Tyrosine phosphorylation of the Wiskott-Aldrich Syndrome protein by Lyn and Btk is regulated by CDC42

Rodolphe Guinamard[a], Pontus Aspenström[b], Michel Fougereau[a], Philippe Chavrier[a,*], Jean-Claude Guillemot[a]

[a]*Centre d'Immunologie INSERM-CNRS de Marseille-Luminy, Case 906, 13288 Marseille Cedex 9, France*
[b]*Ludwig Institute for Cancer Research, Biomedical Center, Box 595, 751 24 Uppsala, Sweden*

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Abstract The Wiskott-Aldrich syndrome (WAS) is a rare immunodeficiency disease affecting mainly platelets and lymphocytes. Here, we show that the WAS gene product, WASp, is tyrosine phosphorylated upon aggregation of the high affinity IgE receptor (FcεRI) at the surface of RBL-2H3 rat tumor mast cells. Lyn and the Bruton's tyrosine kinase (Btk), two protein tyrosine kinases involved in FcεRI-signaling phosphorylate WASp and interact with WASp in vivo. Interestingly, expression of a GTPase defective mutant form of CDC42, that interacts with WASp, is accompanied by a substantial increase in WASp tyrosine phosphorylation. This study suggests that activated CDC42 recruits WASp to the plasma membrane where it becomes phosphorylated by Lyn and Btk. We conclude that WASp represents a connection between protein tyrosine kinase signaling pathways and CDC42 function in cytoskeleton and cell growth regulation in hematopoietic cells.

Key words: Tyrosine phosphorylation; Wiskott-Aldrich syndrome gene product; CDC42; FcεRI

1. Introduction

The Wiskott-Aldrich Syndrome (WAS) is a severe disease that results in thrombocytopenia and abnormally sized platelets as well as susceptibily to recurrent infections and eczema [1]. The WAS gene has been recently cloned [2] and its product, WASp, interacts with the GTP-bound form of CDC42, a member of the Rho GTPase family [3–5]. Rho GTPases regulate actin cytoskeleton reorganization, G1 cell cycle progression and transcriptional regulation in response to several stimuli (for review see [6]) and WASp serves as an effector for CDC42 function in actin remodeling [4,7]. In vitro studies have described interactions between WASp and various protein tyrosine kinases (PTKs) [8–10]. Although tyrosine phosphorylation of WASp has not been reported yet, these different interactions point at an important function for WASp in hematopoietic cell signaling.

In mast cells, aggregation of FcεRI, the high affinity receptor for IgE, triggers various responses including release of inflammatory mediators of allergy and a dramatic reorganization of the actin cytoskeleton [11]. We have recently established that in RBL-2H3 rat tumor mast cells, two Rho GTPases, Rac1 and CDC42, control specific pathways leading to actin remodeling and serotonin release in response to FcεRI engagement [12]. As in other systems such as induction of stress fibers by lysophosphatidic acid [13], FcεRI-induced actin reorganization depends on tyrosine phosphorylation events and is blocked by tyrosine kinase inhibitors [14,15]. At least three classes of non-receptor tyrosine kinases are sequentially engaged during FcεRI activation: the Src-family kinases Src, yes and lyn [16]; Syk, a member of the Syk/Zap70-family kinases [17,18]; and finally, members of the Tec-family kinases such as Btk [19] which is defective in human X-linked agammaglobulinemia (XLA) [20] and murine X-linked immunodeficiency (Xid) [21].

An important question remains as to how PTK engagement couples to Rho GTPase activation and then to actin remodeling. On the one hand, PTKs may regulate the activity of guanine nucleotide exchange factors (GEF), i.e. the proteins that promote GDP/GTP exchange on Rho GTPases and thereby stimulate their transition to an active conformation. This hypothesis is supported by the finding that p95Vav, which becomes rapidly tyrosine phosphorylated upon FcεRI-crosslinking [22], is a Rac1-specific GEF whose activity is controlled by tyrosine phosphorylation [23]. p95Vav may therefore link early phosphorylation events to Rho GTPase (Rac1) activation. On the other hand, PTKs may also regulate the function/activity of Rho GTPase downstream effectors including the product of the WAS gene which interacts with isolated SH3 domains from Src- and Tec-family PTKs in vitro [8–10].

We have investigated the participation of WASp in FcεRI signaling. We demonstrate that WASp is constitutively tyrosine phosphorylated in RBL-2H3 cells and that phosphorylation is increased in response to FcεRI-aggregation. Overexpression of Lyn or Btk is sufficient to induce the phosphorylation of WASp and both PTKs are found complexed to WASp in RBL-2H3 cells. Finally, tyrosine phosphorylation of WASp by Lyn or Btk is enhanced upon coexpression of a GTPase-defective mutant form of CDC42, suggesting that interaction with activated CDC42 at the membrane facilitates tyrosine phosphorylation of WASp.

2. Materials and methods

2.1. Antibodies and reagents

Rat anti-DNP IgE (clone LO-DNP30) was obtained from LO-IMEX (Brussels, Belgium). Anti-rat FcεRIα chain (clone BC4) was kindly provided by Dr. R.P. Siraganian (NIH, Bethesda, MD, USA). The monoclonal anti-phosphotyrosine antibody 4G10 was purchased from Upstate Biotechnology. Anti-p53/p56 Lyn rabbit polyclonal antibodies were from Santa Cruz Biotechnology. FITC-configurated anti-CD16 mAb was obtained from Coulter-Immunotech (Marseille, France). The anti-BTK antibody is a rabbit affinity purified antiserum raised against amino acid residues 71–93 [24]. Anti-WASp rabbit antibodies were raised against amino acid residues 201–321 of human

*Corresponding author. Fax: (33) 491 269 430.
E-mail: chavrier@ciml.univ-mrs.fr

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WASp expressed as a fusion protein with GST after cleavage with thrombin and removal of the GST moiety. Horseradish peroxidase (HRP) conjugated goat anti-mouse and HRP conjugated protein A were purchased from Sigma. Unless indicated, chemicals were purchased from Sigma.

2.2. Expression constructs

The myc tag was inserted at the C-terminal end of WASp as follows. A 180-bp PCR fragment corresponding to the carboxy terminus of WASp was generated between a 5′ primer (5′-CAGGGAATTCAGCTGAAC-3′) spanning an internal *Eco*RI site in the WAS cDNA (underlined) and a 3′ primer (5′-CTACTACTTACCCTACTGCTTGTTTTTGAGTAGAGCTTCTCCTAGACACTTTCGAATTCAACTA-3′) coding for the myc epitope (double underlined) and including a stop codon and a *Hin*dIII restriction site (underlined). This fragment was subcloned into pGEM1 (Promega, Madison, WI, USA), sequenced and finally inserted in place of the wild-type sequence in the WAS cDNA cloned into pGEM1. The pGEM1-V12CDC42VHs construct has been already described [25].

2.3. Cell culture, vaccinia infection and transfection

Rat basophil leukemia (RBL-2H3) cells and baby hamster kidney (BHK-21) cells were maintained as described [12,26]. Transient expression studies were carried out using the vaccinia recombinant T7 RNA polymerase virus system (VT7, [27]). Cells seeded at a density of 1.5×10^6 cells/9-cm^2 well were infected the following day with VT7 at 3–5 pfu/cell. Coexpression of PTKs was obtained by coinfection with recombinant vaccinia viruses expressing the PTKs Btk, Btk*, BtkΔSH3, Lyn (a gift from D.J. Rawlings, UCLA, CA, USA) or with a vaccinia virus expressing a CD16/7/Syk chimera [28]. After 45 min infection at 37°C, cells were transfected with pGEM1 constructs using the Lipofectamine Reagent (Gibco-BRL) as previously described [12]. Cells were cultured for 6 h in DMEM supplemented with 10 mM HEPES, pH 7.2, and then lysed in ice cold buffer (1% NP-40, 10 mM Tris-HCl (pH 7.4), 150 mM NaCl, 100 mM sodium orthovanadate, 1 mM EDTA and protease inhibitors). After 20 min at 4°C lysates were centrifuged at 4°C for 20 min at 15 000 rpm. Stable WASp-myc transfected cell lines were generated by electroporation of the WASp-myc encoding sequence inserted into pNT-Neo as described [12].

2.4. GST fusion constructs, affinity purification and immunoblotting analysis

The GST-SH3 Btk construct (amino acid positions 217–277 of human Btk) and the GST-SH3 Btk construct with a proline-265 to leucine substitution have been described [24]. The GST-ProSH3 Btk construct was generated by PCR amplification of a cDNA fragment corresponding to amino acids 185–277 of human BTK using the following primers: 5′-GAGGGATCCACTAGTGAGCTGAAAAAGGTTGTGG-3′ and 5′-TAACCCGGGGATTCAATTGAGTCTTCTGCTTCAGGACAT-3′). The PCR product was subcloned into the pGEX-2T expression vector (Pharmacia Biotech) and selected clones were confirmed by sequencing. Production of GST fusion proteins was performed as described by the manufacturer (Pharmacia Biotech).

Immunoprecipitation or affinity purification with GST fusion proteins were performed at 4°C for 2 h in the presence of either specific antibodies bound to protein A-Sepharose (Pharmacia Biotech) or GST constructs bound to glutathione-Sepharose (Pharmacia Biotech). Purified proteins were extensively washed in lysis buffer, subjected to SDS-PAGE, and transferred to Immobilon-P membranes (Millipore). Membranes were blocked with 5% non-fat milk or 0.5% BSA in PBS/0.05% Tween 20, followed by incubation with the indicated antibodies. Bound antibodies were revealed with HRP-conjugated secondary antibodies or HRP-protein A and visualized by chemiluminescence with the ECL system (Amersham).

3. Results

3.1. FcεRI aggregation induces tyrosine phosphorylation of WASp

In order to purify WASp from cell lysates we made use of the observation that the isolated SH3 domain of Btk interacts with WASp in vitro [9,10]. Lysates of unstimulated RBL-2H3 cells were incubated with GST fusion proteins comprising the Btk SH3 domain alone (GST-SH3) or extended N-terminally to a proline-rich region of Btk (GST-proSH3). We chose this strategy because the rabbit polyclonal antibodies we have raised against WASp were inefficient for immunoprecipitation analysis. After extensive washing, immunoblotting analysis of bound proteins with anti-WASp antibodies showed that both GST recombinant proteins interacted with WASp (Fig. 1A, lanes 1 and 3). As we previously described [24], the introduction of a point mutation in the Btk SH3 domain (Pro^{265} to Leu) prevented this interaction (Fig. 1A, lanes 2 and 4). Since binding appeared more specific with the proline-rich containing fusion protein ([24] and data not shown), the proSH3 construct was used throughout for the subsequent experiments.

As shown in Fig. 1B immunoblotting analysis with anti-phosphotyrosine mAb (4G10 blot, upper panel) revealed that following crosslinking of FcεRI, three phosphotyrosine containing proteins migrating with an apparent molecular weight of ∼65, ∼75 and ∼125 kDa could be purified from RBL-2H3 cell lysate using the Btk GST-proSH3 construct (lane 3). We identified the 125-kDa molecular weight species (asterisk) as Cbl (data not shown). The identity of the ∼75-kDa (arrow) protein is presently unknown. Analysis of unstimulated cell lysate detected only a very low amount of tyrosine phosphorylated 65- and 125-kDa proteins (Fig. 1B, lane 1). The blot was stripped and reprobed with Anti-WASp that revealed a protein comigrating with the 65-kDa phosphotyrosine containing species (lanes 1 and 3, middle panel). The identity of the 65-kDa protein was further confirmed by transiently expressing C-terminally myc-tagged WASp. Transfected RBL-2H3 cells were lysed prior or 5 min after FcεRI crosslinking and lysates were incubated with the GST-proSH3 construct. Immunoblotting analysis with anti-phosphotyrosine mAb revealed an additional polypeptide that migrated slightly slower than endogenous WASp (upper panel, lanes 2 and 4) and which was detected by both anti-WASp and anti-myc tag antibodies (middle and lower panels, respectively). As a final proof of the identity of the 65-kDa phosphotyrosine protein as WASp, stably transfected RBL cells expressing WASp-myc were lyzed without or after pervanadate treatment and WASp-myc was immunoprecipitated with anti-myc tag mAb. Anti-phosphotyrosine immunoblotting analysis detected the presence of tyrosine phosphorylated WASp-myc in the anti-myc immunoprecipitate from stimulated cells (Fig. 1C, lane 4).

Altogether, these results demonstrate that the 65-kDa WASp protein exhibits constitutive tyrosine phosphorylation that is strongly increased upon FcεRI stimulation in RBL-2H3 cells.

3.1.1. Overexpression of Lyn induces tyrosine phosphorylation of WASp. In an attempt to identify the PTK(s) responsible for WASp phosphorylation, we overexpressed Lyn, Btk and Syk that are involved in FcεRI signaling [29]. Expression of the PTKs was achieved upon infection of RBL-2H3 cells with recombinant vaccinia viruses (see Fig. 2B). Anti-phosphotyrosine immunoblot of total cell lysates showed that overexpressed Syk and Lyn induced the tyrosine phosphorylation of a number of cellular proteins (Fig. 2A, lanes 1–2 and 5–6), while Btk or an activated PH domain mutant form of Btk, Btk*, [30] were inactive (lanes 3–4 and data not shown). Anti-phosphotyrosine immunoblot analysis of the proteins

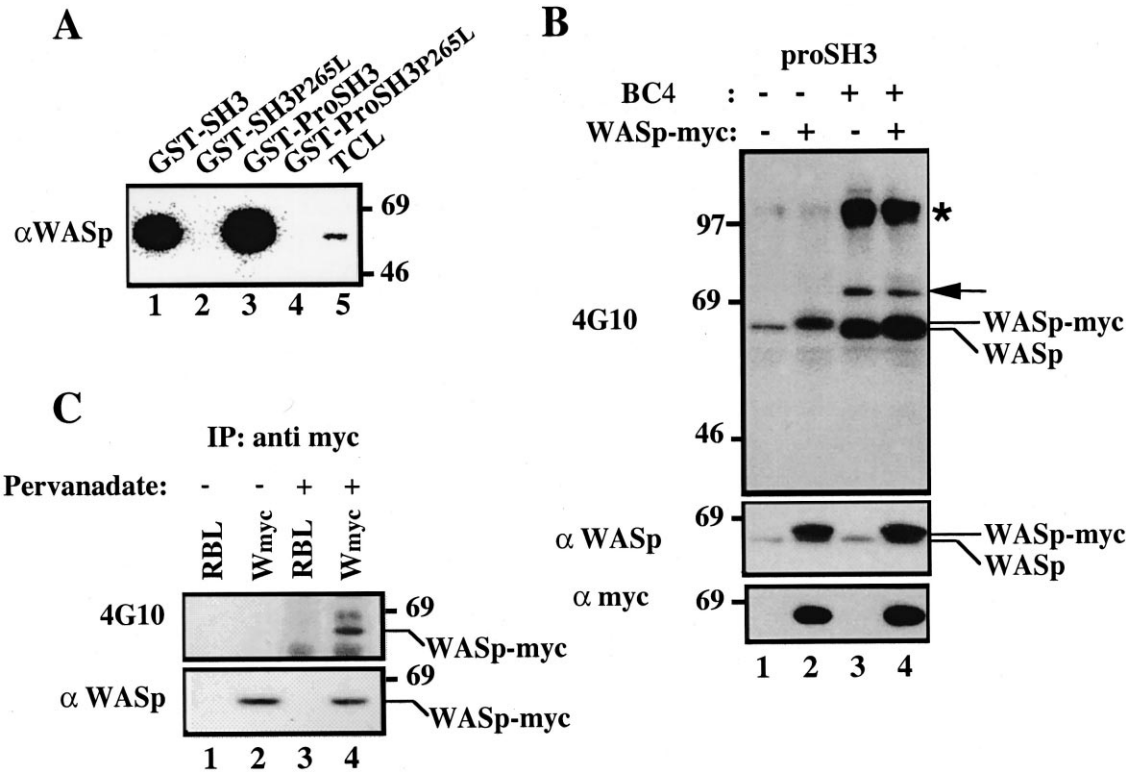

Fig. 1. WASp is tyrosine phosphorylated in RBL-2H3 cells. A: RBL-2H3 cell lysates were incubated with GST constructs corresponding to different domains of Btk bound to gluthatione-Sepharose beads: isolated Btk SH3 domain (lane 1); Pro^{265} to Leu substitution in the Btk SH3 domain (lane 2); Btk SH3 domain extended with the amino-terminal proline-rich region of Btk (lane 3); and mutated GST-ProSH3P265L (lane 4). The bound proteins were separated by SDS-PAGE, electrotransferred and immunoblotted with anti-WASp antibodies (αWASp). In lane 5, total cell lysate corresponding to one fiftieth of the material used for affinity purification was immunoblotted with anti-WASp. B: RBL-2H3 cells either non-stimulated (lane 1) or stimulated for 5 min by FcεRI crosslinking with anti-FcεRI α chain mAb (clone BC4, lane 3). RBL cells transiently overexpressing C-terminally myc-tagged WASp unstimulated (lane 2) or after FcεRI crosslinking (lane 4). Cell lysates were incubated with Btk GST-ProSH3 gluthatione-Sepharose beads. Bound proteins were separated by SDS-PAGE and immunoblotted with anti-phosphotyrosine mAb (4G10). The asterisk represents a 125-kDa tyrosine phosphorylated protein further identified as Cbl (data not shown). Arrow: unidentified 75-kDa species. The blot was stripped and successively probed with anti-WASp antibodies (αWASp) and then with anti myc-tag mAb (αmyc). C: Lysates of control RBL cells (RBL) or RBL cells stably transfected with WASp-myc construct (Wmyc) were stimulated with pervanadate for 10 min. WASp-myc was immunoprecipitated with anti-myc tag mAb and immunoblotted with anti-phosphotyrosine mAb (4G10). After stripping, the blot was reprobed with anti-WASp antibodies (αWASp). Molecular weight standards are indicated (kDa).

bound to the Btk GST-proSH3 construct showed that overexpression of Lyn was sufficient to induce phosphorylation of both endogenous and myc-tagged WASp (Fig. 2C, lanes 5 and 6). In contrast, overexpression of Syk did not result in any detectable phosphorylation of WASp (lanes 1–4). In agreement with the absence of overall phosphorylation in total cell lysates, WASp was not phosphorylated in Btk overexpressing RBL-2H3 cells (lanes 3 and 4).

3.1.2. CDC42 modulates WASp phosphorylation by Lyn and Btk. In contrast to RBL-2H3 cells, infection of BHK cells with recombinant vaccinia viruses encoding Btk induced the phosphorylation of several cellular proteins and this phosphorylation pattern was further increased upon expression of Btk* (data not shown). In order to examine whether WASp may be a substrate for Btk, BHK cells which do not express detectable endogenous levels of WASp were transiently transfected with a WASp-myc construct in combination with Btk, Btk* or Lyn as a control. As shown in Fig. 3, anti-phosphotyrosine immunoblotting analysis (4G10) demonstrated that Btk (lane 5) and to a greater extent the activated Btk* (lane 10) induced tyrosine phosphorylation of WASp-myc, although they were less potent than Lyn (lane 8).

Rho GTPases are believed to regulate the translocation of specific effectors to target membrane sites of the cell. Therefore, we tested the possibility that overexpression of V12CDC42, a GTPase-defective mutant form which should bind to WASp, may modulate WASp phosphorylation by facilitating its membrane recruitment. WASp-myc and V12CDC42 were coexpressed in combination with the various PTKs and WASp-myc was purified with the GSTproSH3 construct. Anti-myc tag immunoblotting analysis demonstrated that equal amounts of WASp-myc were recovered from the different cell lysates (αmyc blot, middle panel). In addition, anti-CDC42 antibodies could detect V12CDC42 in the material recovered with the GSTproSH3 beads (αCdc42 blot, lower panel). Recovery of V12CDC42 on the Btk GSTproSH3 beads did not occur in the absence of WASp-myc coexpression (data not shown). These findings indicate that WASp-myc and V12CDC42 were in a complex. Interestingly, expression of V12CDC42 resulted in a 3–5-fold increase in WASp-myc tyrosine phosphorylation by Btk, Lyn or Btk* (compare lanes 5 and 6; 8 and 9; 10 and 11, respectively).

3.1.3. Btk and Lyn directly associate with WASp. The above results clearly show that Lyn and Btk play a crucial role in WASp tyrosine phosphorylation and that this event is regulated by CDC42. The interaction of WASp to the isolated

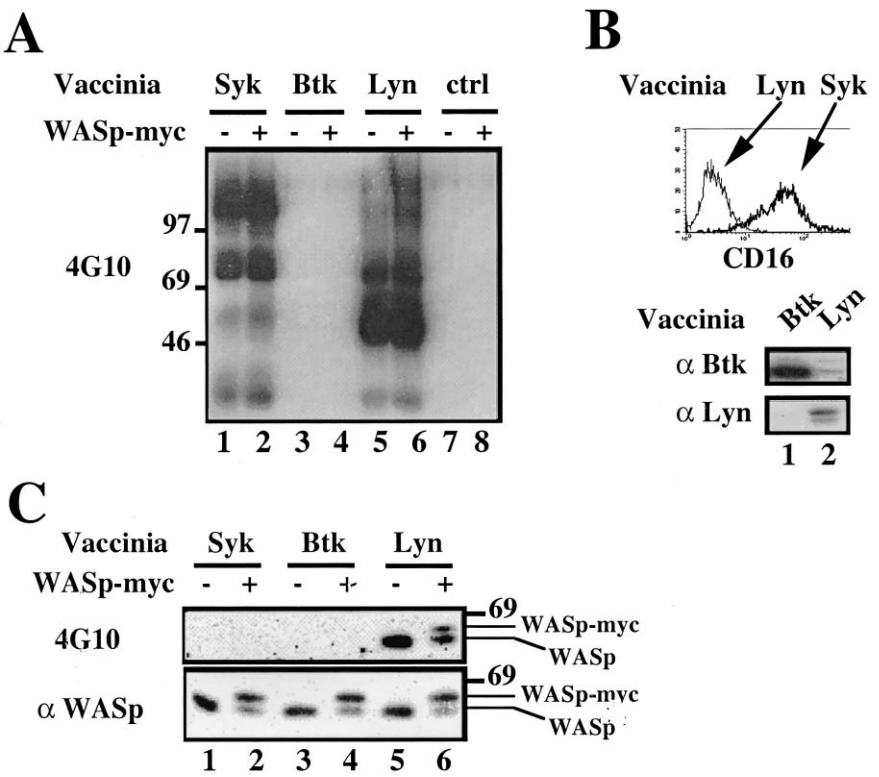

Fig. 2. Lyn-induced phosphorylation of WASp. A: RBL-2H3 cells (1.5×10^6) were infected with recombinant vaccinia viruses expressing the PTKs Syk, expressed as a transmembrane CD16/7/Syk chimeric protein [28] (Syk) or native Btk or Lyn. Total cell lysates were subjected to anti-phosphotyrosine immunoblotting analysis (4G10). B: Control for PTK expression. Upper panel: Surface expression of the CD16-containing chimera was demonstrated by FACS analysis with anti-CD16 mAb of CD16/7/Syk-infected RBL-2H3 cells (Syk) or Lyn-infected cells as a negative control (Lyn). Lyn and Btk expression was determined by immunoblotting analysis of Btk-(lane 1) or Lyn-(lane 2) infected cell lysates with anti-Btk (αBtk) or anti-Lyn antibodies (αLyn). C: RBL-2H3 cells expressing the CD16/7/Syk (Syk), Btk or Lyn in combination with myc-tagged WASp (+) or without (−) were lysed and incubated with the Btk GST-proSH3 fusion protein immobilized on glutathione-Sepharose beads. Bound proteins were separated by SDS-PAGE and after transfer were analyzed by immunoblotting with anti-phosphotyrosine mAb (4G10). After stripping, the membrane was reprobed with anti-WASp antibodies (αWASp) to demonstrate that endogenous and myc-tagged WASp were recovered in equal amounts from the different samples.

SH3 domain of Lyn or Btk in vitro ([8,10] and this study) suggests that WASp may directly associate with the PTKs in vivo. In order to investigate this possibility, RBL-2H3 cells expressing WASp-myc were infected with recombinant vaccinia viruses expressing the different PTKs Lyn, Btk or an SH3 domain deleted form of Btk (Btk-ΔSH3). Overexpressed Lyn or Btk were immunoprecipitated using specific antibodies and the immunoprecipitates were analyzed for the presence of myc

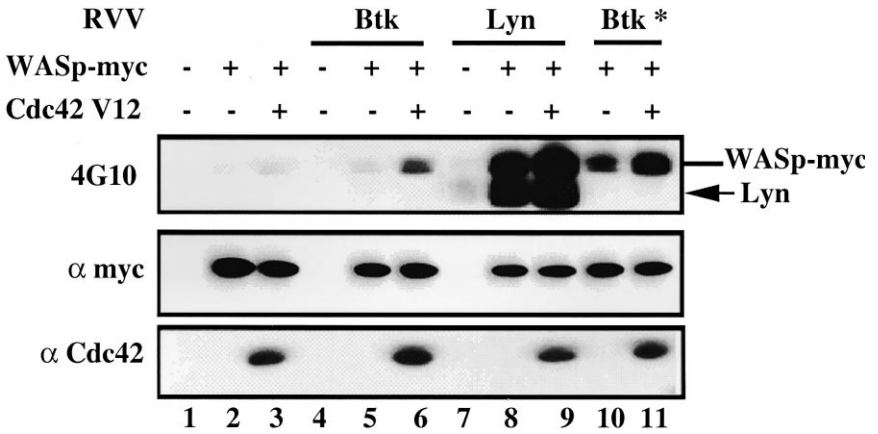

Fig. 3. V12CDC42 expression increases tyrosine phosphorylation of WASp by Lyn and Btk. BHK cells expressing WASp-myc alone or in combination with V12CDC42 were infected with recombinant vaccinia viruses coding for wild-type Btk, Lyn or the active PH domain mutant form Btk*. WASp-myc was recovered after binding to the Btk proSH3 construct and tyrosine phosphorylation was examined by immunoblotting with 4G10 mAb (upper panel). The lower molecular weight species which is detected in the Lyn-infected samples corresponds to Lyn (data not shown). Middle panel: the 4G10 blot was stripped and reprobed with anti-myc tag mAb to show relative recovery level. Lower panel: the blot was restripped and reprobed with anti-CDC42. Anti-Lyn and anti-Btk immunoblots demonstrated that the expression levels of the PTKs in total cell lysates were equal (data not shown).

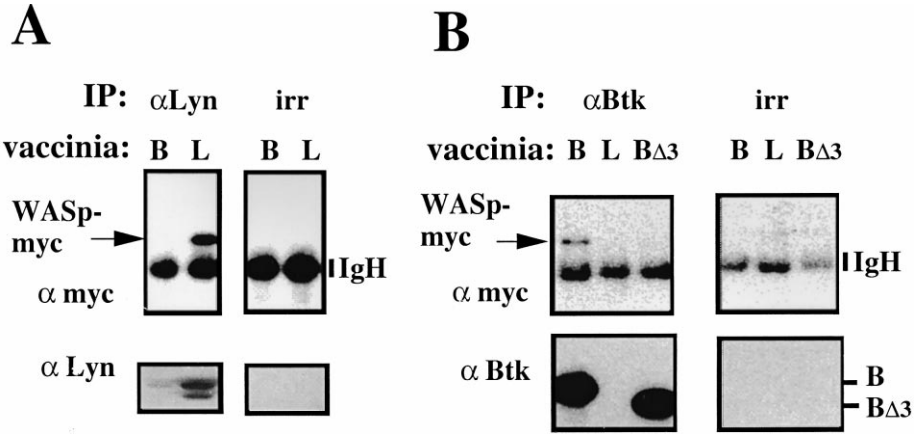

Fig. 4. Btk and Lyn directly associate with WASp in vivo. RBL-2H3 cells (1.5×10^6) overexpressing C-terminally myc-tagged WASp in combination with Lyn (L), Btk (B), or an SH3-deleted form of Btk (BΔ3) were lysed and Lyn or Btk were immunoprecipitated using anti-Lyn (αLyn, panel A) or anti-Btk (αBtk, panel B) specific rabbit antibodies. Specificity of the antibodies was demonstrated by immunoprecipitation using irrelevant rabbit antibodies (irr). After SDS-PAGE and electrotransfer the presence of WASp-myc (arrow) in the immunoprecipitates was assessed by immunoblotting analysis using anti-myc tag mAb (αmyc). The position of the rabbit IgG heavy chain that cross-reacted with the anti-mouse IgG secondary antibodies used for the detection is indicated (IgH). The lower panels show that Lyn, Btk and SH3-deleted Btk overexpressed proteins were effectively immunoprecipitated with the corresponding antibodies. The positions of native Btk (B) and SH3-deleted form of Btk (BΔ3) are indicated.

tagged WASp. As shown in Fig. 4A, WASp-myc was present in the anti-Lyn immunoprecipitate. Similarly, overexpressed wild-type Btk could be co-immunoprecipitated with the WASp-myc protein (Fig. 4B). In contrast, deletion of the SH3 domain of Btk totally prevented the formation of this complex. Therefore, we conclude that Lyn and Btk are both capable of interacting with WASp in vivo, and that, as suggested by in vitro analysis with isolated domains [9,10], interaction with Btk requires the presence of an intact SH3 domain.

4. Discussion

In this study, we demonstrate that WASp is tyrosine phosphorylated at a basal level in unstimulated RBL-2H3 cells and that phosphorylation is further increased upon FcεRI-aggregation (Fig. 1). Using vaccinia-driven expression of Syk, Lyn and Btk, which are involved in FcεRI-signaling, we observe that only Lyn and Btk could phosphorylate WASp (Figs. 2 and 3). Lyn, which belongs to the Src-family PTKs, has been implicated in cell signaling through a number of hematopoietic cell specific surface receptors including FcεRI [16]. The current model based on much experimental data is that Lyn is bound to FcεRIβ and becomes activated upon FcεRI-aggregation. Activated Lyn phosphorylates the ITAMs of the FcεRIβ and γ subunits allowing SH2-mediated recruitment of Syk and signal propagation (reviewed in [29]). Expression of the CD16/7/Syk chimera which mimics an activated Syk at the plasma membrane [28], was not able to trigger WASp phosphorylation although it induced an overall increase of tyrosine phosphorylation in RBL-2H3 cells (Fig. 2). This result suggests that Lyn-induced phosphorylation of WASp is very likely independent of Syk activation.

A different situation was observed in the case of Btk which also becomes activated in response to FcεRI-crosslinking [19]. As in the case of Syk, Lyn is very likely instrumental for Btk activation since Src-family PTKs have been shown to phosphorylate tyrosine 551 of Btk, an event that leads to Btk activation [31]. Overexpression of Btk resulted in phosphorylation of WASp on tyrosine residues (Fig. 3). These results identify WASp as the third known substrate of Btk together with BAP-135, a protein of unknown function [32] and PLCγ that links Btk to the regulation of intracellular Ca^{2+} mobilization [33]. Defects in the gene encoding Btk result in a severe block in B cell development and are associated with immunological diseases in human (XLA) [20] and mouse (Xid) [21]. Interestingly, WAS, which is associated with a range of immune defects culminating in a profound thrombocytopenia, affects also B cell responses such as the response to polysaccharide antigens [1]. Therefore, the observation that Lyn and Btk possibly act upstream of WASp adds further evidence to the critical involvement of WASp in immune receptor signaling.

An interesting finding revealed by this study is that the interaction of CDC42 with its effector modulates WASp phosphorylation by Lyn and Btk (Fig. 3). WASp has been shown to interact with the GTP-bound form of CDC42 through a region, the CDC42/Rac1 interacting binding (CRIB) domain, which is conserved in several potential target effectors for both CDC42 and Rac1 [34]. Overexpression of V12CDC42, an activated mutant form which mimics GTP-bound CDC42, resulted in a substantial increase of Btk- or Lyn-mediated phosphorylation of WASp in BHK cells (Fig. 3). In mast cells Lyn is constitutively bound to the cytoplasmic portion of FcεRIβ [35] and FcεRI-aggregation causes the activation of Btk which translocates to the membrane [19]. Therefore, a possible scenario is that GTP-bound CDC42 interacts with and recruits WASp to the plasma membrane in the vicinity of activated membrane-associated PTKs. Membrane association of WASp could be further stabilized by its amino-terminal PH domain. Many mutations found in WAS patients map within this N-terminal portion of the WAS gene product [2] indicating that the PH domain is crucial for WASp function. This assumption is further supported by the observation that Btk*, a PH domain mutant with increased membrane binding [30], is more active than native Btk in phosphorylating WASp (Fig. 3).

What might be the functional consequences of these inter-

actions and of WASp tyrosine phosphorylation which link Lyn, Btk, WASp and its partner CDC42 into a common signaling pathway in mast cells? We have previously shown that Rac1 and CDC42 control FcεRI-induced actin cytoskeleton reorganization and exocytosis of inflammatory mediators in RBL-2H3 cells [12], two phenomena which require PTK activity [14,15]. Therefore, WASp represents an attractive candidate effector for mediating CDC42 function in FcεRI-dependent cytoskeletal changes. Overexpression of WASp in Jurkat T lymphocytes induces the formation of F-actin rich clusters that also contain WASp [4]. N-WASp, a brain isoform, is capable of depolymerizing actin filaments and this activity requires the presence of a carboxy-terminal cofilin-homology region which is conserved in WASp [36]. Tyrosine phosphorylation of WASp may regulate its actin filament organization activity. Another, non-exclusive, possibility is that tyrosine phosphorylation of WASp may modulate the affinity of the WASp/CDC42 complex. In this respect, it is worth noting that the consensus Lyn-phosphorylation site which has been recently defined (L/I-Y-D/E-X-L [37]), is represented once in the primary sequence of human and mouse WAS proteins (I-Y^{291}-D-F-I [2,38]) and is also conserved in N-WASp [36]. Interestingly, this site is adjacent to the CRIB domain (positions 236–253 of human WASp [2]) and the possibility exists that phosphorylation of Tyr^{291} may modulate, either positively or negatively, the affinity of WASp for its target Rho GTPase. Finally, tyrosine phosphorylation of PSTPIP, a cytoskeletal protein, has been recently shown to regulate interaction of PSTPIP with WASp, supporting the conclusion that tyrosine kinase activity is actually involved in controlling WASp function in cytoskeleton organization [39].

WAS is a pleiotropic immunodeficiency that affects platelet architecture and function but also B and T cell responses. Our findings provide evidence for the pivotal role of WASp in hematopoietic cell signaling by coordinating Rho GTPase functions in cytoskeleton and cell proliferation with tyrosine kinase signaling pathways.

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