

Effects of interferon-alpha (IFN- α) administration on leucocytes in healthy humans

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SUMMARY

Plasma concentrations of IFN- α are increased in several inflammatory conditions. Several lines of evidence indicate that IFN- α has anti-inflammatory properties. To study the effects of IFN- α on leucocyte subsets and activation and on cytokines, we administered IFN- α (rhIFN- α 2b; 5×10^6 U/m²) to eight healthy human subjects in a randomized controlled cross-over study and analysed changes in circulating leucocytes and parameters for neutrophil and monocyte activation. After administration of IFN- α , neutrophil counts increased, monocyte counts decreased transiently, whereas the number of lymphocytes, basophils and eosinophils showed a sustained decrease. IFN- α administration was also associated with neutrophil activation, reflected in an increase in the plasma concentrations of elastase- α_1 -antitrypsin complexes and lactoferrin. Serum neopterin, a marker for monocyte activation, was significantly increased 10 h after administration of IFN- α . IFN- α significantly increased plasma concentrations of IL-6, IL-8 and IL-10. Although IL-1 and tumour necrosis factor (TNF) remained undetectable, plasma concentrations of soluble TNF receptors p55 and p75 increased after IFN- α administration. We conclude that IFN- α induces multiple alterations in the distribution and functional properties of leucocytes. IFN- α exerts pro- as well as anti-inflammatory effects within the cytokine network.

Keywords cytokines neopterin lactoferrin interferons leucocytes

INTRODUCTION

Leucocytes play a major role in host defence against invading microorganisms. These cells produce, among other cytokines, interferons, which are low molecular weight proteins that play a role by the natural defensive responses of the body to foreign structures, such as microorganisms, antigens and tumours [1,2]. According to differences in their primary structure, interferons have been subdivided into classes (IFN- α , IFN- β , IFN- γ) with distinct production sites and cell surface receptors [3]. IFN- α is produced by leucocytes in response to viral infections and to multiple stimulating agents [4–6]. Several lines of evidence indicate that IFN- α has anti-inflammatory properties. Tzung *et al.* reported that IFN- α prevents endotoxin-induced mortality in mice [7]. There is increasing evidence that IFN- α interferes with the synthesis of various cytokines. IFN- α was shown to induce IL-1 receptor antagonist (IL-1Ra) *in vitro* and *in vivo* [8] and to suppress IL-1-induced IL-1 synthesis by peripheral blood mononuclear cells (PMBC) [9]. Constitutive IL-8 mRNA expression in PMBC from patients with chronic myelogenous leukaemia is also down-

regulated during IFN- α therapy [10]. Several reports show that IFN- α may suppress tumour necrosis factor-alpha (TNF- α) gene suppression and protein synthesis [11]. Therefore, it has been postulated that part of the effects of interferons on the immune system may be mediated by altered functional properties of leucocytes. To document this issue in further detail, we studied the effects of subcutaneous administration of recombinant human IFN- α on leucocyte numbers and leucocyte functions reflected in neutrophil and monocyte activation and in cytokine release in eight healthy humans in a randomized, controlled cross-over study.

PATIENTS AND METHODS

Study design

The study was performed as a randomized, placebo-controlled cross-over study. Eight healthy males (age 23 ± 1 years (mean \pm s.e.m.); weight 79 ± 4 kg; height 1.83 ± 0.03 m) participated in the study. The clinical effects and the effects of IFN- α on thyroid hormone metabolism in the same subjects have been reported previously [16]. None of the subjects had any abnormality on physical examination or routine laboratory investigation. They did not use any medication and had not experienced a

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febrile disease in the month before the study. The study of the immunological effects of IFN- α was approved by the Research Committee and the Medical Ethical Committee of the Academic Medical Centre, Amsterdam. Written informed consent was obtained from all subjects before their participation.

Each subject was studied twice with an interval of at least 4 weeks. On one occasion, a bolus subcutaneous injection of rhIFN- α 2b (5×10^6 U/m 2 ; Schering-Plough BV, Amstelveen, The Netherlands) dissolved in 1 ml of isotonic saline was given; on the other occasion an equivalent volume of isotonic saline was administered (10:00 h; $t = 0$ h). The order in which recombinant IFN- α and isotonic saline were administered to each subject was determined by randomized, balanced assignment.

The volunteers were fasted overnight (from 18:00 h) until the end of each study period (22:00 h). Blood was collected by separate venipunctures from an antecubital vein, directly before and every 2 h until 12 h after administration of IFN- α , for analysis of leucocyte numbers and differentials, for leucocyte activation tests (elastase- α_1 -antitrypsin (α_1 -AT) complexes, lactoferrin and neopterin) and cytokine concentrations (IFN- α , TNF, IL-1, IL-6, IL-8, IL-10, sTNF-p55 and sTNFR-p75). Oral temperature, heart rate and blood pressure were measured at 2-h intervals. Subjects took a standard meal at the end of study days (22:00 h). The next morning (24 h after the administration of IFN- α), before breakfast, the last blood samples were taken for leucocyte counts and differentials.

Assays

Leucocyte counts and differentials were determined in blood anticoagulated with K $_3$ -EDTA with the use of a flow cytometer (Technicon H1 system; Technicon Instruments, Tarrytown, NY). Blood for the determination of elastase- α_1 -AT complexes and lactoferrin was collected in siliconized Vacutainer tubes (Becton Dickinson, Plymouth, UK) to which EDTA (10 mM) and Polybrene (0.05%, w/v) were added to prevent any *in vitro* complex formation. Blood samples were centrifuged at 4°C for 20 min at 1600 g and stored at -70°C until further analysis. The plasma concentrations of elastase- α_1 -AT complexes and lactoferrin were measured with radioimmunoassays (RIAs) [12]. Serum for the determination of neopterin was prepared by centrifugation of clotted blood for 20 min at 1600 g (room temperature). The serum concentrations of neopterin were measured by RIA (IMMUtest Neopterin; Henning, Berlin, Germany). Plasma IFN- α concentration was measured by RIA (Medgenix, Amersfoort, The Netherlands) with a detection limit of 5 U/ml, TNF- α concentration by IRMA (Medgenix) with a detection limit of 1 pg/ml, IL-6 concentrations by ELISA [17] with a detection limit of 2 pg/ml, IL-1 β concentrations by IRMA (Medgenix) with a detection limit of 4 pg/ml. Plasma IL-8 concentrations were measured with a sandwich ELISA modified from that described previously [13]: anti-IL-8 MoAb (MoAB CLB-IL-8/1) and biotinylated affinity-purified sheep anti-IL-8 antibodies were used as capture and detecting antibodies, respectively. Polymerized horseradish peroxidase conjugated to streptavidin (Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, The Netherlands) was used to quantify both biotinylated antibodies. Results were compared with those obtained with dilutions of recombinant human IL-8 and expressed as pg/ml. Plasma IL-10 was measured by ELISA (Schering Plough, Kenilworth, NJ). sTNFR-p55 and sTNFR-p75 were measured by enzyme-linked immunological binding assays (ELISA; Hoffmann-La Roche, Basel, Switzerland), as described previously [14,15].

Statistical analysis

Data are presented as mean \pm s.e.m. Differences within experiments (differences compared with $t = 0$) were tested by analysis of variance and Fisher's least square difference (LSD) test for multiple comparison, when indicated. Data between experiments (IFN- α and control data) were tested by Wilcoxon test. $P < 0.05$ was considered significant.

RESULTS

Leucocyte counts

Baseline leucocyte counts were within normal limits and not different between both study periods (Fig. 1). IFN- α administration was associated with a biphasic change in total leucocyte counts, the initial decrease ($P < 0.03$ versus control) reflecting the change in the circulating number of lymphocytes, monocytes, basophils and eosinophils, the subsequent increase reflecting the change in circulating neutrophils ($P = 0.06$ versus control) and monocytes ($P < 0.02$ versus control). The total leucocyte count reached its nadir ($4.5 \pm 0.5 \times 10^9/l$) after 4 h and peaked ($7.3 \pm 1.1 \times 10^9/l$) at 8 h. Total leucocyte count had returned to baseline values after 24 h. IFN- α administration increased the total neutrophil count ($P < 0.02$ versus control), with peak values after 8 h ($6.31 \pm 1.02 \times 10^9/l$). After 24 h, the total neutrophil count had returned to control values. IFN- α administration also elicited a sustained decrease of lymphocytes ($P < 0.02$ versus control), basophils ($P < 0.05$ versus control) and eosinophils ($P < 0.05$ versus control). IFN- α induced an initial monopenia lasting 6 h post-injection ($P < 0.03$ versus control), followed by a sustained increase ($P < 0.05$ versus control).

Neutrophil activation

Activation of neutrophils was assessed by measuring plasma concentrations of elastase- α_1 -AT complexes and lactoferrin. Preinjection

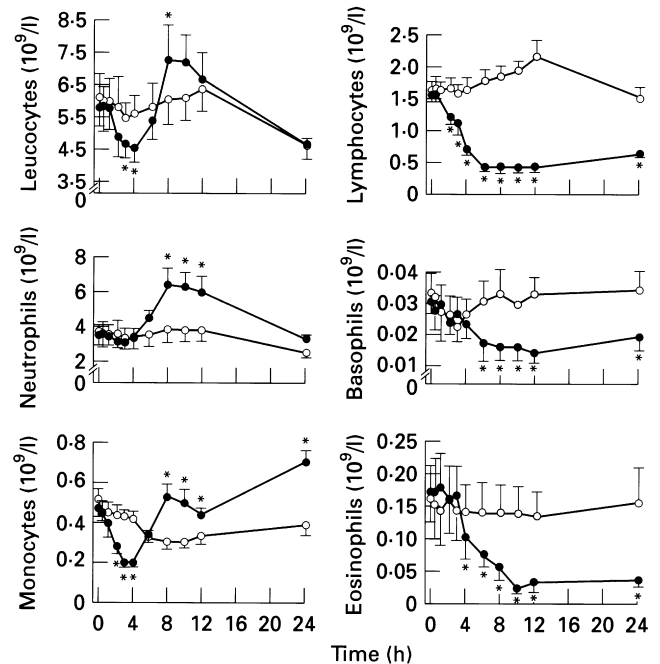


Fig. 1. Effects of IFN- α (5×10^6 U/m 2) (●) or saline (○) on differential leucocyte counts. Values are mean \pm s.e.m. * $P < 0.05$ versus the corresponding value of the control study.

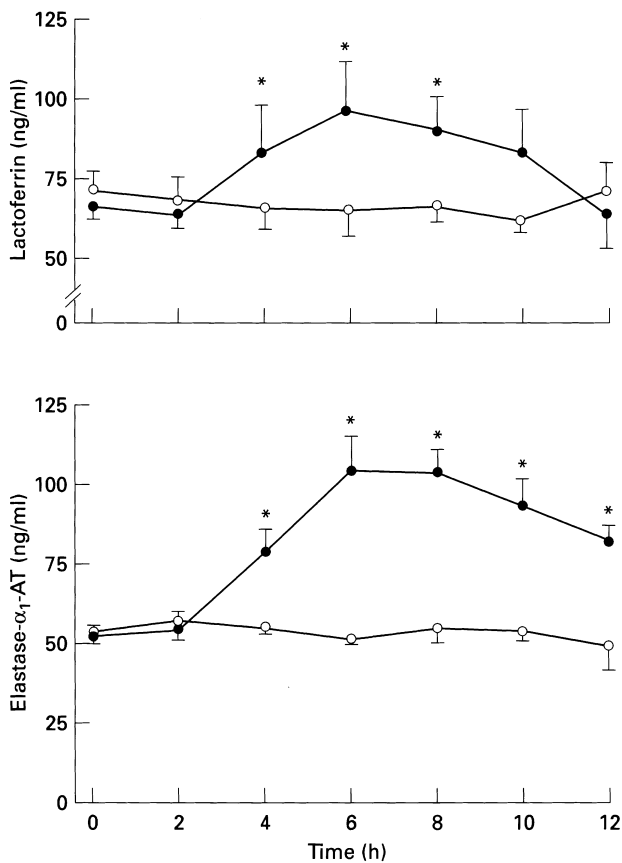


Fig. 2. Effects of IFN- α (5×10^6 U/m 2) (●) or saline (○) on plasma concentrations of elastase- α_1 -antitrypsin (α_1 -AT) complexes and lactoferrin. Values are mean \pm s.e.m. * $P < 0.05$ versus the corresponding value of the control study.

values were not different between both studies (Fig. 2). IFN- α induced sharp increases in the plasma levels of elastase- α_1 -AT complexes and lactoferrin, both becoming significant after 4 h and peaking after 6 h. Elastase- α_1 -AT complexes rose from 54 ± 2 ng/ml at baseline to 104 ± 11 ng/ml ($P < 0.03$ versus control); lactoferrin rose from 66 ± 8 to 96 ± 15 ng/ml ($P < 0.05$ versus control). From 6 h onwards gradual decreases in elastase- α_1 -AT complexes and lactoferrin were observed, elastase- α_1 -AT complexes remaining elevated until the end of the 12-h observation period.

Monocyte activation

Serum concentrations of neopterin were assayed as a measure of monocyte activation. Baseline neopterin levels were not different between both studies (4.36 ± 0.28 nmol/l before IFN- α ; 4.50 ± 0.24 nmol/l before saline), and they remained unchanged until 10 h after the initial injections, after which they steadily increased to values of 9.41 ± 0.90 nmol/l at the end of the study ($P < 0.052$ versus control) (Fig. 3).

Cytokines

On the control day, IFN- α concentrations were below the detection limit in all subjects (Fig. 4). After subcutaneous administration of rhIFN- α , IFN- α levels increased to peak levels of 24 ± 1.6 U/ml after 8 h to remain constant until the end of the study ($t = 12$ h). Concentrations of TNF- α and IL-1 β were below the detection limit

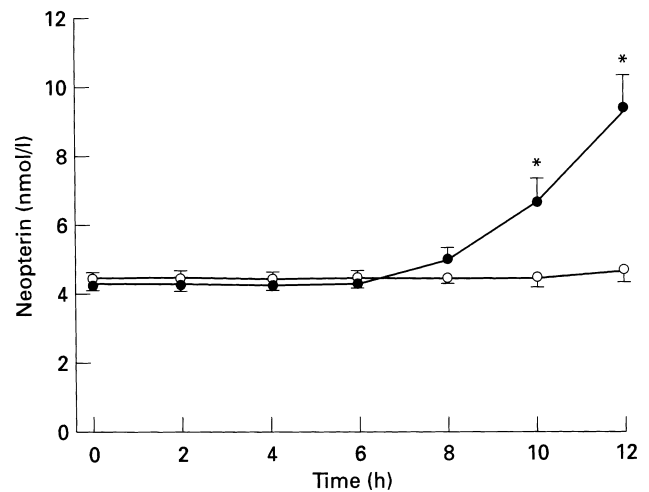


Fig. 3. Effects of IFN- α (5×10^6 U/m 2) (●) or saline (○) on plasma concentrations of neopterin. Values are mean \pm s.e.m. * $P < 0.02$ versus the corresponding value of the control study.

in all subjects both on the control and on the IFN- α study day. On the control day, IL-6 concentrations were below the detection limit in all subjects. After subcutaneous administration of IFN- α , there was a slight though steady increase in plasma IL-6 concentrations ($P < 0.02$ versus control day). Peak concentrations were reached after 8 h (9.4 ± 1.1 pg/ml). Preinjection values of IL-8, IL-10, sTNF-p55 and sTNFR-p75 were not different between the two studies. Plasma IL-8 increased to higher values than in the control study after 6 h, plasma IL-10 after 8 h, plasma sTNF-p55 after 4 h

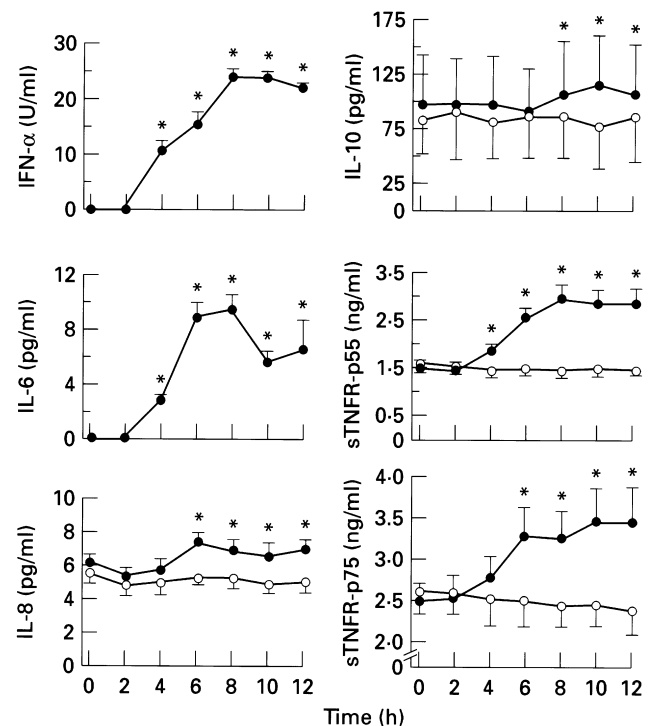


Fig. 4. Effects of IFN- α (5×10^6 U/m 2) (●) or saline (○) on plasma concentrations of cytokines (IFN- α , IL-6, IL-8, IL-10, sTNFR-p55, sTNFR-p75). Values are mean \pm s.e.m. On the control day, concentrations of IFN- α and IL-6 were below the detection limit in all subjects. * $P < 0.05$ versus the corresponding value of the control study.

and sTNFR-p75 after 6 h, all remaining elevated until the end of the study.

DISCUSSION

This study demonstrates that IFN- α , in addition to its known antiviral and anti-proliferative activities, influences human leucocytes *in vivo* with regard to numbers and functional properties. A single subcutaneous injection of IFN- α to human subjects induced marked changes in peripheral blood leucocyte numbers, and activation of neutrophils and monocytes, as reflected by the rises in the plasma concentrations of elastase- α 1-AT complexes and lactoferrin (neutrophils) and serum concentrations of neopterin (monocytes). In addition, IFN- α induced release of the pro-inflammatory cytokines IL-6 and IL-8, of the anti-inflammatory cytokine IL-10 and of the soluble TNF receptors p55 and p75.

IFN- α induced an increase in neutrophil counts, which might be explained by the increase in cortisol levels which was observed in our study (see [16] for data, [17]). IFN- α injections provoked sustained lymphopenia, which might also be the result of hypercortisolaemia [17]. Since IFN- α has only little effect on the expression of the adhesion molecule intercellular adhesion molecule-1 (ICAM-1) on the cell surface of lymphocytes [18,19], increased adherence of lymphocytes to the vascular endothelium probably plays only a minor role in the decrease in lymphocytes. Since IFN- α increased plasma IL-6 concentrations in our study, which does not influence the adhesive capacity of endothelial cells [20] but which induces neutrophilia and a mild lymphopenia in rats *in vivo* [21], IL-6 may have contributed to the altered neutrophil and lymphocyte counts.

Neutrophils secrete elastase and lactoferrin upon stimulation with various agents *in vitro*. As neutrophils are the predominant source of circulating elastase [22] and the exclusive source of circulating lactoferrin [23], the plasma concentrations of these proteins are commonly used as a measure of neutrophil activation *in vivo*. Since elastase is a very potent proteinase, degrading almost all components of the extracellular matrix as well as a variety of plasma proteins [22], the host's primary defence against uncontrolled action of elastase is the proteinase inhibitor α ₁-AT, which rapidly and irreversibly binds to elastase, forming elastase- α ₁-AT complexes. Lactoferrin is a glycoprotein from neutrophil-specific granules. Several pro-inflammatory functions have been postulated for lactoferrin, including antimicrobial activity and regulation of neutrophil adhesiveness. IFN- α induced marked increases in the plasma concentrations of both neutrophil markers, reaching significance after 4 h and peaking after 6 h. IFN- α may have provoked this neutrophil degranulation directly, although no data are present on this issue, or in concert with IL-6 [24] or IL-8 [25].

Neopterin is exclusively released by activated monocytes and macrophages [26]. Our study shows a late though definite increase in serum neopterin after IFN- α administration. This delayed activation of monocytes contrasts with the rapid activation of neutrophils.

IFN- α injection resulted in the release of multiple pro-inflammatory factors (like IL-6 and IL-8) and of anti-inflammatory factors (IL-10) and the soluble TNF receptors p55 and p75. In line with our results are the (mainly *in vitro*) reports from Rosztoczy & Content, who demonstrated that IFN- α enhances lipopolysaccharide (LPS)-induced IL-6 production in the adherent cell fraction of human PBMC [27], and of Tilg *et al.*, who showed that IFN- α induces the circulating TNF receptor p55 in healthy

human volunteers [28]. Although Aman *et al.* reported down-regulation by IFN- α of IL-8 mRNA expression in patients with chronic myelogenous leukaemia [10], Ohashi *et al.* reported IFN- α augmented IL-8 secretion from latently HIV-1-infected U937 cells [29]. Although suppression of IL-1-induced IL-1 synthesis by PMBC [9] and of TNF- α gene expression and protein synthesis [11] were reported, we found no effect of IFN- α on the release of TNF and IL-1 *in vivo*.

The results of the present study are of interest for the interpretation of the inflammatory response to tissue injury. The plasma levels of IFN- α were within the range of the values observed in AIDS patients (17 ± 3 U/ml) [30] and in other viral infections (18 ± 2 U/ml) [31]. In systemic vasculitis even considerably higher IFN- α concentrations were measured by the same assay (120 ± 19 U/ml) [32]. Therefore, the effects of IFN- α in the present study are not merely the result of a pharmacological dose of IFN- α , but rather of a dose of IFN- α that mimics the concentrations of IFN- α observed in human diseases.

IFN- α is used as a therapy for several unrelated conditions such as chronic viral hepatitis [33] and malignant disorders of the haematopoietic system [34]. The effects of IFN- α in hepatitis are thought to be related to inhibition of viral replication, whereas the exact mechanism of the anti-tumour action of IFN- α is unknown. It is unclear whether and to what extent the short-term effects of IFN- α observed in the present study are involved in the therapeutic effects of prolonged IFN- α treatment.

In conclusion, this controlled study in healthy humans shows that a single subcutaneous injection of IFN- α induces marked alterations in the peripheral blood counts and functional properties of leucocytes. IFN- α exerts a pro- as well as anti-inflammatory effects as an immune modulating factor within the cytokine network.

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