Interferon α Increases the Frequency of Interferon γ-producing Human CD4+ T Cells

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Summary

An increased ratio of T helper type 2 (Th2)- vs Th1-like cells contributes to the immune dysregulation in allergic disease situations and in many chronic infections, including AIDS. Th2-type immune responses are characterized by Th cells that produce increased levels of interleukin-4 (IL-4) and decreased levels of interferon γ (IFN-γ). The induction of either a Th1- or a Th2-like phenotype may be critically controlled by the antigen-presenting cells and their cytokines, e.g., IFN-α. In this study we have determined the frequencies of potential IL-4- and/or IFN-γ-producing T cells in the peripheral blood of randomly selected healthy individuals, and analyzed whether IFN-α controls IL-4 and/or IFN-γ production. Purified CD4+ or CD8+ T cells were stimulated for 24 h via the T cell receptor/CD3 complex in the presence or absence of IFN-α, and single IL-4- and IFN-γ-secreting cells were detected in enzyme-linked immunospot assays. In the absence of IFN-α, CD4 cells produced IFN-γ at frequencies of 1:50–300, and produced IL-4 at frequencies of 1:110–<1:100,000. Addition of IFN-α during the activation of CD4 cells increased the levels of IFN-γ mRNA. As a consequence, the numbers of IFN-γ-producing CD4 cells and the amounts of secreted IFN-γ increased 10-fold. In contrast, IFN-α did not increase the frequency of IL-4–secreting CD4 cells. In the absence of IFN-α, addition of exogenous IL-4 to cultures of CD4 cells suppressed IFN-γ secretion by 70%. However, in the presence of IFN-α, IL-4 did not display any suppressive effect. Compared with CD4 cells, CD8 cells produced IFN-γ more frequently (1:5–10) but IL-4 less frequently (1:5,300 to <1:100,000). IFN-α did not display any effect on the frequency of either IFN-γ or IL-4 production by CD8 cells. Taken together the results indicate that IFN-α increases the frequency of IFN-γ-secreting CD4 Th cells and antagonizes the suppressive effect of IL-4 on IFN-γ production. As a consequence, IFN-α may favor the induction and maintenance of Th1-like cells and thereby counteract Th2-driven allergic immune responses.

IFN-α has a wide range of immunomodulatory activities (1, 2). The cytokine has been used successfully for the treatment of viral infections (3–8), including AIDS (7, 8), and also displays beneficial effects in several tumor-associated diseases (9–12), and in allergic disorders (13, 14). Despite the broad indication range for IFN-α, the detailed mechanism of IFN-α action in vivo as well as its target cells have not been well defined. It has been found that IFN-α modulates the immunoglobulin isotype selection process, since it suppresses IgE production in an isotype-specific fashion in vitro (15) as well as in vivo (13, 16, 17). A direct effect of IFN-α on B cells is unlikely, since IFN-α does not inhibit IgE switching of human B cells upon physical interaction with preactivated helper cells in the absence of IFN-γ and in the presence of exogenously added IL-2 and IL-4 (18). It is tempting to speculate that IFN-α suppresses IgE production by modulating lymphokine expression by Th cells, since the immunoglobulin switch of B cells to IgE is induced by IL-4 (19–22), and antagonized by IFN-γ (15, 21, 22). This interpretation is supported by the finding that, in mice stimulated with a foreign anti-IgD antibody, IFN-α reduced the IgE and IgG1 response as well as the levels of splenic IL-4 mRNA, but increased the levels of splenic IFN-γ mRNA (16). However, since the mRNA levels were quantified in unfractionated spleen cells, it was not clear whether IFN-α acts on the level of IL-4 or IFN-γ gene expression, or whether the decreased levels of IL-4 mRNA reflect a selective accumulation of IFN-γ-producing cells in the spleen. With human cells it has been found that addition of IFN-α to cultures of PBMC before long-term T cell cloning favored the development of T cell clones that express a Th1, rather than a Th2, cytokine profile (23). Th1 cells mainly release IFN-γ and IL-2, while Th2 cells release IL-4, IL-5, and IL-10, the lymphokines involved in the control of allergic responses

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(24–29). The target cell as well as the mode of IFN-α action have not been clearly defined in either human or murine systems.

In this report we analyzed the effect of IFN-α on the expression of IL-4 and IFN-γ by freshly isolated human CD4⁺ Th cells and CD8⁺ cytotoxic-suppressive T cells. Since the secretion of IL-4 by T cells is generally low, and IL-4 is barely detectable in the culture supernatants due to rapid consumption of the lymphokine by the growing cells, we have established enzyme-linked immunospot (ELISPOT)[1] assays to detect single IL-4- and IFN-γ-secreting cells within 24 h of culture. Several reports from us and others have attested to the versatility of this methodological principle for enumerating cells that secrete antibody or lymphokines (30–35). We found that IFN-α increased the number of IFN-γ–secreting CD4 Th cells 10-fold within 24 h of culture, but that it did not regulate IFN-γ production by CD8 cells. Furthermore, IFN-α antagonized the suppressive effect of IL-4 on IFN-γ production, but did not affect the secretion of IL-4 by either CD4 or CD8 T cells. Based on this finding we propose that IFN-α favors Th1-driven immune responses by increasing IFN-γ production in the Th cell compartment.

Materials and Methods

IL-4. Recombinant human IL-4 was purified by gel filtration from extracts of *Escherichia coli* cells transfected with the human IL-4 gene as described elsewhere (36). The specific activity of 2.5 × 10^7 U/mg was comparable to preparations commercially available.

IFN-α. Recombinant human IFN-α B (alpha 8; Ciba-Geigy, Basel, Switzerland) with a specific activity of 2.5 × 10^7 IU/mg was used in all experiments. HuIFN-α B was calibrated against the National Institutes of Health standard G 023-901-527 of HuIFN-α by determining plaque reduction of vesicular stomatitis virus Indiana serotype on human embryonic foreskin diploid cells (37, 38).

T Cell Separation. PBMC were isolated by Ficoll-Hypaque centrifugation (39). CD4⁺ or CD8⁺ T cells were negatively selected on FACS® (Becton Dickinson & Co., Mountain View, CA) by depleting cells that express either CD8 or CD4, respectively, as well as CD14, CD16, and CD19. The sorted populations were >98% pure. The antibodies used for staining (all from Becton Dickinson & Co.) were FITC-labeled Leu3a (anti-CD4), Leu2a (anti-CD8), LeuM3 (anti-CD14), Leu12 (anti-CD19), and Leu11c (anti-CD16).

Detection of Single IL-4-secreting Cells (IL-4-SC) or IFN-γ-SC in ELISPOT Assays. mAbs to IL-4 (8F12, 3H4) (40, 41) and IFN-γ (23.9, 76.18) were produced inhouse. To perform ELISPOT assays, 96-well microtiter plates (Maxisorp; Nunc, Roskilde, Denmark) were coated with a mixture of anti-CD3 mAb OKT3 (American Type Culture Center; 1 μg/ml) plus either mouse anti–human IL-4 mAb 8F12 or mouse anti–human IFNγ mAb 23.9 (1 μg/ml). Plates were blocked with 2% BSA in PBS containing 0.05% NaN_3 (1 h, 37°C, 150 μl/well). Plates were washed, and isolated cells added in appropriate dilutions (10^5 to 10^3 cells/0.2 ml per well) into the coated ELISPOT plates. Plates were centrifuged (3 min, 50 *g*) and incubated at 37°C for 24 h to detect IFN-γ-SC and IL-4-SC. A longer incubation did not increase the numbers of either IL-4 or IFN-γ-spots. Cells were removed and developing antibodies added to the plates (IL-4: biotin-labeled mouse anti–human IL-4 mAb 3H4, 0.2 μg/well, 2 h, 37°C; IFN-γ: biotin-labeled mouse anti–human IFN-γ mAb 76.18, 0.2 μg/well, 2 h, 37°C). Plates were washed, and avidin-AP was added (0.2 μg/well, 2 h, 37°C; Zymed Labs., Inc., San Francisco, CA). After additional washing, substrate (bromo-4-chloro-3-indolylphospat, 0.1 μg/well; Sigma Chemical Co., St. Louis, MO) was added, and the development of visible ELISPOTS followed microscopically. After 30–50 min, plates were rinsed with H_2O and air dried. ELISPOTS were counted either by microscopy or by using an automated ELISPOT counter (ASBA, Basel, Switzerland).

Detection of IFN-γ in Culture Supernatants by ELISA. 96-well microtiter plates (Maxisorp) were coated with mouse anti–human IFN-γ mAb 23.9 (1 μg/ml). All plates were blocked with 2% BSA in PBS containing 0.05% NaN_3 (1 h, 37°C, 150 μl/well). After washing with PBS, test samples or control human IFN-γ were added in blocking buffer (100 μl/well) and incubated for 16 h at room temperature. After washing, mouse anti–human IFN-γ mAb 76.18 was added (0.2 μg/well, 2 h, 37°C). Plates were washed and incubated with goat anti–mouse Ig coupled to alkaline phosphatase (0.1 μg/well; Tago, Inc., Burlingame, CA). Plates were washed and phosphatase substrate was added (0.1 mg/well; Sigma Chemical Co.).

PCR Analysis. 48-well culture plates were coated for 24 h with anti-CD3 mAb (OKT3, 50 μg/ml) and washed. CD4 T cells (2 × 10^6/ml) were added and cultured for 12–48 h in the presence of IL-2 (50 U/ml) and various concentrations of IFN-α. Preactivated cells were lysed with 600 μl of guanidinium thiocyanate buffer (4 M guanidinium thiocyanate, 25 mM sodium citrate, pH 7, 0.5% sacrosyl, 0.1 M mercaptoethanol) and the cellular RNA was isolated by two cycles of acidic phenol extractions (42). The RNA pellet was dissolved in diethylpyrocarbonate-treated water and the concentration was determined by measuring the OD_{260} nm. The PCR amplifications were performed with the Gene Amp PCR kit (Perkin-Elmer Cetus, Norwalk, CT) and commercially available primers for IFN-γ, and beta-Actin (Clontech, Palo Alto, CA) according to the manufacturer's instructions. Briefly, 100 ng of total RNA were reverse transcribed with 5 U of reverse transcriptase at 37°C for 20 min, using as primers the specific PCR primers. Amplifications were performed for 30 cycles (1 min at 60°C, 1 min at 72°C, 1 min at 94°C) using a thermal reactor from Hybaid^R, Middlesex, UK. The amplified DNA was separated on a 1% agarose gel together with DNA size markers. The gel was stained with ethidium bromide and the bands were visualized by UV transillumination at 366 nM. The relative intensities of the bands were determined by scanning the polaroid picture of the gel with a video densitometer (Bio-Rad Laboratories, Richmond, CA) in the reflection mode. The values obtained with IFN-γ were standardized with respect to beta actin RNA levels, to correct for variations in RNA amount.

Northern Blot Analysis. 10 μg of RNA from each sample was separated on 1% agarose/6.6% formaldehyde gels (43) and transferred to gene screen membranes using a posiblotter (Stratagene, Inc., La Jolla, CA). After baking the filters at 80°C for 2 h and prehybridization for 6 h, the filters were hybridized (43) with the labeled probes for IFN-γ and 28S ribosomal RNA for 12–16 h at 42°C. The probes were prepared using standard techniques (43). Labeling was carried out using the random primer technique (44). The filters were washed three times at 55°C in 0.1× SSPE, 0.1% sodium dodecyl sulfate (20× SSPE = 3.6 M sodium chloride, 0.2 M sodium phosphate, 0.02 M EDTA, pH 7.4) and exposed to Kodak

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[1] *Abbreviations used in this paper:* ELISPOT, enzyme-linked immunospot; SC, secreting cells.

XAR X-ray films for 12–16 h. The relative intensities of the bands were determined by scanning the autoradiograph with a densitometer.

Results

Detection of Single IL-4- and IFN-γ-secreting Cells by ELISPOT Assay. Allergic immune responses in vivo depend on activated Th2 cells that produce increased levels of IL-4 and decreased levels of IFN-γ (45–48). To determine the frequency of T cells present in the peripheral blood that are capable of secreting IL-4 or IFN-γ upon stimulation via the TCR/CD3 complex, we developed ELISPOT assays that allow the detection of single IL-4–SC and of IFN-γ–SC within 24 h of culture. CD4 or CD8 T cells (10^3–10^5/well) were activated for 24 h in ELISPOT plates coated with a mixture of anti-CD3 mAb plus either anti-IFN-γ or anti-IL-4 mAb. This approach allowed simultaneous stimulation of the T cells and binding of the secreted lymphokine to the plate. As demonstrated in Fig. 1, neither IL-4- nor IFN-γ–SC were detectable, if the plates were only coated with the activating anti-CD3 mAb in the absence of specific mAb to IL-4 or IFN-γ, or if the plates were coated with anti-CD3 mAb plus an irrelevant antibody. In contrast, IL-4–SC (1:120) and IFN-γ–SC (1:70) were found, if plates were coated with anti-CD3 plus either anti-IL-4 or anti-IFN-γ mAb, respectively. The frequency of responding cells was independent of the cell density within concentrations between 10^5 and 10^3 cells/0.2 ml per well. A persistent stimulation of the T cells by anti-CD3 mAb was essential to maintain lymphokine secretion, since only low numbers of IFN-γ–SC (1:10,000) and IL-4–SC (1:100,000) were detectable in the absence of anti-CD3 triggering. Active secretion of IFN-γ or IL-4 was revealed by the addition of cycloheximide during culture, a treatment that completely abolished ELISPOT formation (data not shown). Addition of graded amounts of free soluble anti-IL-4 mAb 8F12 or anti-IFN-γ mAb 23–9 during the cell culture inhibited, in a dose-dependent manner, the formation of IL-4 or IFN-γ spots, respectively (data not shown). Furthermore, addition of unlabeled anti-IL-4 mAb 3H4 or anti-IFN-γ mAb 76–18 after the cell incubation period prevented the development of spots after subsequent exposure of ELISPOT plates to biotinylated anti-IL-4 mAb 3H4 or anti-IFN-γ mAb 76–18. Together these observations attest to the high degree of specificity of this assay, and show that the applied system provides a powerful tool to induce and to quantify potential IL-4- and IFN-γ–producing T cells within 24 h in vitro.

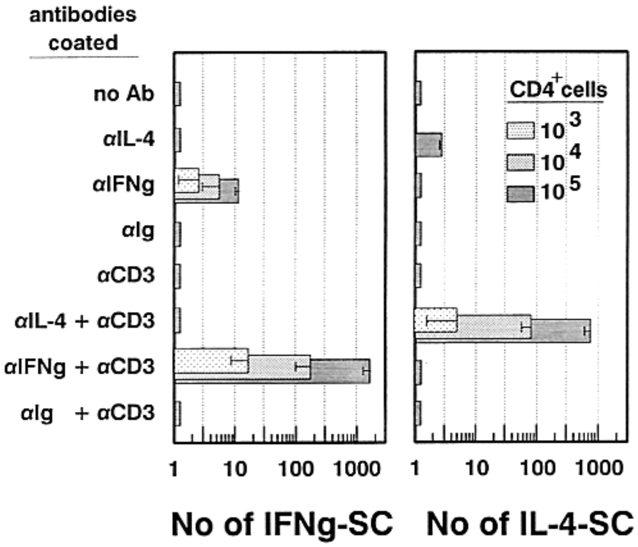

Figure 1. Detection of single IL-4–SC or IFN-γ–SC in ELISPOT assays. To detect IL-4–SC or IFN-γ–SC, ELISPOT plates were coated as indicated with a mixture of anti-CD3 mAb OKT3 (1 μg/ml) plus either mouse anti–human IL-4 mAb 8F12 or mouse anti–human IFN-γ mAb 23.9 (1 μg/ml). Control culture wells were coated with either anti-IL-4, anti-IFN-γ, goat anti–human Ig, or anti-CD3 alone, or with a mixture of anti-CD3 plus goat anti–human Ig, or anti-CD3 alone, or with a mixture of anti-CD3 plus goat anti–human Ig. Plates were blocked with BSA. Purified CD4 cells were added in appropriate dilutions (10^5–10^3 cells/0.2 ml per well). Plates were incubated at 37°C for 24 h. Cells were removed, developing antibodies added, and plates incubated for 2 h. (IL-4, biotin-labeled mouse anti–human IL-4 mAb 3H4, 0.2 μg; IFN-γ, biotin-labeled mouse anti–human IFN-γ mAb 76.18, 0.2 μg). Plates were washed, avidin-AP was added (0.2 μg), and plates were incubated for 2 h. Phosphatase substrate (bromo-4-chloro-3-indolylphospat, 0.1 μg) was added, and the development of visible ELISPOTS followed microscopically. After 30–50 min, plates were rinsed with H_2O and air dried. ELISPOTS were counted either by microscopy or by using an automated ELISPOT counter.

Frequency of IL-4- and IFN-γ-producing CD4 and CD8 T Cells in the Peripheral Blood of Healthy Individuals. In the next set of experiments we determined the frequency of peripheral blood T cells that immediately respond with IL-4 and/or IFN-γ production upon T cell receptor–CD3 triggering. Within randomly selected healthy individuals, the frequencies of IFN-γ–secreting CD4 T cells were comparable (1:50–300), whereas the frequency of IL-4-producing cells varied considerably between 1:110 (Table 1) and <1:100,000 (data not shown). The different numbers of IL-4–SC found in different individuals were not related to experimental variation. Within three independent experiments, the numbers of IL-4–SC detected with CD4 T cells from the same individual varied between 1:110 and 1:300 (donor 1, Table 1). In the same donor, IFN-γ production varied in CD4 cells from 1:80 to 1:110, and in CD8 cells from 1:5 to 1:6. Therefore the ELISPOT system can be used to determine frequencies of potential lymphokine secreting cells. However, all experiments presented were performed within a 6 mo period. We do not know if the frequencies may change in response to antigen–allergen exposure and therefore vary with the time of the year.

To study the effect of IFN-α on IL-4 and IFN-γ production, T cells of three preselected donors were used (Table 1). CD4 T cells of these donors secreted detectable amounts of IL-4 at a frequency of 1:110–8,000 and IFN-γ at frequencies of 1:50–110. CD8 T cells from these donors secreted IL-4 at frequencies between 1:5,300 and <1:100,000, but produced IFN-γ at frequencies of 1:4–8.

IFN-α Increases the Number of IFN-γ-producing CD4 T Cells. We have recently demonstrated that IFN-α inhibits the potential of T cells to help antibody production by B cells by interfering with the initial T cell activation process

Table 1. *Frequency of IL-4 and IFN-γ-secreting CD4 and CD8 T Cells in the Peripheral Blood*

Exp.	Donor	Frequency of CD4⁺ T cells that produce: IL-4	IFN-γ	Frequency of CD8⁺ T cells that produce: IL-4	IFN-γ
1	1	1:110	1:80	1:5,000	1:5
2	1	1:300	1:90	1:8,000	1:5
3	1	1:230	1:110	1:6,200	1:6
4	2	1:150	1:50	1:3,300	1:4
5	3	1:8,000	1:100	<1:100,000	1:8

CD4- or CD8-positive T cells were sorted by FACS® from PBMC of individuals that had been preselected and mounted satisfactory IL-4 responses. Cells (10^3–10^5/well) were cultured for 24 h with IL-2 in ELISPOT plates coated with either anti-CD3 plus anti-IL-4, or with anti-CD3 plus anti-IFN-γ, to detect IL-4- or IFN-γ-secreting cells, respectively. After 24 h, cells were removed, and the lymphokine bound to the plate was detected as described in the legend to Fig. 1.

(18). To determine whether IFN-α affects lymphokine production of T cells, we isolated CD4⁺ and CD8⁺ T cells by FACS®, and cultured them (10^3/well) for 24 h with IL-2, but with or without CD14 monocytes (100/well) and/or IFN-α. Cultures were performed in plates coated with a mixture of anti-CD3 and anti-IFN-γ mAb. In the absence of IFN-α, IFN-γ production was more frequent in CD8 cells (1:5) than in CD4 cells (1:100; Fig. 2). In the presence of IFN-α, the number of IFN-γ–producing CD4 T cells increased 10-fold within 24 h. Addition of CD14 monocytes to the cultures had no effect. In contrast to CD4 cells, the frequency of IFN-γ–producing CD8 cells (1:5) was not changed by IFN-α. The results indicate that IFN-α increases IFN-γ production by CD4 but not by CD8 T cells.

IFN-α Does Not Increase the Number of IL-4-producing CD4 Cells. In the preceding section we have shown that IFN-α increases IFN-γ production in CD4 T cells. To test whether IFN-α also affects IL-4 production, we determined the number of IL-4-SC under identical conditions. As demonstrated in Fig. 3, IL-4 production occurred in 1:110 CD4 and in a 1:5,300 CD8 T cells. IFN-α did not increase the frequency of IL-4 producers within either the CD4 or the CD8 population. The results indicate that IFN-α favors the expression of a Th1 phenotype by T cells.

The Numbers of IFN-γ–SC Are Proportional to the Amounts of IFN-γ Secreted Into the Culture Supernatant. To analyze whether the increased number of IFN-γ–SC induced by IFN-α correlates to increased amounts of IFN-γ secreted into the culture supernatant, we stimulated T cells as described above in the presence of various concentrations of IFN-α. Cells were cultured at 10^4/0.2 ml per well to determine the number of IFN-γ–SC, and at 10^6/1 ml per well to determine the amount of IFN-γ secreted into the culture supernatant. As

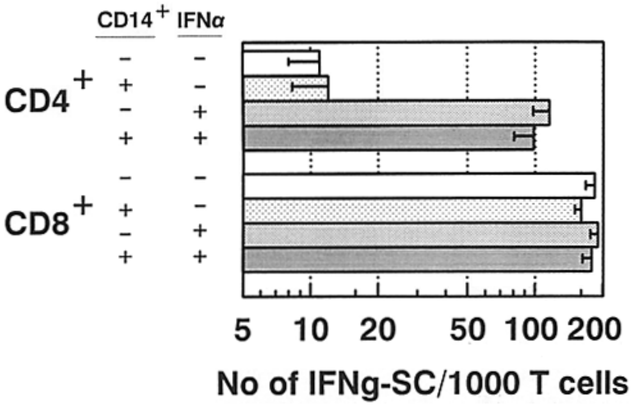

Figure 2. IFN-α increases the frequency of IFN-γ–secreting CD4 but not CD8 T cells. Culture plates were coated with anti-CD3 plus anti-IFN-γ mAb. Purified CD4 or CD8 T cells (10^3/well) were added and cultured for 24 h with IL-2 (50 U/ml) with or without CD14 monocytes (100/well) in the presence or absence of IFN-α (10 ng/ml). After 24 h, cells were removed, and IFN-γ spots were developed as described in the legend to Fig. 1.

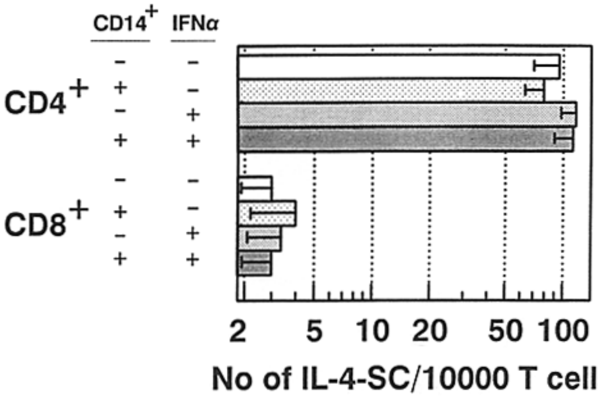

Figure 3. IFN-α does not increase the frequency of IL-4-secreting CD4 T cells. Culture plates were coated with anti-CD3 plus anti-IL-4 mAb. Purified CD4 or CD8 T cells (10^4/well) were added and cultured for 24 h with IL-2 (50 U/ml) with or without CD14 monocytes (10^3/well) in the presence or absence of IFN-α (10 ng/ml). After 24 h, cells were removed, and the IL-4 spots were developed as described in the legend to Fig. 1.

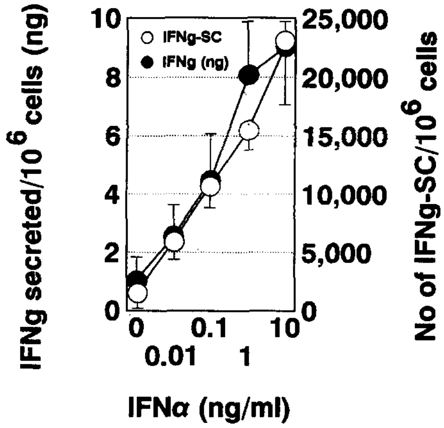

Figure 4. Correlation between the number of IFN-γ–SC and the amount of secreted IFN-γ. To determine the numbers of IFN-γ–SC, culture plates were coated with anti-CD3 plus anti-IFN-γ mAb. Purified CD4 T cells (10^4/well) were added and cultured for 24 h with IL-2 (50 U/ml) in the presence of increasing concentrations of IFN-α. After 24 h, cells were removed, and IFN-γ spots were developed as described in the legend to Fig. 1. To determine the amounts of IFN-γ secreted into the culture supernatant, CD4 T cells (10^6/ml) were cultured in IL-2 (50 U/ml) in the presence of increasing concentrations of IFN-α. After 24 h, culture supernatants were harvested and analyzed for IFN-γ by ELISA.

shown in Fig. 4, IFN-α increased the numbers of IFN-γ–SC and the amounts of IFN-γ secreted into the culture supernatant ~10-fold and in a dose-dependent fashion. Single cells secreted ~0.4 pg/24 h of IFN-γ independent of the presence or absence of IFN-α. This indicates that IFN-α increases the frequency of IFN-γ–SC rather than increasing the rate of IFN-γ production per single cell.

IFN-α Increases the Expression of IFN-γ mRNA. In the preceding experiment we have demonstrated that IFN-α specifically promotes the secretion of IFN-γ but not of IL-4 by CD4 T cells. To analyze whether IFN-α induces IFN-γ gene expression, we stimulated CD4 T cells by plate-bound anti-CD3 mAb in the presence or absence of IFN-α, and determined mRNA for IFN-γ by PCR (42) after 12–48 h (Fig. 5 *A*). We found that anti-CD3 triggering induced expression of IFN-γ mRNA, and addition of IFN-α to the cultures prolonged the expression of IFN-γ message. Quantification of IFN-γ mRNA by Northern Blot analysis revealed that about three- to fourfold higher amounts of IFN-γ mRNA were present after 24 h stimulation in the presence of IFN-α (Fig. 5 *B*). The results may indicate that IFN-α increases–stabilizes the expression of IFN-γ mRNA in CD4 T cells.

IFN-α Antagonizes the Suppressive Effect of IL-4 on the Secretion of IFN-γ. It has recently been demonstrated that IL-4 suppresses the production of IFN-γ by PBMC (49, 50). To analyze whether IFN-α could antagonize the suppressive activity of IL-4, we stimulated CD4 T cells (10^6/ml) for 24–72 h by plate-bound anti-CD3 mAb in the presence of either IL-4 or IFN-α, or in the presence of IL-4 plus IFN-α. In the absence of exogenously added lymphokines, 4 ng of IFN-γ was produced within 72 h of culture (Fig. 6). Addition of IL-4 reduced IFN-γ production to 1 ng, whereas addition of IFN-α increased IFN-γ production to 10 ng. In the presence of IL-4 plus IFN-α, the levels of secreted IFN-γ were comparable to those observed in the presence of IFN-α alone. This demonstrates that IFN-α completely antagonizes the suppressive effect of IL-4 on IFN-γ secretion by CD4 T cells. We speculate that IFN-α may favor the generation of Th1-like cells by inducing IFN-γ and by antagonizing the suppressive effect of IL-4 on IFN-γ production.

IFN-α Increases IFN-γ Production by Allostimulated T Cells. In the preceding section we have shown that IFN-α critically increases the production of IFN-γ in CD4 T cells stimulated by plate-bound anti-CD3 mAb. To further prove the physiological relevance of the finding, we analyzed the effect of IFN-α in mixed lymphocyte culture. Therefore, PBMC of two different donors (2 × 10^6/ml) were cultured in the presence of increasing concentrations of IFN-α either separately without TCR triggering, or together to induce allostimulation. Culture supernatants were harvested after 24 h and analyzed for IFN-γ by ELISA. Without allostimulation, IFN-α did not increase IFN-γ production significantly (Fig.

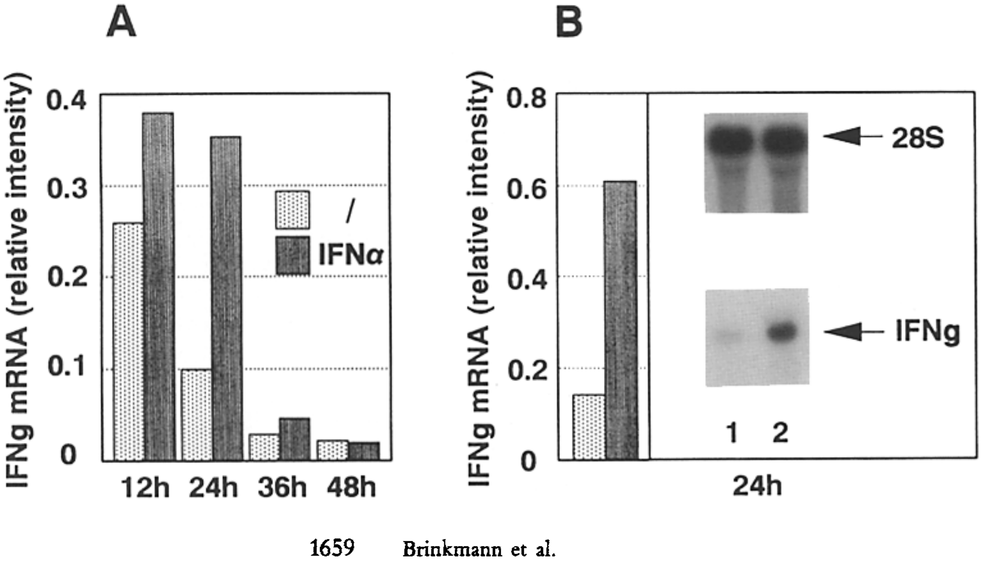

Figure 5. IFN-α increases the expression of mRNA for IFN-γ. CD4 T cells (2 × 10^6/ml) were stimulated by plate-bound anti-CD3 antibody in the presence of IL-2 (50 U/ml) with or without IFN-α (10 ng/ml) as indicated. After 12–48 h, cells were harvested and mRNA was detected by PCR (*A*) (representative of three experiments with three different donors). Furthermore, mRNA was quantified after 24 h by Northern Blot analysis (*B*) (lane *1*, no IFN-α; lane *2*), IFN-α 10 ng/ml). The relative intensities of the bands were determined by scanning the polaroid picture of the gel with a video densitometer.

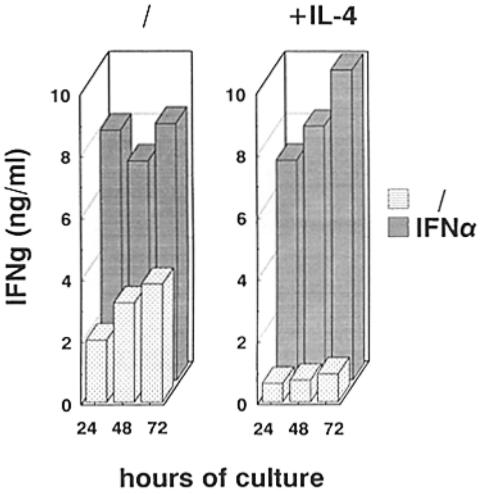

Figure 6. IFN-α antagonizes the suppressive effect of IL-4 on the production of IFN-γ. CD4 T cells (10^6/ml) were cultured for 3 d in anti-CD3 coated plates with either IL-4 (50 ng/ml) or IFN-α (10 ng/ml) alone, or with IL-4 plus IFN-α. Culture supernatants were harvested after 24, 48, and 72 h, and analyzed for IFN-γ by ELISA (means of duplicates).

7). In contrast, IFN-α increased the production of IFN-γ in a dose-dependent fashion, and up to sixfold within 24 h in mixed leukocyte cultures. The results may indicate that stimulation of IFN-γ production by IFN-α represents a physiological mechanism to favor the development of Th1-like cells in vivo.

Discussion

In this study we have determined the relative frequency of potential IL-4- and IFN-γ–producing T cells present in the peripheral blood of healthy individuals, and analyzed the effect of IFN-α on the secretion of IFN-γ and IL-4. To minimize effects of endogenously produced lymphokines and cell–cell interactions in vitro, highly purified $CD4^+$ and $CD8^+$ T cells were stimulated by plate-bound anti-CD3 mAb at low cell density and in the absence of accessory cells, and lymphokine secretion was accessed during the initial 24 h of culture. Within randomly selected normal individuals, IFN-γ was produced by 1:50–300 CD4 cells and by 1:5–10 CD8 cells. The number of IL-4–SC ranged from undetectable levels to 1:110 in CD4 cells and to 1:5,300 in CD8 cells. Since it has been found that IFN-γ inhibits the clonal expansion of Th2-like cells in response to antigen (51), the relative constant number of IFN-γ–SC found in different healthy individuals may strictly control Th2-like cells and thereby counteract excessive IL-4 production. We do not favor a direct action of IFN-γ on the expression of the IL-4 gene, since exogenously added IFN-γ neither increased nor decreased the frequency of IL-4–SC (data not shown).

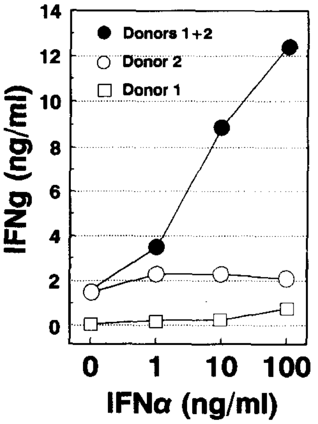

Figure 7. IFN-α increases IFN-γ production by allostimulated PBMC. PBMC of two donors (10^6/ml) were cultured separately (○ and □), or were allostimulated in MLC (10^6 plus 10^6/ml; ●) in the presence of IFN-α as indicated. Culture supernatants were analyzed for IFN-γ after 24 h (means of duplicates).

The signals inducing the production of either IL-4 or IFN-γ in T cells remain largely unknown. It has been suggested that unique cofactors are necessary for optimal stimulation of Th1 and Th2 clones, and that different cofactors may be produced by different antigen-presenting cells. It was found that B cells presenting OVA stimulated optimal proliferation of Th2 clones, whereas adherent cells stimulated optimal proliferation of Th1 cells (52). Since antigen-presenting macrophages but not B cells produce large amounts of IFN-α (53) and stimulate Th1 clones (52), we analyzed whether IFN-α would favor the expression of a Th1 phenotype in T cells. We found that IFN-α increased the expression of mRNA for IFN-γ and as a consequence the frequency of IFN-γ–producing CD4 T cells and the amounts of secreted IFN-γ. In contrast, IFN-α did not increase the frequency of IFN-γ–producing CD8 T cells. The fact that similar results were obtained with anti-CD3 or allostimulated T cells may point to the physiologic relevance of the immunomodulatory mechanism. The results strongly suggest that IFN-α favors the generation of IFN-γ–producing Th1-like cells. It is reasonable that, given the capacity of viruses and intracellular bacteria to stimulate macrophage production of IFN-α (53), Th cells may be simultaneously presented with processed antigen and IFN-α, which induces them to differentiate towards a Th1-like phenotype. It might be speculated that allergens inducing Th2 rather than Th1 cells (29) may be poor stimulators of IFN-α production by macrophages, or may rather be presented by APC, which do not produce IFN-α. Therefore, desensitization of allergic patients with allergen in combination with Th2-suppressive agents like IFN-α could induce allergen-specific Th1- rather than Th2-like cells in vivo. It is likely that an allergen-specific Th1 memory would protect against future allergic reactions, since the increased levels of IFN-γ and the decreased levels of IL-4 produced by Th1 cells would reduce the clonal expansion of Th2 cells (51) as well as the switch of B cells to IgE (15, 19).

The described mode of action may also explain the beneficial effects of IFN-α in the treatment of HIV infection (7, 8). It has been discussed that the progression to AIDS is characterized by loss of IL-2 and IFN-γ production and increases in IL-4 and IL-10 (54). In contrast, many seronegative, HIV-exposed individuals generate strong Th1-type responses to HIV antigens. It is tempting to speculate that protective Th1 responses to HIV antigens may prevent seroconversion and the development of AIDS even after multiple exposures to HIV (54). Interestingly, HIV infection of monocytes–macrophages results in a diminished production of IFN-α (55). From our data it may be concluded that the presentation of HIV to T cells by IFN-α–deficient macrophages would favor the development of HIV-specific Th2-like cells. Therefore, the progression of HIV infection to AIDS may at least be delayed with drugs like IFN-α that stabilize Th1 responses. Future vaccine protocols could aim on the specific induction of Th1 immune responses through vaccination with a cocktail of antigen plus agents like IFN-α, anti-IL-4 mAb, or soluble IL-4 receptor.

In our system, IFN-α increased the production of IFN-γ but not of IL-4 by CD4 T cells. However, it has been demonstrated in mice that IFN-α treatment during immunization with foreign anti-IgD antibodies reduces message for IL-4 and increases message for IFN-γ in total splenic T cells (17). In the light of our data, IFN-α may not deactivate the IL-4 gene, but may rather increase the relative number of IFN-γ–producing cells in the spleen by activating the IFN-γ gene in the CD4 Th helper cell compartment. The increased expression of IFN-γ would inhibit proliferation of Th2- but not of Th1-like cells (51), and thereby indirectly control the expression of IL-4. The profound effect of IFN-α on IFN-γ production by Th cells may also explain its suppressive effect on IgE production in vitro (15) as well as in vivo (13, 16, 17). It is unlikely that IFN-α displays a direct effect on the B cell level, since it does not block the IgE switch in human B cells that are stimulated by preactivated helper cells in the absence of IFN-γ and in the presence of exogenously added IL-2 and IL-4 (18).

Recently it has been described that a 15-kD protein that induces IFN-γ production is released by human monocytes and lymphocytes after IFN-α treatment (56). However, this protein should induce IFN-γ production in both CD4 and CD8 T cells, which was not observed (56). In our hands, IFN-α only acted on CD4 T cells, and addition of monocytes to purified CD4 or CD8 T cells did not alter their responsiveness to IFN-α.

From the data we conclude that IFN-α, which can be released in large amounts by antigen-presenting macrophages (52, 53), may favor the generation of TH1-like cells by activating the gene for IFN-γ in CD4 T cells, and by antagonizing the suppressive effect of IL-4 on IFN-γ production by these cells. The described mechanism may explain the beneficial effects of IFN-α in the treatment of allergic disease situations (13, 14) and of HIV infections (7, 8). These diseases are, at least at certain stages, characterized by a defective production of IFN-γ and/or an increased production of IL-4 (29, 47, 54, 57).

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