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Signaling through the interleukin 2 receptor β chain activates a STAT-5-like DNA-binding activity

SARAH L. GAFFEN*, STEPHEN Y. LAI*, WEIDUAN XU*, FABRICE GOUILLEUX†, BERND GRONER†, MARK A. GOLDSMITH*‡, AND WARNER C. GREENE*‡§¶

*Gladstone Institute of Virology and Immunology, Departments of ‡Medicine and §Microbiology and Immunology, School of Medicine, University of California, San Francisco, CA 94143; and †Institute for Experimental Cancer Research, Tumor Biology Center, Freiburg, Germany

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ABSTRACT **To explore the possible involvement of STAT factors ("signal transducers and activators of transcription") in the interleukin 2 receptor (IL-2R) signaling cascade, murine HT-2 cells expressing chimeric receptors composed of the extracellular domain of the erythropoietin receptor fused to the cytoplasmic domains of the IL-2Rβ or -γ_c chains were prepared. Erythropoietin or IL-2 activation of these cells resulted in rapid nuclear expression of a DNA-binding activity that reacted with select STAT response elements. Based on reactivity with specific anti-STAT antibodies, this DNA-binding activity was identified as a murine homologue of STAT-5. Induction of nuclear expression of this STAT-5-like factor was blocked by the addition of herbimycin A, a tyrosine kinase inhibitor, but not by rapamycin, an immunophilin-binding antagonist of IL-2-induced proliferation. The IL-2Rβ chain appeared critical for IL-2-induced activation of STAT-5, since a mutant β chain lacking all cytoplasmic tyrosine residues was incapable of inducing this DNA binding. In contrast, a γ_c mutant lacking all of its cytoplasmic tyrosine residues proved fully competent for the induction of STAT-5. Physical binding of STAT-5 to functionally important tyrosine residues within IL-2Rβ was supported by the finding that phosphorylated, but not nonphosphorylated, peptides corresponding to sequences spanning Y392 and Y510 of the IL-2Rβ tail specifically inhibited STAT-5 DNA binding.**

The cytokine interleukin 2 (IL-2) plays a central role in the immune response, serving as a primary regulator of T-cell proliferation and differentiation as well as a mediator of NK- and B-cell development and differentiation (1, 2). The IL-2 receptor (IL-2R) is a multisubunit complex composed of three recognized proteins, α, β, and a common chain, γ_c, shared with the IL-4, IL -7, IL-9, and IL-15 receptors (3–6). The IL-2Rβ and -γ_c chains contain cytoplasmic domains of 385 and 86 amino acids, respectively, and are sufficient to transduce signals after IL-2-mediated dimerization (7–9). In particular, the cytoplasmic domain of the IL-2Rβ chain is associated with the Janus-family kinase JAK1, while γ_c is found with JAK3 (10, 11). Thus, a major early signaling event produced by IL-2 binding is tyrosine phosphorylation of several substrates, including the IL-2Rβ and -γ_c chains themselves as well as JAK1 and JAK3 (12–15).

Cytokine receptors and their associated JAKs have been shown to mediate tyrosine phosphorylation and concomitant activation of STAT factors ("signal transducers and activators of transcription") (reviewed in ref. 16), which are found in a latent, unphosphorylated state in the cytoplasm (17, 18). Upon receptor stimulation, they likely physically associate with the activated receptor tail through their SH2 domains (19, 20), become phosphorylated, dissociate from the receptor, and then dimerize via these SH2 domains (21). Dimerization is thought to allow nuclear import, where the STAT factors subsequently regulate transcription of target genes. At least four STAT response elements are the targets of a rapidly induced DNA-binding activity after IL-2R stimulation (22–26). Thus, to study the role of STAT factors in IL-2 signaling, we made use of an IL-2R signaling system in the IL-2-dependent, $CD4^+$ murine T-cell line HT-2. In this system, chimeric receptors containing the extracellular domain of the erythropoietin (EPO) receptor fused to the transmembrane and cytoplasmic domains of the IL-2Rβ and -γ_c chains (EPOβ and EPOγ) are stably expressed in HT-2 cells. Heterodimerization of EPOβ/EPOγ results in proliferation and induced phosphorylation of the IL-2R and JAK1 and JAK3 (M.A.G., S.Y.L., W.X., and W.C.G., unpublished data). With this system, mutations in the β and γ_c receptors can be readily introduced into the cellular context of the IL-2-dependent HT-2 cells and their functional consequences assessed.

The present studies demonstrate that IL-2R ligation in HT-2 cells induces binding of a STAT-5-like factor to specific STAT response elements. STAT-5 induction is strictly dependent on the IL-2Rβ chain; however, its induction appears to be insufficient to trigger proliferation signaling.

MATERIALS AND METHODS

Cell Culture, Preparation of Cell Lines, and Cytokine Stimulations. HT-2 transfectant cell lines were prepared as will be reported elsewhere. HT-2 cells were cultured in RPMI 1640 medium, 10% fetal bovine serum (FBS) (GIBCO/BRL), 2 mM glutamine, 0.05 mM 2-mercaptoethanol, and 1 nM recombinant IL-2 (rIL-2) (Chiron). HT-2EPOβγ, HT-2EPOβ/γYF, HT-2EPOβΔA/γ, and HT-2EPOβΔAB/γ cells were grown in this medium with 5 units of EPO per ml (Amgen) in place of IL-2. HT-2EPOβYF/γ stable transfectants were obtained by selection in hygromycin B (Boehringer Mannheim) and clones were selected by limiting dilution. HeLa cells were grown in Dulbecco's modified Eagle medium with 10% FBS. For cytokine stimulations, cells were washed twice in phosphate-buffered saline (PBS), stripped by a 30-sec incubation in 10 mM sodium citrate and 140 mM NaCl, and incubated for 4 hr in serum- and growth factor-free RPMI 1640 medium containing 1% bovine serum albumin (fraction V, Sigma). Stimulations were for various periods of time (typically 15 min) with 10 ng of interferon γ per ml (IFN-γ; PharMingen), 10 nM IL-2, or 50 units of EPO per ml in serum- and growth factor-free media. Herbimycin A (HA; BRL) and rapamycin (Calbiochem) were added 4 hr prior to and maintained during cytokine stimulations.

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Abbreviations: IL, interleukin; R, receptor; EMSA, electrophoretic mobility shift assay; HA, herbimycin A; PRL, prolactin; STAT, signal transducer and activator of transcription; EPO, erythropoietin; IFN, interferon; JAK, Janus kinase; FBS, fetal bovine serum; r, recombinant.

¶To whom reprint requests should be addressed.

Nuclear Extracts. Ten to 50 million cells were stimulated as described above and washed in PBS. Nuclear extracts were prepared as described (27) in the presence of 1 mM sodium orthovanadate and the following protease inhibitors: antipain, 0.5 mg/ml; aprotinin, 0.5 mg/ml; bestatin, 0.75 mg/ml; leupeptin, 0.5 mg/ml; pepstatin A, 0.05 mg/ml; phosphoramidon, 1.4 mg/ml; and soybean trypsin inhibitor, 0.5 mg/ml (Sigma).

Electrophoretic Mobility Shift Assay (EMSA). Oligonucleotide probes were end-labeled with [γ-^{32}P]dATP (Amersham) and polynucleotide kinase (New England Biolabs), and binding assays were performed with 10^5 cpm of probe, 2 μg of poly[d(I-C)], and 10 μg of nuclear extract as described (28). Preincubations of nuclear extracts with different antibodies were performed in the absence of poly[d(I-C)] and binding buffer for 45 min on ice prior to initiation of the binding assay by addition of radiolabeled probe. Preincubations with peptides were performed for 45 min at 25°C with all binding reagents except probe. STAT-1 and STAT-5 C-terminal antibodies were obtained from Transduction Laboratories (Lexington, KY); STAT-2, -3, and -6 antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA); 4G10 and anti-Shc (poly-IgG control) were from Upstate Biotechnology (Lake Placid, NY); MOPC 195 (IgG2b control) and UPC10 (ascites control) were from Cappel; and phosphoserine and phosphothreonine antibodies were from Sigma. Phosphopeptides were synthesized by C. Turck (University of California, San Francisco) and solubilized in PBS at a final pH of 7–8.

Preparation of STAT-5 N-Terminal Antisera. Anti-STAT-5 was produced in chickens against a glutathione *S*-transferase (GST)–STAT-5 fusion protein containing an N-terminal fragment spanning residues 6–160 (29). Antibodies from yolk egg extracts were precipitated and purified with polyethylene glycol and resuspended in 10 mM phosphate buffer, pH 7.5/100 mM NaCl/0.01% sodium azide as described (30).

RESULTS

Identification of IL-2-Inducible DNA-Binding Complexes in HT-2EPO$\beta\gamma$ Cells. To identify an IL-2-inducible DNA-binding activity in wild-type HT-2 and HT-2EPO$\beta\gamma$ cells, cells maintained in IL-2 or EPO were cultured in the absence of these factors for 4 hr and then stimulated for 15 min with saturating quantities of IL-2 (10 nM) or EPO (50 units/ml). Nuclear extracts were prepared and EMSAs were performed with ^{32}P-radiolabeled oligonucleotides (19–30 bp in length) corresponding to STAT-responsive elements found in the promoters of FcγR1, IRF-1, Ly6E, and SIE (16, 22, 24, 25, 31, 32). These probes revealed a specific pattern of IL-2-inducible DNA-binding activity. Specific binding to the FcγR1 element was observed in wild-type HT-2 cells induced with IL-2 as well as in HT-2EPO$\beta\gamma$ cells induced with EPO (Fig. 1). This DNA binding activity was not detected in HT-2 cells expressing EPOβ or EPOγ alone (data not shown), and allowed to react with both the FcγR1 and the IRF-1 probes, since the addition of a 100-fold molar excess of either unlabeled oligonucleotide as a competitor blocked binding to either probe (lanes 5 and 6). In contrast, oligonucleotides containing two other STAT-responsive elements, Ly6E and SIE, previously shown to bind IL-2-induced nuclear factors in other cell types (22, 25), did not significantly compete for binding (lanes 7 and 8). An additional, faster migrating DNA-binding complex was variably observed that was not reproducibly competitively inhibited by any of the unlabeled competitor oligonucleotides (Fig. 1 and data not shown) and thus appeared to reflect an extract-dependent, nonspecific protein–DNA interaction.

Characterization of IL-2-Binding Activity. To assess the role of tyrosine phosphorylation in the IL-2 induction of the observed DNA-binding activity, HT-2EPO$\beta\gamma$ cells were stimulated with EPO in the presence of a protein tyrosine kinase inhibitor, HA, which was added 4 hr prior to EPO stimulation

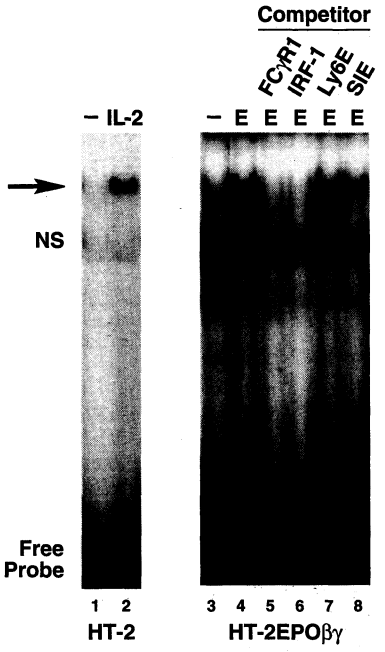


FIG. 1. IL-2-induced DNA-binding activity is selective for specific STAT response elements. HT-2EPO$\beta\gamma$ cells were incubated in medium alone and stimulated for 15 min with 10 nM IL-2 (lane 2) or 50 units of EPO (E) per ml (lane 4), and nuclear extracts were prepared. The specific probe employed was ^{32}P-end-labeled FcγRI. Binding assays and EMSAs were performed in the presence or absence of 50 ng of unlabeled competitor oligonucleotides (lanes 5–8). Sequences of oligonucleotides: FcγRI, GTATTTCCCAGAAAAAGGAC; IRF-1, GCCTGATTTCCCCGAAATGACGG; Ly6E, GATCATATTCCTGTAAGTG; SIE, GTGCATGGTTCAGCCATCTTGTCTACAATTC. Arrow, inducible band; NS, nonspecific band.

(Fig. 2*A*). The presence of HA completely blocked the induction of this DNA-binding activity (lane 2), whereas equivalent concentrations of dimethyl sulfoxide used to dissolve the HA had no effect on its induction (lane 2). In contrast to HA, treatment of HT-2EPO$\beta\gamma$ cells with the IL-2 signaling antagonist rapamycin, at a concentration known to inhibit T-cell proliferation (refs. 33 and 34 and data not shown), did not impair the activation of this DNA-binding activity (lane 4).

To examine further the requirements for tyrosine phosphorylation, nuclear extracts were incubated with monoclonal antibodies specific for phosphotyrosine for 45 min prior to EMSA. As shown in Fig. 2*B* (lane 4), the addition of 4G10 anti-phosphotyrosine antibody almost completely abrogated DNA-binding activity. In contrast, addition of anti-phosphoserine or anti-phosphothreonine antibodies had no effect on DNA binding (lanes 6 and 7). The critical involvement of phosphotyrosine residues in DNA-binding competence is another characteristic feature of STAT factors.

The kinetics of induction of this DNA-binding activity were next examined. Maximal activity was observed within 15 min of EPO stimulation of the HT-2EPO$\beta\gamma$ cells, while a 70% decline in total binding occurred over the ensuing 4 hr (Fig. 2*C*, lanes 2–5). These activation kinetics are similar to those observed with many other STAT factors, in which binding to the target promoter sequences occurs quickly and decays over time (31, 35).

Identification of the IL-2-Induced DNA-Binding Activity as a Murine STAT-5 Homologue. Studies were next performed to assess whether the detected IL-2-induced DNA-binding activity could be attributed to any of the six, currently identified members of the STAT family. It has been previously demonstrated that HT-2 cells do not express STAT-4 (36). To screen the remainder of the STAT proteins, nuclear extracts from EPO-stimulated HT-2EPO$\beta\gamma$ cells were incubated with

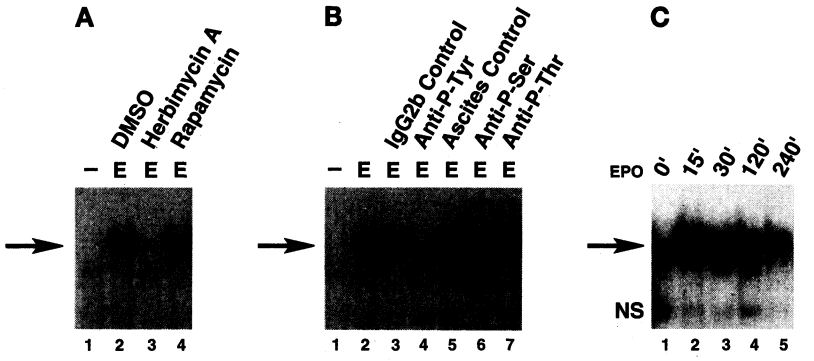


FIG. 2. IL-2 receptor-induced DNA-binding activity shares characteristics of a STAT factor. (*A*) HT-2EPOβγ cells were stimulated with EPO (E) and nuclear extracts were prepared as detailed in the legend to Fig. 1 after a prior 4-hr incubation in 0.5% dimethyl sulfoxide (DMSO), HA (0.5 μg/ml), or rapamycin (2 ng/ml). HA and rapamycin were dissolved in DMSO to a final concentration of 0.5%. (*B*) HT-2EPOβγ cells were stimulated with EPO and nuclear extracts were prepared (see legend to Fig. 1). Nuclear extracts were incubated with 3 μg of MOPC 195 IgG2b control, 3 μg of 4G10 anti-phosphotyrosine, 5 μg of UPC10 ascites control, 5 μg of anti-phosphoserine, or 5 μg anti-phosphothreonine antibodies for 45 min at 4°C prior to EMSA. (*C*) Time course of IL-2R-induced STAT expression. HT-2EPOβγ cells were stimulated with EPO for the indicated times and nuclear extracts were prepared (see legend to Fig. 1). Arrows and NS are as in Fig. 1.

STAT-specific antibodies prior to EMSA. Fig. 3 shows that the EPO-induced complex was neither competitively inhibited nor supershifted by preincubation with antibodies to STAT-1, -2, -3, and -6 (lanes 3–8) or ISGF3γ (p48) (data not shown). However, antibodies specific for an N-terminal fragment of sheep STAT-5 resulted in a new DNA protein complex with a slower mobility in IL-2-induced HT-2EPOβγ cells (lanes 12 and 13) but had no effect on IFNγ-induced STAT-1 in HeLa cells (data not shown). Notably, the original DNA-binding complex was not completely shifted by addition of anti-STAT-5 antibodies, suggesting that there may be a second protein involved in the complex that is antigenically distinct from the known STAT factors. Interestingly, antibodies raised against a C-terminal protein fragment of sheep STAT-5 had no effect on the DNA-binding complex (lane 14). This finding raises the possibility that this factor may be a distinct protein that shares STAT-5 homology at its N terminus. Thus, the DNA-binding activity induced in HT-2EPOβγ cells appears to correspond to a murine STAT-5-like protein.

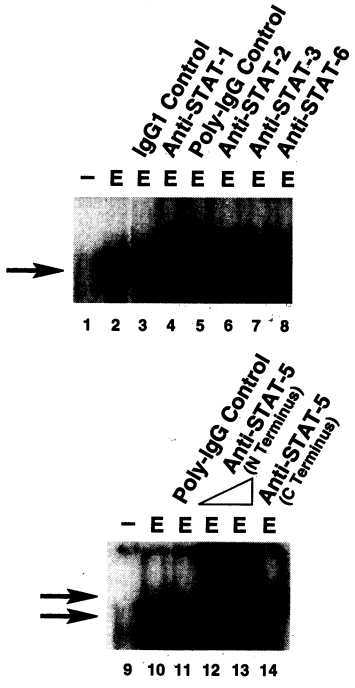


FIG. 3. The IL-2-induced DNA-binding complex is a STAT-5-like protein. HT-2EPOβγ cells were stimulated with EPO (E) for 15 min and nuclear extracts prepared (see legend to Fig. 1). Nuclear extracts were incubated with 2 μg of LFA-1β IgG1 control, 2 μg of anti-human STAT-1, 3 μg of anti-human Shc poly-IgG control, 3 μg of anti-human STAT-2, 3 μg of anti-mouse STAT-3, 3 μg of anti-human STAT-6, 1 μl (lane 12) or 2 μl (lane 13) of anti-sheep STAT-5 (N terminus), or 2 μg of anti-sheep STAT-5 (C terminus) as detailed in the legend to Fig. 2*B*. Arrows, specific, cytokine-inducible band (*Lower*) and supershifted band (*Upper*).

STAT-5 Induction Is Mediated Through the IL-2Rβ Chain. The HT-2 EPOβγ chimera system allows testing of β and γ_c mutants in the context of endogenous, wild-type β and γ_c chains. To determine whether the tyrosine residues within the γ_c subunit play a role in the induction of the STAT-5-like activity, nuclear extracts were prepared from HT-2EPOβ/γYF cells, a cell line in which all four of the cytoplasmic tyrosines within EPOγ had been substituted with phenylalanine residues (M.A.G., *et al.*, unpublished data). As shown in Fig. 4*A*, these cells remained fully capable of inducing STAT-5 in response to EPO (lane 4).

To determine whether the tyrosine residues of the IL-2Rβ chain were necessary for STAT-5 induction, a chimeric EPOβ fusion gene in which all cytoplasmic tyrosine residues of β were replaced with phenylalanine (M.A.G., *et al.*, unpublished data) was introduced into HT-2 cells stably expressing the EPOγ construct (HT-2EPOβYF/γ). Transgene expression was confirmed by RNA Northern analysis (data not shown). In marked contrast to cells expressing the γYF mutation, six independently derived HT-2EPOβYF/γ cell lines were incapable of activating STAT-5 DNA-binding activity in response to EPO (two representative transfectants are shown, Fig. 4*B*, *Upper*). As a control for the integrity of the nuclear extracts, DNA-binding activity to the ubiquitous transcription factor SP-1 was assessed and found to be equivalent in all extracts (Fig. 4*B*, *Lower*). This result provided strong evidence that the IL-2Rβ chain was critical for induction of the STAT-5-like factor.

To localize further the region within the IL-2Rβ chain involved in STAT-5 activation, nuclear extracts were prepared from cells expressing EPOγ and either of two deletion mutants in the β chain, EPOβΔA and EPOβΔAB (38). Both of these mutant β chains were competent to induce STAT-5 fully (Fig. 4*A*), indicating that sequences in the A and B regions of the IL-2Rβ subunit are unnecessary for STAT-5 induction, thus implicating the most distal tyrosine (Y510) in inducible STAT-5 DNA binding.

STAT-5 DNA Binding Is Inhibited by Phosphotyrosine-Containing Peptides from the IL-2Rβ Chain. In previously described cytokine-induced STAT activation systems, direct association of the cytokine receptor with its cognate STAT factor through SH2-phosphotyrosyl interactions has been pro-

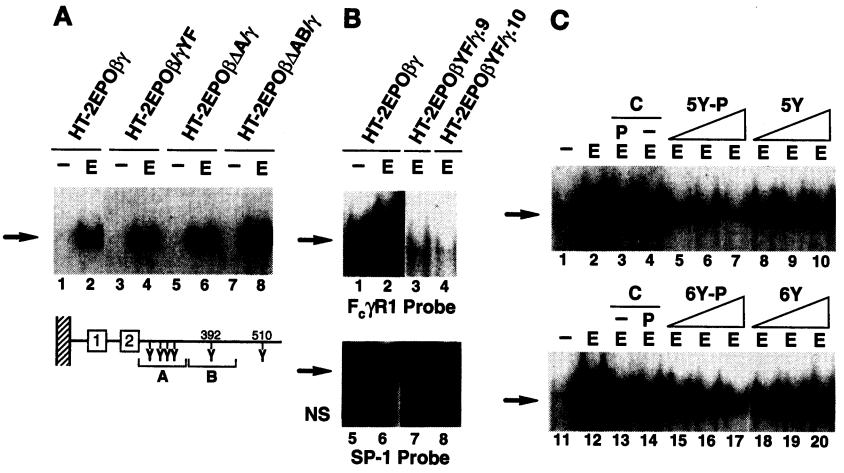


FIG. 4. STAT-5 induction is mediated through the IL-2Rβ chain. (*A*) STAT-5 induction is independent of γ_c tyrosine residues and the IL-2Rβ AB region. Indicated cell lines were stimulated with EPO (E), nuclear extracts were prepared, and EMSAs were performed as described in the legend to Fig. 1. The IL-2Rβ chain transmembrane and cytoplasmic regions are represented. Conserved box 1 and box 2 regions (37), tyrosine residues (Y), and A and B regions are indicated. (*B*) Tyrosine residues of the IL-2Rβ chain are required for STAT-5 induction. Indicated cell lines were stimulated with EPO and nuclear extracts were prepared. EMSA was performed with the FcγR1 or SP-1 probe (TCGAGAGGTGGGTGGAGTTTCGCG), as indicated. (*C*) Certain tyrosine-phosphopeptides derived from the IL-2Rβ cytoplasmic tail inhibit STAT-5 DNA binding. HT-2EPOβγ cells were stimulated with EPO and nuclear extracts were prepared. Nuclear extracts were incubated with phosphorylated (P) or unphosphorylated (−) peptides at concentrations of 680 μM (lanes 3, 4, 7, 10, 13, 14, 17, and 20), 425 μM (lanes 6, 9, 16, and 19), or 250 μM (lanes 5, 8, 15, and 18). Peptide sequences: C, TPSLLEYDQNAL; 5Y, SGEDDAYCTFPS; 6Y, PLNTDAYLSLQE. Arrows, specific, cytokine-induced bands. NS, nonspecific band.

posed (32, 39). Together, the HT-2EPOβΔAB/γ and HT-2EPOβYF/γ mutants suggested that Y510 was critical for STAT-5 induction. However, previous studies of receptor mutants have implicated a redundant function for Y392 and Y510 in proliferation signaling in the Ba/F3 cell line (M.A.G., *et al.*, unpublished data). Further, Y392 has been clearly shown to be phosphorylated *in vivo* (M.A.G., *et al.*, unpublished data). Therefore, to delineate the potential role of phosphorylated tyrosine residues within the IL-2Rβ tail in STAT-5 activation, synthetic phosphopeptides corresponding to these receptor sites and their nonphosphorylated counterparts were added to EMSA reactions. A peptide corresponding to a random rearrangement of the amino acids surrounding IL-2Rβ Y510 was employed as a control (C). Peptides derived from the IL-2Rβ Y392 and Y510 were designated 5Y and 6Y, respectively. Fig. 4*C* shows that neither the control peptides nor nonphosphorylated 5Y and 6Y peptides affected STAT-5 DNA binding. However, addition of the phosphorylated 5Y and 6Y peptides markedly inhibited DNA binding in a dose-dependent fashion (lanes 5–7 and 15–17). Since the amino acid sequences surrounding these tyrosines are similar (*Discussion*), it is not unexpected that both sequences when phosphorylated can form binding motifs recognized by STAT-5. These data suggest that STAT-5 activation likely involves physical assembly at Y392 and/or Y510 of the IL-2Rβ chain, further supporting a central role for IL-2Rβ in the activation of STAT-5.

DISCUSSION

The present studies identify an IL-2-inducible DNA-binding activity in murine T cells that exhibits activation kinetics and tyrosine phosphorylation dependence characteristic of a STAT factor. These observations are fully consistent with its identification as a murine homologue of STAT-5, based on reactivity with anti-sheep STAT-5 antibodies.

STAT-5 was originally identified as a prolactin (PRL)-induced DNA-binding activity present in sheep and murine mammary epithelial cells (29, 40, 41). PRL and IL-2 induce a STAT-like DNA-binding activity in rat Nb2 cells (24), and it has been suggested that nuclear PRL may be required for IL-2-induced proliferation in this cell line (42). The fact that the IL-2 and PRL receptors utilize a common or closely related STAT factor raises the intriguing notion of convergent signaling through these disparate receptors in specific cell types.

The pattern of STAT activation by IL-2 varies considerably, depending upon the specific cellular environment and STAT response element tested. For example, in HT-2 cells, IL-2 induces STAT-5 binding to some (FcγR1 and IRF-1), but not all (Ly6E and SIE), STAT response sequences (Fig. 1). Similarly, in human T cells and NK cells, IL-2 induces STAT-5 binding to the FcγR1 element (32). In human lymphocytes, IL-2 also induces STAT-1 binding with the FcγR1 site (22, 32), while constitutive binding of STAT-3 is observed with this element (32). Using the Ly6E and SIE elements as probes, STAT-1-binding activity has been detected in human T cells and promonocytes (22, 25) but is lacking in HT-2 cells. We conclude that multiple STAT factors may be involved in IL-2 signaling and that different patterns of STAT factor induction may occur in different cellular environments.

The emerging picture of STAT factor activation suggests a direct physical association of the factor with its inducing receptor mediated through the same SH2-phosphotyrosyl interactions that subsequently allow for its dimerization and nuclear import (21, 32, 39). This model was strengthened by the finding that tyrosine-phosphorylated peptides derived from the IL-4R block STAT-6 binding to DNA, presumably by preventing its dimerization (32). We now describe studies in the IL-2R system that are consistent with this paradigm. Notably, our studies indicate that the IL-2Rβ chain, and not γ_c, is principally responsible for STAT-5 induction. Indeed, a γ_c mutant chain lacking all four cytoplasmic tyrosine residues continued to promote normal STAT-5 induction when paired with IL-2Rβ (Fig. 4A). In contrast, an IL-2Rβ mutant receptor lacking all cytoplasmic tyrosines failed to induce STAT-5 activity (Fig. 4). Further evidence indicating that STAT-5 activation is mediated exclusively through the β chain was obtained in studies of a hybrid receptor containing wild-type EPOR and EPOβ chains without γ_c; this hybrid complex, but not the EPOR homodimer, induced STAT-5 identically to the HT-2EPOβγ cells. Data supporting the direct physical assembly of STAT-5 with the IL-2Rβ chain were obtained in the present peptide competition experiments in which phosphor-

ylated, but not nonphosphorylated, peptides spanning either Y392 or Y510 of IL-2Rβ specifically blocked STAT-5 DNA binding (Fig. 4*C*). The ability of the EPOβΔAB mutant receptor (which lacks Y392 and four proximal cytoplasmic tyrosines) to induce STAT-5 compared to the inability of the EPOβYF (lacking all six tyrosines) to induce STAT-5 suggests that there may be functional redundancy of these two tyrosine residues. Such a functional redundancy in Y392 and Y510 has also been found for growth signaling in pro-B cells. In addition, Y392 has been documented as a site for IL-2-induced phosphorylation *in vivo* (M.A.G., *et al.*, unpublished data). Together, these observations strongly suggest that the specificity of IL-2-induced activation of STAT-5 lies in the IL-2Rβ cytoplasmic domain and that specific phosphotyrosines within this chain mediate binding of STAT-5 to the receptor.

Recent studies have led to the identification of a tyrosine-containing motif within the gp130-containing family of cytokine receptors that regulates the specificity of STAT factor binding (20). In a parallel fashion, we note that the sequences surrounding Y392 and Y510 of the IL-2Rβ share certain similarities with each other as well as with sequences present in other select cytokine receptors such as the EPO, IL-3, IL-4, and growth hormone receptors. This general sequence, DAYXS/TLX/P, may represent a second motif for cytokine receptor–STAT association. Consistent with this model, the EPO and growth hormone receptors have been shown to induce STAT-5 (unpublished data; ref. 43). However, the tyrosine-containing regions within the PRLR fail to conform to the proposed IL-2Rβ motif, yet STAT-5 is induced through this receptor. Thus, more than one STAT-5 motif may exist, or different STAT-5 isoforms may display different receptor specificities. With sufficient knowledge of the range of such motifs, it may be possible to predict on the basis of a receptor's primary sequence and state of phosphorylation *in vivo* which STAT factors will be induced following receptor ligation.

In conclusion, the role of STAT-5 in signal transduction through the IL-2R as described in this work adds to the rapidly expanding picture of STAT factor diversity in cytokine receptor signaling and reinforces the importance of the JAK–STAT pathway in IL-2R signaling. At present, the precise targets of STAT-5 action remain unknown. Further understanding of the range of STAT factors induced and the identity of the cellular genes that they regulate will help dissect at a molecular level the underlying basis for IL-2 signaling.

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