Differential effects of IFN-α on the expression of various T_H2 cytokines in human $CD4^+$ T cells

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Background: In both human subjects and mice, T helper cells are classified into 2 subsets, T_H1 and T_H2 cells, on the basis of the cytokines they produce. Although IFN-α has been shown to enhance human T_H1 responses, its influences on human T_H2 responses have not yet been fully characterized. In addition, the mechanism for induction of T_H1 responses by IFN-α has not been fully delineated.

Objective: The present study was undertaken to explore the direct effects of IFN-α on the expression of various cytokines in human $CD4^+$ T cells with a system using immobilized anti-CD3, which permits activation of $CD4^+$ T cells in the complete absence of accessory cells.

Methods: Highly purified $CD4^+$ T cells obtained from healthy donors were stimulated with immobilized anti-CD3 with or without IFN-α and IL-12 in the complete absence of accessory cells. The production of cytokines was estimated by means of ELISA. The expression of mRNA for various cytokines, as well as transcription factors, was evaluated by using quantitative PCR.

Results: IFN-α enhanced IL-4 protein and mRNA expression in immobilized anti-CD3–stimulated $CD4^+$ T cells, irrespective of the presence of IL-12, whereas IFN-α suppressed the expression of IL-5 and IL-13. Of note, IFN-α enhanced the expression of mRNA for c-Maf, T-bet, and Fox-P3, irrespective of the presence of IL-12, but not that for GATA-3, in anti-CD3–stimulated $CD4^+$ T cells.

Conclusion: These results indicate that IFN-α enhances the induction of T_H1 responses through upregulation of T-bet mRNA expression, as well as the induction of T_H2 responses through upregulation of c-Maf mRNA expression, followed by IL-4 expression. Moreover, the data also suggest that IFN-α might suppress the expression of IL-5 and IL-13 in differentiated T_H2 cells. (J Allergy Clin Immunol 2005;116:205-12.)

Key words: *Human, T_H1, T_H2, IL-4, IL-5, IL-13, c-Maf, GATA-3, T-bet, Fox-P3*

Abbreviations used
IL-12Rβ2: IL-12 receptor β2
NK: Natural killer

In both human subjects and mice, activated $CD4^+$ T cells can be classified into 2 subsets, T_H1 and T_H2 cells, on the basis of the cytokines they produce.[1-4] Thus T_H1 cells produce IFN-γ and IL-2, which are involved in cell-mediated immune responses, whereas T_H2 cells produce mainly IL-4, IL-5, and IL-13, which are involved in humoral immune responses.[5,6] IL-4 has a major role in B-cell activation and isotype switching, particularly in IgE production.[7] On the other hand, IL-5 activates mature eosinophils, prolongs their survival, and contributes to their accumulation at sites of inflammation.[8] In T_H2 cells the transcription factors GATA-3 and c-Maf are selectively expressed and have been shown to regulate T_H2 cytokine expression.[9,10] Thus c-Maf is required for the expression of IL-4,[11] whereas GATA-3 is involved in the expression of IL-5 and IL-13.[12,13]

IFN-α presents potent antiviral actions, as well as immunoregulatory activities, including enhancement of cytotoxic activity of T cells and natural killer (NK) cells.[14] IFN-α has been shown to enhance human T_H1 responses, which is reflected by IFN-γ production in the presence of accessory cells with stimulation by PHA.[15-17] We have recently revealed that IFN-α by itself did not enhance IFN-γ production or mRNA expression in anti-CD3–stimulated human $CD4^+$ T cells in the absence of accessory cells or exogenous IL-12.[18] Consistently, IFN-α enhanced IL-12 receptor β2 (IL-12Rβ2) mRNA expression in $CD4^+$ T cells.[18] It is therefore indicated that the induction of human T_H1 responses by IFN-α requires the presence of IL-12.[18] As for human T_H2 responses, it was previously shown that IFN-α inhibits IL-5 production and mRNA expression in $CD4^+$ T cells.[19] However, the effects of IFN-α on the production of IL-4 in human $CD4^+$ T cells have been uncertain, possibly because of contaminating accessory cells or other supplemental cell lines to cross-link CD3 molecules through soluble anti-CD3. In addition, IL-13 is one of the T_H2 cytokines that has very similar biologic actions of IL-4.[20] Although the regulation of IL-13 production is pivotal in the function of T_H2 cells, the effects of IFN-α on the production of IL-13 have not been determined. The current studies were therefore undertaken to explore the direct effects of IFN-α on the expression of the T_H2 cytokines IL-4, IL-5, and IL-13 in activated human $CD4^+$ T cells by using a system with immobilized anti-CD3, which permits stimulation of T cells in the complete absence of accessory cells or other supplemental cell lines. Special attention was paid to the effects of IFN-α on the expression of mRNA for a variety of transcription factors that regulate the polarization of

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T_H1 and T_H2 cells. The results demonstrate that IFN-α suppresses the expression of IL-5 and IL-13 and enhances the expression of IL-4 in $CD4^+$ T cells. More importantly, IFN-α enhanced the expression of mRNA for c-Maf, T-bet, and Fox-P3, but not for GATA-3, in $CD4^+$ T cells. The data indicate that IFN-α exerts a variety of effects on human T_H1 and T_H2 responses through regulation of mRNA for various transcription factors.

METHODS

mAbs and reagents

Anti-CD3 mAb 64.1 (an IgG2a mAb directed at the CD3 molecule on mature T cells) was a gift of Dr P. E. Lipsky (National Institute of Health, Bethesda, Md). Recombinant human IL-12 was purchased from PeproTech (Rocky Hill, NJ). Recombinant human IFN-α2a was a gift of Nippon Roche (Tokyo, Japan).

Culture medium

RPMI 1640 medium (Life Technologies, Grand Island, NY) supplemented with 100 U/mL penicillin G, 100 μg/mL streptomycin, 0.3 mg/mL L-glutamine, and 10% FBS (Life Technologies) was used for all cultures.

Cell preparation

PBMCs were obtained from healthy adult volunteers by means of centrifugation of heparinized venous blood over sodium diatrizoate-Ficoll gradients (Histopaque; Sigma Chemical Co, St Louis, Mo). PBMCs were depleted of monocytes and NK cells by means of incubation with 5 mM L-leucine methyl ester HCl (Sigma) in serum-free RPMI 1640, as described elsewhere.[21] T cells were obtained from the treated cell population by rosetting with neuraminidase-treated sheep red blood cells, as previously described.[22] Purified $CD4^+$ T cells were further prepared by means of positive selection with anti-CD4 microbeads and MACS (Miltenyi Biotec, Auburn, Calif). The $CD4^+$ T-cell population obtained in this manner contained less than 0.1% esterase-positive cells, less than 0.1% NK cells, less than 0.1% $CD19^+$ cells, and greater than 96% $CD4^+$ T cells.

Cell cultures

Anti-CD3 mAb 64.1 was diluted in RPMI 1640 (2 μg/mL), and 50 μL was placed in each well of 96-well flat-bottomed microtiter plates (no. 3596; Costar, Cambridge, Mass) and incubated at room temperature for 1 hour.[21] The wells were then washed once with culture medium to remove nonadherent mAb before the cells were added. Purified $CD4^+$ T cells (2×10^5/well) were cultured in wells with immobilized anti-CD3 with or without IFN-α (1×10^5 IU/mL) and IL-12 (10 ng/mL). The cells were incubated at 37°C in a humidified atmosphere of 5% CO_2 and 95% air.

RNA isolation and real-time quantitative PCR

Total RNA was isolated from cultured cells with Trizol reagent (Life Technologies), according to the manufacturer's application protocol, and quantified spectrophotometrically. cDNA samples were prepared from 1 μg of total RNA by using the SuperScript reverse transcriptase preamplification system (Life Technologies) with oligo (dT) primer and subjected to real-time quantitative PCR.

Real-time quantitative PCR was performed with the LightCycler rapid thermal cycler system (Roche Diagnostics Ltd, Lewes, United Kingdom), with ready-made primer sets for human IFN-γ, IL-4, IL-5, IL-12Rβ2, GATA-3, T-bet, Fox-P3, or β-actin (LightCycler-Primer Set; Roche Diagnostics GmbH, Heidelberg, Germany) and LightCycler-Fast Start DNA Master SYBR Green I (Roche Diagnostics Ltd). The primers for human c-Maf were designed as follows: forward, 5′-GGTCAGCAAGGAGGAGGT-3′; reverse, 5′-TCTCCTGCTTGAGGTGGTC-3′. PCR reaction condition was identical for all genes except for c-Maf (shown in parentheses): incubation at 95°C for 10 minutes, followed by 35 cycles (40 cycles) of 95°C for 10 seconds, 68°C (60°C for c-Maf) for 10 seconds, and 72°C for 16 seconds (6 seconds for c-Maf). Melting-curve analysis was then carried out to confirm the quality of the performance of the PCR by using 1 cycle of 95°C for 0 seconds and 58°C for 10 seconds (70°C for 15 seconds for c-Maf), with continuous increase to 95°C (rate, 0.1°C/s), followed by cooling at 40°C for 30 seconds. A standard curve was generated in each experiment by using a standard solution in each primer set, and quantitative analysis was performed with LightCycler Software version 3.5. All results were calibrated to the copy number (copies per microliter) of β-actin from each cDNA sample.

Measurement of IL-4, IL-5, and IL-13

IL-4 and IL-13 contents in the supernatants were measured with ELISA kits (Cytoscreen; BioSource International, Camarillo, Calif). The detection limits of the assays were approximately 2.0 and 12.0 pg/mL for IL-4 and IL-13, respectively. The assay is specific for natural and recombinant human IL-4 and IL-13. IL-5 contents in the supernatants were measured with a Human IL-5 ELISA development kit (PeproTech). The detection limit of the assay was approximately 2.0 pg/mL IL-5.

Statistical analysis

The results were analyzed for statistical significance by using the Wilcoxon signed-rank test.

RESULTS

The induction of T_H1 responses in immobilized anti-CD3–activated $CD4^+$ T cells by IFN-α totally depends on the presence of IL-12

We have previously shown that IFN-α enhanced IFN-γ production and mRNA expression only in the presence of IL-12.[18] Initial experiments were carried out to reexamine these direct effects of IFN-α on IFN-γ mRNA expression in activated human $CD4^+$ T cells. As summarized in Table I, IFN-α did not enhance IFN-γ mRNA expression of immobilized anti-CD3–activated $CD4^+$ T cells throughout the cultures, whereas IL-12 significantly enhanced this expression. Of note, IFN-α significantly enhanced IFN-γ mRNA expression in the presence of exogenous IL-12, but not in the absence of IL-12, as early as 3 hours of culture. The results therefore confirm the conclusion that upregulation of the expression of IFN-γ mRNA by IFN-α totally depends on the presence of IL-12.[18] Of note, IFN-α enhanced the expression of IL-12Rβ2 mRNA in anti-CD3–activated $CD4^+$ T cells, irrespective of the presence of IL-12 as early as 3 hours of culture (Table I), as is consistent with the results of a previous study.[18] Taken together, these data suggest that the induction of T_H1 responses by IFN-α is mediated through upregulation of the expression of functional IL-12R, although the precise

TABLE I. Effects of IFN-α and IL-12 on the expression of mRNA for IFN-γ and IL-12Rβ2 in immobilized anti-CD3–activated $CD4^+$ T cells

		× 10^{-2} to β-actin mRNA copies (mean ± SD)			
mRNA	**Incubation**	**Nil**	**IFN-α**	**IL-12**	**IFN-α + IL-12**
IFN-γ	3 h	9.8 ± 4.6	10.7 ± 5.1	14.6 ± 6.8*	20.9 ± 6.0*†
	24 h	6.9 ± 3.8	6.4 ± 3.1	27.8 ± 15.0*	38.4 ± 14.7*†
IL-12Rβ2	3 h	0.091 ± 0.072	0.473 ± 0.302*	0.133 ± 0.122*	0.613 ± 0.348*†
	24 h	1.017 ± 0.722	2.497 ± 1.149*	1.728 ± 1.279*	3.673 ± 2.187*†

$CD4^+$ T cells (2×10^5/well) from 6 healthy individuals were cultured in wells with immobilized anti-CD3 (mAb 64.1, 100 ng/well) with or without IFN-α (1×10^5 IU/mL) and IL-12 (10 ng/mL). After 3 or 24 hours of incubation, total RNA was isolated, and real-time quantitative PCR was performed with specific primers for IFN-γ, IL-12Rβ2, and β-actin. All results were calibrated to the copy number of β-actin (copies per microliter) from each cDNA sample.
*$P < .05$ compared with cultures without cytokines *(Nil)*.
†$P < .05$ compared with cultures with IL-12.

mechanism for the upregulation of IL-2R2 mRNA by IFN-α remains unclear.

Differential effects of IFN-α on the expression of T_H2 cytokines in anti-CD3–activated $CD4^+$ T cells

It was previously shown that IFN-α inhibits IL-5 production and mRNA expression in human $CD4^+$ T cells.[19] However, the effects of IFN-α on the production of IL-4 in human $CD4^+$ T cells have been unclear in these studies.[19] It was possible that contaminating accessory cells or supplemental cells to facilitate cross-linkage of CD3 with soluble anti-CD3 might influence the results.[19] The next experiments therefore compared the direct effects of IFN-α on the production of the T_H2 cytokines IL-4, IL-5, and IL-13 in immobilized anti-CD3–activated $CD4^+$ T cells in the complete absence of accessory cells or other supplemental cells. As shown in Fig 1, the production of IL-4 appeared to reach its peak at 24 hours of culture, whereas that of IL-5 and IL-13 markedly increased between 24 and 72 hours. More importantly, IFN-α appeared to enhance the production of IL-4 as early as 24 hours of culture in a dose-dependent manner. By contrast, IFN-α seemed to decrease the production of IL-5 and IL-13 at 72 hours of culture.

The next experiments were carried out to confirm the effects of IFN-α on the expression of IL-4, IL-5, and IL-13 in anti-CD3–stimulated $CD4^+$ T cells. In accordance with previous studies,[19] IFN-α markedly suppressed IL-5 production at 72 hours of culture and IL-5 mRNA expression at 24 hours of culture, irrespective of the presence of IL-12 (Table II). Of note, IFN-α also significantly suppressed the production of IL-13 by $CD4^+$ T cells stimulated with immobilized anti-CD3 for 24 and 72 hours, irrespective of the presence of IL-12 (Table III). The data therefore indicate that IFN-α suppresses the expression of IL-5, as well as that of IL-13.

In contrast with IL-5 and IL-13, the production of IL-4 was increased very modestly between 24 and 72 hours of culture (Fig 1). More importantly, IFN-α significantly enhanced IL-4 production and mRNA expression of anti-CD3–activated $CD4^+$ T cells, irrespective of the presence of IL-12 (Table IV). The results indicate that IFN-α by itself promotes the expression of IL-4 protein and mRNA in spite of its enhancing effects on IL-12Rβ2 mRNA expression.

Effects of IFN-α on the expression of mRNA for a variety of transcription factors in anti-CD3–activated $CD4^+$ T cells

It has been revealed that the transcription factors c-Maf and GATA-3 are expressed exclusively in T_H2 cells and regulate T_H2 cytokine expression.[9,10] Thus c-Maf is required for the expression of IL-4,[11] whereas GATA-3 is critical for the expression of IL-5.[12] It was therefore possible that the effects of IFN-α on the mRNA expression of IL-4 and IL-5 in anti-CD3–activated $CD4^+$ T cells might result from changes in the expression of these transcription factors. On the other hand, previous studies have demonstrated that T-bet plays a critical role in the induction of T_H1 responses.[23] In addition, recent studies have demonstrated that Fox-P3 is required for the development of $CD4^+CD25^+$ regulatory T cells.[24] To examine the effects of IFN-α on the expression of mRNA for these transcription factors, total RNA was isolated from cultured cells, and real-time quantitative PCR was performed with specific primers for c-Maf, GATA-3, T-bet, Fox-P3, and β-actin.

As shown in Fig 2, IFN-α enhanced the expression of mRNA for c-Maf, T-bet, and Fox-P3 in a dose-response manner, whereas it did not appear to affect the expression of GATA-3 mRNA in $CD4^+$ T cells stimulated with immobilized anti-CD3 for 3 hours. Consistently, as can be seen in Fig 3, IFN-α significantly enhanced the expression of mRNA for c-Maf, T-bet, and Fox-P3 in anti-CD3–activated $CD4^+$ T cells, irrespective of the presence of IL-12, whereas IFN-α did not significantly affect the expression of GATA-3 mRNA. Of note, IL-12 also significantly upregulated the expression of mRNA for T-bet and Fox-P3. IFN-α further enhanced their expression in the presence of IL-12. These results suggest that IFN-α might enhance the production of IL-4 in anti-CD3–activated $CD4^+$ T cells by upregulating the expression of c-Maf mRNA. Moreover, the data indicate that IFN-α induces T_H1 responses through upregulation of T-bet. Finally, it is unlikely that the suppression of the expression of IL-5 and IL-13 by IFN-α might be accounted for by the downregulation of GATA-3 mRNA expression. Because

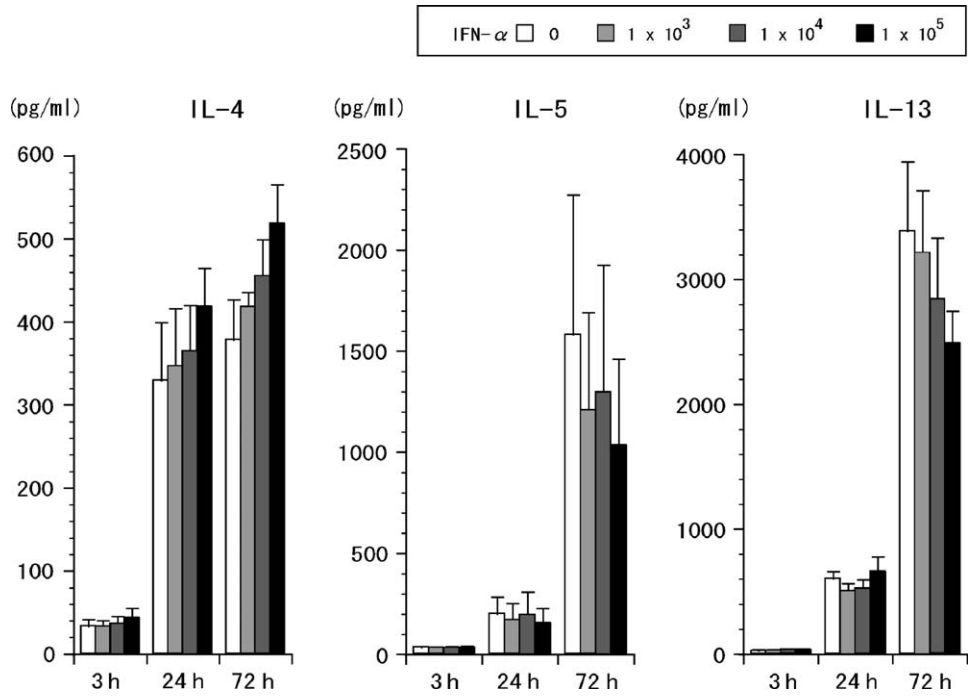

FIG 1. Effects of IFN-α on the production of IL-4, IL-5, and IL-13 by immobilized anti-CD3–activated $CD4^+$ T cells. $CD4^+$ T cells (2×10^5/well) were cultured in wells with immobilized anti-CD3 (mAb 64.1, 100 ng/well) with various concentrations of IFN-α. After 3, 24, or 72 hours of incubation, the supernatants were assayed for IL-4, IL-5, and IL-13 contents by means of ELISA. Mean values of 2 independent experiments are shown. *Error bars* represent the SD of 2 independent experiments.

TABLE II. Effects of IFN-α and IL-12 on IL-15 protein production and mRNA expression in immobilized anti-CD3–activated $CD4^+$ T cells

IL-13 production, pg/mL (mean ± SD)	IL-5 production, pg/mL (mean ± SD)	IL-5 mRNA (mean ± SD)
Nil	77.3 ± 52.3	5.183 ± 5.323
IFN-α	16.2 ± 26.5*	1.402 ± 1.671*
IL-12	87.2 ± 61.8	4.510 ± 3.995
IFN-α + IL-12	20.4 ± 11.6*†	1.643 ± 1.400*†

$CD4^+$ T cells (2×10^5/well) from 6 healthy individuals were cultured in wells with immobilized anti-CD3 (mAb 64.1, 100 ng/well) with or without IFN-α (1×10^5 IU/mL) and IL-12 (10 ng/mL). After 72 hours of incubation, the supernatants were assayed for IL-5 contents by means of ELISA. After 24 hours of incubation, total RNA was isolated, and real-time quantitative PCR was performed with specific primers for IL-5 and β-actin. All results were calibrated to the copy number of β-actin (copies per microliter) from each cDNA sample.
*$P < .05$ compared with cultures without cytokines *(Nil)*.
†$P < .05$ compared with cultures with IL-12.

IFN-α also enhanced Fox-P3 and T-bet mRNA expression, it is more likely that induction of T_H1-like regulatory cells that express Fox-P3[25] might be involved in suppression of the expression of IL-5 and IL-13.

DISCUSSION

Previous studies have reported that type 1 interferons (IFN-α/β) act directly on human, but not mouse, T cells to drive T_H1 development, bypassing the need for IL-12–induced signaling.[15] However, our previous and present studies demonstrated that IFN-α did not affect IFN-γ protein and mRNA expression in $CD4^+$ T cells unless IL-12 was present, confirming that IFN-α by itself might not be sufficient for the optimal induction of T_H1 responses.[18] It should be noted that previous studies explored IFN-α–induced T_H1 responses in the presence of accessory cells with stimulation by PHA.[15-17] Because accessory cells produce IL-12, it is likely that the induction of T_H1 responses by IFN-α in PHA-stimulated cultures might be mediated by cooperative actions of IFN-α and IL-12. In fact, no IL-12 could be detected in the culture supernatants of anti-CD3–activated $CD4^+$ T cells in our system.[18] These findings confirm that the presence of IL-12 is essential for the upregulation of IFN-γ expression by IFN-α. Consistently, we and others have demonstrated that IFN-α enhances the expression of IL-12Rβ2 mRNA in anti-CD3–activated $CD4^+$ T cells.[16,18] It was therefore most likely that IFN-α–induced T_H1 responses observed in the previous studies[15-17] might be mediated through upregulation of the responsiveness to IL-12 secreted from accessory cells. In fact, a recent study also suggests that IFN-α might enhance IFN-γ production in human T cells through IL-12–dependent mechanisms.[26]

We have shown that IFN-α enhanced the expression of T-bet mRNA in immobilized anti-CD3–activated $CD4^+$ T cells. In this regard it has recently been disclosed that IL-27 and IFN-α activate signal transducer and activator

TABLE III. Effects of IFN-α and IL-12 on IL-13 production by immobilized anti-CD3–activated $CD4^+$ T cells

	IL-13 production, pg/mL (mean ± SD)			
Incubation	**Nil***	**IFN-α**	**IL-12**	**IFN-α + IL-12**
24 h	137.6 ± 131.5	71.5 ± 59.5*	113.1 ± 88.5	62.4 ± 45.6*†
72 h	1278.8 ± 640.8	650.9 ± 429.0*	1310.8 ± 797.6	685.0 ± 496.0*†

$CD4^+$ T cells (2×10^5/well) from 7 healthy individuals were cultured in wells with immobilized anti-CD3 (mAb 64.1, 100 ng/well) with or without IFN-α (1×10^5 IU/mL) and IL-12 (10 ng/mL). After 24 or 72 hours of incubation, the supernatants were assayed for IL-13 contents by means of ELISA.
*$P < .05$ compared with cultures without cytokines *(Nil)*.
†$P < .05$ compared with cultures with IL-12.

TABLE IV. Effects of IFN-α and IL-12 on IL-4 protein production and mRNA expression in immobilized anti-CD3–activated $CD4^+$ T cells

		Addition			
Assays	**Length of incubation**	**Nil**	**IFN-α**	**IL-12**	**IFN-α ± IL-12**
IL-4 protein production (pg/mL) (mean ± SD)	24 h	46.9 ± 42.3	75.1 ± 58.8*	53.5 ± 49.1	77.1 ± 57.3*†
	72 h	103.1 ± 69.4	121.2 ± 81.6*	121.4 ± 71.8*	143.4 ± 86.5*†
IL-4 mRNA expression ($\times 10^{-3}$ to β-actin mRNA copies) (mean ± SD)	3 h	2.172 ± 1.662	4.602 ± 2.718*	1.403 ± 0.835*	4.145 ± 2.445*†
	24 h	1.478 ± 1.052	1.943 ± 1.044*	1.213 ± 0.781*	1.688 ± 1.242†

$CD4^+$ T cells (2×10^5/well) from 8 healthy individuals were cultured in wells with immobilized anti-CD3 (mAb 64.1, 100 ng/well) with or without IFN-α (1×10^5 IU/mL) and IL-12 (10 ng/mL). After 24 or 72 hours of incubation, the supernatants were assayed for IL-4 contents by means of ELISA. After 3 or 24 hours of incubation, total RNA was isolated, and real-time quantitative PCR was performed with specific primers for IL-4 and β-actin. All results were calibrated to the copy number of β-actin (copies per microliter) from each cDNA sample.
*$P < .05$ compared with cultures without cytokines *(Nil)*.
†$P < .05$ compared with cultures with IL-12.

of transcription 1 and 3 to induce T-bet mRNA in naive T cells.[27] Induction of T-bet resulted in upregulation of IL-12Rβ2 on naive T cells.[27] It was thus possible that T-bet might act upstream of IL-12Rβ2 in early T_H1 differentiation. However, IFN-γ induced expression of T-bet, but not IL-12Rβ2, in naive T cells.[27] It is therefore most likely that T-bet and IL-12Rβ2 might be regulated by independent mechanisms. In addition, our data indicate that T-bet and IL-12Rβ2 are not sufficient for the induction of optimal T_H1 responses, although they are important for early T_H1 commitment.[27]

Several studies showed that the T_H2 cytokines IL-4, IL-5, and IL-13 are regulated by a coordinated mechanism.[28,29] On the other hand, a number of other studies showed evidence for differential regulation of the expression of IL-4 and IL-5 in murine and human T cells.[30-32] The results in the current studies have disclosed that IFN-α displays differential effects on the expression of these T_H2 cytokines. Thus IFN-α suppressed the expression of IL-5 and IL-13, whereas it enhanced the expression of IL-4 in anti-CD3–activated $CD4^+$ cells. Of note, in the report by Cousins et al,[29] the expression of IL-5 is well correlated with that of IL-13, but not with that of IL-4. Taken together, it is most likely that the expression of various T_H2 cytokines might be differentially regulated in $CD4^+$ T cells.

Some studies disclosed that IFN-α inhibited the differentiation of T_H2 cells producing IL-4 and IL-5 in bulk cultures of PBMCs,[33,34] whereas other studies showed that IFN-α enhanced the production of IL-4 by PBMCs from patients with chronic hepatitis C.[35] Of note, it has been also demonstrated that IFN-α by itself directly inhibited the production of IL-5 by $CD4^+$ T cells stimulated with PMA and anti-CD28.[19] Although IL-5 production was strongly inhibited in this study, IL-4 production was either upregulated or unchanged by IFN-α.[19] It was thus suggested that the effects of IFN-α on IL-4 production might depend on the system considered. Moreover, it was also possible that the contaminating non-T cells[33-35] or supplemental fibroblasts[19] might result in conflicting results as to the effects of IFN-α on IL-4 expression. In this regard the results in the current studies have clearly demonstrated that IFN-α directly upregulates IL-4 production and mRNA expression in $CD4^+$ T cells stimulated with immobilized anti-CD3 in the complete absence of other cell components, such as NK cells, accessory cells, and B cells.

The time kinetics of IL-5 and IL-13 expression were quite different from those of IL-4 expression in cultures of immobilized anti-CD3–stimulated $CD4^+$ T cells. Thus the production of IL-4 appeared to reach its peak at 24 hours of culture, whereas that of IL-5 and IL-13 markedly increased between 24 and 72 hours, during which the

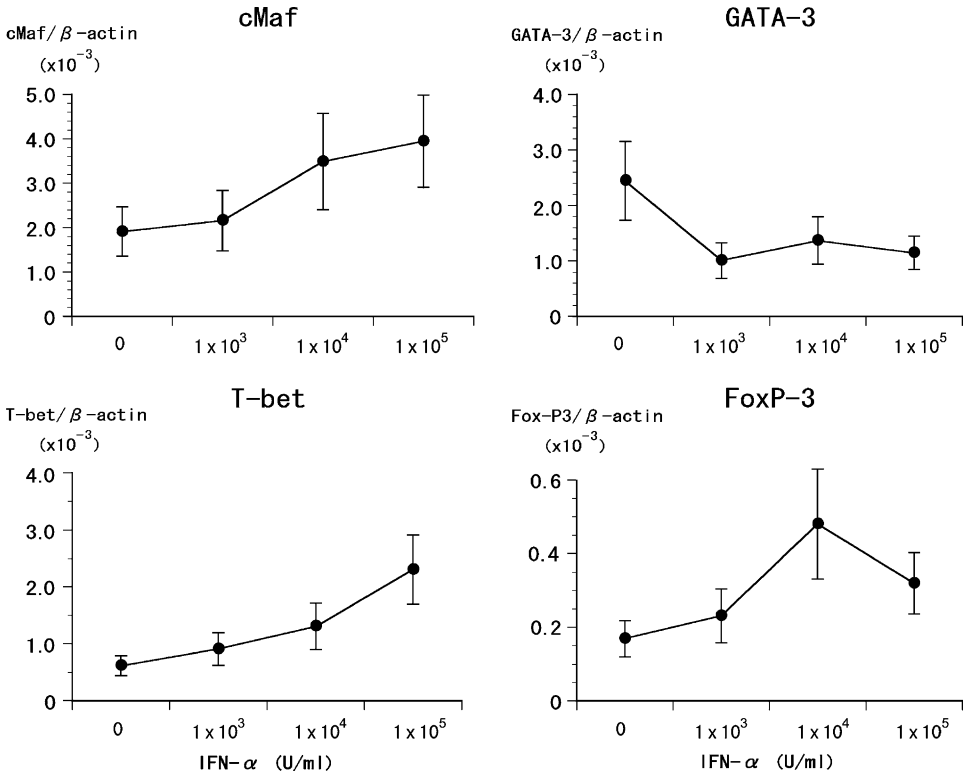

FIG 2. Effects of IFN-α on the expression of mRNA for various transcription factors in anti-CD3–activated $CD4^+$ T cells. $CD4^+$ T cells (2×10^5/well) were cultured in wells with immobilized anti-CD3 (mAb 64.1, 100 ng/well) with various concentrations of IFN-α. After 3 hours of incubation, total RNA was isolated, and real-time quantitative PCR was performed with specific primers for c-Maf, GATA-3, T-bet, Fox-P3, and β-actin. All results were calibrated to the copy number of β-actin (copies per microliter) from each cDNA sample. *Error bars* indicate the SD of duplicated determinations.

production of IL-4 was almost unchanged. Moreover, the upregulation of IL-4 production by IFN-α was clearly observed as early as 24 hours of culture, whereas the downregulation of IL-5/IL-13 production by IFN-α became evident at 72 hours of culture, when the effect of IFN-α on IL-4 production was less marked. These results suggest that IL-4 and IL-5/IL-13 might be expressed at different stages of activation of $CD4^+$ T cells or be expressed in different subsets of $CD4^+$ T cells. In fact, previous studies showed that IL-4 is prominently produced by naive T_H0 cells in contrast to IL-5 and IL-13, which are generally limited to T_H2-like effector-memory cells.[36] Moreover, it has been revealed that the presence of IL-4 in initial priming of $CD4^+$ T cells directs the development of T_H2-like effector cells, although the source of IL-4 initiating this process is debated.[37,38] It is therefore possible that IFN-α might facilitate the development of T_H2 cells through upregulation of IL-4 expression in naive T_H0 cells. Further studies are required to clarify this point.

It has been revealed that the transcription factors c-Maf and GATA-3 are selectively expressed in T_H2 cells and have been shown to regulate T_H2 cytokine expression.[9,10] Thus c-Maf is required for the expression of IL-4,[11] whereas GATA-3 is critical for the expression of IL-5[12] and IL-13.[13] It was therefore possible that the differential effects of IFN-α on the expression of IL-4, IL-5, and IL-13 in anti-CD3–activated $CD4^+$ T cells might result from changes in the expression of these transcription factors. Of note, we demonstrate that IFN-α enhances the expression of c-Maf mRNA, whereas it does not affect the expression of GATA-3 mRNA in $CD4^+$ T cells stimulated with immobilized anti-CD3. These results therefore suggest that IFN-α might enhance the expression of IL-4 in anti-CD3–activated $CD4^+$ T cells by upregulating the expression of c-Maf mRNA. Moreover, it is also suggested that IFN-α might suppress the expression of IL-5 and IL-13 through unknown mechanisms that do not involve the expression of GATA-3 mRNA.

Recent studies have demonstrated the presence of a population of regulatory T cells that developed from naive $CD4^+CD25^-$ T cells during a T_H1 response distinct from $CD25^+$ regulatory T cells.[25] These regulatory T cells expressed Fox-P3 and T-bet and potently inhibited the development of airway hyperreactivity.[25] Of note, we have also shown in the present study that IFN-α upregulates the expression of mRNA for Fox-P3, as well as T-bet, in immobilized anti-CD3–stimulated $CD4^+$ T cells. It is

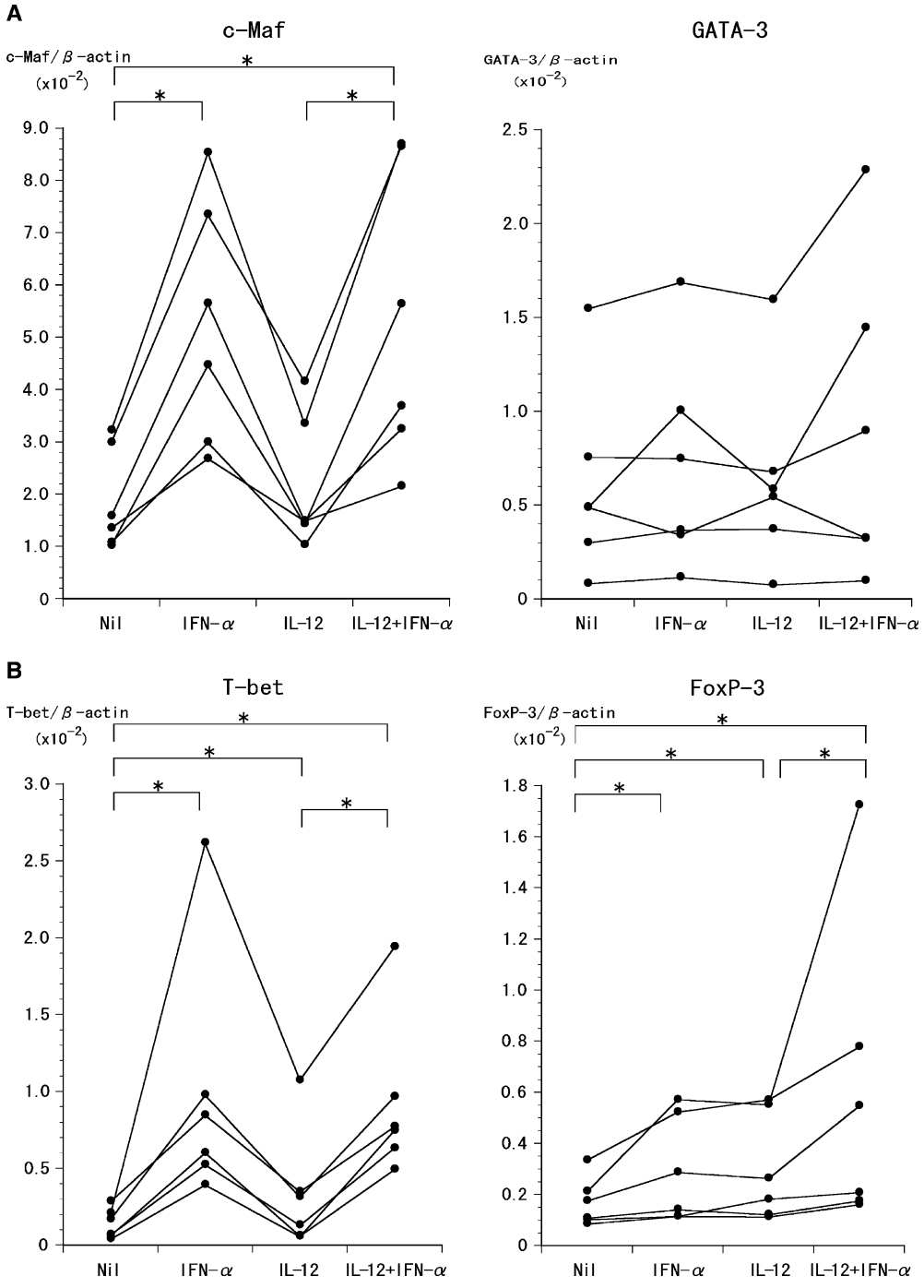

FIG 3. Effects of IFN-α and IL-12 on the expression of mRNA for various transcription factors in anti-CD3–activated $CD4^+$ T cells. $CD4^+$ T cells (2×10^5/well) were cultured in wells with immobilized anti-CD3 (mAb 64.1, 100 ng/well) with or without IFN-α (1×10^5 IU/mL) and IL-12 (10 ng/mL). After 3 hours of incubation, total RNA was isolated, and real-time quantitative PCR was performed with specific primers for c-Maf, GATA-3, T-bet, FoxP3, and β-actin. All results were calibrated to the copy number of β-actin (copies per microliter) from each cDNA sample. Each *line* on the graph is representative of the same cell preparation from the same donor. $*P < .05$.

therefore possible that IFN-α might promote the development of T_H1-like regulatory T cells, which might suppress T_H2 responses, including the expression of IL-5 and IL-13.

In summary, taken together with data from previous studies,[17,19,33] the data in the present study provide a rational basis for therapeutic use of IFN-α therapy in T cell–mediated disorders associated with IL-5 hyperproduction, such as hypereosinophilic syndrome.[39] Of note, it has been recently reported that IFN-α treatment rapidly improved the clinical condition of patients with

corticosteroid-resistant asthma.[40] The establishment of a correct T_H1/T_H2 balance and the induction of the IL-10 gene have been suggested as potential mechanisms of action.[40] It is also possible that induction of T_H1-like regulatory T cells might be involved in the IFN-α–mediated improvement of corticosteroid-resistant asthma. Further studies designed to explore the capacity of IFN-α to induce T_H1-like regulatory T cells would be important for a complete understanding of its role in the treatment of bronchial asthma and atopic diseases.

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