Tyrosine Phosphorylation of SLP-76 Is Downstream of Syk following Stimulation of the Collagen Receptor in Platelets*

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Collagen-related peptide (CRP), a collagen homologue, induces platelet activation through a tyrosine kinase-dependent pathway, leading to sequential tyrosine phosphorylation of Fc receptor (FcR) γ-chain, Syk, and phospholipase C-γ2. Here we report that CRP and the platelet low affinity immune receptor FcγRIIA stimulate tyrosine phosphorylation of the T cell adapter SLP-76, whereas the G protein-coupled receptor agonist thrombin induces only minor tyrosine phosphorylation. This suggests that SLP-76 has a specific role downstream of receptors that signal via an immunoreceptor tyrosine-based activation motif. Immunoprecipitation studies demonstrate association of SLP-76 with SLAP-130, Vav, Fyn, Lyn, and the FcR γ-chain in CRP-stimulated platelets. Several of these proteins, including SLP-76, undergo tyrosine phosphorylation in in vitro kinase assays performed on SLP-76 immunoprecipitates. Tyrosine phosphorylation of all of these proteins in the in vitro kinase assay was abrogated by the Src family kinase inhibitor PP1, suggesting that it is mediated by either Fyn or Lyn. The physiological significance of this is uncertain, however, since tyrosine phosphorylation of SLP-76 in vivo is not altered in either Fyn- or Lyn-deficient platelets. CRP stimulation of Syk-deficient platelets demonstrated that in vivo tyrosine phosphorylation of SLP-76 is downstream of Syk. The absence of Syk in the SLP-76 immunoprecipitates raises the possibility that another protein is responsible for bringing SLP-76 to Syk. Candidates for this include those proteins that co-immunoprecipitate with SLP-76, including the FcR γ-chain. Tyrosine phosphorylation of PLC-γ2 and Ca2+-mobilization is markedly attenuated in SLP-76-deficient platelets following CRP stimulation, suggesting that the adapter plays a critical role in the regulation of the phospholipase. The increase in tyrosine phosphorylation of SLAP-130 in response to CRP is also inhibited in SLP-76-deficient platelets, placing it downstream of SLP-76. This work identifies SLP-76 as an important adapter molecule that is regulated by Syk and lies upstream of SLAP-130 and PLC-γ2 in CRP-stimulated platelets.

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PLC-γ2 (20).

SLP-76 was recently reported to be a crucial adapter protein in collagen-stimulated platelets, since aggregation and tyrosine phosphorylation of PLC-γ2 in response to collagen is abolished in SLP-76-deficient platelets.2 In this study, we have investigated the mechanism of tyrosine phosphorylation of SLP-76 and the function of SLP-76 in platelets following stimulation by a collagen-related peptide (CRP), through the specific binding to GPVI (22). CRP is a synthetic, triple helical peptide composed of Gly-Pro-hydroxyproline repeats, crosslinked by cysteines at the N and C termini (23). CRP activates platelets through the GPVI but, in contrast to collagen, is unable to bind the integrin αIIβ3 (23, 24). It is a more powerful agonist than collagen and exhibits less variation in response between individuals.

MATERIALS AND METHODS

Antibodies and Reagents—A CRP (GCP–GPP)3GCP–G; single amino acid code P represents hydroxyproline) was cross-linked via cysteine residues as described previously (23); CRP was kindly donated by Dr. M. Barnes (Cambridge, UK). Collagen (native collagen fibrils from equine tendons) was from Nycemod (Munich, Germany). Fc-yRIIa-specific monoclonal antibody (mAb) was purchased from Madarex Inc (Annandale, NJ). Sheep F(ab)2 raised against mouse IgG (M-1522) and thrombin were purchased from Sigma (Poole, UK). Fura-2 AM was from Molecular Probes, Inc. (Eugene, OR). Anti-phosphotyrosine mAb 4G10 was purchased from Upstate Biotechnology, Inc. (TCS Biologicals Ltd., Botolph Claydon, UK); polyclonal anti-Lyn antibody, Lyn(44), and polyclonal anti-Fyn antibody, Fyn(FYN3), were from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Anti-SLP-76 mAb and the GST-SLP-SH2 were described previously (1), and polyclonal anti-SLAP-130 rabbit antibody (23, 24). It is a more powerful agonist for GPVI but is unable to bind to αIIβ3 (23, 24). Tyrosine phosphorylation of SLP-76 in CRP-stimulated mouse platelets was shown by sequential immunoprecipitation (25).

RESULTS

SLP-76 Is Involved in Early Signaling Events Induced by CRP—We have previously reported that FcγRIIA cross-linking stimulates marked tyrosine phosphorylation of SLP-76 in human platelets (15), a result that is confirmed in the present study (Fig. 1A). Collagen was also observed to stimulate marked tyrosine phosphorylation of SLP-76, in agreement with the observation in mouse platelets,2 whereas the G protein-coupled receptor agonist thrombin induced only a low level of tyrosine phosphorylation of SLP-76 (Fig. 1A). CRP, a synthetic peptide based on the triple-helical structure of collagen, also stimulated tyrosine phosphorylation of SLP-76 (Fig. 1A).

Further studies were performed with CRP, since this is a powerful agonist for GPVI but is unable to bind to αIIβ3 (23, 24). Tyrosine phosphorylation of SLP-76 by CRP occurred within 10 s and reached a maximum at 60 s, being sustained for up to 10 min (Fig. 1B). The time course showing tyrosine phosphorylation of total platelet protein indicated that a major band of 75 kDa displayed the same tyrosine phosphorylation pattern as SLP-76 (not shown). SLP-76 was identified as a component of this band. We have previously shown that Syk is also a component of this band. Pretreatment of platelets with the Ca2 +/Mg2+ chelator bis(O-aminophenoxy)N,N,N′,N′-tetraacetic acid and the protein kinase C antagonists Ro 31-8220 inhibited the action on the second messengers produced by PLC-γ2 indicated that tyrosine phosphorylation of SLP-76 is independent of PLC-γ2 activation (Fig. 1A).

In Vitro Kinase Assay—Protein immunoprecipitations or precipitations were submitted to kinase assay as described (16). Proteins were separated by SDS-PAGE and transferred to PVDF membranes. Membranes were treated with 1 μM KOH for 1 h at 55 °C to cleave serine/threonine phosphorylation and then subjected to autoradiography and immunoblotting.

GST Fusion Proteins—Wild type SLP-76 (1) and the triple mutant SLP-76 YYF/FFF (5) were used as template for polymerase chain reaction using the following primers: SLP-γ2 (5′-ATT GGA TCC GCC GCT TCC TCG TCA TTG GAG GG-3′) and SLP-γ2 (5′-ATT CCC GGC GCT TCC TCG TCA TTG GAG GAG-3′). The polymerase chain reaction products were expressed in pGEX-2T (Amersham Pharmacia Biotech, St. Albans, UK) as described previously (30).

Measurements of Cytosolic Ca2+ Levels—Mouse platelets (106 cells/ml) were loaded with 1 μM Fura-2/AM for 30 min at 37 °C in RPMI 1640 medium containing 1% fetal calf serum and resuspended at 2 × 108 cells/ml in Hepes-buffered salt solution (135 mM NaCl, 5 mM KCl, 10 mM Hepes, 1.2 mM CaCl2, 1.2 mM MgCl2) containing 1% fetal calf serum. Stimulation and measurements were performed at 37 °C. Cytosolic Ca2+ levels were measured as described previously (31). The intracellular Ca2+ concentration was estimated using the equation described by Grynkiewicz et al. (32): \[ [Ca^{2+}]_i = \frac{[R - R_{	ext{min}}][R_{	ext{max}} - R]}{K_w} \] where K represents the value K × S/2N2.

The experimentally determined value of K used in this study is 3.6 μM.

SLP-76 was immunoprecipitated from precleared lysate using the anti-SLP-76 mAb. Immunoprecipitated proteins were separated by 10% SDS-PAGE, electroblotted to CRP stimulation. Stimulation was stopped by the addition of an equal volume of Nonidet P-40 lysis buffer. SLP-76 was immunoprecipitated with an anti-SLP-76 mAb covalently linked to Protein A-Sepharose, and proteins were separated on 10% SDS-PAGE and immunoblotted using the anti-phosphotyrosine mAb 4G10. Membranes were stripped and re-probed for SLP-76 using the anti-SLP-76 mAb (bottom panel). Reprobing revealed Lyn and SLAP-130 as components of these bands, respectively (Fig. 2). There was a small increase in association with SLP-76 following CRP stimulation, although this was less than the increase in tyrosine phosphorylation. Immunoblotting for Grb2 revealed the presence of a similar level of the adapter protein co-immunoprecipitating with SLP-76 in resting and CRP-stimulated platelets (not shown). This is likely to be mediated through the SH3 domain of Grb2 as previously shown in FcγRIIA-stimulated platelets (15).

SLP-76 Is Associated with Lyn and SLAP-130 through Its SH2 Domain—We used a fusion protein containing the SH2 domain of SLP-76, GST-SLP-SH2, to locate the binding site of Lyn and the component(s) of the 130-kDa protein. GST alone, used as control, did not bind any tyrosine-phosphorylated proteins (Fig. 2). GST-SLP-SH2 precipitates two major tyrosine-phosphorylated proteins of 55 and 130 kDa (Fig. 2) in resting platelets. Stimulation of platelets by CRP strongly increased the degree of tyrosine phosphorylation of 130-kDa protein that is precipitated by GST-SLP-SH2, whereas the level of phosphorylation of the 55-kDa protein underwent a small increase. Reprobing revealed Lyn and SLAP-130 as components of these bands, respectively (Fig. 2). There was a small increase in the association of Lyn and SLAP-130 in CRP-stimulated lanes (Fig. 2). Association of SLAP-130 to SLP-76 under basal conditions was also reported in T cells (8). Minor tyrosine-phosphorylated proteins of 90 and 75 kDa were also associated with GST-SLP-SH2 in CRP-stimulated platelets. The 75-kDa band was not identified as Syk or Btk by immunoblotting, although either or both may be below the detection sensitivity of the antibodies.

SLP-76 Tyrosine-phosphorylated in Vitro by a Member of the Src Kinase Family—Comparison of autoradiographs of kinase assays of immunoprecipitated SLP-76 before and after KOH treatment indicated the absence of serine/threonine kinase co-immunoprecipitating with SLP-76 in resting and CRP-stimulated platelets (not shown). Three major bands of 130, 75, and 55 were phosphorylated in SLP-76 immunoprecipitates from resting platelets (Fig. 3A). The three bands of 130, 75, and 55 kDa correspond to the major tyrosine-phosphorylated bands observed in SLP-76 immunoprecipitates when immunoblotted for phosphotyrosine (Fig. 3A). The level of tyrosine phosphorylation of the 130-, 75-, and 55-kDa bands increased dramatically in SLP-76 immunoprecipitates from CRP-stimulated platelets.

Precipitation conditions. The 55-kDa band was present in resting platelets, and its level of tyrosine phosphorylation increased with stimulation of platelets by CRP. It was identified as the tyrosine kinase Lyn by immunoblotting (Fig. 1C). There was a small increase in association with SLP-76 following CRP stimulation, although this was less than the increase in tyrosine phosphorylation. Immunoblotting for Grb2 revealed the presence of a similar level of the adapter protein co-immunoprecipitating with SLP-76 in resting and CRP-stimulated platelets (not shown). This is likely to be mediated through the SH3 domain of Grb2 as previously shown in FcγRIIA-stimulated platelets (15).
CRP stimulation also induced the appearance of three other minor tyrosine-phosphorylated bands of 90, 60, and 38 kDa following kinase assay. On a longer exposure, a doublet of 13/11.5 kDa could also be seen in CRP-stimulated samples (not shown). The 90-kDa band was also seen by anti-phosphotyrosine immunoblotting in Fig. 3A, while the 60-kDa band could be seen in a longer exposure. The 75-kDa band was identified as SLP-76 by immunoblotting. The remaining proteins were identified through sequential immunoprecipitation. Following in vitro kinase assay, the proteins co-immunoprecipitating with SLP-76 were dissociated by boiling in the presence of 2% SDS. Supernatant was diluted down to 0.1% SDS, and proteins from the supernatant were immunoprecipitated with specific antibodies. Components of the 13/11.5-, 55-, 60-, 90-, and 130-kDa radiolabeled bands were identified as FcRγ-chain (Fig. 4A), Lyn (Fig. 4B), Fyn (Fig. 4C), Vav (Fig. 4D), and SLP-130 (Fig. 4E), respectively. A more prominent association with Fyn was seen in some studies, was dramatically reduced in Lyn-deficient mouse platelets (Fig. 6, A and B). In vitro phosphorylation of SLP-76, following immunoprecipitation of these samples, was dramatically reduced in Lyn-deficient

Immunoprecipitation of SLP-76, followed by kinase assay, was performed on platelet lysates using a milder detergent, Brij 96 (compared with Nonidet P-40). This revealed a marked increase in in vitro tyrosine phosphorylation of FcRγ-chain (Fig. 5) in CRP-stimulated samples. The intensity of the band corresponding to Lyn in Brij 96 immunoprecipitation is lower than with Nonidet P-40, in agreement with the decrease of Lyn as shown by immunoblotting with anti-Lyn antibody (not shown). A similar result is seen for Fyn. The reduction in the level of these two kinases is in contrast to the increase in tyrosine phosphorylation of the FcRγ-chain, suggesting that the latter is a consequence of a greater amount of protein in the immunoprecipitate.

SLP-76 co-immunoprecipitates with two members of the Src kinase family, Lyn and Fyn, either or both of which could mediate the increase in tyrosine phosphorylation observed in the in vitro kinase assays. In order to investigate this, PP1, an inhibitor specific to Src kinases (34), was added to the kinase assay. In the presence of 10 μM PP1, in vitro tyrosine phosphorylation of SLP-76 and its co-immunoprecipitated proteins was abrogated under basal and CRP-stimulated conditions in Nonidet P-40 or Brij 96 lysates (Fig. 5).

Tyrosine Phosphorylation of SLP-76 Is Abolished in Syk-deficient Platelets—The identity of the kinases underlying phosphorylation of SLP-76 was investigated in knock-out mouse platelets. Immunoprecipitation studies showed that SLP-76 is phosphorylated in mouse platelets and that it associates with the same profile of tyrosine-phosphorylated proteins as seen with human SLP-76 (not shown). The increase in tyrosine phosphorylation of SLP-76 by CRP was not altered in Lyn- or Fyn-deficient mouse platelets (Fig. 6, A and B). In vitro phosphorylation of SLP-76, following immunoprecipitation of these samples, was dramatically reduced in Lyn-deficient

**Fig. 3.** In vitro tyrosine kinase activity is detected in SLP-76 immunoprecipitates and GST-SLP-SH2 precipitates. A, SLP-76 was immunoprecipitated from resting and stimulated platelets with 3 μg/ml CRP for 90 s. B, 5 μg of GST-SLP-SH2 were used for protein precipitation from lysate of resting or CRP-stimulated platelets. Immunoprecipitated and precipitated proteins were submitted to in vitro kinase assay and separated on 10–18% gradient SDS-PAGE and transferred to PVDF membranes. Membranes were immunoblotted using the anti-phosphotyrosine mAb 4G10. Membranes were then incubated in 1% KOH for 1 h at 55 °C before autoradiography.

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**A**

**IP:SLP-76**

- **CRP**
- **+**

**Blot:4G10**

- **Autoradiograph**

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**B**

**GST-SLP-SH2**

- **CRP**
- **+**

**Blot:4G10**

- **Autoradiograph**

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platelets (Fig. 6A) but hardly altered in Fyn-deficient platelets (Fig. 6B). This suggests that in vitro tyrosine phosphorylation is mainly caused by Lyn but that this kinase is unlikely to be responsible for mediating in vivo phosphorylation of SLP-76 following stimulation by CRP.

The role of Syk in SLP-76 tyrosine phosphorylation was carried out by the study of platelets from Syk-deficient mice. In platelets of control mice, SLP-76 exhibited an increase in tyrosine phosphorylation following CRP stimulation, which was abrogated in CRP-stimulated platelets from Syk-deficient mice (Fig. 7). An increase in tyrosine phosphorylation of the 130-kDa protein upon CRP activation could also be seen with longer exposures, and this was also lost in the Syk-depleted cells (data not shown).

SLP-76 possesses three N-terminal tyrosine phosphorylation sites, Tyr\(^{113}\), Tyr\(^{128}\), and Tyr\(^{145}\). This N-terminal region (amino acids 103–154) was expressed as a GST fusion protein (GST-Tyr-WT) and was used as a substrate in kinase assays performed on Lyn, Fyn, and Syk immunoprecipitates. A fusion protein with the three tyrosine phosphorylation sites mutated to phenylalanine (GST-3Tyr-Mut) was used as a control. Two proteins of 75 and 32 kDa were tyrosine-phosphorylated in the Syk kinase assay, corresponding to Syk and GST-Tyr-WT, respectively (Fig. 8A). A small increase in Syk autophosphorylation and tyrosine phosphorylation of the fusion protein was observed in stimulated conditions relative to basal level. A similar level of tyrosine phosphorylation of the two proteins was observed in the presence of PP1 (not shown). No tyrosine phosphorylation of GST-3Tyr-Mut was detected in the Syk kinase assay from resting and CRP-stimulated platelets (Fig. 8A). This indicates that tyrosine phosphorylation of GST-Tyr-WT by Syk is specific to one or more of Tyr\(^{113}\), Tyr\(^{128}\), and Tyr\(^{145}\). In contrast, GST-Tyr-WT was weakly phosphorylated by Lyn and Fyn, whereas both kinases underwent dramatic autophosphorylation (Fig. 8, B and C), which was abolished in the presence of PP1 (not shown). GST-3Tyr-Mut was not tyrosine-phosphorylated in the Lyn and Fyn kinase assays. This indicates that tyrosine phosphorylation of the N-terminal ty-
Tyrosine Phosphorylation of both PLC-\(\gamma_2\) and SLAP-130 Is Lost in SLP-76-deficient Mouse Platelets—The role of SLP-76 in tyrosine phosphorylation of PLC-\(\gamma_2\) and SLAP-130 was investigated in SLP-76-deficient platelets. A similar profile of tyrosine phosphorylation was observed in basal and CRP-stimulated samples from wild-type (+/+) and heterozygous (+/−) platelets, whereas there was a marked decrease in phosphorylation of proteins of 130 and 75 kDa in the SLP-76-deficient (−/−) cells (not shown). The reduction in the 75-kDa protein is likely to be due to the absence of SLP-76, which migrates in this area. The remaining increase in phosphorylation of this band is likely due to phosphorylation of Syk, which co-migrates with SLP-76. This was confirmed by immunoprecipitation of the kinase (Fig. 9A). The 130-kDa band migrates in the region of SLP-130 and PLC-\(\gamma_2\). Phosphorylation of PLC-\(\gamma_2\) was dramatically reduced in response to CRP in SLP-76-deficient platelets (Fig. 9B), although a residual increase could be seen on a longer exposure (not shown). SLAP-130 was tyrosine-phosphorylated in resting platelets, and the level of phosphorylation increased following stimulation by CRP. The level of phosphorylation of SLAP-130 under basal conditions was not altered in the SLP-76-deficient platelets, whereas the increase induced by CRP was abolished (Fig. 9C).

The functional consequence of the reduction in PLC-\(\gamma_2\) phosphorylation was monitored through measurement of intracellular Ca\(^{2+}\) in Fura-2/AM-loaded platelets. CRP was unable to elevate Ca\(^{2+}\) in SLP-76-deficient platelets (Fig. 10A) in contrast to the robust response in control cells. Ca\(^{2+}\) mobilization in response to the G protein-coupled receptor agonist thrombin was unaltered (Fig. 10B) in SLP-76-deficient platelets, suggesting that SLP-76 plays a crucial function downstream of immunoreceptor tyrosine-based activation motif-containing receptor.

**DISCUSSION**

Here we report that Fc\(yRIIA\), CRP, and collagen stimulate dramatic tyrosine phosphorylation of SLP-76 in contrast to the G protein-coupled receptor agonist thrombin. This suggests that this adapter protein has a specific role in immunoreceptor tyrosine-based activation motif-mediated signaling. This is consistent with the absence of other reports of phosphorylation of SLP-76 by G protein-coupled receptor agonists.

Tyrosine phosphorylation of SLP-76 is one of the earliest of the events following CRP stimulation and is sustained for up to 10 min. This indicates that SLP-76 may be involved in initial events of the signal transduction pathway induced by CRP. This is consistent with the fact that tyrosine phosphorylation of SLP-76 was maintained in the presence of the Ca\(^{2+}\) chelator bis(\(\text{O}-\text{aminophenoxy})\)-\(\text{N},\text{N},\text{N}^\prime,\text{N}^\prime\)-tetraacetic acid and a protein kinase C antagonist Ro 31-8220, a combination designed to inhibit events downstream of PLC-\(\gamma_2\), following CRP stimulation.

SLP-76 was reported to be tyrosine-phosphorylated by ZAP-70 or Syk in T cells and rat basophilic leukemia cells (9, 14), respectively. Consistent with this, tyrosine phosphorylation of SLP-76 induced by CRP was abrogated in Syk-deficient platelets. However, we were not able to detect in vivo association between Syk and SLP-76 following immunoprecipitation, suggesting an indirect, unstable, or weak interaction between the two proteins.

SLP-76 contains three N-terminal tyrosine phosphorylation sites including two consensus sequences (pYESP, where pY represents phosphotyrosine) for association to the SH2 domain of Vav. SLP-76 also contains a proline-rich region that associates with the SH3 domain of Grb2 and an SH2 domain that associates with tyrosine-phosphorylated proteins. Immunoprecipitation of SLP-76 revealed a marked association with the Src kinase Lyn and a phosphotyrosyl protein of 130 kDa. One component of this 130-kDa phosphoprotein was identified as SLP-130 through sequential immunoprecipitation following kinase assay. This strategy also revealed a lower level of binding of other proteins with SLP-76, namely Vav, Fyn, and FcR \(\gamma\)-chain. SLP-76 has been reported to co-immunoprecipitate with SLAP-130 and an uncharacterized protein of 62 kDa, as well as a serine/threonine kinase in rat basophilic leukemia cells (14) and T cells (7, 8). No evidence for association of the latter two proteins was found in the present study. However, SLP-130, Lyn, and Fyn were found to interact with the SH2 domain of SLP-76.
domain of SLP-76 expressed as a fusion protein. It is unclear whether the association of FcRγ-chain also occurs through this region, because radiolabeled incomplete GST fusion protein products co-migrated in this region of the gel. Vav did not associate with the SH2 domain of SLP-76, consistent with an interaction occurring between the SH2 domain of Vav and the N-terminal tyrosine-phosphorylated sites as shown in stimulated T cells (2–5).

Kinase assays performed on immunoprecipitated SLP-76 from CRP-stimulated platelets lysed with the mild detergent Brij 96 exhibited a stronger tyrosine phosphorylation of FcRγ-chain than in Nonidet P-40 lysates following CRP stimulation. This is in contrast with the lower level of co-immunoprecipitating Lyn and Fyn found in Brij 96 lysates. The interaction with the FcR γ-chain could be direct or indirect, since Fyn and Lyn were recently reported to associate with FcRγ-chain irrespective of stimulation by collagen (35). In the latter case, the increase in the level of FcR γ-chain in Brij 96 suggests either that the interaction is more stable in Brij 96 compared with Nonidet P-40 or that there is a selective solubilization of a pool of Lyn and/or Fyn associated with FcR γ-chain. In Nonidet P-40, SLP-76 could co-immunoprecipitate at least two pools of Src tyrosine kinases, a pool associated with FcRγ-chain and a second pool possibly associated with downstream events. Lyn and Fyn are likely to tyrosine-phosphorylate FcR γ-chain in the

were immunoprecipitated under basal or CRP-stimulated conditions and subjected to an in vitro kinase assay. 5 μg of GST-Tyr-WT, GST-3Tyr-Mut, or GST alone were added in the kinase assay. Kinase reactions were analyzed on 10% SDS-PAGE and electroblotted on PVDF membranes. Ser/Thr phosphorylation was removed by treating the membranes with 1 M KOH at 55 °C for 1 h. The upper part of each panel shows an autoradiograph of the kinase assay. Membranes were immunoblotted using anti-Syk (A, middle part), anti-PLC-γ2 (B, middle part), and anti-SLAP-130 rabbit antiserum (C, middle part).
in vitro kinase assay, since the addition of PP1, the Src kinase inhibitor, abolished tyrosine phosphorylation.

In vitro tyrosine phosphorylation of SLP-76 was also abrogated in the presence of PP1 in both Nonidet P-40 and Brij 96 lysates following CRP stimulation. Lck was reported to be able to tyrosine-phosphorylate Tyr423/Tyr426 within the SH2 domain of SLP-76, but this does not correlate with physiological mapping of tyrosine phosphorylation sites (Tyr113, Tyr128, and Tyr137) of SLP-76 following TCR stimulation (11). This cannot be attributed to Blnk, since this is not expressed in platelets.3 This might occur through a direct interaction between Syk and PLC-γ2 similar to the interaction seen in B-cells (21, 37) or indirectly through LAT as suggested for residual phosphorylation of PLC-γ1 in T cells (11).

SLAP-130 associated with the SH2 domain of SLP-76 in platelets. The function of SLAP-130 is not known. The relationship of the association between the SH2 domain of SLP-76 and SLAP-130 was investigated in SLP-76-deficient platelets. Residual tyrosine phosphorylation of PLC-γ2 could be seen in CRP-stimulated SLP-76-deficient platelets, indicating the existence of a minor pathway leading to tyrosine phosphorylation of PLC-γ2. This may be through the recruitment of PLC-γ1 in T cells lacking SLP-76 (11).

SLAP-130 is brought to Syk via binding to either Fyn or Lyn (15). A novel possibility is that PLC-γ1 may phosphorylate this region of SLP-76, but this does not correlate with physiological evidence that PLC-γ1 is recruited to Syk in B-cells (11). PLC-γ1 may also play a role in the requirement for Syk activation and PLC-γ1 regulation, although further work is required to establish a complete understanding of this pathway, including the role of SLAP-130.

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2. SLP-76 co-immunoprecipitates with the tyrosine kinases Lyn and Fyn, and this interaction may be important for downstream events. This work confirms SLP-76 as an important link between Syk activation and PLC-γ1 regulation, although further work is required to establish a complete understanding of this pathway, including the role of SLAP-130.

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