separated these populations using a magnetic micro bead separation system. Each population was stimulated with either HSV or Sendai virus in the presence or absence of hIL-10, and the frequency of NIPC and the amount of secreted IFN-α were determined. IL-10 inhibited the frequency of IFN-α-producing cells from both CD14+ and CD14- populations as well as bulk IFN-α secreted by these cells (Table II). The CD14- cells failed to produce IFN-α in response to HSV but responded well to Sendai virus stimulation. Since the monocytes were very pure (97% CD14+), this suggests that the IL-10 may be acting directly on this population to suppress Sendai virus-induced IFN-α production. A direct effect of IL-10 on NIPC in the CD14- population cannot be inferred from this data since the NIPC represent only a small fraction of the CD14- cells. Therefore, studies were undertaken to determine whether peripheral DCS, to which the NIPC belong, express the IL-10 receptor.

**Identification of IL-10 receptors on DCs**

Recently, human and mouse IL-10 receptors have been identified and characterized on several cell lines, including human B cell, T cell, monocytes, promonocytes, and NK cell lines (35–38). Cross-linking studies have indicated a molecular size of 90 to 120 kDa for the hIL-10 receptor, with a few hundred receptors detected per cell with affinities in the 50 to 250 pm range. However, the presence of hIL-10 receptor on human peripheral blood DC has not been reported. We therefore used biotinylated IL-10 followed by avidin-FITC to detect the presence of IL-10 receptor on DC. DC were identified among PBMC by their expression of HLA-DR but failure to express CD3, CD14, CD16, CD19, or CD20 (Fig. 7B). Of the cells identified as DC (region R1 in Fig. 7B), 90% were found to bind IL-10 and therefore express the IL-10 receptor (Fig. 7C). These IL-10 receptor positive cells had a forward and side scatter typical of DC (Fig. 7D).

**Discussion**

Cytokines exert effects within the context of a network in which up- and down-regulation of cytokine production contributes to communication between different cell types. In this report, we have shown that IL-10 inhibits the production of IFN-α by PBMC stimulated with either HSV or Sendai virus. The inhibition of IFN-α by IL-10 was dose dependent and reversed by addition of a neutralizing anti-IL-10 mAb, illustrating the specificity of IL-10 effects. The inhibitory effect of IL-10 was partial, even at saturating concentrations, and ranged from 60% to 85%. IL-10 also inhibited the frequency of NIPC and bulk IFN-α production by PBMC that were...
Oswald et al. have shown the synergistic effect of IL-10 with IL-4. rIL-10 affects an early step of IFN-α production by PBMC (39). The frequency of IFN-α-producing cells and the amount of secreted IFN-α were determined by ELISPOT and IFN bioassay, respectively. Statistical comparisons were related to HSV- or Sendai virus-induced samples. An asterisk indicates a significant effect (by paired t test) of IL-10 or anti-IL-10 on IFN-α production.

stimulated with two other viruses, NDV and VSV. It remains to be determined whether the observed IL-10 effects are direct or mediated via different factors, indirectly. However, the presence of the IL-10 receptor on both monocytes (38) and peripheral blood DCs, the kinetics of inhibition of IFN-α production, and the IL-10 suppression of IFN-α production in response to Sendai virus in highly purified monocytes all suggest that the IL-10 acts directly on the IFN-α-producing cells.

Time course studies demonstrated that hIL-10 added at 4 h and 2 h prior or 2 h poststimulation but not after 4 h postinduction, inhibited the IFN-α production by PBMC stimulated with either HSV or Sendai virus. However, pretreatment of the cells with IL-10 did not enhance the inhibitory effect of IL-10, whereas PBMC stimulated with viruses for 4 h were subsequently resistant to the suppressive effects of IL-10. These results demonstrate that rIL-10 affects an early step of IFN-α induction. These kinetics are consistent with those reported for the inhibitory effects of IL-10 on the production of the proinflammatory cytokines IL-1β, IL-6, and TNF-α by LPS-stimulated PBMC (39).

IL-4 shares many common effects with IL-10 on macrophages. Oswald et al. have shown the synergistic effect of IL-10 with IL-4 and TGF-β to inhibit macrophage cytotoxic activity (19). Hart et al. have shown that IL-10 and IL-4 inhibit the production of IL-1β by synovial fluid macrophages and blood monocytes from patients with inflammatory arthritis (31). These observations prompted us to study whether IL-4, similar to IL-10, has an effect on IFN-α production by PBMC in response to HSV or Sendai virus. In PBMC from healthy donors, we have confirmed the work of Gobl et al., who demonstrated that IFN-α produced in response to Sendai virus but not HSV is inhibited by IL-4 (30). In contrast to the selective inhibition by IL-4, IL-10 significantly inhibited IFN-α produced by PBMC in response to either HSV or Sendai virus, suggesting unique pathways for these inhibitory cytokines. Moreover, neither TGF-β nor IL-13 affected IFN-α production in response to either stimulus. Several laboratories, including our own, have shown the presence of two major IFN-α-producing cell populations among PBMC: the dendritic NIPC and monocytes (5, 6, 26). The early events in the production of IFN-α by NIPC and monocytes in response to HSV and Sendai virus are very different. In addition to Sendai virus inducing a more frequent monocytic IPC population, this IFN-α production is insensitive to inhibition with the lysosomotropic drug, chloroquine, whereas production of IFN-α by NIPC in response to a number of viruses, including HSV, is clearly dependent on endosomal acidification (Ref. 40; and M. Milone and P. Fitzgerald-Bocarsly, manuscript in preparation). Moreover, several lines of evidence have implicated the mannose receptor as important in recognition and probably uptake of virus into endosomal compartments by the NIPC but not by the monocyte. Since IL-10 affects IFN production by both the monocytes and NIPC, it seems likely that this inhibition may affect events downstream of the initial recognition of viruses by the IPC. Our previous observations of signaling events during IFN-α induction have shown that protein kinases C and A and tyrosine kinase activity are involved in the regulation of IFN-α production in response to HSV-1 (33). Therefore, the effect of IL-10 on these kinase activities will be the target of future investigations in our laboratory.

The mechanism by which IL-10 inhibits the production of other cytokines by LPS-activated monocytes has been shown to be via

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Table II. Effect of IL-10 on IFN-α production from PBMC, CD14<sup>+</sup>, and CD14<sup>−</sup> cells stimulated with either HSV or Sendai virus<sup>a</sup>

<table>
<thead>
<tr>
<th>Cells</th>
<th>Virus</th>
<th>IL-10 % CD14&lt;sup&gt;+&lt;/sup&gt;</th>
<th>IPC/10,000 PBMC</th>
<th>IFN (IU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBMC</td>
<td>Unstim.</td>
<td>–</td>
<td>23.1%</td>
<td>0</td>
</tr>
<tr>
<td>HSV</td>
<td>–</td>
<td>8.7</td>
<td>6500</td>
<td></td>
</tr>
<tr>
<td>Sendai</td>
<td>–</td>
<td>161.5</td>
<td>3000</td>
<td></td>
</tr>
<tr>
<td>HSV</td>
<td>+</td>
<td>3.1</td>
<td>1000</td>
<td></td>
</tr>
<tr>
<td>Sendai</td>
<td>+</td>
<td>65.7</td>
<td>1000</td>
<td></td>
</tr>
<tr>
<td>HSV</td>
<td>+</td>
<td>173</td>
<td>3000</td>
<td></td>
</tr>
<tr>
<td>Sendai</td>
<td>+</td>
<td>70.1</td>
<td>600</td>
<td></td>
</tr>
<tr>
<td>CD14&lt;sup&gt;−&lt;/sup&gt;</td>
<td>Unstim.</td>
<td>–</td>
<td>97.6%</td>
<td>0</td>
</tr>
<tr>
<td>HSV</td>
<td>–</td>
<td>10</td>
<td>3000</td>
<td></td>
</tr>
<tr>
<td>Sendai</td>
<td>+</td>
<td>41.3</td>
<td>3000</td>
<td></td>
</tr>
<tr>
<td>HSV</td>
<td>+</td>
<td>1.9</td>
<td>1000</td>
<td></td>
</tr>
<tr>
<td>Sendai</td>
<td>+</td>
<td>15</td>
<td>1000</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> PBMC from healthy donors were stimulated with either HSV at an MOI of 1 or Sendai virus at 16 HAU/ml at a final concentration of 1 × 10<sup>5</sup> cell/ml in the presence or absence of hIL-10 at 10 ng/ml. The frequency of IPC and the amount of secreted IFN-α were determined by ELISPOT and IFN bioassay, respectively, as described in Materials and Methods. Percent CD14<sup>−</sup> was determined by flow cytometry.
degradation of cytokine mRNA and at the transcription level (41). The work by Wang et al. has demonstrated that hIL-10 inhibits expression of IL-1β, IL-6, and TNF-α by acting mainly at the level of cytokine gene transcription (18). Using RT-PCR, we have demonstrated that hIL-10 inhibits the steady state mRNA levels for IFN-α1, -α2 and -β in PBMC induced either with HSV or Sendai virus. However, nuclear run-on transcription assays and stability assays will be required to determine whether hIL-10 inhibits transcription of the IFN-α and IFN-β genes or effects the stability or processing of the mRNA involved. In preliminary studies we have found that IL-10 inhibited the elevated level of NF-κB in monocytes stimulated with Sendai virus (our unpublished results). Since at least the IFN-β promoter contains NF-κB binding sites, this observation clearly warrants further investigation.

Roles for endogenously produced IL-10 in host defense have previously been reported. Endogenous IL-10 was found to protect mice from death in a septic peritonitis model (34). Recent data also suggest that the loss of T helper cell function in HIV+ individuals results, at least in part, from changes in immunoregulatory cytokine profiles. It was observed that IL-10 was up-regulated at the mRNA and protein level by PBMC from HIV+ individuals and that addition of anti-IL-10 reversed the defective Ag-specific Th cell function (20, 42). We investigated the effect of endogenous IL-10 on IFN-α production by PBMC stimulated with HSV and Sendai virus. Neutralizing Ab to IL-10 significantly increased the frequency of NIPC and the amount of IFN-α produced by PBMC stimulated with HSV, which suggests a role for endogenous IL-10 in the regulation of IFN-α production in NIPC. Contrary to its effect on HSV-treated PBMC, anti-IL-10 did not affect the level of IFN-α production by PBMC in response to Sendai virus. We have found that HSV induces IL-10 message and protein at higher levels than those induced by Sendai virus (F. Payvandi and P. Bocarsly, manuscript in preparation). Thus, the differential effects of endogenous IL-10 may be explained by response of PBMC to these viruses and suggest an important role for endogenous IL-10 regulation of NIPC. This induction of IL-10 by virus is also likely to play a major role in down-regulating IL-12 and TNF-α synthesis in addition to IFN-α. In the absence of regulators such as IL-10, secretion of these cytokines might lead to excessive IFN-γ synthesis by NK cells and T cells, which, in turn, could activate macrophages and lead to host pathology. These predictions are supported by the previous observations that mRNA levels for IL-1β, IL-12, TNF-α, and IFN-γ in the lung tissue of Toxoplasma-infected IL-10-deficient animals were severalfold higher than control animals, which suggests a role for endogenous IL-10 in down-regulating cytokine responses to acute intracellular infection, thereby preventing host pathogenesis (43).

Further, inhibition of IFN-α production by IL-10 may play a role in the regulation of the natural immune response since IFN-α has been found to stimulate NK cell activation and IFN-γ production (44–46). IFN-α is proposed to be important for switching Th cells toward of the Th1 cytokine profile (IL-2 and IFN-γ) and has been suggested to enhance cell-mediated immunity (45, 47). On the basis of these observations, it is reasonable to hypothesize that inhibition of IFN-α production by IL-10 could contribute to changing the cytokine profile from Th1 to Th2 and away from cell-mediated immunity.

In conclusion, hIL-10 decreases the number of IFN-α-producing cells and the amount of IFN-α produced by PBMC stimulated by viruses, including HSV, Sendai virus, VSV, and NDV. This inhibition is specific for IL-10, since it can be blocked by neutralizing Ab. IL-10 also inhibits IFN-α by both major populations of IFN-producing cells, monocytes, and NIPC. Furthermore, endogenous IL-10 also plays a role in inhibition of IFN-α produced by PBMC.
Stimulated with HSV, suggesting that IL-10 may be an important physiologic regulator of IFN production by the dendritic NIPC.

Acknowledgments

We gratefully acknowledge Dr. Sidney Smith of Schering-Plough for providing the hIL-10 and anti-IL-10 Ab, Glaxo-Wellcome for providing the bovine anti-IFN-α antiseraum, Drs. Brita Cederblad and Gunnar Alm of Uppsala, Sweden for monoclonal anti-IFN-α Abs, Martin Feuerman for statistical work, Dana Stein and Zenaida Garcia for help with flow cytometry, and Michael Milone for valuable comments.

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