Bi-phasic Effect of Interferon (IFN)-α

IFN-α UP- AND DOWN-REGULATES INTERLEUKIN-4 SIGNALING IN HUMAN T CELLS*

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Interferon (IFN)-α/β is produced by virally infected cells and is believed to play an important role in early phases of the innate immune response. In addition, IFN-α/β inhibits interleukin (IL)-4 signaling in B cells and monocytes, suggesting that IFN-γ is a Th1 cytokine. Here, we study cross-talk between IFN-α and IL-4 in human T cells. As expected, stimulation with IFN-α for 12–24 h inhibits IL-4 signaling. Surprisingly, however, IFN-α has the opposite effect on IL-4 signaling at earlier time points (up to 6 h). Thus, IFN-α enhances IL-4-mediated STAT6 activation in both CD4+ and CD8+ human T cells. The effect is specific because (i) another interferon, IFN-γ, does not enhance IL-4-mediated STAT6 activation, (ii) IFN-α-mediated STAT1 and STAT2 activation is not modulated by IL-4, and (iii) activation of Janus kinases is not enhanced or prolonged by simultaneous stimulation with IFN-α and IL-4. Moreover, co-stimulation results in a selective increased STAT6/STAT2 association and an association between IFNAR/IL-4R components, suggesting that the IFNAR provides a cooperative increase in nuclear translocation, DNA binding, transcriptional activity, and mRNA expression of STAT6 target genes (IL-4Ra and IL-15Ra). In conclusion, we provide evidence that IFN-α both up- and down-regulates IL-4-mediated STAT6 signaling and thereby regulates the sensitivity to IL-4 in human T lymphocytes. Thus, our findings suggest that IFN-α has a complex regulatory role in adaptive immunity, which is different from the “classical” Th1 profile of IFN-γ.

Interferon-α (IFN-α) plays a crucial role in the innate immune response against viral infections. Thus, in addition to the direct anti-viral effect, IFN-α has several important regulatory effects on multiple cell types involved in the innate immune defense. For example, IFN-α stimulates the cytolytic capacity and function of NK cells, the phagocytic functions and production of cytokines by macrophages, and the expression of major histocompatibility complex molecules in most immune cells as well as many other types of somatic cells (1–3). In contrast to the well established functions in innate immunity, less is known about the functions of IFN-α in adaptive immunity. Yet some studies indicate that IFN-α promotes Th1 differentiation (4, 5) and inhibits interleukin-4 (IL-4)-induced IgE production and CD23 expression (6–9), indicating that IFN-α is a Th1 cytokine similar to IFN-γ.

IL-4 is a Th2 cytokine that plays an important role in (i) B cell activation, isotype switching, and production of IgE; (ii) commitment and differentiation of Th2 cells; (iii) inhibition of Th1 cell-mediated immune responses; and (iv) development of allergic diseases such as atopic dermatitis, allergic rhinitis and asthma (reviewed in Ref. 10). IL-4 and IFN-α transduce signals directly to the nucleus through rapid activation of the Janus kinase (JAK)/STAT signaling pathway. By binding of IL-4 and IFN-α to their high affinity receptors, receptor-associated JAKs become activated by tyrosine phosphorylation. Once activated, the JAKs phosphorylate key tyrosine residues in the cytoplasmic receptor tails, creating docking sites for STAT transcription factors. Upon tyrosine phosphorylation by JAKs, the recruited STATs dimerize and translocate to the nucleus where they activate transcription of cytokine-inducible genes (11–13). IL-4 induces activation of JAK1/JAK3 bound to the IL-4Ra chain (common γ chain, respectively). In contrast, IL-4 and IFN-α stimulation thereby lead to activation of STAT6 and STAT1, respectively, and the importance of STAT6/STAT1 in IL-4/IFN-α-signaling is clearly demonstrated in studies with STAT-deficient mice; thus, in STAT6-deficient mice, almost all of the IL-4-mediated functions in B and T cells are impaired (14–16), whereas STAT1-deficient mice show no response to IFN-α and are highly sensitive to infection by viruses (17). Once activated, STAT6 homodimers and or STAT1/STAT2 heterodimer in complex with the p48 protein (the IFN-stimulated gene factor 3 complex) bind to specific STAT-binding sequences in promotor regions of IL-4.

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‡ The abbreviations used are: IFN, interferon; IL, interleukin; JAK, Janus kinase; IFNAR, IFN-αR; γc, common γ chain; CTCL, cutaneous T cell lymphoma; STAT, signal transducers and activators of transcription; IL-4R, IL-4 receptor.
and IFN-α-inducible genes, respectively, thereby assisting in their transcriptional activation (3, 10).

It is a central dogma in immunology that IL-4 and IFN-α have opposing effects on the regulation of adaptive immune responses (reviewed in Refs. 18 and 19). As a type I IFN, IFN-α shares the same receptor with and induces indistinguishable signals from the other type I IFN, IFN-β, and has partly overlapping signaling mechanisms with the type II IFN, IFN-γ (3). It was therefore expected that IL-4 and IFN-α/IFN-β have opposing effects in adaptive immune responses. Indeed, several studies on monocytes and B cells have shown that IFN-α/IFN-β have negative regulatory effects on IL-4-mediated signaling (9, 20–24). In the present study, however, we provide the first evidence that IFN-α both up- and down-regulates IL-4-mediated signaling in human T cells, suggesting that IFN-α has a complex role in immune regulation that is different from the well established role of IFN-γ.

**EXPERIMENTAL PROCEDURES**

**Antibodies and Other Reagents**—Recombinant IFN-α (Introna) was from Schering-Plough (Kenilworth, NJ). Recombinant IL-4 and IFN-γ was from Leinco Technologies (St. Louis, MO). Recombinant IL-2 (Proleukin) was from Chiron (Emeryville, CA). Phospho-specific STAT1 (Tyr^701) and STAT6 (Tyr^641) polyclonal antibody was from New England Biolabs (Beverly, MA). Phospho-specific STAT2 (Tyr^689) polyclonal antibody was from Upstate Biotechnology, Inc. (Lake Placid, NY). Phospho-specific JAK1 (Tyr^1022/1023) was from BIOSOURCE (Camarillo, CA). STAT1, STAT2, STAT6, JAK1, JAK3, TYK2, IL-4Rα, IFNAR-1, and IFNAR-2 polyclonal antibody was from Santa Cruz Biotechnology (Santa Cruz, CA). The ECL kit was from Amersham Biosciences. The biotinylated double-stranded oligonucleotide probes used for affinity purification were: IgE-3 (5′-Bio-CGACTTCCCAAGAACGTGCTTCCCAA-3′).
...A, CTCL cells were preincubated with IFN-α (5,000 units/ml) at the indicated periods of time prior to stimulation with IL-4 (0.25 ng/ml) as indicated for 10 min. B, CTCL cells were preincubated with IL-4 (0.25 ng/ml) at the indicated periods of time, prior to stimulation with IFN-α (5,000 units/ml) as indicated for 10 min. C, CTCL cells were simultaneously stimulated with IFN-α (5,000 units/ml) and IL-4 (0.25 ng/ml) for the indicated periods of time. A–C, total cell lysates were subjected Western blotting using the indicated antibodies.

Glyceraldehyde-3-phosphate dehydrogenase was used as an internal standard, and the relative cytokine receptor expression was calculated as cytokine receptor/glyceraldehyde-3-phosphate dehydrogenase.

**RESULTS**

It is well known that IFN-α and IL-4 induce tyrosine phosphorylation of STAT1 and STAT6, respectively (3, 10, 35). This tyrosine phosphorylation is a critical step in STAT homodimerization, DNA binding, and transcriptional activation of IFN-α- and IL-4-inducible genes. Thus, to study the early signaling events during cross-talk between IL-4 and IFN-α, we first analyzed the IFN-α- and IL-4-mediated tyrosine phosphorylation of STAT proteins in a human CD8+ T cell line, MySi. As shown in Fig. 1A, stimulation with IFN-α induced tyrosine phosphorylation of STAT1 in a dose-dependent manner with an optimum of 10,000 units/ml and a lower detection level between 500 and 1,000 units/ml. Likewise, IL-4 induced tyrosine phosphorylation of STAT6 in a dose-dependent manner with an optimum of 25 ng/ml and a lower detection level of ~0.05–0.1 ng/ml (Fig. 1B). IFN-α at 10,000 units/ml induced a weak tyrosine phosphorylation of STAT6 (Fig. 1C, lane 2), whereas optimal concentrations of IL-4 (25 ng/ml) did not induce detectable levels of tyrosine-phosphorylated STAT1 (Fig. 1C, lane 1). Simultaneous stimulation with IFN-α (10,000 units/ml) and nonstimulatory concentrations of IL-4 (0.05–0.1 ng/ml) triggered a significantly enhanced tyrosine phosphorylation of STAT6 (Fig. 1C, top row, lane 4 versus lane 3 and lane 6 versus lane 5), indicating a synergistic effect of IFN-α and IL-4 at these concentrations. IFN-α also increased IL-4-mediated tyrosine phosphorylation of STAT6 at higher concentrations of IL-4 (Fig. 1C, top row, lane 8...
versus lane 7 and lane 10 versus lane 9). In contrast, IFN-α-induced tyrosine phosphorylation of STAT1 was not enhanced by simultaneous stimulation with IFN-α and IL-4 (Fig. 1C, third row), indicating that IFN-α had a selective, co-stimulatory effect on IL-4-mediated STAT6 activation, whereas IL-4 did not affect the IFN-α-induced STAT1 phosphorylation.

To address whether these observations were a unique finding for the CD8 T cell line or a general feature for T cells, we tested the effects of IFN-α and IL-4 in alloreactive CD4 T cell lines and CD4 CTCL cells. As shown in Fig. 2A, IL-4 induced tyrosine phosphorylation of STAT6 in CD4 alloreactive T cells with a profile similar to CD8 T cells. More importantly, the combined stimulation with IL-4 and IFN-α had a synergistic effect on STAT6 phosphorylation (Fig. 2A) that was almost identical to that observed in CD8 T cells (Fig. 1C). Again, the combined stimulation with the two cytokines had no effect on tyrosine phosphorylation of STAT1 (Fig. 2A, third row, lane 2 versus lanes 4, 6, 8, and 10). IFN-α also enhanced IL-4-mediated tyrosine phosphorylation of STAT6 in a CTCL cell line (Fig. 2B). Indeed, the synergistic effect of IFN-α and IL-4 became stronger at suboptimal concentrations of IFN-α (Fig. 2B, top row, lane 6 versus lane 5 and lane 8 versus lane 7), supporting the observation above that IFN-α increased the IL-4 sensitivity or, in other words, lowered the threshold for IL-4-mediated STAT6 activation. Essentially identical findings were observed in other CD4 T cell lines and CTCL lines (cf. below and data not shown). In contrast to IFN-α, IFN-γ had no positive stimulatory effect on IL-4-mediated STAT6 activation (Fig. 2C), indicating that the different classes of interferons differ in their immunoregulatory effects.

Recent studies indicated that IFN-α and IFN-β, in fact, had a negative regulatory effect on IL-4-mediated STAT6 activation in B cells and monocytes, respectively (22–24). In these studies, the cells were preincubated with IFN-α or IFN-β for 45 min to 24 h prior to stimulation with IL-4, suggesting that the kinetics play a critical role for the outcome of cytokine cross-talk. Accordingly, experiments were performed to study the kinetics of cross-talk between IL-4 and IFN-α. T cells were incubated with IFN-α for various periods of time prior to stimulation with IL-4. As shown in Fig. 3A, the strongest co-stimulatory effect was seen at the simultaneous addition of cytokines and after short periods of preincubation, whereas the co-stimulatory effect declined following extended preincubations with IFN-α. Almost identical results were obtained when preincubating the T cells with IL-4 prior to stimulation with IFN-α. Here, the co-stimulatory effect declined after only 10 min of preincubations with IL-4 (Fig. 3B). Simultaneous stimulation with the two cytokines triggered a rapid synergistic effect, which became stronger with time (Fig. 3C, top row), suggesting that the effect was accumulating over time. Because JAK1 is associated with the high affinity receptors for both IL-4 and IFN-α, we addressed whether the co-stimulatory effect was mediated through a synergistic effect on JAK1 activation. As judged from induction of tyrosine phosphorylation, co-stimulation with IFN-α and suboptimal concentrations of IL-4 had no significant co-stimulatory effect (Fig. 3C, second row). Likewise, simultaneous stimulation with IFN-α and IL-4 did not induce an enhanced tyrosine phosphorylation of JAK3 or TYK2 (data not shown), indicating that the co-stimulatory effect was not mediated through a combinatorial effect on JAK activation per se.

In human B cells, IFN-α has been shown to induce tyrosine phosphorylation of STAT6 leading to complex formation with STAT2 (36). In agreement with this, we found that IFN-α also induced STAT6 phosphorylation in human T cell lines (Figs. 1C, 2A and B; and 3). Given the ability of STAT6 to both homodimerize and to form complexes with STAT2, a simultaneous stimulation with IL-4 and IFN-α might lead to a faster dissociation of phosphorylated STAT6 proteins from their dock-
ing sites on the IL-4/IFN-α receptors. As a result, more STAT6 proteins will bind and become phosphorylated, which in turn might lead to the observed synergistic increase in STAT6 phosphorylation. To investigate this hypothesis, the association between STAT6 and STAT2 was first examined by co-immunoprecipitation during co-stimulation with IL-4 and IFN-α. As shown in Fig. 4A, immunoprecipitation with anti-STAT2 followed by stimulation with IFN-α did indeed result in an increased association between STAT2 and STAT6 (top panel, first row, lane 3). More importantly, the combined stimulation with IL-4 and IFN-α lead to a significant increase in this association (lane 4 versus lanes 2 and 3). In contrast, the association between STAT2 and STAT1 induced by IFN-α was not affected by IL-4 (second row, upper band, lane 4 versus lane 3). Almost identical results were obtained during co-stimulation with the two cytokines, when immunoprecipitating with anti-STAT6 (Fig. 4A, middle panel). However, STAT6 has never been reported to form complexes with STAT1, and no association between these STAT proteins was observed in our study (second row (no upper band)).

Total lysates from the stimulated cells were also subjected to Western blotting (Fig. 4A, bottom panel). Interestingly, whereas the co-stimulatory effect on the STAT6/STAT2 interaction correlated with increased STAT6 phosphorylation (top row, lane 4 versus lanes 2 and 3), phosphorylation of STAT2 was not affected (second row). Thus, whereas stimulation with IFN-α alone leads to increased STAT2/STAT6 and STAT2/STAT1 association, a simultaneous stimulation with IFN-α and IL-4 only increases the STAT2/STAT6 association and STAT6 phosphorylation, supporting the conclusions above that the co-stimulatory effect was selective for the IL-4-mediated STAT6 signal pathway. In conclusion, these results indicate that IFN-α-activated STAT2 participates in an increased IL-4-mediated STAT6 activation without being further activated itself. Thus, STAT2 seems to act as an additional docking and phosphorylation site for STAT6 during IL-4-mediated STAT6 activation, which ultimately results in a more efficient STAT6 dimerization and thereby increased STAT6 phosphorylation/activation.

STAT2 is known to bind to the IFNAR-1 chain (3). To further investigate the co-stimulatory effect on the STAT6 activation and support the suggested role of STAT2, we examined the IL-4R components, IL-4Rα and γc, for their association with the IFNAR-1 chain. Simultaneous stimulation with IL-4 and IFN-α clearly increased the association of IL-4Rα and both STAT6 and the γc chain (Fig. 4B, upper panel, first and second rows, lane 4 versus lanes 2 and 3). This indicates that only in combination with IFN-α does the non-stimulatory concentration of IL-4 result in effective oligomerization of the IL-4 high affinity receptor and recruitment of STAT6 to the IL-4Rα chain. However, this did not seem to be mediated through a direct association between the receptor subunits IL-4Rα and IFNAR-1, because no clear association was observed when replotting with anti-IFNAR-1 (third row). In contrast, a clear association between the γc and IFNAR-1 chains is observed during simultaneous stimulation with IL-4 and IFN-α (Fig. 4B, lower panel, top row), whereas no recruitment of STAT6 to the γc chain was observed (middle row). Finally, the IFN-α-induced STAT2 association to the IFNAR-1 chain was not increased by co-stimulation with IL-4, and no clear association was observed between the IFNAR-2 chain and the IL-4Rα or γc chain during cytokine stimulation (data not shown). These findings therefore suggest that the co-stimulatory effect on STAT6 activation may result from close proximity between the IL-4 and IFN-α receptors, which in turn is mediated by γc/IFNAR-1 association, leading to a more efficient STAT6 activation via its association with the IL-4Rα chain and STAT2.

To address whether co-stimulation with IFN-α and IL-4 had a functional impact on downstream events, we examined nuclear translocation of STAT6. Accordingly, cytoplasmic and nuclear fractions from stimulated cells were isolated and subjected to Western blotting analysis. As shown in Fig. 5A, higher amounts of STAT6 were detected in the nuclear fraction following stimulation with both cytokines as compared with separate stimulation with either cytokine (Fig. 5A, lower panel, top row, lane 4 versus lanes 2 and 3). In contrast, stimulation with both cytokines did not enhance nuclear STAT1 (Fig. 5A, lower panel, bottom row, lane 4 versus lanes 2 and 3), supporting the conclusions above that the co-stimulatory effect is selective for the STAT6 signal pathway. Simultaneous stimulation with IFN-α and IL-4 also induced an enhanced binding of STAT6 to a DNA oligonucleotide probe representing the STAT6-binding domain of the IgE promoter (Fig. 5B, top row, lane 4 versus lanes 2 and 3), whereas there was no increase in binding of STAT1 to the STAT1-binding site in the human serum-inducible element (Fig. 5B, bottom row).

To address whether the increased nuclear translocation and DNA binding of STAT6 was associated with an increased transcription of STAT6 target genes, RNase protection assays were performed on T cells stimulated with cytokines for 3 h. As shown in Fig. 6, combined IL-4 and IFN-α stimulation produced an enhanced expression of IL-4Rα mRNA and IL-15Rα (Fig. 6, A and B, lane 4 versus lanes 2 and 3) when compared with each cytokine alone.
The kinetics of transcriptional activity of STAT6 during stimulation with IFN-\(\alpha\) and IL-4 was investigated using a STAT6-responsive luciferase expression construct encoding the oligonucleotide sequence (IgE-3 (5)) used above. As shown previously (31) and confirmed in Fig. 7B, IL-4 induced a strong luciferase expression in Jurkat J-TAg cells after 24 h. As a control, the cells were co-transfected with an expression vector encoding a mutated STAT6 carrying amino acid substitutions within the DNA-binding domain (see “Experimental Procedures”), which completely blocked the IL-4-induced luciferase expression (data not shown). In agreement with the results from RNase protection assays (Fig. 6), co-stimulation with IFN-\(\alpha\) and IL-4 for 3–6 h increased luciferase expression when compared with the effect of each cytokine alone (Fig. 7). In contrast, IFN-\(\alpha\) inhibited IL-4 transcriptional activity after 12–24 h of stimulation (Fig. 7), indicating that IFN-\(\alpha\) at later time points inhibited IL-4-mediated transcription. In conclusion, these results indicate that IFN-\(\alpha\) is able to both up- and down-regulate IL-4-mediated signaling.

DISCUSSION

In the present study we provide the first evidence that IFN-\(\alpha\) both up- and down-regulates IL-4-mediated STAT6 signaling in human T and CTCL cells. Thus, co-stimulation with IFN-\(\alpha\) for 12 h and above clearly inhibited IL-4-mediated transcription, whereas IFN-\(\alpha\) at earlier time points (up to 6 h) triggered an enhanced IL-4-mediated tyrosine phosphorylation, nuclear translocation, DNA binding, and transcription of STAT6 target genes. Indeed, even at nonstimulatory concentrations of each cytokine, a clear co-stimulatory effect was seen, indicating that IFN-\(\alpha\) lowers the threshold for IL-4-mediated STAT6 signaling in human T lymphocytes. These findings were quite unexpected because IFN-\(\alpha\) was previously reported only to down-regulate IL-4R signaling. Thus, IFN-\(\alpha\) and IFN-\(\beta\) were reported as strong inhibitors of IL-4-mediated STAT6 activation and IL-4Ra mRNA expression in B cells and monocytes (22–24). It is possible that the difference between the present and previous studies reflect cell type-specific differences between T cells and antigen presenting cells such as B cells and monocytes. An alternative explanation might be that the earlier studies focused on the effects seen after longer periods (days) of incubation with IFN-\(\alpha\), whereas the enhanced IL-4-mediated signaling by IFN-\(\alpha\) in the present study on T cells was seen as an early effect of simultaneous cytokine stimulation. In support of this explanation, we found that IFN-\(\alpha\) inhibited IL-4-signaling after 12–24 h of co-stimulation.

It is unlikely that protein synthesis is a prerequisite for the co-stimulatory effect observed at early time points when cytokines are added simultaneously. In contrast, it is highly likely that the inhibitory effect seen after hours or days of preincubation with IFN-\(\alpha\) involves protein synthesis, modulation of
IL-4R expression, and/or changes in the cellular metabolism modifying the subsequent IL-4 response. Indeed, a recent report stressed the involvement of protein synthesis and induction of IFN-α-target genes as a key event in IFN-α-mediated inhibition of IL-4 signaling (22). Moreover, IFN-α and/or IFN-β induce expression of suppressors of cytokine signaling in many cell types including B and T cells (24, 37–39), and it was recently proposed that the inhibitory effect of the IFN-γ and IFN-β was mediated through the induction of suppressor of cytokine signaling 1 (24, 40).

Our observation that cytokine co-stimulation at early time points triggered a synergistic up-regulation of STAT6 activation but not STAT1 and STAT2 activation indicates that cross-talk between IFN-α and IL-4 receptors is an asymmetrical event favoring IL-4-mediated signaling. Accordingly, we investigated whether IFN-α modulated the expression of IL-4 receptors and/or the activation of IL-4R-associated JAKs (JAK1 and JAK3). However, IFN-α and IL-4 lacked co-stimulatory effects on receptor expression (data not shown) and activation of JAKs (Fig. 3B and data not shown), indicating that cross-talk between IFN-α and IL-4 was not mediated via a modulation of receptor expression or through an enhanced activation of JAK1 and JAK3 per se. However, at suboptimal concentrations of IL-4, tyrosine-phosphorylated JAK1 was barely detectable which coincided with a weak induction of tyrosine-phosphorylated STAT6. It is therefore possible that the IL-4R at suboptimal concentrations of IL-4 provide docking sites for STAT6, which coincided with a weak induction of tyrosine-phosphorylation of STAT6.

When further investigating the contribution of IFN-α-mediated signaling to the synergistic up-regulation of STAT6 activation, we found that co-stimulation with IFN-α resulted in increased association between STAT6/STAT2, STAT6/IL-4Rα, and IL-4Rα/γc, and a clear association between γc and IFNAR-1, without further increase in STAT2 or STAT1 activation. These observations prompted us to suggest a mechanism for the co-stimulatory effect on STAT6 activation during simultaneous stimulation with suboptimal concentrations of IL-4 and IFN-α (Fig. 8). According to this mechanism, binding of the cytokines to their high affinity receptors leads to association between γc and IFNAR-1, resulting in a multimeric complex consisting of IL-4Rα/γc/IFNAR-1/IFNAR-2. An excess of IFNAR-associated, activated JAK kinases ensures sufficient phosphorylation of STAT6 docking sites on IL-4Rα and STAT2. Recruited and phosphorylated STAT6 proteins, situated in close proximity to each other on IL-4Rα and STAT2, efficiently homodimerize and dissociate from the docking sites, allowing recruitment of more STAT6 proteins. A synergistically increased amount of STAT6 proteins become phosphorylated and activated without affecting the activation and dimerization of STAT6/STAT2 (or STAT1/STAT2) heterodimers.

**Fig. 8. Hypothetical mechanism for IL-4- and IFN-α-induced synergistic up-regulation of STAT6 activation.** Part 1, stimulation with suboptimal concentrations of IL-4. Binding of IL-4 to IL-4R, consisting of IL-4Rα and γc, leads to oligomerization of the receptor chains and tyrosine (auto)phosphorylation (P) of the receptor-associated JAK1 and JAK3. Following phosphorylation of receptor tyrosines (Y) by the JAKs, latent cytoplasmic STAT6 proteins are recruited to the receptor via their SH2 domains (L). STAT6 then becomes activated by tyrosine phosphorylation by the JAKs, followed by homodimerization with another STAT6 protein. Part 2, stimulation with suboptimal concentrations of IFN-α. Binding of IFN-α to the IFNAR, consisting of IFNAR-1 and -2 (α and β chains, respectively) leads to activation of TYK2 and JAK1 and phosphorylation of receptor tyrosines. Receptor-bound STAT2 becomes activated by phosphorylation by the JAKs, followed by heterodimerization with STAT6 (or STAT1). Part 3, simultaneous stimulation with suboptimal concentrations of IL-4 and IFN-α. Binding of the cytokines to their high affinity receptors leads to association between γc and IFNAR-1, resulting in a multimeric complex consisting of IL-4Rα/γc/IFNAR-1/IFNAR-2. An excess of IFNAR-associated, activated JAK kinases ensures sufficient phosphorylation of STAT6 docking sites on IL-4Rα and STAT2. Recruited and phosphorylated STAT6 proteins, situated in close proximity to each other on IL-4Rα and STAT2, efficiently homodimerize and dissociate from the docking sites, allowing recruitment of more STAT6 proteins. A synergistically increased amount of STAT6 proteins become phosphorylated and activated without affecting the activation and dimerization of STAT6/STAT2 (or STAT1/STAT2) heterodimers.
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and STAT2. Recruited and phosphorylated STAT6 proteins, situated in close proximity to each other on IL-4Rα and STAT2, efficiently homodimerize and dissociate from the docking sites, allowing recruitment of more STAT6 proteins. A synergistically increased amount of STAT6 proteins became phosphorylated and activated without affecting the activation and dimerization of STAT6/STAT2 (or STAT1/STAT2) heterodimers.

Such unidirectional cooperative signaling cross-talk may also occur between IFN-α and other cytokines using STAT proteins capable of docking to sites provided by the IFNAR. Indeed, IFN-α was shown to contribute to efficient IFN-γ- and IL-6-signaling, with IFNAR-1 associating with the receptor components, IFNGR-2 and gp130, respectively, and thereby providing docking sites for IFN-γ-IL-6-activated STAT proteins (41, 42).

The most important findings in the present work are the observations that short exposure with IFN-α increases the sensitivity of T cells to IL-4 and enhances the induction of STAT6 activation and expression of the STAT6 target genes, such as the IL-4Rα chain. At the same time, longer exposure to IFN-α results in inhibition of IL-4-mediated gene expression in T cells. STAT6 and IL-4Rα play a well-established role in the induction of Th2-like immune responses. Having the ability of IFN-α to both up- and down-regulate IL-4 signaling might be an important characteristic in in vivo situations where both cytokines are present at the same, for example during viral infection of allergenic individuals exposed to allergens, where both Th1- and Th2-like responses are required. The present findings thus indicate that IFN-α has a complex regulatory role in adaptive immunity that is different from the clearly defined role of IFN-γ as a Th1 cytokine.

REFERENCES

1. Wallach, D. (1983) Cell Immunol. 75, 390–395
2. Garotta, G., Talmadge, K. W., Pink, J. R., Dewald, B., and Baggioni, M. (1986) Biochem. Biophys. Res. Commun. 140, 948–954
3. Postka, S. (1997) Semin. Oncol. 24, 18–40
4. Brinkmann, V., Geiger, T., Alkan, S., and Heusser, C. H. (1993) J. Exp. Med. 178, 1655–1663
5. Roggero, L., D’Ambrosio, D., Roff, M.,Penn, G., Minelli, L. J., Presky, D. H., Adorni, L., and Sinigaglia, F. (1998) J. Immunol. 161, 6567–6574
6. Pene, J., Rouset, F., Briere, F., Chretien, I., Bonnafoy, J. Y., Spits, H., Yokota, T., Arai, N., Aral, K., and Banchereau, J. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 6880–6884
7. Pannonen, J., Pannonen, K., Jansen, C. T., and Kalimo, K. (1993) Allergy 48, 189–195
8. Delepeisse, G., Sarfati, M., and Peleman, R. (1989) J. Immunol. 142, 134–138
9. Denovoy, M. C., Yodoi, J., and Banchereau, J. (1990) Mol. Immunol. 27, 129–136
10. Nelms, K., Keegan, A. D., Zamorano, J., Ryan, J. J., and Paul, W. E. (1999) Annu. Rev. Immunol. 17, 701–728
11. Darnell, J. E. J. (1997) Science 277, 1630–1635
12. Ihle, J. N. (1996) Nature 379, 591–594
13. O’Shea, J. J. (1997) Immunity 7, 1–11
14. Kuhn, R., Rajewsky, K., and Muller, W. (1991) Science 254, 707–710
15. Kopf, M., Le Gros, G., Bachmann, M., Lamers, M. C., Bluthmann, H., and Kohler, G. (1993) Nature 362, 245–248
16. Kaplan, M. H., Schindler, U., Smiley, S. T., and Grubay, M. J. (1996) Immunity 4, 313–319
17. Meraz, M. A., White, J. M., Sheehan, K. C., Bach, E. A., Rodig, S. J., Dighe, A. S., Kaplan, D. H., Riley, J. K., Greenlund, A. C., Campbell, D., Carver-Moore, K., Dubois, R. N., Clark, R., Aguet, M., and Schreiber, R. D. (1996) Cell 84, 431–442
18. Paul, W. E. (1999) Fundamental Immunology, 4th Ed., pp. 879–908, Raven Press, New York
19. Paludan, S. R. (1998) Scand. J. Immunol. 48, 459–468
20. Alderson, M. R., Armitage, R. J., Trough, T. W., and Zeigler, S. F. (1994) Cytokine 6, 407–413
21. te, V. A., Rousset, F., Peronne, C., de Vries, J. E., and Figdor, C. G. (1990) J. Immunol. 144, 3052–3059
22. So, E. Y., Park, H. H., and Lee, C. E. (2000) J. Immunol. 165, 5472–5479
23. Dickensheets, H. L., and Donnelly, R. P. (1999) J. Leukocyte Biol. 65, 307–312
24. Dickensheets, H. L., Venkataraman, C., Schindler, U., and Donnelly, R. P. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 10800–10805
25. Eriksen, K. W., Nielsen, M., Kaltoft, K., Sveggaard, A., Nissen, M. H., Ropke, C., and Odum, N. (2001) Exp. Clin. Immunogenet. 18, 233–241
26. Wagner, B. J., Hayes, T. E., Hoban, C. J., and Cochran, B. H. (1990) EMBO J. 9, 4477–4484
27. Kaltoft, K., Bishalle, S., Dyberg, T., Boel, R., Rasmussen, P. B., and Thestrup-Pedersen, K. (1992) In Vitro Cell Dev. Biol. 28A, 161–167
28. Kaltoft, K., Hansen, B. H., and Thestrup-Pedersen, K. (1994) Dermatol. Clin. 12, 295–304
29. Nielsen, M., Odum, N., Bendtzen, K., Ryder, L. P., Jakobsen, B. K., and Sveggaard, A. (1994) Exp. Clin. Immunogenet. 11, 23–32
30. Geisler, C., Scholler, J., Wahi, M. A., Rubin, B., and Weiss, A. (1999) J. Immunol. 163, 1761–1767
31. Wiedmann, A., Brockdorff, J., Lovato, P., Nielsen, M., Leich, V., Rieck, K., Sveggaard, A., Geisler, C., and Odum, N. (2003) J. Biol. Chem. 278, 2787–2791
32. Mikita, T., Campbell, D., Wu, P., Williamson, K., and Schindler, U. (1996) Mol. Cell. Biol. 16, 5811–5820
33. Eriksen, K. W., Kaltoft, K., Mikkelsen, G., Nielsen, M., Zhang, Q., Geisler, C., Nissen, M. H., Ropke, C., Wasik, M. A., and Odum, N. (2001) Leukemia 15, 787–793
34. Nielsen, M., Kaltoft, K., Nordsla, M., Ropke, C., Geisler, C., Mustelin, T., Dobson, P., Sveggaard, A., and Odum, N. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 6764–6769
35. Queille, P. W., Shimoda, K., Thierfelder, W., Fischer, C., Kim, A., Ruben, S. M., Cleveland, J. L., Pierce, J. H., Keegan, A. D., and Nelms, K. (1995) Mol. Cell. Biol. 15, 3336–3343
36. Gupta, S., Jiang, M., and Pernis, A. B. (1999) J. Immunol. 163, 3834–3841
37. Wang, Q., Miyakawa, Y., Fox, N., and Kaushansky, K. (2000) Blood 96, 2093–2099
38. Crespo, A., Fillia, M. B., Russell, S. W., and Murphy, W. J. (2000) Biochem. J. 349, 99–104
39. Brender, C., Nielsen, M., Ropke, C., Nissen, M. H., Sveggaard, A., Billestrup, N., Geisler, C., and Odum, N. (2001) Exp. Clin. Immunogenet. 18, 80–85
40. Venkataraman, C., Leung, S., Salvekar, A., Mano, H., and Schindler, U. (1999) J. Immunol. 162, 4053–4061
41. Takaoaka, A., Mitani, Y., Suenori, H., Sato, M., Yokohi, T., Noguchi, S., Tanaka, N., and Taniguchi, T. (2000) Science 288, 2357–2360
42. Mitani, Y., Takaoaka, A., Kim, S. H., Kato, Y., Yokohi, T., Tanaka, N., and Taniguchi, T. (2001) Genes Cells 6, 631–640