Deletion of the acidic-rich domain of the IL-2Rβ chain increases receptor-associated PI3K activity

Angel Ciprés, Silvia Carrasco, Isabel Mérida*

Department of Immunology and Oncology, Centro Nacional de Biotecnología, Consejo Superior de Investigaciones Científicas, Campus de Cantoblanco, 28049 Madrid, Spain

Received 14 May 2001; accepted 30 May 2001

First published online 15 June 2001

Edited by Julio Celis

Abstract Interleukin-2 (IL-2) regulates the proliferation and homeostasis of lymphocytes through the coordinated activation of distinct signaling pathways. Deletion of the acidic-rich domain of the IL-2 receptor β chain (IL-2Rβ) prevents association of Src tyrosine kinases to the receptor, as well as IL-2-induced Akt activation. Cells bearing this deletion (BafβΔA) maintain full proliferation in response to IL-2 both in vivo and in vitro, suggesting that those pathways are dispensable for this important function of IL-2. In this study, we re-examined phosphatidylinositol-3 kinase (PI3K) activation in BafβΔA cells and found that, in BaF/3 IL-2RβΔA cells, deletion of the acidic domain induced constitutive activation of the receptor-associated PI3K activity. This, in turn, was responsible for the higher basal Akt activity observed in cells expressing this deletion. Based on these data, and since pharmacological abrogation of PI3K activity prevented IL-2-driven cell proliferation of BafβΔA cells, we conclude that the PI3K/Akt pathway is still functionally relevant in cells bearing this mutation. Moreover, we show that the PI3K-induced signals are, at least in part, responsible for c-myc expression. In conclusion, we have used this model to better identify those signals that are integral components of the molecular mechanisms responsible for IL-2-regulated cell proliferation. © 2001 Published by Elsevier Science B.V. on behalf of the Federation of European Biochemical Societies.

Key words: Interleukin-2 receptor; Phosphatidylinositol-3 kinase; Src family tyrosine kinase; Signal transduction

1. Introduction

Interleukin-2 (IL-2) is a cytokine essential for the proliferation of activated T lymphocytes, through its binding to the heterotrimeric IL-2 receptor (IL-2R) [1]. This receptor is composed of three different molecules termed α, β and γ. IL-2 binding to the IL-2R induces the rapid heterotrimerization of the receptor molecules and consequent activation of tyrosine kinases of the Jak and Src families [2,3]; these, in turn, induce the association/activation of other signaling molecules to the IL-2R complex. The identification of the role of the different domains in the IL-2Rβ and γ chains involved in IL-2-regulated signaling has been made possible by the generation of cell lines bearing truncations and/or mutations of these receptor components. Early studies of the IL-2Rβ subunit indicated the existence of three distinct and well-defined subdomains, conserved in the human and mouse molecules, which are important for the activation of different signaling molecules [4]. The membrane proximal domain of IL-2Rβ, also called the S-domain, is essential for IL-2 action and is responsible for the association of Jak1 kinase [5–7]. The function of the other two regions seems less essential, since truncations of either domain do not impair IL-2-dependent proliferation [4]. The proline-rich region, also termed the H-region, contains the tyrosine residues responsible for recruiting Stat 5 and Tat 3 transcription factors [8]. The acidic-rich domain (A-domain) of IL-2Rβ comprises amino acids 314–381 and is responsible for direct association of Src family tyrosine kinases and Shc [9,10]. Cells expressing receptors with the A-domain deleted proliferate in response to IL-2, indicating that direct association of Src family tyrosine kinases is unnecessary for the mitogenic action of IL-2.

IL-2-induced activation of phosphatidylinositol-3 kinase (PI3K) was demonstrated some time ago [11–13]. As is the case for other growth factor receptors, generation of 3′ phosphorylated lipids through PI3K activation is important for the IL-2-regulated cell cycle, as well as for IL-2 prevention of apoptosis [14]. The IL-2Rβ chain is essential for IL-2-induced PI3K activation [15], but the exact nature of the association/activation of this lipid kinase following IL-2 binding has not been fully determined. It has recently been demonstrated that the IL-2Rβ chain and Jak1 cooperate functionally in the association of the regulatory subunit of p85 [16]. In those studies, deletion of the A-domain was shown to prevent not only the phosphorylation on tyrosine residues of p85, but also Akt activation by IL-2 in cells expressing the deleted receptor. Proliferation of the cells expressing the A-deleted mutant suggested that IL-2-induced Akt activation was not required for IL-2 regulation of cell proliferation.

Given the relevance of the PI3K/Akt pathway in the control of cell proliferation by several mitogens in other systems, we decided that a more detailed examination was required of the IL-2 regulation of these pathways in cells expressing the A-mutant IL-2Rβ chain. Here we have investigated the consequences of IL-2Rβ chain A-domain deletion on IL-2 activation of the PI3K/Akt pathway. Our results indicate that deletion of the A-domain induces higher PI3K activity constitutively associated to the IL-2Rβ chain compared to that associated to wild type IL-2Rβ. Addition of IL-2 to cells expressing the mutant receptor diminished this activity, indicating that those mechanisms responsible for downregulation of this activity remained IL-2-regulated. Constitutive activation of PI3K induced, in turn, an increase in Akt activity in cells expressing the mutant receptor. Based on these studies,

*Corresponding author. Fax: (34)-91-372 0493.
E-mail: imerida@cnb.uam.es

0014-5793/01/$20.00
PII: S0014-5793(01)02594-7

we conclude that deletion of the A-domain in IL-2Rβ alters the time course and intensity of PI3K/Akt pathway activation. Binding of the cytokine to the mutated receptor nonetheless regulates this important signaling pathway, suggesting that it is required for the correct delivery of IL-2 proliferative signals.

2. Materials and methods

2.1. Cells

Murine BaF/3 cells stably transfected with cDNA encoding either the wild type (Bafβwt) or the A-deleted form (BafβΔA) of human IL-2Rβ have been previously described [4]. Both cell lines were maintained in RPMI 1640 medium supplemented with 10% fetal calf serum, β-mercaptoethanol (50 μM), and either 50 U/ml of human recombinant IL-2 or 5% WEHI.3B cell-conditioned media and gentamicin (50 μg/ml). For analysis of IL-2-induced signals, cells in exponential growth were washed extensively to remove growth factors and resuspended in growth factor- and serum-free media (basal media) for 6 h to induce maximum synchronization. Cells were then stimulated with IL-2 (50 U/ml) for the times indicated in each experiment. Cell viability of synchronized cells was 95% in all experiments.

2.2. Reagents

Human recombinant IL-2 was from Hoffmann-La Roche (Nutley, NJ, USA), wortmannin was from Sigma (St. Louis, MO, USA), LY294002 was from Biomol Research Labs, Inc. (Plymouth Meeting, PA, USA). [γ-^{32}P]ATP and the ECL-Plus kit were from Amersham (Piscataway, NJ, USA). Anti-PI3K(p85), anti-Akt and anti-phosphotyrosine horseradish peroxidase (HRPO)-conjugated antibodies were from Upstate Biotechnology (Lake Placid, NY, USA), anti-human IL-2Rβ chain was from Pharmingen (San Diego, CA, USA), anti-myc was from Oncogene (Boston, MA, USA).

2.3. Plasmids and transfections

The expression cDNA constructs pEF-IL-2Rβ and pEF-IL-2RβΔA were kindly donated by T. Taniguchi (University of Tokyo, Japan). Plasmids encoding the constitutively active form of PI3K (p110-CAAX) and Akt were a gift of J. Downward (ICRF, UK). The c-myc promoter-driven luciferase gene was a gift from K. Sugamura (Tohoku University, Japan). For transfection experiments, cells were electroporated as described [17].

2.4. Analysis of cell growth and apoptosis

The [^{3}H]thymidine incorporation assay was performed as described [15]. Briefly, cells were seeded in 96-well plates at an initial concentration of 10^5 cells/ml. After 16 h, cells were pulsed with 1 μCi of [^{3}H]thymidine for 6 h prior to harvest. For cell cycle analyses, the fluorescence of propidium iodide-stained DNA was quantitated on a per cell basis with an EPIC-XL flow cytofluorometer (Coulter, Hialeah, FL, USA).

2.5. Immunoprecipitation and immunoblotting

Immunoprecipitation and immunoblotting were carried out as described [15]. In brief, after stimulation, cells were washed with ice-cold phosphate-buffered saline and resuspended in lysis buffer (1% Nonidet P-40 (NP-40), 50 mM Tris–HCl pH 7.4, 150 mM NaCl, 10 mM NaF, 10 mM $Na_4P_2O_7$, 1 mM Na_3VO_4, 1 mM PMSF, 10 μg/ml leupeptin and 10 μg/ml aprotinin). Cell lysates were cleared by centrifugation (12 000 rpm for 15 min) and the supernatants pre-cleared with an excess of protein Gamma Bind G-Protein Sepharose (Pharmacia). Pre-cleared lysates were incubated with the indicated antibodies and the immune complex precipitated with G-protein Sepharose. For c-myc detection, cells were collected, resuspended in hypotonic buffer (10 mM HEPES pH 7.9, 10 mM KCl, 0.1 mM EDTA, 0.1 mM EGTA, 2.5 mM dithiothreitol (DTT), 0.6% NP-40 and protease inhibitors) and incubated on ice for 15 min. Cell extracts were vortexed vigorously and centrifuged for 1 min. The supernatants were removed and ice-cold nuclear extract buffer (20 mM HEPES pH 7.9, 25% glycerol, 0.4 M NaCl, 1 mM EDTA, 1 mM EGTA, 2.5 mM DTT and protease inhibitors) was added to the pellets. Samples were rocked (15 min, 4°C), centrifuged for 5 min and the supernatants collected. For Western blot analysis, samples were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE), then transferred to nitrocellulose membranes (Bio-Rad). Proteins were detected by incubation with the appropriate antibodies followed by anti-mouse or anti-rabbit HRPO-conjugated antibodies (Amersham) or G-protein–HRPO conjugate (Bio-Rad). Western blots were developed using ECL-Plus (Amersham) according to the manufacturer's instructions.

2.6. PI3K assay

The assay of PI3K activity in pellets was performed as described [12]. Briefly, immunoprecipitates were washed three times with lysis buffer, once with 0.5 M LiCl and three times with 50 mM Tris–HCl (pH 7.5), then resuspended in 20 μl of 50 mM Tris–HCl pH 7.5, containing PI micelles (Avanti Polar Lipids, Alabaster, AL, USA) at 0.2 mg/ml. Kinase reaction was initiated by addition of 10 μM [γ-^{32}P]ATP (10 μCi), 25 μM $MgCl_2$ and 20 μM unlabeled ATP and allowed to proceed at 25°C for 10 min. The reaction was terminated by addition of 100 μl of 1 M HCl and 200 μl of a methanol:chloroform mixture (1:1 v/v). The extracted phospholipids were resolved by thin layer chromatography (Silica Gel 60; Merck) on plates coated with 1% potassium oxalate and developed in chloroform:methanol:4.5 M ammonia (9:7:2 v/v/v), as described [12]. The radioactive products were visualized by autoradiography and quantified by scanning laser densitometry.

2.7. Immunoprecipitation and Akt kinase assay

IL-2-stimulated cells were lysed and Akt was immunoprecipitated with a specific antibody as described [17]. Pellets were washed twice with lysis buffer, twice with high salt buffer (0.1 M Tris–HCl pH 8, 0.5 M LiCl, 10 mM $MgCl_2$) and once with kinase buffer (50 mM Tris–HCl pH 7.5, 10 mM $MgCl_2$). The reaction was initiated by addition of 25 μl of the following mixture: 50 mM Tris–HCl pH 7.5, 10 mM $MgCl_2$, 1 mM DTT, 25 μg histone H2B, 1 μM phosphokinase inhibitor (Sigma), 50 μM ATP and 5 μCi [γ-^{32}P]ATP. The reaction was continued for 30 min at 25°C and terminated by addition of 25 μl of Laemmli sample buffer. Samples were resolved in SDS–PAGE, and the radioactive products detected by autoradiography.

2.8. Luciferase assays

Bafβwt cells were transfected by electroporation with 30 μg each of plasmids encoding a constitutive active form of p110 (p110-CAAX) or Akt, together with pXLuc and GFP to evaluate the transfection efficiency. After 24 h in IL-3-supplemented medium, cells were washed twice with RPMI 1640 and incubated in basal medium at a final concentration of 5×10^6 cells/ml for 8 h, then lysed and assayed for luciferase activity. To test the effect of inhibitors, cells were electroporated with the pXLuc plasmid and incubated in complete medium plus or minus IL-2 (50 U/ml) in the presence of the PI3K inhibitor LY294002 (10 μM). Luciferase activity was determined 18 h after transfection.

3. Results

3.1. PI3K-specific inhibitors prevent IL-2-dependent proliferation in Bafβwt and BafβΔA cells

Our previous experiments and those of other laboratories indicated that PI3K activation is essential for IL-2-driven proliferation. Nonetheless, it has been shown that deletion of the A-domain not only abolishes association of Src family tyrosine kinases to the receptor, but also prevents IL-2-dependent activation of the PI3K-regulated serine–threonine kinase Akt [16]. We thus assessed the role of PI3K-regulated signaling pathways in IL-2-dependent proliferation by measuring [^{3}H]thymidine incorporation into cells stably transfected with the wild type IL-2Rβ chain (Bafβwt) or the A-region deletion mutant (BafβΔA), in the presence of a pharmacological inhibitor for this kinase. Treatment with the PI3K inhibitor wortmannin diminished proliferation of BafβΔA cells to the same extent (about 50%) as it blocked proliferation of cells expressing the wild type IL-2Rβ (Fig. 1).

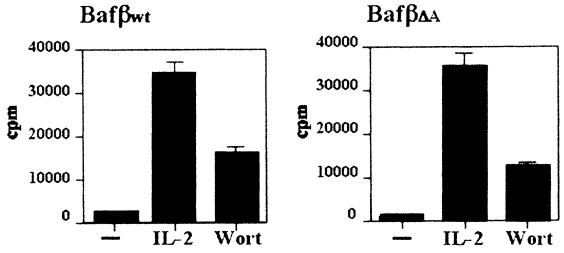

Fig. 1. Pharmacological inhibition of PI3K activity prevents IL-2-induced proliferation of Bafβwt and Bafβ∆A cells. Bafβwt or Bafβ∆A cells were synchronized in basal medium, then cultured in complete medium with or without IL-2 (50 U/ml). Where indicated, cells were preincubated with wortmannin (0.1 μM) for 1 h before IL-2 addition. Cell proliferation was determined by measuring [^{3}H]thymidine incorporation (see Section 2).

3.2. A-domain deletion activates the PI3K/Akt pathway in the absence of IL-2

Binding of IL-2 to its high affinity receptor is known to induce the transient activation of PI3K, which can be measured in immunoprecipitates of the IL-2Rβ protein. To determine the effect of the IL-2Rβ A-domain deletion on receptor-associated PI3K activity, we performed in vitro PI3K assays in immunoprecipitated IL-2Rβ molecules. As predicted, IL-2 increased the PI3K activity measured in immunoprecipitates of the IL-2Rβ chain (Fig. 2A). When Bafβ∆A cells were stimulated with IL-2, there was no apparent increase of IL-2Rβ-associated PI3K activity, but rather a decrease with respect to time zero was observed. We examined the lipid kinase activity associated with the receptor following more prolonged cell starvation and, surprisingly, found that the receptor-associated PI3K activity was higher when starvation time was increased (Fig. 2B). Consistent with this, in all experiments using Bafβ∆A cells, IL-2 was found to induce a rapid decrease of PI3K activity measured in receptor immunoprecipitates. Akt is a serine–threonine kinase that depends on 3′ phosphorylated lipids generation for activation. This protein kinase thus represents a reliable parameter for determining changes on PI3K activity. When a phosphorylation assay was performed in anti-Akt immunoprecipitates from Bafβwt cells after IL-2 stimulation, we found, as expected, IL-2-induced Akt activation (Fig. 2C). No similar effect was detected in cells expressing the A-deleted mutant. As was the case for receptor-associated PI3K, basal Akt activity was higher in cells expressing the A-deletion compared to that of cells expressing the wild type receptor. This suggests that the higher basal PI3K activity associated to the A-deleted IL-2Rβ chain induces an elevation in the basal Akt activity in these cells.

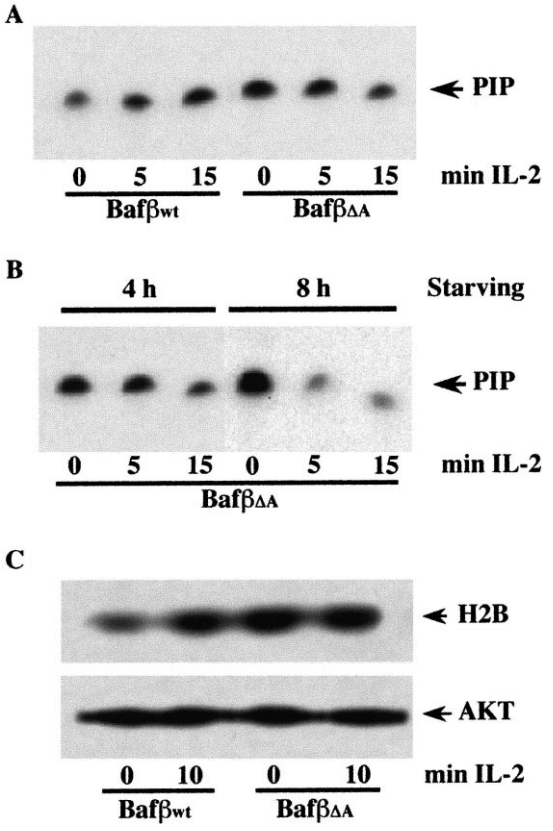

Fig. 2. Constitutive activation of receptor-associated PI3K/Akt activity in Bafβ∆A cells. (A) Synchronized Bafβwt or Bafβ∆A cells were stimulated with IL-2 for the times indicated. IL-2Rβ-associated PI3K activity was determined as described (Section 2). The figure shows [^{32}P]PI-3-phosphate production. (B) IL-2-induced PI3K activity was measured in Bafβ∆A immunoprecipitates following 4 or 8 h starvation in basal medium, as described for A. (C) Synchronized Bafβwt and Bafβ∆A cells were stimulated with IL-2 and Akt activity determined in immunoprecipitates (see Section 2).

3.3. Transient expression of the IL-2Rβ∆A chain activates the PI3K/Akt pathway

Bafβwt and Bafβ∆A are cell lines stably transfected with cDNA encoding the wild type β chain or bearing a deletion of the A-domain. To discard possible differences due to clonal variability between transfected cell lines, we also assessed both IL-2Rβ-associated PI3K activity and total Akt activity in cells transiently expressing both receptor forms. Parental cells were electroporated with plasmids encoding cDNA for both IL-2Rβwt and Bafβ∆A and analyzed 48 h after transfection. PI3K activity associated with IL-2Rβ∆A was high, and diminished following IL-2 addition (Fig. 3A). Moreover, the same cells had higher Akt activity than those expressing the IL-2Rβwt receptor counterpart. This confirms that the differences between receptor-associated PI3K and total Akt activity are a direct consequence of the deletion of the A-domain of the IL-2Rβ subunit.

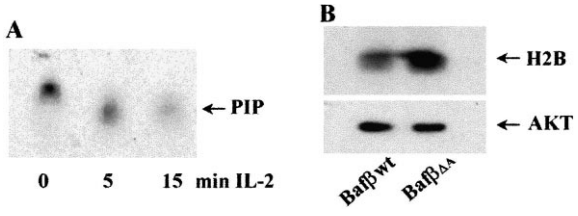

Fig. 3. PI3K/Akt activity is increased in BaF/3 cells transiently transfected with the A-domain deleted IL-2Rβ. (A) BaF/3 cells were transiently transfected with cDNA encoding the IL-2Rβ∆A chain. After transfection, cells were cultured in IL-3-conditioned medium for 24 h, synchronized in basal medium and IL-2-stimulated for the times indicated. IL-2Rβ-associated PI3K activity was determined as described. (B) IL-2Rβwt or IL-2Rβ∆A were transiently transfected into the BaF/3 cell line. Cells were cultured in IL-3-conditioned medium and synchronized in basal medium for 6 h, after which total Akt activity was determined as described.

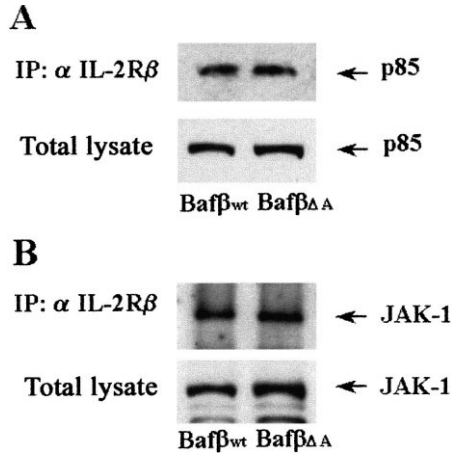

Fig. 4. Deletion of the A-domain has no effect on PI3K association to the IL-2Rβ. (A) Bafβwt or BafβΔA cells were synchronized in basal medium for 6 h, lysed, and the PI3K associated to the IL-2Rβ chain determined by Western blot analysis of IL-2Rβ immunoprecipitates using an antibody specific for the PI3K regulatory subunit p85. 10% of the total cell lysates were also analyzed. (B) The same membranes as in A were reprobed for Jak1 association using a specific antibody.

3.4. A-domain deletion does not affect PI3K association to the IL-2Rβ subunit

Our experiments indicated that deletion of the IL-2Rβ A-domain induced elevated PI3K activity associated with the IL-2Rβ chain in the absence of cytokine stimulation. We therefore analyzed the consequence of this deletion on the basal constitutive association of this lipid kinase with the receptor. Using an antibody specific for p85, the regulatory subunit of PI3K, we found that A-domain deletion did not significantly alter the level of receptor-associated p85 (Fig. 4). As a control, the blots were reprobed with Jak1, the tyrosine kinase constitutively associated with the serine-rich domain of IL-2Rβ. These experiments allow us to conclude that the increased PI3K activity associated with the receptor bearing the A-domain deletion is not caused by greater association of the enzyme with the IL-2Rβ chain.

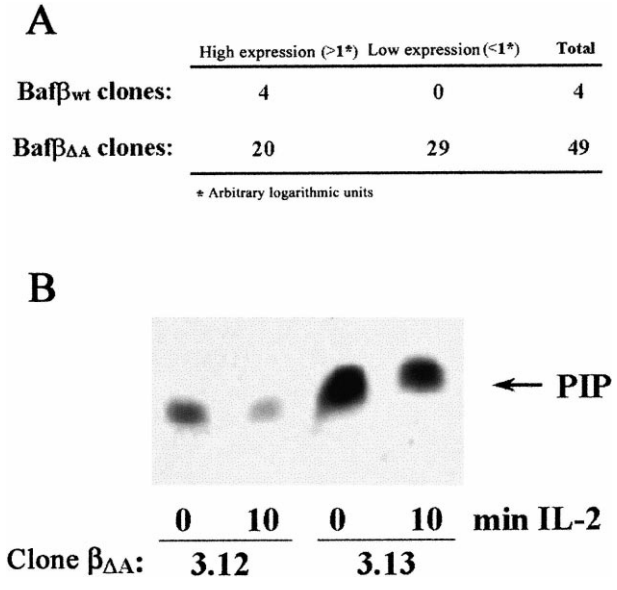

Fig. 5. Transfection of the IL-2RβΔA confers a survival/proliferative advantage on BaF/3 cells. (A) BaF/3 cells were transfected with IL-2Rβwt or βΔA chains. Clonal selection of the cells was performed by limiting dilution; cells were cultured in complete medium with IL-2 (50 U/ml). The number of clones expressing high or low numbers of cell surface IL-2Rβ molecules is shown. (B) Two independent clones expressing low (3.12) or high (3.13) numbers of cell surface receptors were selected and IL-2Rβ-associated PI3K activity was determined as in Fig. 2.

3.5. A-domain deletion confers a survival advantage on transfected cells

Activation of the PI3K/Akt pathway contributes to IL-2-regulated survival and proliferation mechanisms [18]. We reasoned that if deletion of the A-domain induced higher receptor-associated PI3K activity and this in turn increased basal Akt activity, cells expressing the mutated receptor would have a survival or proliferation advantage over cells expressing the wild type receptor. To test this hypothesis, we generated new clones of BaF/3 cells expressing either the wild type or the A-deleted IL-2Rβ chain. The total number of clones obtained for the IL-2RβΔA mutant was 10 times greater than that obtained for cells bearing the IL-2Rβwt (Fig. 5A). Moreover, in IL-2RβΔA cDNA-transfected cells, we consistently found a larger number of clones expressing low levels of the transfected β chain, whereas in the case of transfection with the wild type IL-2Rβ receptor, only cells expressing high levels of IL-2Rβwt molecules were obtained. To further demonstrate that the IL-2R-associated PI3K activity was constitutively higher, independently of the number of receptors expressed on the cell surface, we determined the lipid kinase activity in two clones expressing either high or low numbers of cell surface IL-2RβΔA. The behavior of both clones was similar,

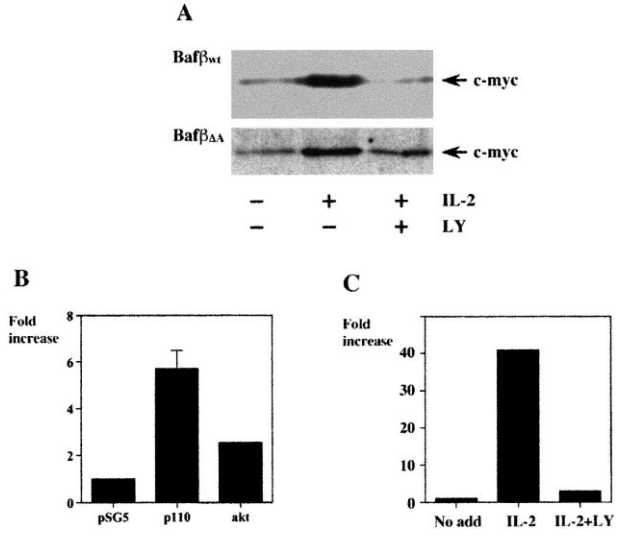

Fig. 6. Regulation of c-myc by the PI3K/Akt pathway. (A) After 4 h starvation in basal medium, cells were preincubated with the inhibitor LY292002 (10 μM) or vehicle, and stimulated with IL-2 for 2 h. Cells were collected, lysed, proteins resolved by SDS–PAGE and c-myc expression was analyzed by Western blot. (B) Bafβwt cells were co-transfected with a c-myc promoter-driven luciferase gene and plasmids encoding a constitutive active form of PI3K (p110-CAAX) or wild type Akt. After transfection, cells were cultured in IL-3-conditioned medium for 24 h, followed by incubation in basal medium for an additional 8 h; luciferase activity was then determined. (C) Bafβwt cells were transfected with the c-myc promoter-driven luciferase gene and cultured in complete medium with or without IL-2 (50 U/ml) for 18 h. The inhibitor LY292002 (LY, 10 μM) was added where indicated.

and in both cases IL-2 addition resulted in a decrease in the associated PI3K activity (Fig. 5B).

3.6. Involvement of PI3K-regulated pathways in c-myc induction

Activation of the PI3K/Akt pathway by growth factors has been shown to regulate several signals required for cell proliferation. Addition of IL-2 to lymphocytes results in the rapid expression of c-myc, and induction of this proto-oncogene is essential for IL-2-induced cell proliferation [28]. Pharmacological inhibition of PI3K prevents the induction of c-myc that follows IL-2 addition to both IL-2Rβwt and IL-2RβΔA cells (Fig. 6A). To further evaluate the role of the PI3K/Akt pathway on c-myc regulation, we examined the effect of constitutive activation of this pathway on the transcriptional regulation of the c-myc promoter. Overexpression of 3′ phosphorylated derivatives of PI or Akt overexpression are both sufficient to induce a detectable increase in the transcriptional activity of the c-myc promoter (Fig. 6B). Treatment of the cells with a pharmacological inhibitor of PI3K prevents IL-2 induction of the c-myc promoter (Fig. 6C). Taken together, these data demonstrate that PI3K regulation by IL-2 is essential for c-myc induction, confirming the importance of this lipid kinase in the control of proliferation.

4. Discussion

Activation of PI3K has been implicated in two important functions for IL-2, cell proliferation and prevention of apoptosis [14,18,19]. The lack of IL-2-induced Akt activation in cells transfected with the IL-2RβA deletion mutant suggested, however, that regulation of the PI3K/Akt pathway by IL-2 is not required for proliferation [16]. The detailed analysis, presented here, of the IL-2 regulation of PI3K-derived signals in BafβΔA cells, has allowed re-evaluation of the role of this pathway in IL-2-mediated proliferation. Deletion of the IL-2Rβ chain A-domain induces a concomitant increase in the PI3K activity constitutively associated to this molecule. This basal activation of the PI3K/Akt pathway is important for IL-2-induced cell proliferation, since inhibition of PI3K activity, either by addition of pharmacological inhibitors or following transfection of a construct with dominant negative properties (data not shown), has a profound effect on cell proliferation in response to this cytokine. These data confirm our previous observation [17] that an early increase in 3′ phosphorylated PI derivatives is necessary, but not sufficient, for IL-2-induced proliferation. Moreover, we demonstrate downregulation of receptor-associated PI3K activity in response to IL-2 addition, confirming that not only the activation, but also the downregulation of PI3K activity by IL-2-induced signals are important for cytokine-regulated cell proliferation. These data explain the lack of activation of the Akt pathway by IL-2 previously described in BafβΔA cells [16].

Our experiments demonstrate constitutive association of PI3K activity to the β subunit of the IL-2R. These results concur with recent studies by Migone et al., who showed that p85 associates to Jak1, which is in turn associated to the S-rich domain of IL-2Rβ [16]. In those studies, the authors elegantly demonstrated that the A-domain was required for a more effective association of p85 to the receptor, but the activity of the p85/p110 heterodimer was not investigated. The regulatory subunit of PI3K has been shown to negatively modulate the catalytic activity of p110 both in vitro and in vivo [20,21]. The activity of p85/p110 dimers is increased when the SH2 domains of p85 bind to phosphorylated residues in the proteins that contain the appropriate sequence motifs [22]. This suggests that the increased activity of the heterodimer when bound to phosphoproteins is not a true activation, but rather a disinhibition of the p85/p110 heterodimer. p85/p110 dimer activity may also be affected by other mechanisms, such as binding to GTP-bound ras and CDC42 [23,24], SH3 domain from Src family kinases [25] or proline-rich proteins that bind to the p85 SH3 domain [26]. Although not examined in detail, it would be interesting to determine whether activation of p85/p110 dimers in these cases also reflects a transition between inhibited and disinhibited states. Our experiments suggest that deletion of the A-rich domain of the IL-2Rβ chain induces disinhibition of the effect of the regulatory subunit on the p110 catalytic activity by a mechanism that remains to be defined.

We have shown that while activity of receptor-associated PI3K activity is enhanced in the absence of IL-2, addition of this cytokine to BafβΔA cells results in rapid downregulation of associated PI3K activity. Activation of PI3K in response to IL-2, as is the case for the majority of growth factor receptors, is rapid and transient, suggesting rapid downregulation of this activity following those signals responsible for PI3K association/activation. Although the mechanisms underlying PI3K activation in response to growth factors have been reviewed extensively, few data in the literature have addressed the downregulation of this activity. The recent discovery of the profound effect on cell transformation caused by mutations in the lipid phosphatase PTEN underlines the importance of a regulated balance, not only in the production but also in the degradation of 3-phosphorylated lipids for correct cell homeostasis [27]. This IL-2Rβ chain mutation represents an invaluable model to study the mechanisms by which IL-2 downregulates PI3K activity.

Activation of PI3K/Akt by IL-2 has been related to the anti-apoptotic effects of this cytokine [18]. Our experiments demonstrate that inhibition of PI3K prevents IL-2-regulated proliferation without affecting IL-2-induced survival, confirming our previous observation of the existence of alternative mechanisms of IL-2 protection from apoptosis [17]. IL-2-mediated expression of the cell cycle regulator E2F has been shown to be mediated by PI3K activity, suggesting that this pathway serves as a link between the IL-2R and the cell cycle machinery [14]. Here we show that IL-2-mediated PI3K activation can also regulate c-myc expression, suggesting the existence of multiple cell cycle control mechanisms regulated by IL-2R-triggered 3′ phosphorylated lipid products. Induction of c-myc has been demonstrated to be essential for IL-2-induced proliferation [28], nevertheless very little is known about the early signals that regulate IL-2-induced c-myc expression. Our experiments demonstrate for the first time a direct correlation between PI3K and c-myc induction.

The role of the A-domain deletion has recently been evaluated in primary lymphocyte populations through generation of mice expressing the IL-2RβA transgene on an IL-2Rβ null background [29]. T cells expressing the IL-2RβΔA mutant exhibited increased IL-2-mediated proliferation, indicating that the lack of this domain results in enhanced proliferation of primary T cells. This enhancement was accompanied by the prolonged phosphorylation of IL-2Rβ, suggesting that dele-

tion of the A-region results in the loss of signals involved in negative regulation. Examination of PI3K activity in these cells should provide further evidence of the role of these pathways in IL-2-mediated cell proliferation.

Acknowledgements: We are grateful to Dr. T. Taniguchi for reagents, suggestions and critical comments, to Drs. J. Downward, A. Carrera and K. Sugamura for the gift of reagents, and to Drs. D. Jones and E. Díez for critical reading of the manuscript. We thank Hoffmann-La Roche for the gift of recombinant human IL-2. This work was partially supported by Grants PM97-0132 from the Dirección General de Enseñanza Superior e Investigación Científica and 08.3/0016.1/99 from CAM to I.M. The Department of Immunology and Oncology was founded and is supported by the Spanish National Research Council (CSIC) and the Pharmacia Corporation.

References

[1] Taniguchi, T. and Minami, Y. (1993) Cell 73, 5–8.
[2] Miyazaki, T., Kawahara, A., Fuji, H., Nakagawa, Y., Minami, Y., Liu, Z.J., Oishi, I., Silvennoinen, O., Wittuhn, B. and Ihle, J. (1994) Science 266, 1045–1047.
[3] Taniguchi, T., Miyazaki, T., Minami, Y., Kawahara, A., Fujii, H., Nakagawa, Y., Hakateyama, M. and Liu, Z.J. (1995) Ann. N.Y. Acad. Sci. 766, 235–244.
[4] Hakateyama, M., Mori, H., Doi, T. and Taniguchi, T. (1989) Cell 59, 837–845.
[5] Leonard, W.J., Noguchi, M., Russell, S.M. and McBride, O.W. (1994) Immunol. Rev. 138, 61–86.
[6] Ihle, J.N. (1995) Nature 377, 591–594.
[7] Taniguchi, T. (1995) Science 268, 251–253.
[8] Fujii, H., Nakagawa, Y., Schindler, U., Kawahara, A., Mori, H., Gouilleux, F., Groner, B., Ihle, J.N., Minami, Y. and Miyazaki, T. (1995) Proc. Natl. Acad. Sci. USA 92, 5482–5486.
[9] Friedmann, M.C., Migone, T.S., Russell, S.M. and Leonard, W.J. (1996) Proc. Natl. Acad. Sci. USA 93, 2077–2082.
[10] Ravichandran, K.S., Igras, V., Shoelson, S.E., Fesik, S.W. and Burakoff, S.J. (1996) Proc. Natl. Acad. Sci. USA 93, 5275–5280.
[11] Remillard, B., Petrillo, R., Maslinski, W., Tsudo, M., Strom, T.B., Cantley, L. and Varticovski, L. (1991) J. Biol. Chem. 256, 14167–14170.
[12] Mérida, I., Díez, E. and Gaulton, G.N. (1991) J. Immunol. 147, 2202–2207.
[13] Augustine, J.A., Sutor, R.L. and Abraham, R.T. (1991) Mol. Cell. Biol. 11, 4431–4440.
[14] Brennan, P., Babbage, J.W., Burgering, B.M.T., Groner, B., Reif, K. and Cantrell, D. (1997) Immunity 7, 679–689.
[15] Mérida, I., Williamson, P., Kuziel, W.A., Greene, W.C. and Gaulton, G.N. (1993) J. Biol. Chem. 268, 6765–6770.
[16] Migone, T., Rodig, S., Cacalano, N.A., Berg, M., Schreiber, R.D. and Leonard, W.J. (1998) Mol. Cell. Biol. 18, 6416–6422.
[17] Ciprés, A., Gala, S., Martínez-A, C., Mérida, I. and Williamson, P. (1999) Eur. J. Immunol. 29, 1157–1158.
[18] Parijs, L.V., Refaeli, Y., Lord, J.D., Nelson, B.H., Abbas, A.K. and Baltimore, D. (1999) Immunity 11, 281–288.
[19] Ahmed, N.N., Grimes, H.L., Bellacosa, A., Chang, O.T. and Tsichlis, P.N. (1997) Proc. Natl. Acad. Sci. USA 94, 3627–3632.
[20] Yu, J., Zhang, Y., McIlroy, J., Rordorf-Nikolic, T., Orr, G.A. and Backer, J.M. (1998) Mol. Cell. Biol. 18, 1379–1387.
[21] Ueki, K., Algenstaedt, P., Mauvais-Jarvis, F. and Kahn, R. (2000) Mol. Cell. Biol. 20, 8035–8046.
[22] Carpenter, L.C., Auger, K.R., Chanudhuri, M., Yoakim, M., Schaffhausen, B., Shoelshon, S. and Cantley, L.C. (1993) J. Biol. Chem. 268, 9478–9483.
[23] Rodríguez-Viciana, P., Warne, P.H., Vanhaesebroeck, B., Waterfield, M.D. and Downward, J. (1996) EMBO J. 15, 2442–2451.
[24] Zheng, Y., Bagrdia, S. and Cerione, R. (1994) J. Biol. Chem. 269, 18727–18730.
[25] Pleiman, C.M., Hertz, W.M. and Cambier, J.C. (1994) Science 263, 1609–1612.
[26] Guinebault, C., Payastre, B., Racaud-Sultan, C., Mazarguil, H., Breton, M., Mauco, G., Plantavid, M. and Chap, H. (1995) J. Cell. Biol. 129, 831–842.
[27] Di Cristofano, A. and Pandolfi, P.P. (2000) Cell 100, 387–390.
[28] Shibuya, H., Yoneyama, M., Ninomiya-Tsuji, J., Matsumoto, K. and Taniguchi, T. (1992) Cell 70, 57–67.
[29] Fujii, H., Ogasawara, K., Otsuka, H., Suzuki, M., Yamamura, K., Yokochi, T., Miyazaki, T., Suzuki, H., Mak, T.W., Taki, S. and Taniguchi, T. (1998) EMBO J. 17, 6551–6557.