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Effect of interferon-alpha on neutrophil functions

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SUMMARY

Incubation of neutrophils with interferon-alpha (IFN-α) for 5 min and subsequent stimulation with the calcium ionophore A23187 enhanced the production of oxygen radicals, while a suppression was obtained with FMLP-stimulated cells. Similar data were observed for the direct G-protein activator sodium fluoride (NaF). Incubation of the cells with IFN-α and subsequent stimulation with FMLP (in the presence of cytochalasin b) reduced the generation of the chemotactic active leukotriene B_4 (LTB_4). The metabolism of LTB_4 was significantly inhibited. IFN-α decreased the specific binding sites for LTB_4 and increased the number of binding sites for FMLP. The GTPase activity as a parameter for the activation of G-proteins was enhanced by IFN-α. Preincubation of the cells with IFN-α and subsequent stimulation with NaF increased the GTPase activity synergistically, whereas co-incubation of IFN-α with FMLP showed additive effects. Our results clearly demonstrate the modulatory effects of IFN-α on granulocyte functions with regard to the receptor-mediated signal transduction.

INTRODUCTION

Human polymorphonuclear granulocytes (PMN) play a major role in host defence against invading micro-organisms. In this regard, the transformation of the arachidonic acid via the 5-lipo-oxygenase pathway is followed by the production and release of pro-inflammatory mediators (e.g. leukotrienes), inducing the classical signs of inflammation, allergic reactions and activation of different cell types.[1-3] Among them leukotriene B_4 (LTB_4) is strongly chemotactic and leads to lysosomal enzyme release. Commonly, PMN are activated as a result of an interaction between ligands and membrane receptors by receptor-dependent agonists such as LTB_4 or the synthetic analogue of bacterial peptides, the formyl-methionyl-leucyl-phenylalanine (FMLP). This interaction is mediated by nucleotide regulatory proteins (G-proteins) and is followed by processes of transmembrane signalling, e.g. as an increase in the intracellular Ca^{2+} concentration, translocation of protein kinase C, and phosphoinosite turnover.[4-6] The consequence of these events leads to the activation and phosphorylation of a variety of enzymes initiating the cell-specific response, e.g. oxygen radical production, generation of lipid mediators and increased formation of actin filaments.[7,8] Recently, it has been shown that fluoride ions (F^-) activate G-proteins coupled to adenylate cyclase or to phospholipase C without occupying the receptor.[9]

Interferon-alpha (IFN-α) is produced by leucocytes in response to viral infections and to multiple stimulating agents.[10-12] Besides inducing the synthesis of enzymes that inhibit virus multiplication, IFN-α exerts a variety of cellular functions, such as inhibition of cell growth, enzyme activities, differentiation and immune functions.[13-15] More recently, attention has been focused on the anti-proliferative and immunomodulatory activities of interferons to treat human cancers.[16,17] IFN-α induces the differentiation of myeloid precursor cells into mature granulocytes.[18,19]

Since other differentiating cytokines (e.g. GM-CSF) modulate the function of mature granulocytes[11] and since IFN-α is commonly used for the treatment of patients with chronic myeloid leukaemia (CML), it was the purpose of the study to investigate the effects of IFN-α on human neutrophils of normal donors. In this regard oxygen radical production, the expression of receptor sites for FMLP and LTB_4 and the generation of LTB_4 were analysed. Since these events are mediated by membrane biochemical events, defined components of the signal transduction pathway were studied.

Abbreviations: FMLP, formyl-methionyl-leucyl-phenylalanine; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; HPLC, high-pressure liquid chromatography; IFN-α, interferon-alpha; IFN-γ, interferon-gamma; LTA_4, leukotriene A_4; LTB_4, leukotriene B_4; NaF, sodium fluoride; PBS, phosphate-buffered saline; PMN, polymorphonuclear granulocyte.

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MATERIALS AND METHODS

Materials

The reagents were from the following sources. Ficoll 400 was obtained from Pharmacia, Uppsala, Sweden; Macrodex (6%) from Knoll, Ludwigshafen, Germany; sodium metrizoate solu-

tion (75%) from Nycomed, Oslo, Norway; calcium-ionophore A23187, zymosan, cytochalasin b, arachidonic acid, heparin, FMLP, NaF, adenylylimidodiphosphate [App(NH)p], ATP, oubain, EDTA, EGTA, leupeptin, dithiothreitol, charcoal, sucrose, dextran, FITC phalloidin and lysophosphatidyl choline from Sigma, Deisenhofen, Germany. IFN-α was from Essex Pharma, München, Germany. Unlabelled LTB_4, 20-OH-LTB_4 and 20-COOH-LTB_4 were kindly provided by Merck-Frosst, Pointe Claire, Canada. ^{32}P-GTP (specific activity 185 TBq/mmol), [^{3}H]FMLP (specific activity 1·48–2·22 TBq/mmol) and [^{3}H]LTB_4 (specific activity 0·74–2·22 TBq/mmol) were supplied by New England Nuclear, Dreieich, Germany. Methanol and chloroform (analytical grade) were from Riedel de Häen (Seelze, Germany). All other chemicals were from Merck, Darmstadt, Germany.

Preparation of the cells

Human PMN were isolated from 200 ml of heparinized blood (15 U/ml) of healthy donors and separated on a Ficoll-metrizoate gradient followed by dextran sedimentation, as described previously.[21] The erythrocytes were lysed by exposing the cell suspension to hypotonic conditions. This method revealed more than 97% pure PMN. Less than 1% of the total cells was platelets. The PMN were suspended to a final concentration of 2×10^7 cells per ml in PBS.

Preparation of membrane fractions

Isolation of the membrane fractions were carried out as previously described.[22] Stimulated neutrophils (4×10^7 cells/0·5 ml) and purified neutrophils (1×10^8 cells/ml) were resuspended in Tris-buffer (0·05 M, pH 7·5) supplemented with sucrose (0·25 M). EDTA (1 mM), EGTA (1 mM), dithiothreitol (1 mM) and leupeptin (100 μg/ml) were added and cell disruption was carried out by sonication in three periods over 10 seconds (energy output 40 W, sonifier 250 W; Branson, Danbury, CT). Light microscopy revealed complete cell breakage. The granules, nuclei and unbroken cells were removed by centrifugation at 10,000 *g* for 20 min (J2-21, rotor JA-20; Beckman, Palo Alto, CA). The crude membrane fractions were collected from the supernatant fraction by centrifugation at 100,000 *g* for 60 min (Beckman-centrifuge L8-70, rotor SW60TI). The protein content was assayed as described previously.[23]

Oxygen radical production

The production of oxygen radicals was measured as described previously.[24] Briefly, 1×10^6 cells (final volume, 300 μl) were incubated for 10 min at 37° in the presence of luminol (25 μM) and Ca^{2+}/Mg^{2+} (1/0.5 mM). Subsequently, the various stimuli were added and the luminol-enhanced photo-emission was monitored for an additional 20 min (Lumacounter M2080; Lumac, Schaesburg, The Netherlands). The bacterial peptide FMLP, the direct G-protein activator NaF, the calcium ionophore A23187, as an activator of the protein kinase C, and opsonized zymosan (part of the yeast cell wall) were used as stimuli.

Conversion of LTB_4

The conversion of LTB_4 (3·7 kBq = 2·8 pmol) was performed in PBS buffer and in the presence of Ca^{2+}/Mg^{2+} (1/0·5 mM). The cells were incubated with [^{3}H]LTB_4 for the appropriate time. After the incubation the reaction was stopped by addition of methanol/acetonitril (1 : 1, v/v). After storage for 24 hr at −20° the samples were centrifuged (1200 *g*, 5 min) and the supernatant evaporated under a stream of nitrogen. The residue was resuspended in 150 μl methanol/water (30/70) and analysed by HPLC.

Analysis of LTB_4 metabolites

The ^{3}H-labelled metabolites of LTB_4 were separated on a reversed phase column (Nucleosil 5C18, Macherey & Nagel, Düren, Germany; 4 × 250 mm, Bischoff, Leonberg, Germany) using a gradient system. Solvent A consisted of methanol/acetonitril/water 20/20/60 (v/v) to which 0·1 g EDTA/1 H_2O and 0·75 g K_2HPO_4 were added. The pH was titrated with H_3PO_4 to 5·0. Solvent B consisted of 30/30/40 methanol/acetonitril/H_2O (v/v), pH set to 5·0. The flow rate was set at 0·9 ml/min at 40°. A linear gradient was chosen starting at $t = 0$ (A 100%) to 20 min (B 100%). After $t = 30$ min the eluent was reset to A 100%. The technical equipment was purchased from LDC Milton Roy (HPLC gradient pump CM4000, Milton Roy, Hasselroth, Germany) and Spectraphysics (autosampler 8780 XR, Darmstadt, Germany). The radioactivity was monitored by a HPLC radiomonitor (Ramona, Raytest, Essen, Germany). Prior to the entrance into the monitor (containing a 500 μl cell), scintillator fluid (Rotiszint 2211, Roth, Karlsruhe, Germany) was mixed to the eluent in a ratio of 0·9/1·25 v/v. Peak identification and quantification as well as control of the HPLC unit was performed with the Nelson Analytical Software (chromatography system 2600, Semrau, Spockhövel, Germany).

Generation of LTB_4

LTB_4 generation was determined by incubation of PMN (1×10^7) with IFN-α (10–1000 U) for 5 min in the presence of Ca^{2+}/Mg^{2+} (1/0·5 mM) and subsequently the respective stimuli were added. After 20 min the incubation was stopped by addition of 2 ml methanol/acetonitril (1 : 1, v/v) and the samples were stored at −70°.

After centrifugation at 1900 *g* for 15 min (Cryofuge 6-4, Heraeus Christ, Osterode, Germany) the supernatants were evaporated to dryness by lyophilization (Modulyo, Edwards-Kniese, Marburg, Germany). The residues were dissolved in 600 μl of methanol/water (30:70, v/v) and 200 μl were analysed by reversed-phase HPLC. The column (4·6 × 200 mm) was packed with Nucleosil C18 (particle size 5 μm; Machery-Nagel, Düren, Germany). HPLC equipment consisted of a CM4000 pump, a SM4000 detector (both Laboratory Data control/Milton Roy, Hasselroth, Germany), and an automatic sample injector (WISP 710B, Waters, Eschborn, Germany). Peak integration was carried out by using a chromatographic software (system 2600, Nelson Analytical, Cupertino, CA). LTB_4 was analysed using a mobile phase consisting of methanol/water/acetonitrile/phosphoric acid (48:24:28:0·03, v/v), including 0·04% EDTA and 0·15% K_2HPO_4, pH 5·0. The flow rate was maintained at 0·9 ml/min; LTB_4 was detected at 270 nm. The overall recoveries ranged between 81% and 86%.

LTB_4 and FMLP receptor assay

Neutrophils were preincubated with IFN-α (10–1000 U/ml) for 5 min at 37° in the presence of Ca^{2+}/Mg^{2+} (1/0·5 mM). After the preincubation period the cells were washed by short term centrifugation, resuspended in calcium-free buffer and placed on ice. The binding assays were carried out using 96-well

filtration plates with 5 μm pore-size polyvinylidene fluoride membranes (Millipore, Eschborn, Germany).

Specific binding of LTB_4. Each well contained [^{3}H]LTB_4 (1·6 nM) and 40 μg of bovine serum albumin. Non-specific binding was determined in the presence of 220 nM of unlabelled LTB_4.

Specific binding of FMLP. The total concentration of [^{3}H]FMLP/FMLP into each well was 50 pmol; the non-specific binding component was determined in the presence of a 500-fold excess of unlabelled FMLP.

Neutrophils at a concentration of 4×10^6 cells/200 μl PBS were added; after 45 min at 4° the incubation was terminated by rapid filtration using the Millititer vacuum holder. The filters were transferred to scintillation vials; methanol (0·5 ml) and 4 ml of Rotiscint 2211 (Roth, Karlsruhe, Germany) were added and the radioactivity was measured by liquid scintillation counting (Rack beta 1209, LKB, Turku, Finland). All experiments were carried out in triplicate. Specific binding was expressed as total binding minus non-specific binding.

Scatchard plot analysis was carried out with various concentrations of [^{3}H]LTB_4 ([^{3}H]fMLP). The binding data were analysed as described previously.

Determination of GTPase activity

GTPase activity was measured as described elsewhere.[25] The membrane fraction (10 μg protein) was incubated in 20 mM Tris-buffer (pH 7·5) containing 150 mM NaCl, 5 mM $MgCl_2$, 0·1 mM EGTA, 1·14 mM ATP, 0·5 mM App(NH)p, 0·25 mM ouabain and 0·5 μM ^{32}P-gamma-GTP. After incubation for 60 min at room temperature in the absence or presence of the respective stimuli, the reaction was stopped by addition of activated charcoal in 20 mM phosphate buffer (pH 7·5). The samples were then centrifuged at 9600 *g* for 8 min and the ^{32}P-phosphate content of the supernatant fraction was assessed.

Measurement of actin polymerization

Purified PMN (5×10^6 cells/500 μl) were incubated with the respective stimuli for the indicated time periods at 37°. The reaction was stopped by addition of 100 μl of formaldehyde. Cells were fixed for 15 min at room temperature before staining for F-actin, as shown previously.[26] FITC-labelled phalloidin (0·6 μg) and lysophosphatidyl choline (100 μg) were added to the fixed cell suspensions, which were mixed and incubated for further 30 min at room temperature in the dark. The cell suspensions were then centrifuged at 9600 *g* for 1 min, the supernatants removed and the pellets resuspended in 500 μl PBS buffer.

Flow cytometry

Flow cytometry was performed on the FACStar plus (Becton-Dickinson, Heidelberg, Germany). Cells were excited with an argon ion laser at 488 nm and the emission was recorded at 520 nm. Gating was performed on the forward angle and right angle light scatter to exclude contaminating platelets and lymphocytes. A minimum of 10,000 cells was analysed per individual experiment.

Enzyme release

IFN-α was used within a concentration range of 10 and 1000 U/ml. The release of lysozyme, glucuronidase and lactate dehydrogenase was determined after 5 and 30 min of incubation as a percentage of total activity of the sonicated cell fractions.

RESULTS

Human neutrophils (1×10^7) were preincubated with IFN-α (10–1000 U/ml) or PBS buffer for 5 min and stimulated subsequently with FMLP (10^{-5} M), NaF (20 mM), the Ca-ionophore A23187 ($6{\cdot}8 \times 10^{-6}$ μM), opsonized zymosan (2 mg) or PBS buffer for an additional 20 min.

IFN-α itself had no effects on oxygen radical production (data not shown). Pretreatment of the cells with various concentrations of IFN-α and subsequent stimulation with the Ca-ionophore A23187 resulted in a dose-dependent increase of oxygen radical production; a maximal response was obtained after 2 min of incubation (Fig. 1a). The oxygen radical production induced by opsonized zymosan was not modulated by IFN-α (data not shown). In contrast, the chemiluminescence response to NaF was inhibited after preincubation of the cells with IFN-α in a dose-dependent manner (Fig. 1b) and completely blocked at a concentration of 1000 U/ml of IFN-α. IFN-α at 10 U/ml caused a maximal inhibition of the FMLP response (Fig. 1c).

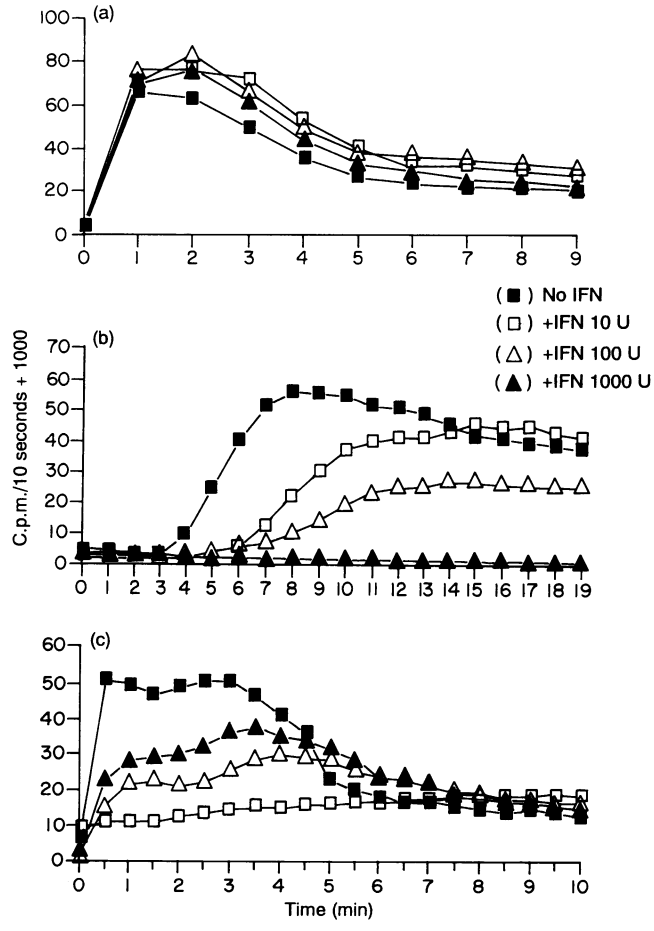

Figure 1. Chemiluminescence response in human neutrophils (1×10^6 cells) after pretreatment with IFN-α (10–1000 U) and subsequent stimulation with (a) the Ca-ionophore, (b) NaF and (c) FMLP in the presence of Ca^{2+}/Mg^{2+} (1/0·5 mM). Results of one of three typical experiments from three different donors are shown.

Table 1. Generation of LTB_4

	LTB_4 and omega-oxidated products (ng)			
	PBS	IFN-α (10 U)	IFN-α (100 U)	IFN-α (1000 U)
PBS	0	0	0	0
Arachidonic acid/ Ca-ionophore A23187	326±33	320±18	313±54	382±92
Opsonized zymosan	18±14	21±15	35±23	36±27
Cytochalasin b/FMLP	59±14	34±22	34±7*	38±26

Leukotriene synthesis was determined by incubation of PMN (1×10^7) with IFN-α (10–1000 U) for 5 min in the presence of Ca^{2+}/Mg^{2+} (1/0·5 mM) and subsequent stimulation with (a) PBS buffer, (b) arachidonic acid (60 μM)/the Ca-ionophore 23187 ($6{\cdot}8 \times 10^{-6}$ μM), (c) opsonized zymosan (2 mg/1×10^7 cells) or (d) cytochalasin b (5×10^{-6} M)/FMLP over 20 min. The data represent means±SD ($n=3$; * $P<0{\cdot}05$).

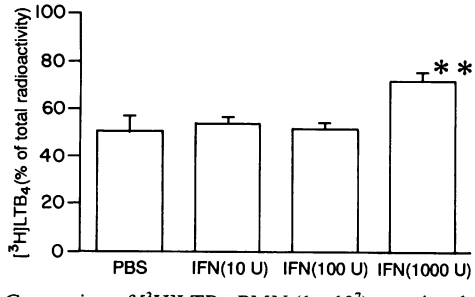

Figure 2. Conversion of $[^3H]LTB_4$. PMN (1×10^7) were incubated with IFN-α (10–1000 U) for 5 min in the presence of Ca^{2+}/Mg^{2+} (1/0·5 mM), subsequently stimulated with LTB_4 (2·8 pmol), and the metabolism of LTB_4 was carried out over 20 min. The values are means±SD ($n=3$; * $P<0{\cdot}05$).

Effect of IFN-α on the generation and metabolism of LTB_4

IFN-α (10–1000 U/ml) itself did not cause LTB_4 generation (20 min incubation at 37°). To study the effect of IFN-α on the release of LTB_4, PMN (1×10^7) were pretreated with IFN-α (10–1000 U/ml) in the presence of Ca^{2+}/Mg^{2+} (1/0·5 mM) and subsequently incubated with various stimuli (Table 1). IFN-α inhibited the generation of LTB_4 after subsequent stimulation with FMLP (10^{-5} in the presence of cytochalasin b (5×10^{-6} M), whereas the LTB_4-inducing activity of other stimuli, such as opsonized zymosan, arachidonic acid or the Ca-ionophore A23187, was not affected significantly by IFN-α pretreatment.

Further studies were directed to the question whether the metabolism of $[^3H]LTB_4$ was modulated by IFN-α. Therefore, PMN (1×10^7) were incubated with IFN-α (10–1000 U/ml) for 5 min in the presence of Ca^{2+}/Mg^{2+} (1/0·5 mM) and subsequently incubated for 20 min with $[^3H]LTB_4$ (0·37 kBq=2·8 pmol).

As is apparent from Fig. 2, the concentration of 1000 U/ml of IFN-α significantly inhibited the metabolism of LTB_4: only 25% of LTB_4 was converted into omega-oxidated products (20-OH- and 20-COOH LTB_4) in contrast to the buffer control (50%

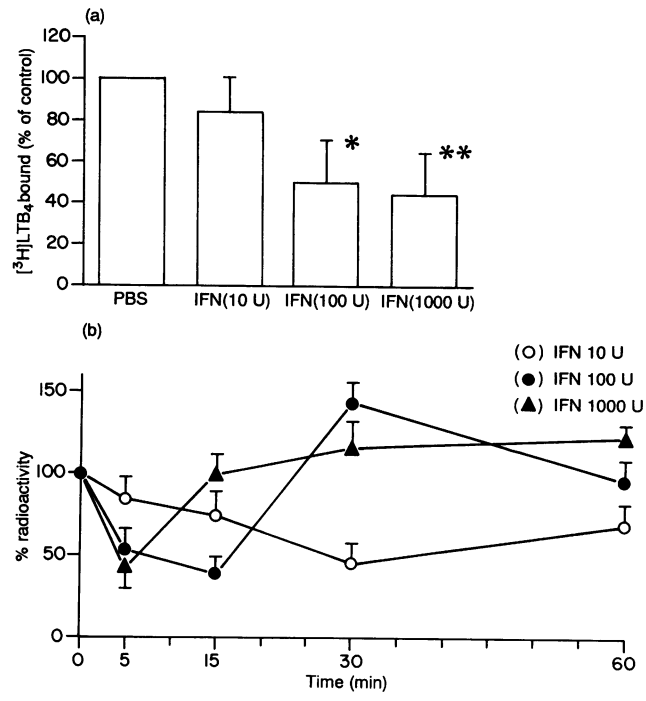

Figure 3. Dose- and time-dependent effects of IFN-α on $[^3H]LTB_4$ binding to human neutrophils. Purified human PMN were preincubated with various concentrations of IFN-α (10–1000 U) for 5 min (a) or with IFN-α (10–1000 U) for the indicated time periods at 37° (b). Cells preincubated with PBS, which was defined as 100%, served as control. Each value represents the mean±SD of four individual experiments (* $P<0{\cdot}05$; ** $P<0{\cdot}01$).

Table 2. Scatchard plot analysis for the binding of ^3H-LTB_4 and ^3H-FMLP

	Scatchard plot analysis			
	High-affinity receptor population		Low-affinity receptor population	
LTB_4	Binding sites	K_d (nM)	Binding sites	K_d (nM)
PBS	814±102	0·2	5600±700	3·7
IFN-α (10^3 U)	254±31	0·33	3818±450	7·5
FMLP				
PBS	1003±150	77·0	25,585±3838	377·0
IFN-α (10^2 U)	24,080±2590		40·0	

Scatchard plot analysis for the binding of $[^3H]LTB_4$ and $[^3H]$ FMLP. PMN were incubated with IFN-α (1000 U for $[^3H]LTB_4$, 100 U for $[^3H]$FMLP) or PBS for 5 min, washed and the specific binding of various concentrations of $[^3H]LTB_4$/$[^3H]$FMLP was determined. The data represent the result of two individual experiments±SD.

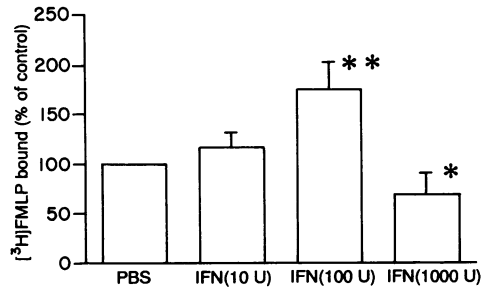

Figure 4. Dose-dependent effects of IFN-α (10–1000 U) on [^{3}H]FMLP binding to human neutrophils. PMN were incubated with IFN-α for 5 min at 37°. As a control served PMN incubated with PBS buffer, which was defined as 100%. The values are means ± SD ($n=4$; * $P<0.05$; ** $P<0.01$).

of [^{3}H]LTB_4 metabolized). Lower concentrations of IFN-α (10–100 U/ml) had no significant effects.

The data suggest that IFN-α modulates the signal transduction in PMN with regard to the metabolism of LTB_4 induced after stimulation with FMLP and NaF. We further studied the receptor expression for FMLP and LTB_4 as well as the G-protein activity after treatment with IFN-α.

Effect of IFN-α on the receptor expression for LTB_4 and FMLP

Treatment of PMN (1×10^7) with IFN-α (10–1000 U/ml) for 5 min decreased the expression of specific binding sites for LTB_4 in a dose-dependent manner (Fig. 3a).

Time–course experiments indicated that the inhibition was maximal after 30 min for 10 U/ml, after 15 min for 100 U/ml and after 5 min for 1000 U/ml (Fig. 3b).

Subsequently the influence of IFN-α on the affinity state of the LTB_4 receptor was analysed: PMN (4×10^6) were incubated with various concentrations of [^{3}H]LTB_4 (0·3–4·8 nM) in the presence of unlabelled LTB_4 (220 nM) for 45 min at 4°. The specific binding was calculated and the data were analysed by Scatchard plot. PMN incubated with PBS buffer showed a heterogenous receptor population with high- and low-affinity receptors (Table 2); pretreatment of the cells with IFN-α (10–1000 U/ml) revealed evidence for a decreased expression of high affinity receptors. At 1000 U/ml of IFN-α only 68% of low-affinity and 31% of high-affinity binding sites were detected.

These results were in contrast to the expression of specific binding sites for FMLP.

Incubation of PMN with IFN-α at a concentration of 10 and 100 U/ml for 5 min increased the expression of specific binding sites for FMLP; a concentration of 100 U/ml had the most pronounced effect on FMLP receptor expression (Fig. 4); a concentration of 1000 U/ml decreased the FMLP receptor expression. Scatchard plot analysis for FMLP indicated two receptor populations, including high- and low-affinity subsets for cells pretreated with PBS, whereas IFN-α-treated cells showed only one receptor population (Table 2).

Involvement of G-proteins

We analysed the effects of IFN-α on the receptor-mediated stimulation of the GTPase activity.

For this purpose PMN (1×10^7) were incubated with either IFN-α (10–1000 U/ml), FMLP (10^{-5} M) or NaF (20 mM) for 20 min. Subsequently, the neutrophil membrane fractions were isolated by differential centrifugation and 10 μg of protein were incubated with 0·5 μM ^{32}P-gamma-GTP.

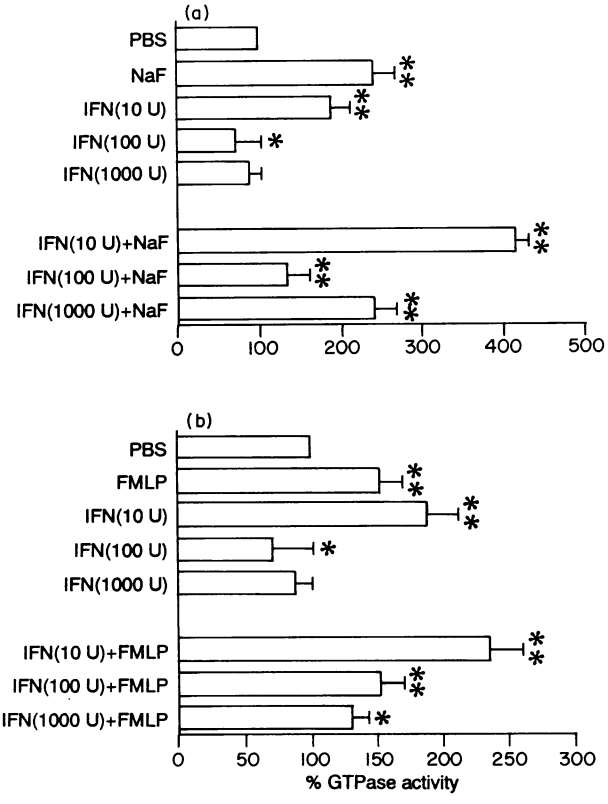

Figure 5. Modulation of GTPase activity by IFN-α. Isolated membrane fractions (10 μg of protein) from stimulated neutrophils (4×10^6) were incubated with PBS buffer, NaF (20 mM) (a) or FMLP (10^{-5}) (b) or simultaneously incubated with various concentrations of IFN-α (10–1000 U) in the presence of 0·5 μM ^{32}P-gamma-GTP. The release of P_i was corrected against the spontaneous ^{32}P-gamma-GTP hydrolysis in the absence of the membrane fractions. The basal GTPase activity (stimulation with PBS buffer) was defined as 100%. All values are means ± SD ($n=3$; * $P<0.05$; ** $P<0.01$).

Unstimulated membrane fractions showed a GTPase activity of 14·84 ± 4·35 pmol/mg × min (defined as 100%), which was enhanced by 140% in the presence of NaF, by 50% in the presence of FMLP and by 90% in the presence of IFN-α (10 U); at concentrations of 100 and 1000 U/ml of IFN-α the GTPase activity was slightly reduced (Fig. 5a, b).

Pretreatment of the cells (4×10^7) with 10 U IFN-α for 5 min and subsequent addition of NaF (20 mM) synergistically enhanced the GTPase activity compared to the action of each stimulus alone. Higher doses of IFN-α (100 and 1000 U/ml) in combination with NaF additively enhanced the enzymatic activity (Fig. 5a).

Incubation of IFN-α (10–1000 U/ml) in combination with FMLP (10^{-5} M) showed an additive increase of the enzymatic activity (Fig. 5b).

Further experiments were directed to the interaction of IFN-α and the GTPase activity of neutrophil membrane fractions. For this purpose membrane fractions (10 μg protein) of unstimulated neutrophils were incubated under the same conditions as mentioned above for intact cells in the presence of 0·5

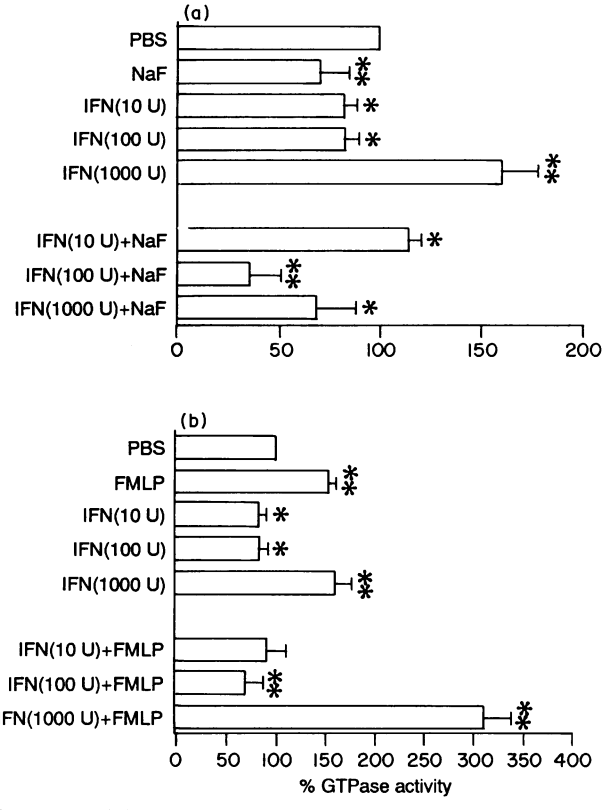

Figure 6. Modulation of GTPase activity by IFN-α. Isolated membrane fractions from unstimulated neutrophils (10 μg of protein) were incubated under the same conditions as indicated in Fig. 5. All values are means ± SD ($n = 3$; * $P < 0.05$; ** $P < 0.01$).

μM ^{32}P-gamma-GTP. The results are summarized in Fig. 6a, b. FMLP and IFN-α (1000 U/ml) alone enhanced the GTPase activity significantly, whereas IFN-α (10 and 100 U/ml) as well as NaF had inhibitory effects. Co-incubation of PMN with IFN-α (100, 1000 U/ml) and NaF significantly blocked the GTPase activity, whereas IFN-α (1000 U/ml) in combination with FMLP synergistically enhanced the enzymatic activity.

In subsequent experiments the effect of IFN-α on cytoscelatal functions was studied. For this purpose PMN were stimulated with IFN-α (10–1000 U/ml) in the presence of Ca^{2+}/Mg^{2+} (1/0·5 mM) for 15 seconds at 37°; subsequently, the polymerization of F-actin was determined by FACS analysis using FITC-labelled phalloidin. No differences in the F-actin polymerization could be observed in IFN-α-treated cells compared to the buffer control (data not shown).

DISCUSSION

Our data clearly demonstrate that IFN-α in addition to its known anti-viral and anti-proliferative activities, influences human granulocytes with regard to several important cellular functions (e.g. receptor expression, oxygen radical production) and signal transduction.

IFN-α itself did not augment the production of oxygen radicals but altered the chemiluminescence induced by FMLP, NaF and the Ca ionophore A23187. These results confirm those of Larrick *et al.*[27] and Kapp *et al.*,[28] who showed that only IFN-γ, in contrast to IFN-α, triggered oxygen radical production.

Few data exist about the role of interferons on lipid mediator generation and metabolism. Recent studies[29] revealed evidence for an enhanced hydrolysis of LTA_4 to LTB_4 in endothelial cells. One may suggest that by this mechanism LTB_4 is produced in large quantities by non-haematopoetic cells at acute sites of inflammation. Our results indicate a differential role of IFN-α on the chemotactically active LTB_4. Pretreatment of granulocytes with IFN-α and subsequent stimulation with FMLP resulted in a reduction of LTB_4 production. Studies on the metabolism of LTB_4 indicated an inhibition of metabolization to the biologically inactive omega-oxidated products. This may then result in increased LTB_4 concentrations at sites of inflammation.

Many efforts have been directed to study the influence of cytokines on the receptor expression of human granulocytes. IFN-γ, GM-CSF and G-CSF enhance the membrane expression of FcR3,[20] furthermore it was demonstrated by Atkinson *et al.*[30] that preincubation of neutrophils with TNF-α resulted in the modulation of the FMLP receptor, including a change from a biphasic receptor population to a homogenous receptor population. Similar results were obtained by measuring the affinity state of the LTB_4 receptor after stimulation of human PMN with TNF-β.[31] Our data show that IFN-α acts differently on the expression of receptors for LTB_4 compared to receptor expression for FMLP. Preincubation of PMN with IFN-α resulted in a decreased LTB_4 receptor expression with reduced values for high and low affinity binding sites.

Resting PMN express high-affinity receptors which mediate the chemotactic response, as well as low-affinity receptors which are responsible for degranulation. The loss of high-affinity receptors may explain the fact why IFN-α alters the biological responsiveness of neutrophils. The fact that the affinity state of the receptor is regulated by guanine nucleotide-binding proteins and guanine nucleotides[32] may suggest that changes in the affinity state of the receptor are a consequence of an interaction with guanine nucleotide-binding proteins.

The receptor expression of FMLP on human PMN is still discussed controversially. In contrast to intact cells, membrane fractions from PMNs suggested two receptor populations including high and low affinity subsets.[33] Different results were obtained by Atkinson *et al.*[30] who described high- as well as low-receptor populations for FMLP on human neutrophils. These different findings have also been attributed to a cellular activation during PMN preparation and to washing steps involved in the filtration technique.[34] However, our results reveal evidence for two receptor populations on unstimulated cells, whereas IFN-α-treated cells showed only one receptor population.

To analyse the biochemical mechanisms of the altered cell functions induced by IFN-α we studied the involvement of G-proteins. For this purpose the intrinsic GTPase activity was analysed. An enhanced GTPase activity was obtained when (i) membrane fractions were stimulated with IFN-α or (ii) when membrane fractions isolated from IFN-α-stimulated intact cells were studied. These effects were even enhanced after subsequent stimulation with FMLP or NaF.

The receptor-associated G-proteins are a heterogeneous family including diverse α- and β-subunits. Small GTP-binding proteins, such as ras, rho, rap or smg proteins, which have been shown to bind guanine nucleotides and to hydrolyse GTP, have been detected recently; however, little is known about their

cellular functions.[35] In this regard our data probably emphasize the functions of a variety of G-proteins and not of a distinct class of GTP-binding proteins.

In mammalian cells, preliminary evidence was obtained for the involvement of proteins regulating GTP-binding proteins. GAP (G-protein activating protein) was detected in human platelets and neutrophils promoting the hydrolysis of GTP.[36] Whether IFN-α acts on the level of GAP regulation has to be analysed.

Thus, our data indicate alterations in PMN function after treatment with IFN-α. To explain the various biological responses further experiments are directed to analyse the components of the signal transduction pathway.

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