Published in final edited form as:
J Immunol. 2010 July 15; 185(2): 813–817. doi:10.4049/jimmunol.1000469.

Type I interferon reverses human Th2 commitment and stability by suppressing GATA3[1]

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Abstract

Th2 cells regulate inflammatory responses to helminth infections while also mediating pathological processes of asthma and allergy. IL-4 promotes Th2 development by inducing the expression of the GATA3 transcription factor, and the Th2 phenotype is stabilized by a GATA3-dependent auto-regulatory loop. In this study, we found that type I interferon (IFN-α/β) blocked human Th2 development and inhibited cytokine secretion from committed Th2 cells. This negative regulatory pathway was operative in human but not mouse $CD4^+$ T cells and was selective to type I interferon as neither IFN-γ nor IL-12 mediated such inhibition. IFN-α/β blocked Th2 cytokine secretion through the inhibition of GATA3 during Th2 development and in fully committed Th2 cells. Ectopic expression of GATA3 via retrovirus did not overcome IFN-α/β-mediated inhibition of Th2 commitment. Thus, we demonstrate a novel role for IFN-α/β in blocking Th2 cells, suggesting its potential as a promising therapy for atopy and asthma.

Introduction

$CD4^+$ Th2 cells regulate both humoral and cell-mediated inflammatory responses to helminth pathogens as well as toxins and other foreign soluble molecules (1). In genetically predisposed individuals, Th2 cells also mediate atopic responses to normally innocuous materials such as animal dander, pollens, and pollutants. Th2 cells mediate these processes through the selective secretion of a subset of cytokines that include IL-4, IL-5, IL-13, and in some cases IL-9 and IL-10 (2). Together, these cytokines lead to a cascade of events that culminate in driving inflammation at sites of allergen contact, such as the lungs in cases of allergic asthma.

Various signals contribute to a “Th2 environment” such as thymic stromal lymphopoietin (TSLP), OX40L, Notch (3), and IL-25 (4); however, IL-4 remains the key signal that

[1]This work was supported by the NIH/NIAID (AI056222 awarded to J.D.F., and AI59379 awarded to M.A.G.) and by the ExxonMobil Corporation (M.A.G.). J.P.H. was supported by a training grant from the NIH/NIAID (AI005284), and H.J.R. was supported by a pre-doctoral fellowship from the NIH/NIAID (AI068622).

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Disclosure
The authors have no financial conflict of interest.

directly drives Th2 commitment. IL-4 receptor signaling promotes STAT6 recruitment and phosphorylation (5), which ultimately regulates induction of GATA3 transcription (6). GATA3 is a member of the 2-zinc finger GATA-binding family of transcription factors, and its expression is critical for both T cell thymic development and Th2 commitment (7). Once induced by IL-4, the GATA3 protein positively regulates Th2 cytokine gene expression and negatively regulates various aspects of Th1 commitment such as inhibiting the expression of the IL-12Rβ2 subunit (8). Further, GATA3 positively regulates its own expression through an auto-activation loop, thereby maintaining Th2 stability in the absence of further IL-4 signaling (9,10). The inherent stability of Th2 cells poses a significant challenge to treating allergic diseases. However, in this study, we have found that type I interferon (IFN-α/β) potently inhibits IL-4-driven Th2 commitment in human CD4+ T cells and destabilizes the Th2 phenotype by inhibiting GATA3 expression. Considering that IFN-α/β is used routinely to treat various diseases including multiple sclerosis and hepatitis C, we propose that IFN-α/β may represent a novel and readily available therapy for atopic conditions such as allergic asthma.

Materials and Methods

Human Subjects

Peripheral blood was collected from healthy adult donors, and informed consent was obtained from each donor according to the guidelines approved by the Institutional Review Board at UT Southwestern Medical Center (Dallas, TX).

T cell cultures

Naïve $CD4^+$/$CD45RA^+$ T cells were purified (>90%) from buffy coats either by flow cytometric sorting or by magnetic bead separation (BD Biosciences) as previously described (11). $CD4^+$ cells were activated with plate-bound anti-CD3/anti-CD28 and IL-2 in complete IMDM containing 10% FBS (cIMDM) in the presence of cytokines (rhIL-4, rhIL-12, rhIFN-γ, rhIFN-α(A), rhIFN-β (R & D Systems), or IL-29 (Peprotech)), or with neutralizing anti-cytokine antibodies (anti-IL-4 (R & D Systems) anti-IFN-γ (4S.B3), anti-IL-12 (20C2), or anti-hIFNAR2 (Millipore)) as indicated. Cytokines and antibodies were used at the following concentrations: anti-CD3 (5.0 μg/ml), anti-CD28 (5.0 μg/ml), IL-2 (50 U/ml), IL-4 (20 ng/ml), IL-12 (10 ng/ml), IFN-γ (5 ng/ml), IFN-α (1000 U/ml), IFN-β (1000 U/ml), IL-29 (100 ng/ml), anti-IL-4 (2 μg/ml), anti-IL-12 (5 μg/ml), anti-IFN-γ (5 μg/ml), and anti-IFNAR2 (2 μg/ml). Cells were cultured for either 7 days, or restimulated with anti-CD3/anti-CD28 with various cytokine conditions for an additional 7 days prior to analysis.

Flow Cytometry

Intracellular cytokine staining was performed as described (12) with the following antibodies: hCD4-PE, hIFNγ-FITC, Streptavidin-Qdot655 (Invitrogen);hCD45RA -FITC, hIL-4-PE, hIFNγ-PE-Cy7, hGATA3-Alexa647, hCD294 (CRTH2)-Alexa647, pY641-STAT6, Streptavidin-PerCp, (BD Biosciences); hSTAT6 (Santa Cruz Biotechnology); hIL-4-FITC, hIL-5-PE, hIL-13-APC (Biolegend). Data were collected on an LSR II or FACS Calibur (BD Biosciences) and processed using FlowJo (Tree Star).

Quantitative real-time PCR analysis

Total RNA was extracted using the RNeasy Mini Kit (Qiagen) or Pico Pure Kit (Arcturus) according to the manufacturer's instructions followed by reverse transcription. The resultant cDNA was subjected to qPCR analysis with SYBR Green Master Mix (Stratagene) on an ABI7300 real-time thermocycler (Applied Biosystems). Human GAPDH was used as a reference. Relative changes in mRNA expression were calculated by the $2^{-\Delta\Delta CT}$ method

(13), and all treatment groups were referenced to the neutralized control. The primers used to detect mRNA transcripts are as follows: hGATA3, 5′-AGGGACGTCCTGTGCGAACT-3′ and 5′-GGTCTGGATGCCTTCCTTCTTCAT-3′ hT-bet, 5′-GTCCAACAATGTGACCCAG-3′, 5′-GCAGTCACGGCAATGAACTG-3′ hGAPDH, 5′-CTGCACCACCAACTGCTTAGC-3′ and 5′-TCATGTTCTGGAGAGCCCCG-3′.

Quantification of secreted cytokines

Standard cytokine ELISAs were performed as previously described (14). Alternatively, multiple cytokine concentrations were quantified by Cytometic Bead Array (BD Biosciences) or Meso Scale discovery platform according to the manufacturer's instructions (Meso Scale Discovery).

Retroviral transduction

Retroviral supernatants were generated by transfection of the Phoenix am- photropic cell line as described (10). Purified $CD4^+/CD45RA^+$ cells were activated with plate-bound anti-CD3/anti-CD28 in cIMDM with IL-2, IL-4, anti-IL-12 and anti-IFN-γ for 7 days. The cells were then reactivated with anti-CD3/anti-CD28 plus IL-2 and transduced with retroviral constructs expressing either GFP only (GFPRV) or hGATA3 (GATA3-GFP) (10). At day 14, transduction efficiency averaged between 3–5%, and GFP^+ cells were sorted to > 90% purity. These GFP^+ cells were then reactivated with anti-CD3/anti-CD28 plus IL-2 in the presence or absence of IFN-α.

Statistical analysis

All bar graphs are shown as mean +/− SEM. Statistical analysis was performed by one-way ANOVA using Prism software (GraphPad). Values of $p < 0.05$ were considered significant.

Results and Discussion

Selective inhibition of human Th2 development by IFN-α/β

IL-4 is a critical regulator of Th2 development, and the downstream signaling events in this pathway dominate over the effects of other Th1-inducing signals such as IFN-γ and IL-12. While early reports suggested that IFN-α/β could decrease baseline Th2 cytokine secretion in naïve human $CD4^+$ T cells (15,16), the role of IFN-α during Th2 priming in response to IL-4 has not been investigated in human cells. Previous studies with mouse DO11.10 T cells ruled out IFN-α/β as a negative regulator of Th2 commitment (17,18). Indeed, we confirmed these previous observations by demonstrating that IFN-α does not inhibit IL-4-driven Th2 development in murine OT-II transgenic T cells (Supplementary Fig. S1). However, in the present study, we tested the role of IFN-α/β in regulating human Th2 development by activating purified naïve human $CD4^+/CD45RA^+$ T cells with plate-bound anti-CD3/anti-CD28 in the presence of cytokines or neutralizing anti-cytokine antibodies for 7 days. Cells were then restimulated and assessed for cytokine production. IL-4 potently induced Th2 development by promoting high levels of IL-4 expression (Fig. 1A, lower panel), as well as IL-4 and IL-5 secretion (Fig. 1B). Moreover, IL-12, but not IFN-α, promoted Th1 commitment as revealed by high levels of IFN-γ expression (Fig. 1A, upper panel). Neither IL-12 nor IFN-γ inhibited IL-4 expression (Fig. 1A, lower panel and Fig. 1B, upper panel), while IL-12 did inhibit IL-5 secretion by approximately 50% (Fig. 1B, lower panel). In contrast, we found that IFN-α markedly blocked IL-4-driven Th2 development in human $CD4^+$ T cells (Fig. 1A, lower panel and Fig. 1B). This inhibition was specific to Th2 cells as IFN-α did not block IL-12-mediated Th1 development (Fig. 1A, upper panel), and IFN-α consistently inhibited Th2 development in cells isolated from each of 10 healthy adult

donors (Fig. 1C). IFN-α did not attenuate proliferation during priming, suggesting that IFN-α/β did not interfere with Th2 commitment by inhibiting Th2 cell expansion (Supplementary Fig. S2). Finally, both IFN-α and IFN-β blocked Th2 development in a dose-dependent manner (Supplementary Fig. S3), suggesting that Th2 cross regulation in human $CD4^+$ T cells is a general property of IFN-α/β signaling.

In humans, the prostaglandin D2 receptor, CRTH2, is selectively expressed on Th2 cells (19) and is induced by IL-4 during Th2 development (20). We assessed the expression of CRTH2 in response to cytokine activation during Th2 commitment in human $CD4^+$ T cells. IL-4 promoted the development of cells expressing CRTH2; however, IFN-α markedly blocked IL-4-driven CRTH2 expression (Fig. 1D). A recent report suggested that IL-28/29 (IFN-γ, type III interferon) could inhibit human Th2 cells (21). We compared IL-29 (IFN-γ1) with IFN-α and observed a modest decrease in IL-4-driven CRTH2 expression in response to IL-29 that was not significantly different compared to treatment with IL-4 alone (Fig. 1D, compare conditions 2 and 6). However, we found that IFN-α significantly blocked IL-4-mediated CRTH2 expression compared to IL-29 (Fig. 1D). Therefore, IFN-α/β inhibits both Th2 cytokine secretion and expression of the inflammatory receptor, CRTH2 that mediates recruitment and cytokine secretion from committed Th2 cells (22). Collectively, these data demonstrate a unique and species-specific inhibition of human Th2 development by IFN-α/β.

IFN-α/β destabilizes committed human Th2 cells by repressing GATA3

IL-4 regulates Th2 commitment via activation of STAT6, which drives expression of the Th2-specific transcription factor GATA3 (5,6,23). GATA3 stabilizes the Th2 phenotype by a feedback loop that uncouples the requirement for further IL-4 signaling (9,10). We tested the ability of IFN-α to disrupt the stability of committed Th2 cells by first activating naïve T cells for 1 week with IL-4 (Fig. 2). These cells were then re-activated for an additional 7 days with either IL-4, anti-IL-4, IFN-α, or IL-4 + IFN-α followed by analysis of Th2 cytokine production. IL-4 efficiently promoted the development of Th2 cells, and, as expected, their stability was maintained during secondary culture regardless if the cells were activated with additional IL-4 or with anti-IL-4 (Fig. 2, *A* and *B*, conditions 1–3). However, IFN-α markedly inhibited Th2 cytokine expression during secondary activation even in the presence of additional IL-4. Further, IFN-α inhibited IL-4 secretion to levels observed in non-Th2 cells cultured under neutralizing conditions for 2 consecutive weeks (Fig. 2*B*).

A recent study by Löhning and colleagues demonstrated that a combination of IL-12 + IFN-γ and IFN-α could promote IFN-γ secretion from fully committed murine Th2 cells and give rise to IL-4/IFN-γ dual cytokine-secreting cells (24). However, we found that in human, IFN-α alone suppressed IL-4 secretion both during priming (Fig. 1) and after secondary activation (Fig. 2) even in the absence of IL-12. Moreover, we found that in human Th2 cells, the combination of IFN-γ + IFN-α + IL-12 only marginally increased IFN-γ secretion during secondary priming (Supplementary Fig. 4). Thus, in human $CD4^+$ T cells, IFN-α suppresses Th2 commitment and stability and is dominant over the Th2-promoting signal of IL-4. Importantly, IFN-α does not promote significant IFN-γ secretion either during priming or during secondary redirection, making IFN-α an attractive therapeutic candidate for atopic diseases.

GATA3 is required for Th2 cell stability and cytokine production. $CD4^+$ cells from mice with a T cell-specific deletion of GATA3 produce increased levels of IFN-γ but greatly reduced levels of IL-4, IL-5, and IL-13 (7). Additionally, the deletion of GATA3 specifically in Th2 cells significantly impairs their ability to produce these cytokines (7,25). Based on these observations, we tested the ability of IFN-α to modulate the induction of GATA3 by IL-4 (Fig. 3). IL-4 induced GATA3 mRNA in naïve human $CD4^+$ T cells (Fig.

3A, compare conditions 1–2). However, cells cultured with IFN-α showed significantly reduced GATA3 transcript levels (Fig. 3A, conditions 3–4). The block in GATA3 mRNA also resulted in a significant inhibition of IL-4-induced GATA3 protein by IFN-α (Fig. 3, B and C). As previously reported (26,27), naïve $CD4^+$ T cells expressed low levels of GATA3 that was induced by TCR activation and further elevated in response to IL-4 (Fig. 3C, conditions 1–3). In line with the observed decrease in GATA3 mRNA (Fig. 3A), IFN-α significantly inhibited IL-4-induced GATA3 protein expression that was not observed in response to either IL-12 or IFN-γ (Fig. 3C). Since we have demonstrated that IFN-α inhibits the development of $CRTH2^+$ cells (Fig. 1D), it is not surprising that IFN-α controls GATA3 as well, considering that GATA3 has been shown to control the expression of CRTH2 (28). Thus, the ability of IFN-α to regulate a key Th2 transcription factor correlates with its ability to regulate CRTH2 and Th2 cytokine expression.

A variety of GATA binding proteins, such as Runx, Fog, and T-bet, have been shown to inhibit the transcriptional regulatory activity of GATA family members, including GATA3. We tested whether any of the select members of these GATA binding factors were induced by IFN-α during priming. However, we found that neither Runx3, Fog1 (data not shown), nor T-bet (Fig. 3D) were significantly induced by IFN-α during Th2 development suggesting a novel negative regulatory mechanism for GATA3 inhibition.

IFN-α/β overrides GATA3-mediated Th2 stability

Since IFN-α limits cytokine production from committed Th2 cells, we considered whether IFN-α bypassed IL-4 signaling and inhibited GATA3 expression directly. While induction of GATA3 by IL-4 depends upon the activation of STAT6 (23), we tested this early signaling event and found that IFN-α did not significantly alter acute IL-4-driven STAT6 phosphorylation (Supplementary Fig. S5). Thus, we determined if IFN-α could inhibit GATA3 protein levels in committed Th2 cells that have already upregulated the protein (Fig. 4A). Polarization of naïve T cells with IL-4 for 7 days led to a stable increase in GATA3 protein levels (Fig. 4A, conditions 1–3). However, IFN-α markedly inhibited GATA3 protein expression (Fig. 4A, conditions 4–5), and these data strongly suggest that IFN-α destabilizes human Th2 cells by directly interfering with GATA3 expression.

GATA3 is considered a “master regulator” of Th2 development based on its ability to promote Th2 commitment when ectopically expressed in cells under various counter-regulatory situations. The GATA3 feedback loop was originally discovered when cells transduced with GATA3RV subsequently upregulated the endogenous GATA3 mRNA (9). We hypothesized that ectopic expression of GATA3 in human Th2 cells would elevate GATA3 protein levels and thereby reverse the inhibition normally mediated by IFN-α. To test this hypothesis, we expressed GATA3 in human Th2 cells via retroviral transduction (Fig. 4). GFP^+ cells were reactivated for 3 days and the culture supernatants were analyzed for IL-5 and IL-13 (Fig. 4B). As expected, the levels of both cytokines increased significantly in cells transduced with GATA3-GFP compared to the control retrovirus (Fig. 4B). The addition of IFN-α to the culture inhibited the secretion of both IL-5 and IL-13 from cells transduced with empty retrovirus as well as in cells transduced with GATA3-GFP. Purified GFP^+ cells were cultured for seven days at which time the cells were washed, reactivated, and analyzed for cytokine secretion (Fig. 4C). Again, addition of IFN-α to the culture inhibited the secretion of Th2 cytokines even in cells ectopically expressing GATA3. Thus, IFN-α is able to overcome ectopic expression of GATA3 and suppress the secretion of Th2 cytokines.

To confirm that GATA3 was actually expressed in the transduced cells, we analyzed GATA3 protein levels by intracellular staining at day 5 following sorting (Fig. 4D). Indeed, GATA3 protein levels increased in cells transduced with GATA3-GFP compared to the

GFPRV control. However, IFN-α treatment reduced GATA3 protein levels in cells transduced with either the empty retrovirus or with GATA3-expressing retrovirus. IFN-α treatment did not affect the retroviral mRNA levels (data not shown), further supporting the conclusion that IFN-α inhibits GATA3 protein levels.

In summary, we demonstrate a novel function for type I interferon to block Th2 development and to destabilize committed Th2 cells via inhibition of GATA3. This function is lacking in mice but may be important for ensuring proper differentiation of $CD4^+$ T cells activated during viral infections in humans. As IFN-α/β has already been demonstrated to inhibit the development of Th17 but not Th1 cells (12,29,30), IFN-α/β may restrict alternate differentiation pathways for newly activated $CD4^+$ T cells. Further, both Th2 and Th17 responses have been proposed to contribute to the pathology of allergic diseases (31,32). Thus, since IFN-α/β inhibits both Th17 and Th2 pathways but does not promote Th1 cytokine expression, we propose that IFN-α/β may be useful as a therapy for the treatment of allergies and asthma.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We acknowledge Angela Mobley, Elizabeth Curry, and the UT Southwestern Flow Cytometry Core Facility for help with cell sorting and flow cytometric analysis. We thank Drs. Rebecca Gruchalla and James Forman for helpful discussion and critical review of the manuscript.

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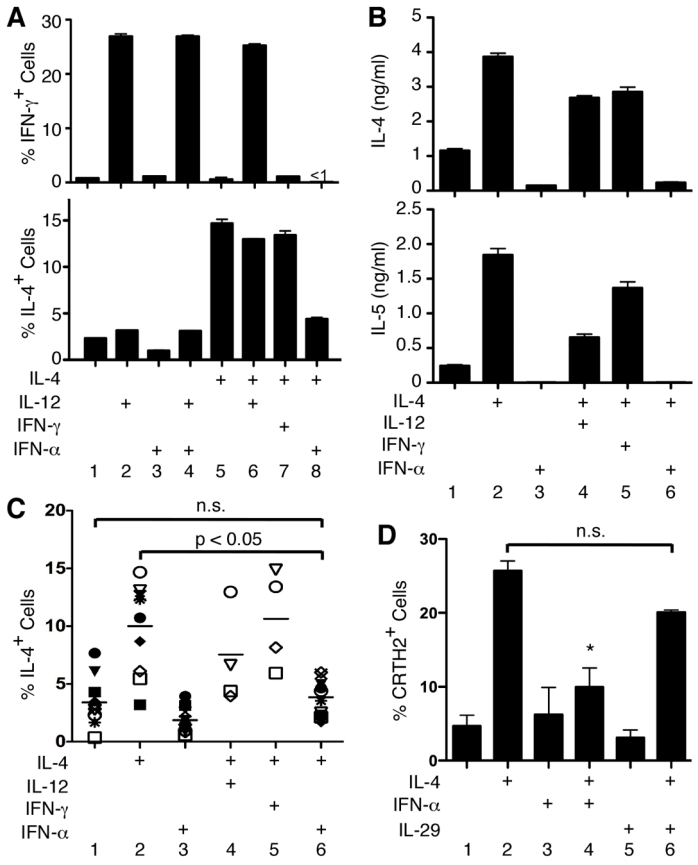

FIGURE 1.

IFN-α/β inhibits human $CD4^+$ Th2 development. Purified human $CD4^+/CD45RA^+$ cells were activated with plate bound anti-CD3/anti-CD28 under defined cytokine conditions. Cytokines were either neutralized with anti-cytokine antibodies (no symbol), or cytokines were added as indicated by the "+" symbol. *A*, Cells were restimulated with PMA + ionomycin, and IFN-γ and IL-4 expression were measured by intracellular staining. Data are gated on live events. *B*, Cells were restimulated with PMA + ionomycin for 24 h, and IL-4 and IL-5 were quantified by cytometric bead array from the culture supernatants. *C*, IL-4 expression was measured by intracellular staining of samples from multiple donors. 10 donors were assessed with conditions 1–3 and 6, while 4 of the 10 donors were also assessed with conditions 4–5. Each symbol represents a separate donor. *D*, Induction of CRTH2 expression was assessed by flow cytometric analysis. *, $p < 0.05$ compared to IL-4 and to IL-4 + IL-29 (conditions 2 and 6).

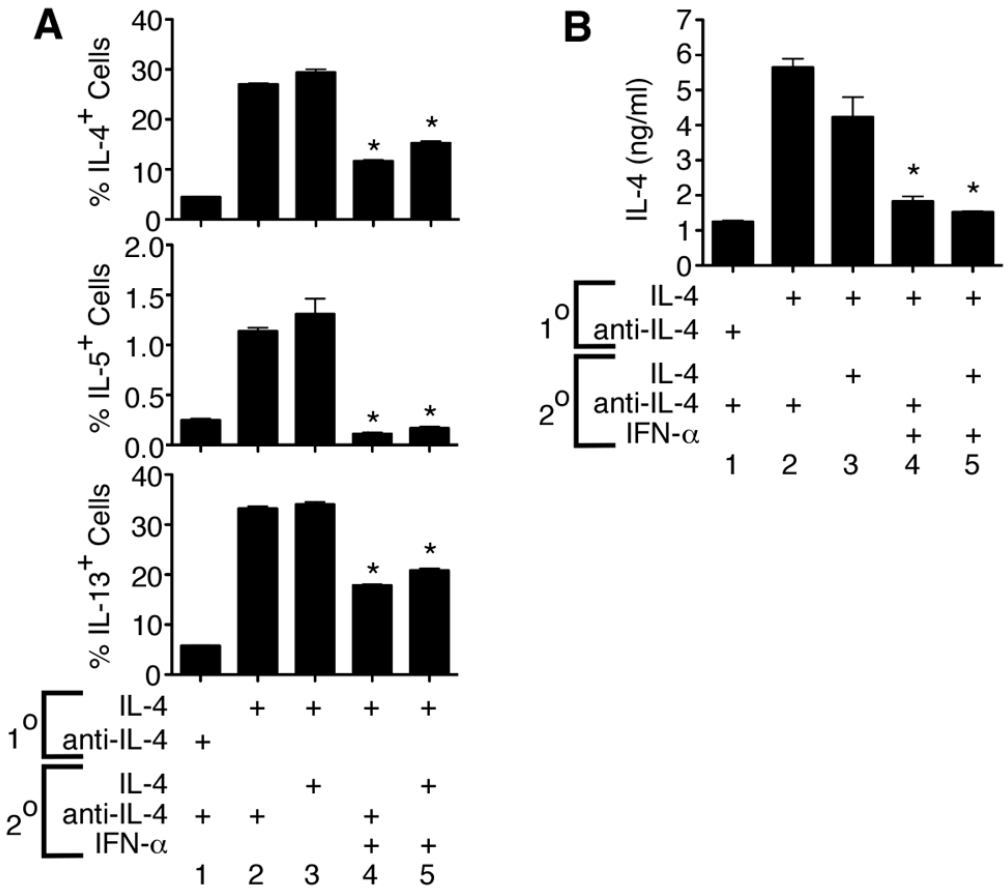

FIGURE 2.

IFN-α/β suppresses Th2 cytokine expression in committed Th2 cells. Purified human $CD4^+$/$CD45RA^+$ cells were activated with plate bound anti-CD3/anti-CD28 for 7 days under neutralizing conditions or with IL-4 to promote Th2 development (1° condition). Cells were then washed and restimulated for an additional 7 days with anti-IL-4, IL-4, IFN-α, or IL-4 + IFN-α (2° conditions). *A*, Cells were stimulated with PMA + ionomycin for 6 h, and IL-4, IL-5, and IL-13 were measured by intracellular staining. *B*, Cells were stimulated with PMA + ionomycin for 48 h, and IL-4 was quantified by ELISA from the culture supernatants. *, $p < 0.05$ compared to IL-4 (*A* and *B*, condition 3).

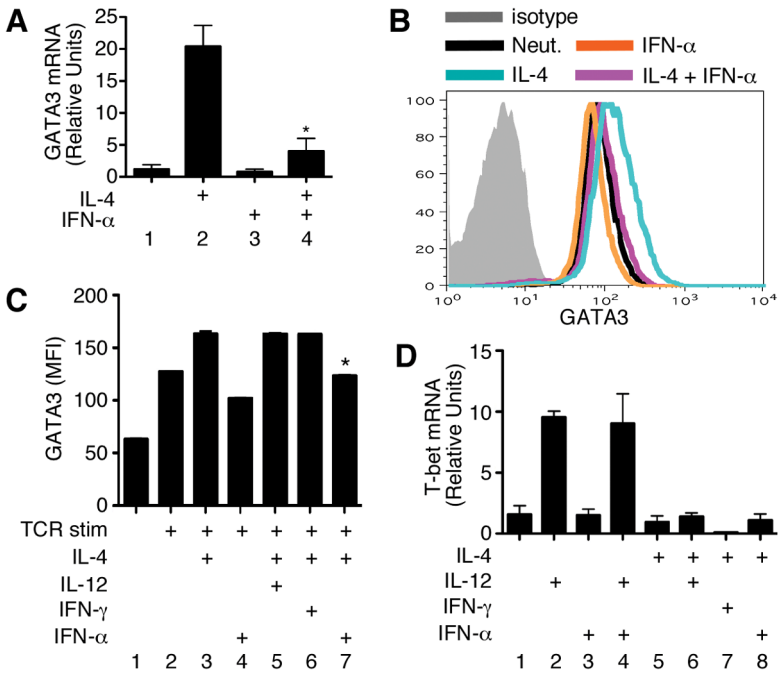

FIGURE 3.

IFN-α/β specifically inhibits GATA3 expression. *A*, Purified human $CD4^+/CD45RA^+$ cells were activated with plate bound anti-CD3/anti-CD28 for 72 h with IL-4, IFN-α, or IL-4 + IFN-α. mRNA was isolated from cells, and relative GATA3 mRNA was quantified by real-time PCR. *, $p < 0.05$ compared to IL-4 (condition 2). *B*, Cells were activated as in A for 6 days, and GATA3 protein was assessed by intracellular staining and flow cytometric detection. *C*, Purified human $CD4^+/CD45RA^+$ cells were left unstimulated or were activated with plate bound anti-CD3/anti-CD28 for 6 days in the presence of the indicated cytokines. GATA3 protein was assessed by intracellular staining and flow cytometric detection, and the data are gated on live cells and expressed as the mean fluorescence intensity of the population. *, $p < 0.05$ compared to IL-4 (condition 3). *D*, Purified human $CD4^+/CD45RA^+$ cells were activated with plate bound anti-CD3/anti-CD28 for 7 days in the presence of the indicated cytokines. mRNA was isolated from cells, and relative T-bet mRNA was quantified by real-time PCR.

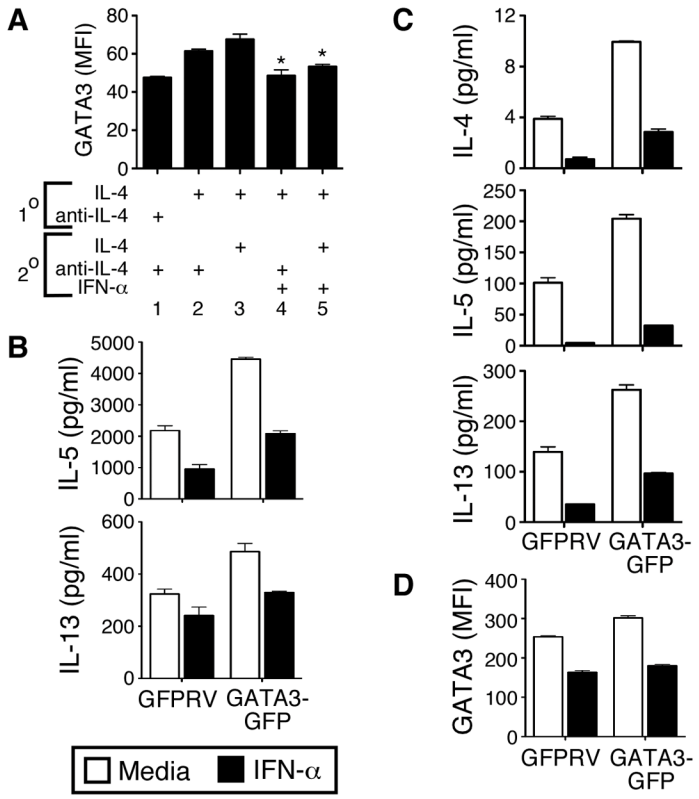

FIGURE 4.

IFN-α suppresses Th2 cytokines by inhibiting GATA3 protein levels in committed Th2 cells. *A*, Cells were activated for two consecutive weeks under 1° and 2° conditions as described in Fig. 2. GATA3 protein was measured on day 14 by intracellular staining. *, $p < 0.05$ compared to IL-4 (condition 3). *B-D*, Purified human $CD4^+/CD45RA^+$ cells were activated with plate bound anti-CD3/anti-CD28 under Th2 conditions for 1 week prior to retroviral transduction with GFPRV or GATA3-GFP. GFP+ cells were sorted on day 14 and reactivated with anti-CD3/anti-CD28 in the presence or absence with IFN-α. *B*, Cell culture supernatants were harvested on day 3 following the GFP sort and analyzed for IL-5 and IL-13 by ELISA. *C*, On day 7 following the GFP sort, cells were washed and restimulated for 48 h with anti-CD3/anti-CD28. IL-4, IL-5 and IL-13 were quantified by ELISA from the culture supernatants. *D*, Cells were harvested on day 5 following the GFP sort and GATA3 protein was measured by intracellular staining and flow cytometric detection. Data are gated on live cells and expressed as the mean fluorescence intensity of the population.