Definition of the Sites of Interaction between the Protein Tyrosine Phosphatase SHP-1 and CD22*

(Received for publication, October 14, 1998)

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CD22 phosphorylation is an early event of B cell antigen receptor engagement and results in the recruitment of the negative regulatory tyrosine phosphatase, SHP-1. Peptides representing the potential phosphorylation sites within the cytoplasmic domain of CD22 have been used to stimulate SHP-1 catalytic activity and to inhibit the binding of SHP-1 to CD22 (Doody, G., Justement, L., Delibrias, C., Matthews, R., Lin, J., Thomas, M., and Fearon, D. (1995) Science 269, 242–244). However, the sites of phosphorylation within the cytoplasmic domain of CD22 and the importance of each for the recruitment and activation of SHP-1 remain unknown. Here we demonstrate that there are multiple sites within the cytoplasmic domain of CD22 that interact with the Src homology 2 domains of SHP-1. Nevertheless, a minimum of two tyrosines in CD22 is required for the association with SHP-1. Furthermore, both Src homology 2 domains of SHP-1 are necessary for efficient binding to CD22.

Engagement of the B cell antigen receptor (BCR) results in an increase in tyrosine phosphorylation. CD22, a B cell-specific transmembrane lectin, is one of the initial substrates phosphorylated upon BCR engagement. The cytoplasmic domain of CD22 contains six tyrosines and has the potential of being a docking site for various Src homology 2 (SH2) domain-containing proteins. Indeed, it has been shown that CD22 binds to multiple enzymes including Syk, p53/56 (Lyn), phosphatidylinositol 3-kinase, phospholipase Cγ, and SHP-1 (1–4). However, neither the sequence nor the mechanism of these interactions has been delineated.

The importance of CD22 in BCR signaling is demonstrated by the development of mice ablated in CD22 gene expression (5–8). These mice exhibit a spontaneous decrease in IgM expression and elevated calcium mobilization in response to BCR cross-linking. This suggests that CD22 is important in the negative regulation of BCR signaling. Interestingly, an elevated calcium response is also observed in mice deficient with SHP-1 (10). BCR negative regulation has been delineated.

SHP-1 is a cytosolic enzyme containing two SH2 domains at the amino terminus. In its native form, SHP-1 has low basal catalytic activity. However, truncation of the SH2 domains or binding to the SH2 domains substantially increases its catalytic activity, suggesting that the SH2 domains regulate the catalytic activity by an allosteric mechanism (3, 14–16). Further supportive evidence for this model comes from the recently solved crystal structure for a highly related phosphatase, SHP-2 (17). The resolution of the crystal structure shows that the first SH2 domain of SHP-2 interacts with the active site of the phosphatase domain to preclude substrate binding. Binding to the first SH2 domain may result in a conformational change allowing access to the catalytic site. Thus, it is likely that the SH2 domains of SHP-1 have two functions, to recruit the enzyme to appropriate locations and to regulate enzymatic activity.

The tyrosine-phosphorylated sequence recognized by the SH2 domains of SHP-1 has been termed an immunoreceptor tyrosine-based inhibitory motif (ITIM) with the consensus sequence (V/L/I)X(p)XXL. The cytoplasmic domain of CD22 has five potential ITIMs (Table I). Tyrosine-phosphorylated peptides representing three of these sequences have been shown to increase SHP-1 activity and inhibit binding to CD22 (3). To determine which of these potential ITIMs are necessary for SHP-1 binding, we co-expressed SHP-1 with a chimeric protein containing mutations of the ITIM sequences. Here we demonstrate that the ITIMs have redundant functions, and furthermore, two intact ITIMs are required for optimal binding to SHP-1. In addition, both SH2 domains of SHP-1 are required for the interaction with the cytoplasmic domain of CD22.

Experimental Procedures

Cell Lines and Antibodies—HeLa cells were obtained from the ATCC (Rockville, MD) and maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% bovine calf serum and l-glutamine. Transfection of HeLa cells was carried out as described previously (18). An antiphosphotyrosine monoclonal antibody 4G10 was purchased from Upstate Biotechnology, Inc. (Lake Placid, NY), and rabbit antivascular stomatitis virus serum was purchased from Access Biomedical (San

* This work was supported by the National Institutes of Health Grant GM56455 and the Human Frontiers Science Program. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ Supported by the Division of Biology and Biomedical Sciences, Washington University, School of Medicine.

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1 The abbreviations used are: BCR, B cell antigen receptor; ITIM, immunoreceptor tyrosine-based inhibitory motif; SH2, Src homology 2; VSVG, vesicular stomatitis virus G protein; PBS, phosphate-buffered saline; PCR, polymerase chain reaction.

2 L. Dustin, D. Plas, T. Hu, and M. L. Thomas, submitted for publication.
Diego, CA). Rabbit anti-SHP-1 antiserum was purchased from Cappel Organon Teknika Corp. (West Chester, PA).

cDNA Reagents—Mouse SHP-1(C453S) cDNA has been described previously (19). Mutations were introduced into the SH2 domains of SHP-1 by polymerase chain reaction (PCR) site overlap extension as described. To construct SHP-1(ΔP), PCR site overlap extension was used to generate a BglII-Ball fragment with a deletion of the phosphotyrosine catalytic domain signature motif (4'VHCSAG4'5); the PCR primers used were 5'-CACGCGATACTGTCGAC-3' (forward, outer), 5'-TCATGGGGGATCCATATGCGGTTCTTCGATGAACTGG-3' (forward, inner), 5'-TATCCAATGACGATGATGGTACCCGTAATGATGGG-3' (reverse, inner), and 5'-GTCGTTGTATGACGGAGGTAC-3' (reverse, outer). This BglII-Ball PCR fragment was cloned into a modified MscI Tagged SHP-1 construct. CD22 constructs were made as a fusion protein comprising the extracellular and transmembrane domains of vesicular stomatitis virus G protein (VSVG) and the cytoplasmic domain of mouse CD22 (amino acids 717–850). Substitutions of the tyrosines into phenylalanines were introduced by PCR site overlap extension (20). All mutations were confirmed by automated DNA sequencing. SHP-1 cDNAs were cloned into pBluescript (Stratagene) behind the T7 promoter; CD22 cDNAs were cloned into pGEM-Z (Promega) behind the T7 promoter.

Interaction Sites between SHP-1 and CD22

To determine which phosphorylated tyrosines in CD22 were required for the interaction between CD22 and SHP-1, a chimeric protein of the intact cytoplasmic domain of CD22, and the extracellular and transmembrane domains of the VSVG protein (G/22) and SHP-1 were expressed in HeLa cells. The G/22 chimeric protein is expressed on the membrane surface as a fusion protein with a peptide of the intact cytoplasmic domain of CD22, and the extracellular and transmembrane domains of the VSVG protein. Immunoprecipitates and crude lysates were resolved on a 7.5% SDS-polyacrylamide gel under reducing conditions. The gel was transferred onto nitrocellulose and blocked in PBS containing 0.05% Tween 20 and 3% bovine serum albumin for 1 h at room temperature. Following the primary antibody, membranes were washed twice in PBS containing 1% Nonidet P-40 and once with PBS containing 0.05% Tween 20 for 5 min, developed with appropriate secondary antibodies in PBS containing 0.05% Tween 20 for 30 min, and washed as described above. Proteins were visualized using the enhanced chemiluminescence kit (Amersham Pharmacia Biotech, Buckinghamshire, United Kingdom).

RESULTS

Phosphorylated CD22 Associates with SHP-1—To define the site(s) of interaction between CD22 and SHP-1, a chimeric protein of the intact cytoplasmic domain of CD22, and the extracellular and transmembrane domains of the VSVG protein (G/22) and SHP-1 were expressed in HeLa cells. The G/22 chimeric protein is expressed on the membrane surface as determined by flow cytometric analysis (data not shown). Co-expression of G/22 with catalytically inactive SHP-1 (SHP-1(C453S)) results in tyrosine phosphorylation of G/22 and association with SHP-1 (Fig. 1). In contrast, under these conditions co-expression of G/22 with wild type SHP-1 results in no detectable tyrosine phosphorylation of G/22 and no association of the two proteins. These data suggest that G/22 is a substrate of SHP-1. In addition, expression of G/22 alone in HeLa cells results in no tyrosine phosphorylation of G/22 compared with that seen when co-expressed with SHP-1(C453S). This indicates that binding of SHP-1 protects G/22 from dephosphorylation by endogenous phosphatases. Of note is the presence of a doublet detected by VSVG immunoblotting; the slower migrating band corresponds to tyrosine-phosphorylated G/22. To define the sites of interaction between SHP-1 and G/22, SHP-1(C453S) was used in our subsequent studies.

Two Tyrosines Are Required for Phosphorylation of CD22 and Association with SHP-1—The cytoplasmic domain of CD22 contains six tyrosine residues; tyrosine 795 does not fit the consensus ITIM sequence for SHP-1 and therefore was left unchanged. The remaining five tyrosines were all mutated to phenylalanine (Table I). To examine whether any single ITIM tyrosine was sufficient for SHP-1 binding, mutations were created such that only a single ITIM was left intact. Co-expression of single ITIM G/22 with SHP-1(C453S) revealed that only tyrosines 765 and 825 were consistently phosphorylated (data not shown). Neither pervanadate treatment nor co-expression with Lyn enhanced the tyrosine phosphorylation levels of the other single ITIM G/22 proteins. Co-expression of the single ITIM G/22 with SHP-1(C453S) resulted in poor and inconsistent association of the two proteins as measured by co-immunoprecipitation (data not shown). These findings suggest that more than one tyrosine is required for efficient tyrosine phosphorylation and binding to SHP-1.

Previously, it was demonstrated that phosphorytide peptides representing the ITIM sequences of tyrosines 765, 825, or 845 but not 799 or 810 were capable of increasing SHP-1 association with SHP-1. In contrast, under these conditions co-expression of G/22 with wild type SHP-1 results in no detectable tyrosine phosphorylation of G/22 and no association of the two proteins. These data suggest that G/22 is a substrate of SHP-1. In addition, expression of G/22 alone in HeLa cells results in no tyrosine phosphorylation of G/22 compared with that seen when co-expressed with SHP-1(C453S). This indicates that binding of SHP-1 protects G/22 from dephosphorylation by endogenous phosphatases. Of note is the presence of a doublet detected by VSVG immunoblotting; the slower migrating band corresponds to tyrosine-phosphorylated G/22. To define the sites of interaction between SHP-1 and

3 Wang, L., Blasioli, J., Plas, D., Thomas, M., L., and Yokoyama, W., (1999) J. Immunol., in press.
TABLE I

| Tyrosine residue | ITIM sequencea |
|------------------|----------------|
| 765              | YYYY          |
| 799              | YYYYF         |
| 810              | YYTFY         |
| 825              | YFFFY         |
| 845              | FFFFF         |

a The CD22 consensus ITIM sequence is (V/I/L)(p)YXX(L/I/V).

Fig. 2. Two phosphotyrosines within the cytoplasmic domain of CD22 are required for SHP-1 binding. Double (A) or triple (B) ITIM G/22 chimeric proteins were co-expressed with SHP-1(C453S) in HeLa cells. The nonmutated tyrosines were: A, tyrosines 765 and 825 (lane 3), 825 and 845 (lane 4), 765 and 845 (lane 5), 799 and 810 (lane 6); or B, tyrosines 765, 799, and 825 (lane 3) and 765, 825, and 845 (lane 4). The relative position of the intact ITIM sequence is indicated by Y, and the position of the mutated ITIM is indicated by F. The wild type G/22 protein (YYYYY) (lane 1) and the non-ITIM-containing G/22 protein (FFFFF) (lane 2) were also expressed in HeLa cells with SHP-1(C453S). Lysates were immunoprecipitated with anti-VSVG. Immunoprecipitates and crude lysates were resolved on a 7.5% SDS-polyacrylamide gel. Immunoblots were sequentially performed for SHP-1, VSVG, and phosphotyrosine (PTyr).

Fig. 3. Both SH2 domains are required for binding to the cytoplasmic domain of CD22. SHP-1(C453S), SHP-1(R30K, R33E, C453S), SHP-1(R136K, C453S), or SHP-1(R30K, R33E, R136K, C453S) was co-expressed with either wild type G/22 (YYYYY) (lanes 1–5), G/22 in which tyrosines 765 and 825 were mutated to phenylalanine (YFFFY) (lanes 6–10), or G/22 in which all five tyrosines were mutated to phenylalanine (FFFFF) (lanes 11–15) in HeLa cells. Lysates were immunoprecipitated with anti-VSVG. Immunoprecipitates and crude lysates were resolved on a 7.5% SDS-polyacrylamide gel. Immunoblots were performed for SHP-1, VSVG, and phosphotyrosine (PTyr).

It is widely recognized that CD22 Is a Substrate of SHP-1—To determine whether both SH2 domains of SHP-1 were required for binding to CD22, mutations that eliminate phosphotyrosine binding (21) were introduced either into the individual SH2 domains or into both SH2 domains. The mutations were constructed using the cDNA encoding catalytically inactive SHP-1(C453S). When co-expressed with wild type G/22 protein or the double ITIM G/22 containing tyrosines 765 and 825, mutations that disrupted phosphotyrosine binding to both SH2 domains completely prevented the association of SHP-1 with G/22 (Fig. 3). Results were the same for all combinations of double and triple ITIM G/22 proteins containing tyrosines 765, 825, and 845. SHP-1 with a single functional SH2 domain was capable of binding the CD22 cytoplasmic domain; however, the binding was dramatically decreased. Thus, both SH2 domains of SHP-1 are required for efficient binding to the cytoplasmic domain of CD22. The absence of tyrosine phosphorylation and association with SHP-1 to the G/22 protein in which all five tyrosines were mutated to phenylalanines confirmed that phosphorylated tyrosines interact with the SH2 domains of SHP-1.

CD22 Is a Substrate of SHP-1—It is widely recognized that SHP-1(C453S) is a substrate-trapping mutant, whereas SHP-1(ΔP), which lacks the phosphatase catalytic signature motif, is a nontrapping mutant (22). Using these two mutants, one can discriminate between SHP-1 substrates and binding proteins. Substrates bind to the SHP-1(C453S) phosphatase domain, and the phosphorylated tyrosine is protected from dephosphorylation by other endogenous phosphatases. However, this does not occur with SHP-1(ΔP); thus the substrate should exhibit greater tyrosine phosphorylation in the presence of SHP-1(C453S) compared with SHP-1(ΔP) or wild type SHP-1. To this end, the wild type G/22 protein was co-expressed with wild type SHP-1, SHP-1(C453S), and SHP-1(ΔP). The level of tyrosine phosphorylation of G/22 when co-expressed with SHP-1(ΔP) was greatly reduced compared with that co-expressed with SHP-1(C453S) (Fig. 4). In addition, less SHP-1 co-immunoprecipitated with G/22 in the presence of SHP-1(ΔP) compared with SHP-1(C453S). These results indicate that SHP-1 binds to G/22 via its phosphatase domain as well as the SH2 domains, thus confirming the earlier results that CD22 is a target for the phosphatase domain of SHP-1.

The triple ITIM G/22 protein containing tyrosines 765, 825, and 845 confirmed the results from the double ITIM G/22 proteins. Compared with wild type G/22, this triple ITIM G/22 protein was tyrosine-phosphorylated and bound SHP-1 at an equivalent amount (Fig. 2B). However, the triple ITIM G/22 protein containing tyrosines 765, 799, and 825 was equally tyrosine-phosphorylated as wild type G/22 but associated with less SHP-1. One possible explanation is that in the absence of tyrosine 845, tyrosine 799 destabilizes the complex and thus does not allow for efficient binding of SHP-1.

Both SH2 Domains of SHP-1 Associate with CD22—To determine whether both SH2 domains of SHP-1 were required for binding to CD22, mutations that eliminate phosphotyrosine binding (21) were introduced either into the individual SH2 domains or into both SH2 domains. The mutations were constructed using the cDNA encoding catalytically inactive SHP-1(C453S). When co-expressed with wild type G/22 protein or the double ITIM G/22 containing tyrosines 765 and 825, mutations that disrupted phosphotyrosine binding to both SH2 domains completely prevented the association of SHP-1 with G/22 (Fig. 3). Results were the same for all combinations of double and triple ITIM G/22 proteins containing tyrosines 765, 825, and 845. SHP-1 with a single functional SH2 domain was capable of binding the CD22 cytoplasmic domain; however, the binding was dramatically decreased. Thus, both SH2 domains of SHP-1 are required for efficient binding to the cytoplasmic domain of CD22. The absence of tyrosine phosphorylation and association with SHP-1 to the G/22 protein in which all five tyrosines were mutated to phenylalanines confirmed that phosphorylated tyrosines interact with the SH2 domains of SHP-1.

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**FIG. 4.** CD22 is a substrate of SHP-1. Wild type SHP-1 (lane 3), SHP-1(C453S) (lane 2), or SHP-1(ΔP) (lane 4) was co-expressed with wild type G/22 in HeLa cells. Lysates were immunoprecipitated with anti-VSVG. Immunoprecipitates and crude lysates were resolved on a 7.5% SDS-polyacrylamide gel. Immunoblots were performed for SHP-1, VSVG, and phosphotyrosine (PTyr).

substrate of SHP-1. Nonetheless, the lack of SHP-1(C453S) binding to CD22 when both SH2 domains are mutated demonstrates that the SH2 domains are required for substrate trapping to occur (Fig. 3).

**DISCUSSION**

CD22 is required for the negative regulation of the BCR. This effect is mediated by the recruitment and activation of SHP-1 to the cytoplasmic domain of CD22. Here, we demonstrate that at least two functional ITIMs and both SH2 domains of SHP-1 are required for efficient binding to the cytoplasmic domain of CD22. The requirement for both SHP-1 SH2 domains likely reflects the increased affinity achieved by binding tandem SH2 domains (23). Thus, it is conceivable that the tandem SH2 domains of SHP-1 allow for SHP-1 to preferentially bind to the cytoplasmic domain of CD22 because of the increased binding affinity achieved by tandem SH2 domains compared with proteins with single SH2 domains. This phenomenon may explain why the two triple ITIM G/22 proteins exhibited a similar level of tyrosine phosphorylation but bound different amounts of SHP-1. Interestingly, CD22 is a potential substrate for SHP-1, and thus, it appears that there is an inherent feedback mechanism allowing for the disengagement of SHP-1 from CD22. This is supported by our finding that under the conditions we used, SHP-1(C453S) co-immunoprecipitates with G/22, which is tyrosine-phosphorylated. However, wild type SHP-1 does not co-immunoprecipitate, and G/22 is not tyrosine-phosphorylated. Thus, dephosphorylation of G/22 causes disassociation of the two proteins. Therefore, the requirement for both SHP-1 SH2 domains and at least two phosphorylated ITIM tyrosines may also reflect the necessity of maintaining SHP-1 binding to CD22 for a period of time sufficient to allow dephosphorylation of substrates involved in BCR signal transduction. In addition, the requirement for two ITIM sequences adds to the biological specificity, localizing SHP-1 activity to appropriate sites to control BCR signal transduction.

The cytoplasmic domain of CD22 contains six tyrosines, of which five are potential ITIM sequences. Lyn has recently been shown to be required for the phosphorylation of CD22 cytoplasmic domain (10–13) and the subsequent recruitment of SHP-1. CD22 also binds Syk, phospholipase Cγ, and phosphatidylinositol 3-kinase (1, 4). It is not known, however, which tyrosines are phosphorylated by Lyn and to which tyrosines these other molecules may bind. However, we have been unable to demonstrate binding of Syk or phosphatidylinositol 3-kinase to G/22 under similar conditions. In this study, we focused on mapping the interaction between CD22 and SHP-1. We have shown that three tyrosines, 765, 825, and 845, are capable of associating with SHP-1. Moreover, our results implicate tyrosine 825 as the primary SHP-1 binding site because the amount of SHP-1 associated with wild type G/22 was equal to that containing only tyrosines 765 and 825 or tyrosines 825 and 845. In addition, our results implicate tyrosine 765 as the target dephosphorylation site of SHP-1 because G/22 was maximally tyrosine-phosphorylated in the presence of tyrosines 765 and 825.

That CD22 is a substrate of SHP-1 was confirmed by co-expression of SHP-1(ΔP) with wild type G/22. SHP-1(ΔP) acts as a substrate-trapping mutant but needs the SH2 domains to bring CD22 to within close proximity prior to dephosphorylation (or binding via its catalytically inactive phosphatase domain). In contrast, SHP-1(ΔP) does not act as a substrate-trapping mutant as it does not have a functional phosphatase domain, and thus it only interacts with CD22 via its SH2 domains. SHP-1 substrates should exhibit greater tyrosine phosphorylation in the presence of SHP-1(C453S) compared with SHP-1(ΔP). Indeed, this was observed when wild type G/22 was co-expressed with SHP-1(C453S) and SHP-1(ΔP) (Fig. 4).

Interestingly, not all the single ITIM G/22 mutants were consistently tyrosine-phosphorylated; this could be because of a specific order required for phosphorylation. Recently this phenomenon has been described for the T cell receptor γ chain (24). Our results show that tyrosines 765 and 825 are tyrosine-phosphorylated as single ITIMs, whereas tyrosine 845 is not and requires a second tyrosine as demonstrated by the double ITIM G/22 protein containing tyrosines 825 and 845. It may be likely that tyrosines 765 and 825 also act to recruit a tyrosine kinase that then phosphorylates tyrosine 845, enabling complete binding and activation of SHP-1. Our results also show that there is specificity to which tyrosine SHP-1 binds, because the double ITIM G/22 protein containing tyrosines 799 and 810 did not bind to SHP-1. It remains to be determined whether the spacing between the tyrosines is critical for SHP-1 binding, as is the case for the immunoreceptor tyrosine-based activation motif and tyrosine kinases (25).

Several recent studies have shown that SHP-1 binds to several potentially biphosphorylated proteins, such as signaling inhibitory receptor proteins, paired immunoglobulin-like receptor B, and killer inhibitory receptors (22, 26–31). Thus, the requirement for two ITIM sequences may be a general mechanism directing biological specificity and duration of SHP-1 activity. The recently solved crystal structure for SHP-2 has provided important insights as to how the tandem SH2 domains may work in unison to regulate the catalytic activity of the enzyme (17). The amino SH2 domain interacts with the phosphatase domain preventing access to the catalytic site and preventing optimal phosphotyrosine binding. The carboxyl SH2 domain, in contrast, is freely available. Thus, as previously proposed (21), it is possible that the carboxyl SH2 domain acts to recruit SHP-1 to appropriate locations placing the amino SH2 domain in close proximity to a second ITIM sequence, which under these conditions engages, allowing for the conformation change needed for high affinity binding and enzymatic activity. Our data support this model for the recruitment and activation of SHP-1, by showing that for efficient binding, both SHP-1 SH2 domains are required. However, we were not able to determine whether there is any difference in the specificity of each SH2 domain, that is whether there are

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X. He, D. Plas, and M. L. Thomas, unpublished data.
ITIM preferences for each SH2 domain. This may be important in spatially orienting SHP-1 correctly to dephosphorylate appropriate substrates.

Acknowledgments—We thank David Plas, Tanya Ulyanova, and Andy Chan for comments on the manuscript.

REFERENCES
1. Tuscano, J. M., Engel, P., Tedder, T. F., Agarwal, A., and Kehrl, J. H. (1996) Eur. J. Immunol. 26, 1246–1252
2. Campbell, M., and Klinman, N. (1995) Eur. J. Immunol. 25, 1573–1579
3. Doody, G., Justement, L. B., Delibrias, C. C., Matthews, R. J., Lin, J., Thomas, M. L., and Fearon, D. T. (1995) Science 269, 242–244
4. Law, C.-L., Sidorenko, S. P., Chandran, K. A., Zhao, Z., Shen, S. H., Fischer, E. H., and Clark, E. A. (1996) J. Exp. Med. 183, 547–560
5. O'Keefe, T. L., Williams, G. T., Davies, S. L., and Neuberger, M. S. (1996) Science 274, 798–801
6. Otipoby, K. L., Anderson, K. B., Draves, K. E., Klaus, S. J., Farr, A. G., Goodnow, C. C. (1994) J. Exp. Med. 180, 509–517
7. Pei, D., Lorenz, U., Klingmüller, U., Neel, B. G., and Walsh, C. T. (1994) Biochemistry 33, 15483–15493
8. Kharitonkov, A., Chen, Z., Sures, I., Wang, H., Schilling, J., and Ulrich, A. (1997) Nature 386, 181–186
9. Gupta, N., Scharenberg, A. M., Burshtyn, D. N., Wagtmann, N., Limbach, M. N., Rohrschneider, L. R., Kinet, J. P., and Long, E. O. (1997) J. Exp. Med. 186, 473–478
10. Blery, M., Kubagawa, H., Chen, C. C., Vely, F., Cooper, M. D., and Vivier, E. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 2446–2451
11. Maeda, A., Kurnosaki, M., Oka, M., Takai, T., and Kurosaki, T. (1998) J. Exp. Med. 187, 807–811
12. Roy, G., Matthews, J., Woodford-Thomas, T., and Thomas, M. L. (1995) Adv. Protein Phosphatases 9, 121–139
13. Vely, F., Olivero, S., Olcese, L., Moretta, A., Damen, J. E., Liu, L., Krystal, G., Cambier, J. C., Daeron, M., and Vivier, E. (1997) Eur. J. Immunol. 27, 1994–2000

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J. Biol. Chem. 1999, 274:2303-2307.
doi: 10.1074/jbc.274.4.2303

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