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Immunology

Different interleukin 2 receptor β-chain tyrosines couple to at least two signaling pathways and synergistically mediate interleukin 2-induced proliferation

(Jak1/Jak3/Stat5/protein phosphorylation)

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ABSTRACT **One of the earliest events induced by interleukin 2 (IL-2) is tyrosine phosphorylation of cellular proteins, including the IL-2 receptor β chain (IL-2Rβ). Simultaneous mutation of three tyrosines (Y338, Y392, and Y510) in the IL-2Rβ cytoplasmic domain abrogated IL-2-induced proliferation, whereas mutation of only Y338 or of Y392 and Y510 inhibited proliferation only partially. While Y392 and Y510 were critical for IL-2-induced activation of signal transducers and activators of transcription (STAT proteins), Y338 was required for Shc–IL-2Rβ association and for IL-2-induced tyrosine phosphorylation of Shc. Thus, activation of both Jak–STAT and Shc-coupled signaling pathways requires specific IL-2Rβ tyrosines that together act in concert to mediate maximal proliferation. In COS-7 cells, overexpression of Jak1 augmented phosphorylation of Y338 as well as Y392 and Y510, suggesting that the role for this Jak kinase may extend beyond the Jak–STAT pathway.**

Receptor tyrosine phosphorylation can create docking sites for downstream signaling molecules with Src homology 2 (SH2) domains, including, for example, Shc, Src, Grb2, phosphatidylinositol (PI) 3-kinase, GTPase-activating protein (GAP), protein-tyrosine-phosphatase 1D (PTP-1D), and phospholipase C-γ (PLC-γ) in the case of the epidermal growth factor receptor (1), and Zap70 and Syk tyrosine kinases in the case of the T-cell and B-cell antigen receptors (2). In this study, we have investigated the role of interleukin 2 receptor (IL-2R) phosphotyrosine docking sites in mediating IL-2-induced proliferation. IL-2 signaling requires the heterodimerization of the IL-2R β- and γ-chain cytoplasmic domains (3, 4), which brings receptor-associated molecules into closer proximity and activates signaling pathways. The γ chain is a shared component of the receptors for IL-2 (5), IL-4 (6, 7), IL-7 (8, 9), IL-9 (10, 11), and IL-15 (12) (and hence is termed the common cytokine receptor γ chain, γ_c) (7, 8), whereas IL-2Rβ is shared by the receptors for IL-2 and IL-15 (12, 13). Mutation of γ_c results in X chromosome-linked severe combined immunodeficiency in humans (14–16). Like other members of the cytokine receptor superfamily, neither β nor γ_c has known intrinsic catalytic activity; however, Jak3 interacts with γ_c (10, 17, 18), and many signaling molecules, including Src family kinases (19, 20), Syk kinase (20), PI 3-kinase (21), Shc (22), and Jak1 (10, 17, 18) have been reported to associate with IL-2Rβ.

To further understand the basis for IL-2-mediated proliferation, we have investigated the role of tyrosine residues in IL-2Rβ. IL-2Rβ is known to be a target for tyrosine phosphorylation (23–26), but the importance of this phosphorylation has been unclear. We find that Y338 (the cytoplasmic tyrosine most proximal to the transmembrane domain), which we demonstrate to be required for tyrosine phosphorylation of Shc, and Y392 and Y510 (the two most distal tyrosines), which mediate activation of signal transducers and activators of transcription (STAT proteins), together are required for full proliferation. In contrast, Y355, Y358, and Y361 do not appear to be required for IL-2-induced proliferation.

MATERIALS AND METHODS

Reagents and Antibodies. Immunoprecipitations were performed with the anti-IL-2Rβ monoclonal antibodies (mAbs) TU11 (5) and hMikβ1 (humanized form of Mikβ1) (27) and protein A-agarose (Pharmacia). Immunoblots were performed with antisera to Jak1 (Transduction Labs, Lexington, KY), IL-2Rβ (ErdA antiserum; ref. 3), or Shc (Upstate Biotechnology) or with 4G10 mAb to phosphotyrosine (Upstate Biotechnology).

Vectors and *In Vitro* Mutagenesis. cDNA encoding human IL-2Rβ was cloned into the vector pME18S, in which expression is driven by the SRα promoter (28), and the murine Jak1 cDNA was cloned into pMLCMV (provided by J. Ihle). IL-2Rβ was mutated with the Altered Sites *in vitro* mutagenesis system (Promega) using oligonucleotides designed to change tyrosine (TAC) to phenylalanine (TTC, underlined) codons at Y338, 5′-ACCAACCAGGGT<u>TTC</u>TTCTTCTTC-CAC-3′; Y355, Y358, and Y361, 5′-ATAGAGGCCTGCCAG-GTG<u>TTC</u>TTTACTT<u>TC</u>GACCCC<u>TTC</u>TCA-GAGGAAGACCCTGATG-3′; Y392, 5′-GAGGAC-GACGCC<u>TTC</u>TGCACCTTCCCCTCCAGG-3′; or Y510, 5′-AACACTGATGCC<u>TTC</u>TTGTCCCTCCAA-3′. Oligonucleotides were also combined to create multiple mutations. Mutants were confirmed by sequencing (Sequenase; United States Biochemical), and subcloned into pME18S.

Cell Culture and Transfections. 32D cells were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS), 10 μM 2-mercaptoethanol, 5% WEHI-3B conditioned medium (WEHI-CM) as a source of IL-3, 2 mM glutamine, and penicillin (100 units/ml) and streptomycin sulfate (100 μg/ml). Transfectants expressing wild-type and mutated IL-2Rβ were generated by electroporating cells (5×10^6 cells in 400 μl) with linearized plasmids containing IL-2Rβ constructs with pCDNA3neo (InVitrogen), using a Gene Pulser (Bio-Rad; 300 V, 960 μF; average time constant, 30 ms). After 24 hr, cells were aliquoted into a 24-well plate and selected in medium with G418 (GIBCO/BRL) at 1 mg/ml. Resistant clones were stained for IL-2Rβ expression by flow cytometry with fluorescein isothiocyanate (FITC)-conjugated anti-IL-2Rβ mAb (Endogen, Cambridge, MA) or with FITC-conjugated control IgG2a (Becton Dickinson) on a FACSort FST (Becton Dickinson). COS-7 cells were maintained in

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Abbreviations: IL, interleukin; IL-2R, IL-2 receptor; mAb, monoclonal antibody; CM, conditioned medium.

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Dulbecco's modified Eagle's medium with 10% FBS, penicillin (100 units/ml), and streptomycin (100 μg/ml) and were transfected with the indicated plasmids by the DEAE dextran method (29).

Tyrosine Phosphorylation of IL-2Rβ in COS-7 Cells. COS-7 cells transfected with combinations of plasmids driving expression of wild-type or mutant IL-2Rβ and Jak1 were cultured for 48–72 hr, washed in phosphate-buffered saline, lysed in lysis buffer [10 mM Tris, pH 7.5/150 mM NaCl/2 mM EDTA/0.875% Brij 96/0.125% Nonidet P-40/1 mM Na_3VO_4/5 mM NaF/1 mM 4-(2-aminoethyl)benzenesulfonyl fluoride (AEBSF) with leupeptin and aprotonin each at 10 μg/ml] and centrifuged at 18,000 × *g* at 4°C for 15 min. Supernatants were boiled in SDS reducing sample buffer and immunoblotted; alternatively they were immunoprecipitated with hMikβ1 or TU11 IL-2Rβ mAb and protein A-agarose beads for 2 hr at 4°C. The beads were washed four times in lysis buffer, three times in kinase buffer (50 mM Hepes, pH 7.9/0.1 mM EDTA/0.01% Brij 96/10 mM dithiothreitol/150 mM NaCl/0.1 mM Na_3VO_4/10 mM NaF), and resuspended in 20 μl of kinase buffer containing 1 mM AEBSF and leupeptin and aprotonin each at 10 μg/ml. Ten microliters of ATP solution (0.3 mM in 5 mM $MnCl_2$/5 mM $MgCl_2$) was added and the samples were incubated at room temperature for 20 min. Reactions were stopped by washing with 0.5 ml of ice-cold kinase buffer and the pellets were boiled in 50 μl of 2× SDS reducing sample buffer. Samples were electrophoresed in SDS/10% polyacrylamide gels (Novex), transferred onto poly(vinylidene difluoride) membranes (Millipore), and immunoblotted with either anti-phosphotyrosine mAb 4G10 (Upstate Biotechnology) or antiserum to Jak1 or IL-2Rβ. Blots were visualized by enhanced chemiluminescence (ECL; Amersham) after incubation with horseradish peroxidase-conjugated secondary antibodies (Amersham).

Tyrosine Phosphorylation of IL-2Rβ and Shc in 32D Cells. 32D cells (5–7 × 10^7) were washed and starved of growth factor for 4 hr in RPMI 1640 medium supplemented with 1% FBS and 10 μM 2-mercaptoethanol, washed, and then treated with 10 nM IL-2 for 15 min at 37°C. Cells were washed once with ice-cold phosphate-buffered saline containing 0.1 mM Na_3VO_4 and 2 mM EDTA and then lysed in lysis buffer. For IL-2Rβ, lysates were immunoprecipitated with TU11 or hMikβ1 mAb and immunoblotted with 4G10. For Shc, lysates were either immunoprecipitated with antiserum to Shc and immunoblotted with 4G10 or immunoprecipitated with 4G10 mAb and immunoblotted with anti-Shc.

Shc Association with IL-2Rβ in 32D Cells. For Shc association experiments, 32D cells were treated with 2 nM IL-2 and lysates immunoprecipitated with hMikβ1 or TU11 mAb to IL-2Rβ were immunoblotted with anti-Shc.

Thymidine Incorporation Assays. 32D cells were washed and starved of growth factor for 4 hr in RPMI 1640 supplemented with 10% FBS and 10 μM 2-mercaptoethanol. The cells were aliquoted at 2–4 × 10^4 cells per well in a 96-well plate and treated in triplicate for 20 hr in 200 μl (final volume) with medium alone or medium containing 10 nM IL-2 or 5% WEHI-CM. One microcurie of [^{3}H]thymidine (6.7 Ci/mmol; DuPont/NEN; 1 Ci = 37 GBq) was then added, the cells were incubated for 4 hr and harvested with a cell harvester (Tom Tec; Wallac, Gaithersburg, MD) and thymidine incorporation was assayed with a Betaplate 1205 counter (Wallac, Gaithersburg, MD).

Electrophoretic Mobility-Shift Assays. 32D cells (1 × 10^7) were washed, starved of growth factor for 4 hr in RPMI 1640 supplemented with 1% FBS and 10 μM 2-mercaptoethanol, washed, and treated with 2 nM IL-2 for 30 min at 37°C. Cells were then washed with ice-cold phosphate-buffered saline containing 1 mM Na_3VO_4 and 5 mM NaF, nuclear extracts were prepared as described (30), and protein concentrations

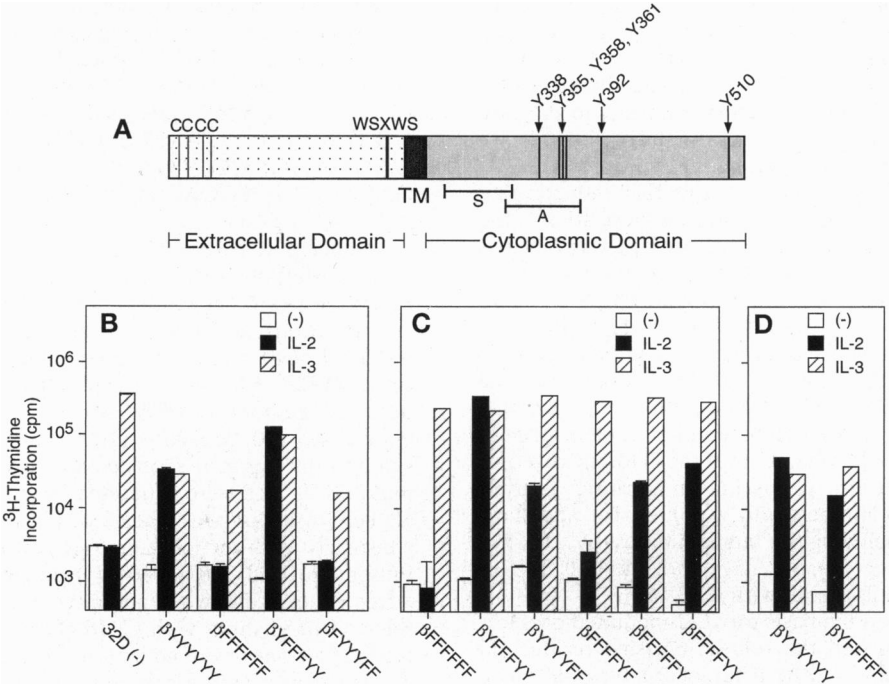

FIG. 1. Requirement of Y338, Y392, and Y510 for full IL-2-induced proliferation. (*A*) Schematic of IL-2Rβ, showing the four conserved cysteines and WSXWS motif in the extracellular domain typical of cytokine receptor superfamily members, the transmembrane (TM) domain, the regions deleted in the IL-2Rβ S-region and A-region mutants, and the positions of tyrosines in the cytoplasmic domain. All tyrosines but Y361 are conserved in murine IL-2Rβ. βYYYYYY and βFFFFFF, respectively, indicate IL-2Rβ constructs with tyrosines or phenylalanines at Y338, Y355, Y358, Y361, Y392, and Y510; similar nomenclature was used for other IL-2Rβ constructs. (*B–D*) 32D cells not transfected [32D(−)] or transfected with the indicated constructs were treated with medium alone (open bars), 10 nM IL-2 (solid bars), or 5% WEHI-CM as a source of IL-3 (an amount sufficient to induce maximal proliferation) (hatched bars), and thymidine incorporation was determined. At least three high-expressing clones were assayed for each construct, except for βYFFFFF, for which two clones were tested. Results of representative experiments are shown.

were quantitated (Bio-Rad protein assay kit). Binding reactions were performed with 5–10 μg of nuclear extract, 1 μg of poly(dI-dC) as nonspecific competitor, and 15,000 cpm of ^{32}P-labeled double-stranded oligonucleotide containing a γ-interferon-activated site (GAS) derived from the Fc_γ receptor I gene, a motif capable of binding IL-2-activated STAT proteins (30, 31). The reaction products were separated in 5% polyacrylamide gels in 0.5× TBE (1× is 100 mM Tris/100 mM boric acid/0.2 mM EDTA) and autoradiographed.

RESULTS

To determine whether tyrosines in the IL-2Rβ cytoplasmic domain (Fig. 1*A*) were required for IL-2-induced proliferation, we transfected wild-type or mutated forms of IL-2Rβ into IL-3-dependent 32D cells, which lack IL-2Rβ but can proliferate in response to IL-2 after IL-2Rβ is transfected and expressed on these cells (32, 33). Mutation of all six tyrosines in the IL-2Rβ cytoplasmic domain to phenylalanines abolished the proliferative response to IL-2 (compare βYYYYYY and βFFFFFF, Fig. 1 *B–D*; Table 1). Mutation of the three clustered tyrosines (Y355, Y358, and Y361) did not decrease thymidine incorporation (βYFFFYY; Fig. 1 *B* and *C*); in contrast, mutation of the other three tyrosines (Y338, Y392, and Y510) abolished IL-2 responsiveness (βFYYYFF; Fig. 1*B*). Thus, one or more of Y338, Y392, and Y510 contributed to IL-2-induced proliferation. Mutation of Y392 or Y510 (βYYYYFY and βYYYYYF) had little effect (data not shown); however, when both Y392 and Y510 were mutated (βYYYYFF), IL-2-induced proliferation was <10% of IL-3-induced proliferation (Fig. 1*C*). Y510 appeared to have a stronger effect on proliferation than did Y392, since cells expressing a construct that retained only Y510 (βFFFFFY) proliferated better than cells expressing a construct that retained only Y392 (βFFFFYF) (Fig. 1*C*). The fact that cells expressing βYYYYFF proliferated to some extent suggested that at least one of the first four tyrosines also could mediate proliferation. Indeed, the most membrane-proximal tyrosine, Y338, was important, since βFYYYFF did not mediate proliferation (Fig. 1*B*). βFFFFYY (Fig. 1*C*), βYYYYFF (Fig. 1*C*), and βYFFFFF (Fig. 1*D*) mediated suboptimal proliferation whereas βYFFFYY (Fig. 1*C*) mediated full proliferation, consistent with the model that Y338, Y392, and Y510 together coordinate two or more cooperating signaling pathways.

Having established the importance of Y338, Y392, and Y510, we examined whether these tyrosines were targets for phosphorylation. Immunoblotting with 4G10 anti-phosphotyrosine mAb showed basal tyrosine phosphorylation of IL-2Rβ when 32D cells were transfected with wild-type IL-2Rβ (data not shown) or with a construct containing Y338, Y392, and Y510 (βYFFFYY) (Fig. 2*A*, lane 3). This phosphorylation slightly increased after treatment with IL-2 (lane 4). As expected, no signal was detected with lysates from cells expressing a construct in which all tyrosines were mutated (βFFFFFF; Fig. 2*A*, lanes 1 and 2; Fig. 2*B*, lane 2). Moreover, of the constructs tested, only those retaining one or more of Y338, Y392, and Y510 were phosphorylated (βYYYYFF, βFFFFYY, βYFFFYY, and βYYYYYY; Fig. 2*B*, lanes 3–6), whereas a transfectant containing only Y355, Y358, and Y361 was not (βFYYYFF; Fig. 2*B*, lane 1).

Since Jak1 physically associates with IL-2Rβ (10, 17, 18), we investigated whether Jak1 contributed to the phosphorylation of Y338, Y392, and Y510. COS-7 cells were transfected with Jak1 and IL-2Rβ constructs and lysed, and IL-2Rβ was immunoprecipitated under conditions allowing coprecipitation of Jak1 (10). After *in vitro* kinase assays using unlabeled ATP, IL-2Rβ phosphorylation was assessed by immunoblotting with 4G10. Mutating all six tyrosines (βFFFFFF) or just Y338, Y392, and Y510 (βFYYYFF) eliminated IL-2Rβ phosphorylation (Fig. 2*C*, lanes 2 and 6). However, a construct additionally retaining Y338 (βYYYYFF) was phosphorylated when Jak1 was transfected (lane 5 vs. lane 4). Although mutants retaining only Y392 (βFFFFYF) or Y510 (βFFFFFY) were poorly phosphorylated (data not shown), a construct retaining both Y392 and Y510 was more heavily phosphorylated (lane 7), suggesting a possible synergistic effect in the phosphorylation of Y392 and Y510, or perhaps that these tyrosines are less susceptible to phosphatase activity when both are phosphorylated. Thus, in COS-7 cells, transfection of Jak1 enhanced the phosphorylation of Y338, Y392, and Y510.

IL-2 activates both the Ras (34–36) and Jak–STAT (10, 30, 31, 37–42) pathways. It was striking that Y338, Y392, and Y510 differentially coupled to these two pathways. Shc, an adaptor protein that can initiate activation of the Ras pathway (35), associates with IL-2Rβ (22) and is tyrosine-phosphorylated in response to IL-2 (22, 43). Using 32D transfectants, we found

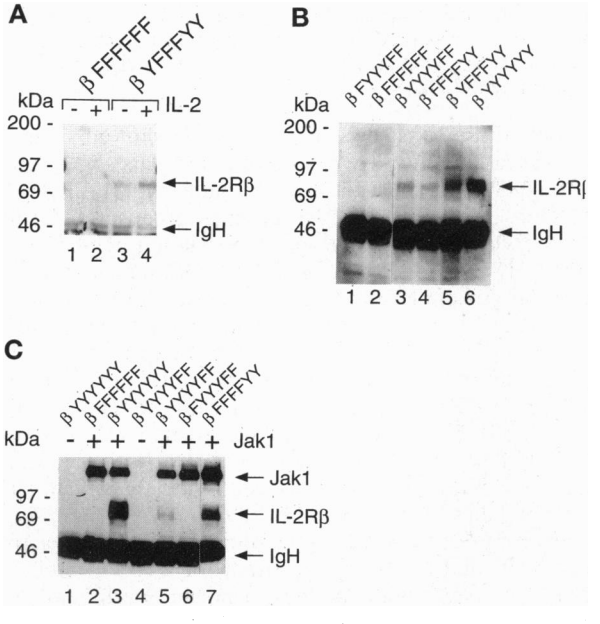

FIG. 2. Phosphorylation of Y338, Y392, and Y510 of IL-2Rβ. (*A*) Fifty million 32D cells transfected with βFFFFFF (lanes 1 and 2) or βYFFFYY (lanes 3 and 4) were incubated in the absence (lanes 1 and 3) or presence (lanes 2 and 4) of 2 nM IL-2 for 15 min at 37°C. Cells were lysed, immunoprecipitated with hMikβ1 anti-IL-2Rβ mAb, and immunoblotted with 4G10 and ECL. (*B*) 32D cells transfected with the indicated constructs were treated with 10 nM IL-2 for 15 min, lysed, immunoprecipitated with TU11 mAb against IL-2Rβ, and immunoblotted as in *A*. (*C*) COS-7 cells were cotransfected with the indicated IL-2Rβ construct and with an expression plasmid without insert (lanes 1 and 4) or with Jak1 insert (lanes 2, 3, 5, 6, and 7). After 48–72 hr, cells were lysed, clarified extracts were immunoprecipitated with hMikβ1, and *in vitro* kinase assays were performed, followed by immunoblotting with 4G10. Equivalent expression of transfected components was confirmed by immunoblotting with antisera to IL-2Rβ and Jak1.

Table 1. Summary of the IL-2-induced functional responses of 32D cells expressing wild-type or mutated forms of IL-2Rβ

IL-2Rβ mutant	Proliferation	IL-2Rβ phosphorylation	STAT activation	Shc phosphorylation
βYYYYYY	+++	+	+	+
βFFFFFF	–	–	–	–
βYFFFYY	+++	+	+	+
βFYYYFF	–	–	–	–
βFFFFYY	+	+	+	–
βYYYYFF	+	+	–	+

In the second column, +++ indicates maximum proliferation and + indicates suboptimal proliferation.

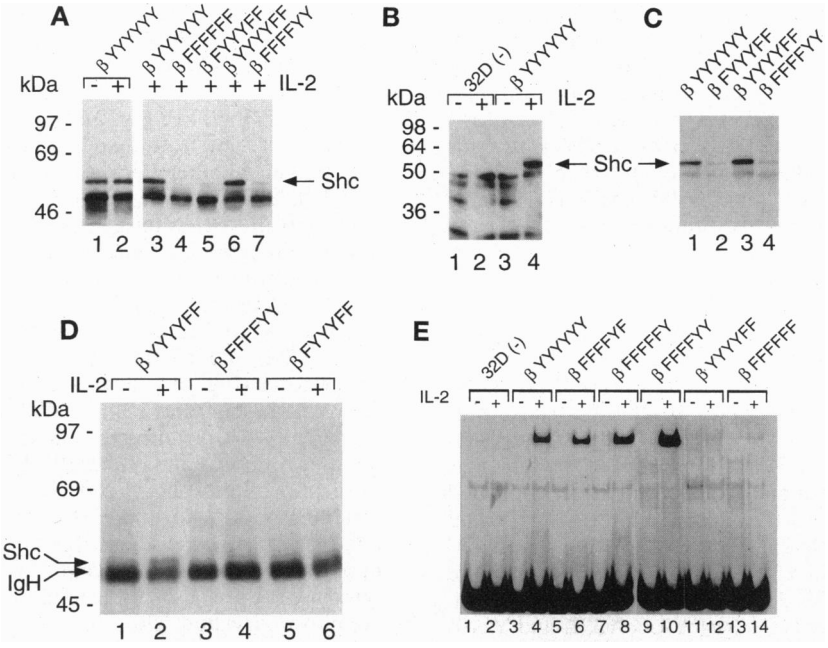

FIG. 3. Coupling of Y338 to the Shc pathway and Y392 and Y510 to the Jak–STAT pathway. (*A*) 32D cells transfected with the indicated IL-2Rβ constructs were lysed and immunoprecipitated with hMikβ1 (lanes 1 and 2) or TU11 (lanes 3–7) anti-IL-2Rβ mAb, and the immunoprecipitates were immunoblotted with anti-Shc. Cells expressing wild-type IL-2Rβ were analyzed both before and after stimulation with either 2 nM IL-2 (lanes 1 and 2) or 10 nM IL-2 (lanes 3–7) for 15 min. (*B*) Parental 32D cells [32D(−)] or cells expressing wild-type IL-2Rβ were not stimulated or were stimulated with 2 nM IL-2 for 15 min, followed by immunoprecipitation with 4G10 and immunoblotting with anti-Shc. (*C*) 32D cells expressing different IL-2Rβ mutants were stimulated with 2 nM IL-2 for 15 min, lysates were immunoprecipitated with 4G10, and immunoprecipitates were immunoblotted with anti-Shc. (*D*) 32D cells expressing different IL-2Rβ mutants were stimulated with 2 nM IL-2 for 15 min, lysates were immunoprecipitated with anti-Shc, and immunoprecipitates were immunoblotted with 4G10. Positions of tyrosine-phosphorylated Shc and immunoglobulin heavy chain (IgH) are indicated. (*E*) Wild-type 32D cells (lanes 1 and 2) or cells transfected with the indicated IL-2Rβ constructs (lanes 3–14) were washed, starved for growth factor, and then left untreated or treated with 2 nM IL-2 for 30 min at 37°C, as indicated. Total cell extracts were analyzed by electrophoretic mobility-shift assays using a ^{32}P-labeled γ-interferon-activated site (GAS) probe derived from the Fc_γ receptor I gene (30).

that only those constructs retaining Y338 showed significant Shc association (Fig. 3*A*, lanes 3 and 6 vs. lanes 4, 5, and 7). Although Shc was constitutively associated with IL-2Rβ in 32D cells (Fig. 3*A*, lanes 1 and 2), perhaps because of the basal phosphorylation of IL-2Rβ, phosphorylation of Shc was detected only after IL-2 stimulation (Fig. 3*B*, lane 4 vs. lane 3). The requirement of Y338 for IL-2-induced phosphorylation of Shc (Fig. 3*C*, lanes 1–4; Fig. 3*D*, lanes 1–6) may explain why Y338 is important for IL-2-induced proliferation.

IL-2 induces STAT activity in 32D cells transfected with wild-type IL-2Rβ but not in untransfected cells (Fig. 3*E*, lanes 1–4; ref. 30). Constructs containing either Y392 or Y510 or both could mediate IL-2-induced STAT DNA-binding activity (lanes 5–10; ref. 30), whereas constructs in which all six tyrosines (lanes 13 and 14) or just Y392 and Y510 (lanes 11 and 12) were mutated had markedly diminished binding activity. Thus, Y392 and Y510 are important for STAT protein activation. Consistent with findings in normal peripheral blood lymphocytes (30), an anti-Stat5 antibody could supershift the IL-2-induced complex in 32D cells transfected with IL-2Rβ (data not shown).

DISCUSSION

In this study, we have investigated the roles of tyrosine residues in the IL-2Rβ cytoplasmic domain for IL-2-induced proliferation. Our data suggest that Y338, Y392, and Y510 each contribute to a proliferative signal and together can mediate a full proliferative response (Fig. 1 *B* and *C*; Table 1). Y392 and Y510 mediate STAT protein activation, whereas Y338 mediates tyrosine phosphorylation of Shc (Table 1; Fig. 4).

The correlation between the large decreases in proliferation and STAT protein activation resulting from mutation of Y392 and Y510 (βYYYYFF; Figs. 1*C* and 3*D*) suggests that the Jak–STAT pathway is essential for maximal IL-2-induced proliferation. Interestingly, whereas an IL-2Rβ mutant retaining only Y392 (βFFFFYF) showed almost as potent STAT protein activation as one retaining only Y510 (βFFFFFY) (Fig. 3*E*), it mediated a weaker proliferative signal (Fig. 1*C*). This suggests either that a critical threshold concentration of activated STAT protein is required to mediate proliferation or perhaps that, as compared with Y392, Y510 may be involved in additional signaling pathway(s) critical for proliferation. The weak DNA-binding activity sometimes seen when both Y392 and Y510 (lanes 11 and 12) or all six tyrosines (lanes 13 and 14) were mutated raises the possibility of weaker secondary contact points between IL-2Rβ and STAT proteins or direct Jak–STAT interactions; however, the low (Fig. 1*C*; βYYYYFF) or absent (Fig. 1 *B* and *C*; βFFFFFF) proliferation of cells expressing these constructs emphasizes the importance of the Y392 and Y510 docking sites.

Recently, Fujii *et al.* (44) concluded that the region of IL-2Rβ containing Y392 and Y510 was not required for proliferation in BAF-B03 cells transfected with IL-2Rβ constructs. In fact, their data indicate a small but statistically significant decrease in proliferation when this region is deleted, whereas we reproducibly see a substantial decrease in proliferation in 32D cells when Y392 and Y510 are mutated and STAT protein activation is inhibited. The differences presumably are due to the different cell lines used. Nevertheless, even their data are consistent with Y392 and Y510 contributing to proliferation that is maximal when Y338 is also present. Interestingly, constitutive STAT protein activation has been shown to correlate with cellular transformation by human T-lymphotropic virus type I (45), c-*src* (46), and v-*abl*

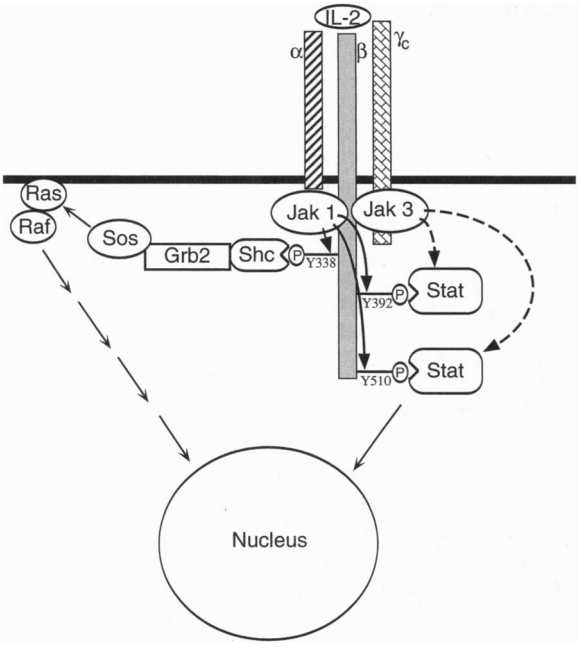

FIG. 4. Schematic of IL-2Rβ coupling to the Shc and Jak–STAT pathways. Jak1 associates with IL-2Rβ, and we hypothesize that Jak1 may be directly responsible for the phosphorylation of Y338, Y392, and Y510 (bold solid arrows). Shc binds to Y338 and couples to Grb2 and Sos, thereby activating Ras (22, 35); STAT proteins dock at Y392 and Y510. Jak3 primarily associates with γ_c (10, 17, 18) but also interacts with IL-2Rβ (10). It is unclear which kinase phosphorylates the STAT proteins; we hypothesize that Jak3 plays a role (48) (bold dashed arrows; see text). Not shown are Y355, Y358, and Y361, since their roles, if any, in contributing to IL-2-induced proliferation remain unclear. Lck and Syk have been reported to associate with IL-2Rβ (20); they are omitted from this schematic to focus on the pathways we demonstrate to be involved in IL-2-induced proliferation.

(47), consistent with the possibility that STAT proteins play a role in proliferation.

Lck and other Src family kinases also have been reported to associate with IL-2Rβ (19, 20). Cells transfected with an IL-2Rβ mutant lacking the "A region" (residues 313–382) cannot activate Lck but they still proliferate (19), albeit less than cells expressing wild-type IL-2Rβ (20). That these cells can proliferate may be explained by the retention of Y392 and Y510 in the A-region mutant, allowing for STAT protein activation. That their proliferation is diminished may be due to the absence of Y338, the IL-2Rβ docking site for Shc rather than due to a lack of Lck activation. *In vitro* kinase experiments using a COS overexpression system have suggested that Y355 and Y358 are substrates for Lck (19); however, in 32D cells, which lack Lck but express other Src family kinases, a construct retaining only Y355, Y358, and Y361 was not phosphorylated and a construct lacking these tyrosines could mediate proliferation as well as wild-type IL-2Rβ. Additional studies are needed to clarify whether Src family kinases play a role in mediating IL-2-induced proliferation and whether Y355, Y358, and Y361 have physiologically important roles.

Phosphorylation of Y338, Y392, and Y510 was greatly facilitated in COS-7 cells when Jak1 was transfected, consistent with Jak1 being the kinase that phosphorylates these tyrosines, but the role of Jak1 in phosphorylating some or all of these IL-2Rβ residues in T cells requires additional investigation. Nevertheless, the Jak1-dependent phosphorylation of Y338 suggests that the role of Jak1 may not be limited to the STAT pathway. Consistent with this possibility, activation of the Ras pathway requires both the IL-2Rβ S (residues 267–323) and A regions (34). This may be explained by the observations that Jak1, which is required for the phosphorylation of Y338, associates with the S region (18) and that Y338, which we show is the docking site for Shc, is in the A region. Although the catalytic activity of Jak1 is increased in response to IL-2, basal Jak1 activity is sometimes detected (10). This raises the possibility that this kinase might physiologically mediate a basal level of IL-2Rβ phosphorylation. If this were to occur, signaling molecules could bind to IL-2Rβ via SH2 domains, facilitating the rapid triggering of signals once γ_c brings Jak3 into closer proximity as a result of IL-2 binding. We hypothesize that Jak3 may phosphorylate STAT proteins after they dock (Fig. 4), a possibility consistent with the absence of IL-4-induced tyrosine phosphorylation of Stat6 in Jak3-deficient cells (47).

This study correlates the effect on cellular proliferation with mutation of specific tyrosine residues in IL-2Rβ and defines at least some of the signaling pathways dependent on these tyrosines. The ability to selectively mutate Y338 or Y392 plus Y510 should help to clarify the roles of the Shc-coupled and Jak–STAT pathways, and perhaps of other signaling molecules that can associate with IL-2 receptor chains. By analysis of cells transfected with IL-2Rβ constructs in which specific IL-2Rβ tyrosines were mutated, we have also provided direct evidence for the synergistic contributions of distinct signaling pathways to IL-2 mediated proliferation and found that Jak kinases may have actions beyond mediating the activation of STAT proteins.

Note Added in Proof. Another study has also demonstrated the requirement for IL-2RB tyrosines Y392 and Y510 for proliferation (49).

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