Interferons and interferon (IFN)-inducible protein 10 during highly active anti-retroviral therapy (HAART)—possible immunosuppressive role of IFN-α in HIV infection

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SUMMARY

Interferons play an important, but incompletely understood role in HIV-related disease. We investigated the effect of HAART on plasma levels of IFN-α, IFN-γ, neopterin and interferon-inducible protein 10 (IP-10) in 41 HIV-infected patients during 78 weeks of therapy. At baseline HIV-infected patients had raised levels of both IP-10 and IFN-α compared with healthy controls ($n = 19$), with particularly high levels in advanced disease. HAART induced a marked decrease in levels of both IFN-α, neopterin and IP-10, though not to normal concentrations. In contrast, IFN-γ levels were low throughout the study, and not different from controls. While neopterin and IP-10 remained significantly decreased compared with baseline levels throughout the study, IFN-α levels returned to baseline at the end of the study. Persistently high IP-10 and IFN-α levels were associated with immunological treatment failure and even high baseline levels of IFN-α appeared to predict immunological relapse. Furthermore, we found a markedly suppressive effect of exogenously added IFN-α on phytohaemagglutinin-stimulated lymphocyte proliferation in both patients and controls, and this suppressive effect seemed not to involve enhanced lymphocyte apoptosis. Our findings suggest a pathogenic role of IFN-α in HIV infection, which may be a potential target for immunomodulating therapy in combination with HAART.

Keywords HIV infection IFN-α IFN-γ neopterin IP-10

INTRODUCTION

Interferons (IFN) are classified into two distinct superfamilies based on their biochemical structure, their cellular receptors and biological effects [1,2]. Type I IFNs, such as IFN-α, are mainly produced by monocytes/macrophages and are thought to be key participants in the anti-viral defence in humans [1–4]. Type II IFN or IFN-γ, which is only produced by activated T and natural killer (NK) cells, has a cellular receptor distinct from type I IFNs. Although IFN-γ is endowed with little direct anti-viral activity, this cytokine plays an important role in the recruitment and activation of cytotoxic T cells, NK cells and phagocytes directed against virus- or parasite-infected cells and tumour cells [1,2,5].

There are several studies supporting a role for IFNs in the pathogenesis of HIV infection [2,6–8]. IFN-γ production in T cells has been found to decline along with disease progression in

HIV-infected patients, possibly reflecting a gradual impairment in the cellular immune response against intracellular microbes [9,10]. As for IFN-α, decreased stimulated production in peripheral blood mononuclear cells (PBMC) has been reported during HIV infection, with particularly low levels in advanced disease [6,11]. Furthermore, based on its ability to block different steps in HIV replication *in vitro*, IFN-α has been given for therapeutic purposes, both alone and in combination with other agents [12–14]. On the other hand, high rather than low levels of IFN-α have been found in sera of HIV-infected patients, and levels increase with disease progression [6,15,16]. Moreover, it has recently been suggested that IFN-α may impair production of chemokines with anti-viral effects in these patients [17]. Thus, the exact role of IFNs, and particularly IFN-α, in the pathogenesis of HIV infection is at present unclear.

Current standard therapy for HIV infection consists of HIV protease inhibitor-containing multidrug regimens. Although such HAART has been shown to have a profound down-regulatory effect on HIV replication with concomitant increases in CD4+ and CD8+ T cell counts in a considerable proportion of patients [18],

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full immunological normalization may not be achieved during such therapy [19]. Considering the important role of IFNs and related proteins in the anti-viral immune defence, we examined the effect of HAART on circulating levels of IFN-α, IFN-γ and neopterin, a surrogate marker thought to reflect IFN-γ activity. We also measured plasma levels of IFN-inducible protein 10 (IP-10), a CXC-chemokine with several immunomodulatory effects with potential relevance to HIV infection, such as granuloma formation and maintenance of a Th1-like immune response [20,21]. At present there is to our knowledge no information of IP-10 levels in HIV-infected individuals.

PATIENTS AND METHODS

Patients and controls

Forty-one HIV-infected patients (median age 38 years, range 15–57 years; 31 males and 10 females) were included in the study. Clinically, 22 subjects were classified as having asymptomatic HIV infection (CDC group A), six as having symptomatic non-AIDS HIV infection (CDC group B), and 13 as having AIDS (CDC group C) [22]. All patients received one protease inhibitor (indinavir, 800 mg tid, $n = 19$; ritonavir, 600 mg 2 bid, $n = 7$; saquinavir, 600 mg tid, $n = 12$; or nelfinavir 750 mg tid, $n = 2$) or one non-nucleoside analogue (nevirapine; 200 mg bid, $n = 1$) in combination with two nucleoside analogues (zidovudine 250 mg bid + lamivudine 150 mg bid, $n = 30$; stavudine 40 mg bid + lamivudine 150 mg bid, $n = 9$; or stavudine 40 mg bid + didanosine 400 mg qd, $n = 2$). Clinical, immunological and virologic characteristics of the study group at baseline are given in Table 1. Only blood samples taken in periods without any acute complication or exacerbation of chronic infection were included. Nineteen HIV$^-$, healthy volunteer blood donors (median age 41 years, range 25–64 years; 14 males and five females) were used as controls.

Blood sampling protocol

Blood was drawn into pyrogen-free vacuum blood collection tubes without any additives (serum) or with EDTA as anticoagulant (plasma). Tubes were immediately immersed in melting ice, centrifuged within 15 min at $600\,g$ for 10 min (plasma) or allowed to clot for 1 h before centrifugation at $1000\,g$ for 10 min (serum). Both plasma and serum were stored at $-80°C$. Samples were thawed less than three times before assay, which appeared not to influence the measured cytokine levels [23]. Moreover, all samples assayed for any one cytokine/chemokine were treated in the same way, i.e. equal numbers of freeze–thaw cycles.

Isolation and stimulation of PBMC

PBMC were obtained from heparinized blood by Isopaque–Ficoll (Lymphoprep; Nycomed Pharma, Oslo, Norway) gradient centrifugation within 45 min as previously described [24]. Mononuclear cells were resuspended in RPMI 1640 (GIBCO, Paisley, UK) with 2 mmol/l L-glutamine and 25 mmol/l HEPES buffer (GIBCO) and 10% heat-inactivated pooled human AB$^+$ serum, hereafter referred to as culture medium. Unstimulated or stimulated (phytohaemagglutinin (PHA); Murex Diagnostics Ltd, Dartford, UK; final concentration 1:100) PBMC (10^6 cells/ml; 0.2 ml/well) were incubated in flat-bottomed 96-well trays (Costar, Cambridge, MA) with or without different concentrations of recombinant human IP-10 (R&D Systems, Minneapolis, MN) or recombinant human IFN-α (Pepro Tech EC Ltd, London, UK). In some experiments neutralizing rabbit polyclonal anti-IFN-α antibody (final concentration 3000 U/ml; Biosource Int., Camarillo, CA) was also added to cell culture before stimulation. After 48 h of culture, 1 μCi of ^{3}H-thymidine was added for 16 h before the cultures were harvested onto glass filter strips, using an automated multisample harvester (Skatron, Lier, Norway). ^{3}H-thymidine incorporation was determined by liquid scintillation counting. In all preparations of medium, the endotoxin levels were < 10 pg/ml (limulus amoebocyte lysate test; Bio Whittaker, Walkersville, MD).

Detection of apoptosis in PBMC cultures

1. The TUNEL assay was carried out according to [25,26] with some modifications. PBMC (2×10^6 cells/ml) were seeded in 24-well plates (Costar; 1 ml/well) in culture medium with PHA (final concentration 1:100) or with PHA + 10^4 pg/ml IFN-α for 48 h, washed once in PBS, preincubated in PBS with human IgG (Octagam; Octapharma, Vienna, Austria; 5 g/l), 5% mouse serum (Sigma, St Louis, MO), 2% bovine serum albumin (BSA; Calbiochem, La Jolla, CA) and 0.1% sodium azide (Sigma) for 15 min at room temperature. Cells were stained for 30 min at 4°C with anti-CD3 (clone SK7) PE or the isotype-matched PE conjugate (both from Becton Dickinson, San Jose,

Table 1. Immunologic and virologic parameters in the study group

| | HIV-infected patients | | |
	Asymptomatic	Symptomatic	Controls
Number of subjects	22	19	19
Age, years	39 (22–56)	38 (16–57)	41 (25–64)
Sex (male/female)	18/4	13/6	14/5
CD4$^+$ T cells ($\times 10^6/l$)	183* (120–210)	78* (15–175)	610 (510–720)
CD8$^+$ T cells ($\times 10^6/l$)	610* (500–740)	400 (180–750)	385 (250–450)
HIV-1 RNA (10^3 copies/ml)	19 (6–70)	134 (34–1003)	–
Neopterin (nM)	17.5* (7.7–21.4)	16.5* (10.0–23.0)	7.9 (5.0–9.4)

Data are given as medians and 25–75th percentiles except for age, which is given as median and ranges.
Symptomatic HIV-infected patients: CDC group B, $n = 6$ + CDC group C, $n = 13$.
*$P < 0.001$ versus controls.

CA), washed twice in PBS, fixed in 4% paraformaldehyde (Sigma) for 30 min at room temperature, washed once in PBS and resuspended in permeabilization buffer (0·1% sodium citrate, 0·1% Triton X-100 (Sigma) in PBS) for 2 min at 4°C. Cells were then washed twice in PBS and incubated in 50 μl reaction mixture consisting of 10 nM biotin-16-dUTP (Boehringer Mannheim, Mannheim, Germany), 0·25 mg/ml BSA, 0·1 mM dithiothreitol (Sigma), 2 mM cobalt chloride (Sigma), 200 mM sodium cacodylate (Sigma), 25 mM Tris–HCl (Sigma) and 5 U terminal deoxynucleotidyl transferase (TdT; Boehringer Mannheim) for 30 min at 37°C. Control cells were incubated in the same mixture, but without TdT. After one wash in 0·1% Triton X-100 and 5% BSA in PBS (washing buffer), cells were incubated in FITC–streptavidin (1:50; Amersham Pharmacia Biotech AB, Uppsala, Sweden), 0·1% Triton X-100 and 3% non-fat dried milk (Sigma) in PBS for 30 min at 20°C. Cells were washed and analysed by flow cytometry (FACScan; Becton Dickinson) with CellQuest software (Becton Dickinson). List mode files were collected for 10 000 cells from each sample. The gate for apoptotic cells was set so that < 1% of cells were positive in the control samples (without TdT).

2. Apoptotic cells expressing negatively charged phospholipids were detected by staining with annexin V–FITC conjugate, whereas non-viable cells were stained by propidium iodide (TACS Annexin V-FITC Apoptosis Detection Kit; Genzyme Corp., Cambridge, MA) according to the manufacturer's instructions. Briefly, PBMC (2×10^6 cells/ml) were seeded in 96-well plates (Costar; 0·2 ml/well) in culture medium with PHA (final concentration 1:100) or with PHA + 10^4 pg/ml IFN-α for 48 h, washed once in PBS and stained with annexin V–FITC and propidium iodide in binding buffer (Genzyme) for 15 min at room temperature and immediately analysed by flow cytometry as described above.

Quantification of IFN-α, IFN-γ and IP-10 by enzyme immunoassay

Plasma levels of IFN-α were quantified by enzyme immunoassay (EIA; detection limit 3 pg/ml; Biosource International). Plasma levels of IFN-γ were quantified by two different EIAs using microtitre wells coated with one (detection limit 3 pg/ml; R&D Systems) or several (detection limit 0·03 U/ml; Biosource) MoAbs against distinct epitopes of the IFN-γ molecule. For the IP-10 EIA, wells were coated overnight with monoclonal mouse anti-human IP-10 antibody (R&D Systems; clone 33036.211; 4 μg/ml in sterile PBS). Standard was recombinant IP-10 (R&D Systems; 16·6–1000 pg/ml). Subsequent steps included biotinylated polyclonal goat anti-human IP-10 (R&D Systems; 25 ng/ml), alkaline phosphatase-conjugated streptavidin (Zymed Labs, San Francisco, CA; 1:2000) and *p*-nitrophenyl disodium phosphate as substrate (Sigma; 1 mg/ml in diethanolamine buffer, pH 9·8). Detection limit for IP-10 EIA was 16·5 pg/ml. The intra- and interassay coefficients of variations were < 10% for all EIAs. All samples from a given patient were analysed in the same microtitre plate to minimize run-to-run variability.

Miscellaneous

Plasma neopterin levels were measured using radioimmunoassay (IMMUtest Neopterin; Henning Berlin GMBH, Berlin, Germany). CD4$^+$ and CD8$^+$ T cell counts were determined by immunomagnetic quantification [27]. Quantification of ^{125}I-IFN-α-binding IgG was carried out by protein-G affinity chromatography, as described [28]. Plasma HIV RNA levels were measured by quantitative reverse polymerase chain reaction (detection limit 200 copies/ml; Amplicor Monitor; Roche Diagnostic Systems, Branchburg, NY).

Statistical analysis

When comparing two groups of individuals we used the two-tailed Mann–Whitney *U*-test. When comparing more than two groups, the Kruskal–Wallis test was used *a priori*. If a significant difference was found, the Mann–Whitney *U*-test was used to determine differences between each pair of groups. Coefficients of correlations were calculated by the Spearman rank test. Responses within the same individuals were compared by the (two-tailed) Wilcoxon signed rank test for paired data. Data are given as medians and 25–75th percentiles if not otherwise stated. *P* values were two-sided and considered significant when < 0·05.

RESULTS

Plasma levels of IFN-α, IP-10 and IFN-γ levels before initiating HAART

At baseline 31 patients received nucleoside analogues (combination of zidovudine and lamivudine, *n* = 28; zidovudine, *n* = 2; didanosine, *n* = 1). As shown in Table 2, HIV-infected patients had markedly elevated plasma levels of both IFN-α and IP-10 compared with healthy controls, and as for IP-10, the highest levels were found in those with symptomatic HIV infection (CDC group C and B). Furthermore, IP-10 levels showed a significant negative correlation with both CD4$^+$ ($r = -0.33$, $P < 0.04$) and CD8$^+$ ($r = -0.32$, $P < 0.05$) T cell counts in peripheral blood. IP-10 also showed a significant positive correlation with HIV RNA copy numbers in plasma ($r = 0.39$, $P < 0.02$). No such correlations were found for IFN-α (data not shown). In contrast to the high IFN-α and IP-10 levels, IFN-γ was detected only in six patients and was

Table 2. Plasma levels of IFN-α and IP-10 before initiating HAART

	IFN-α (pg/ml)	IP-10 (pg/ml)
Controls (*n* = 19)	0 (0–16·5)	0 (0–0)
Asymptomatic HIV infection (*n* = 22)	3·4* (0–17·0)	38* (0–213)
Symptomatic HIV infection (*n* = 19)	9·8** (3·2–16·5)	247**† (103–449)

Data are given as medians and 25–75th percentiles. Symptomatic HIV-infected patients: CDC group B, *n* = 6 + CDC group C, *n* = 13.

†*P* < 0·05 *versus* asymptomatic HIV infection; **P* < 0·05 and ***P* < 0·001 *versus* controls.

not different from concentrations in healthy controls as assessed by two different EIAs (data not shown).

IFN-α, IP-10, IFN-γ and neopterin levels during HAART

Blood samples were taken at baseline ($n = 41$) and 8 ($n = 38$), 26 ($n = 37$), 52 ($n = 38$) and 78 ($n = 28$) weeks after initiating HAART. During therapy there was a significant increase in CD4$^+$ T cell counts (maximum increase 137 (70–188) $\times 10^6/l$, $P < 0.001$) and a pronounced fall in HIV RNA copy numbers in plasma (maximum decrease 4.49 (2.74–5.03)$\log_{10}$ copies/ml, $P < 0.002$). Also CD8$^+$ T cell counts increased during HAART, but the rise was more modest than that for CD4$^+$ T cells, reaching significance only at 78 weeks of therapy ($P < 0.002$). Concomitant with the increase in T cell counts and decrease in viral load, HAART induced a significant decrease in plasma levels of both IFN-α, neopterin and IP-10 (Fig. 1). Interestingly, there was a highly significant correlation between IFN-α and IP-10 levels at all time points during the study period, but no such correlation was found between neopterin and IP-10 or IFN-α (data not shown). Naturally occurring neutralizing antibodies against IFN-α may influence both the measurement and the activity of this cytokine, but such antibodies were not detected either at baseline or after initiating HAART when analysing levels in 25 HIV-infected patients (data not shown). Although there was a marked decline in IFN-α, neopterin and IP-10 levels during HAART, there was no normalization of these parameters, and the levels were significantly elevated compared with healthy controls throughout the study period (Fig. 1). Furthermore, while neopterin and IP-10 levels were significantly decreased compared with baseline throughout the study, IFN-α levels tended to return to baseline at the end of the study (Fig. 1). In contrast to the pronounced decrease in IFN-α, neopterin and IP-10, the IFN-γ concentrations were low or undetectable throughout the study (data not shown).

IFN-α, neopterin and IP-10 levels in relation to immunological and virologic response during HAART

During HAART there was a significant negative correlation between CD4$^+$ T cell counts and plasma levels of IP-10 and IFN-α at both 26 and 52 weeks of therapy (data not shown). To examine further the relationship between immunological response and IFN-α, IP-10

and neopterin levels during HAART, the patients were classified as immunological responders ($n = 28$) or non-responders ($n = 10$) after 52 weeks of therapy. Since no standardized criteria for immunological response exist we chose to define patients as immunological non-responders on the basis of the following criteria: < 20% increase in CD4$^+$ T cells compared with baseline (patients with $> 100 \times 10^6/l$ CD4$^+$ cells at baseline) or $< 20 \times 10^6/l$ increase in CD4$^+$ T cells compared with baseline (patients with $< 100 \times 10^6/l$ CD4$^+$ cells at baseline). As shown in Fig. 2, immunological non-responders had significantly higher levels of both IFN-α and IP-10 at several time points during the first 52 weeks of therapy compared with other HIV-infected patients. In fact, non-responders had markedly increased IFN-α levels even before initiating therapy (Fig. 2). Moreover, while responders had a significant decrease in both IP-l0 and IFN-α levels after initiating HAART, non-responders had only a minor non-significant decrease (IFN-α) or even an increase (IP-10) in concentration after start of therapy (Fig. 2). A similar pattern of correlations was found also after 78 weeks on HAART. As for neopterin, more modest differences were found between immunological responders and non-responders at baseline. However, while neopterin levels significantly decreased in responders ($P < 0.001$), this was not seen in patients with immunological treatment failure (data not shown).

In contrast to the correlation with immunological response, no significant relationships were found between virologic response and IFN-α, neopterin or IP-10 levels during HAART (data not shown).

Effect of exogenously added IFN-α and IP-10 on PBMC proliferation *in vitro*

Our findings suggest that a persistent elevation of IP-10 and IFN-α may be associated with lack of immunological response during HAART. It is noteworthy that these trends were seen long before manifestations of treatment failure, and as for IFN-α, even high baseline levels appear to predict immunological treatment failure. To examine further the possible immunosuppressive effect of IFN-α and IP-10 during HIV infection, we measured the effect of different concentrations of these cytokines on PHA-stimulated proliferation of PBMC in six HIV-infected patients on HAART (five immunological responders and one non-responder) and five
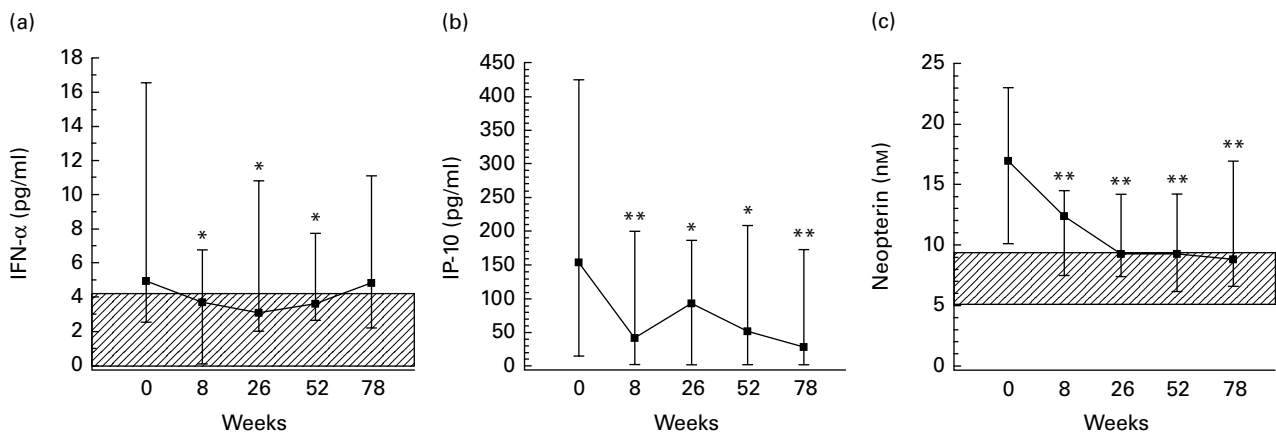

Fig. 1. Plasma levels of IFN-α (a), IP-10 (b) and neopterin (c) after start of HAART. Forty-one HIV-infected patients were studied; blood samples were taken at 8 ($n = 38$), 26 ($n = 37$), 52 ($n = 38$), and 78 ($n = 28$) weeks after start of therapy. Data are medians and 25–75th percentiles. *$P < 0.05$ and **$P < 0.01$ *versus* baseline. Hatched areas indicate 25–75th percentiles in 19 healthy controls. As for IP-10 (b), both 25th and the 75th percentiles in healthy controls were below the detection limit of the assay (see Table 2).
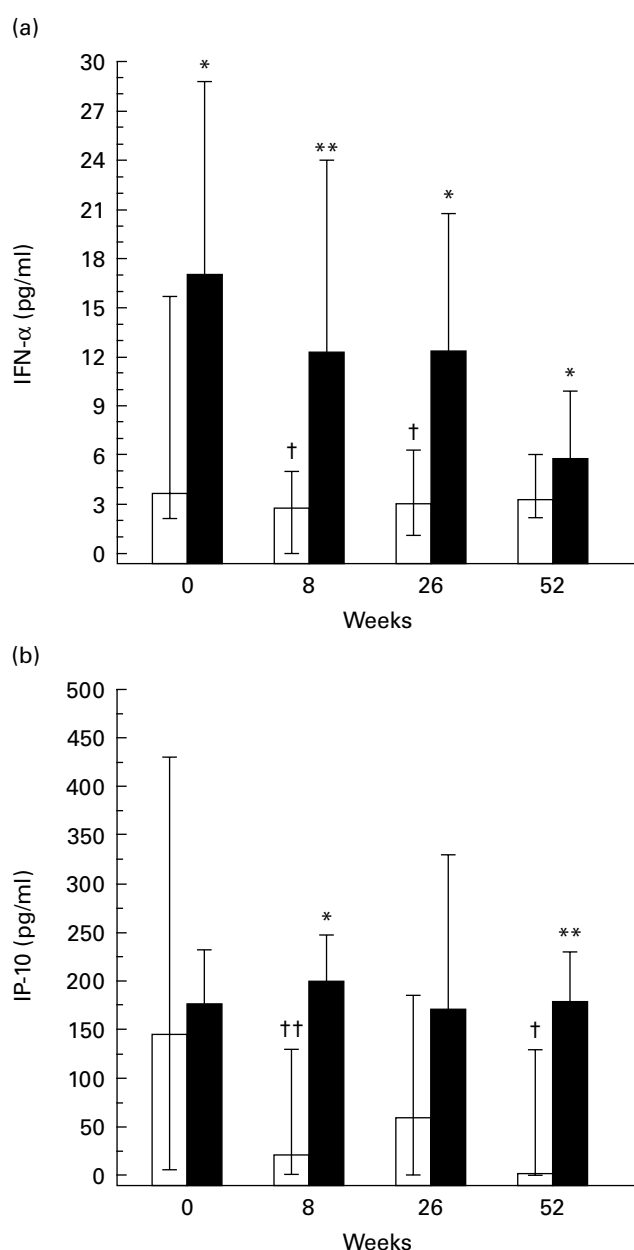
(a)

(b)

Fig. 2. IFN-α and IP-10 levels in relation to immunological treatment failure after 52 weeks of HAART. Plasma levels of IFN-α (a) and IP-10 (b) at different times after initiation of HAART in 10 patients classified as immunological non-responders (■) and in 28 immunological responders (□). Immunologic treatment failure was defined as < 20% increase in CD4+ T cells compared with baseline (patients with > $100 \times 10^6/l$ CD4+ cells at baseline) or < $20 \times 10^6/l$ increase in CD4+ T cells compared with baseline (patients with < $100 \times 10^6/l$ CD4+ cells at baseline) after 52 weeks of therapy. Data are medians and 25–75th percentiles. *$P < 0.05$ and **$P < 0.01$ *versus* responders; †$P < 0.05$ and ††$P < 0.01$ *versus* baseline.

healthy controls. Low IFN-α doses (< 100 pg/ml) exhibited a modest and non-significant stimulatory effect on PHA-stimulated PBMC proliferation (~10% increase). In contrast, a marked dose-dependent suppressive effect was observed in all patients and controls with higher doses, reaching ~40% of baseline at the highest concentration tested (100 ng/ml) (Table 3). This suppressive effect was blocked by neutralizing IFN-α antibodies added

before stimulation (data not shown). Notably, the suppressive effect was already detectable at doses as low as 100 pg/ml, comparable with IFN-α plasma levels in some HIV-infected patients. In contrast, IP-10 even at high doses exhibited only a slight suppressive effect on PHA proliferation in PBMC in some HIV-infected patients and controls (Table 3). As we found for IFN-α, addition of low IP-10 doses caused a modest and non-significant increase (~10%) in proliferation (data not shown).

The effect of IFN-α on PHA-stimulated apoptosis
To elucidate the mechanisms for the inhibitory effect of IFN-α on lymphocyte proliferation, we tested whether IFN-α influenced PHA-induced apoptosis in PBMC from two blood donors and two HIV-infected patients on HAART. The HIV-infected patients had markedly enhanced PHA-stimulated lymphocyte apoptosis (annexin labelling and TUNEL assay, data not shown). However, in neither patients nor controls did IFN-α change the percentage of viable lymphocytes (propidium iodide staining) or the percentage of lymphocytes undergoing apoptosis (annexin labelling and TUNEL assay, data not shown).

DISCUSSION

HIV infection is associated with significant abnormalities of cytokine production [7,29–31], and there are reasons to believe that this dysregulated cytokine network may play an important pathogenic role in the progression of HIV-related disease [32]. The present study, by demonstrating high levels of both IP-10 and IFN-α in HIV-infected patients, with particularly high levels in those with most advanced clinical and immunological disease, further supports this hypothesis.

Treatment of HIV-infected patients with HAART has a profound down-regulatory effect on HIV replication *in vivo* and is associated with increases in CD4+ and CD8+ T cell counts [18]. In the present study we found that HAART induced a marked decrease in plasma concentrations of IFN-α, neopterin and IP-10, though not to normal levels. Furthermore, while the neopterin and IP-10 levels were significantly decreased throughout the study period, IFN-α levels returned to baseline at the end of the study. These observations support the notion that full immunological normalization may not be achieved during HAART. Even more importantly, our findings suggest that persistently high levels of IP-10, and particularly IFN-α, are associated with lack of immunological response as reflected by CD4+ cell increase. Notably, a lack of decline or even a rise in these cytokines was an early indicator of immunological treatment failure, and as for IFN-α, even high baseline levels appear to predict such treatment failure. Furthermore, our finding of a marked suppressive effect of IFN-α on PHA-induced PBMC proliferation, even in concentrations comparable to circulating levels in HIV-infected patients, suggests that the association between immunological treatment failure and persistently high IFN-α levels are not only parallel phenomena, but rather reflect an important immunopathogenic role of IFN-α associated with impaired numerical and functional restoration of CD4+ T cells during HAART.

IFN-α has previously been reported to impair proliferation of various cell types [1,33], and in the present study we found a markedly suppressive effect of exogenously added IFN-α on PHA-stimulated lymphocyte proliferation in both HIV-infected patients and controls, which appears not only to reflect a decreased proportion of viable cells when thymidine was added to cell cultures.

Table 3. Effect of exogenously added IFN-α on phytohaemagglutinin (PHA)-stimulated proliferation of peripheral blood mononuclear cells

	PHA-stimulated proliferation (ct/min)	Maximal percent decrease with addition of IFN-α (100 ng/ml)
HIV-infected patients ($n = 6$)	77 600 (61 300–151 800)	41* (31–50)%
Controls ($n = 5$)	172 600 (145 300–216 900)	38* (29–45)%

Data are given as medians and 25–75th percentiles.

*$P < 0.05$ *versus* PHA-stimulated proliferation. As for IP-10, a median reduction in PHA stimulated proliferation of 4% and 1% (patients and controls, respectively) was seen at the highest concentration used (10 ng/ml).

Although IFN-α has been reported to promote apoptosis in some cell lines [34–36], this was not observed in PHA-stimulated PBMC. The mechanisms for the suppressive effect of IFN-α on lymphocyte proliferation are therefore unclear, but may involve factors such as enhanced production of cytokines with anti-proliferative effects (e.g. IL-10) [37], and/or disturbances of specific components of the cell cycle control apparatus (e.g. cyclins and cyclin-dependent kinases) [34,35]. While the plasma concentrations of IFN-α in the majority of patients were below the concentration which would be expected to have suppressive effects on lymphocyte proliferation, concentrations of both IFN-α and other relevant cytokines are probably considerable higher in lymphoid tissue. Thus whatever the mechanisms, our findings, in addition to the recent demonstration of inhibitory effects of IFN-α on production of chemokines [17] and IL-12 [38], suggest a role for IFN-α in HIV-related disease and immunodeficiency.

The present study is to our knowledge the first report of elevated IP-10 levels during HIV infection. IP-10 has been shown to be produced by a variety of cell types [20,21], and although it was named as a protein inducible by IFN-γ, other agents including IFN-α have been found to induce IP-10 expression *in vitro* [20,39]. Interestingly, we found a significant correlation between IFN-α and IP-10 levels at all time points during HAART, possibly reflecting *in vivo* induction of IP-10 production by IFN-α. Furthermore, and with relevance to HIV infection, IP-10 seems to be predominantly chemotactic to activated T cells and enhanced IP-10 levels have been suggested to play a pathogenic role in various inflammatory disorders [20,40,41]. Similar mechanisms may also be operating in HIV infection, possibly favouring a persistent 'inflammatory stress' which again may be related to disease progression. On the other hand, IP-10 may have potential beneficial effects in HIV-infected patients, e.g. by impairing the development of Kaposi's sarcoma [42]. Although the biological significance of raised IP-10 levels in HIV infection is unclear, IP-10 may represent a 'new' chemokine involved in the pathogenesis of HIV infection.

The role of IFN-α in HIV infection is complex and only incompletely understood. Several *in vitro* studies have shown that IFN-α has significant anti-retroviral activities affecting the ability of HIV to infect and replicate in its target cell [2,6–8]. Moreover, beneficial effects of IFN-α in the treatment of early HIV-infection with stabilizing effect on CD4$^+$ T cell counts have also been reported in addition to its effect on Kaposi's sarcoma [7,14,43]. On the other hand, elevated serum levels of IFN-α and its surrogate markers are found in patients with advanced HIV disease, suggesting an association with adverse clinical outcome [6,15,16].

Moreover, although IFN-α may have anti-viral effects against HIV, Gingeri *et al.* demonstrated clinical and immunological beneficial effects of anti-IFN-α immunization in HIV-infected patients [44]. Furthermore, as also suggested by the present study, IFN-α has been found to mediate immunosuppression rather than immunostimulation in HIV-infected patients [17]. Thus, any anti-viral effects IFN-α may have in HIV infection may be overshadowed by detrimental effects on the immune system.

The failure of state-of-the-art HAART regimens to totally eradicate the virus, the emergence of drug-resistant strains, serious long-term side effects and in particular the persistence of HIV-associated immunodeficiency, call for additional immunomodulating therapy in HIV infection. The present study suggests that further exploration of IFN-α as target for immunomodulating therapy in HIV-infected patients might be rewarding.

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