Differential regulation of antigen-induced IL-4 and IL-13 generation from T lymphocytes by IFN-α

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Background: IL-4 and IL-13 are related cytokines with similar functional properties. Differential regulation of IL-4 and IL-13 has not been described.
Objective: We have examined the effects of IFN-α on antigen-driven proliferation, IL-4 generation, and IL-13 generation from human PBMCs and T-cell clones.
Methods: Proliferation was assessed by ^{3}H-thymidine incorporation. Cytokine generation was assessed by reverse transcription PCR and ELISA. Messenger RNA stability was assessed in the presence of actinomycin D.
Results: IFN-α induced a concentration-dependent inhibition of antigen-driven proliferation of T_{H1} and T_{H2} clones (median effective concentration, 150 to 200 U/mL); the sensitivity of T_{H1} and T_{H2} clones to IFN-α was not significantly different (P = .6). IFN-α induced an analogous concentration-dependent inhibition of antigen-driven IL-13 generation from T_{H1} and T_{H2} clones (median effective concentration, 100 U/mL); this effect was evident by 12 hours of culture and persisted beyond 48 hours. However, IL-4 generation from T_{H2} clones was insensitive to IFN-α at all concentrations and times tested (1 to 10,000 U/mL). A similar inhibitory effect of IFN-α on mitogen-driven proliferation and IL-13 generation from PBMCs was demonstrated; once again, IL-4 generation from PBMCs was insensitive to IFN-α. IL-13 mRNA stability was unaffected by IFN-α, suggesting transcriptional regulation.
Conclusion: IFN-α differentially regulates antigen-stimulated IL-4 and IL-13 generation. (J Allergy Clin Immunol 1999;103:451-7.)

Key words: *Human, T lymphocyte, cytokine, IL-4, IL-13, IFN-α*

IL-4 and IL-13 are T cell–derived cytokines that share many structural and functional properties. Both are 4 helix bundle cytokines with more than 30% amino acid identity.[1]

Abbreviation used
APC: Antigen-presenting cell
PMA: Phorbol 12-myristate 13-acetate
RT: Reverse transcription
RW: Ragweed extract

IL-4 and IL-13 each use the IL-4Rα chain, and JAK1 and STAT6, for signaling.[2,3] Both cytokines induce B-cell class switching to IgE, upregulation of the low-affinity IgE receptor (FcεRII/CD23), adhesion molecule expression, and MHC class II expression on both B cells and monocytes.[4,5] Both IL-4 and IL-13 map to the q31.1 locus of chromosome 5, a region known to be linked to genetic regulation of total serum IgE and the atopic phenotype.[6] Finally, both IL-4 and IL-13 expression are enhanced at sites of allergic inflammation,[7,8] and both cytokines are downregulated by antiinflammatory agents, such as steroids and cyclic nucleotide phosphodiesterase–4 inhibitors.[9-11]

Despite these similarities, IL-4 and IL-13 display different kinetics, cellular origins, and specific effector functions. We and others have reported rapid, transient IL-4 generation but delayed, prolonged IL-13 generation, after antigen stimulation of T cells.[12,13] Moreover, IL-13, but not IL-4, is a product of human T_{H1} clones.[12] Berkman et al[14] demonstrated the ability of IL-4, but not IL-13, to downregulate human airway epithelial cell RANTES expression. Horie et al[15] have shown that IL-13, but not IL-4, is capable of inducing dose-dependent prolongation of eosinophil survival and eosinophil chemotaxis. Finally, Hasegawa et al[16] demonstrated that serum IL-13 levels, and not IL-4 levels, correlated with erythrocyte sedimentation rates and C-reactive protein levels in patients with systemic sclerosis. Therefore the ability to differentially regulate antigen-induced IL-4 and IL-13 generation would be of significant interest.

IFN-α is a member of the type I interferon family, produced by immune and accessory cells and possessing potent antiproliferative and immunomodulatory properties.[17] We have previously described the ability of IFN-α to downregulate T-cell proliferation and expression of eosinophil-activating cytokines.[18] We describe the differential regulation by IFN-α of IL-4 and IL-13 generation from human PBMCs and a set of well-characterized Amb

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a 1 (a major allergen of short ragweed, *Ambrosia artemisiifolia*)–specific human T-cell clones. Furthermore, these data suggest that IFN-α inhibits IL-13 gene expression through transcriptional regulation.

METHODS

PBMC isolation and derivation of antigen-specific T-cell clones

PBMCs were isolated by gradient centrifugation on Ficoll-Paque (Pharmacia Inc, Piscataway, NJ) and resuspended in RPMI (BioWhittaker, Walkersville, Md) supplemented with 1% penicillin/streptomycin and 5% human AB serum (Gibco/BRL, Gaithersburg, Md), as previously described.[18,19] Platelet contamination of these preparations was less than 1%; viability by trypan blue exclusion was uniformly greater than or equal to 99%. These cells were either used immediately for PBMC studies or irradiated for use as antigen-presenting cells (APCs) in conjunction with the T_{H1} and T_{H2} clones.

Antigen-specific T-cell clones were derived and characterized as previously described, with PBMCs from a subject with asthma/rhinitis (subject MST) and a subject with rhinitis (subject JMW), each with epicutaneous skin test reactivity to short ragweed (ALK Laboratories Inc, Milford, Conn).[12] Subjects took no medications for 1 month preceding the study; none of the subjects had undergone allergen immunotherapy. PBMCs were cultured in the presence of 10 mg/mL short ragweed antigen. This primary culture underwent 2 successive biweekly restimulations of the antigen-specific T cells with a major short ragweed antigen, Amb a 1, in the presence of irradiated autologous PBMCs as APCs. The resulting antigen-specific T-cell line was cloned and subcloned by the limiting dilution technique. All T-cell clones were $CD3^+CD4^+CD8^-$ by flow cytometry. Proliferative responses restricted to Amb a 1 confirmed antigen specificity. Cytokine profiles were determined by both reverse transcription PCR (RT-PCR) and ELISA for secreted protein; based on these data, phenotypic assignment to T_{H0}, T_{H1}, or T_{H2} was made.[12] A total of 8 clones from subject MST and 7 clones from subject JMW were used in these studies.

Proliferation assays

Proliferation assays were performed as previously described with conditions optimized for cell number, clone/APC ratio, antigen concentration, and kinetics.[11,19] Briefly, 2×10^4 clonal T cells with 1.5×10^5 APCs were cultured in 96-well flat-bottom plates in the absence or presence of ragweed 10 μg/mL and various concentrations of IFN-α (lot 3616; specific activity 2×10^8 IU/mg; Amarillo Cell Culture, Amarillo, Tex). PBMCs (2×10^5) were cultured in 96-well flat-bottom plates in the absence or presence of a mitogen, PHA (5 μg/mL), and various concentrations of IFN-α. Cells were preincubated with IFN-α for 1 hour before the addition of antigen or mitogen, although neither longer preincubations nor simultaneous addition of IFN-α significantly altered these results (data not shown). Negative (media alone) and positive (antigen or mitogen alone) control assays were performed with each culture. All conditions were performed in triplicate and incubated for 72 hours at 37°C with 5% carbon dioxide. Each culture was pulsed with 1 μCi of tritiated thymidine, incubated for an additional 20 hours, harvested onto glass-fiber filters in a multichannel cell harvester (Cambridge Technologies Inc, Watertown, Mass), and counted for 1 minute in an automated beta counter (Beckman Instruments Inc, Fullerton, Calif).

Cytokine gene expression assay

Cytokine gene expression assays were performed as previously described with conditions optimized for cell number, clone/APC ratio, antigen concentration, and kinetics.[11,20-21] Briefly, 2×10^5 clonal T cells with 3×10^6 APCs were cultured in the absence or presence of antigen (ragweed, 10 mg/mL) and various concentrations of IFN-α. Cells were preincubated with IFN-α for 1 hour before the addition of antigen. After a 12-hour incubation with antigen in slanted polypropylene tubes, the cells were pelletted, washed, and subjected to RNA isolation by the RNAzol[B] technique, according to the manufacturer's instructions (Tel-Test Inc, Friendswood, Tex). Diethylpyrocarbonate-treated water without SDS was used for the final resuspension step. RNA was stored at –80°C until studied. Normalization of RNA to approximately 100 ng/mL was achieved with a combination of spectrophotometry, ethidium bromide-stained gel electrophoresis, and RT-PCR for a constitutive marker gene, β-actin. $A_{260/280}$ values more than 1.7 were uniformly obtained. Semiquantitative RT-PCR was performed with 5 mmol/L magnesium and oligo dT priming with standard reagents (Perkin Elmer Cetus, Norwalk, Conn) and cytokine-specific primer pairs designed in our laboratory and made at the DNA Core Facility of The Johns Hopkins University.[11] Strict RNase-free conditions were maintained throughout these experiments. All PCR products were visualized by ethidium bromide–stained gel electrophoresis and photographed.

Cytokine protein secretion assays

Cytokine protein secretions from antigen-stimulated T-cell clones or mitogen-stimulated PBMCs were assessed by ELISA, with Cytoscreen Immunoassay kits (Biosource Int, Camarillo, Calif) or Immunoassay kits from R&D Systems (Minneapolis, Minn) according to the manufacturers' instructions. Quantitation was achieved with the World Health Organization standards provided by the company. Clonal T cells (2×10^5) with 3×10^6 APCs were cultured in slanted polypropylene tubes in the presence of 10 mg/mL ragweed and various concentrations of IFN-α. PBMCs (2×10^6) were cultured in 24-well flat-bottom plates in the presence of PHA (2 μg/mL) and various concentrations of IFN-α. Culture supernatants were collected after 12 hours or at extended time points as indicated, centrifuged free of debris, and stored at –20°C until assayed. Dilutions of samples, when necessary, were performed in culture medium. All standards and samples were tested in duplicate. Most samples were analyzed at 2 different dilutions and compared for internal consistency.

RNA-stability assay

The effect of IFN-α on RNA stability was assessed in the presence of actinomycin D with a modification of the method of Iademarco et al.[22] Briefly, Jurkat T cells were cultured in RPMI-1640 medium supplemented with 1% penicillin/streptomycin and 10% fetal bovine serum (Gibco/BRL, Gaithersburg, Md). Cells in log growth phase were stimulated for 3 hours with either anti-CD3, PHA (2 μg/mL), or a combination of phorbol 12-myristate 13-acetate (PMA; 1 ng/mL) and ionomycin (1 μmol/L; Sigma Chemical Co, St Louis, Mo); stimulation was followed by preincubation with actinomycin D for 30 minutes and the subsequent addition of IFN-α (1000 U/mL). After 0.5, 2, 4, and 8 hours of culture, cells were pelletted, washed, and subjected to RNA isolation and RT-PCR for β-actin and IL-13, as previously described. Concentrations of actinomycin D from 0.1 to 10 μg/mL were evaluated (data not shown); results are shown with 3 μg/mL.

Statistical analysis

Mean and standard error values and *t* test comparisons were derived with the use of StatView (BrainPower, Inc, Calabasas, Calif) on a Macintosh computer (Apple Computer Inc, Cupertino, Calif). *P* values are paired and 2-tailed. Percent inhibition for each condition was calculated on the basis of inhibition relative to stimulated, drug-free mean counts and was corrected in each case for

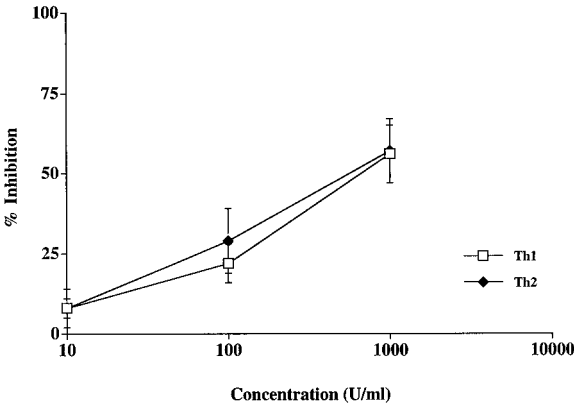

FIG 1. Modulation of clonal T_{H1} and T_{H2} proliferative responses by IFN-α. Data are presented as percent inhibition ± SEM relative to stimulated IFN-α–free cultures, corrected for background with media alone (15,240 ± 3109 cpm and 421 ± 22 cpm, respectively, for T_{H1}; 16,283 ± 9665 cpm and 453 ± 100 cpm, respectively, for T_{H2}). Each clone was used in 3 separate replicate experiments.

background counts with media alone. IC_{50} values represent the concentration of drug at 50% inhibition; EC_{50} values represent the concentration of drug at 50% efficacy.

RESULTS

Antigen-driven proliferation of T_{H1} and T_{H2} clones

Fig 1 depicts the modulation of ragweed-driven proliferation of T_{H1} and T_{H2} clones in the presence of varying concentrations of IFN-α. IFN-α produced a significant, concentration-dependent inhibition of ragweed-driven proliferation; IC_{50} and EC_{50} values were nearly identical for the 2 T-cell phenotypes (IC_{50}, 800 and 750 U/mL; EC_{50}, 200 and 150 U/mL, respectively, for T_{H1} and T_{H2} clones; $P = .6$). These data suggest equivalent efficacy of IFN-α in the modulation of antigen-driven proliferative responses in human T_{H1} and T_{H2} clones.

Antigen-driven cytokine gene expression from T_{H1} and T_{H2} clones

Fig 2 shows the β-actin– and cytokine-specific RT-PCR amplification products from a representative study of T_{H1} and T_{H2} clones cultured with APCs alone, with APCs in the presence of antigen and with APCs in the presence of antigen and 1000 U/mL of IFN-α. Control assays in the absence of RT were uniformly negative (data not shown). Adequate normalization of RNA for each clone was confirmed by the equality of RT-PCR amplification products for β-actin gene expression at subsaturating cycle number (30 cycles). Resting clonal cells cultured with APCs in the absence of antigen did not express significant message for proinflammatory cytokines; the addition of antigen induced gene expression for both IL-4 and IL-13. Exposure to 1000 U/mL of IFN-α induced a marked downregulation of IL-13 gene expression from both T_{H1} and T_{H2} clones but was ineffective in modulating IL-4 gene expression from the T_{H2} clone. These data suggest differential regulation of IL-4 and IL-13 gene expression from antigen-stimulated T-cell clones by IFN-α.

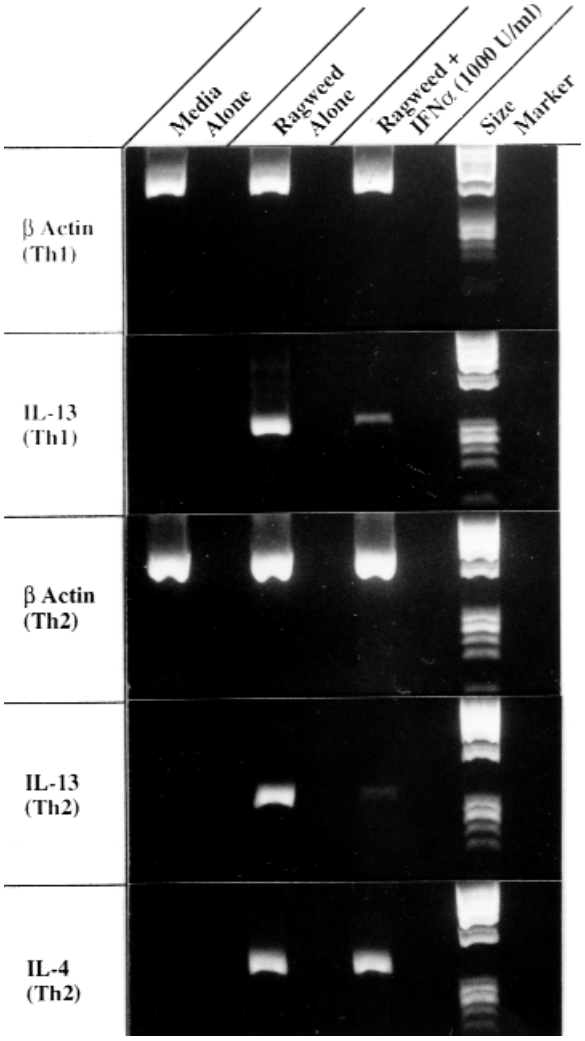

FIG 2. Modulation of cytokine gene expression by IFN-α. Representative results of RT-PCR products for β-actin, IL-4, and IL-13 are shown for antigen-stimulated T-cell clones under the specific culture conditions indicated. Normalization by equivalent β-actin gene expression at a subsaturating cycle number is depicted for each condition in the first and third rows. The DNA size marker is shown in the fourth column. Each clone was used in 3 separate replicate experiments.

Antigen-driven cytokine secretion from T_{H1} and T_{H2} clones

Fig 3 shows the percent inhibition of IL-13 secretion from T_{H1} clones cultured with antigen and APCs in the presence of varying concentrations of IFN-α for varying periods of time. For this and all subsequent protein secretion data, the accuracy of individual values for each of the T-cell clones was confirmed by both replicate culture experiments and replicate ELISA assays at different dilu-

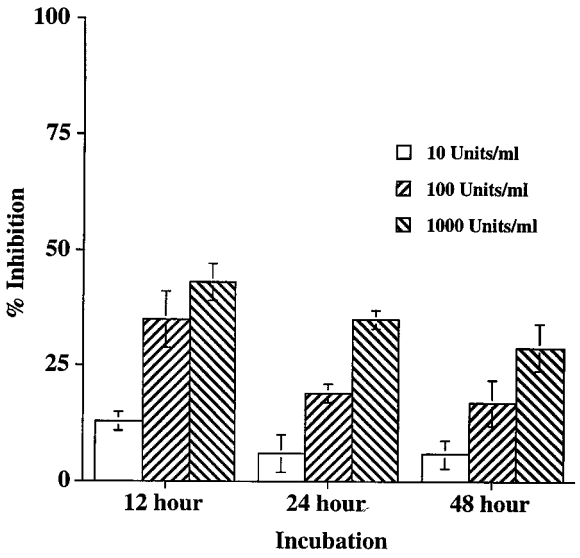

FIG 3. Modulation of cytokine secretion by IFN-α in T_{H1} clones. Secretion of IL-13 is shown for the specific culture conditions indicated. Data are presented as percent inhibition ± SEM relative to stimulated IFN-α–free cultures (1249 ± 690 pg/mL, 1453 ± 449 pg/mL, and 1652 ± 329 pg/mL, respectively, for the 3 time points). Each clone was used in 3 separate replicate experiments.

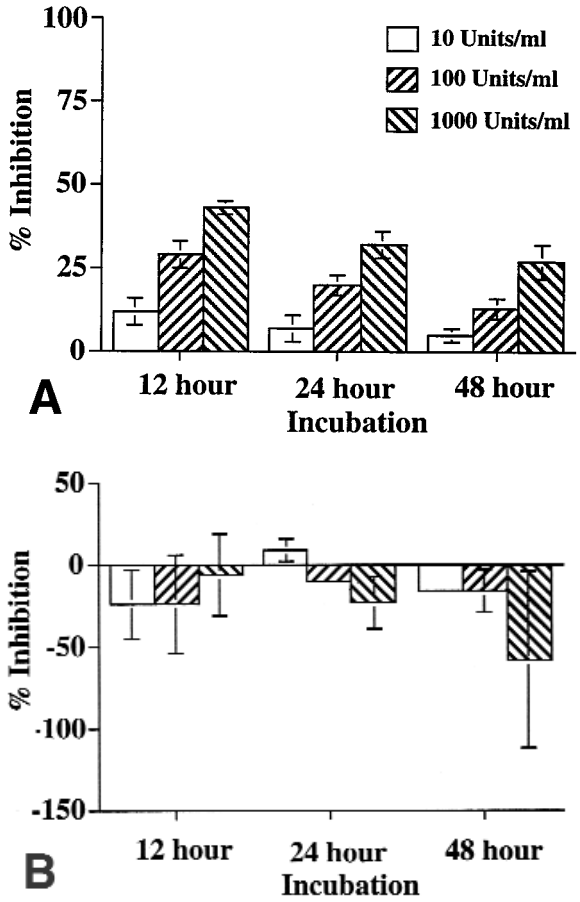

FIG 4. Modulation of cytokine secretion by IFN-α in T_{H2} clones. Secretion of IL-13 (**A**) and IL-4 (**B**) are shown for the specific culture conditions indicated. Data are presented as percent inhibition ± SEM relative to stimulated IFN-α–free cultures (11,616 ± 3654 pg/mL, 12,328 ± 3335 pg/mL, and 12,194 ± 3016 pg/mL, respectively, for IL-13 for the 3 time points; 1357 ± 350 pg/mL, 1208 ± 228 pg/mL, and 795 ± 160 pg/mL, respectively, for IL-4 for the 3 time points). Each clone was used in 3 separate replicate experiments.

tions of individual culture supernatants (data not shown). IFN-α produced a significant, concentration-dependent inhibition of IL-13 production that was persistent over time (10 vs 1000 U/mL; $P < .05$). Fig 4 shows the percent inhibition of IL-13 and IL-4 secretion from T_{H2} clones cultured with antigen and APCs in the presence of varying concentrations of IFN-α for varying periods of time. Once again, IFN-α produced a significant, concentration-dependent inhibition of IL-13 production that was persistent over time (10 vs 1000 U/mL; $P < .03$). Furthermore, although the absolute quantities of IL-13 from the T_{H2} clones were nearly 10-fold greater than those produced by the T_{H1} clones, the normalized dose-response was nearly identical for the 2 T-cell phenotypes. However, IFN-α was ineffective in downregulating antigen-stimulated IL-4 generation from the T_{H2} clones. Although IFN-α produced significant enhancement at some concentrations and some time points, neither clear dose-response nor kinetics was apparent. These data corroborate the gene expression data that show differential regulation of IL-4 and IL-13 from antigen-stimulated T-cell clones by IFN-α and further define the dose-response and kinetics of this effect.

Fig 5 shows the percent inhibition of IL-4 and IL-13 secretion from each of 15 independent T-cell clones cultured with antigen and APCs, each at 2 different time points and each in the absence and presence of 1000 U/mL of IFN-α. The 2 sets of clones, isolated from 2 different individuals, are each comprised of T_{H0}, T_{H1}, and T_{H2} phenotypes. In all patients, IFN-α induced downregulation of IL-13 secretion without effecting IL-4 secretion; significant enhancement of IL-4 secretion with IFN-α was not seen in this set of experiments. These data suggest the generalizability of the differential effect of IFN-α on IL-4 and IL-13 generation.

Mitogen-driven proliferation and cytokine secretion from fresh PBMCs

Fig 6, *A* and *B*, shows the effects of IFN-α on both proliferative responses and IL-13 generation, respectively, from freshly isolated PBMCs from nonatopic individuals after stimulation with PHA. Stimulation of atopic or nonatopic PBMCs with ragweed did not yield significant levels of cytokines for detection in our assay systems. IFN-α at 1000 and 10,000 U/mL produced significant inhibition of PHA-induced proliferation of PBMCs ($P = .02$ and $P = .006$, respectively). Furthermore, significant inhibition of IL-13 production from PBMCs by IFN-α was seen at 1000 and 10,000 U/mL ($P = .006$ and $P =$

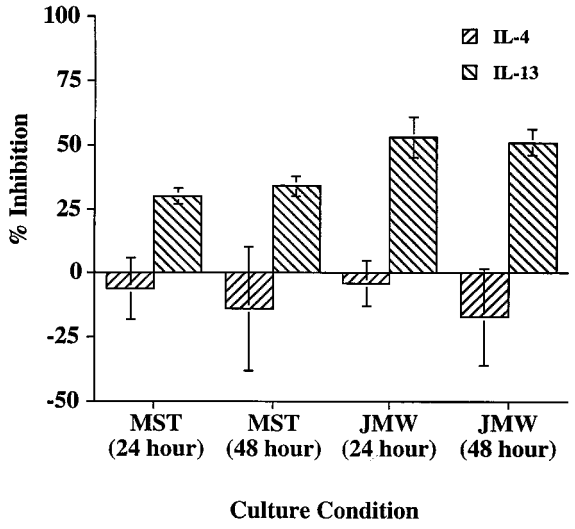

FIG 5. Modulation of cytokine secretion by IFN-α in 2 groups of phenotypically heterogeneous T-cell clones. Secretion of IL-4 and IL-13 are shown with and without 1000 U/mL of IFN-α. Data are presented as percent inhibition ± SEM relative to stimulated IFN-α–free cultures (1016 ± 295 pg/mL and 574 ± 219 pg/mL, respectively, for IL-4 from all clones at the 2 time points; 8524 ± 3466 pg/mL and 7965 ± 3026 pg/mL, respectively, for IL-13 from all clones at the 2 time points). There were 8 clones for subject MST and 7 clones for subject JMW.

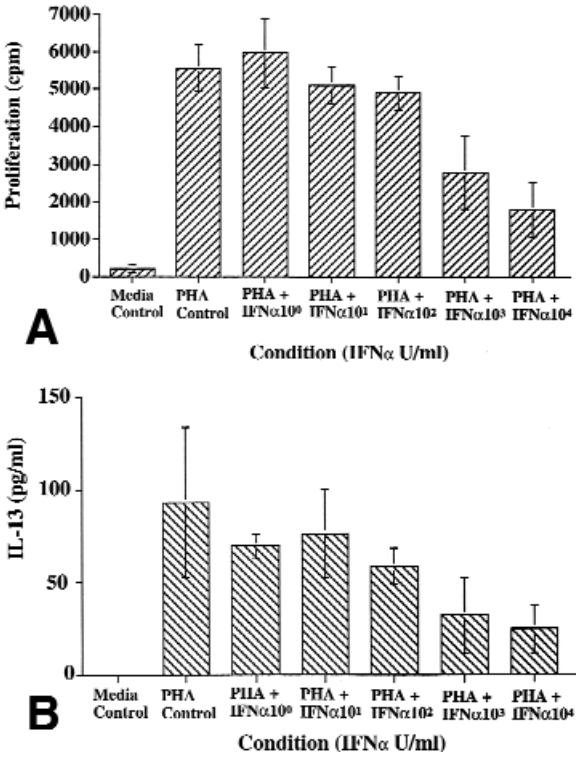

FIG 6. A, Inhibition of PHA-induced proliferative response of PBMCs by IFN-α. Data are presented as mean counts per minute ± SEM for culture conditions as indicated. There were 3 separate replicate experiments. **B**, Inhibition of PHA-induced IL-13 generation from PBMCs by IFN-α. Data are presented as picograms per milliliter of secreted IL-13 ± SEM for culture conditions as indicated. There were 3 separate replicate experiments.

.001, respectively); once again, IL-4 generation from PBMCs was insensitive to IFN-α (data not shown). These results suggest that IFN-α regulates both long-term cultured T-cell clones and freshly isolated PBMC T cells in a similar manner.

RNA stability assays in the Jurkat cell line

Because of the excessive requirement for cells, RNA stability assays could not be performed with the antigen-specific T-cell clones; Jurkat T cells, known to express mRNA for both IL-4 and IL-13 on activation with PMA and ionomycin, were used as a surrogate. Once again, although IFN-α downregulated IL-13 gene expression from Jurkat T cells, IL-4 gene expression was insensitive to IFN-α (data not shown). Fig 7 shows the β-actin– and IL-13–specific RT-PCR amplification products from a representative study of Jurkat cells cultured in the absence and presence of 3 μg/mL of actinomycin D. Adequate normalization of RNA is demonstrated by the equivalent expression of β-actin at subsaturating cycle numbers for all culture conditions (30 cycles). Resting clonal cells cultured in the absence of stimulus expressed low levels of message for proinflammatory cytokines; control assays in the absence of RT were uniformly negative (data not shown). Negative control conditions with IFN-α alone and actinomycin D alone showed low level gene expression for IL-13 that was not different from that seen with media alone. In the absence of actinomycin D, stimulation with PMA and ionomycin induced enhanced gene expression for IL-13, which was downregulated in

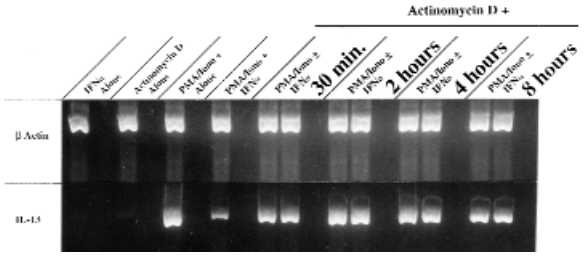

FIG 7. Effects of IFN-α on IL-13 RNA survival. Representative results (from 3 separate replicate experiments) of RT-PCR products for β-actin and IL-13 are shown for mitogen-stimulated Jurkat cells under the specific culture conditions indicated. Normalization by equivalent β-actin gene expression at subsaturating cycle number is depicted for each condition in the first row.

the presence of IFN-α. Although the level of gene expression for IL-13 in the presence of actinomycin D decreased slightly over the duration of the experiment, the presence of IFN-α had no discernible effect on the level of gene expression at any of the time points in the presence of actinomycin D. Similar results were seen after stimulation of Jurkat cells with PHA or anti-CD3 (data not shown). These data suggest transcriptional regulation of IL-13 gene expression by IFN-α.

DISCUSSION

We have provided evidence that induction in activated T cells of IL-13, but not IL-4, is sensitive to downregulation by IFN-α. These data are the first to describe differential regulation of these 2 cytokines in antigen-driven human T cells; moreover, these data suggest transcriptional regulation as the mechanism for this effect.

The ability to differentially regulate IL-4 and IL-13 has been suggested previously in a number of experimental models. Brinkmann and Kristofic[23] have shown that primed $CD4^+$ $CD45RO^-$ cells produce large quantities of IL-13, IL-5, and IFN-γ, but no IL-4, whereas primed $CD4^+$ $CD45RO^+$ produce IL-13, IL-5, IFN-γ, and IL-4; both populations were capable of inducing IgE production. However, priming and stimulation in this model were achieved with plate-bound anti-CD3 and exogenous IL-2 (100 U/mL). Van der Pouw Kraan et al[24] have shown that T cells stimulated with PMA and anti-CD28 produce large quantities of IL-13 and modest amounts of IL-4. Ligation of CD3 decreased IL-13 production and increased IL-4 production; these effects were reversed by the addition of cyclosporine A. Once again, these studies used mitogen-stimulated T cells to demonstrate a pharmacologic effect. Previous studies with antigen-stimulated T cells have failed to identify differential regulation of IL-4 and IL-13 by various anti-inflammatory agents.[9-11] This report is the first to show such an effect.

Recent evidence suggests that IFN-α promotes production of T_{H1} responses.[25] Parronchi et al[26] demonstrated that T-cell clones generated from a subject who was allergic to grass pollen in the presence of grass pollen allergen without IFN-α were predominantly (17 of 37) T_{H2} in phenotype, although those generated in the presence of grass pollen allergen with IFN-α were predominantly (36 of 36) T_{H0}/T_{H1}. Wenner et al[27] showed that IFN-α can augment IL-12 priming for subsequent IFN-γ production and the generation of T_{H1} responses. Furthermore, IFN-α use in vivo may worsen psoriasis (thought to be mediated by a T_{H1} response) but improve idiopathic hypereosinophilic syndrome (thought to be mediated by a T_{H2} response).[17] The apparent resistance of IL-4 to modulation by IFN-α in the present study suggests the presence of additional pathways for the shift toward T_{H1} responsiveness. Two clinical studies have suggested the therapeutic benefit of IFN-α for subjects with systemic sclerosis (Sjogren's syndrome).[28,29] This effect may be mediated in part by the efficacy of IFN-α in decreasing procollagen synthesis from fibroblasts of subjects with this disease.[30] Because elevated levels of serum and salivary gland IL-4 and IL-13 have been demonstrated in subjects with systemic sclerosis, a primary role for IL-13 in this disease process may be suggested.[16,31,32]

In conclusion, IFN-α downregulates IL-13, but not IL-4, generation by transcriptional regulation from activated T cells. Further elucidation of the signaling pathways regulating this effect may provide insights into a variety of inflammatory syndromes.

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