Phosphatidylinositol 3-kinase is recruited to a specific site in the activated IL-1 receptor I

Sandra Marmiroli[a,*], Alberto Bavelloni[b], Irene Faenza[a], Alessandra Sirri[b], Andrea Ognibene[b], Vittoria Cenni[a], Junichi Tsukada[c], Yoshinobu Koyama[c], Maria Ruzzene[d], Alberto Ferri[e], Philip E. Auron[c], Alex Toker[f], Nadir M. Maraldi[a,b]

[a] *Istituto di Citomorfologia Normale e Patologica del C.N.R., via di Barbiano 1/10, 40136 Bologna, Italy*
[b] *Laboratorio Biologia Cellulare I.O.R., Bologna, Italy*
[c] *Department of Pathology, Harvard Medical School, Boston, MA, USA*
[d] *Department of Biochemistry, University of Padova, Padova, Italy*
[e] *Department of Biochemistry, University of Ferrara, Ferrara, Italy*
[f] *Boston Biomedical Research Institute, Boston, MA, USA*

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Abstract Interleukin 1 (IL-1) delivers a stimulatory signal which increases the expression of a set of genes by modulating the transcription factor NF-κB. The IL-1 receptors are transmembrane glycoproteins which lack a catalytic domain. The C-terminal portion of the type I IL-1 receptor (IL-1RI) is essential for IL-1 signalling and for IL-1 dependent activation of NF-κB. This portion contains a putative phosphatidylinositol 3-kinase (PI 3-kinase) binding domain (Tyr-E-X-Met), which is highly conserved between the human, mouse and chicken sequences, as well as the related cytoplasmic domain of the *Drosophila* receptor Toll. This observation prompted us to investigate the role of PI 3-kinase in IL-1 signalling. Here we report evidence that PI 3-kinase is recruited by the activated IL-1RI, causing rapid and transient activation of PI 3-kinase. We also show that the receptor is tyrosine phosphorylated in response to IL-1. Expression of a receptor mutant lacking the putative binding site for p85 demonstrates that Tyr^{479} in the receptor cytoplasmic domain is essential for PI 3-kinase activation by IL-1. Our results indicate that PI 3-kinase is likely to be an important mediator of some IL-1 effects, providing docking sites for additional signalling molecules.

Key words: Phosphatidylinositol 3-kinase; Interleukin-1 receptor; Nuclear factor κB; Human osteosarcoma cell line

1. Introduction

Interleukin 1 (IL-1) constitutes a family of polypeptide cytokines secreted in response to inflammatory and immunological diseases and exhibiting a wide range of biological activities [11]. The most consistent property of IL-1 is to upregulate the expression of a set of genes through the modulation of the transcription factors NF-κB and AP-1. Although the biological effects of IL-1 are well documented, the molecular mechanism of signalling by IL-1 is complex. IL-1 binds to transmembrane glycoprotein receptors which lack a catalytic domain [8,40], and are therefore supposed to interact with accessory signalling components. Two receptor proteins have been identified so far, type I and type II IL-1 receptors (IL-1Rs). The 80-kDa IL-1RI possesses a 200 amino acid cytoplasmic tail critical for IL-1 signalling; conversely, the 68-kDa type II IL-1R lacks this cytoplasmic tail almost completely, and it appears to act as a decoy receptor. While both receptor types are coexpressed on many cell types, they appear to be functionally independent and do not bind IL-1 as a heterodimer complex. Cloning and sequencing of the IL-1 receptors provided very few clues as to what the post-receptor events may be [40]. It is clear, however, that IL-1 is a pleiotropic molecule, able to generate a number of signals through more than one signalling pathway. Consequently, a wide variety of post-receptor events have been reported, which include generation of second messengers, such as cyclic AMP and ceramide [23], as well as activation of protein kinase C and G-proteins [11]. However, these effects are often almost anecdotal, being cell-type dependent, and their physiological significance for signalling by IL-1 remains to be established.

Deletion of most of the cytoplasmic region of the murine IL-1R does not affect the binding of IL-1 to the receptor, and still allows internalisation and nuclear localisation of IL-1 [17]. Furthermore, mutant IL-1R genes carrying different degrees of truncations in the cytoplasmic COOH-terminal [7,26,36] have allowed the identification of a domain, between amino acids 486–527, required by IL-1 to activate NF-κB. Much effort has been focused in this direction, in consideration of the fact that most of the well known biological effects of IL-1 are mediated by NF-κB transcriptional activity [33,39]. In fact, many cytokines activate the transcription factor NF-κB through hitherto unrelated signalling pathways. Recently, the cloning and characterisation of a second subunit of the IL-1 receptor, the IL-1 receptor accessory protein (IL-1RAcP), associating with IL-1RI, has been reported [16]. IL-1RAcP increases the binding affinity of IL-1RI when the two are coexpressed [16], and it plays an important role in IL-1 signalling restoring IL-1 induced responses in an IL-1 non-responsive cell line expressing IL-1RI, but lacking constitutive IL-1RAcP [24,25,43,44]. IL-1 stimulation evokes the aggregation of the IL-1RI with the accessory protein,

*Corresponding author. Fax: (39) (51) 583593.
E-mail: marmiroli@area.bo.cnr.it

Abbreviations: IL-1, interleukin 1; IL-1RI, IL-1 receptor I; IL-1RAcP, IL-1 receptor accessory protein; NF-κB, nuclear factor κB; TNF, tumour necrosis factor; TRAF, TNF receptor associated factor; IRAK, IL-1 receptor associated kinase; PI 3-kinase, phosphatidylinositol 3-kinase; CAT, chloramphenicol acetyltransferase; NIK, NF-κB inducing kinase

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which in turn associates with the serine/threonine kinase IRAK. IRAK is therefore recruited to the activated IL-1RI [5,20], together with the adaptor protein TRAF6 [6]. However, neither TRAF6 nor IL-1RI/AcP are substrates of IRAK. A dominant-negative mutant of TRAF6 blocks IL-1 dependent activation of NF-κB [6], resembling another member of the TRAF family, TRAF2, which is required for NF-κB activation through the TNF receptors. TRAF6, as well as TRAF2, interacts with the NF-κB-inducing kinase NIK [6,29], leading to activation of NF-κB. At this point, signals from entirely unrelated receptors converge, hinting at a pathway common to cytokine receptors belonging to different families. Another unifying phenomenon connecting the signalling mechanism of the IL-1RI to other cytokine receptors has been the recent identification of a STAT-like factor activated by IL-1, LPS and IL-6, able to bind to GAS-like sequences within the IL-1B gene [42]. The STAT pathway signals directly to the nucleus, thus providing a direct connection with the transcriptional machinery. The STAT-like protein is phosphorylated on tyrosine and this phosphorylation is essential for DNA binding. Canonical STAT proteins are known to be activated through phosphorylation by Jak tyrosine kinases which, upon ligand binding, associate with the cytoplasmic domain of the activated receptor. Therefore, a STAT-like protein would require the presence of a tyrosine kinase activated by IL-1 and possibly associating to the receptor. However, tyrosine phosphorylation of the receptor has not been demonstrated yet, although an increase in the phosphotyrosine profile of cells treated with IL-1 has been observed. Several laboratories have recently demonstrated that phosphatidylinositol 3-kinase (PI 3-kinase) is a target for a variety of cell surface receptors [4], including cytokine receptors [35]. The identification, within the cytoplasmic domain of the IL-1RI, of the putative binding site for PI 3-kinase (Tyr-X-X-Met) prompted us to investigate whether PI 3-kinase is part of the signalling mechanism of IL-1.

In this article, we present evidence that PI 3-kinase is recruited and activated by IL-1RI through binding of the SH2 domains to the consensus sequence on the C-terminal tail of the receptor, and that Tyr^{479} in the IL-1 receptor is essential for PI 3-kinase activation.

2. Materials and methods

2.1. Materials

All cell culture products were from Flow. Radiochemicals, nitrocellulose membrane and ECL reagents were from Amersham. Protease inhibitors were from Calbiochem, Protein A-Sepharose CL-4B was from Pharmacia LKB. Anti-P-tyrosine (clone 4G10) and anti-p85 (06-195) were from UBI. Anti-IL-1 type I receptor (C-20) was from Santa Cruz (sc-687). Protein concentration was determined with a Bradford protein assay kit (Bio-Rad). All other products were from Sigma, unless specified otherwise.

2.2. Cell culture

Saos2 cells were obtained from the American Type Culture Collection and were cultured as described previously [30,37]. For experiments requiring growth factor-deprived cells, sub-confluent cells were maintained 48 h in serum-free medium, in the presence of 0.5% BSA, then cells were treated with IL-1β (2 ng/ml) for the indicated times.

2.3. Plasmid constructs

The wild-type human IL-1 type I expression vector (CNOD-IL-1R) was the kind gift of Peter R. Young (SmithKline Beecham, King of Prussia, PA, USA). Mutations were introduced by using recombinant polymerase chain reaction synthesis employing primers that contained a single base-pair mismatch [18]. Mutation specific primers were either 24 or 25 bp long and contained a single mismatch at a central position in which a TAT codon was changed to TTT for Y479F and a TAC was changed to a TTC for Y519F. Flanking primers contained upstream and downstream *Eco*RI restriction sites to permit cloning into an appropriately digested wild-type CNOD-IL-1R vector. The numbering convention used for the IL-1RI protein corresponds to that of the 552 amino acid mature protein which does not contain the 17 amino acid secretory signal [12].

2.4. Transfections and CAT assay

Stable clones carrying either WT, Y479F or Y519F receptors were obtained transfecting Saos2 cells that had been plated at 50% confluency the day before, using 10 μg of the expression vectors described above. Forty-eight hours after transfection, transformants were selected in medium containing the neomycin analog G418 (Sigma, St. Louis, MO, USA) at a concentration of 500 μg/ml. Clones were harvested and expanded separately 40 days in the presence of G418. Receptor expression was analysed by Western blotting.

For transient transfections, cells were grown in 175-cm^2 flasks to 70% confluence, then transfections were performed with the calcium-phosphate coprecipitation technique [1] in 10% FCS for 5 h. After transfection, cells were serum starved for 48 h and then treated with IL-1 (2 ng/ml). Total amount of DNA was kept constant by using empty vectors, and results were normalised to the level of β-galactosidase activity as described [1,2].

For CAT assay, cells were transfected with a reporter plasmid carrying four *rel* repeats upstream of the CAT gene [28]. After 48 h in serum-free medium, cells were treated for 6 h with 2 ng/ml of IL-1 and 50 μg of protein extracts, prepared in TEN solution (40 mM Tris-HCl, pH 7.5, 1 mM EDTA, 150 mM NaCl) with the freeze-thaw method [1], were assayed. The acetylated and non-acetylated forms of [^{14}C]chloramphenicol were separated by thin layer chromatography (TLC). Data is presented as percentage of acetylation and is the average of at least four independent transfection experiments, quantified using a Bio-Rad Molecular Imager.

2.5. Immunoprecipitation

To assay for PI 3-kinase activity, cell monolayers were washed twice with cold phosphate-buffered saline (PBS) and lysed directly in IP buffer (20 mM HEPES, pH 7.5, 1% NP-40, 50 mM NaF, 100 mM NaCl, 1 mM Na_3VO_4, 0.5 mM PMSF, 10 μg/ml leupeptin, 10 μg/ml aprotinin, 10 μg/ml pepstatin, 5 μg/ml calpain inhibitors), then cleared at $7000\times g$ in microfuge for 15 min at 4°C. Lysates were precleared by centrifugation and then immunoprecipitation was carried out incubating 1 mg lysates with anti-P-tyr (5 μg), anti-IL-1RI (2.5 μg) or anti-p85 (1 μl) antibodies for 2 h at 4°C with constant agitation, followed by 1 h incubation with 50 μl of Protein A-Sepharose (10% w/v). Pellets were washed three times in PBS containing 1% NP-40, twice in 0.1 M Tris, 0.5 M LiCl and twice in TNE. All buffers contained 100 μM Na_3VO_4. Pellets, resuspended in 50 mM HEPES, pH 7.5, 25 mM $MgCl_2$, were preincubated at room temperature (r.t.) for 10 min with PI vesicles (10 μg/sample), then [γ-^{32}P]ATP was added (10 μCi/sample) and pellets were further incubated at r.t. for 20 min. The reaction was stopped with 1 M HCl and methanol/chloroform (1:1). The organic phase was resolved on oxalate-coated TLC silica plates (Silica Gel 60, Merck) and the results quantified using a Bio-Rad GS-250 Molecular Imager.

2.6. Binding assay

Cells were lysed in IP buffer as described above. Lysates of unstimulated and IL-1 stimulated cells were boiled in 1% SDS, diluted 10 times with lysis buffer, and incubated for 2 h at 4°C with 2.5 μg of the p85 C-terminal or N-terminal SH2-GST fusion protein conjugated with agarose beads (UBI), with constant agitation. Beads were washed as described for immunoprecipitation procedures, resuspended in 30 μl of 2× Laemmli's buffer containing 10 mM glutathione and boiled for 10 min at 100°C. Pellets were then separated by centrifugation and proteins resolved by 7.5% SDS-PAGE, transferred to nitrocellulose filter and revealed using anti-p85 or anti-pTyr antibody (4G10). In some experiments, the filters were stripped by exposing them to 62.5 mM Tris (pH 6.8), 0.1 M β-mercaptoethanol, 2% SDS at 50°C for 30 min. Filters were then reprobed with different antibodies as described.

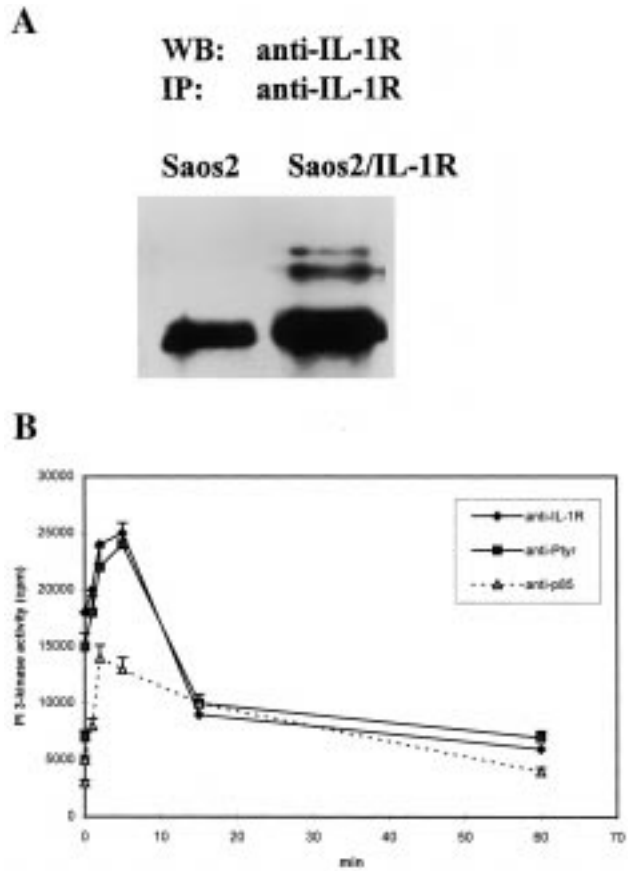

Fig. 1. IL-1 activates PI 3-kinase in Saos2/IL-1RI cells. A: Saos2 cells were stably transfected with wild-type IL-1RI expression vector (Saos2/IL-1R), or untransfected (Saos2) and 500 μg of cell lysate were immunoprecipitated with 2.5 μg of anti-IL-1RI (C-terminal, Santa Cruz). B: Serum-starved Saos2 or Saos2/IL-1R cells were either left untreated (time 0) or were treated with IL-1 (2 ng/ml) for the indicated times. PI 3-kinase activity was immunoprecipitated from 1 mg of Saos2/IL-1R cleared lysates using anti-IL-1R, 2.5 μg, or anti-Tyr(P), 5 μg antibodies, and from Saos2 using anti-p85 (1 μl) antibody, and assayed using exogenous PtdIns as a substrate. $n = 3$ experiments.

3. Results

3.1. Association of p85 with the activated IL-1 receptor

Initial studies of the biochemical basis of responses to IL-1 have been hampered by the low number of receptors, which renders their detection by conventional immunochemical techniques very difficult. To increase the number of IL-1RI, Saos2 cells were stably transfected with a plasmid containing the full length human cDNA for type I receptor (Saos2/IL-1RI).

The IL-1RI can be easily immunoprecipitated from Saos2/IL-1RI (Fig. 1A). Conversely, from cells which do not overexpress IL-1RI, the band corresponding to the receptor is undetectable. In the transfected cells, the receptor is detected as a doublet representing both the non-glycosylated 60-kDa and the larger, 80-kDa glycosylated forms [12].

IL-1 treatment of Saos2/IL-1RI cells led to a very rapid increase of PI 3-kinase activity, as detected by immune-complex kinase assay using anti-IL-1RI as the immunoprecipitating antibody (Fig. 1B). The time course of IL-1-dependent PI-3 kinase activity in IP from Saos2 cells was compared using anti-p85, anti-IL-1R and anti-P-Tyr antibodies. In all cases the activity was near maximal between 2 and 5 min. Furthermore, the relative increase in PI 3-kinase activity in the anti-IL-1R IP was similar to the relative increase observed in the anti-P-Tyr IP. A sustained PI 3-kinase activity was maintained for at least 30 min after exposure to IL-1. In untransfected Saos2 cells, the effect of IL-1 stimulation on PI 3-kinase activity is less pronounced, although still detectable. The effect was observed at a physiological dose of IL-1 (2 ng/ml), and doses up to 100-fold higher than this did not significantly modify this response (not shown).

IL-1R and p85 coprecipitate from lysates of Saos2/IL-1RI cells stimulated with IL-1, using the 4G10 anti-phosphotyrosine or anti-receptor antibodies, as shown in Fig. 2A. The amount of p85 recruited by IL-1R, as detected by immunoprecipitation with the anti-receptor antibody, was already detectable 2 min after stimulation with IL-1, and increased at 5 min. p85 was also detectable, with the same time course, in anti-phosphotyrosine immunoprecipitates, showing the association of p85 with some tyrosyl-phosphorylated protein complex. The IL-1 induced association of PI 3-kinase with the

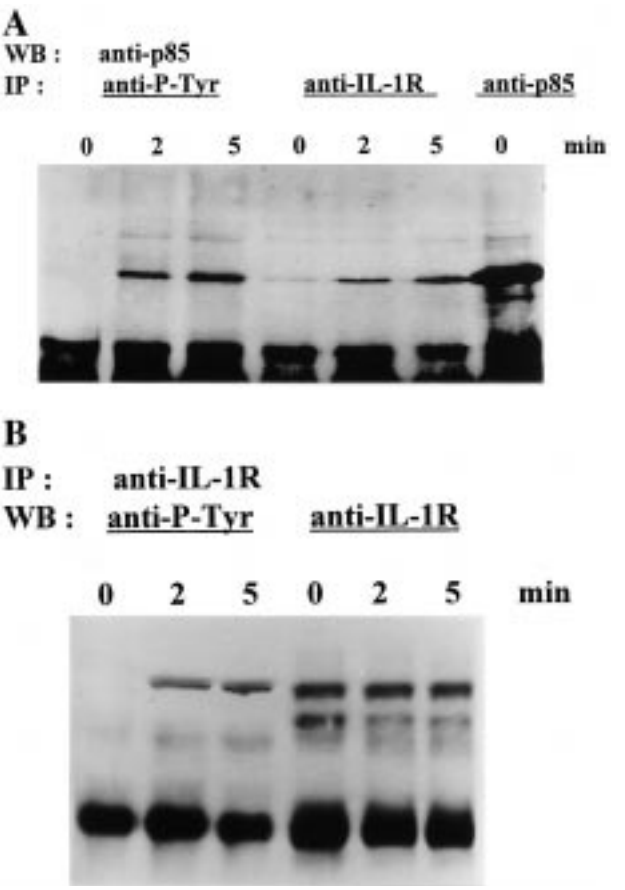

Fig. 2. A: IL-1 stimulates the association of PI 3-kinase with the IL-1R. Serum-starved Saos2/IL-1R cells were untreated (lanes 1, 4) or treated with IL-1 (2 ng/ml) for 2 (lanes 2, 5) or 5 (lanes 3, 6) min. Cleared lysates (1 mg) were immunoprecipitated with anti-P-Tyr (5 μg) (lanes 1, 2, 3), anti-IL-1R (2.5 μg) (lanes 4, 5, 6), or anti-p85 (1 μl) (lane 7). Then proteins were resolved by SDS-PAGE (7.5%), transferred to nitrocellulose and probed with anti-p85 antibody (1:3000). B: IL-1R is tyrosine phosphorylated in response to IL-1. Lysates were immunoprecipitated with anti-IL-1R (2.5 μg), and nitrocellulose was probed with anti-phosphotyrosine antibody (clone 4G10, UBI) (1:1000), or anti-IL-1R (1:750), as indicated.

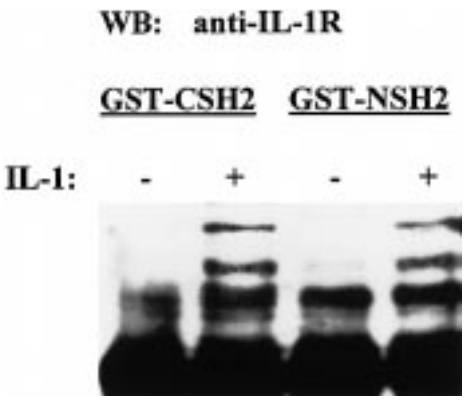

Fig. 3. Association of IL-1RI with GST-SH2 domains. Serum-starved Saos2/IL-1R cells were untreated or treated with IL-1 (2 ng/ml), as indicated. Cleared lysates were boiled in 1% SDS, diluted 10 times in lysis buffer and incubated with 5 μg of the C-terminal or the N-terminal GST-SH2 fusion proteins, conjugated with agarose beads. Pellets were boiled in Laemmli buffer plus 10 mM glutathione, subjected to SDS-PAGE and immunoblotted using anti-IL-1RI antibody (1:750 dilution).

activated IL-1R most likely occurs through an interaction between the SH2 domains of p85 and either one or more phosphorylated tyrosines within the IL-1R cytoplasmic tail or a phosphorylated protein intermediate. Here we show that the receptor can be rapidly Tyr-phosphorylated in response to IL-1 (Fig. 2B), thus creating binding sites for p85 SH2 domains. The association of the phosphorylated receptor with p85 could be direct or indirect. To test for direct binding, we utilised the fact that SH2 domains recognise linear phosphopeptide sequences that persist after the three-dimensional structure of the proteins has been disrupted by denaturation [27]. Therefore, lysates from Saos2/IL-1RI cells were boiled in 1% SDS, diluted 10 times in lysis buffer, then incubated with the immobilised p85 C-terminal and N-terminal SH2 domains GST fusion proteins. Although with slightly different affinity, both domains were able to associate with the receptor in denaturing conditions, thus showing that the interaction is direct, in an IL-1 dependent manner (Fig. 3). The data reported so far show that IL-1 stimulates PI 3-kinase activity, and that the activated IL-1 receptor recruits PI 3-kinase, presumably through the formation of a phosphotyrosyl complex with p85-SH2 domains, as suggested by in vitro binding data. The precise mapping of the p85 binding site on the IL-1 receptor, however, requires the expression of receptor mutants lacking the motif predicted to bind this domain. For this purpose, the Y479F receptor mutant, which contains a Tyr to Phe point mutation in the IL-1 receptor p85 consensus sequence, and the Y519F mutant, containing a Tyr to Phe mutation at an irrelevant position, were generated. The wild-type and mutant receptors were expressed in Saos2 cells, and cells were stimulated with IL-1. While cells expressing the wild-type or the Y519F mutated receptors retained their ability to induce activation of PI 3-kinase in response to IL-1, expression of the Y479F receptor mutant completely prevented this activation (Fig. 4A). Moreover, we assayed the IL-1-induced association of p85 with IL-1RI in cells expressing the Y479F receptor. IL-1 treatment of these cells yielded no detectable p85 coprecipitated with the receptor (Fig. 4C), clearly indicating that Tyr^{479} is required for p85 recruitment by the activated IL-1RI.

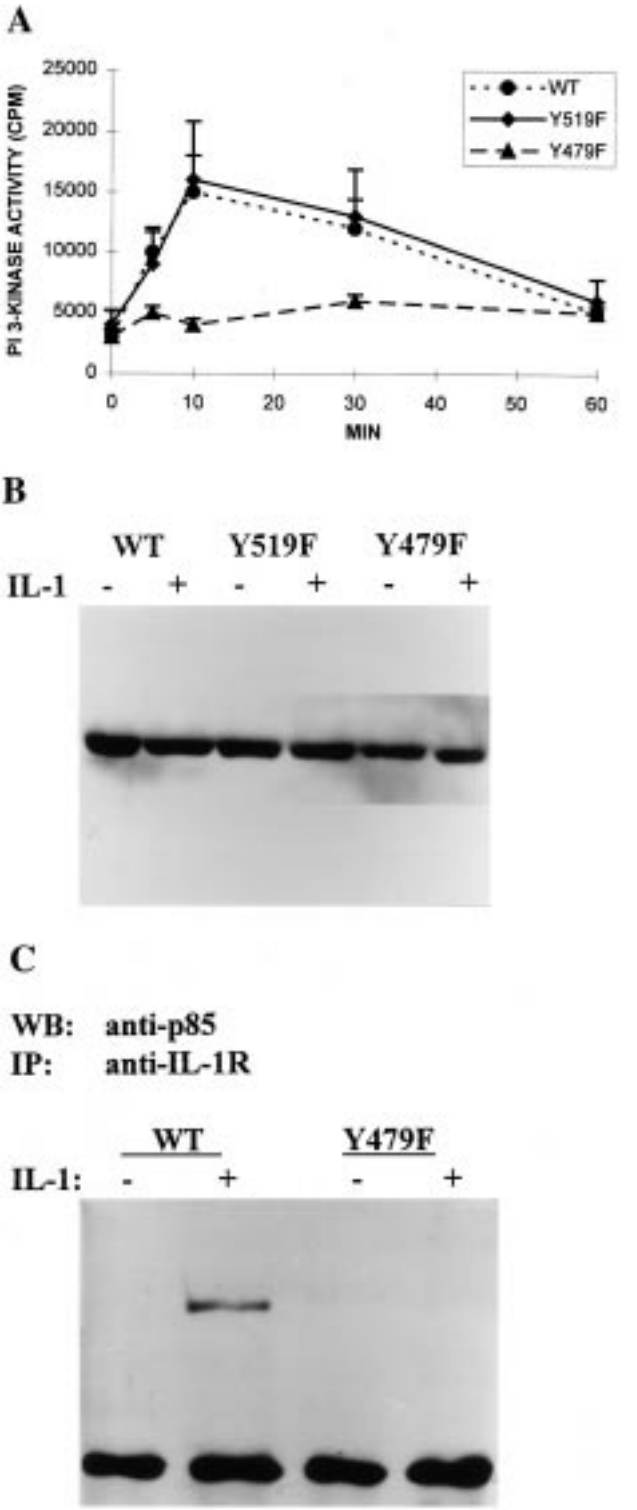

Fig. 4. Effect of receptor mutants on PI 3-kinase activity and association. Human Saos2 cells were transiently transfected with wild-type (WT), mutant $Tyr^{519}Phe$ or $Tyr^{479}Phe$ IL-1RI expression vectors. Then, cells were serum-starved for 48 h and treated with IL-1 (2 ng/ml) for 5 min. Cleared lysates immunoprecipitated with anti-IL-1R antibody were split and used for PI 3-kinase activity (A), Western blotting with anti-IL-1R (B), or anti-p85 (C).

3.2. Induction of transcriptional activation

IL-1 can induce specific genes through the transcription

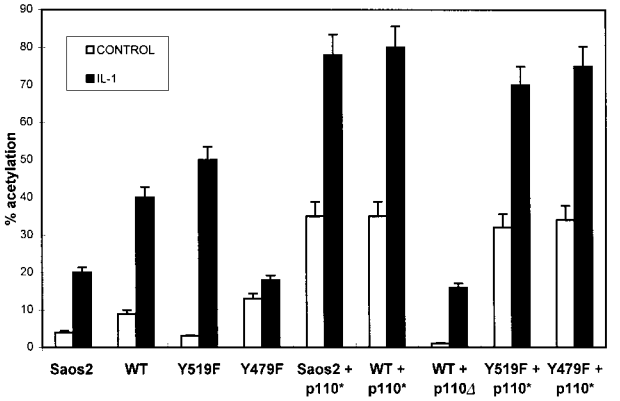

Fig. 5. Effect of IL-1 and activated PI 3-kinase on gene expression. Cells were co-transfected with a 4× NF-κB-CAT reporter plasmid and the different expression vectors indicated in the figure (10 μg each), plus 4 μg of the β-galactosidase vector pCH110 (Pharmacia Biotech); the amount of total DNA was kept constant by using empty vectors. Where indicated, 48-h serum-starved cells were stimulated for 6 h with IL-1 (2 ng/ml) before extraction for CAT assay. Results represent five different transfections, and are normalised to the β-galactosidase activity present in the extracts.

factor NF-κB [33,39], suggesting that complex, cell-specific molecular mechanisms operate to transduce a signal from the activated IL-1 receptor to the nucleus.

We performed transient transfection experiments using a κB-CAT reporter plasmid, carrying four *rel* repeats in front of the CAT cDNA, which had been previously shown to respond specifically to NF-κB activation [28]. Expression of κB-CAT in Saos2/IL-1R leads to an IL-1-dependent increase in the transcriptional activation of NF-κB. Similar results were obtained with the Tyr^{519} receptor mutant. However, expression of the κB-CAT reporter in cells carrying the Tyr^{479} mutant failed to enhance transactivation significantly (Fig. 5). This data shows that the Tyr^{479} residue is important to trigger NF-κB transcriptional activity by IL-1, and suggests an involvement of PI 3-kinase in this pathway. However, since the use of receptor mutants may affect signalling pathways other than one mediated by PI 3-kinase, the role of PI 3-kinase in IL-1-mediated induction of NF-κB transcriptional activity was investigated by co-expression of the κB-CAT reporter with a constitutively active form of p110 (p110*) [19] in Saos2 cells. Expression of p110* with κB-CAT in serum-starved Saos2 cells resulted in a striking induction of CAT activity in the absence of stimulus, and stimulation of CAT activity was completely independent of the receptor type (Fig. 5). Activation of CAT by p110* expression was further enhanced by IL-1 treatment; conversely, in Saos2 cells expressing a kinase dead mutant p110 (p110Δ kin) [19], basal CAT activity was extremely low and the response to IL-1 was almost completely lost, confirming specificity.

It has been recently demonstrated that the transactivation domain of the p65 subunit undergoes phosphorylation [32,38]. Although the precise function of this phosphorylation is still unclear, it features a further checkpoint in the regulation of NF-κB transcriptional activity, which may be under control of the IL-1 stimulated PI 3-kinase pathway. Alternatively, the target of PI 3-kinase could be a different member of the NF-κB transcriptional complex, which can interact with NF-κB in a DNA-independent manner, rather than NF-κB itself [3,13,41].

4. Discussion

Intracellular events linking the activation of IL-1 receptors to specific cellular responses are unclear. IL-1 activates the transcription factors NF-κB and AP-1 within minutes, transmitting a signal from the cell surface receptor to the nucleus. Depending on the nature of the target cell, IL-1 can elicit proliferative, differentiative, or metabolic responses [11]. How the specificity of each effect is controlled, is poorly understood. One level of control may lie in the specificity of the receptor-signalling complex formation. A number of components of the IL-1 receptor complex have recently emerged: activation of IL-1R induces association of the receptor with an accessory membrane-spanning protein, the IL-1RAcP, essential for full activation [16]. The activated IL-1RAcP complex recruits the serine/threonine kinase IRAK, through binding to the AcP cytoplasmic tail [20], and the adaptor protein TRAF6 [6]. The activated complex signals to a downstream kinase, NIK, which in turn induces the phosphorylation of I-κB by the I-κB kinase IKK-α [10,21,37], followed by NF-κB activation. Therefore, NIK activation by the TRAFs represents the point at which signals from IL-1 and TNF, although triggered by entirely unrelated receptors, converge. PI 3-kinase has been implicated recently in signal transduction from some cytokine receptors, such as the IL-2, IL-4, IL-7 and, more recently, IL-1 receptor [22,38,35]. In the case of IL-1, IL-2 and IL-4, recruitment of PI 3-kinase by the receptor was reported [38]. The observation that the IL-1RI cytoplasmic domain possesses a PI 3-kinase consensus sequence suggests involvement of PI 3-kinase in IL-1 signalling. Remarkably, the Y-E-X-M motif is highly conserved between human, mouse, chicken and *Drosophila* Toll [15]. It is worth mentioning that the IL-1R accessory protein [16], as well as MyD88 [31], contain a putative binding site for PI 3-kinase, analogous to the binding site on the erythropoietin receptor [9], which may represent a second binding site for p85. Here, we present evidence that PI 3-kinase is recruited by the activated, Tyr-phosphorylated IL-1R, as shown by coprecipitation experiments (Fig. 2). Furthermore, PI 3-kinase is rapidly and transiently activated following IL-1 treatment (Fig. 1). Binding experiments with recombinant GST fusion proteins containing the C- and N-terminal SH2 domains of p85 show that both domains are able to bind the activated IL-1 receptor in vitro (Fig. 3). Taken together, these data indicate that PI 3-kinase is recruited to the activated IL-1 receptor through binding of the SH2 domains of p85 to the C-terminal tail of the receptor. Our observations using receptors with a Tyr to Phe mutation in the putative PI 3-kinase binding domain clearly demonstrate that Tyr^{479} is essential for IL-1 induced PI 3-kinase activation and receptor association (Fig. 4). It is tempting to speculate about the physiological function of the IL-1 induced PI 3-kinase pathway in cellular responses to IL-1. The 3′-phosphoinositides have been shown to activate a growing number of kinases by binding to specific protein modules [34,14]. These kinases are therefore possible candidates as regulators of the multiple transcriptional effects driven by IL-1. Indeed, it is worth remembering that phosphorylation of NF-κB is required to trigger the response to the inducer [32], and it has been demonstrated that the activity of NF-κB can be modulated by phosphorylation of its active nuclear form at both p65 transactivation domains [38].

Furthermore, we demonstrate that activated PI 3-kinase

(p110*) induces NF-κB transactivation in the absence of stimuli, and that Tyr^{479}, in the PI 3-kinase binding site, is important for NF-κB transcriptional activity; coexpression of a κB-CAT reporter and the mutated receptors leads to a loss of IL-1 dependent CAT activity only in cells carrying the Tyr^{479}Phe receptor (Fig. 5). This strongly suggests that PI 3-kinase is involved in triggering this event by IL-1. The data obtained in our system suggest that expression of the Tyr^{479}Phe mutant impairs receptor-associated signals and blocks transcriptional activity preventing either the phosphorylation of NF-κB subunit or the activation of some distal factor interacting with NF-κB. Moreover, given its multifunctional structure, p85 association with the activated IL-1 receptor may serve as a platform for recruitment of additional proteins and this pathway, in combination with the known components of the IL-1 receptor complex, which depends upon a distinct part of the IL-1RI cytoplasmic domain, may generate the well known complexity of IL-1 biological functions. Despite many open questions, the identification of PI 3-kinase as a member of the IL-1 signalling pathway in Saos2 cells represents an important step towards a better understanding of the pleiotropic biological effects evoked by IL-1.

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