Recombinant Interferon-Alpha Selectively Inhibits the Production of Interleukin-5 by Human CD4+ T Cells

Liliane Schandené,* GianFranco F. Del Prete,‡ Elie Cogan,§ Patrick Stordeur,* Alain Crusiaux,* Bernard Kennes,‖ Sergio Romagnani,‡ and Michel Goldman*

**Department of Immunology and §Department of Internal Medicine, Hôpital Erasme-Cliniques Universitaires de Bruxelles, B-1070 Brussels, Belgium; ‡Division of Clinical Immunology and Allergology, Istituto di Clinica Medica, University of Florence, I-50134, Italy; and ‖Centre Hospitalo-Universitaire Vésale, B-6110 Montigny-le Tilleul, Belgium*

Abstract

The effects of recombinant IFN-α on the production of IL-5 by human CD4+ T cells were first analyzed on resting CD4+ T cells purified from normal PBMC and stimulated either with a combination of PMA and anti-CD28 mAb or anti-CD3 mAb cross-linked on B7-1/CD32-transfected mouse fibroblasts. We found that IFN-α profoundly inhibited in a dose-dependent manner IL-5 production by resting CD4+ T cells whereas IL-10 was upregulated in both systems. The addition of a neutralizing anti–IL-10 mAb to PMA and anti-CD28 mAb upregulated IL-5 production by resting CD4+ T cells but did not prevent IFN-α–induced IL-5 inhibition. We then analyzed the effect of IFN-α on the production of cytokines by differentiated type 2 helper (Th2) CD4+CD3− cells isolated from peripheral blood of two patients with the hypereosinophilic syndrome. In both cases, IFN-α markedly inhibited IL-5 production while it induced mild upregulation of IL-4 and IL-10. Finally, the inhibitory effect of IFN-α on IL-5 production was confirmed on a panel of Th2 and Th0 clones generated in vitro. In 2 out of 6 clones, IL-5 inhibition was associated with upregulation of IL-4 and IL-10. We conclude that IFN-α selectively downregulates IL-5 synthesis by human CD4+ T cells. (*J. Clin. Invest.* 1996. 97: 309–315.) Key words: interferon-alpha • interleukin-5 • interleukin-10 • eosinophilia • T-lymphocyte-subsets

Introduction

Recombinant IFN-α therapy has been successfully used in diseases characterized by accumulation of eosinophils such as the hypereosinophilic syndrome (1–3) and atopic dermatitis (4, 5). In these disorders, IFN-α might act directly on eosinophils by inhibiting their differentiation and proliferation (6) as well as their recruitment into tissues (7). Another possible target of IFN-α is represented by the CD4+ T cells secreting interleukin IL-5. Indeed, IL-5 emerged as the major cytokine involved in the production, activation and recruitment of eosinophils (reviewed in 8, 9). In vivo, IL-5 was found responsible for the development of eosinophilia in murine models of parasitic diseases (10, 11) and increased production of IL-5 was observed in patients with the hypereosinophilic syndrome (12, 13). IL-5 is produced together with IL-4 and IL-10 by type 2 helper (Th2)[1] T cells but can also be secreted by unpolarized resting or type 0 helper (Th0) CD4+ T cells (14–17). IFN-α was previously shown in mice to inhibit IL-4 and enhance IFN-γ production and thereby to promote type 1 helper (Th1) responses (18). In man, addition of IFN-α to cultures of PBMC before long-term cloning was found to favor the development of T cell clones with a Th1-type (IL-2, IFN-γ) rather than a Th2-type (IL-4, IL-5) cytokine profile (19). Along the same line, addition of IFN-α to neonatal T cells during stimulation with anti-CD3 mAb inhibited their production of IL-4 and IL-5 upon restimulation with PMA and ionomycin (20). However, the effects of IFN-α on the production of Th2-type cytokines by resting CD4+ T cells or by differentiated Th2 cells have not been specified so far.

In the present study, we determined the effects of IFN-α on the production of IL-4, IL-5, and IL-10 by resting CD4+ T cells purified from normal PBMC, by in vivo differentiated Th2 cells isolated from two patients with the hypereosinophilic syndrome, and by Th2 and Th0 cell clones generated in vitro. As we found that the inhibition of IL-5 production by IFN-α was often associated with an enhanced IL-10 production and since IL-10 was recently shown to inhibit IL-5 production (17), we evaluated the possible role of IL-10 in the control of IL-5 production by IFN-α.

Methods

Reagents. Human recombinant IFN-α 2b with a specific activity of 1.10^6 IU/ml was obtained from Schering-Plough (Innishannon, Ireland). Human recombinant IFN-γ with a specific activity of 2.10^6 IU/ml was provided by Boehringer Ingelheim Inc. (Brussels, Belgium). Anti-CD56, -CD19, -DR, -CD14, -CD8 monoclonal antibodies (mAb) were all purchased from Becton Dickinson (Mountain View, CA). Anti-CD28 mAb (clone CD28. 2) was obtained from Immunotech (Marseille, France). The CLB-T3/4.1 anti-CD3 mAb (IgG1) was a gift from Dr. R. van Lier (Central Laboratory of the Netherlands, Red Cross, Amsterdam, The Netherlands). The 1F9 neutralizing anti–human IL-10 mouse IgG1 mAb was kindly provided by Dr. D. De Groote (Medgenix, Fleurus, Belgium). PMA (Sigma Chemical Co., St. Louis, MO) was dissolved in dimethylsulfoxide (Sigma Chemical Co.) to obtain a 1 mg/ml stock solution.

Transfectant cell lines. The mouse fibroblast cell line 3T6 expressing human CD32 (FcγRII) in association with the B7-1/BB1 antigen was kindly provided by Dr. M de Boer (Innogenetics, Ghent, Belgium). Transfected cells were incubated for 5 h with 10 μg/ml mitomycin-C (Kyowa; Christiaens, Brussels, Belgium), then washed and

Address correspondence to Michel Goldman, M.D., Hôpital Erasme - Department of Immunology, 808, route de Lennik, B-1070 BRUSSELS, Belgium. Phone: 2.555.39.25; FAX: 2.555.44.99.

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1. *Abbreviation used in this paper:* Th2, type 2 helper T cell.

used in co-culture with resting $CD4^+$ T cells, as previously described (17).

Isolation of resting $CD4^+$ T cells from peripheral blood of normal donors. Whole PBMC were isolated from buffy coats of healthy donors by density gradient centrifugation on Lymphoprep (Nycomed, Oslo, Norway). After three washings in Hank's balanced salt solution (HBSS) (GIBCO, Life Technologies, Paisley, Scotland), cells were resuspended in tissue culture medium RPMI 1640 (GIBCO) supplemented with 2 mM L-glutamine, gentamicin (20 mg/ml) and 10% FCS (GIBCO). T lymphocytes were purified using one cycle of Lymphokwik-T treatment (One Lambda, Los Angeles, CA). The T cell suspensions were further incubated with anti-CD56, anti-CD19, anti-DR, anti-CD14, and anti-CD8 mAbs for 30 min at 4°C, washed and incubated with immunomagnetic particles coated with goat anti–mouse IgG (Immunotech, Marseille, France). After 1 h incubation at 4°C, uncoated cells were removed using a magnet. The resulting cell preparations routinely contained > 98% viable $CD4^+$ T cells as shown by flow cytometry.

Isolation of $CD4^+$ $CD3^-$ cells of two patients with the hypereosinophilic syndrome. Two patients with the hypereosinophilic syndrome were included in this study. Patient 1 who presented a monoclonal $CD4^+$ $CD3^-$ Th2-like population in peripheral blood was described in a previous report (21). Patient 2 was a 20-yr-old girl who presented massive hypereosinophilia (blood eosinophil count 8910/mm^3) and ankle tenosynovitis associated with eosinophilic infiltration. Immunophenotyping of PBMC revealed an abnormal $CD4^+CD3^-CD2^+$ T cell population representing 85% of total $CD4^+$ cells (10590/mm^3). This population differed from that of patient 1 in that no monoclonality was detected by Southern blot analysis for TCR genes. $CD4^+$ $CD3^-$ T cells of both patients were prepared from $CD4^+$ cells by the selective depletion of $CD3^+$ cells through incubation with anti-CD3 mAb (Ortho Biotech, Raritan, NJ) and immunomagnetic particles coated with goat anti–mouse IgG (Immunotech, Marseille, France), as previously described (21). The resulting cell preparations contained < 1% $CD3^+$ cells as determined by flow cytometry. To study the in vitro effects of IFN-α, cells from patient 1 were collected when he was on low dose methylprednisolone (16 mg/d) while patient 2 was not treated at the time of blood sampling.

Human Th2 and Th0 clones generated in vitro. Th2 and Th0 T cell clones were generated in vitro by antigen-specific stimulation, as previously described (22). The effects of IFN-α were studied on the following clones: GTT6 and DKT8, Th2 clones specific for tetanus toxoid, NDT39 and NDT67, Th2 clones specific for the excretory/secretory antigen of Toxocara canis, and DUP 23 and AR37, Th0 clones specific for tetanus toxoid and purified Lolium perenne group I allergen, respectively.

Procedure of T cell stimulation for induction of cytokine production. T cells were incubated at 37°C and 5% CO_2 in flat-bottomed 96-well plates with either PMA (1 ng/ml) and anti-CD28 mAb (1 μg/ml) or anti-CD3 mAb (100 ng/ml) cross-linked on B7-1/CD32 transfectants (10^4 cells/well) in a total volume of 200 μl. T cells were seeded at 1.10^5/well when stimulated with PMA and anti-CD28 mAb, and at 2.10^5/well in the experiments using transfectants. These procedures were chosen because they were previously found to induce optimal IL-5 secretion (17). After 48 h of incubation, culture supernatants were collected and stored at −20°C until assayed for cytokine determinations. In preliminary experiments (not shown), this timing was found the most appropriate for the determination of IL-4, IL-5, and IL-10 production.

Determinations of cytokine levels. As previously described (17), IL-5 levels in culture supernatants were determined by sandwich ELISA using the following anti–human IL-5 mAbs: H30 rat anti–human IL-5 mAb IgG2b (Roche Research, Ghent, Belgium) as coating mAb and mAb7 mouse anti–human IL-5 mAb IgG1 (kindly provided by Glaxo Group Research Limited, Greenford, Middlesex, UK) as second mAb. IL-5 levels in serum were determined by an ELISA obtained from Pharmingen (San Diego, CA). This assay for serum IL-5 was found more reliable that the one we previously used (21) as it correlates more closely with a bioassay on cells transfected with the human IL-5 receptor (kindly provided by Dr. R. Devos, Roche Research Institute, Ghent, Belgium). Commercially available kits were used for determination of IL-4 and IL-10 (Medgenix, Fleurus, Belgium). Lower limits of detection were 10 pg/ml for IL-5, 16 pg/ml for IL-4, and 11 U/ml for IL-10.

PCR analysis of IL-4, IL-5, and IL-10 gene expression. Total RNA from purified $CD4^+$ T cells was extracted using the acid guanidinium thiocyanate-phenol-chloroform extraction method and analyzed for IL-4, IL-5, IL-10, and β-actin mRNA by a reverse PCR method, as previously described (23). Briefly, 1 μg of total RNA was reverse-transcribed and 5 μl of the resulting cDNA were subjected to 35 PCR cycles. Each cycle was performed at 94°C for 1 min, at 55°C for 1 min and at 72°C for 1 min. IL-4, IL-5, IL-10, and β-actin primers were synthetized according to the human cDNA sequences. The primers had the following sequences: IL-4, 5′-TGCCTCCAAGAACACAACTG-3′ (sense), 5′-AACGTACTCTGGTTGGTCTTC-3′ (anti-sense); IL-5, 5′-CTTGGCACTGCTTTCTACTC-3′ (sense) and 5′-GCAGGTAGTCTAGGAATTGG-3′ (anti-sense); IL-10, 5′-AAATTTGGTTCTAGGCCGGG-3′ (sense) and 5′-GAGTACAGGGGCATGATATC-3′ (anti-sense); β-actin, 5′-GGGTCA-

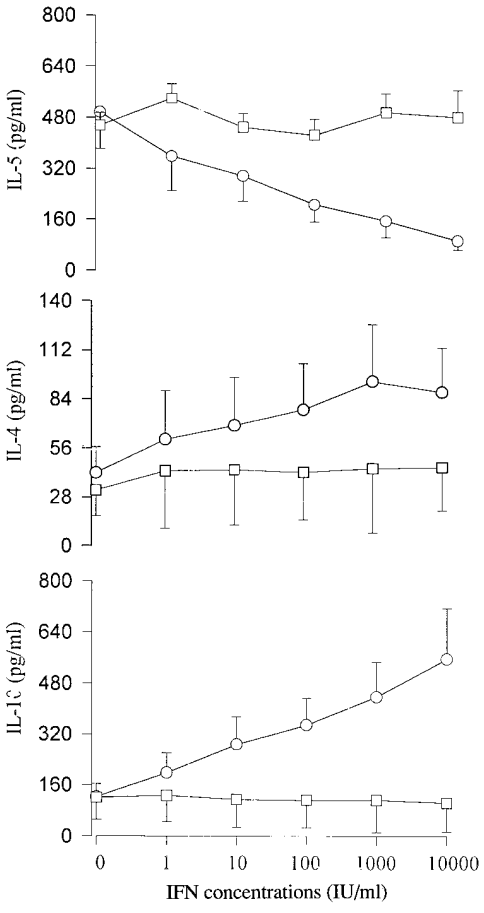

Figure 1. Differential effects of IFN-α on the production of IL-5, IL-4 and IL-10 by resting $CD4^+$ T cells from healthy individuals. Resting $CD4^+$ T cells (10^5/well) were stimulated with PMA (1 ng/ml) and anti-CD28 mAb (1 μg/ml) in the absence or presence of graded concentrations of IFN-α (○) or IFN-γ (□). After 48 h, IL-5, IL-4, and IL-10 levels in culture supernatants were determined by ELISA. Data are shown as mean±SEM of five independent experiments on five different individuals for IFN-α and three experiments on three different individuals for IFN-γ.

GAAGGATTCCTATG-3′ (sense) and 5′-GGTCTCAAACATGATCTGGG-3′ (anti-sense). The expected size of the PCR fragments for IL-4, IL-5, IL-10 and β-actin was 224, 259, 264, and 237 bp, respectively.

Results

IFN-α inhibits IL-5 but enhances IL-4 and IL-10 secretion by human resting CD4⁺ cells. We have recently demonstrated that resting T cells secreted significant levels of IL-5 provided that they are costimulated by B7/CD28 signalling (17). Therefore, we first evaluated the effects of IFN-α on resting CD4⁺ cells stimulated with PMA in conjunction with anti-CD28 mAb. As shown in Fig. 1, IFN-α inhibited in a dose-dependent manner the production of IL-5. Substantial inhibition of >25% was already observed with 1 U/ml IFN-α, a maximal inhibition of 80% being obtained at a concentration of 1.10^4 IU/ml IFN-α. In the same system, IFN-α upregulated IL-10 production in a dose-dependent manner with a fourfold increase in IL-10 levels in the presence of 10^4 IU IFN-α. Only low levels of IL-4 were produced under this condition and this low IL-4 production was slightly enhanced by IFN-α. These effects of IFN-α

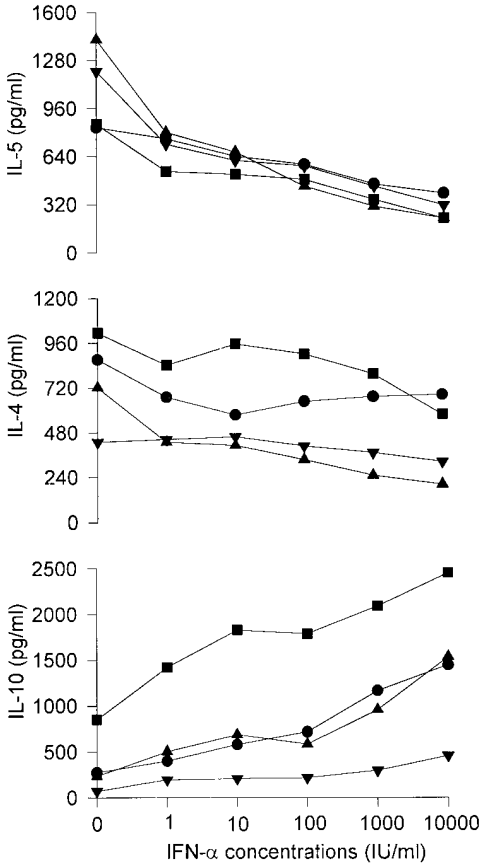

Figure 2. Differential effects of IFN-α on the production of IL-5, IL-4, and IL-10 by resting CD4⁺ T cells from healthy individuals. Resting CD4⁺ T cells (2.10^5/well) obtained from 4 different individuals were stimulated with the CLB-T3/4.1 anti-CD3 mAb (100 ng/ml) cross-linked on B7-1/CD32 transfected fibroblasts (10^4/well) in the absence or presence of graded concentrations of IFN-α. After 48 h, IL-5, IL-4, and IL-10 levels in culture supernatants were determined by ELISA.

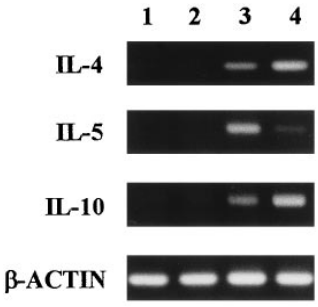

Figure 3. Differential effects of IFN-α on IL-4, IL-5, and IL-10 mRNA accumulation induced by PMA (1 ng/ml) and anti-CD28 mAb (1 μg/ml) in resting CD4⁺ T cells. Resting CD4⁺ T cells (10^6/ml) were incubated in medium alone (lane *1*), in IFN-α alone (lane *2*), or stimulated with PMA (1 ng/ml) and anti-CD28 mAb (1 μg/ml) in the absence (lane *3*), or presence of 1.10^4 IU/ml IFN-α (lane *4*). After 24 h, mRNA was extracted and analyzed by reverse PCR for IL-4, IL-5, IL-10, and β-actin mRNA.

in five independent experiments on five different donors were statistically significant using the one-way ANOVA test ($P < 0.005$ for each cytokine). In parallel, we evaluated in three experiments the effects of IFN-γ. As shown in Fig. 1, even high doses of IFN-γ (up to 10^4 IU/ml) did not affect neither IL-5 nor IL-10 and IL-4 production (Fig. 1).

We also evaluated the influence of IFN-α on the secretion of cytokines by resting CD4⁺ T cells stimulated by anti-CD3 mAb cross-linked on B7-1/CD32 transfectants. As illustrated in Fig. 2, the differential effects of IFN-α on IL-5 and IL-10 production were also clearly apparent in this system while no consistent effect on IL-4 production was observed.

IFN-a reduces IL-5 but increases IL-4 and IL-10 mRNA accumulation in CD4⁺ T cells activated by PMA and anti-CD28 mAb. To analyze whether IFN-α interfered with IL-5, IL-4, and IL-10 gene expression, we stimulated CD4⁺ T cells with PMA and anti-CD28 mAb in the absence or presence of IFN-α (10^4 IU/ml) and determined IL-5, IL-4, and IL-10 mRNA accumulation by reverse PCR after 24 h of culture. As shown in Fig. 3, IFN-α clearly inhibited IL-5 mRNA accumulation while it increased IL-10 mRNA and IL-4 mRNA accumulation in this system. These effects of IFN-α were observed in three independent experiments on CD4⁺ T cells obtained from 3 different individuals.

The inhibitory effect of IFN-α on IL-5 production is not mediated by IL-10. As we recently demonstrated that IL-10 downregulates IL-5 production by human resting T cells (17), we evaluated the possible role of IL-10 upregulation in the inhibition of IL-5 synthesis by IFN-α. In these experiments, we added a neutralizing anti–IL-10 mAb or an isotype-matched control mAb to CD4⁺ T cells stimulated with PMA and anti-CD28 mAb in the absence or presence or IFN-α. The effective neutralization of endogenous IL-10 in this system was verified by the disappearance of immunoreactive IL-10. Moreover, we confirmed that in the absence of IFN-α, addition of anti–IL-10 mAb resulted in an increased IL-5 production (Fig. 4). As shown in Fig. 4, addition of the neutralizing anti–IL-10 mAb did not prevent the inhibitory effect of IFN-α, indicating that IFN-α–induced IL-5 downregulation is independent of IL-10 upregulation.

IFN-α inhibits IL-5 but not IL-4 production by CD41 cells from two patients with the hypereosinophilic syndrome. To further analyze the regulation of IL-5 production by IFN-α, we examined the effects of IFN-α on the production of cytokines by CD4⁺ CD3⁻ cells isolated from two patients with the hyper-

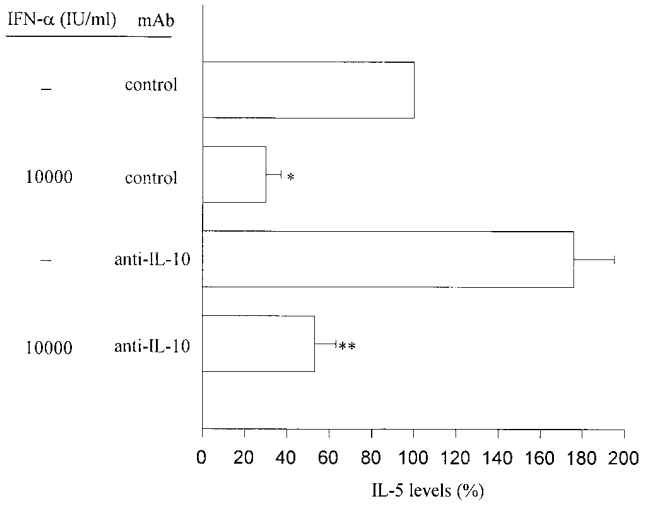

Figure 4. Neutralization of endogenous IL-10 does not prevent the inhibitory effect of IFN-α on IL-5 production. Resting $CD4^+$ T cells were cultured with PMA (1 ng/ml) and anti-CD28 mAb (1 μg/ml) with or without IFN-α (1.10^4 IU/ml), in presence of either a neutralizing anti–human IL-10 mAb (25 μg/ml) or an isotype-matched control mAb (25 μg/ml). After 48 h, IL-5 levels in culture supernatants were determined by ELISA. Results were expressed as percentages of IL-5 levels (mean±SEM in five independent experiments) by reference to control condition (PMA and anti-CD28 mAb together with control mAb). $*P < 0.005$ vs. control mAb in absence of IFN-α; $**P < 0.005$ vs. anti–IL-10 mAb in absence of IFN-α (one way ANOVA test).

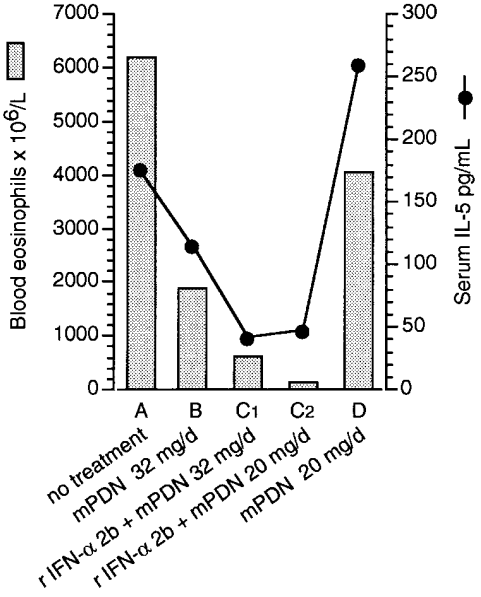

Figure 5. In vivo effects of IFN-α on serum IL-5, blood eosinophils and IgE serum levels in a patient with a clonal Th2 disease. Eosinophil blood count, serum IL-5 and serum IgE levels were measured in patient 1 before any treatment (period A), during administration of methylprednisolone (mPDN) alone (periods B and D), and under mPDN and IFN-α therapy (periods C1 and C2). Each value is the average of 2 to 4 determinations. Period B corresponds to the 10 d period immediately preceding IFN-α administration and period C1 corresponds to the first 4 wk of IFN-α administration.

eosinophilic syndrome. When stimulated by PMA + anti-CD28 mAb, $CD4^+$ $CD3^-$ cells from both patients produced much higher levels of IL-4 and IL-5 than $CD4^+$ cells from healthy individuals whereas they were profoundly deficient in IFN-γ production (Table I). They can thus be considered as Th2-like cells although they did not secrete high levels of IL-10 (Table I). A shown in Table I, IFN-α selectively inhibited IL-5 secretion by those cells, while IL-4 and IL-10 production tended to be increased. The defect in IFN-γ production was not corrected by the addition of IFN-α.

As IL-5 (but neither IL-4 nor IL-10) was detected in the serum of the patient with the monoclonal Th2 cell population (patient 1), it was possible to assess the effects of IFN-α on IL-5 production in vivo when this patient received IFN-α therapy. This treatment was initiated because methylprednisolone alone (mPDS) up to 32 mg/d did not allow to control the hypereosinophilia (period B). As shown in Fig. 5, IFN-α administration was clearly associated with a decrease in both blood eosinophil counts and serum IL-5 (period C1) allowing to taper mPDS daily dose to 20 mg (period C2). When IFN-α therapy had to be withdrawn because of leucopenia (period D), both IL-5 and blood eosinophil count rapidly increased to levels higher than before IFN-α administration (Fig. 5).

Effects of IFN-α on the production of IL-4, IL-5, and IL-10 by Th2 and Th0 clones generated in vitro. In a final series of experiments, we evaluated the effects of IFN-α on the production of IL-5, IL-4, and IL-10 by a panel of Th2 and Th0 clones stimulated by PMA and anti-CD28 mAb. As shown in Fig. 6,

Table I. Differential Effects of IFN-α on the Production of Cytokines by $CD4^+$ $CD3^-$ T Cells in Two Patients (P1 and P2) with the Hypereosinophilic Syndrome

	IFN-γ (IU/ml)‡			IL-4 (pg/ml)‡			IL-5 (pg/ml)‡			IL-10 (pg/ml)‡		
IFN-α added*	Controls ($n = 3$)	P1	P2	Controls ($n = 3$)	P1	P2	Controls ($n = 3$)	P1	P2	Controls ($n = 3$)	P1	P2
–	59 (±34)	< 6	6	23 (±16)	355	827	456 (±66)	4260	10400	121 (±65)	20	55
+	221 (±128)	< 6	18	44 (±20)	390	1138	64 (±27)	520	4420	317 (±69)	95	366

*$CD4^+$ T cells (10^5/well) from three healthy individuals (controls) and $CD4^+$ $CD3^-$ T cells from two patients (P1 and P2) with the hypereosinophilic syndrome were stimulated with PMA (1 ng/ml) and anti-CD28 mAb (1 μg/ml) in the absence or presence of 1.10^4 IU/ml IFN-α. ‡IFN-γ, IL-4, IL-5, and IL-10 levels (mean±SEM for controls) in 48h culture supernatants as determined by ELISA. Data from one out of three different experiments which gave similar results.

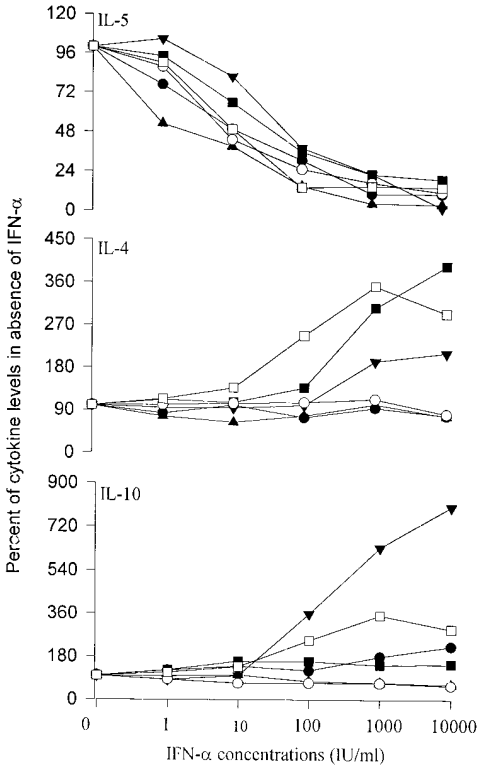

Figure 6. Effects of IFN-α on the production of IL-5, IL-4, and IL-10 by Th2 and Th0 clones. Four Th2 (*closed symbols*) and 2 Th0 (*open symbols*) clones were stimulated with PMA (1 ng/ml) and anti-CD28 mAb (1 μg/ml) in the absence or presence of graded concentrations of IFN-α. After 48 h, IL-5, IL-4, and IL-10 levels in culture supernatants were determined by ELISA.

IFN-α dramatically inhibited in a dose-dependent manner the production of IL-5 by the 6 clones that we tested. On the other hand, the effects of IFN-α on the production of IL-4 and IL-10 was variable from clone to clone, the levels of these cytokines being either unchanged or increased by IFN-α. Indeed, both IL-4 and IL-10 were upregulated by IFN-α in 1 out of 4 Th2 clones and in 1 out of 2 Th0 clones (Fig. 6). In one Th2 clone, IL-4 was upregulated without significant changes in IL-10 levels while IL-4 and IL-10 levels were unaffected by IFN-α in the remaining clones.

Discussion

So far, the effects of IFN-α on the profile of cytokines secreted by human T cells has been mainly studied in systems where IFN-α was added in bulk cultures of PBMC before in vitro restimulation. In these systems, IFN-α clearly inhibited the differentiation of Th2 cells producing IL-4 and IL-5 (19, 20). As far as IL-10 production is concerned, experiments on neonatal T cells indicated that IFN-α primed those cells for IL-10 synthesis (20). In the present study, we first compared the direct effects of IFN-α and IFN-γ on the production of Th2-type cytokines by resting $CD4^+$ cells stimulated by PMA and anti-CD28 mAb, a system previously shown to elicit optimal production of IL-5 (17). In contrast with IFN-γ which did not significantly influence the secretion of the three cytokines considered, IFN-α profoundly inhibited IL-5 while it upregulated IL-4 and IL-10 production. The differential modulation of the production of IL-5, IL-4 and IL-10 by IFN-α was also apparent at the mRNA level, indicating that IFN-α influences either the transcription or the mRNA stability of corresponding genes. Both mechanisms were previously shown to be operative in the inhibition of IL-8 production by IFN-α (24). The opposite effects of IFN-α on the production of IL-5 and IL-10 were also observed when resting $CD4^+$ T cells were stimulated by anti-CD3 mAb cross-linked on B7-1/CD32 transfectants, a system which mimics antigenic stimulation in presence of B7-mediated costimulation. Moreover, IFN-α was found to inhibit IL-5 production by differentiated Th2 cells obtained from the two patients with the hypereosinophilic syndrome, as well as by Th2 and Th0 clones generated in vitro. Although the inhibition of IL-5 production was observed with all clones, upregulation of both IL-4 and IL-10 was observed in only 2 out of 6 clones. In the other clones, IFN-α either upregulated IL-4 alone or had no significant effects neither on IL-4 nor on IL-10. The main conclusions of these experiments are twofold: (1) among the Th2-type cytokines, IFN-α selectively inhibits IL-5 and (2) this effect of IFN-α is independent on the differentiation/activation stage of $CD4^+$ T cells. This might be relevant to the therapeutic effects of IFN-α in hypereosinophilic syndrome. Indeed, the clinical benefit of IFN-α administration in patient 1 was associated with a decrease in serum IL-5 levels. Besides its action on IL-5-producing $CD4^+$ T cells, IFN-α might also act in the hypereosinophilic syndrome by its direct inhibitory effects on eosinophil precursors (6) and on the recruitment of eosinophils into tissue (7).

Since IL-10 was recently shown to inhibit IL-5 expression by resting T cells (17), we evaluated the role of endogenous IL-10 in the control of IL-5 by IFN-α. Our finding that the inhibition of IL-5 production by IFN-α was not modified by addition of a neutralizing anti–IL-10 mAb indicated that this effect of IFN-α was not related to IL-10 upregulation. This was confirmed in the experiments with T cell clones since IL-5 inhibition without IL-10 upregulation was observed in four of them. However, the IL-10 upregulation observed on resting $CD4^+$ T cells and on some T cell clones suggest that IL-10 could mediate some of the antiinflammatory properties of IFN-α, together with its ability to inhibit IL-8 production by PBMC (24) and to induce the IL-1 receptor antagonist (25). As a matter of fact, IL-10 is a potent deactivating macrophage cytokine that inhibits the release of several inflammatory mediators (reviewed in 26). Along this line, it is interesting that preliminary results indicate that IFN-α might be effective in Crohn's disease (27), a disease that might be linked to IL-10 deficiency, as suggested by the intestinal pathology of IL-10 knock-out mice (28). As IFN-β binds to the same type-1 receptors than IFN-α (reviewed in 29) and since recent reports indicate that IL-10 is protective in experimental autoimmune encephalomyelitis (30), one can also speculate that the beneficial effects of IFN-β in multiple sclerosis (31) involve IL-10 upregulation.

Although the production of IL-4 is parallel to that of IL-5 in several settings, our data indicate that these two cytokines can be differentially regulated by IFN-α: IL-5 production was strongly inhibited whereas IL-4 production was either upregulated or unchanged by IFN-α. The differential regulation of IL-4 and IL-5 production was previously observed in other experimental systems. For example, cycloheximide was found to inhibit the expression of IL-5 mRNA but not of IL-4 mRNA

induced by anti-CD3 mAb in murine TH2 clones while cyclosporin A had opposite effects in the same experimental setting (32). As far as IFN-α is concerned, its effects on IL-4 production appear much less predictable than on IL-5 and depend on the system considered. In previous studies, IFN-α was shown to inhibit IL-4 mRNA accumulation in spleen cells after injection of mice with anti-IgD antibody (18), to prevent the priming of neonatal T cells for IL-4 production (20) and to slightly down-regulate the hyperproduction of IL-4 by PBMC from patients with Sezary syndrome (33). On the other hand, IFN-α did not influence the frequency of IL-4 producing cells upon polyclonal activation of normal human $CD4^+$ T cells while it increased the frequency of IFN-γ producing cells (34). The molecular basis for these variable effects of IFN-α on IL-4 production remains to be specified.

Taken together with previous observations (7, 18–20), our data provide a rationale basis for the therapeutic use of IFN-α therapy in T cell–mediated disorders associated with IL-5 hyperproduction. These disorders might involve clonal or nonclonal expansion of Th2 cells such as in cutaneous lymphomas (35), bronchial asthma (36), and other atopic diseases (37, 38), and certain forms of the hypereosinophilic syndrome (21). In these diseases, IFN-α could exert inhibitory effects at multiple levels including $CD4^+$ T cell activation and proliferation (39), Th2 cell differentiation (18–20), $CD4^+$ T cell recruitment in tissues (7), and IL-5 secretion by $CD4^+$ T cells as shown in the present paper.

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