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Intermediate Affinity Interleukin-2 Receptor Mediates Survival via a Phosphatidylinositol 3-Kinase-dependent Pathway*

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Peripheral blood T lymphocytes require two signals to enter and progress along the cell cycle from their natural quiescent state. The first activation signal is provided by the stimulation through the T cell receptor, which induces the synthesis of cyclins and the expression of the high affinity interleukin-2 receptor. The second signal, required to enter the S phase, is generated upon binding of interleukin-2 to the high affinity $\alpha\beta\gamma$ interleukin-2 receptor. However, resting T cells already express intermediate affinity $\beta\gamma$ interleukin-2 receptors. As shown here, T cell stimulation through intermediate affinity receptors is capable of inducing cell rescue from the apoptosis suffered in the absence of stimulation. Characterization of the signaling pathways utilized by $\beta\gamma$ interleukin-2 receptors in resting T cells, indicated that pp56^{lck}, but not Jak1 or Jak3, is activated upon receptor triggering. Compelling evidence is presented indicating that phosphatidylinositol 3-kinase associates with the intermediate affinity interleukin-2 receptor and is activated upon interleukin-2 addition. Bcl-x_L gene was also found to be induced upon $\beta\gamma$ interleukin-2 receptor stimulation. Finally, pharmacological inhibition of phosphatidylinositol 3-kinase blocked both interleukin-2-mediated bcl-x_L induction and cell survival. We conclude that $\beta\gamma$ interleukin-2 receptor mediates T-cell survival *via* a phosphatidylinositol 3-kinase-dependent pathway, possibly involving pp56^{lck} and bcl-x_L as upstream and downstream effectors, respectively.

Interleukin-2 (IL-2)[1] plays a major role in T cell biology, as activated T cells depend on this cytokine for their proliferation and effector functions. IL-2 effects are mediated through interaction with a specific transmembrane receptor (IL-2R) composed of three different molecules, α, β, and γ (1). The β and γ chains are constitutively expressed in T lymphocytes and bind IL-2 with intermediate affinity. The α chain, in contrast, is only expressed upon T cell activation and, together with the β and γ chains, forms the high affinity IL-2 receptor (1). It has been shown that heterodimerization of the IL-2R β and γ chains is required for IL-2-induced cellular proliferation (2, 3). The IL-2R has no intrinsic enzymatic activity; therefore, its ability to transmit intracellular signals relies on its association with signaling molecules. IL-2R ligation triggers the activation of several tyrosine kinases, including src-kinases (4, 5), Jak kinases (6–8), and pp72^{syk} (9). While Jak kinases are essential for IL-2-induced proliferation, the involvement of src-kinases on T cell proliferation following IL-2R binding is not so clear (4). IL-2 also regulates phosphatidylinositol 3-kinase (PI3K) activation (10–12) and induces p21^{ras} (13), c-*raf* (14), and pp70S6K (15). After the initial phase mediated by these set of cytoplasmic molecules, several transcription factors including Stat3, Stat5, c-*myc*, c-*fos*, and c-*jun* (16, 17) are also induced that putatively control gene expression leading to cell division. All these signaling cascades have been described to be triggered by high affinity IL-2 receptors and are involved in inducing cell proliferation. However, it has been recently shown that the intermediate affinity IL-2 receptor mediates protection against radiation-induced cell death (18, 19). We confirm here that although unable to promote cell division, the intermediate affinity IL-2R induced cell survival. In fact, IL-2 treatment rescued resting T cells from the spontaneous cell death observed when these cells are cultured without stimulus. We subsequently analyzed the signaling pathways triggered by intermediate affinity IL-2R in resting T cells and found that pp56^{lck} is induced upon $\beta\gamma$ IL-2R stimulation. In addition, data is presented indicating that the activation of PI3K is required for IL-2-mediated cell survival and that bcl-x_L behaves as a downstream effector of this enzyme.

EXPERIMENTAL PROCEDURES

Antibodies, Reagents, and Cells—Rapamycin was kindly donated by Dr. J. Luengo (SmithKline Beecham); LY294002 was purchased from Biomol (Plymouth Meeting, PA). The following polyclonal antisera were used: rabbit anti-N-terminal pp56^{lck}, rabbit anti-Jak3 (kindly donated by Dr. J. O'Shea, Frederick Cancer Research and Development Center, MD), rabbit anti-Jak1 (obtained from UBI, Lake Placid, NY), and rabbit anti-$\alpha\beta$-p85 (kindly donated by Dr. B. Schaffhausen, Tufts University School of Medicine, Boston, MA). The following monoclonal Abs were used: 4G10 anti-phosphotyrosine (donated by Dr. B. Drucker, D.F.C.I., Harvard Medical School, Boston, MA) and SPVT3b anti-human CD3 (kindly donated by Dr. F. Sanchez-Madrid, S. Immunología, H. Princesa, Madrid, Spain). Horseradish peroxidase-conjugated Abs and chemiluminiscence developing kit were from Amersham Corp. (UK). Reagents for cell cycle analysis were from Coulter Corp. (Miami, FL). Peripheral blood lymphocytes (PBL) were isolated from healthy donor buffy coats as described (20). Cells were grown in RPMI containing 10% fetal calf serum, 2 mM L-glutamine, 10 mM Hepes, pH 8, 50 units/ml penicillin, and 50 units/ml streptomycin (RPMIc) at 37 °C, 5% CO_2, in a humid atmosphere.

Cellular Activation—For cell cycle analysis, PBL were seeded at 10^6 cell/ml and cultured for either 24 or 72 h in RPMIc, in the presence or absence of recombinant IL-2 (kindly donated by Hoffman La Roche, Switzerland). Activated T cells were prepared by culturing purified T

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[1] The abbreviations used are: IL-2, interleukin-2; IL-2R, interleukin-2 receptor; TCR, T cell receptor; PI3K, phosphatidylinositol 3-kinase; Abs, antibodies; PBL, peripheral blood lymphocytes; PAGE, polyacrylamide gel electrophoresis.

cells in wells coated with SPVT3b, anti-human CD3 (30 ng/cm^2) during 72 h. For experiments using LY294002, cells were preincubated for 1.5 h with this inhibitor prior to incubation with 100 units/ml recombinant IL-2. After induction, cells were collected, washed in PBS, permeabilized, and stained with propidium iodide using a DNA-prep kit (Coulter). The percentage of subdiploid DNA-containing cells was determined by flow cytometry (Coulter).

For DNA synthesis analysis, cells were cultured for 72 h in 96-well plates with or without recombinant IL-2 at the indicated doses or in 96-well plates previously coated with 10 ng/well of the SPVT3b mAb. Then, 1 μCi/well [^{3}H]thymidine (2 Ci/mmol, Amersham) was added for the last 24 h of culture, and [^{3}H]thymidine incorporation was estimated by scintillation counting, as described previously (20).

For biochemical analysis, freshly isolated PBL or T cells activated during three days (see above) were washed, resuspended in RPMI with 1% bovine serum albumin at 10^7 cells/ml, and incubated for 1 h at 37 °C. Cells were subsequently washed and resuspended in RPMI at 10^7 cells/ml, and aliquots of 5×10^6 cells were incubated with 500 units/ml IL-2 for the indicated periods of time before lysis.

Immunoprecipitations and Kinase Assays—Cells were lysed in Triton X-100 lysis buffer (50 mM HEPES, pH 8.0, 150 mM NaCl, and 1% Triton X-100 with protease inhibitors) as described (21). Cleared lysates were used for Western blot analysis or immunoprecipitated with the appropriate Abs as described (21). For pp56^{lck}, Jak1, and Jak3 assays, cellular extracts were prepared in radioimmune precipitation buffer (22). pp56^{lck} *in vitro* kinase assays in immunoprecipitates were performed, as described previously (21), using enolase as exogenous substrate. The PI3K *in vitro* kinase assay was performed as described elsewhere (11), using PI as exogenous substrate. Phospholipids were resolved in thin layer chromatography (Silica Gel 60, Merck, Germany) and developed in chloroform:methanol:ammonia (9:7:2 v/v). Radioactive products were visualized by autoradiography.

RESULTS

IL-2 Promotes T Cell Survival through Intermediate Affinity IL-2 Receptors—Fresh *ex vivo* isolated resting T cells undergo apoptotic cell death upon *in vitro* culture in the absence of stimulation (Fig. 1*A*). This apoptotic process was significant at 24 h and affecting more than 50% of the cells at 72 h (Fig. 1*A*). Addition of IL-2 to the cultures prevented apoptotic cell death in a dose-dependent manner (Fig. 1*A*). In contrast, only a small increase in the percentage of cells in S+G$_2$/M, indicative of cell division, was observed upon IL-2 addition (from 2% in medium alone to 12.6% with the higher IL-2 dose, Fig. 1*A*). To further demonstrate that prevention of cell death in resting T cells is independent of DNA synthesis, we tested whether or not the same IL-2 doses that promote cell survival (see Fig. 1*A*) were able to trigger significant DNA synthesis. The highest IL-2 dose used (100 units/ml) induced only an [^{3}H]thymidine incorporation of approximately 10% of the value obtained using anti-TCR antibodies (in the presence or absence of exogenous IL-2, Fig. 1*B*). In all the samples analyzed, a small percentage of the population ranging from 5 to 8%, depending on the donor, are already activated T cells, expressing the IL-2R α chain (data not shown). Therefore, proliferation observed in the presence of IL-2 is likely to correspond to these cells. These results indicate that, although lacking the α chain, intermediate affinity IL-2Rs are capable of delivering a signal that prevents the apoptotic cell death of resting T cells cultured in the absence of TCR stimulation.

pp56^{lck} Is Activated upon IL-2 Binding to $\beta\gamma$ IL-2R—Previous reports have been dedicated to characterizing the signaling pathways triggered by high affinity IL-2R (for a review, see Ref. 23). These studies have been carried out either in T cells stimulated *via* the TCR or in cell lines (CTLL2 or transfected BaF/3 cells). However, all these cells differ from resting T cells in the expression of several signaling molecules such as c-*raf*, c-*myc*, and others (14, 24) (our data not shown). To identify the signaling pathways triggered by the intermediate affinity IL-2R in resting T cells and implicated in cell survival, we have carried out a systematic analysis of which of the previously

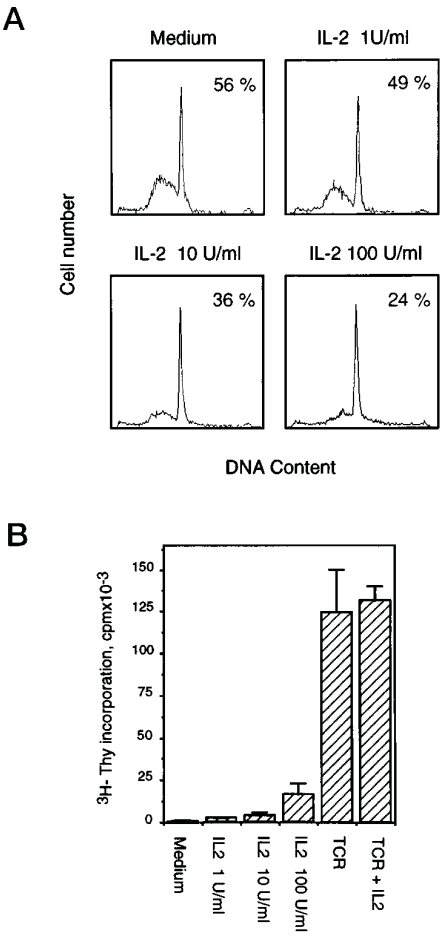

FIG. 1. **IL-2 promotes survival in resting T cells.** *A*, cells were cultured in medium alone or in medium containing the indicated doses of recombinant IL-2. After 72 h in culture, cells were collected and stained with propidium iodide, and cell cycle distribution was determined by flow cytometry. The percentage of cells with sub-G1 DNA content (apoptotic cells) is shown in the *upper right* corner. Cells in G_0/G_1 represents 40.6% in cells incubated with medium alone, 46.5% for cells treated with 1 unit/ml IL-2, 57.9% for 10 units/ml IL-2, and 61.9% in cells treated with 100 units/ml IL-2. The percentage of cells in S + G_2 + M is 2% for cells incubated in medium alone, 3% in the presence of 1 unit/ml IL-2, 4.9% for 10 units/ml IL-2, and 12.6% for cells incubated with 100 units/ml IL-2. A representative experiment of five performed with similar results is shown. *B*, 2×10^5 resting T cells were incubated with IL-2 (different doses as indicated) or anti-TCR complex Abs (in the presence or absence of 100 units/ml IL-2 as indicated) for 72 h. [^{3}H]Thymidine incorporation was estimated in the different samples as described under "Experimental Procedures."

described high affinity IL-2R-induced events are observed upon ligation of the intermediate affinity IL-2R.

We first compared the induction of tyrosine kinases in resting T cells (expressing intermediate affinity IL-2R) and in previously activated T cells (expressing high affinity IL-2R). To this end, tyrosine phosphorylation of cellular proteins was analyzed in anti-phosphotyrosine Western blot. As shown in Fig. 2, activation of tyrosine kinases was essentially different in the two cell types analyzed. While in activated cells, the activation of tyrosine kinases was faster and induced the phosphorylation of two major bands of 90 (25) and 130 kDa; in resting T cells, the activation was slower and induced the phosphorylation of two bands of approximately 56 and 92 kDa. These results suggest that stimulation of tyrosine kinases triggered by the intermediate affinity IL-2R differs from that observed upon high affinity IL-2R stimulation.

Considering that the anti-phosphotyrosine Western blot does not address the activation of a particular tyrosine kinase, we

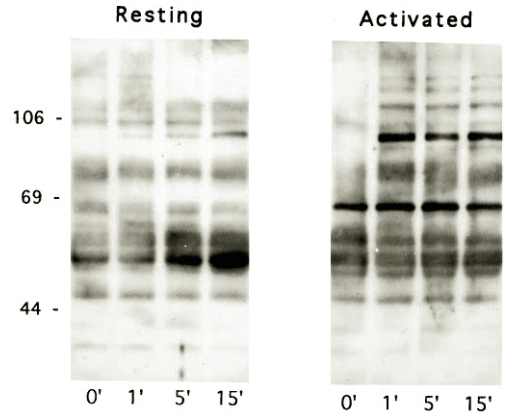

FIG. 2. **Differential tyrosine kinase triggering upon IL-2R stimulation in resting and activated cells.** Induction of tyrosine phosphorylation upon IL-2R ligation in resting (*left panel*) or activated (*right panel*) T cells. Resting or activated T cells were incubated with 500 units/ml IL-2 for the indicated periods of time. Upon induction, cells were lysed, lysates were normalized for protein content, and 300 μg of protein were subjected to immunoprecipitation using anti-phosphotyrosine Abs. Immunoprecipitated proteins were resolved by 10% SDS-PAGE. Gels were transferred onto nitrocellulose, and Western blot was performed using anti-phosphotyrosine Abs.

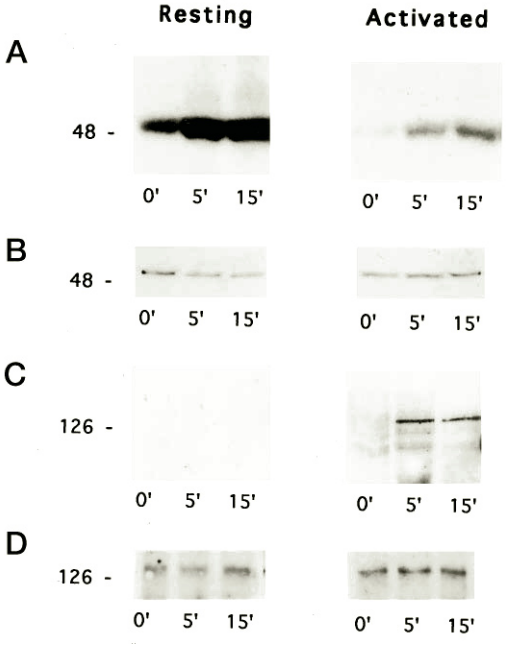

FIG. 3. **pp56^{lck}but not Jak1 is induced upon IL-2R stimulation in resting T cells.** *A*, *in vitro* kinase activity of pp56^{lck} immunoprecipitated from resting (*left panel*) or activated (*right panel*) T cells upon IL-2R stimulation. Cells were incubated with 500 units/ml IL-2 for the indicated periods of time and lysed, and cellular lysates were normalized for protein content. pp56^{lck} was immunoprecipitated from the different cell extracts (see above), and samples were subjected to *in vitro* kinase assay in the presence of 10 μg of acid-denatured enolase, as detailed under "Experimental Procedures." The same exposure time for both cells types is shown. *B*, amount of pp56^{lck} present in samples equal to those analyzed in (*A*) was checked by Western blot using anti-pp56^{lck}. *C*, tyrosine phosphorylation of Jak1 upon IL-2 addition in cells treated as in (*A*). Jak1 protein was immunoprecipitated from an amount of lysate containing 600 μg of protein, and immunoprecipitates were analyzed by Western blot using anti-phosphotyrosine Abs. *D*, Jak1 content in immunoprecipitates prepared as in (*C*) was analyzed by Western blot using anti-Jak1 Abs.

subsequently examined individually the activation of pp56^{lck}, Jak1, and Jak3, previously implicated in high affinity IL-2R signaling (4–9). pp56^{lck} expression levels in resting or activated T cells was analyzed by Western blotting comparing the amount of pp56^{lck} present in similar volumes of both lysates (normalized for protein concentration). This analysis indicated that pp56^{lck} is three-fold more abundant in lysates of activated T cells (data not shown). 150 μg of protein from lysates of resting cells and 50 μg of protein from lysates of activated T cells were used to compare the specific enzymatic activity of pp56^{lck} in both cell types. Using this amount of cellular protein, we immunoprecipitated similar amounts of pp56^{lck} from the two cell types (Fig. 3*B*). Under these conditions, pp56^{lck} from resting cells was consistently found to be much more active than that obtained from activated T cells (Fig. 3*A*). Interestingly, pp56^{lck} kinase activity increased upon IL-2 treatment not only in activated T cells (4) (Fig. 3*A*) but also very significantly in IL-2-treated resting T cells (Fig. 3*A*), indicating that, as was the case with high affinity IL-2R, intermediate affinity IL-2R triggers pp56^{lck} activation.

We then analyzed the role of the constitutively expressed Jak1 tyrosine kinase (26). We tested whether the intermediate affinity IL-2R expressed in resting T cells would induce Jak1 phosphorylation, as reported for the high affinity IL-2R (6, 7). As shown in Fig. 3*C*, Jak1 was tyrosine phosphorylated upon IL-2 treatment in activated T cells but was not modified in IL-2-treated resting T cells, even though both cells contain similar amounts of Jak1 (Fig. 3D). With regard to Jak3 kinase, it has been described that resting T cells do not virtually express this enzyme (26). To confirm these data, we have compared the amount of Jak3 present in resting and in activated T cells, which require Jak3 to proliferate in response to IL-2 (27, 28). As shown in Fig. 4*A*, while resting T cells express similar amounts of p85-PI3K than activated cells, as much as 2×10^8 resting T cells are required to slightly detect Jak3 expression under overexposure conditions. Therefore, resting T cells only express less than 5% of the amount of Jak3 expressed by activated T cells. This Jak3 most likely corresponds to that expressed by the 5–8% of preactivated cells present in the pool of resting T cells (see above). Since in resting T cells IL-2 drives survival of approximately 50% of the population, the low amounts of Jak3 expressed in these cells do not prove, but strongly suggest, that Jak3 is not involved in the early signals triggered by $\beta\gamma$ IL-2R in resting T cells. Finally, to make sure that Jak3 does not participate in $\beta\gamma$ IL-2R-mediated survival at a later step of the activation process, we also analyzed whether or not IL-2 could trigger its expression in resting cells. As shown in Fig. 4*B*, no induction of Jak3 expression was observed in resting T cells upon IL-2 treatment (analyzed at 24 and 48 h post-induction). In contrast, Jak3 expression was induced by TCR stimulation in resting T cells at 24 and 48 h (Fig. 4*B*) (6). As expected, when TCR-stimulated T cells were induced via high affinity IL-2R, Jak3 underwent the described tyrosine phosphorylation (data not shown) (6, 7). Thus, Jak3 does not seem to be involved in IL-2-mediated cell survival. The lack of Jak3 involvement in $\beta\gamma$ IL-2R signaling in resting T cells is consistent with lack of Jak1 phosphorylation (Fig. 3*C*) since both kinases are thought to be regulated by transphosphorylation (27). In conclusion, of the tyrosine kinases analyzed, only pp56^{lck}, but not Jak1 or Jak3, seems to be induced by $\beta\gamma$ intermediate affinity IL-2R in resting T cells.

IL-2-mediated Cell Survival Requires PI3K Activity—pp56^{lck} has been shown to regulate PI3K activation (29, 30). The fact that $\beta\gamma$ IL-2R triggered pp56^{lck} induction (Fig. 3*A*) and the observation that PI3K mediates cell survival triggered by neural growth factor receptor (31) and insulin growth factor receptor (32) prompted us to investigate whether or not PI3K activation could mediate the survival of resting T cells induced through $\beta\gamma$ IL-2R. We first tested whether incubation of resting T cells with IL-2 would induce the early association/activation

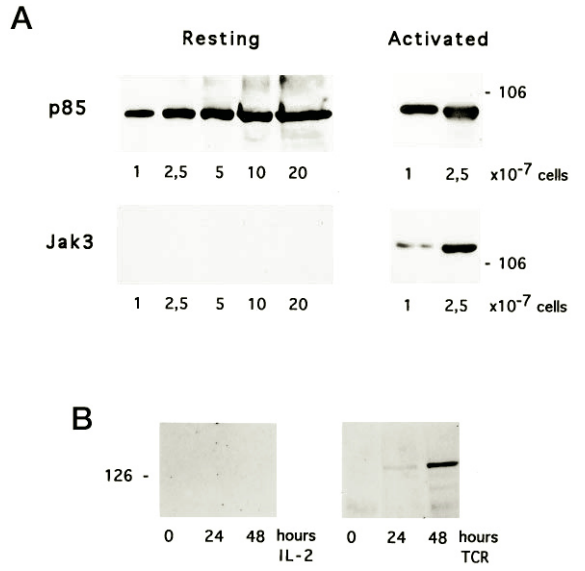

FIG. 4. **Jak3 is barely expressed in resting T lymphocytes.** *A*, p85-PI3K (*upper panel*) or Jak3 (*lower panel*) were immunoprecipitated from cellular lysates corresponding to different numbers of resting or activated cells (indicated) Immunoprecipitates were analyzed by Western blot using the appropiate Abs. *B*, induction of Jak3 expression upon ligation of either IL-2R or TCR. Human resting T cells (15×10^6) were incubated for 0, 24, or 48 h in the presence of 100 units/ml recombinant IL-2 or in plates previously coated with anti-CD3 antibodies (30 ng/cm^2). After induction, cells were lysed, and an amount of lysate containing 600 μg of protein was used to immunoprecipitate Jak 3. Immunoprecipitates were resolved by SDS-PAGE and analyzed by Western blot using anti-Jak3 Abs.

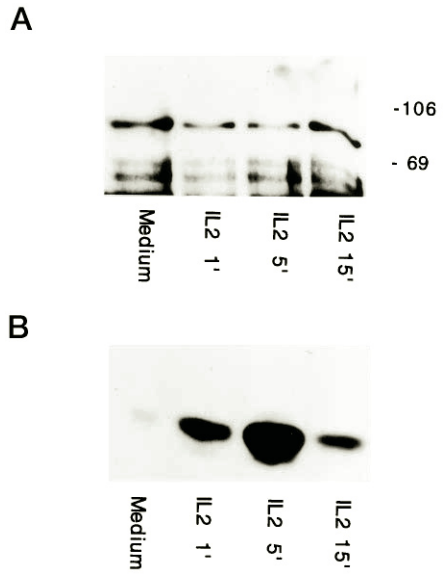

FIG. 5. **IL-2-induced survival correlates with the induction of PI3K activity.** *A*, association of PI3K to the IL-2R β chain. Cells (15×10^6) were treated with 500 units/ml recombinant IL-2 for the indicated periods of time. IL-2R β chain was immunoprecipitated from an amount of lysate containing 450 μg of protein. Immunoprecipitates were resolved by 10% SDS-PAGE and analyzed by Western blot using anti-p85-PI3K Abs. *B*, PBL (15×10^6) were incubated as in (*A*), and PI3K activity was determined *in vitro* on immunoprecipitates prepared using anti-IL-2Rβ Abs as detailed under "Experimental Procedures."

of PI3K with the IL-2R. The presence of PI3K on IL-2Rβ immunoprecipitates was analyzed by Western blot using an antibody against p85, the regulatory subunit of PI3K (33). As shown in Fig. 5*A*, p85-PI3K was associated with the IL-2Rβ chain, even before IL-2 addition to the cells, and became early and transiently activated upon IL-2 binding to the $\beta\gamma$ IL-2R (Fig. 5*B*). Therefore, stimulation of intermediate affinity IL-2R mediates the activation of its associated PI3K.

To analyze whether or not PI3K activation in response to $\beta\gamma$ IL-2R ligation was related to the IL-2-induced survival effect in resting T cells, cells were incubated with IL-2 in the presence of PI3K inhibitors. Given that wortmannin has been shown to inhibit other enzymes besides PI3K (34, 35), we utilized LY294002 (36). In contrast with the PI3K pool associated to $\beta\gamma$ IL-2R (Fig. 5*B*), the total cellular PI3K present a steady-state kinase activity as evaluated by an *in vitro* lipid kinase assay in anti-p85-PI3K immunoprecipitates (Fig. 6*A*). A slight increase in PI3K activity was observed upon IL-2 treatment, consistently with the small percentage of total PI3K associated with IL-2R. Under these experimental conditions, high LY294002 concentrations blocked virtually all cellular PI3K activity (Fig. 6*A*). Inhibition of PI3K activity by LY294002 was similarly observed in the presence or absence of IL-2 (not shown). We subsequently tested the effect of LY294002 for IL-2-mediated T cell survival. In the absence of IL-2, LY294002 induced the apoptotic cell death of approximately 10% of the population. This 10% may represent a toxic effect or reflect, as other authors propose (31), that basal PI3K activity is required for cellular survival (Fig. 6*B*). In the presence of 100 units/ml IL-2, a dose-response inhibition by LY294002 of IL-2-mediated survival was observed (a representative experiment is shown in Fig. 6*B*). In fact, in the absence of any exogenous additives, the percentage of cell death was 40%. In the presence of 100 units/ml IL-2, this number decreases to 15%, but incubation with 100 units/ml IL-2 and 50 μM LY294002 induces an increase in cell death from 15% to 45%. This result demonstrates the ability of LY294002 to block IL-2-induced survival beyond its putative toxic effect, affecting only up to 10% of the population. Representation of residual PI3K activity (at 24 h), in comparison with IL-2-induced survival upon LY294002, shows a clear correlation between both parameters (Fig. 6*C*). While 5 μM LY294002 only partially blocks PI3-kinase activity and IL-2-induced survival, 50 μM is able to block almost totally IL-2-induced survival and PI3K activity. At the intermediate doses, we consistently found in different experiments a partial effect in both LY294002 ability to block IL-2-induced-cell survival and PI3K activity. Therefore, abrogation of PI3K activity and inhibition of IL-2-induced cell survival are observed in parallel, indicating that intermediate affinity IL-2R mediate cell survival via a PI3K-dependent pathway.

Bcl-x_L Is a Downstream Effector of PI3K—In an attempt to characterize putative downstream effectors of PI3K in our system, we first considered pp70S6K. This enzyme has been shown to be necessary for S phase transition and is known to be blocked by PI3K inhibitors (37). We analyzed its putative involvement in $\beta\gamma$ IL-2R-mediated survival by using a pp70S6K inhibitor, rapamycin (15). Under conditions of pharmacological inhibition of pp70S6K that block 60% of IL-2-induced cell division, rapamycin did not abrogate IL-2-induced survival (Fig. 7), suggesting that pp70S6K does not participate in cell survival. Another interpretation of this result is that more pp70S6K activity is required for cell proliferation than for cell survival. However, data from Yao and Cooper (38), as well as other reports showing PI3K-mediated cellular responses independent of pp70S6K (39, 40), support that, most likely, this enzyme is not involved in $\beta\gamma$ IL-2R-mediated survival but is required for cell cycle progression.

Bcl-2-related proteins have also been found to be regulated by $\alpha\beta\gamma$ IL-2R stimulation (41–43). These genes have been involved as survival mediators (44) and related to PI3K activation in other cellular systems (45). The possible involvement of these proteins in intermediate affinity IL-2R-mediated cell

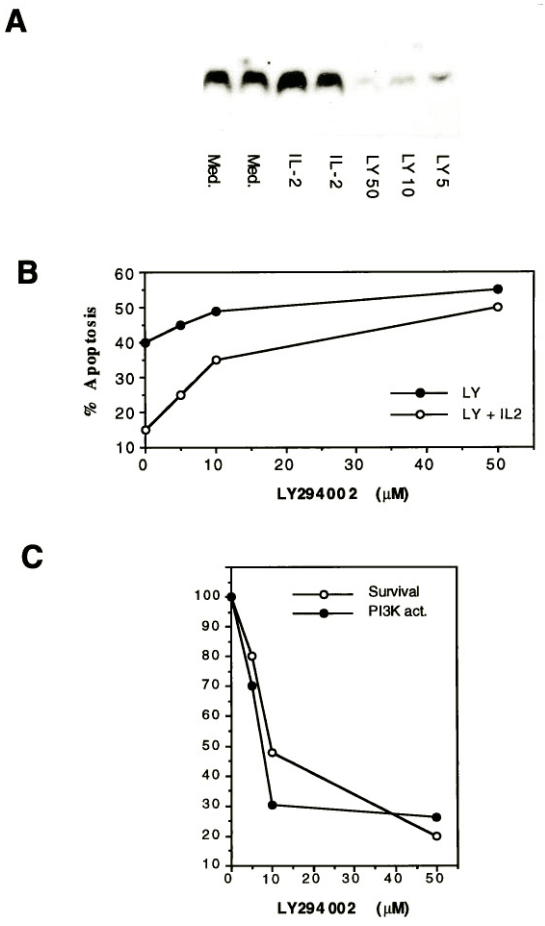

FIG. 6. **IL-2 mediates survival via a PI3K-dependent pathway.** *A*, cells were preincubated for 1.5 h with LY294002 (10–50 μM). After this incubation period, 100 units/ml recombinant IL-2 was added and maintained for 3 h before cells were harvested and lysed. 100 μg of total cellular protein was then immunoprecipitated using anti-p85-PI3K Ab, and *in vitro* kinase activity was estimated as described under "Experimental Procedures." *B*, LY294002 blocked IL-2-mediated survival in a dose-dependent manner. Cells were preincubated with LY294002 for 1.5 h and additionally incubated in the presence or in the absence of 100 units/ml recombinant IL-2 for 24 h. After this incubation period, cell cycle was analyzed. The percentage of cells with sub-G_1 DNA content (apoptotic cells) is represented. A representative experiment of five performed with similar results is shown. *C*, correlation between IL-2-induced survival and PI3K activity. The amount of cellular PI3K activity (*PI3K act.*) present in anti-p85-PI3K immunoprecipitates (*filled circles*) obtained for the different samples 24 h after induction (experiment represented in *B*) was estimated upon *in vitro* lipid kinase assay by scintillation counting of the band corresponding to phosphatidyl 3-phosphoinositol. Maximal PI3K activity (100%) was considered to be the value of counts/minute obtained in the absence of LY29004. The percentage of IL-2-mediated survival (*open circles*) was referred to the maximal cell survival observed when the cells were incubated with IL-2 (100 units/ml) in the absence of LY29004.

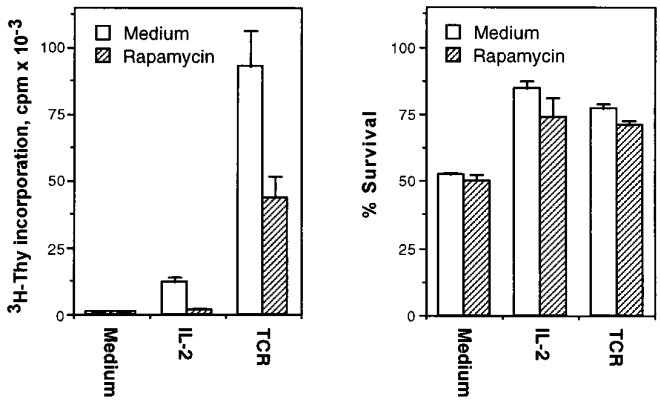

FIG. 7. **IL-2-mediated survival is independent of pp70S6K.** Cells were preincubated with 1 nM rapamycin for 30 min before adding 100 units/ml recombinant IL-2 or transferring cells to plates previously coated with anti-CD3 antibodies. 72 h later, the rate of DNA synthesis (*left panel*) or apoptotic cell death (*right panel*) was determined as described under "Experimental Procedures." Percentage of survival was calculated subtracting to one hundred the percentage of apoptotic cell death.

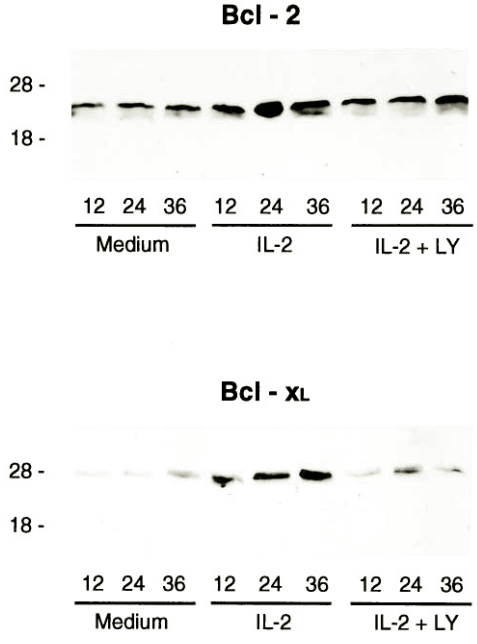

FIG. 8. **Bcl-X_L is induced in response to IL-2 treatment in resting T cells.** Cells (10^7) were incubated for 12, 24, or 36 h in medium alone or in medium containing either 100 units/ml IL-2 alone or 100 units/ml IL-2 plus 50 μM LY290004. After induction, cells were lysed, and an amount of cellular lysate containing 100 μg of protein was resolved by SDS-PAGE and analyzed by Western blot using either anti-bcl-2 Abs (*upper panel*) or anti-bcl-x_L Abs (*lower panel*).

survival, was therefore analyzed. A marginal increase in bcl-2 protein levels was observed in IL-2-treated cells (Fig. 8), making unlikely a significant involvement of this protein in IL-2-mediated survival. Another member of this family, bcl-x_L, has also been shown to negatively regulate apoptotic processes (46). Analysis of bcl-x_L expression by Western blot in IL-2-treated resting cells indicated that this protein is significantly induced upon IL-2 addition (Fig. 8). Moreover, while bcl-2 expression was poorly affected, bcl-x_L up-regulation was abrogated in the presence of the PI3K inhibitor LY294002 (Fig. 8). Thus, bcl-x_L behaves as a putative downstream effector of PI3K. The expression of bax was also analyzed and found to be not significantly modulated in response to IL-2 (not shown). In conclusion, while bcl-2 may play a partial role in mediating $\beta\gamma$ IL-2R-induced survival, bcl-x_L is most likely an effector of this cellular response.

DISCUSSION

The results presented in this report indicate that IL-2 binding to the intermediate affinity IL-2R promotes rescue from apoptosis of resting T cells cultured *in vitro* in the absence of TCR stimulation. Analysis of the signaling molecules implicated in $\beta\gamma$ IL-2R-mediated survival, addressed here for the first time, indicates that pp56^{lck}, but not Jak kinases, is induced upon $\beta\gamma$ receptor ligation. We have previously described that pp56^{lck} is required for activation of TCR-associated PI3K (30) and regulates $\alpha\beta\gamma$ IL-2R-induced PI3K activation (29). Thus, we have also analyzed whether PI3K was stimulated in this system. We found that PI3K is constitutively associated to

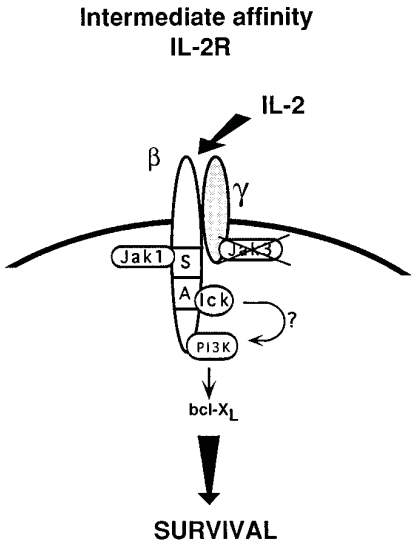

FIG. 9. **Schematic representation of a putative model for IL-2-mediated cell survival in resting T cells.** The stimulation of intermediate affinity IL-2R induces pp56^{lck} activation, PI3K induction, and bcl-x$_L$ expression. Several observations (see Refs. 29, 30) suggest that the activation of pp56^{lck} could be involved in inducing PI3K activation. PI3K activation seems to be in turn required for bcl-x$_L$ increased expression and for cell survival.

$\beta\gamma$ IL-2R and became activated in response to IL-2 binding. The fact that pharmacological inhibition of PI3K blocked IL-2-mediated survival strongly suggests that PI3K activation is required for the ability of IL-2 to abrogate cell death. Finally, we also show here that bcl-x$_L$ is induced upon $\beta\gamma$ IL-2R stimulation (Fig. 8) and that this induction is blocked upon PI3K inhibition (Fig. 8). Together these results allow the proposal of the model depicted in Fig. 9: ligation of intermediate affinity $\beta\gamma$ IL-2R induces both pp56^{lck} and PI3K activation. PI3K activity is then required for the induction of bcl-x$_L$, an important mediator of cell survival.

To the best of our knowledge, no previous publication has characterized the effect of $\beta\gamma$ IL-2R ligation on the death of normal T cells in the absence of TCR stimulation. However, at least three previous reports have analyzed the IL-2 involvement in T cell rescue from γ radiation-induced cell death (18, 19, 47). Boise *et al.* (18) describe that preincubation of normal human peripheral T cells with IL-2, IL-4, or IL-7, but not with IL-1, IL-3, or IL-6, decreases the apoptosis observed in response to γ radiation. Mor and Cohen (47), have reported that IL-2 mediates protection to γ radiation in antigen-specific T cells; however, they do not detect protection in resting T cells. Finally, Seki *et al.* (19) show that CD8$^+$ and NK cells are the most radiosensitive of resting T cells but could be rescued by IL-2 treatment. These observations, together with the description here that IL-2 protects from the cell death suffered by normal T cells when cultured in the absence of TCR stimulation (Fig. 1), support the conclusion that intermediate affinity IL-2R is capable of inducing T cell survival. In addition to this mechanism, others are likely to mediate survival of resting T cells, given that a significant proportion of the resting cells survive in the absence of IL-2 (Fig. 1*A*).

We have performed a systematic study of $\beta\gamma$ IL-2R-induced signaling pathways. As proposed in the model in Fig. 9, these data suggest that pp56^{lck} is one of the signals involved in IL-2R-mediated survival in resting T cells. This is in agreement with the observation that constitutive active pp56^{lck} mutants decrease the apoptosis induced by growth factor removal (42). In contrast, the involvement of pp56^{lck} on IL-2-induced cell division is controversial, since a mutant of the β chain lacking the acidic region (which does not associate pp56^{lck}) is, however, capable of mediating a proliferative signal (4). The other tyrosine kinases involved in high affinity IL-2R signaling are the Jak kinases (6–8), which have been shown to be required for IL-2-mediated proliferation (27, 28). The mechanism by which these enzymes are activated entails their phosphorylation on tyrosine residues. The current model proposes that Jak1 and Jak3 transphosphorylate each other (27). The low levels of Jak3 expression, and the concomitant lack of Jak1 and Jak3 activation in our system suggest that these proteins do not participate in IL-2-mediated survival. In addition to IL-2-mediated survival, bcl-2 induction by IL-2 in BAF/3 also seems to be independent of Jak3 as bcl-2 up-regulation is not altered by overexpression of a dominant negative Jak3 in these cells (28).

Our conclusion on PI3K activation involvement in T cell rescue from apoptosis (Fig. 6*C*) concurs with the implication of PI3K in survival discussed by Ward *et al.* (45) for CD28-mediated signals. Moreover, studies in rodent fibroblast (38), in neural growth factor receptor signaling in PC12 cells (29), and insulin growth factor receptor signaling in myeloid progenitors cells (32), in which activation of PI3K prevents apoptosis, also support this view. The mechanism through which PI3K is activated is not evident. In this regard, several cytokine receptors, such as IL-4R and IL-7R that share the γ chain with the IL-2R (48, 49) have been shown to enhance the resistance of resting T cells to radiation-induced cell death as effectively as IL-2 (18, 19). Studies with the IL-7 receptor show that PI3K activation is mediated by the γ chain and its associated Jak3 tyrosine kinase (50). However, the low levels of Jak3 expression in our system and the fact that this protein is not up-regulated upon IL-2 treatment makes unlikely the implication of this mechanism in PI3K activation. Another possible mechanism for PI3K activation involves the IL-2R β chain and src-kinases (29, 51). We previously described that pp56^{lck} activation regulates PI3K activation upon IL-2 triggering of high affinity IL-2R (29). In addition, the serine-rich region required for pp56^{lck} activation is also required for activation of PI3K (52). Finally, the fact that pp56^{lck} tyrosine kinase and correlatively PI3K are activated upon IL-2 binding to $\beta\gamma$ IL-2R (Fig. 3*A*) suggests that pp56^{lck} may contribute to induce PI3K activation.

Bcl-2 and bcl-x$_L$ have been shown to mediate survival (44) and to be induced upon high affinity IL-2R ligation (41–43, 47). We therefore tested the putative involvement of bcl-2 and bcl-x$_L$ in IL-2-mediated survival. Induction of bcl-x$_L$/bcl-2 in irradiated cells upon $\beta\gamma$ IL-2R ligation has not been previously detected (19). We found a slight increase in bcl-2 expression that could suggest a partial role of this protein for IL-2-mediated survival. More clearly, the induction of bcl-x$_L$ upon $\beta\gamma$ IL-2R stimulation and the fact that blocking IL-2-induced survival with LY294002 also results in abrogation of bcl-x$_L$ up-regulation suggest that PI3K activation precedes bcl-x$_L$ expression and that bcl-x$_L$ is probably involved in $\beta\gamma$ IL-2R-induced survival.

IL-2 mediates three different cellular responses. First, it has been involved in mediating the proliferation of cells preactivated via T cell receptor and expressing $\alpha\beta\gamma$ IL-2R (53). Second, it has been suggested to mediate survival (Fig. 1) (18, 19), and finally, it has been recently proposed to program T lymphocytes for apoptosis (54). Regarding the dual role of IL-2 at promoting either survival or cell proliferation, we describe here that only a set of signals (pp56^{lck}, PI3K, and bcl-x$_L$) of those triggered for IL-2-induced cell division are involved in mediating cell survival. The reason for high affinity IL-2R to trigger different early signals than that derived from intermediate affinity IL-2R in resting T cells could be the presence in the high affinity IL-2R of the α chain. However, chimeric β and γ IL-2R

chains transfected in cell lines lacking the α chain behave as high affinity IL-2R, inducing proliferation (2, 3). Therefore, it seems that the lack of IL-2R α chain expression is not responsible for the partial signaling of $\beta\gamma$ IL-2R in resting T cells. Instead, other proteins not expressed in resting T cells but expressed in cell lines or induced upon TCR cross-linking in normal T cells could be responsible for the different biological responses following TCR ligation. Candidates for these molecules could be c-*myc*, Jak3, or c-*raf*, relatively abundant in activated T cell and cell lines but not detectable in resting T cells (our data not shown) (14, 26).

Regarding the ability of IL-2 to program T lymphocytes for apoptosis, it has been described that incubation of T cells in IL-2 or IL-4 is required for ulterior TCR-mediated cell death (54). Moreover, IL-$2^{-/-}$ mice developed splenomegaly and lymphoadenophaty (55), similar to IL-2R$\beta^{-/-}$ mice (56). We sought to investigate the signaling pathways responsible for the ability of IL-2 to induce susceptibility to TCR-triggered apoptosis. However, in our hands, the conditions that induce susceptibility to apoptosis also trigger cell division. Thus, we favor the hypothesis that the same intracellular signaling program induces both cellular responses.

The fact that intermediate affinity IL-2 receptors transmit intracellular signals in resting cells, opens the possibility of a biological role for the $\beta\gamma$ IL-2R. The circulating levels of IL-2 are normally low. However, encounter with antigen primes T cells for IL-2 production, locally increasing the concentration of this cytokine. Cells that have not been stimulated by the antigen, and therefore do not express the IL-2R α chain, could use IL-2 to increase their survival potential.

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