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SHORT REPORT

Interleukin-2 induces tyrosine phosphorylation of SHP-2 through IL-2 receptor β chain

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Coupling of interleukin-2 (IL-2) to the IL-2 receptor (IL-2R) induces rapid increase in tyrosine phosphorylation of cellular substrates through activation of non-receptor protein tyrosine kinases. Here, we report that stimulation through the IL-2R induced tyrosine phosphorylation of the SH2-containing protein-tyrosine phosphatase SHP-2 in F7, a hematopoietic BAF-B03 transfectant clone expressing the IL-2Rβ chain. The tyrosine phosphorylation of SHP-2 was specific since another protein-tyrosine phosphatase SHP-1, which is structurally homologous to SHP-2, was not tyrosine phosphorylated. The IL-2-induced tyrosine phosphorylation of SHP-2 required the acidic region within the IL-2Rβ chain where Src-family PTKs interact. Though the serine-rich region within IL-2Rβ chain was also required for the phosphorylation of SHP-2, Jak3 activation was dispensable. In COS-7 cells, co-expression of SHP-2 with Lyn resulted in increased tyrosine phosphorylation levels of SHP-2, whereas co-expression of SHP-2 with Fyn failed to alter the levels significantly. Considering that Lyn and Fyn are major Src-family PTKs expressed in BAF-B03 cells, our data suggest that Lyn may be principally responsible for the tyrosine phosphorylation of SHP-2 in F7 cells. Furthermore, the IL-2 stimulation also induced tyrosine phosphorylation of SHP-2 in the human IL-2-dependent T-cell line ILT-Mat. Taken together, these studies demonstrate an involvement of SHP-2 in the IL-2-mediated signaling events through the activation of specific PTKs.

Keywords: SHP-2; IL-2; tyrosine phosphorylation; signal transduction

In addition to the proliferation of T-cells, the ligand-induced activation of IL-2 receptor (IL-2R) induces several biological activities, such as proliferation and differentiation of B-cells, and activation of killing activity in natural killer cells. IL-2R consists of three distinct subunits, IL-2Rα, IL-2Rβ, and IL-2Rγ (Takeshita *et al*., 1992). The cytoplasmic domain of the IL-2Rβ chain carries at least two distinct regions crucial for their signalings (Hatakeyama, *et al*., 1989). One is the 'serine-rich' (S) region which is indispensable for IL-2-induced mitotic signaling and is required for the induction of c-*myc* and *bcl*-2 genes (Shibuya, *et al*., 1992; Miyazaki *et al*., 1995). The other is the 'acidic' (A) region which is responsible for its physical association with Src-family protein tyrosine kinases (PTKs), such as $p56^{lck}$ $p59^{fyn}$ or $p56^{lyn}$, and is required for the induction of c-*fos*/c-*jun* (Shibuya, *et al*., 1992) as well as a cytoplasmic protein tyrosine phosphatase (PTP) LC–PTP (Adachi *et al*., 1995). There is an increase in the tyrosine phosphorylation immediately after the binding of IL-2 to its receptor. This signaling is initiated by the activation of non-receptor PTKs, such as Src family, Syk-family and Jak family PTKs associated with the IL-2Rβ and γ chains (Taniguchi, 1995). So far, little is known about substrates of the PTKs and the consequences of the tyrosine phosphorylation during IL-2R-mediated signalings.

SHP-2 is a cytoplasmic PTP carrying two SH2 domains (Adachi *et al*., 1992, 1996; Freeman *et al*., 1992; Ahmad *et al*., 1993). SHP-2 binds directly to some activated growth factor receptors, such as platelet-derived growth factor (PDGF) and epidermal growth factor (EGF) receptors (Feng *et al*., 1993; Vogel *et al*., 1993). Furthermore, SHP-2 also binds to the signaling adaptor Grb2 and insulin receptor substrate-1 (IRS-1) upon activated tyrosine phosphorylation in response to PDGF and insulin, respectively (Lechleider *et al*., 1993; Kuhne *et al*., 1993), and transduces positive growth signals through the activation of Ras (Bennett *et al*., 1994; Noguchi *et al*., 1994). In addition, SHP-2 can be phosphorylated by the Src PTK *in vitro* (Peng *et al*., 1995). Recently, it has been found that stimulation of the cytokine/growth hormone receptor family such as erythropoietin, prolactin, IL-11, IL-3 and GM-CSF-mediated signals results in tyrosine phosphorylation of SHP-2 (Tauchi *et al*., 1996; Ali *et al*., 1996; Fuhrer *et al*., 1995; Welham *et al*., 1994). These accumulating data strongly suggest that SHP-2 may function in the regulation of tyrosine phosphorylation levels in a wide variety of signal transductions, including signalings mediated by receptor PTKs and Src family PTKs. Here, we show that SHP-2 is involved in signaling through IL-2R. SHP-2 is tyrosine phosphorylated by the stimulation of IL-2 in B- and T-cells, and this signaling requires both the serine and the acidic regions within the IL-2Rβ chain.

IL-2-induced tyrosine phosphorylation of SHP-2, but not SHP-1 in F7 cells

F7 is a murine hematopoietic cell BAF-B03 transfectant clone expressing the human IL-2Rβ chain. The parental

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BAF-B03 cells normally lack IL-2Rβ expression, but express endogenous IL-2Rα and IL-2Rγ chains at relatively high levels and they neither bind nor respond to IL-2. On the other hand, F7 cells bind IL-2 with a high affinity and proliferate in response to IL-2 (Hatakeyama *et al*., 1989). Since IL-2 stimulation induces tyrosine phosphorylation of a series of proteins through the activation of non-receptor PTKs (Saltzman *et al*., 1988), the IL-2-induced tyrosine phosphorylation of SHP-2 was examined by anti-SHP-2 immunoprecipitation followed by an anti-phosphotyrosine immunoblotting. IL-2 stimulation induced tyrosine phosphorylation of SHP-2, peaked at 10 min and declined thereafter, whereas the tyrosine phosphorylation of SHP-2 was undetectable in factor-deprived F7 cells (Figure 1a). Figure 1b shows representative experiments in which F7 cells were treated with IL-2 for 10 min with a range of concentrations of IL-2. A concentration-dependent increase in tyrosine phosphorylation levels of SHP-2 was clearly observed. In addition, 90 kD proteins (p90) and 130 kD proteins (p130) were co-immunoprecipitated with SHP-2 and the elevation of tyrosine phosphorylation levels of these associated proteins was also detected. The increase might reflect either an actual increase of tyrosine phosphorylation levels or an increase of their physical association with SHP-2.

Another SH2-containing protein-tyrosine phosphatase SHP-1 is predominantly expressed in hematopoietic cells and highly homologous to SHP-2 in its structure. To investigate whether IL-2 stimulation can also induce tyrosine phosphorylation of SHP-1, their tyrosine phosphorylation levels were monitored. When F7 cells were stimulated with IL-2 for 10 min, the tyrosine phosphorylation of SHP-2 and its associated proteins p90 and p130, were detected (Figure 2a). In contrast, tyrosine phosphorylation of SHP-1 could not be detected while that of SHP-1-associated p90 protein(s) was clearly detected (Figure 2a). Thus, IL-2 seemed to induce tyrosine phosphorylation of SHP-2, but not SHP-1, in F7 cells. This suggests that the IL-2-induced tyrosine phosphorylation of SHP-2 is selective, and that phosphorylation of SHP-2 may play a role in the regulation of the IL-2-mediated signal transduction. In addition, tyrosine-phosphorylated p90 proteins co-immunoprecipitated with SHP-1 or SHP-2 may also be involved in IL-2-mediated signalings. It is of interest whether these p90 proteins are the same protein and how the proteins function in IL-2-mediated signaling.

Tyrosine phosphorylation of SHP-2 by IL-2 stimulation in human T-cell line

ILT-Mat is a human adult T-cell leukemia cell line which can proliferate depending upon IL-2 stimulation. When IL-2-deprived ILT-Mat cells were stimulated with IL-2, tyrosine phosphorylation of SHP-2 increased, peaked at 10–30 min and declined thereafter (Figure 2b). As seen in F7 cells, IL-2 stimulation elevated tyrosine phosphorylation levels of SHP-2-associated p90 and p130 proteins (Figure 2b). These data demonstrate that IL-2-induced tyrosine phosphorylation of SHP-2 and its co-immunoprecipitated proteins is also detected in human T-cell line, and is not a limited event in a specific murine hematopoietic cell line.

Two distinct regions of IL-2Rβ are required for tyrosine phosphorylation of SHP-2

At least two functional regions which are cririca l for IL-2 signaling in the IL-2Rβ chain have been identified. The A region (313–382 aa) is a crucial site for the association with Src-family PTKs, and is required for induction of the c-*fos*/c-*jun* and LC–PTP genes by IL-2 (Shibuya *et al*., 1992; Adachi *et al*., 1995). Another region, S (267–322 aa) is a crucial site for association

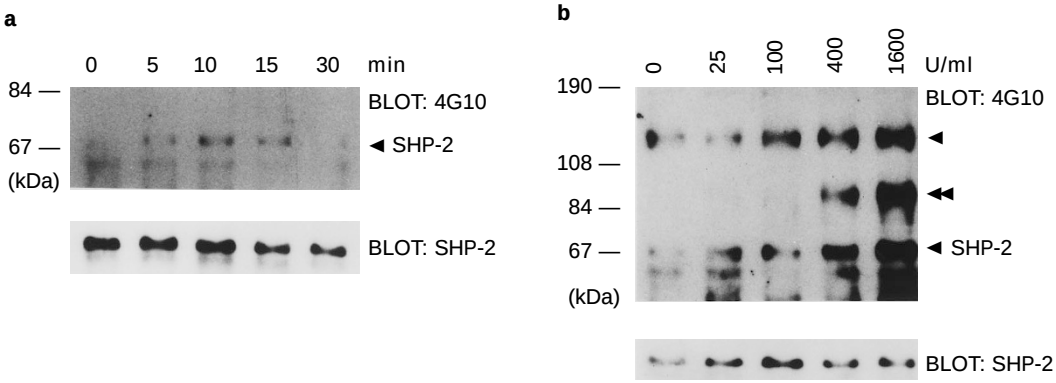

Figure 1 Time course and dose dependence of IL-2 for tyrosine phosphorylation of SHP-2 in BAF-B03 F7 transfectant. (**a**) BAF-B03 transfectants are IL-3-dependent, and are maintained in RPMI-1640 with 10% fetal calf serum (FCS) and 10% WEHI-3B conditoned medium (as a source of murine IL-3). F7 is a BAF-B03-derived transfectant expressing the wild type IL-2Rβ chain. Following factor-deprivation, F7 cells were washed twice with PBS, and resuspended in a medium without the cytokine for 12–18 h. The factor-deprived F7 cells were then treated with recombinant human IL-2 (400 U/ml, kindly provided by Shionogi Chemical Pharmacy, JAPAN), and total cell lysates were extracted from the cells at the indicated time points. SHP-2 was immunoprecipitated from the lysates of the F7 cells (1×10^7 cells) by using 4 μg of an anti-SHP-2 antibody (Santa Cruz) and subsequently analysed by immunoblotting using an anti-SHP-2 antibody or an anti-phosphotyrosine antibody 4G10 (Upstate Biotechnology) at 1 μg/ml. (**b**) The factor-deprived F7 cells were treated with IL-2 at the indicated concentrations (U/ml) for 10 min. Subsequent procedures were performed as described in the legend of (**a**). The positions of SHP-2 and associated tyrosine-phosphorylated proteins, p90 and p130, are indicated. Western blot analysis was performed by a standard ECL method (Amersham) as described previously (Adachi *et al*., 1995)

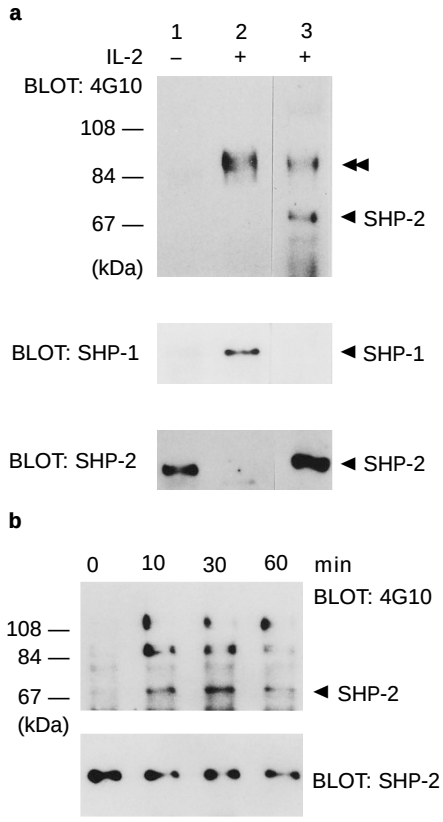

Figure 2 Tyrosine phosphorylation of SHP-2. (**a**) IL-2 induces tyrosine phosphorylation of SHP-2 but not SHP-1 in BAF-B03 F7 transfectant. The cytokine-deprived F7 cells were treated with IL-2 (400 U/ml), and total cell lysates were extracted from the cells at 10 min after the treatment. The lysates from the F7 cells (1×10^7 cells) were immunoprecipitated with either anti-SHP-2 antibody (lanes 1 and 3) or anti-SHP-1 antibody (lane 2) (Matozaki *et al*., 1994). The tyrosine phosphorylation of the immunoprecipitates was analysed by immunoblotting using 4G10. The positions of SHP-1, SHP-2 and their associated tyrosine-phosphorylated protein(s) p90 are indicated by closed triangles. (**b**) IL-2 induces tyrosine phosphorylation of SHP-2 in human T-cells. The IL-2-deprived ILT-Mat cells were treated with IL-2 (400 U/ml), and total cell lysates were extracted from the cells at the indicated time points. SHP-2 was immunoprecipitated from the lysates of ILT-Mat cells (1×10^7 cells) by using 4 μg of an anti-SHP-2 antibody (Santa Cruz) and subsequently analysed by immunoblotting using an anti-SHP-2 antibody or 4G10 (Upstate Biotechnology) at 1 μg/ml

with Syk (Minami *et al*., 1995) and Jak1 (Miyazaki *et al*., 1994), and is required for the c-*myc* gene induction and subsequent cellular proliferation (Hatakeyama *et al*., 1989). The S region-mediated signaling is known to be required to activate the A region-mediated signaling. To identify the region(s) of the IL-2Rβ chain responsible for the tyrosine phosphorylation of SHP-2, we employed two BAF-B03-derived transfectants expressing the mutant IL-2Rβs. A15 cells express the human IL-2Rβ chain lacking the A region and S25 cells express the IL-2Rβ chain lacking the S region (Hatakeyama *et al*., 1989). IL-2 stimulation in both clones failed to induce tyrosine phosphorylation of SHP-2 (Figure 3a). These findings therefore indicate that tyrosine phosphorylation of SHP-2 is specifically induced by IL-2 stimulation, and requires both the A and S regions of the IL-2Rβ chain.

IL-2-induced tyrosine phosphorylation of SHP-2 occurs independent of Jak3 kinase-mediated pathway

It has been shown that Jak1 and Jak3 PTKs are involved in the IL-2-induced signal transduction (Taniguchi, 1995). Jak1 and Jak3 are associated with the S region of the IL-2Rβ and IL-2Rγ chains, respectively, and their PTK activation is essential for the proliferative responses upon IL-2-stimulation. We examined tyrosine phosphorylation of SHP-2 in IL-2-deprived F7 transfectants expressing the wild-type Jak3 (FJ3-4) or a Jak3 mutant lacking the kinase domain

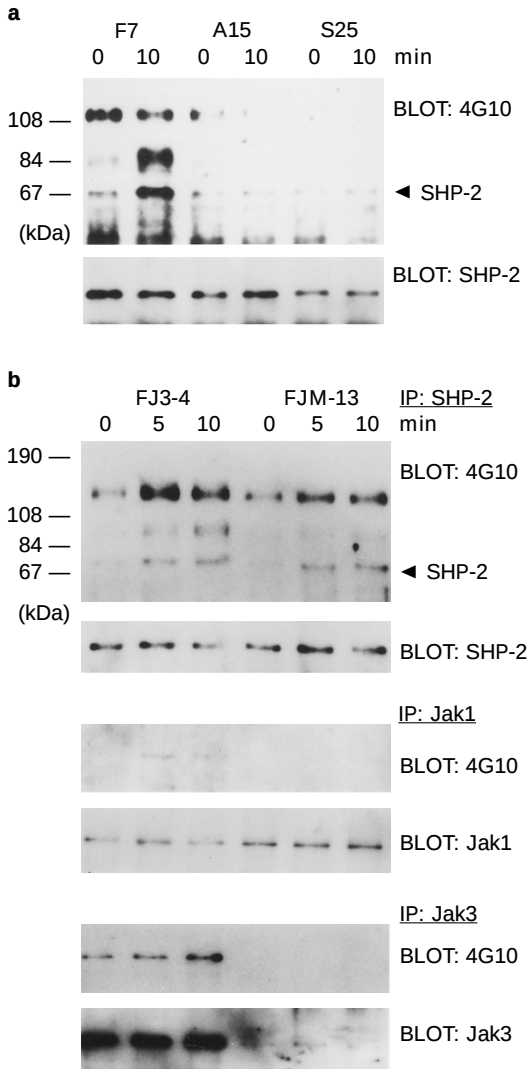

Figure 3 Tyrosine phosphorylation of SHP-2 by IL-2 in BAF-B03 transfectants. (**a**) The cytokine-deprived BAF-B03 transfectants (F7, A15 and S25) expressing the wild-type or mutant IL-2Rβ chain were treated with IL-2 for 10 min. The cell lysates were immunoprecipitated with anti-SHP-2 antibody, and subjected to immunoblot analysis using anti-SHP-2 antibody or 4G10. The position of SHP-2 is indicated. (**b**) The cytokine-deprived BAF-B03 transfectants (FJ3-4 and FJM13) were treated with IL-2 for 10 min, and SHP-2 was immunoprecipitated from the total cell lysates with anti-SHP-2 antibody. Using anti-Jak1 monoclonal antibody (Transduction Laboratories) and anti Jak3 antibody (Upstate Biotechnology), Jak1 or Jak3 was immunoprecipitated from the lysates of both transfectants (8×10^7 cells) which were treated with IL-2 for 10 min. Subsequent procedures were performed as described for (**a**)

(FJM-13) which can act as a dominant negative form of Jak3 following IL-2 stimulation (Kawahara *et al*., 1995). Overexpression of a Jak3 mutant strongly suppressed an increase of tyrosine phosphorylation levels of Jak1 and Jak3 after IL-2 stimulation in FJM-13 cells (Figure 3b). When the FJ3-4 cells were stimulated with IL-2, tyrosine phosphorylation levels of SHP-2 increased and reached peak levels at 10 min after the stimulation (Figure 3b). Although tyrosine phosphorylation levels of p130 and p90 were significantly decreased in FJM-13 cells, similar levels of IL-2-induced tyrosine phosphorylation of SHP-2 were observed in FJM-13 cells when compared to FJ3-4 cells. Moreover, the kinetics of the tyrosine phosphorylation of SHP-2 revealed no apparent difference between these clones. These data suggest that the activation of Jak3 kinase is not required for IL-2-induced tyrosine phosphorylation of SHP-2. In contrast, tyrosine phosphorylation of SHP-2-associated proteins, p130 and p90, in turn, could be mediated at least partially through Jak-family PTKs. However, the possibility cannot be excluded that endogenous Jak3 and Jak1 may still be active in FJM-13 cells, and their suppressed activation could be enough to induce tyrosine phosphorylation of SHP-2, but not of SHP-2-associated proteins.

SHP-2 is preferentially tyrosine phosphorylated by Lyn in COS cells

Src-family PTKs are associated with the A region of the IL-2Rβ chain which is crucial for the IL-2-induced tyrosine phosphorylation of SHP-2. Among Src-family PTKs, Fyn and Lyn are major PTKs expressed in F7 cells (Torigoe *et al*., 1992b), and IL-2 induces activation of both Src-PTKs (Kobayashi *et al*., 1993). To assess the possibility of tyrosine phosphorylation of SHP-2 by the Src family PTKs, co-transfection experiments of SHP-2 with the respective Src-family PTKs in COS cells were performed. From lysates of COS cells, SHP-2 was immunoprecipitated with anti-SHP-2 antibody and their tyrosine phosphorylation was analysed. Though protein levels of SHP-2 expressed in the COS-7 cells were comparable, the tyrosine phosphorylation levels of SHP-2 were quite different between *fyn*-transfected cells and *lyn*-transfected cells. A significant level of tyrosine phosphorylation of SHP-2 was seen in COS-7 cells co-transfected with *lyn*, whereas in contrast no significant level of tyrosine phosphorylation was seen in COS-7 cells co-transfected with *fyn* (Figure 4). Since both the protein levels and the autophosphorylation levels of the $p59^{fyn}$ and $p56^{lyn}$ revealed no significant difference between these COS-7 transfectants, this implies that SHP-2 may serve as a preferential substrate for Lyn rather than Fyn. Since Lyn is the most abundant Src-family PTK in F7 cells, SHP-2 may be tyrosine phosphorylated by Lyn in F7 cells upon IL-2 stimulation.

The A region-mediated signaling such as an activation of Src-family PTKs and an induction of c-*junc*/c-*fos* has been shown to require the S region of the IL-2Rβ chain. Jak-family PTKs are one of the signaling components responsible for the S region-mediated signaling. Overexpression of the dominant negative form of Jak3 abolish Jak1/Jak3 activation and thus resulting in inhibition of gene expression of c-*myc*

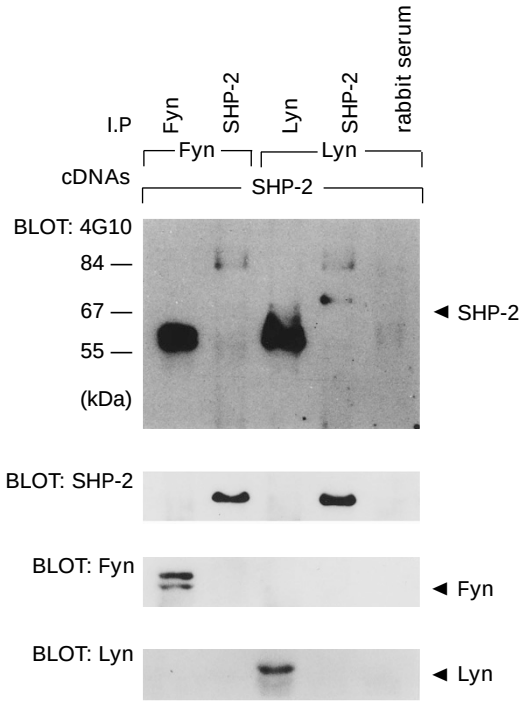

Figure 4 Tyrosine phosphorylation of SHP-2 by Src family kinases. COS7 cells were transfected with the following cDNAs: SHP-2 plus *fyn* or SHP-2 plus *lyn* (a kind gift from Dr Joan Bruggie). The lysates were immunoprecipitated with rabbit polyclonal anti-Fyn (Ishino *et al*., 1995), anti-Lyn antibodies (Torigoe *et al*., 1992a), anti-SHP-2 antibody, or preimmunized rabbit serum as indicated. The same filter was immunoblotted with 4G10, anti-SHP-2 anti-Fyn (Santa Cruz) and Lyn 8 (Yamanashi *et al*., 1992) antibodies. 5.0 μg of cDNAs was transfected to monolayers of COS-7 cells at a density of 3×10^4 cells/cm^2 by a DEAE-dextran method in the presence of 0.1 mM chloroquine (Sompayrack and Danna, 1981). After 60–72 h, the medium was removed and the cell lysates were subjected to an immunoprecipitation and immunoblot

and c-*jun*/c-*fos* as well as tyrosine phosphorylation of STAT3/5 (Miyazaki *et al*., 1995). Our data showing Jak-independent tyrosine phosphorylation of SHP-2 suggest that activation of Src-family PTKs may also be independent of the activation of Jak-family PTKs. There should be still unknown Jak-independent signaling molecules or PTKs associated with the S region in the IL-2Rβ chain, which may be responsible for the activation of Src-family PTKs and the phosphorylation of SHP-2. However, it cannot be ruled out that unidentified PTK(s) other than Src-family PTKs may be responsible for the tyrosine phosphorylation of SHP-2.

In normal T-cells, Lck but not Lyn is expressed and functions in IL-2 signaling pathways. SHP-2 is tyrosine phosphorylated by IL-2 stimulation in normal T-cells and Lck appears to be responsible for the phosphorylation since SHP-2 is tyrosine phosphorylated substantially when co-expressed with Lck in COS-7 cells (data not shown). In the IL-2-dependent human T-cell line ILT-Mat, SHP-2 was tyrosine phosphorylated by IL-2 stimulation (Figure 2b), probably through Lck. These data suggest that SHP-2 may participate in the regulation of several biological responses functionally linked to Src-family PTKs in lymphokine-dependent hematolymphoid cells. It has been shown that Lck-

mediated signaling has a crucial role in the cell-mediated cytotoxic function of T-cells (Karnitz *et al*., 1992), and activation of Src-family PTKs in T-cells resulted in enhanced adhesive capacity to ICAM-1 and enhanced cytotoxic activity (Torigoe *et al*., 1994). Furthermore, it has recently been demonstrated that Lck is involved in IL-2-induced cell proliferation (Miyazaki *et al*., 1995). As shown in our previous reports (Shibuya *et al*., 1992; Adachi *et al*., 1995), the rate of cell growth is slightly delayed in A15 cells in comparison with F7 cells. In this context, tyrosine phosphorylation of SHP-2 may also be involved in IL-2-induced cell proliferation.

Our data highlight the involvement of SHP-2 in IL-2 signaling, although the functional consequence of tyrosine phosphorylation of SHP-2 remains to be determined. We could not detect any significant increase of phosphatase activity of SHP-2 by its tyrosine phosphorylation (data not shown). Recent studies have demonstrated that SHP-2 can function as an adaptor protein in the signal transduction of some receptor PTKs (Bennet, *et al*., 1994). The tyrosine-phosphorylated SHP-2 associates with activated EGF, PDGF and Insulin receptors, and the blocking of this interaction inhibits their mitogenic signal transductions (Feng *et al*., 1993; Kazlauskas *et al*., 1993; Lechleider *et al*., 1993; Case *et al*., 1994; Xiao *et al*., 1994). Thus, SHP-2 may function in the recruitment of signal adaptors to activate Ras-mediated signaling pathway in the IL-2 signalings. Identification of the substrate proteins should further reveal a functional significance of SHP-2 in the IL-2 signal transduction.

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