

# Interleukin 12 induces tyrosine phosphorylation and activation of STAT4 in human lymphocytes

(cytokine signal transduction/transcription factor)

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**ABSTRACT** Interleukin 12 (IL-12) is an important immunoregulatory cytokine whose receptor is a member of the hematopoietin receptor superfamily. We have recently demonstrated that stimulation of human T and natural killer cells with IL-12 induces tyrosine phosphorylation of the Janus family tyrosine kinases JAK2 and Tyk2, implicating these kinases in the immediate biochemical response to IL-12. Recently, transcription factors known as STATs (signal transducers and activators of transcription) have been shown to be tyrosine phosphorylated and activated in response to a number of cytokines that bind hematopoietin receptors and activate JAK kinases. In this report we demonstrate that IL-12 induces tyrosine phosphorylation of a recently identified STAT family member, STAT4, and show that STAT4 expression is regulated by T-cell activation. Furthermore, we show that IL-12 stimulates formation of a DNA-binding complex that recognizes a DNA sequence previously shown to bind STAT proteins and that this complex contains STAT4. These data, and the recent demonstration of JAK phosphorylation by IL-12, identify a rapid signal-transduction pathway likely to mediate IL-12-induced gene expression.

Interleukin 12 (IL-12) is a monocyte/macrophage-derived cytokine (1, 2), which, through its many effects on natural killer (NK) and T lymphocytes, plays a central role in the initiation and control of cell-mediated immune responses (3, 4). The receptor for IL-12 (IL-12R) is incompletely characterized, although a low-affinity subunit has recently been cloned (5). This subunit is a member of the hematopoietin receptor family, closely related to gp130. Like other family members, binding of IL-12 to the IL-12R induces rapid tyrosine phosphorylation of a range of intracellular substrates (6, 7). However, hematopoietin receptors do not possess intrinsic tyrosine kinase activity but instead associate with and activate members of the Janus (JAK) family of cytoplasmic protein tyrosine kinases (8–12). We have recently demonstrated that IL-12 treatment of human T and NK cells leads to the rapid tyrosine phosphorylation of both JAK2 and Tyk2 kinases, implicating these kinases in the immediate biochemical response to IL-12 (6).

The biological effects of IL-12 include the rapid activation of early-response genes such as interferon  $\gamma$  (IFN- $\gamma$ ) and perforin (3, 4), but the molecular mechanisms by which IL-12 might stimulate transcription are not known. A number of recent studies have identified a family of transcription factors called STATs (signal transducers and activators of transcription), which are involved in the signal-transduction cascades of

many cytokines known to activate JAK kinases (8, 13–15). Originally described as mediators of IFN-induced transcription, STATs are latent cytoplasmic transcription factors that, after tyrosine phosphorylation, translocate to the nucleus and bind specific, but related, DNA sequences to promote transcription of cytokine-responsive genes (13). IFN- $\alpha$  induces tyrosine phosphorylation of STAT1 and STAT2, which associate with a nuclear 48-kDa DNA-binding protein to form a multiprotein transcriptional activator known as interferon-stimulated gene factor 3 (16, 17). In contrast, IFN- $\gamma$  promotes tyrosine phosphorylation and homodimerization of STAT1, which translocates to the nucleus and directly binds to a conserved sequence motif termed the IFN- $\gamma$ -activation site (18). Ligand binding to many hematopoietin receptors has now been shown to induce tyrosine phosphorylation of STAT family proteins, thereby promoting their ability to bind IFN- $\gamma$ -activation site-related DNA sequences: the IL-6 family of cytokines activate both STAT1 and STAT3 (19, 20), prolactin activates STAT1 (21) and STAT5 (22), and IL-4 activates a STAT protein designated IL-4-STAT (STAT6) (23). STAT4 is another family member with restricted distribution, being expressed mainly by myeloid cells and developing spermatogonia and also in thymus and spleen (24, 25). To date, no STAT4-activating ligand has been identified.

In this study we sought to determine whether STAT proteins could be identified in the signal-transduction pathway of IL-12. Stimulation of human T cells with IL-12 induced early tyrosine phosphorylation of STAT4 but not of STAT1 or STAT2. Moreover, electrophoretic mobility-shift assays (EMSAs) identified an IL-12-induced DNA-binding complex that contained STAT4. These results suggest that IL-12 might activate transcription by inducing tyrosine phosphorylation of STAT4.

## MATERIALS AND METHODS

**Cytokines and Antibodies.** Recombinant human IL-12 ( $4.5 \times 10^6$  units/mg) was provided by Stanley Wolf (Genetics Institute; Cambridge, MA); recombinant human IL-2 ( $3 \times 10^6$  Cetus units/mg) by Cetus Oncology; recombinant human IFN- $\alpha$  ( $2.4 \times 10^8$  units/mg) by Hoffmann-La Roche; and recombinant human IL-4 and IL-6 by PeproTech (Rocky Hill, NJ). Polyclonal rabbit antisera against STAT4 (C-20 and L-18) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Polyclonal rabbit antisera against STAT1 and STAT2 were provided by Chris Schindler (Columbia University, New

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Abbreviations: NK, natural killer; STAT, signal transducer and activator of transcription; IL, interleukin; IFN, interferon; GRR, IFN- $\gamma$  response element; PHA, phytohemagglutinin; EMSA, electrophoretic mobility-shift assay; IL-12SF, IL-12-stimulated factor; IL-12R, IL-12 receptor; ISRE, IFN- $\alpha$ -stimulated response element.

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York). Monoclonal anti-phosphotyrosine antibody, clone 4G10, was purchased from Upstate Biotechnology (Lake Placid, NY).

**Cell Culture and Activation.** Normal human T cells ( $94 \pm 5\%$  CD3<sup>+</sup>) were isolated from the peripheral blood of healthy donors by Percoll gradient centrifugation as described (26). Fresh NK cells were negatively selected from low-density Percoll fractions using anti-CD3 antibodies coupled to magnetic beads (MACS; Miltenyi Biotechnology, Sunnyvale, CA). To induce IL-12 responsiveness, T cells were cultured for 3 days in RPMI 1640 medium/10% fetal calf serum/phytohemagglutinin (PHA) at 2  $\mu$ g/ml (Sigma), split 1:1 with fresh medium, and then cultured for an additional day with IL-2 at 50 international units/ml (27). The human T-cell line Kit 225 (subclone K6) (28) was provided by Hoffmann-La Roche and maintained in RPMI 1640 medium/10% fetal calf serum/IL-2 at 100 international units/ml. Before stimulation with cytokines, activated T cells and Kit 225/K6 cells were washed in acidified RPMI 1640 medium (pH 6.4) and rested for 4 hr in RPMI 1640 medium/0.5% human AB serum or 2% fetal calf serum, respectively.

**Immunoprecipitation and Immunoblotting.** Cell lysis in 1% Triton X-100 buffer, immunoprecipitation with anti-STAT antisera, and subsequent SDS/PAGE were done as described (6). Immunoblotting with anti-phosphotyrosine antibody was performed as described (6). For immunoblotting with anti-STAT antisera, membranes were blocked in Tris/borate/saline containing 0.05% Tween 20 and 5% nonfat dried milk, incubated sequentially with primary antibody and horseradish peroxidase-conjugated goat anti-rabbit IgG (Boehringer Mannheim), and detected using enhanced chemiluminescence. When a membrane was reprobed, it was first treated with 15% H<sub>2</sub>O<sub>2</sub>.

**EMSA.** Whole-cell extracts were prepared from cytokine-stimulated cells as described (29). EMSAs were done essentially as described (30) using a <sup>32</sup>P-end-labeled double-stranded oligonucleotide (5'-AGCATGTTTCAAGGATTTGAGATGTATTTCCAGAAAAG-3') corresponding to the IFN- $\gamma$  response element (GRR) of the human Fc $\gamma$  receptor I (Fc $\gamma$ RI) gene (31). Briefly, whole-cell extracts were incubated with labeled probe in binding buffer for 15 min at 4°C before

electrophoresis on 6% polyacrylamide gels and autoradiography. When used, antibodies were incubated with cell extracts for 60 min at 4°C before addition of probe. Competitive inhibition was performed by incubating 100-fold excesses of unlabeled GRR or the IFN- $\alpha$ -stimulated response element (ISRE) of interferon-stimulated gene 15 (5'-GATCCATGCCTCGGGAAAGGGAAACCGAAACTGAAGCC-3') (32, 33) with cell extracts for 10 min at 4°C before addition of probe.

## RESULTS AND DISCUSSION

Like many other members of the hematopoietin receptor family, the IL-12 receptor is functionally coupled to members of the JAK family of protein tyrosine kinases. We have previously reported that IL-12 treatment of PHA-activated human T cells induces rapid tyrosine phosphorylation of both Tyk2 and JAK2, implicating these kinases in the early signal-transduction events after interaction of IL-12 with its receptor (6).

Many cytokines that activate JAK kinases induce the binding of STAT proteins to a series of palindromic IFN- $\gamma$ -activation site-related DNA oligonucleotides with the consensus sequence TTNCNNNA (14, 15). Therefore, we investigated by EMSA whether IL-12 might also induce such DNA-binding complexes in human lymphocytes. Whole-cell extracts from untreated or cytokine-treated PHA-activated T cells were examined for binding to a probe corresponding to the GRR of the human Fc $\gamma$  receptor I gene that has been shown to bind a number of cytokine-activated nuclear factors (14, 31). Extracts from untreated PHA-stimulated human T cells contained three GRR-binding complexes designated here as A, B, and C (Fig. 1A, lane 1). Treatment of T cells with IL-12 induced a more slowly migrating complex, which we have called IL-12-stimulated factor (IL-12SF), which appeared within 5 min of stimulation and was still present after 45 min (Fig. 1A, lanes 2–4). In this experiment, band C was only weakly detectable in untreated cells and was modestly enhanced by IL-12 treatment. However, this was not a consistent finding; in most donors band C was detectable in untreated cells and was unaffected by treatment with IL-12 (data not shown). The significance of complex C to the IL-12 response therefore remains unclear. In contrast, IL-12SF was not

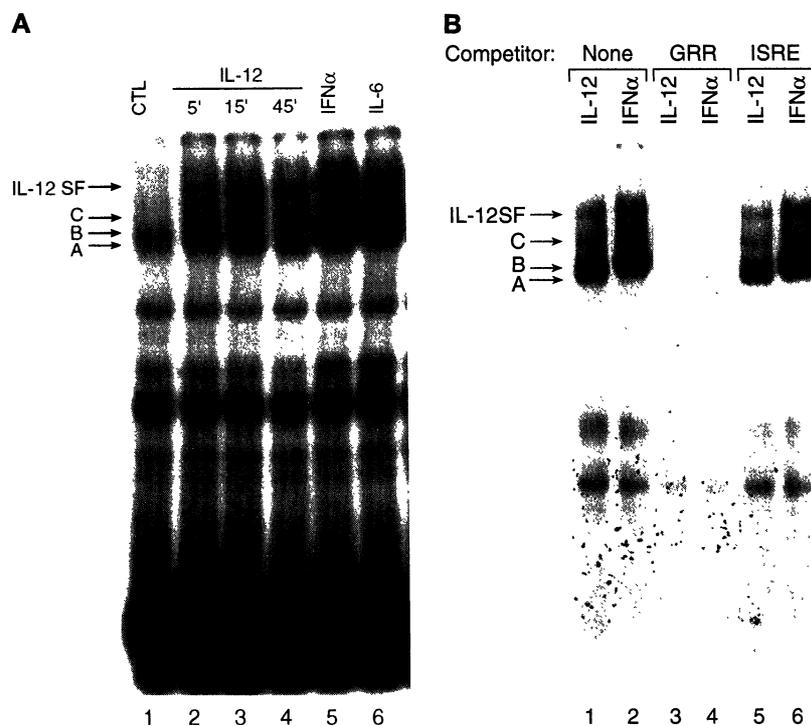


FIG. 1. (A) IL-12 induces a DNA-binding complex (IL-12SF) that recognizes the GRR of the Fc $\gamma$ RI gene. PHA-activated T cells were incubated in medium alone (lane 1), with IL-12 at 100 units/ml for the indicated times (lanes 2–4), with IFN- $\alpha$  at 500 units/ml for 15 min (lane 5), or with IL-6 at 200 ng/ml for 15 min (lane 6). Whole-cell extracts were prepared, and an EMSA was done using the GRR oligonucleotide probe. CTL, control. (B) Competition of IL-12SF using unlabeled oligonucleotides. Whole-cell extracts were prepared from PHA-activated T cells stimulated with IL-12 at 100 units/ml (lanes 1, 3, and 5) or IFN- $\alpha$  at 500 units/ml (lanes 2, 4, and 6). Competition was performed by incubating extracts with a 100-fold molar excess of unlabeled oligonucleotide corresponding to the GRR (lanes 3 and 4) or the ISRE (lanes 5 and 6) before addition of labeled GRR probe and electrophoresis.

present in unstimulated cells from any of the donors examined and was induced in all donors by IL-12 treatment. Stimulation of the same cells with either IFN- $\alpha$  (Fig. 1A, lane 5) or IL-6 (lane 6) induced a complex that migrated at approximately the same position as IL-12SF. IL-12SF, complexes A-C, and the complexes induced by IFN- $\alpha$  and IL-6 each bound specifically to the labeled GRR sequence. These complexes could be displaced by competition with unlabeled GRR oligonucleotide (Fig. 1B, lanes 3 and 4) but not with an unlabeled oligonucleotide corresponding to the ISRE (lanes 5 and 6) or with an oligonucleotide corresponding to the DNA-binding site of the transcription factor AP-1 (data not shown).

The ability of STAT proteins to bind to target DNA elements depends upon their tyrosine phosphorylation (14, 34), and tyrosine-phosphorylated STAT1 binds to GRR in response to IFN- $\gamma$  and other cytokines (14, 30). To determine whether IL-12 could induce tyrosine phosphorylation of STAT1 or STAT2, which might be components of IL-12SF (Fig. 1B), lysates from untreated or cytokine-treated PHA-activated T cells were immunoprecipitated with antiserum to STAT1 or STAT2, resolved by SDS/PAGE, and analyzed by anti-phosphotyrosine immunoblotting (Fig. 2A, *Upper*). As expected, IFN- $\alpha$  induced tyrosine phosphorylation of both STAT1 (lane 3) and STAT2 (lane 6). In response to IFN- $\alpha$ , STAT1 and STAT2 associate in a transcriptional complex known as interferon-stimulated gene factor 3 (16, 17). Consequently, the anti-STAT2 antiserum used was able to coimmunoprecipitate tyrosine-phosphorylated STAT1 from IFN- $\alpha$ -treated cells (Fig. 2A, lane 6) as described (17). In contrast to IFN- $\alpha$ , IL-12 treatment caused tyrosine phosphorylation of neither STAT1 (Fig. 2A, lane 2) nor STAT2 (lane 5). The presence of equal levels of STAT1 and STAT2 in each lane was demonstrated by stripping these blots and reprobing with antiserum against STAT1 and STAT2, respectively (Fig. 2A, *Lower*). These data suggest that STAT1 and STAT2 are not activated by IL-12 in T cells and are unlikely to be components of the IL-12SF complex.

STAT4 is a recently identified STAT family member expressed in hematopoietic tissues, including thymus and spleen, but not in a number of murine T cell lines tested or in many other tissues (24, 25). To investigate whether STAT4 could potentially be a component of the IL-12-induced GRR-binding complex (IL-12SF), we first analyzed by immunoblotting its expression in human T cells. Fresh peripheral T cells from a number of donors expressed either low or undetectable levels of STAT4 protein by immunoblotting (Fig. 2B, lane 1). However, T cells only express IL-12R and respond functionally to IL-12 after activation with agents such as PHA (35). Moreover, components of other cytokine signaling pathways, such as IL-2 receptor  $\alpha$  chain and JAK3, are strongly up-regulated by T-cell activation (9, 36). We therefore examined STAT4 expression in T cells stimulated for various times with PHA. PHA induced expression of STAT4 protein in a time-dependent manner; maximal induction was observed after 72 hr of stimulation (Fig. 2B). Interestingly, this time course of STAT4 expression mirrors closely the kinetics of induction of IL-12R expression on T cells by PHA, as well as the acquisition of IL-12 responsiveness (35), suggesting that STAT4 might be involved in IL-12 signal transduction in activated T cells. Furthermore, STAT4 was found to be expressed in other IL-12-responsive cells: fresh peripheral NK cells, Kit 225/K6 cells, and the NK3.3 cell line (37) (data not shown). In contrast, fresh tonsillar B cells and the NK-like cell line YT, which do not respond to IL-12, did not express STAT4 (data not shown). Together, these data suggested the possible involvement of STAT4 in IL-12 signal transduction.

To more directly establish a role for STAT4 in IL-12 signaling, the ability of IL-12 to stimulate tyrosine phosphorylation of STAT4 was assessed. STAT4 was immunoprecipitated from cytokine-treated or -untreated IL-12-responsive

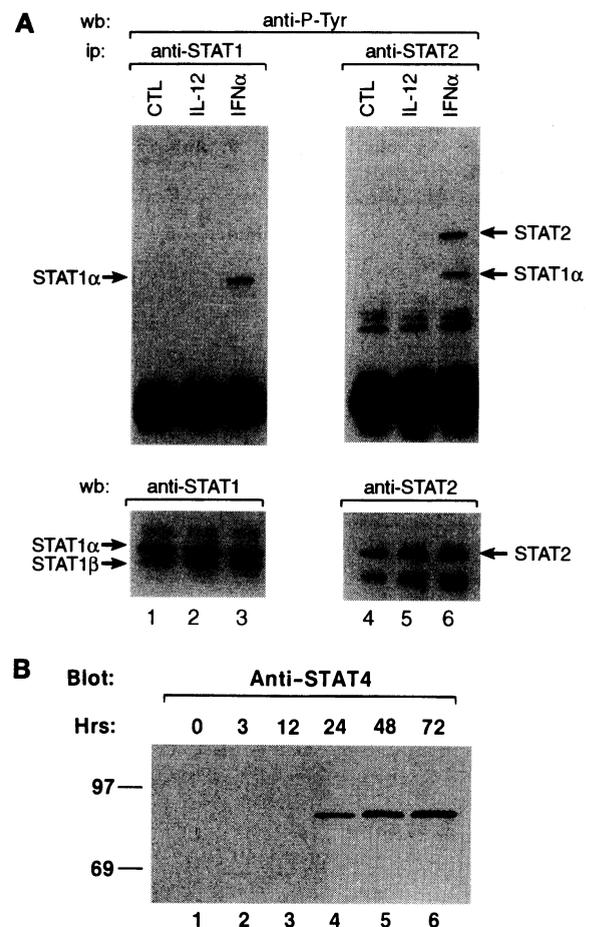
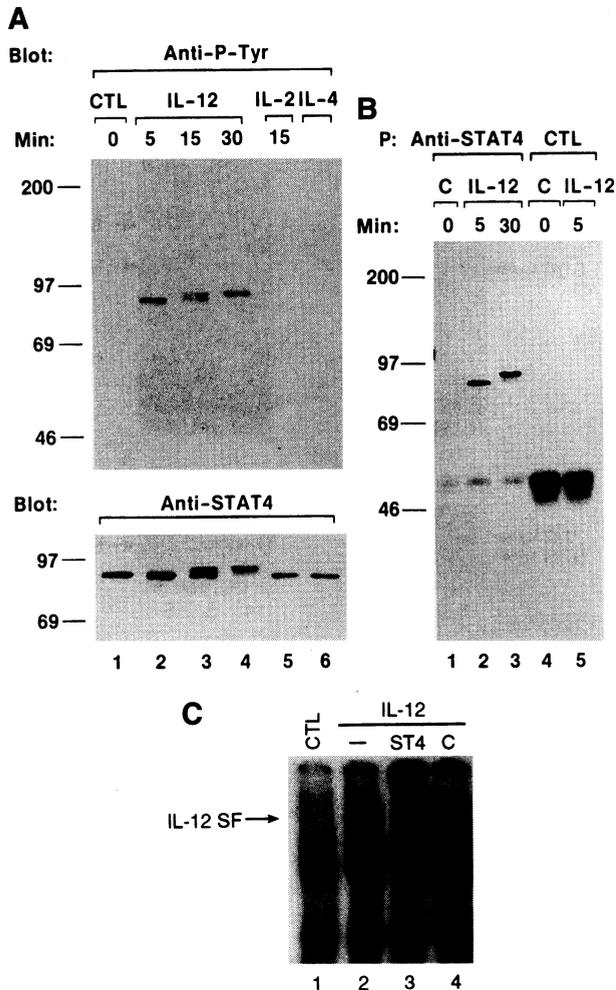


FIG. 2. (A) IL-12 does not induce tyrosine phosphorylation of STAT1 or STAT2. PHA-activated T cells ( $4 \times 10^7$ ) were incubated for 10 min in medium alone (lanes 1 and 4), with IL-12 at 100 units/ml (lanes 2 and 5), or with IFN- $\alpha$  at 1000 units/ml (lanes 3–6). Lysates were immunoprecipitated (ip) with anti-STAT1 (lanes 1–3) or anti-STAT2 (lanes 4–6) antiserum, resolved by SDS/PAGE, transferred to Immobilon, and blotted sequentially with anti-phosphotyrosine (*Upper*), and anti-STAT1 or anti-STAT2 (*Lower*). wb, Western blot. (B) PHA induces STAT4 expression in human T cells. T cells were incubated for the indicated times with PHA at 2  $\mu$ g/ml, and lysates were made. Lysates (100  $\mu$ g) were resolved by SDS/PAGE and analyzed by immunoblotting with anti-STAT4 antiserum.

cells and analyzed by anti-phosphotyrosine immunoblotting (Fig. 3A, *Upper*). Treatment of PHA-activated T cells with IL-12 (lanes 2–4) induced rapid tyrosine phosphorylation of STAT4. Phosphorylation of STAT4 could be detected as early as 5 min and was maintained for >30 min. Neither IL-2 (lane 5), which shares many functional effects with IL-12 (3, 4), nor IL-4 (lane 6) stimulated STAT4 phosphorylation. Equal loading of STAT4 was demonstrated by reprobing the membrane with an antiserum against STAT4 (Fig. 3A, *Lower*). Additionally, IL-12 induced tyrosine phosphorylation of STAT4 in Kit225/K6 cells (Fig. 3B, lanes 2 and 3), whereas no tyrosine-phosphorylated proteins were detected in immunoprecipitates using a control rabbit anti-serum (lanes 4 and 5). Treatment of both fresh peripheral NK cells and the NK cell line NK3.3 with IL-12 also stimulated tyrosine phosphorylation of STAT4 (data not shown).

Interestingly, IL-12 treatment consistently induced a decrease in the mobility of STAT4 protein upon SDS/PAGE, from an apparent molecular mass of  $\approx 90$  kDa to an apparent mass of  $\approx 95$  kDa (Fig. 3A and B). The kinetics of appearance of this 95-kDa species differed markedly from the time course of STAT4 tyrosine phosphorylation (as detected by antibody



**FIG. 3.** (A) IL-12 induces tyrosine phosphorylation of STAT4 in T cells. PHA-activated T cells ( $4 \times 10^7$ ) were incubated in medium alone (lane 1), with IL-12 at 100 units/ml for the indicated times (lanes 2–4), with IL-2 at 1000 units/ml for 15 min (lane 5), or with IL-4 at 1000 units/ml for 15 min (lane 6). Lysates were immunoprecipitated with anti-STAT4 antiserum, resolved by SDS/PAGE, transferred to Immobilon, and blotted sequentially with anti-phosphotyrosine (Anti-P-Tyr) (Upper) and anti-STAT4 (Lower). (B) IL-12 induces tyrosine phosphorylation of STAT4 in Kit 225/K6 cells. Kit 225/K6 cells ( $1 \times 10^7$ ) were incubated in medium alone (lanes 1 and 4) or for the indicated times with IL-12 at 100 units/ml (lanes 2, 3, and 5). Lysates were immunoprecipitated with anti-STAT4 antiserum (lanes 1–3) or with a control antiserum (lanes 4 and 5), resolved by SDS/PAGE, and analyzed by anti-phosphotyrosine immunoblotting. (C) IL-12SF contains STAT4. PHA-activated T cells were incubated in medium alone (lane 1) or with IL-12 at 100 units/ml for 15 min (lanes 2–4). Whole-cell extracts were prepared, and an EMSA was done with the GRR oligonucleotide probe. Antiserum against STAT4 (lane 3) or a control antiserum (C, lane 4) was incubated with the cell extracts for 60 min before addition of the probe. IP, immunoprecipitation; CTL, control.

4G10 immunoblotting). Although tyrosine phosphorylation of STAT4 was already maximal at 5 min of stimulation, only a small fraction, if any, of the STAT4 migrated in the upper band at this time. At 15–20 min approximately half of the STAT4 protein ran at the higher apparent weight, and at 30–45 min of stimulation most, if not all, STAT4 detectable by anti-phosphotyrosine or anti-STAT4 immunoblotting migrated in the upper band. Notably, a cytokine-induced decrease in migration through SDS/PAGE gels has also been observed for STAT1 (15, 38) and STAT3 (39). The protein kinases p56<sup>lck</sup> and c-raf exhibit a similar decrease in electrophoretic mobility after activation of T cells, and this effect has been demonstrated to be the result of serine/threonine phosphorylation

(40, 41). Recently it has been proposed that STAT proteins (39, 42), like a number of other transcription factors (43), may be regulated by serine/threonine phosphorylation, and it is intriguing to consider that phosphorylation of serine/threonine as well as tyrosine residues might contribute to the regulation of STAT4 function.

The tyrosine phosphorylation of STAT4 in response to IL-12 suggests that the IL-12SF detected by EMSA might contain STAT4. To test this directly, EMSAs were done in which antibodies to STAT4 or control antibodies were included in the DNA-binding reaction before addition of  $^{32}$ P-labeled probe (Fig. 3C). Antiserum to STAT4 (lane 3), but not a control antiserum (lane 4), was able to supershift the IL-12SF complex, indicating that IL-12SF contained STAT4. In contrast, and in agreement with the tyrosine phosphorylation data shown in Fig. 2A, antiserum to STAT1 supershifted the IFN- $\alpha$ -induced GRR-binding complex but had no effect on IL-12SF (data not shown). Antiserum to STAT4 had no effect on the constitutive GRR-binding complexes A–C or on the complexes induced by either IL-6 or IFN- $\alpha$  (data not shown), indicating that these do not contain STAT4 and are therefore different in composition from IL-12SF. However, although IL-12 is the only reported inducer of STAT4 activation, the expression of STAT4 in myeloid cells and in developing spermatogonia (24) suggests that other STAT4-activating ligands might exist.

A number of cytokines that signal via JAK kinases have recently been shown to induce activation of STATs (8, 13–15). Analysis of JAK-deficient cell lines has shown that JAK kinases are necessary for phosphorylation of STAT1 and STAT2 in response to IFNs (44, 45), and over-expression of JAKs can activate STAT DNA-binding activity (46). Data such as these suggest that JAK kinases might directly regulate STAT proteins, and the “JAK-STAT” pathway has been proposed as a paradigm for cytokine signaling. In this paper we have shown that IL-12 stimulation of human lymphocytes induces tyrosine phosphorylation of STAT4 but not of STAT1 or STAT2. Moreover, IL-12 stimulates the formation of nuclear complexes that bind to the GRR DNA sequence, and these complexes contain STAT4. Together with our previous finding that IL-12 induces tyrosine phosphorylation of JAK2 and Tyk2 (6), these data suggest that a specific JAK-STAT pathway may transduce rapid and direct IL-12 signals to the nuclei of responsive cells and provide a clear basis for understanding the mechanisms by which IL-12 might regulate gene expression. The mechanisms controlling the induction of genes such as IFN- $\gamma$  and perforin by IL-12 are still very poorly characterized, and it will be interesting to analyze the function of STAT4 in these responses.

Some cytokines, including IL-6 and epidermal growth factor, promote tyrosine phosphorylation and activation of more than one STAT protein (19, 20). Epidermal growth factor, for example, causes activation of STAT1 and STAT3, which can bind DNA as either homo- or heterodimers (20). Whether STAT4 forms homo- or heterodimers upon activation by IL-12 is unclear. Our data suggest that STAT1 and STAT2 are not phosphorylated or activated in response to IL-12 (Fig. 2A), but we cannot rule out that other STAT family members (or unrelated proteins) might also be components of IL-12SF or that IL-12 might induce binding of distinct STATs to other DNA sequences. In fact, preliminary data suggest that in some cell types, IL-12 can also stimulate tyrosine phosphorylation of STAT3 (data not shown). However, the level of STAT3 phosphorylation detected is minimal in comparison with that induced by IL-6 and IFN- $\alpha$ , and the significance of this finding is as yet unclear.

Little STAT4 is expressed in resting human T cells, which express negligible surface levels of IL-12R (35). However, STAT4 protein levels are increased dramatically upon activation of T cells by PHA with a time course similar to the induction of IL-12R expression and the acquisition of IL-12 responsiveness (35). A similar pattern of expression has been

previously described for components of the IL-2 signaling pathway, in which both IL-2R $\alpha$  and JAK3 are induced in T cells with a time course parallel to that of IL-2 responsiveness (9, 36). Together, these data indicate that components of cytokine signaling complexes can be coordinately regulated in T cells. It is possible that such coregulation of signaling molecules is one mechanism by which peripheral blood T cells control cytokine responsiveness during the inflammatory immune response.

IL-2 and IL-12 exert many similar biological effects on NK and T cells, including the induction of common genes such as IFN- $\gamma$  and perforin (3, 4). However, the molecular mechanisms underlying this similarity are unclear. Although JAK2 and Tyk2 are implicated in IL-12 responses (6), IL-2 stimulates the activation of JAK1 and JAK3 (10, 11, 47). Moreover, while IL-12, but not IL-2, triggers activation of STAT4, IL-2 activates distinct STATs (48) which we and others have recently identified as STAT3 and STAT5 (49–51). Similarly, although IL-12 and IFNs  $\alpha$  and  $\gamma$  have many similar effects on lymphocytes, these cytokines differ biochemically in that the IFNs, but not IL-12, activate STAT1. It is likely that the activation of different JAKs and STATs by these cytokines is dictated by the differential ability of these molecules to bind to each cytokine's receptor (52). Further investigation of the ways in which distinct signaling pathways such as these converge to produce common effects should lead to a better understanding of the molecular mechanisms by which cytokines control the immune response.

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