

Tyrosine phosphorylation and activation of STAT5, STAT3, and Janus kinases by interleukins 2 and 15

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ABSTRACT The cytokines interleukin 2 (IL-2) and IL-15 have similar biological effects on T cells and bind common hematopoietin receptor subunits. Pathways that involve Janus kinases (JAKs) and signal transducers and activators of transcription (STATs) have been shown to be important for hematopoietin receptor signaling. In this study we identify the STAT proteins activated by IL-2 and IL-15 in human T cells. IL-2 and IL-15 rapidly induced the tyrosine phosphorylation of STAT3 and STAT5, and DNA-binding complexes containing STAT3 and STAT5 were rapidly activated by these cytokines in T cells. IL-4 induced tyrosine phosphorylation and activation of STAT3 but not STAT5. JAK1 and JAK3 were tyrosine-phosphorylated in response to IL-2 and IL-15. Hence, the JAK and STAT molecules that are activated in response to IL-2 and IL-15 are similar but differ from those induced by IL-4. These observations identify the STAT proteins activated by IL-2 and IL-15 and therefore define signaling pathways by which these T-cell growth factors may regulate gene transcription.

Interleukin 2 (IL-2) is a key growth factor that induces the proliferation and functional differentiation of T lymphocytes and natural killer cells. IL-15 shares many characteristics with IL-2, such as the generation of cytotoxic T cells and lymphokine-activated killer cells (1–3). Two IL-2 receptor subunits, the β chain (p75) and the common γ chain (γ_c), are used by IL-15, and γ_c is also shared by other cytokine receptors, including the IL-4 receptor (4, 5). Studies also suggest the existence of an IL-15 receptor subunit distinct from the IL-2 receptor subunits (3). The finding that IL-15 and IL-2 share receptor subunits and exhibit overlapping biological effects implies that they may activate common intracellular substrates but, as yet, this has not been explored.

Recently, a signal transduction pathway that involves Janus kinases (JAKs) and signal transducers and activators of transcription (STATs) has been found to be utilized by a number of growth factors and cytokines that bind members of the hematopoietin receptor family (6, 7). We and others (8–11) have cloned a member of the Janus family (JAK3) and have shown that JAK3 and JAK1 are functionally coupled to the IL-2 receptor, as well as other receptors that use γ_c (receptors for IL-4, -7, and -9). The requisite role of γ_c in signaling is perhaps best illustrated by the discovery that mutations of this subunit result in X chromosome-linked severe combined immunodeficiency (12). Many of these mutations disrupt JAK3– γ_c interactions, suggesting that this disruption might be important in the pathogenesis of this immunodeficiency (13). Given that IL-15 shares this common receptor subunit, it was

important to examine whether IL-15 might also activate JAK3 and JAK1.

Studies of the mechanism by which hematopoietin receptors activate gene transcription indicate that STAT proteins become rapidly tyrosine-phosphorylated in the receptor complex and directly translocate to the nucleus, where they bind DNA and activate transcription (14–17). A family of STAT proteins has emerged that appears to be involved in cytokine-induced gene activation. STAT1 and -2 were identified as the initial members of this family, six of which have now been cloned. These include STAT3, which is activated by IL-6 (18); STAT5, which is activated by prolactin (19, 20); an IL-4-induced STAT (STAT6) (21); and STAT4, which we have reported to be activated by IL-12 (22). These findings suggest a model in which cytokines, such as IL-15 and IL-2, that bind hematopoietin receptors will activate JAK/STAT family proteins and hence rapidly induce gene transcription.

In the present study we define the STAT proteins involved in IL-2 and IL-15 signaling. Treatment of human T cells with IL-15 and IL-2 resulted in the tyrosine phosphorylation of JAK1 and JAK3. Additionally there was a rapid induction of DNA-binding complexes that contained STAT3 and STAT5, both of which are tyrosine-phosphorylated. We also determined that IL-4 did not activate STAT5. Therefore, while these T-cell growth factors (IL-2, -4, and -15) bind a common receptor subunit and activate identical JAKs, they activate distinct STAT proteins.

MATERIALS AND METHODS

Cells and Reagents. Human T lymphocytes were obtained by Percoll gradient centrifugation from normal donors, following informed consent. The purity of the T-cell population was 93–96%. Peripheral blood T lymphocytes were activated in RPMI 1640 (Advanced Biotechnologies, Columbia, MD) containing gentamicin (100 μ g/ml), 2 mM L-glutamine (GIBCO), 10% heat-inactivated fetal bovine serum (HyClone), and phytohemagglutinin (PHA, 1 μ g/ml) for 3 days. These cells were then rested in 0.5% human serum for 4 hr and washed in CO₂-acidified medium, in order to optimize detection of IL-2 and IL-15 signaling. Recombinant human IL-2 was kindly provided by Cetus. IL-4, IL-7, and IL-9 were obtained from PeproTech (Rocky Hill, NJ). IL-15 was obtained from Immunex. IL-12 was obtained from Stanley Wolf (Genetics Institute, Cambridge, MA). Interferon α (IFN- α) was obtained from Hoffmann–LaRoche. Rabbit polyclonal anti-JAK1 and mouse

monoclonal anti-phosphotyrosine 4G10 were purchased from Upstate Biotechnology (Lake Placid, NY). Rabbit polyclonal anti-JAK3 was made in our laboratory (21). Rabbit polyclonal antisera against STAT1 and STAT2 were kindly provided by Chris Schindler (Columbia University, New York). STAT3 C-terminal antibody was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Rabbit polyclonal anti-STAT5 was a gift from Andrew Larner (Food and Drug Administration, Bethesda) and was also obtained from Transduction Labs (Lexington, KY). p94-STAT5 antiserum, which was raised against aa 515–607 of STAT1 and which immunoprecipitates STAT5 but not STAT1, was prepared as described (23). As the commercially available STAT5 antibodies do not immunoprecipitate STAT5, we have used this antibody for STAT5 immunoprecipitation and electrophoretic mobility-shift assays (EMSAs).

Immunoprecipitation and Immunoblotting. Stimulated T lymphocytes were washed in ice-cold phosphate-buffered saline and lysed in 1% (vol/vol) Triton X-100/300 mM NaCl/50 mM Tris, pH 7.5, with 2.5 mM *p*-nitrophenyl guanidinobenzoate and both leupeptin and aprotinin (Sigma) at 10 μ g/ml. The lysates were centrifuged at 12,000 \times *g* and the postnuclear supernatants were immunoprecipitated with antisera as stated in the text. The immunoprecipitates were resolved by SDS/PAGE and transferred to Immobilon membrane (Millipore) before immunoblotting with anti-phosphotyrosine. Membranes were blocked with 1% (wt/vol) gelatin and sequentially incubated with anti-phosphotyrosine (1:2000), biotinylated goat anti-mouse IgG (Oncogene Science), and horseradish peroxidase-conjugated streptavidin (Oncogene Science). Signal was detected by enhanced chemiluminescence (ECL; Amersham). Membranes that were subsequently reprobed were treated with 15% H₂O₂.

EMSA. After treatment with IL-2, IL-4, or IL-15, cell extracts were prepared as described (24). The EMSA probe was an oligodeoxynucleotide (5'-ACGATGTTTCAAG-GATTTGAGATGTATTTCAGAAAAG-3') corresponding to the IFN- γ response region (GRR) of the Fc γ receptor I gene (25). The extracts were incubated with or without antibody for 60 min on ice prior to the addition of ³²P-labeled probe. After a 10-min incubation with probe, the DNA-binding complexes were resolved by electrophoresis in a nondenaturing 6% polyacrylamide gel that was subsequently dried and exposed to film overnight.

Oligonucleotide Affinity Purification of STATs. Human T cells that had been activated with PHA for 3 days and stimulated with IL-2, IL-4, or IL-15 for 15 min were lysed in 0.1% Triton X-100/10 mM Hepes, pH 7.4/2 mM EDTA/1 mM EGTA/400 mM KCl/1 mM dithiothreitol/10% (vol/vol) glycerol/1 mM Na₃VO₄/200 μ M phenylmethanesulfonyl fluoride containing leupeptin and aprotinin at 10 μ g/ml. Lysates were incubated with biotinylated GRR oligonucleotide bound to streptavidin-coated agarose for 1 hr. The agarose beads were then washed, and the eluted protein was resolved by SDS/PAGE and immunoblotted on Immobilon membrane with antibody to STAT3 or STAT5.

RESULTS

IL-15 Induces Tyrosine Phosphorylation of JAK1 and JAK3. IL-2 and IL-15 have overlapping biological effects on T cells, and both of these lymphokines have been shown to bind the IL-2 receptor β (p75) and γ (γ_c) chains. However, it was unclear whether the biochemical responses induced by these cytokines were similar. We have shown previously that JAK1 and JAK3 are functionally coupled to the IL-2 receptor and are activated by the other cytokines that bind γ_c (IL-4, IL-7, and IL-9) in T cells (9, 13). We therefore assessed whether IL-15 might signal via these or related kinases. We compared tyrosine phosphorylation of JAK1 and JAK3 in response to

IL-2, IL-4, IL-7, IL-9, IL-15, IL-12, and IFN- α (Fig. 1A). Similar levels of tyrosine phosphorylation of JAK3 were observed in response to all stimuli except IL-12 and IFN- α , which do not bind γ_c and which utilize distinct JAKs for signal transduction (26). In parallel experiments elevated levels of JAK1 tyrosine phosphorylation were observed in response to IL-2, IL-4, IL-7, IL-9, and IL-15 but not in response to IL-12 (Fig. 1B). Moreover, as expected, JAK1 was phosphorylated on tyrosine residues in response to IFN- α (27). We did not detect IL-15-induced tyrosine phosphorylation of JAK2 and Tyk2 (results not shown). These data confirm that all cytokines known to signal via γ_c , including IL-15, induce tyrosine phosphorylation of both JAK1 and JAK3 in human peripheral blood T cells.

IL-2 and IL-15 Induce Tyrosine Phosphorylation of STAT5.

We have demonstrated that the known γ_c users induce phosphorylation of JAK1 and JAK3. Cytokines which activate JAKs also induce the tyrosine phosphorylation and activation of transcription factors known as STATs. Indeed evidence for an IL-2-dependent STAT-like transcription factor similar to that activated by prolactin has been reported (28). As prolactin has been shown to activate STAT5, we examined whether cytokines that bind γ_c would induce tyrosine phosphorylation of STAT5. Lysates from cells that had been treated with IL-2, IL-4, or IL-15 for 15 min were immunoprecipitated with anti-phosphotyrosine and the immunoprecipitates were im-

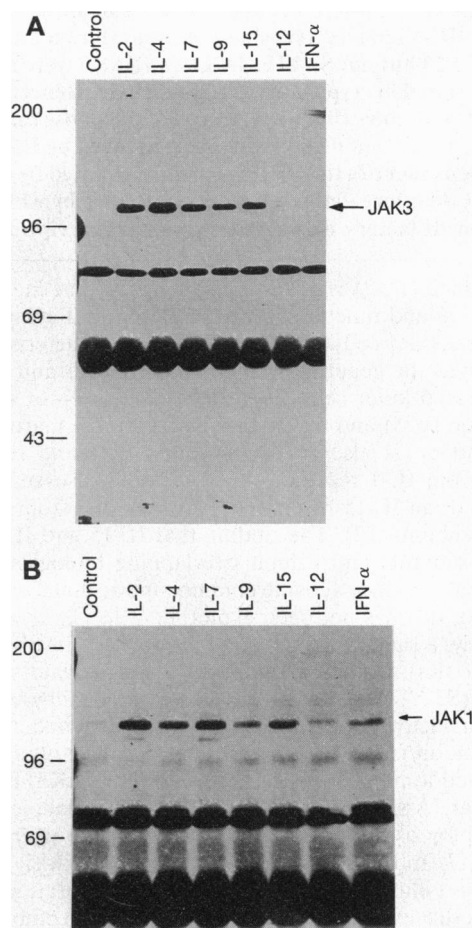


FIG. 1. IL-2, -4, -7, -9, and -15 induce tyrosine phosphorylation of JAK3 (A) and JAK1 (B). T cells were untreated or treated with IL-2 (1000 units/ml), IL-4 (100 units/ml), IL-7 (100 ng/ml), IL-9 (100 ng/ml), IL-15 (5000 units/ml), IL-12 (100 units/ml), or IFN- α (100 units/ml) for 15 min and lysed. Lysates were immunoprecipitated with anti-JAK1 or anti-JAK3 as indicated. These immunoprecipitates were resolved by SDS/PAGE and then immunoblotted with anti-phosphotyrosine. Size markers at left are in kilodaltons.

munoblotted with anti-STAT5. We observed STAT5 tyrosine phosphorylation in response to IL-2 and IL-15 but not in response to IL-4 (Fig. 2A).

Tyrosine phosphorylation of STAT5 induced by IL-2 and IL-15 was also analyzed with a second STAT5 antibody. This antibody immunoprecipitates p94-STAT5 (D.K. and C. Schindler, unpublished work). Lysates from cells that had been treated with IL-2, IL-4, or IL-15 for 15 min were immunoprecipitated with anti-p94-STAT5 and the immunoprecipitates were immunoblotted with anti-phosphotyrosine. Again tyrosine phosphorylation of STAT5 was detected in response to IL-2 and IL-15 but not in response to IL-4 (Fig. 2B Upper). To confirm that the precipitated protein was STAT5, this blot was subsequently stripped and reblotted with a different STAT5 antibody. As shown in Fig. 2B Lower, the apparent molecular weight of the STAT5 protein was increased in cells stimulated by IL-2 or IL-15. This phenomenon has been reported previously for other STATs. The activation of STAT1 by IFN- γ , and of STAT4 by IL-12, results in a similar alteration in mobility (22). The likely explanation for the mobility shift is additional phosphorylation, but this has not been proven. Consistent with the data shown in Fig. 2A, IL-4, which did not induce tyrosine phosphorylation of STAT5, did not induce a change in the apparent mobility of the protein. We have also observed tyrosine phosphorylation of STAT5 in response to IL-7 and IL-9 (data not shown). These data confirmed that, with the exception of IL-4, each cytokine that is known to bind γ_c will also induce tyrosine phosphorylation of STAT5. Together, these results indicate that STAT5 is phosphorylated in response to IL-2 and IL-15, but not IL-4. Therefore, while IL-2, IL-4, and IL-15 activate the same JAK proteins, IL-4 treatment does not result in the phosphorylation of STAT5 in T cells.

IL-2, IL-4, and IL-15 Induce Tyrosine Phosphorylation of STAT3. Cytokines such as IFN- α and IL-6 induce tyrosine phosphorylation of a complex of STAT proteins (16). To determine whether IL-2, IL-4, or IL-15 could stimulate phosphorylation of other STAT proteins, we stimulated human peripheral blood T cells with IL-2, IL-4, or IL-15 and analyzed the cell lysates by immunoprecipitation with antisera specific for STAT1, -2, -3, or -4. While we observed no basal phosphorylation of STAT3, IL-2 and IL-15 induced the phosphorylation of STAT3 in T cells (Fig. 2C Upper). Similarly phos-

phorylation of STAT3 was detected in response to IL-4. We also observed tyrosine phosphorylation of STAT3 in response to IL-7 and IL-9 (data not shown). Upon reblotting, equal amounts of STAT3 were detected in each lane. A shift in the apparent molecular weight of STAT3 was not detected upon tyrosine phosphorylation of this protein in T cells. Thus, STAT3 tyrosine phosphorylation occurs in T cells in response not only to IL-2 and IL-15, but to IL-4 as well.

IL-2 Does Not Induce Tyrosine Phosphorylation of STAT1, STAT2, or STAT4. To investigate whether other STATs were activated by IL-2 or IL-15, PHA-activated human peripheral blood T cells were untreated or stimulated with IL-12, IL-2, and IFN- α ; lysed; and immunoprecipitated with anti-STAT1, anti-STAT2, or anti-p94-STAT5. In human peripheral blood T cells, phosphorylation of STAT1 (Fig. 2D, lane 4) and STAT2 (lane 12) was seen in response to IFN- α . However, these STAT proteins were not phosphorylated in response to IL-2 (lanes 3 and 11), IL-12 (lanes 2 and 10), or IL-15 (data not shown), indicating that although STAT1 and STAT2 were present in these cells, they were not activated by these cytokines. As a positive control we examined tyrosine phosphorylation of STAT5. This was observed in response to IL-2 (lane 7), but not IL-12 (lane 6) or IFN- α (lane 8). No tyrosine phosphorylation of STAT4 was observed in T cells in response to IL-2 or IL-15, although we have detected activation of STAT4 in these cells in response to IL-12 (22).

IL-2 and IL-15 Induce DNA-Binding Activity of STAT3 and STAT5. The cytokine-induced tyrosine phosphorylation of STAT proteins has been shown to be associated with the appearance of DNA-binding complexes containing these phosphorylated STAT proteins. To investigate the activation of DNA-binding complexes induced by IL-2, IL-4, and IL-15 we used EMSAs, with a probe corresponding to the GRR of the Fc γ receptor I gene. This probe binds various STAT proteins that are induced by a number of cytokines (29). PHA-activated T cells were washed and serum-deprived for 4 hr. No basal DNA-binding activity was detected (Fig. 3A, lane 1) in extracts from these serum-deprived cells, but IL-2, IL-4, and IL-15 rapidly induced GRR-binding activity. IL-2 and IL-15 induced complexes with an upper and lower component, contrasting with IL-4, which produced only a single component (Fig. 3A Upper, lanes 2–4). Identical complexes were evident when a

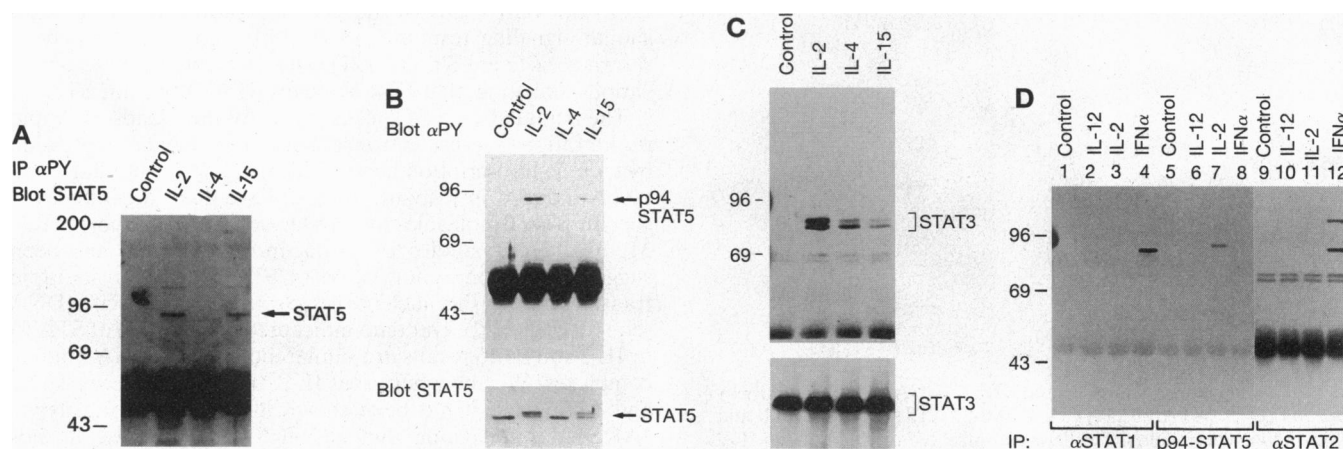


FIG. 2. IL-15 and IL-2 induce tyrosine phosphorylation of STAT proteins. (A) IL-2 and IL-15 induce tyrosine phosphorylation of STAT5. Cells were untreated or treated with IL-2, IL-4, or IL-15 for 15 min and the lysates were immunoprecipitated with anti-phosphotyrosine (IP α PY) and blotted for STAT5. (B) Immunoprecipitation of STAT5 tyrosine-phosphorylated in response to IL-2 and IL-15. Cells were treated with IL-2, IL-4, or IL-15 for 15 min and lysed. STAT5 tyrosine phosphorylation was examined with an antibody that immunoprecipitates STAT5 (p94-STAT5). The immunoprecipitates were immunoblotted with anti-phosphotyrosine (Upper) and subsequently with anti-STAT5 (Lower). (C) IL-15, IL-4, and IL-2 induce tyrosine phosphorylation of STAT3. T cells were untreated or treated with IL-2, IL-4, or IL-15 for 15 min, lysates were immunoprecipitated with anti-STAT3, and the precipitates were immunoblotted first with anti-phosphotyrosine (Upper) and then with anti-STAT3 (Lower). (D) IL-2 does not induce tyrosine phosphorylation of STAT1 or STAT2. T cells were incubated with medium alone (lanes 1, 5, and 7), IL-12 (lanes 2, 6, and 10), IL-2 (lanes 3, 7, and 11) or IFN- α (lanes 4, 8, and 12) for 15 min. Lysates were immunoprecipitated with antisera to STAT1, STAT5, or STAT2. Immunoprecipitates were immunoblotted with anti-phosphotyrosine.

probe from the cytokine response element of the IRF-1 gene (30) was used in place of the GRR, and these complexes were specific as demonstrated by competitive inhibition with unlabeled probe (data not shown).

We next examined whether we could detect specific STAT proteins as components of these DNA-binding complexes induced by IL-2, IL-4, and IL-15. Antiserum to STAT3 supershifted the lower component of the complexes induced in response to IL-2 and IL-15, as well as the single component induced by IL-4 (Fig. 3*A Upper*, lanes 6–8). This observation was in agreement with the tyrosine phosphorylation of STAT3

observed in response to each of these cytokines. Furthermore, antiserum to STAT5, although it did not completely remove any component of the complexes induced by IL-2 and IL-15 (Fig. 3*A Upper*, lanes 10–12), did induce the appearance of a supershifted complex of reduced mobility. This antibody had no effect on the complex induced by IL-4 (Fig. 3*A Upper*, lane 11). Again, this finding is in agreement with the tyrosine phosphorylation data and confirms that IL-2 and IL-4 induce different STAT DNA-binding complexes. We did not detect STAT2 or STAT4 in the IL-2-, IL-4-, or IL-15-induced DNA-binding complexes (Fig. 3*A Lower*). These data provide strong evidence that STAT3 and STAT5 are important components of the DNA-binding complexes induced by IL-2 and IL-15, although with current reagents it is impossible to exclude the possibility that other components may be present.

GRR Affinity Purification of IL-2-Activated STAT3 and STAT5. Although the supershift data provided strong evidence for the presence of STAT3 and STAT5 in the IL-2- and IL-15-induced DNA-binding complexes, we wished to verify this by an oligonucleotide affinity purification technique in which ligand-induced DNA-binding proteins would be precipitated on oligonucleotides bound to agarose. By using GRR affinity purification and Western blotting with specific antisera, we confirmed that the IL-2- and IL-15-induced GRR-binding complexes in peripheral blood T cells contained both STAT3 and STAT5 (Fig. 3*B*). We also confirmed that STAT3 was present in the IL-4-induced GRR-binding complex in these cells. Consistent with the EMSA data, STAT5 was not detected in the IL-4-induced GRR-binding complexes. Thus, we have used several different techniques and reagents to confirm that STAT3 and STAT5 are activated by IL-2 and IL-15 in T cells.

DISCUSSION

IL-2, IL-4, and IL-15 behave as potent T-cell growth factors, but whether they activate common signaling pathways has been unclear. In this report, we demonstrate that IL-15 and IL-2 activate similar JAK/STAT pathways. However, while IL-2 and IL-4 activate JAK1 and JAK3, their pathways diverge at the level of STAT activation. We show that IL-2 and IL-15 induce tyrosine phosphorylation and activation of STAT5 and STAT3 proteins in human peripheral blood T cells. Therefore IL-15 not only binds to the IL-2 receptor, but also induces similar signaling responses to IL-2 in T cells. IL-4 has been shown to activate STAT6 (21). Here we extend these observations and show that IL-4 activates STAT3 but not STAT5.

The stimulation of T cells by cytokines leads to rapid proliferation that results from the activation of gene expression by specific transcription factors. Although DNA-binding complexes induced in response to IL-2 have been described, the specific STAT molecules involved have not been reported (28, 31, 32). From studies of prolactin signaling, it has been suggested that phosphorylation of STAT5 leads to its rapid translocation to the nucleus, where it binds to specific DNA sequences (19, 20). Our data indicate that activation of STAT5 by IL-2 and IL-15 results in a similar induction of DNA-binding complexes. We show here that IL-2 and IL-15 activate JAK1 and JAK3, and it has been shown that prolactin signals via JAK2. It is intriguing that although each of these ligands activates STAT5, the JAK proteins activated differ. Therefore, the phosphorylation of STAT5 is not dependent on the particular JAK that is activated, but as has been described for the IL-6 receptor, may be determined by the STAT or STATs that are recruited to the receptor complex (33).

The demonstration that STAT3 and STAT5 are activated in response to IL-2 and IL-15 does not explain the specific effects of these cytokines. STAT3 has been shown to be phosphorylated in response to epidermal growth factor, IL-6, IFN- α , and IFN- γ , and IL-2 (31). We show here that STAT3 is also

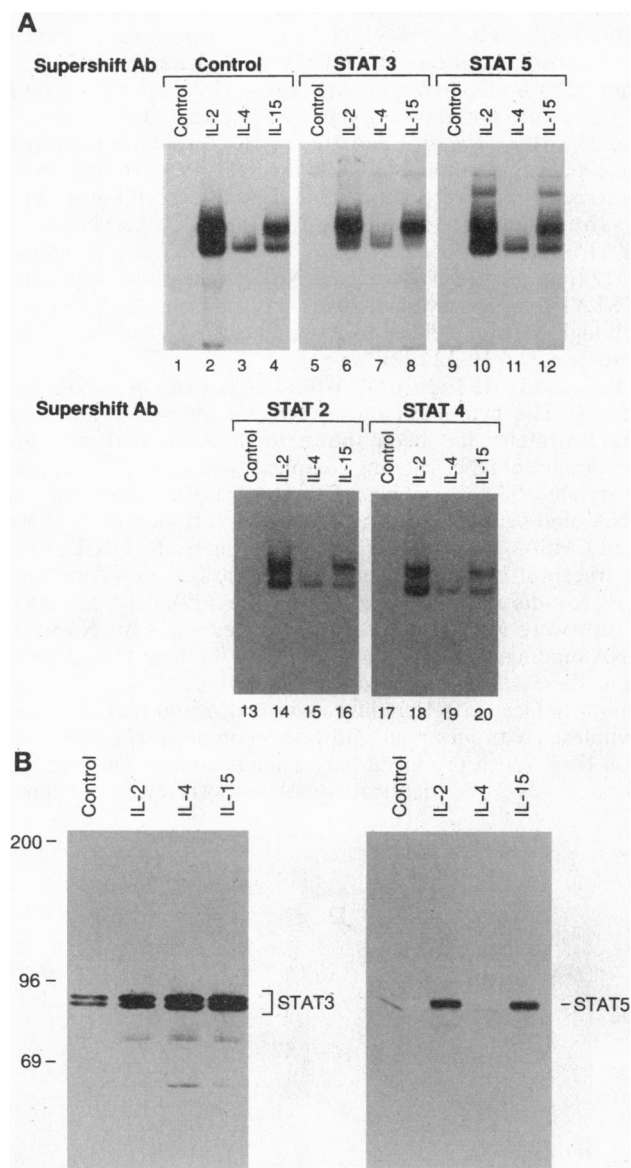


FIG. 3. Activation of STAT3 and STAT5 DNA-binding activity by IL-2 and IL-15. (*A*) Anti-STAT3 and anti-STAT5 supershift IL-2- and IL-15-induced complexes. T cells were untreated or treated with IL-2 (1000 units/ml), IL-4 (100 units/ml), or IL-15 (5000 units/ml) for 15 min, and cell extracts were incubated with or without STAT antibody (Ab) as indicated prior to 32 P-labeled GRR probe. The DNA-binding complexes were resolved by electrophoresis. The gel was then dried and exposed to film overnight. (*B*) IL-2- and IL-15-dependent binding of STAT3 and STAT5 to the GRR probe. Peripheral blood lymphocytes were untreated (control) or treated with IL-2, IL-4, or IL-15 for 15 min, and extracts were incubated with biotinylated GRR oligonucleotide bound to streptavidin-coated agarose. The agarose beads were then washed, and the eluted protein was immunoblotted with antibody to STAT3 (*Left*) or STAT5 (*Right*).

activated by IL-4, IL-7, IL-9, and IL-15. Likewise, we and others have shown that STAT5 can be activated by a number of cytokines, including IL-2, IL-7, IL-9, IL-15, thrombopoietin (unpublished observation), and prolactin. However, these transcription factors may be of importance in many cell types, perhaps activating genes essential for cell growth and proliferation. For example, we find that the megakaryocyte proliferative factor thrombopoietin activates STAT3 and STAT5. We show here that the T-cell growth factors IL-2, IL-7, and IL-15 all activate STAT3 and STAT5. Therefore, transcription complexes that include STAT3 and STAT5 may be of general importance to promote cell proliferation in T cells, megakaryocytes, and perhaps other cells. The challenge remains to relate the activation of specific STATs to the biological effects of these cytokines and to identify their roles in gene activation.

Although much of our evidence clearly demonstrates that the family of cytokines that use γ_c activate common signaling molecules such as JAK1, JAK3, and STAT3, it also suggests that distinct signals are activated by IL-4. IL-4 induces the phosphorylation of a number of unique substrates such as IL-4-STAT (21) and the IRS-1-like molecule 4PS (34). Similarly, IL-4 and IL-7 fail to activate Shc in T cells (35, 36). Again the recruitment of different signaling molecules to the receptors may contribute to the distinct downstream responses to each cytokine.

The activation of distinct STATs provides a mechanism by which STATs could contribute to IL-2- or IL-4-specific gene activation in T cells. STAT3 forms heterodimers with other STAT molecules (18), and perhaps the various STAT complexes formed are an important mechanism by which signal diversity is elicited. An important goal will be to understand how the STATs contribute to gene activation in T cells.

IL-15 binds to subunits of the IL-2 receptor and has biological activity resembling that of IL-2. However, while these cytokines bind the same β and γ receptor subunits, IL-15 may bind a distinct receptor chain. Evidence for this comes from the IL-3-dependent 32D cell line, which expresses the complete IL-2 receptor and proliferates strongly in response to IL-2, but not at all in response to IL-15 (3). Since the precise structure of the IL-15 receptor is unknown, we cannot be certain that IL-2 and IL-15 signal identically in all respects. However, our data indicate that IL-2 and IL-15, consistent with their biological effects, have highly similar signaling substrates in human peripheral blood T cells.

In summary, our results demonstrate that STAT3 and STAT5 are rapidly activated in response to IL-2 and IL-15 but that STAT5 is not activated in response to IL-4. This, coupled with the identification of the JAKs activated by these cytokines, identifies novel pathways by which transcriptional activation may occur. Understanding how IL-2, IL-4, and IL-15 regulate gene expression is a critical issue in T-cell activation. The demonstration that these key cytokines activate STAT3 and STAT5 will provide important insights into the mechanisms of gene regulation by these cytokines.

Note Added in Proof. Our findings agree with those of Lin *et al.* (37), Hou *et al.* (38), and Fujii *et al.* (39) that have been published since submission of this article.

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