Multiple Components of the B Cell Antigen Receptor Complex Associate with the Protein Tyrosine Phosphatase, CD45*

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Signal transduction via the B cell antigen receptor complex is regulated by changes in tyrosine phosphorylation of several proteins. The equilibrium between tyrosine phosphorylation and dephosphorylation is regulated by the combined action of protein tyrosine kinase and protein tyrosine phosphatase enzymes. In particular, the protein tyrosine phosphatase, CD45, has been shown to play an essential role in signal transduction via the B cell antigen receptor. Therefore, experiments were performed to examine the intermolecular associations between CD45 and phosphotyrosine-containing proteins in the B cell to identify potential substrates for CD45. Based on coprecipitation experiments, CD45 was found to be physically associated with multiple components of the B cell antigen receptor complex including the MB-1/B29 heterodimer. Additionally, CD45 was selectively associated with the src family protein tyrosine kinase, lyn. Neither blk nor fyn were observed to interact with CD45 even though they have been implicated in antigen receptor signal transduction. This finding suggests that CD45 may preferentially regulate the phosphorylation of lyn and thus, its activity. In summary, these studies provide evidence to support the hypothesis that CD45 regulates antigen receptor-mediated signal transduction by controlling the tyrosine phosphorylation of multiple components of the antigen receptor complex.

The B cell antigen receptor (AgR) complex is composed of the antigen recognition structure, membrane immunoglobulin (mlg), which is noncovalently associated with transmembrane heterodimers containing subunits encoded by the B cell-specific mb-1 and B29 genes (1–5). The MB-1/B29 heterodimer is believed to function as an accessory structure that physically couples mlg to downstream signal transduction elements (6–8). B cell activation, initiated by ligand binding to mlg, is regulated by changes in tyrosine phosphorylation of key proteins due to the combined action of one or more protein tyrosine kinases (PTK) and at least one protein tyrosine phosphatase, CD45. The finding that either the pharmacologic inhibition of PTK activity (9–11), or a deficiency in the expression of CD45 inhibits several early activation events in the B cell (12, 13), supports the contention that the activities of both PTK and protein tyrosine phosphatase are necessary for AgR-mediated B cell activation. Therefore, it is important to delineate the regulatory interactions between PTK and protein tyrosine phosphatase, as well as to define their common substrates.

Although it is apparent that expression of CD45 is required for signal transduction through the B cell AgR complex following ligand binding (12, 13), relatively little is known concerning the specific substrates that CD45 acts on in order to regulate this process. Previous studies, however, have demonstrated that the subunits of the MB-1/B29 heterodimer are in fact substrates for CD45 (14). Inhibition of protein tyrosine phosphatase function in the B cell by addition of pharmacologic agents, or specific alteration of the function of CD45 by physical cross-linking, revealed that MB-1 and B29 become hyperphosphorylated on tyrosine residues. Thus, CD45 may be important for regulation of the phosphorylation of these mlg-associated proteins, which may in turn maintain the AgR complex in a state that is competent to respond to ligand binding. Alternatively, changes in the phosphotyrosine (PTyr) content of MB-1 and B29 have been predicted to alter, either qualitatively and/or quantitatively, their association with downstream signaling proteins such as the src family PTK, lyn, fyn, and blk (14).

In addition to MB-1 and B29, several proteins have been identified, that are phosphorylated on tyrosine residues in response to mlg cross-linking, including the transmembrane receptors CD22 (15) and CD19 (16), and the intracellular signaling proteins phospholipase Cγ (17–21), phosphatidylinositol 3-kinase (22), microtubule-associated protein kinase (23), GTPase-activating protein (24), and a number of src family PTK (i.e., lyn, fyn, and blk) (25, 26). These proteins either interact with the AgR complex, or are involved in transduction of a signal following AgR cross-linking, or both. Presumably, changes in tyrosine phosphorylation of these proteins are important for regulation of their function. Although it is possible that CD45 may regulate tyrosine phosphorylation of one or more of the above proteins in the B cell, there is no direct evidence that this is the case.

Of the tyrosine-phosphorylated proteins listed above, the src family PTK are particularly attractive candidates for physiologically relevant substrates of CD45. First, multiple src family PTK including lyn, fyn, blk, and lck have been found to associate with the AgR complex in B cells and have also been shown to become activated following cross-linking of mlg (6, 26–28). Additionally, the use of PTK inhibitors has revealed that PTK activation and tyrosine phosphorylation of substrate.
proteins is an essential step in the signal transduction process initiated by AgR cross-linking (9–11). Therefore, the possibility exists that CD45 is required for AgR cell activation because it must dephosphorylate, and thus activate, one or more of the src family PTK associated with the AgR complex. Studies in the T cell demonstrating that CD45 regulates tyrosine phosphorylation of the src family PTK, lck, which in turn alters its enzymatic activity (29–34), suggest a similar role may be played by this protein tyrosine phosphatase in the B cell.

In this report, experiments were performed to identify tyrosine-phosphorylated proteins that are potential substrates for CD45 based on their physical association with this protein tyrosine phosphatase, in order to further define its physiological role in the B cell. Isolation of CD45 from quiescent splenic B cells revealed that CD45 is associated with specific components of the AgR complex, including MB-1, B29, and lyn, thus supporting the possibility that regulation of the P'Tyr content of these proteins by CD45 is important for AgR-mediated signal transduction.

EXPERIMENTAL PROCEDURES

Animals—C57BL/6 X DBA2F1 mice were obtained from Charles River Laboratories (Wilmington, MA). All experiments were performed using mice between the ages of 6 and 8 weeks.

Biologic Reagents and B Cell Preparation—Monoclonal Ab used in these studies included: b76 (rat IgG1, anti-mouse heavy chain (25)), JA12.5 (rat IgG3, anti-mouse 8 heavy chain, from Dr. Joseph Davie, Washington University, St. Louis, MO), I3/2.3 (rat IgG1, common epitope-specific anti-mouse CD45 (36)), R6-5.91 (mouse IgG2a, anti-rat x light chain, from Dr. Timothy Springer, Harvard Medical School, Boston, MA), D3.137.5 (rat IgG1, anti-mouse class II MHC, 14A4 haplotype), M1/42.3.9.8 (rat IgG2b, anti-mouse class I MH, H-2K), and M18/2a (rat IgG1, anti LFA-1 p chain, American Type Culture Collection, Rockville, MD). The anti-PTyr monoclonal Ab PY20 was generously provided by Dr. John R. Glenneny (University of Kentucky College of Medicine, Louisville, KY) (37). Monoclonal Ab were purified using Protein G-Sepharose 4B fast flow (Pharmacia Biotech Inc.) and were coupled to CNBr-activated Sepharose 4B beads (Pharmacia) as recommended by the manufacturer at a concentration of 1–3 mg of protein/ml of packed beads. Polyclonal Ab specific for the src family kinases lyn, fyn, blk, lck, and Zck used in these studies were previously described (25). Peptides from the antigen receptor homology-1 domains of the Ig-associated proteins MB-1 and B29 were used to produce anti-peptide Ab. The murine coding sequences for lyn (38) were cloned into pBMS-1 (39) and recombinant baculoviruses expressing glutathione S-transferase (GST)-lyn fusion proteins were produced. The fusion proteins were purified by glutathione affinity chromatography. GST-lyn fusion proteins and GST alone were produced and purified for use as negative controls.

Resting splenic B cells were purified by previously described (40). Briefly, after preparing a single cell suspension of splenocytes, red blood cells were lysed using Gey's solution, T cells were depleted by complement-mediated lysis, and high density B cells (>1,079 g/ml) were isolated by centrifugation through a discontinuous Percoll (Pharmacia) gradient.

Reimmunoprecipitation of CD45—For each sample, 2–3 x 10⁹ resting splenic B cells were washed once in phosphate-buffered saline and then lysed in 500 μl of lysis buffer (10 mM Tris-HCl, pH 7.3, 150 mM NaCl, 10 mM NaF, 1 mM sodium orthovanadate, 1 mM phenylmethylsulfonyl fluoride, 1 μg/ml aprotinin, 1 μg/ml leupeptin, and 1 μg/ml o-antitrypsin (Sigma)) containing one of the following detergents: 0.2% digitonin, 0.2% Nonidet P-40, 2 mM CHAPS, 0.4% N-octyl-β-D-glucopyranoside, or 0.2% Brij 35. CD45 immune complex-coated beads were then washed and re-equilibrated in reaction buffer (20 mM Tris-HCl, pH 7.2, 10 mM MnCl₂, 0.05% digitonin) and then incubated in 40 μl of reaction buffer supplemented with 10 μCi in [γ³²P]ATP (3000 Ci/mmol) at room temperature for 30 min, and then separated on 4–12% SDS-PAGE (38) were cloned into pBMS-1 (39) and recombinant baculoviruses expressing glutathione S-transferase (GST)-lyn fusion proteins were produced. The fusion proteins were purified by glutathione affinity chromatography. GST-lyn fusion proteins and GST alone were produced and purified for use as negative controls.

Reating splenic B cells were purified as previously described (40). Briefly, after preparing a single cell suspension of splenocytes, red blood cells were lysed using Gey's solution, T cells were depleted by complement-mediated lysis, and high density B cells (>1,079 g/ml) were isolated by centrifugation through a discontinuous Percoll (Pharmacia) gradient.

Reimmunoprecipitation of CD45—For each sample, 2–3 x 10⁹ resting splenic B cells were washed once in phosphate-buffered saline and then lysed in 500 μl of lysis buffer (10 mM Tris-HCl, pH 7.3, 150 mM NaCl, 10 mM NaF, 1 mM sodium orthovanadate, 1 mM phenylmethylsulfonyl fluoride, 1 μg/ml aprotinin, 1 μg/ml leupeptin, and 1 μg/ml o-antitrypsin (Sigma)) containing one of the following detergents: 1% digitonin (Sigma), prepared as previously described (2), 1% Nonidet P-40, 1 mg/ml CHAPS, 2% N-octyl-β-D-glucopyranoside, or 1% Brij 35. Lysis of cells was conducted for 1 h at 4 °C, after which particulate debris was removed by centrifugation at 12,000 x g for 15 min at 4 °C. Lysates were then precleared by incubation with RG7/9.1 coupled to Sepharose 4B for 2 h at 4 °C to minimize nonspecific adsorption of proteins to Sepharose 4B beads. Immunoprecipitation of CD45 was carried out by incubating lysates with anti-CD45 monoclonal Ab (I3/2.3) coupled to Sepharose 4B overnight at 4 °C.

RESULTS

Association of CD45 with a Protein Tyrosine Kinase in Resting B Cells—B cell activation induced by AgR cross-linking is dependent on the function of both src family PTK and the protein tyrosine phosphatase, CD45 (9–13). It has been hypothesized that CD45 may physically associate with one or more src family PTK, thereby facilitating its ability to dephosphorylate the characteristic carboxyl-terminal tyrosine residue that is involved in regulation of kinase activation (42). To examine this possibility, we attempted to demonstrate a physical association between CD45 and one or more PTK. For these experiments, CD45 was immunoprecipitated from quiescent B cells and the resulting CD45 immune complex material was radiolabeled using an in vitro kinase assay. Radiolabeled CD45-associated proteins were then released and reimmunoprecipitated with specific anti-P'Tyr monoclonal Ab.

Initial experiments to examine the putative association of CD45 with a PTK were performed using CD45 immune complex material isolated under different detergent conditions. Based on reimmunoprecipitation of proteins phosphorylated on tyrosine (Fig. 1), it was apparent that CD45 is associated with a PTK, and that the association is stable in the presence of the nonionic detergents, digitonin and Brij-35, and the zwitterionic detergent, CHAPS. Several P'Tyr-containing proteins exhibiting molecular masses of 140, 85, 50–60, and 30–40 kDa were consistently reimmunoprecipitated from CD45 immune complex material isolated under the various detergent conditions above. In contrast, P'Tyr-containing proteins were not detected in CD45 immune complex material isolated in the presence of N-octyl-β-D-glucopyranoside or Nonidet P-40, indicating that the CD45 complex may be unstable under certain detergent conditions. It should be noted that prior to performing the in vitro kinase assay, all CD45 immune complex samples were washed and resuspended in a common reaction buffer with
CD45 and PTK under different detergent conditions. Quiescent B cells (3 x 10^6) were incubated in lysis buffer containing the detergents indicated in lanes 1-5 at the concentrations described under "Experimental Procedures." Lysates were precleared and CD45 was immunoprecipitated using the monoclonal Ah, 13/2.3 (anti-common epitope) coupled to Sepharose 4B. Immune complex proteins were radioiodelabeled using an in vitro kinase assay and tyrosine phosphorylated proteins were reimmunoprecipitated with the anti-PTyr monoclonal Ab, PY20. Radioiodelabeled proteins were run on a 10% acrylamide gel using SDS-PAGE. In lane 1, Dig represents digitonin, and in lane 4, β-OG represents N-octyl-β-D-glucopyranoside. NP-40, Nonidet P-40, 0.05% digitonin. This was done in order to minimize changes in either the conformation of PTK substrates or the activity of CD45-associated PTK that might be caused by suspension of the immune complex material in various detergents. These results suggest that the detergent sensitivity of the CD45-PTK complex in the B cell is in fact similar to that observed for the association between CD45 and lck in the T cell, as reported by Schraven et al. (43). In that study, a stable association between CD45 and lck was demonstrated when digitonin and Brij 58, but not Nonidet P-40, were used for solubilization of cellular proteins.

Several negative controls were utilized to verify that the phosphoproteins observed in Fig. 1 were specifically associated with CD45 as part of a larger multisubunit protein complex (Fig. 2). First, immunoprecipitation of CD45 and associated proteins was inhibited by the presence of excess, soluble anti-CD3 monoclonal Ab during the primary immunoprecipitation step (Fig. 2, lane 2). Second, no PTyr-containing proteins could be reimmunoprecipitated from samples in which Sepharose 4B beads, coated with irrelevant anti-CD3 monoclonal Ab, were used for primary immunoprecipitation (Fig. 2, lane 3). These negative controls, combined with extensive preclearing of lysates, ensured that the radioiodelabeled proteins observed in Figs. 1 and 2 were precipitated by virtue of their association with CD45 and were not precipitated due to nonspecific adsorption to the Sepharose 4B bead matrix. More importantly, most if not all of the phosphoproteins in Fig. 1 are selectively associated with CD45 as opposed to other B cell transmembrane proteins. For example, a unique pattern of PTyr-containing proteins was seen when using anti-LFA-1β to perform the initial immunoprecipitation step (Fig. 2, lane 4). Finally, it has previously been shown that class I and II major histocompatibility complex antigens, immunoprecipitated from quiescent B cells, are not associated with PTyr-containing proteins based on the use of an in vitro kinase assay (14, 27). These findings indicate that in contrast to CD45, many B cell transmembrane proteins either do not associate with a PTK, or do not associate with substrates for a PTK, or both.

The src Family Kinase, lyn, Associates with CD45 in B Cells—Because CD45 was found to associate with at least one PTK, it was of interest to determine whether specific PTK was a member of the src family. This possibility was supported by the observation that a number of PTyr-containing proteins with molecular masses in the 50–60 kDa range coprecipitated with CD45. Moreover, as mentioned above, CD45 has been shown to associate with the src family PTK, lck, in T cells (43–45). As many as five src family PTK, including hck, lck, lyn, lyn, and blk have previously been demonstrated to be present in either normal B cells or B cell lines (25–27), and substantial levels of lyn, lyn, and blk are expressed by quiescent B cells used in the present experiments (14, 26). Initially, studies were conducted in order to confirm the presence of selected src family PTK in resting splenic B cells. Anti-src family PTK polyclonal Ab were used to screen B cell lysates for the presence of hck, lck, lyn, lyn, and blk based on immunoprecipitation and autophosphorylation of kinases using an in vitro kinase assay. As seen in Fig. 3A, the above src family PTK could be detected in resting B cells with the exception of lck. Next, experiments were conducted in an attempt to identify the specific PTK(s) present in the CD45 immune complex by immunoprecipitating CD45 from B cell lysates prepared in the presence of digitonin. Components of the CD45 immune complex were radioiodelabeled using an in vitro kinase assay and polyclonal Ab against the src family PTK were used to reimmunoprecipitate specific PTK. The results depicted in Fig. 3B demonstrate that the polyclonal anti-lyn Ab reimmunoprecipitated a phosphoprotein doublet with molecular masses of 53 and 56 kDa (note doublet in anti-PTyr immunoprecipitate, lane 1). In contrast, none of the other polyclonal Ab specific for src family PTK were observed to reimmunoprecipitate phosphoproteins with an appropriate molecular mass for hck, lck, lyn, lyn, or blk. It is apparent from these studies that the level of lck expression in resting B cells (Fig. 3A) is so low that it is not possible to detect an association of this src family PTK and CD45 (Fig. 3, B and C) as previously reported in the T cell (43).
Precipitate with heads were washed and releasing buffer were surface labeled with specific immunoprecipitated from lysates containing (mAb) was used (mAb) was used for reimmunoprecipitation in lane 1. The radiolabeled proteins were then analyzed on 10% acrylamide gel using SDS-PAGE. C, resting B cells were surface labeled with 125I using the lactoperoxidase method. Radiolabeled cells were lysed in buffer containing 1% digitonin, and specific src family PTK were immunoprecipitated with polyclonal Ab. Following primary immunoprecipitation of src PTK immune complexes, the beads were washed and releasing buffer was added. Anti-CD45 mAb (192-3) coupled to Sepharose 4B was then used to reimmunoprecipitate CD45 from the immune complex material.

Reciprocal experiments were also performed in which B cells were surface labeled with 125I, and the various src family PTK were immunoprecipitated from lysates containing 1% digitonin. Protein-protein interactions were disrupted by the addition of releasing buffer, and anti-CD45 monoclonal Ab was used to reimmunoprecipitate CD45 from the different PTK immune complexes. In agreement with the results depicted in Fig. 3B, CD45 was selectively reimmunoprecipitated from the src immune complex (Fig. 3C). Therefore, it appears that of the src family PTK expressed in the B cell, lyn interacts with CD45 in a selective manner. Thus, it is likely that lyn is responsible for the PTK activity associated with the CD45 immune complex.

The Antigen Receptor Complex Proteins, MB-1 and B29, Co-precipitate with CD45—As seen in Figs. 1–3, several PTPy containing proteins in addition to p536 and p56l6 coprecipitate with CD45. To determine whether these proteins are potential substrates for CD45 and/or play a role in regulation of its function in the B cell, experiments were performed in an attempt to specifically identify selected phosphoproteins. In particular, the phosphoproteins with molecular masses of approximately 32, 34, and 37 kDa were of interest because they exhibited an electrophoretic mobility pattern that was similar to the B cell AgR complex proteins MB-1 and B29 (4–6). Glycosidase treatment of the 32-, 34-, and 37-kDa phosphoproteins generated core proteins with molecular masses of approximately 25 and 26 kDa (data not shown) which were in general agreement with the core molecular masses reported for MB-1 and B29 (23.6 and 24.6 kDa, respectively) (4). Finally, MB-1 and B29 have previously been shown to be substrates for CD45 (14), and thus it is logical to predict that dephosphorylation of these proteins is dependent on their physical interaction with CD45. Following immunoprecipitation of CD45 and radiolabeling of immune complex proteins, polyclonal Ab specific for MB-1 and B29 were used for secondary immunoprecipitation to determine whether these proteins were in fact components of the CD45 immune complex. As can be seen (Fig. 4), both anti-MB-1 and anti-B29 polyclonal Ab recognized phosphoproteins with the appropriate molecular mass, thereby confirming the presence of MB-1 and B29 in the CD45 immune complex. As expected, polyclonal Ab specific for both MB-1 and B29 immunoprecipitated identical phosphoproteins that included MB-1 (32–33 kDa) as well as the two species of B29 (34 and 37 kDa). This is due to the fact that MB-1 and B29 are expressed by the B cell as a disulfide-linked heterodimer (1).
erodimer. CI145 was prepared from immune complex. Reimmunoprecipitated proteins used to reimmunoprecipitate these proteins from the radiolabeled im-

Fig. 4. CD45 is physically associated with the MB-1/B29 heterodimer. CD45 was immunoprecipitated from 1% digitonin lysates prepared from 3 x 10^7 quiescent B cells. The lysates were precleared, and the immune complex material was radiolabeled using an in vitro kinase assay. Polyclonal anti-peptide Ah specific for MR-1 or R29 was used to reimmunoprecipitate these proteins from the radiolabeled im-

Fig. 5. The MB-1/B29 heterodimer and lyn interact with CD45 independently of one another. CD45 was immunoprecipitated from 1% digitonin lysates, and the CD45 immune complex proteins were radiolabeled using an in vitro kinase assay. Following this, buffer containing 1% Nonidet P-40 was added to dissociate radiolabeled proteins from CD45 while maintaining the potential interaction between the MB-1/B29 heterodimer and lyn. Anti-CD45 monoclonal Ab (13/2.3) coupled to Sepharose 4B was then used to preclear any residual CD45 from the lysate prior to reimmunoprecipitation of either lyn or the MB-1/B29 heterodimer. Reimmunoprecipitated proteins were analyzed on 10% acrylamide gels using SDS-PAGE.

was extensively precleared from the lysate in order to eliminate the possibility that it could act as an intermediate bridge between lyn and the MB-1/B29 heterodimer. Subsequently, either lyn or the MB-1/B29 heterodimer were reimmunoprecipitated and separated by SDS-PAGE to examine whether they were associated with one another (Fig. 5). Results from these experiments demonstrated that lyn could be reimmunoprecipitated without recovering significant quantities of the MB-1/B29 heterodimer. Similarly, the MB-1/B29 heterodimer was reimmunoprecipitated in the absence of lyn. Additional evidence to support the hypothesis that lyn can interact with CD45 independently of the MB-1/B29 heterodimer was provided by experiments in which a GST-lyn fusion protein was used to immunoprecipitate CD45 from 125I-
labeled thymocyte lysates prepared in buffer containing 1% digitonin. As depicted in Fig. 6, GST-lyn was observed to bind to CD45 in the absence of the MB-1/B29 heterodimer which is not expressed in thymocytes. Neither GST alone nor a GST-yes fusion protein were observed to bind to CD45 under similar conditions.

Thus, even though it is possible that a small fraction of the lyn in the CD45 immune complex is associated with CD45 by virtue of its interaction with the MB-1/B29 heterodimer or vice versa, it appears that the majority of these proteins interact with CD45 independently of one another. This finding is in agreement with previous results demonstrating that only a small percentage (~1–3%) of the total pool of lyn and MB-1/B29 heterodimers in the resting B cell appear to be physically associated with one another (14).

CD45 Is Associated with the B Cell Antigen Receptor, Membrane Immunoglobulin—The MB-1/B29 heterodimer is expressed in association with mlg on the surface of the B cell (1). Because of this, it is likely that mlg and CD45 may also be physically associated with one another. Such an association could be mediated either by the direct interaction of mlg with CD45 or as a result of their mutual association with the MB-
1/B29 heterodimer. To confirm the association between CD45 and mlg, B cell surface proteins were labeled with \(^{125}\)I using the lactoperoxidase technique. Radiolabeled cells were lysed in buffer containing 1% digitonin, and the lysates were preclari prior to immunoprecipitation of the following proteins: lane 1, mlg (b76 and JA12.5); lane 2, CD45 (13/2.3), lane 3, LFA-1β (M182Aa); lane 4, class I MHC antigen (M1/42.3.9.8); and lane 5, class II MHC antigen (D3.137.5.7). After primary immunoprecipitation, the beads were washed extensively and then incubated in releasing buffer. Reimmunoprecipitation of the following proteins was then performed: lane 1, CD45 (13/2.3); lanes 2-5, mlg (b76 and JA12.5). The reimmunoprecipitated proteins were analyzed on 10% acrylamide gels using SDS-PAGE.

**DISCUSSION**

Recent studies have demonstrated that expression of the protein tyrosine phosphatase, CD45, is necessary for signal transduction via the B cell AgR complex (12, 13). Nevertheless, very little is known concerning the mechanism(s) by which this protein tyrosine phosphatase regulates the transduction of a signal in response to AgR cross-linking. Presumably, CD45 is required to regulate tyrosine phosphorylation of one or more proteins in the B cell that are involved in signal transduction, thereby controlling their functional status. In this regard, it is important to identify physiological substrates for CD45 in order to delineate its functional role in B cell activation. In the present study, experiments were performed to identify tyrosine-phosphorylated proteins that are physically associated with CD45 in quiescent B cells. Based on coprecipitation experiments, several PTyr-containing proteins were consistently found to be associated with CD45 under varied detergent conditions. Further characterization of these proteins revealed that the src family PTK, lyn, and the mlg-associated proteins MB-1 and B29 were present in the CD45 immune complex material. These proteins have been implicated in signal transduction via the AgR complex and exhibit increased tyrosine phosphorylation upon cross-linking of mlg (26, 46). Thus, their association with CD45 in the B cell is suggestive of the fact that they are substrates for CD45 and that their function may be regulated by this protein tyrosine phosphatase.

The above hypothesis is supported by previous studies demonstrating that tyrosine phosphorylation of the mlg-associated MB-1/B29 heterodimer is in fact regulated by CD45 (12, 14). Thus, the functional ability of CD45 to regulate tyrosine phosphorylation of MB-1 and B29 correlates with the physical interaction between these proteins observed in the present study. The effect that alterations in tyrosine phosphorylation of MB-1 and B29 have on their function, and by extension B cell activation, are not well delineated at the present time. However, the tyrosine-containing motifs found in the cytoplasmic domains of MB-1 and B29 all exhibit the characteristic sequence Y-X-X-LII (1), to which src family PTK have been predicted to bind via their SH2 domains (47). Studies have in fact shown that the MB-1/B29 heterodimer couples mlg to one or more src family PTK (7, 14). Therefore, it is possible that alterations in the phosphorylation of MB-1 and/or B29 might affect the recruitment of src family kinases via SH2-PTyr interactions.

In the present study, the src family PTK, lyn, was also observed to be associated with CD45. Evidence from studies in the T cell supports the hypothesis that src family PTK, like the MB-1/B29 heterodimer, are substrates for CD45. Experiments in vitro have in fact demonstrated that lck (29-31), and lyn (48) are substrates for CD45 which preferentially dephosphorylates the carboxyl-terminal regulatory tyrosine residue of each kinase. Interestingly, however, there appears to be a selective physical interaction between CD45 and lck as opposed to lyn, based on coprecipitation experiments (43-45). This selective physical association suggests that CD45 may preferentially regulate the phosphorylation of lck in vivo and thereby control its function. In support of this hypothesis, studies have shown that phosphorylation of lck as opposed to lyn is preferentially regulated, although not exclusively so, in vivo (32-34).

Based on the above findings, it is possible that CD45 selectively associates with one or more of the src family PTK (i.e. lyn, lyn, or blk) implicated in AgR-mediated signaling in the B cell. Such a selective interaction could theoretically result in preferential regulation of PTK phosphorylation and function. In this regard, it was interesting to find that CD45 selectively associates with lyn, as opposed to the other src family PTK normally expressed in quiescent B cells. Therefore, one would predict that CD45 might preferentially regulate tyrosine phosphorylation of lyn as opposed to lyn or blk. If this is the case, then receptor-mediated signal transduction pathways that are dependent on the activation of lyn, as opposed to lyn or blk, might be preferentially regulated by CD45. Although the above hypothesis remains to be confirmed, it potentially complicates the delineation of the relative role that the different src family PTK play in signal transduction via the B cell AgR complex. In this regard, it is quite possible that the different src family PTK may perform unique functions and that their enzymatic activity could be under differential regulatory control as well.
In addition to the potential for regulation of lyn by CD45, the selective association between these proteins could be important from the perspective that lyn may exhibit a unique ability to regulate the function of CD45 via a phosphorylation-dependent mechanism. This possibility is supported by previous studies in which CD45 was observed to become transiently phosphorylated on tyrosine residues during T cell activation (49). Thus, it is possible that specific PTK in T and B cells (i.e. lck and lyn, respectively) may regulate the function of CD45 as part of a dynamic relationship between PTK and protein tyrosine phosphatase in which both are required to transduce a signal due to the need for bidirectional feedback. 

In summary, this study demonstrates that CD45 can exist in the B cell as part of a larger multisubunit complex composed of several proteins that are phosphorylated on tyrosine residues. In particular, it was found that the src family PTK, lyn, and the mlg-associated MB-1/B25 heterodimer were among the CD45-associated proteins. The physical interaction between CD45 and these PTK-containing proteins suggests that regulation of their phosphorylation by CD45 may be important for regulating the signal transduction process initiated by AgR cross-linking.

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