Interferon-α and interferon-γ down-regulate the production of interleukin-8 and ENA-78 in human monocytes

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Abstract: The two chemotactic cytokines interleukin-8 (IL-8) and epithelial neutrophil activating protein 78 (ENA-78) were recently shown to be potent chemoattractants and activators of neutrophil function and to be present in certain inflammatory diseases. We have studied the effects of recombinant and natural interferon-α (IFN-α) and of recombinant interferon gamma (rIFN-γ) on the production of IL-8 and ENA-78 in lipopolysaccharide- and interleukin-1-stimulated human monocytes. Both types of interferons showed a strong, concentration-dependent inhibition of neutrophil-stimulating bioactivity. Similarly, the secretion of IL-8 and ENA-78 was also inhibited by up to 73%. Northern blot experiments demonstrated that IFN-α decreases the steady-state levels of IL-8 and ENA-78 mRNA in monocytes, suggesting that IFN-α as well as IFN-γ may control the expression of neutrophil chemotactic cytokines at the mRNA level. *J. Leukoc. Biol.* 57: 929–935; 1995.

Key Words: *interferon • IL-8 • ENA-78 • chemotactic cytokines • C-X-C chemokines*

INTRODUCTION

Interleukin-8 (IL-8) is one of the best-characterized members of the C-X-C subfamily of the chemotactic cytokines, now called chemokines (for review see refs. 1 and 2). We have isolated and characterized from alveolar type II-like epithelial cells a peptide named epithelial neutrophil activating protein 78 (ENA-78) [3]. ENA-78 is structurally related to IL-8, both sharing the conserved arrangement of four cysteine residues and an ELR motif that is responsible for receptor binding [4]. The two peptides, however, differ markedly in amino acid identity. Both proteins are potent chemoattractants for neutrophils, induce exocytosis and release of intracellular free calcium, and up-regulate surface receptors [3, 5–8]. Monocytes/macrophages appear to be the major producers of IL-8, although a wide variety of cells have been shown to express IL-8 mRNA and to release protein [1]. ENA-78 is produced by epithelial cells, fibroblasts, and monocytes upon induction by interleukin-1 (IL-1) or tumor necrosis factor α [9]. In monocytes, induction of ENA-78 mRNA and protein release differ from those of IL-8. In response to lipopolysaccharide (LPS) stimulation, appearance of ENA-78 mRNA and secretion of protein are delayed by about 12 h, suggesting a different mechanism of induction (S. Schnyder, personal communication).

Chemotactic factors may contribute significantly to the influx of neutrophils into diseased tissue. In fact, elevated levels of IL-8 have been observed in the skin of patients with psoriasis and plantar pustulosis [10–12] and in the synovial fluid of patients with rheumatoid arthritis [13–15]. Furthermore, elevated levels of IL-8 have also been detected in patients with inflammatory lung diseases, such as adult respiratory distress syndrome (ARDS) [16, 17], as well as in patients at risk to develop ARDS [18, 19] and in idiopathic pulmonary fibrosis and sarcoidosis [20]. Very high levels of ENA-78 were seen in patients with rheumatoid arthritis [21].

Leukocyte interferon (IFN-α), fibroblast interferon, and interferon-γ (IFN-γ) have been shown to exert a broad range of antiproliferative bioactivities. IFN-α was predominantly characterized as an antiviral and antiproliferative agent, and IFN-γ was described as a molecule with many immunomodulatory and inflammatory effects on macrophages and other cell types. IFN-γ, which is generally a macrophage activator, was also shown to up-regulate IL-8 in keratinocytes [22], human gastric cancer cell lines [23], and in the human monocytic cell line U937 [24]. IFN-γ also directly induces gene expression of γIP-10 [25] and HuMIG [26], two members of the C-X-C subfamily. In contrast to these results, IFN-γ was shown to down-regulate neutrophil-stimulating activity or IL-8 in blood and synovial fluid mononuclear cells [13, 27] and thymic epithelial cells [28]. Type I interferons were shown to down-regulate IL-8 in fibroblasts [29] and in hematopoietic and bone marrow stromal cells [30]. Because human monocytes are major producers of neutrophil chemotactic cytokines, it is of interest to study possible regulatory effects by IFN-α and IFN-γ on gene expression and secretion of IL-8, ENA-78, and other neutrophil-activating peptides.

Using purified monocytes, we have studied the effects of leukocyte interferon, recombinant IFN-α2a, and IFN-γ on the release of IL-8, ENA-78, and neutrophil-stimulating bioactivity upon induction with LPS and IL-1β. IFN-α and IFN-γ are shown to have potent inhibitory effects on the expression of IL-8 and ENA-78 by down-regulating steady-state levels of mRNA as well as protein secretion from human blood monocytes.

Abbreviations: ARDS, adult respiratory distress syndrome; DEPC, diethylpyrocarbonate; ELISA, enzyme-linked immunosorbent assay; ENA-78, epithelial neutrophil-activating protein 78; fMLP, f-Met-Leu-Phe; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; IFN-α, interferon-α; IL-8, interleukin-8; LPS, lipopolysaccharide; NF-κB, nuclear factor κB; SDS, sodium dodecyl sulfate; TNF-β, tumor necrosis factor β.

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MATERIALS AND METHODS

Reagents

LPS (Escherichia coli 055:B5) was obtained from Difco Laboratories (Detroit, MI) and concanavalin A (Con A) from Boehringer (Mannheim, Germany). IL-1β and IL-8 [31] were kindly provided by Ciba AG (Basel, Switzerland) and Sandoz Research Institute (Vienna, Austria), respectively. IFN-α2a, tumor necrosis factor β (TNF-β), and rIFN-γ were obtained from Hoffmann La Roche AG (Basel, Switzerland). Recombinant ENA-78 was prepared in our laboratory. Leukocyte interferon was prepared from human buffy coat cells by the Cantell procedure [32] with an additional gel permeation chromatography on a 4 × 100 cm Biogel P150 column (Biorad AG, Glattbrugg, Switzerland) in 1 M NaCl, phosphate-buffered saline, pH 7.4. C5a was kindly provided by Dr. C. Dahinden, Department of Clinical Immunology, University of Bern (Bern, Switzerland). Pasteurized human plasma protein solution (PPL, 5%) was obtained from the Swiss Red Cross Laboratory (Bern, Switzerland). Elastase substrate (N-methoxy-succinyl-L-alanyl-L-alanyl-L-prolyl-L-valine-7-amido-4-methylcoumarin) and f-Met-Leu-Phe (fMLP) were obtained from Bachem AG (Bubendorf, Switzerland) and cytochalasin B from Serva GmbH (Heidelberg, Germany). Synthetic oligonucleotides for IL-8, ENA-78, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were prepared on an automatic DNA synthesizer (391; Applied Biosystems, Foster City, CA) and desalted on NAP-10 columns (Pharmacia Biotech AG (Dübendorf, Switzerland). The following antisense oligonucleotide probes were used: IL-8, 5′-GTTGGCGCAGTGTGGTCCACTCTCAATCAC-3′; ENA-78, 5-GCACTGTGGGCCTATGGCGAACACTTGCAGATTACTGATCATT-3′; GAPDH,5′-GGCATCCGGACGTTCTACGG-3′.

Cell culture conditions

Monocytes were isolated from human buffy coats by centrifugation on Ficoll-Paque followed by a one-step discontinuous Percoll gradient [33]. Monocytes (87–95% pure) contained 5–13% lymphocytes and less than 1% neutrophils. Cell were cultivated at 2 × 10^6 cells/ml in minimal essential medium supplemented with 1% PPL and 25 mM HEPES, and stimulated with 100 ng/ml LPS or 10 ng/ml IL-1β in the presence or absence of different concentrations of interferon-α (0.001 to 30,000 U/ml) or interferon-γ (0.001 to 100 U/ml) for 24 h at 37°C, 5% CO_2. Cell-free supernatants were collected and stored at -30°C. Viability of monocytes was tested by trypan blue exclusion.

TABLE 1. Induction of IL-8 and ENA-78 in Human Monocytes by Various Stimuli[a]

Stimulus	IL-8 (ng/ml)	ENA-78 (ng/ml)
Control	12.4 (±4.6)	1.4 (±0.2)
LPS (100 ng/ml)	270 (±51)	21 (±3.2)
TNF-α (100 ng/ml)	119 (±25)	6.8 (±1.9)
TNF-β (100 ng/ml)	35 (±6.8)	3.4 (±0.8)
IL-1β (10 ng/ml)	111 (±7)	10.3 (±1.9)
IL-8 (10 nM)	16 (±3)	2.7 (±1.5)
C5a (0.1 nM)	17.5 (±2.1)	1.6 (±0.3)
fMLP (10 nM)	105 (±11)	12 (±4.5)

[a]Cells at 2 × 10^6 cells/ml were stimulated for 24 h at 37°C, 5% CO_2. IL-8 and ENA-78 were determined in cell-free supernatants by specific ELISA. Mean values (± standard errors) of three independent experiments are shown.

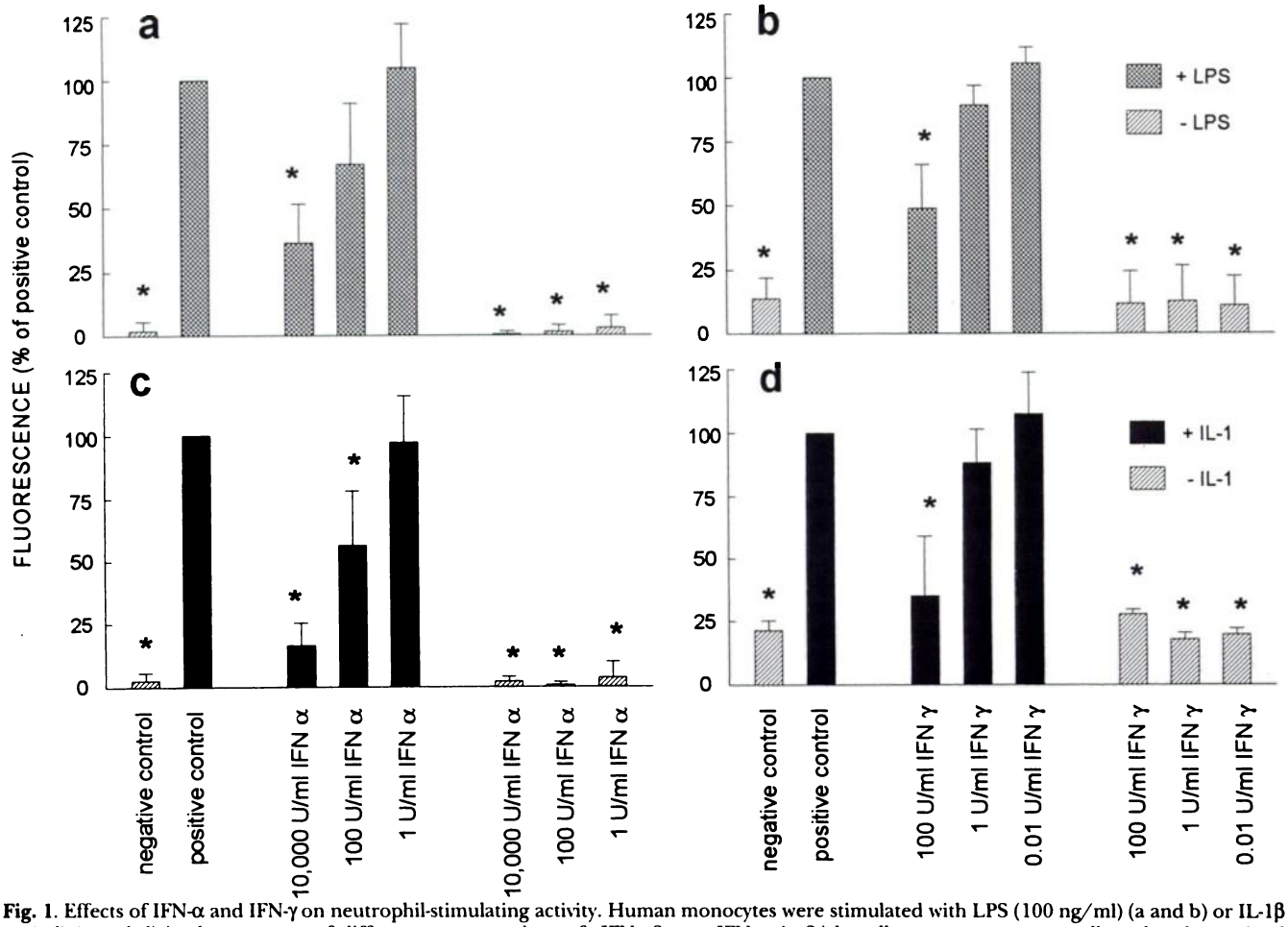

Fig. 1. Effects of IFN-α and IFN-γ on neutrophil-stimulating activity. Human monocytes were stimulated with LPS (100 ng/ml) (a and b) or IL-1β (10 ng/ml) (c and d) in the presence of different concentrations of rIFN-α2a or rIFN-γ. At 24 h, cell supernatants were collected and examined for elastase-releasing activity on cytochalasin B–treated human neutrophils. Data are expressed as % of positive control. Mean values (± standard deviation) of four independent studies are shown. Asteriks indicate a statistically significant difference ($P < .05$) compared with the positive control.

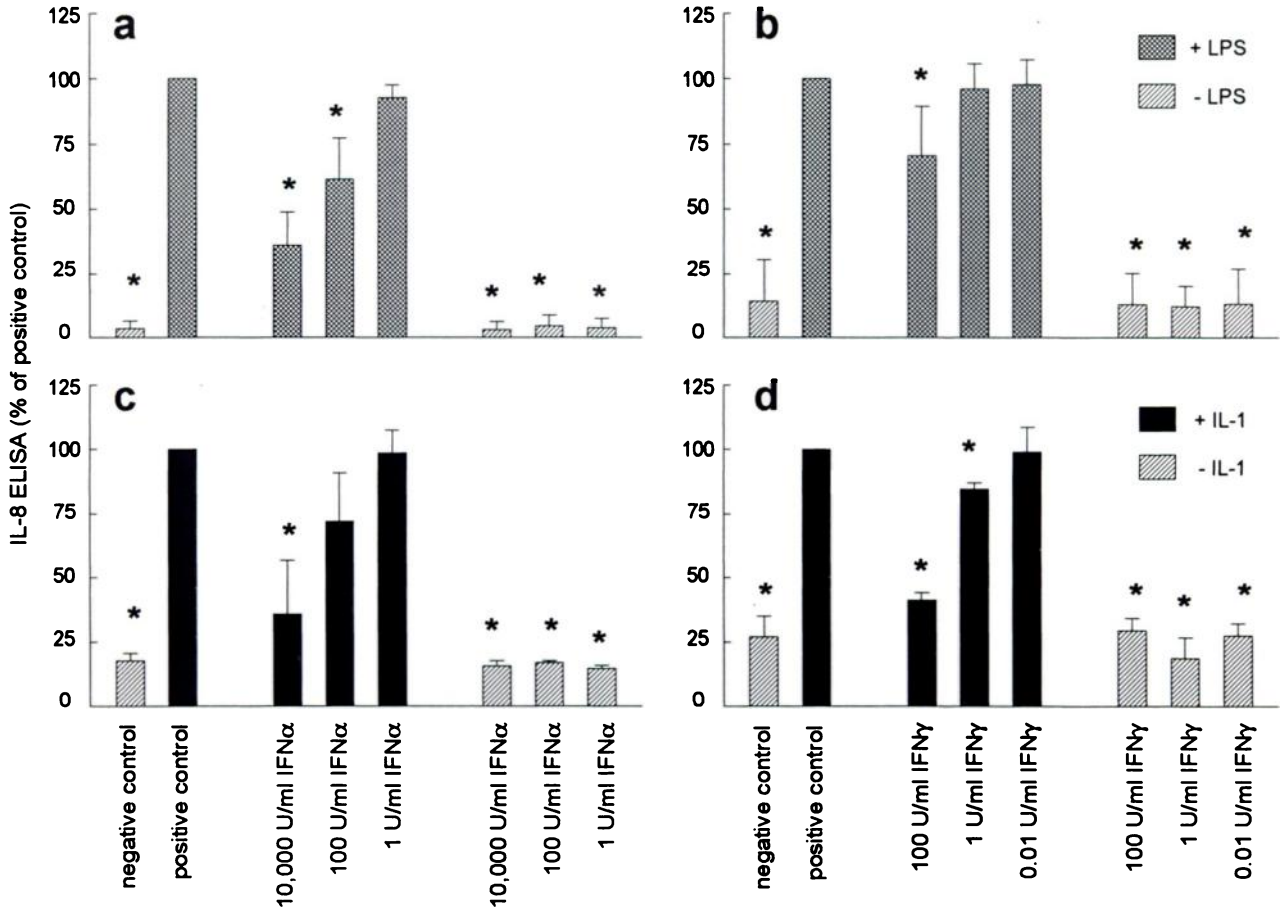

Fig. 2 Effects of IFN-α and IFN-γ on IL-8 induction. Human monocytes were stimulated with LPS (100 ng/ml) (a and b) or IL-1β (10 ng/ml) (c and d) in the presence of different concentrations of rIFN-α2a or rIFN-γ. At 24 h, cell supernatants were collected and examined for IL-8 by specific ELISA. Data are expressed as % of positive control. Mean values (± standard deviation) of four independent studies are shown. Asterisks indicate a statistically significant difference ($P<.05$) compared with the positive control.

Isolation of total mRNA

Total RNA was extracted from monocytes by the sodium dodecyl sulfate (SDS)-guanidinium isothiocyanate method [34]. Briefly, cells were lysed with 3 ml of lysis solution per flask (4 M guanidine isothiocyanate, 0.5% *N*-lauroylsarcosine, 25 mM sodium citrate, and 0.1 M β-mercaptoethanol). After homogenization, 1/10 volume of sodium acetate, pH 4.0, was added and extracted with phenol-chloroform-isoamyl alcohol (250:49:1). RNA was then precipitated with an equal volume of isopropanol, resuspended in the guanidinium isothiocyanate mix, and reprecipitated with isopropanol. The pellet was washed three times with ice-cold 70% ethanol in diethylpyrocarbonate-treated water (DEPC-water) before it was dissolved in DEPC-water.

Northern blot analysis

Equal amounts of RNA (10–15 μg/slot) were separated on a 1% formaldehyde-agarose gel (20 mM 3-morpholinopropanesulfonic acid, pH 6.1, 5 mM sodium acetate, 1 mM EDTA, 1% v/v formaldehyde) and then transferred to a Hybond N membrane (Rahn SA, Dep. Amersham, Zurich, Switzerland) by capillary force in 10× SSC. The RNA was fixed by ultraviolet cross-linking and baking at 120°C for 20–30 min. Membranes were prehybridized for 1 h in 50% formamide, 0.8 M sodium chloride, 50 mM sodium dihydrogen phosphate, pH 7.4, 5 mM EDTA, 0.1% SDS, and 2× Denhardt's solution at 42°C. ^{32}P end labeled antisense oligonucleotides (10^6 cpm/ml) for IL-8, ENA-78, and GAPDH sequences were then added and hybridization continued for 24 h. The filters were washed two times with 2× SSC, 0.1% SDS at room temperature and then at 42°C (IL-8) and at 55°C (ENA-78) for 10 min. Filters were exposed to Kodak X-Omat AR (Eastman Kodak, Rochester, NY) or to Hyperfilm-MP (Rahn SA, Dep. Amersham, Zurich, Switzerland) with intensifying screens at -70°C.

Labeling reaction

The oligonucleotides (20 pmol) were labeled with 50 μCi of [γ^{32}P]ATP (3000 Ci/mmol, NEN Research Prod., Regensdorf, Switzerland) in 20 μl of labeling buffer (70 mM Tris-HCl, pH 7.6, 10 mM sodium chloride, 5 mM dithiothreitol), and 0.5 μl T4-kinase (8000 U/ml, Promega, Madison, WI) at 37°C for 60 min. Free phosphate was removed by Sephadex G50 gelfiltration.

Assays

Established methods were used to assess elastase release activity from cytochalasin B-treated neutrophils [5]. Concentrations of IL-8 and ENA-78 were determined by sandwich enzyme-linked immunosorbent assay (ELISA) as described [9].

RESULTS

Induction of IL-8 and ENA-78 in human monocytes

Various stimuli were tested for their activity to induce IL-8 and ENA-78 in human monocytes (**Table 1**). The highest levels of IL-8 (270 ng/ml) as well as of ENA-78 (21 ng/ml) occurred at 24 h after induction with LPS. Somewhat lower levels of the two chemokines were measured with IL-1β, f-MLP, TNF-β, and TNF-α. No significant levels of IL-8 or ENA-78 were observed upon induction with C5a and IL-8. In agreement with the two ELISA measure-

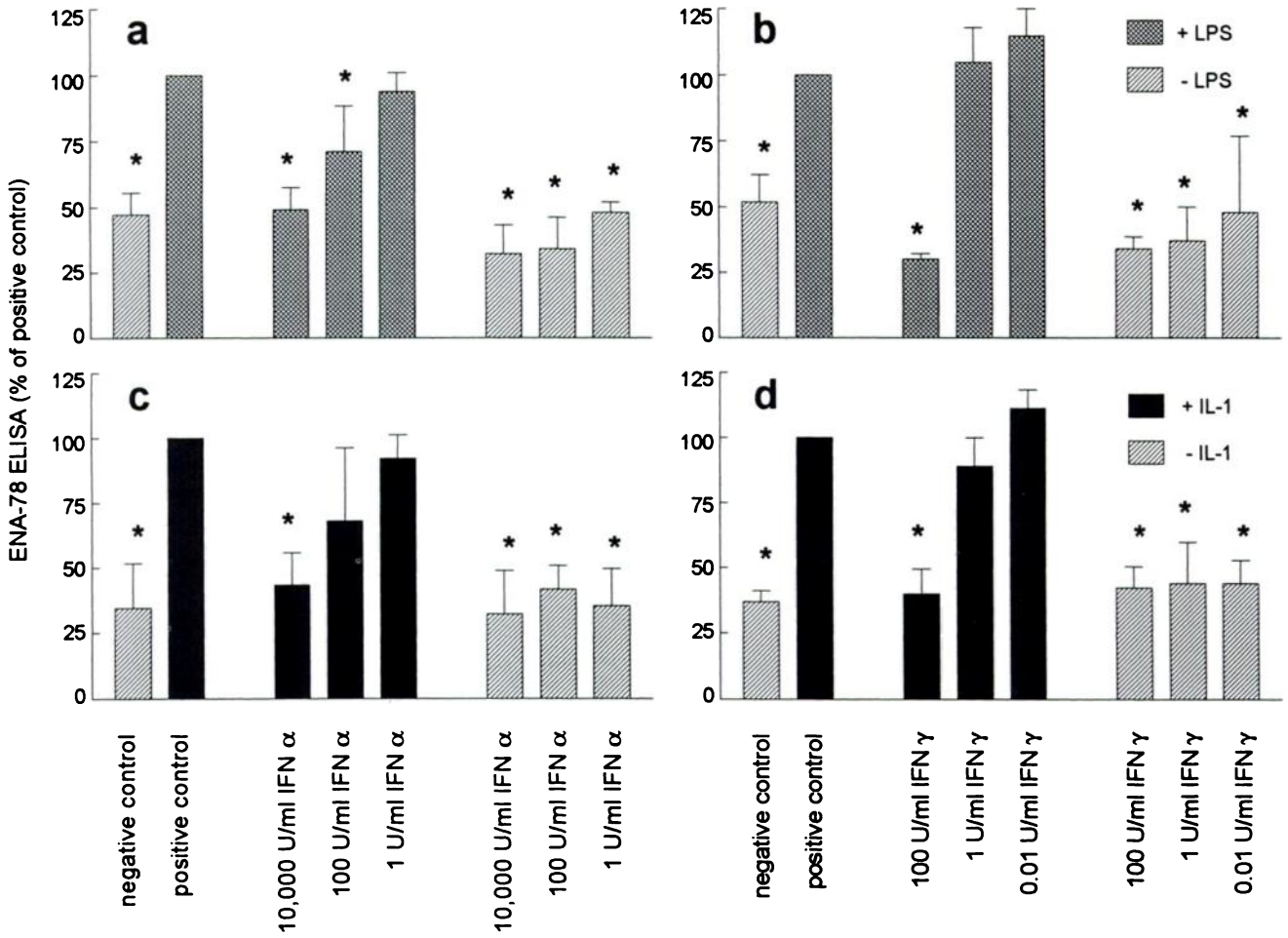

Fig. 3 Effects of IFN-α and IFN-γ on ENA-78 induction. Human monocytes were stimulated with LPS (100 ng/ml) (a and b) or IL-1β (10 ng/ml) (c and d) in the presence of different concentrations of rIFN-α2a or rIFN-γ. At 24 h, cell supernatants were collected and examined for ENA-78 by specific ELISA compared with the positive control. Data are expressed as % of positive control. Mean values (± standard deviation) of four independent studies are shown. Asteriks indicate a statistically significant difference ($P<.05$) compared with the positive control.

ments, no elastase release activity was observed with C5a and IL-8 (not shown). Surprisingly, fMLP at a concentration of 10^{-8} M stimulated monocytes to produce levels of IL-8 and ENA-78 similar to those obtained with IL-1β at 10 ng/ml. In further experiments in which the effect of interferons on IL-8 and ENA-78 expression was studied only IL-1β and LPS were used.

Effects of IFN-α and IFN-γ on neutrophil-stimulating activity

Monocytes were stimulated for 24 h with IL-1β or LPS in the absence or presence of IFN-α (1, 100, 1000, 10,000 U/ml) or IFN-γ (0.01, 1, 10, 100 U/ml). As measured by elastase release from cytochalasin B–treated neutrophils, the induced biological activity was inhibited in a concentration-dependent manner by IFN-α (**Fig. 1a and c**) as well as by IFN-γ (Fig. 1b and d). Maximal inhibition was higher in IL-1β– than in LPS-stimulated monocytes (IFN-α, 83 vs. 63%; IFN-γ, 65 vs. 51%). In comparison to the positive control, statistically significant differences were obtained in all studies with 1000 U/ml (not shown) and 10,000 U/ml IFN-α or 10 U/ml (not shown) and 100 U/ml IFN-γ. IFNs alone did not change basal levels of nonstimulated monocyte cultures.

Effects of IFN-α and IFN-γ on IL-8 and ENA-78 induction

IL-8 release from stimulated monocytes was inhibited in the presence of the highest concentration of IFN-α by 64% in LPS- as well as IL-1β–induced cells (**Fig. 2a and c**). Inhibition by IFN-γ was 30% for LPS and 59% for IL-1β stimulation (Fig. 2b and d). A clear concentration-dependent inhibition of ENA-78 release was observed in either LPS- or IL-1β–induced cells in the presence of IFN-α or IFN-γ (**Fig. 3a–d**). However, in contrast to IL-8, relatively high background levels of ENA-78 were detected, suggesting that a low basal amount of ENA-78 is constitutively produced. The presence of 10,000 U/ml IFN-α was very effective in suppressing ENA-78 release to almost background levels. In comparison to the positive control, statistically significant differences were obtained in all studies with 1000 U/ml (not shown) and 10,000 U/ml IFN-α or 10 U/ml (not shown) and 100 U/ml IFN-γ.

Comparison of recombinant IFN-α2a and natural leukocyte interferon on IL-8 production in human monocytes

Leukocyte interferon purified from human buffy coat cells was compared with recombinant IFN-α2a in its potency to inhibit IL-8 production. Both types of alpha in-

TABLE 2. Comparison of Recombinant IFN-α2a and Leukocyte Interferon on IL-8 production in human monocytes[a]

Inducer		% of positive control		
		None	IL-1β	LPS
IFN–α2a (U/ml)	0	15 (± 0.3)	100	100
	1	15 (± 0.5)	96 (± 2.7)	93 (± 2.6)
	100	14 (± 0.7)	65 (± 3.7)	63 (± 10)
	1000	14 (± 1.2)	49 (± 2.5)	45 (± 6.5)
	10,000	16 (± 2.1)	31 (± 3.2)	34 (± 6.4)
Leukocyte IFN (U/ml)	0	13 (± 7.0)	100	100
	1	16 (± 9.6)	91 (± 6.5)	108 (± 3.0)
	100	12 (± 6.0)	77 (± 11)	92 (± 13.0)
	1000	11 (± 6.2)	56 (± 9.0)	60 (± 4.5)
	10,000	10 (± 4.4)	34 (± 9.1)	45 (± 7.1)

[a]Monocytes at 2×10^6 cells/ml were cultivated for 24 h in the absence or presence of IL-1β (10 ng/ml) or LPS (100 ng/ml) and different concentrations of rIFN-α2a and leukocyte interferon. IL-8 was determined by specific ELISA. Data are expressed as % of positive control. Mean values (± standard errors) of three independent experiments are shown.

terferons had virtually identical inhibitory effects as detected by functional assays (elastase release activity) and by IL-8 ELISA (**Table 2**). As already seen in previous experiments (Figs. 1 and 2), the suppressing effects on IL-1β–stimulated monocytes were more pronounced (at 10,000 U/ml, 69% for IFN-α2a and 66% for leukocyte interferon) than in LPS-stimulated cells (at 10,000 U/ml, 66% for IFN-α2a and 55% for leukocyte interferon).

Effects of IFN-α on IL-8 and ENA-78 mRNA steady-state levels

The effects of IFN-α on IL-8 and ENA-78 mRNA steady-state levels were determined by Northern blot analysis using antisense oligonucleotide probes for the two transcripts. In LPS- as well as in IL-1β–stimulated monocytes, a clear reduction of IL-8 and ENA-78 mRNA was observed with 100 and 10,000 U/ml IFN-α (**Fig. 4**). In the absence of LPS and IL-1β, low levels of both mRNAs were detected in cells incubated at 37°C for 24 h. No IL-8 or ENA-78 mRNA was detected in Northern blots carried out with monocyte mRNA isolated from freshly isolated cells, suggesting that a low-level induction occurred during adherence and cultivation of the cells. A dose-dependent inhibition of mRNA production for GROα was also observed (data not shown).

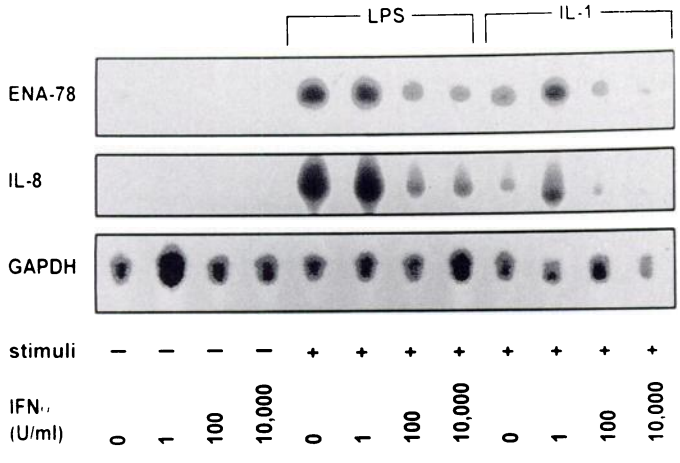

Fig. 4. Effects of IFN-α and IFN-γ on IL-8 and ENA-78 mRNA steady-state levels. Human monocytes were stimulated with LPS (100 ng/ml) or IL-1β (10 ng/ml) in the presence of different concentrations of rIFN–α2a. At 24 h, total RNA was extracted and analyzed by Northern blot analysis. RNA was visualized by hybridization with ^{32}P-labeled antisense oligonucleotides for ENA-78 and IL-8. Control hybridization with GAPDH is shown.

DISCUSSION

In this study we have analyzed the effects of different interferons on IL-1β– or LPS-induced production of IL-8 and ENA-78 by human monocytes. Type I IFN, recombinant IFN-α2a, and natural leukocyte IFN consisting of about 15 different species had suppressing effects similar to those of IFN-γ on the production of neutrophil chemotactic cytokines. At highest concentrations used, IFN suppressed the release of IL-8 and ENA-78 by up to 73%. A similar dose-dependent effect was observed for ENA-78, IL-8, and GROα (data not shown). The inhibition by IFN was more pronounced in IL-1β– than in LPS-stimulated monocytes. Measurement of steady-state mRNA levels suggests that down-regulation of IL-8 and ENA-78 occurs primarily at the transcriptional level.

The pleiotropic nature of IFN has been known for some time. IFN-γ enhances the expression of various functional properties in monocytes, such as nitric oxide production [35] and enhanced killing of intracellular bacteria [36]. In addition, IFNs were shown to enhance the expression of inflammation-associated cytokines, such as IL-6, TNF, and IL-1 [37–39]. Up-regulation of TNF and IL-1 by IFN-γ as well as posttranscriptional mechanisms might

contribute to the up-regulation of IL-8 in certain cell types [22–24].

In agreement with studies of IL-8 [13, 27], our results show that both types of interferons, type I (IFN-α2a and leukocyte IFN) and IFN-γ, strongly inhibit the production of the most abundant neutrophil-stimulating peptides produced by normal blood monocytes. The fact that IFNs strongly suppress IL-8, GRO, and ENA-78 production in human monocytes, in spite of IL-1 and LPS up-regulation, predicts a specific mechanism common for all three chemokines. However, the inhibiting effects of IFN appear to be cell specific. Using human alveolar type II epithelial cells, no change in the expression of IL-8 or ENA-78 has been observed with IFN (S. Schnyder and A. Walz, unpublished observations).

It was demonstrated that induction of IL-8 transcription by IL-1 and TNF-α is dependent on nuclear factor kB (NF-κB)-like binding sites in a fibrosarcoma cell line [40] and on binding sites for transcription factors NF-IL-6-like and NF-κB in the human monocytic cell line U937 [41]. Observations of a gastric cancer and hepatoma cell line suggest the involvement of different *cis*-regulatory enhancer elements for activation of IL-8 by IL-1 or TNF-α [23], suggesting that, depending on the cell type, different sets of nuclear factors are involved in IL-8 induction. There is evidence that IFNs may interact with chemokine gene expression at different levels. While IFN-γ-selective induction of *mig* appears to be dependent on regulatory elements different from NF-κB [42], transcriptional as well as posttranscriptional mechanisms have been described for up-regulation of TNF-α-induced IL-8 expression by IFN-γ [23, 24]. In contrast to IL-8 up-regulation, there are few data available on the nature of IFN suppression of IL-8 or other C-X-C chemokines. Our results obtained by ELISA and by determination of the steady-state mRNA levels predict a similar mechanism for down-regulation of IL-8, ENA-78, and GROα by both IFN-α and IFN-γ. The suppression of steady-state mRNA levels observed suggests that IFNs either decrease the rate of transcription or negatively affect mRNA stability. In human fibroblasts it was demonstrated that inhibition of IL-8 expression by IFN-β is not diminished by inhibitors of protein synthesis, suggesting that de novo protein synthesis is not needed for IL-8 suppression [29]. The molecular mechanism of down-regulation remains unclear and will have to be elucidated.

Transcription of HuMIG and γIP-10, two members of the C-X-C superfamily, was shown to be induced by IFN-γ but not by IFN-α or LPS [26]. In spite of its structural homology to IL-8, γIP-10 was shown to be inactive as a neutrophil activator [43], most likely because of a missing ELR motif in the amino terminus of the protein [44]. However, γIP-10 was shown to function as a chemoattractant for human monocytes and T lymphocytes [45]. Interestingly, MCP-1 and RANTES, two monocyte chemoattractants from the C-C supergene family, were also shown to be up-regulated by IFN-γ [46, 47]. Previous in vivo studies have shown that at early stages of an inflammation, neutrophils predominate and are then gradually replaced by monocytes [48]. IFNs might regulate this switch by suppressing the release of neutrophil chemoattractants in favor of factors attracting monocytes and lymphocytes. The fact that IFNs suppress induction by exogenous (LPS) as well as endogenous (IL-1 and TNF-α) inducers supports their possible role in vivo. In fact, it was demonstrated that the footpad swelling caused by LPS injection in mice was suppressed up to 50% by coinjection of either IFN-γ or IFN-α [49]. Monoclonal antibodies against MuIFN-γ were shown to modify profoundly the observed swelling response, suggesting that endogenous IFN-γ may play a role in this model of local inflammation. Type I interferons, which are readily induced in monocytes and fibroblasts by viruses, may therefore play an important role in modulation of virally induced inflammatory processes.

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