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Biochemistry

Tyrosine phosphorylation of components of the B-cell antigen receptors following receptor crosslinking

(membrane immunoglobulin/membrane immunoglobulin-associated proteins/MB-1 protein)

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ABSTRACT **Crosslinking membrane immunoglobulin (mIg), the B-cell antigen receptor, stimulates tyrosine phosphorylation of a number of proteins. Since many receptors are phosphorylated after ligand binding, we asked if components of the mIg receptor complexes were tyrosine-phosphorylated after mIg crosslinking. Both mIgM and mIgD are noncovalently associated with at least two other proteins. mIgM is associated with the MB-1 protein, which is disulfide-linked to a protein designated Ig-β. mIgD is not associated with MB-1 but is with IgD-α, which is also disulfide-linked to Ig-β. Using immunoprecipitation with a specific anti-MB-1 antiserum followed by anti-phosphotyrosine immunoblotting, we found that crosslinking mIgM stimulated tyrosine phosphorylation of MB-1, Ig-β, and a previously unidentified 54-kDa polypeptide associated with MB-1. In mature splenic B cells that express both mIgM and mIgD, mIgM crosslinking stimulated tyrosine phosphorylation of the 32-kDa MB-1 protein, whereas mIgD crosslinking stimulated tyrosine phosphorylation of MB-1-related proteins of 33 and 34 kDa. The 32-kDa MB-1 protein was only associated with mIgM, whereas the 33- and 34-kDa MB-1-related proteins were specifically associated with mIgD and are most likely IgD-α. Thus, crosslinking either mIgM or mIgD stimulated tyrosine phosphorylation only of the MB-1-related proteins associated with that receptor.**

The B-lymphocyte antigen receptors are membrane forms of immunoglobulins (mIg). Antigen binding by mIgM on immature B cells results in cell death (1) or inactivation (2, 3), while crosslinking mIgM or mIgD on mature B cells leads to proliferation and antibody secretion (4). In both mature and immature B cells, mIg crosslinking activates two intracellular signaling pathways, protein tyrosine phosphorylation (5, 6) and phosphoinositide hydrolysis (7–9). How these signaling events result in activation of mature B cells or inactivation of immature B cells remains to be elucidated.

Since both mIgM and mIgD have cytoplasmic tails of only three amino acids, they are unlikely to trigger intracellular signaling pathways directly. Immunoprecipitation studies have shown that mIgM is noncovalently associated with a disulfide-linked heterodimer composed of the 34-kDa MB-1 protein and a 39-kDa protein called Ig-β (10–12). mIgD is associated with a similar disulfide-linked heterodimer in which MB-1 is replaced by a 35-kDa protein called IgD-α (13).

Since the mIg-associated proteins are likely to be involved in signal transduction by mIg, we asked if these proteins were phosphorylated after receptor stimulation. Many receptors become phosphorylated after ligand binding, and this modification can alter receptor function (14). Tyrosine phosphorylation of tyrosine kinase growth-factor receptors can facilitate signaling (15), whereas serine phosphorylation of receptors often correlates with desensitization or uncoupling from signaling pathways (14, 16). In this study, we show that crosslinking mIgM stimulated tyrosine phosphorylation of MB-1, Ig-β, and a previously unidentified 54-kDa polypeptide. We also found that crosslinking mIgM in splenic B cells stimulated tyrosine phosphorylation of MB-1 but not IgD-α, while crosslinking mIgD increased tyrosine phosphorylation of IgD-α but not MB-1.

MATERIALS AND METHODS

Labeling of Cells. Cells were metabolically labeled with Trans^{35}S-label (34 μCi/ml, ICN; 1 μCi = 37 kBq) in growth medium (9) with 20% of the normal amounts of methionine and cysteine or were surface-iodinated by using sulfosuccinimidyl 3-(4-hydroxyphenyl)propionate (Pierce) (17). Labeled proteins were immunoprecipitated as described (18).

Stimulation of Cells and Preparation of Cell Lysates. WEHI-231 (19) and Bal 17 (ref. 20; our isolate does not express mIgD) cells were resuspended at 5×10^6 cells per ml in growth medium containing 20 mM Hepes. Resting splenic B cells (>93% mIgM$^+$), isolated from (C57BL/6 × DBA/2)F_1 mice as described (5), were resuspended at 16.7×10^6 cells per ml in a modified Hepes-buffered saline solution (9). Cells were stimulated at 37°C, washed, and resuspended at 10^8 cells per ml in lysis buffer [20 mM Tris·HCl, pH 8/137 mM NaCl/10% (wt/vol) glycerol/1% Triton X-100/1 mM Na_3VO_4/2 mM EDTA/1 mM phenylmethylsulfonyl fluoride/20 μM leupeptin/0.15 unit of aprotinin per ml] as described (5). Detergent-insoluble material was removed by centrifugation, and protein concentrations were determined by the BCA (bicinchoninic acid) protein assay (Pierce).

Anti-MB-1 Antiserum. A 34-amino acid peptide from the putative cytoplasmic domain of the murine MB-1 protein [amino acids 187–220 (21); synthesized by the Biomolecular Resource Center, University of California, San Francisco] was coupled to keyhole limpet hemocyanin and used to immunize rabbits (Berkeley Antibody, Richmond, CA).

Anti-MB-1 Immunoprecipitation. Cell lysates were made 0.4% in sodium deoxycholate and 0.3% in sodium dodecyl sulfate (SDS), precleared 30 min with protein A-Sepharose (Sigma), and then incubated with anti-MB-1 antiserum (5 μl per mg of lysate protein) for 3 hr at 4°C. Immunocomplexes were collected on protein A-Sepharose for 1 hr. The beads were suspended in 0.5 ml of lysis buffer containing 50 μM Na_3VO_4, 0.4% deoxycholate, and 0.3% SDS (wash buffer), layered over 0.4 ml of 30% sucrose in 0.5× wash buffer, and centrifuged 5 min at 10,000 × *g*. The beads were then washed once each with wash buffer and 50 μM Na_3VO_4. Proteins were eluted by boiling in nonreducing SDS/PAGE sample

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Abbreviations: mIg, membrane immunoglobulin; anti-Tyr(*P*), anti-phosphotyrosine.

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buffer containing 8 M urea. To reduce disulfide bonds, 2-mercaptoethanol was added to 5% final concentration.

Immunoprecipitation of MB-1–mIg Complexes. To preserve MB-1–mIg complexes, cells were solubilized in 1% digitonin lysis buffer (22) containing 1 mM Na_3VO_4 and the protease inhibitors listed above for 5 min on ice. Detergent-soluble material was immunoprecipitated with anti-MB-1 antiserum, 3:1 rabbit and goat anti-μ antibodies (Jackson ImmunoResearch), or 3:1 Hδa/1 monoclonal anti-mouse δ-chain antibody and goat anti-mouse δ-chain antibody (gifts of F. Finkelman, Uniformed Services University for the Health Sciences, Bethesda, MD). Immunocomplexes were collected on protein A-Sepharose and washed three times with phosphate-buffered saline containing 0.5% digitonin, 2 mM EDTA, 50 μM Na_3VO_4, and protease inhibitors.

Immunoblotting. Proteins were separated by SDS/PAGE and transferred to nitrocellulose for 4.5 hr at 0.5 A. Immunoblotting with the monoclonal anti-phosphotyrosine [anti-Tyr(*P*)] antibody 4G10 (23) and colorimetric detection were performed as described (5). To reprobe blots with anti-MB-1 antiserum, the blots were soaked 10 min in 10 mM Tris·HCl/150 mM NaCl, pH 2.3 to remove bound antibodies, washed with 10 mM Tris·HCl/150 mM NaCl, pH 8, and reblocked as described (5). The blots were incubated 1 hr at 20°C with anti-MB-1 antiserum diluted 1:300 in 10 mM Tris/150 mM NaCl/0.05% Tween-20, pH 8, and then washed and incubated with 2 μCi of ^{125}I-labeled protein A (ICN) for 1 hr.

RESULTS

An antiserum against a 34-amino acid MB-1-derived peptide was produced in rabbits. This antiserum immunoprecipitated a series of bands of molecular mass 32–39 kDa under reducing conditions from ^{35}S-labeled WEHI-231 B lymphoma cells that had been lysed in Triton X-100 (Fig. 1*A*). These bands were not precipitated by preimmune serum (not shown) or by anti-μ chain antibodies. The MB-1 peptide used as the immunogen blocked precipitation of these bands by the anti-MB-1 antiserum but did not block precipitation of mIgM by anti-μ chain. The antiserum precipitated 32- to 39-kDa proteins from a pre-B-cell line (70Z/3) and mature B-cell lines (WEHI-231, 2PK-3) but not from plasmacytoma cell lines (MPC-11, SP2/0) or a T-cell line (EL-4) (Fig. 1*B*), consistent with the reported distribution of MB-1 mRNA (21).

Anti-MB-1 immunoprecipitates from surface-iodinated WEHI-231 cells were analyzed by two-dimensional nonreducing/reducing SDS/PAGE. In these gels, non-disulfide-linked proteins migrate on a diagonal, while the subunits of disulfide-linked proteins fall below the diagonal. Three distinct spots were observed (Fig. 1*C*). Two spots, one of 32–39 kDa (spot a) and the other of 34–51 kDa (spot b), migrated below the diagonal. An additional 32- to 39-kDa species (spot a′) migrated on the diagonal. The large molecular mass ranges reflect variable glycosylation of these proteins (L.M. and R.B.K., unpublished data). To identify which of these spots was MB-1, diagonal gels of anti-MB-1 immunoprecipitates from unlabeled cells were immunoblotted with anti-MB-1. Both of the 32- to 39-kDa spots (a and a′) reacted with anti-MB-1 (Fig. 1*D*). It is unclear whether the non-disulfide-linked form of MB-1 (a′) was present on the cell surface or was generated by reduction after detergent extraction. The higher molecular mass off-diagonal spot (b) did not react with anti-MB-1. However, this polypeptide did have the same mobility in the nonreducing dimension (apparent mass of 68–97 kDa) as the disulfide-linked form of MB-1, suggesting that it was disulfide-linked to MB-1. This is consistent with the 34- to 51-kDa spot being Ig-β (10).

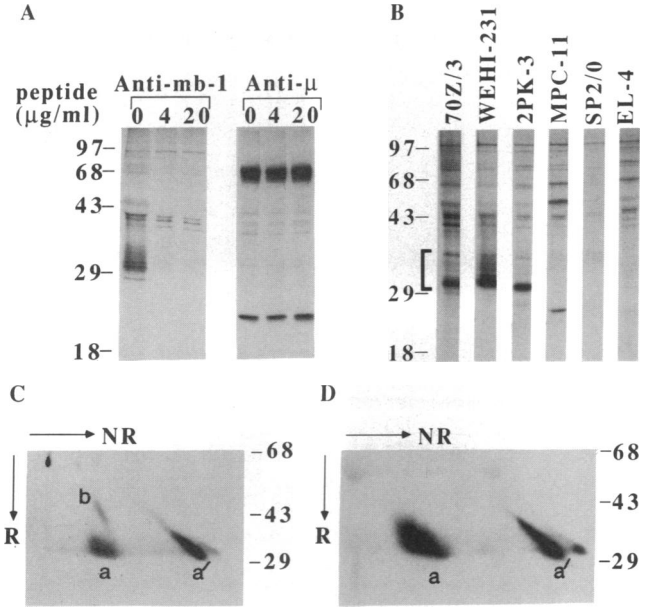

FIG. 1. Specific precipitation of MB-1 and Ig-β by anti-MB-1 antiserum. (*A*) ^{35}S-labeled proteins from WEHI-231 cells were immunoprecipitated with the indicated antibodies in the presence or absence of the 34-amino acid MB-1 peptide and resolved on 12% reducing SDS/PAGE gels. (*B*) Anti-MB-1 immunoprecipitation from ^{35}S-labeled cell lines. The bracket indicates the putative MB-1 and Ig-β proteins identified in *A*. (*C*) Anti-MB-1 immunoprecipitates from lysates of surface-iodinated WEHI-231 cells separated on 10% nonreducing SDS/PAGE gels (NR) followed by electrophoresis in the perpendicular direction on 12% reducing SDS/PAGE gels (R) as in ref. 22. Spots identified by letters are described in the text. (*D*) Unlabeled WEHI-231 cell lysates immunoprecipitated with anti-MB-1, separated as in *C*, and transferred to nitrocellulose. MB-1 polypeptides were detected with anti-MB-1 and ^{125}I-labeled protein A. Molecular masses (in kDa) are indicated.

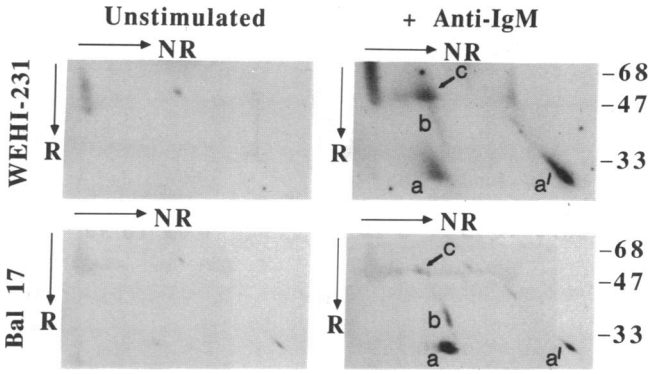

FIG. 2. Anti-Tyr(*P*) immunoblots of proteins precipitated by anti-MB-1. Cells were stimulated with buffer or with optimal concentrations of goat anti-IgM (μ chain-specific; 30 μg/ml for WEHI-231, 5 μg/ml for Bal 17; see Fig. 3) for 10 min. Cell lysates (3.2 mg of protein for WEHI-231 or 2.2 mg of protein for Bal 17) were immunoprecipitated with anti-MB-1, subjected to nonreducing/reducing SDS/PAGE as in Fig. 1*C*, and analyzed by immunoblotting with the monoclonal anti-Tyr(*P*) antibody, 4G10, which was detected with goat anti-mouse IgG-alkaline phosphatase (Bal 17 cells) or ^{125}I-labeled protein A (WEHI-231 cells). When color development was performed, blots were stripped of bound antibody and reprobed with anti-MB-1 and ^{125}I-labeled protein A to localize the MB-1 polypeptides and confirm that equivalent amounts of MB-1 were recovered. Sizes are shown in kDa. Polypeptides showing increased tyrosine phosphorylation after mIgM crosslinking are identified: a and a′ are the two forms of MB-1, b is Ig-β, and c is the 54-kDa polypeptide described in the text. The other spots at 55 kDa in the reducing dimension are the heavy chain of the anti-MB-1 antibody used for immunoprecipitation, which reacts with the developing agents.

To ask whether mIgM crosslinking stimulated tyrosine phosphorylation of MB-1 or Ig-β, anti-MB-1 immunoprecipitates were separated on diagonal gels and analyzed by anti-Tyr(*P*) immunoblotting. In both the immature B-cell line WEHI-231 and the mature B-cell line Bal 17, crosslinking mIgM stimulated tyrosine phosphorylation of four polypeptides that were immunoprecipitated by anti-MB-1 (Fig. 2). Two of these were the on- and off-diagonal forms of MB-1 (a′ and a in Fig. 1), which reacted with anti-MB-1 when the blots were reprobed (data not shown). The polypeptide we had identified as Ig-β (spot b) also showed increased tyrosine phosphorylation, as did a 54-kDa polypeptide (spot c) that was immunoprecipitated by anti-MB-1. The 54-kDa polypeptide did not crossreact with MB-1, since it was not detected when the blots were reprobed with anti-MB-1 (data not shown). The 54-kDa polypeptide migrated below the diagonal and therefore was part of a disulfide-linked complex, although apparently not with MB-1 because the nonreduced molecular mass of the complex containing the 54-kDa polypeptide (95–117 kDa in WEHI-231 cells, 92–108 kDa in Bal 17 cells) was different than that of the MB-1 complex (76–102 kDa in WEHI-231 cells, 68–84 kDa in Bal 17 cells). Coprecipitation of the 54-kDa polypeptide with MB-1 was blocked by the 34-amino acid MB-1 peptide (data not shown), suggesting that it is physically associated with MB-1, even under the harsh detergent conditions used, which disrupt the interaction of mIgM with MB-1. Thus, mIgM crosslinking stimulated tyrosine phosphorylation of MB-1, Ig-β, and a previously unidentified 54-kDa polypeptide associated with MB-1.

Anti-IgM-stimulated tyrosine phosphorylation of MB-1 and Ig-β was analyzed further by one-dimensional SDS/PAGE under reducing conditions. The 54-kDa polypeptide was obscured by the heavy chain of the anti-MB-1 antibody, which reacted with the goat anti-mouse IgG used to detect the binding of the anti-Tyr(*P*) antibody. Tyrosine phosphorylation of MB-1 and Ig-β occurred at much lower concentrations of goat anti-μ chain in Bal 17 cells than in WEHI-231 cells (Fig. 3), even though Bal 17 cells express half as much mIgM as WEHI-231 cells (data not shown). The same difference in dose–response characteristics was seen for tyrosine phosphorylation of other proteins (data not shown). mIgM-stimulated tyrosine phosphorylation of MB-1 and Ig-β was rapid in both Bal 17 (Fig. 4) and WEHI-231 cells (data not shown) and had the same kinetics as tyrosine phosphorylation of other cellular proteins. Increased tyrosine phosphorylation was clearly visible 1 min after addition of anti-IgM antibodies, was maximal at 5–10 min, and then declined to basal levels by 4 hr. Tyrosine phosphorylation of MB-1 and Ig-β was induced by several different anti-IgM antibodies but not by antibodies against major histocompatibility complex class I and class II molecules or the transferrin receptor (data not shown), as observed for tyrosine phosphorylation of other cellular proteins (5).

The bands detected in the anti-Tyr(*P*) blots were tyrosine-phosphorylated species of MB-1 and Ig-β by the following criteria: (*i*) immunoprecipitation of both the anti-Tyr(*P*)-reactive and the anti-MB-1-reactive bands was blocked by the 34-amino acid MB-1 peptide and did not occur when preimmune serum was used (data not shown), and (*ii*) binding of the anti-Tyr(*P*) antibody to the MB-1 and Ig-β bands was completely blocked by 50 mM phosphotyrosine but not by 50 mM phosphoserine or phosphothreonine (data not shown).

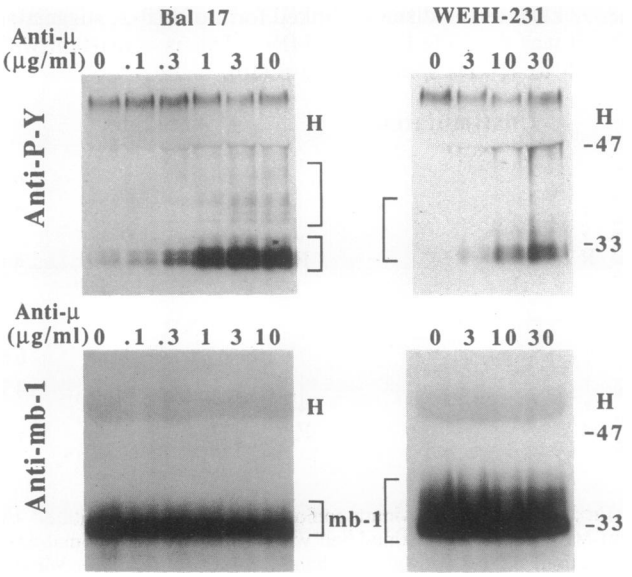

FIG. 3. Dose–response effect for anti-IgM-stimulated tyrosine phosphorylation of MB-1 and Ig-β. Cells were incubated with the indicated concentrations of goat anti-IgM for 10 min. Cell lysates (1 mg of protein for WEHI-231 cells, 0.7 mg of protein for Bal 17 cells) were immunoprecipitated with anti-MB-1 and separated on 10% reducing SDS/PAGE gels. Anti-Tyr(*P*) (anti-P-Y) immunoblotting followed by anti-MB-1 immunoblotting of the same filter was done as in Fig. 2. H indicates the heavy chain of the anti-MB-1 antibody. The lower bracket on the *Upper Left* anti-Tyr(*P*) immunoblot and the bracket on the *Upper Right* immunoblot indicate the polypeptides that comigrated with MB-1 as determined by anti-MB-1 immunoblotting (*Lower*). The upper bracket on the Bal 17 anti-Tyr(*P*) *Upper Left* immunoblot indicates bands that did not comigrate with MB-1 and that are presumably Ig-β. The Ig-β bands were clearly resolved from MB-1 in Bal 17 cells but not in WEHI-231 cells, where MB-1 is more extensively glycosylated. Sizes are shown in kDa.

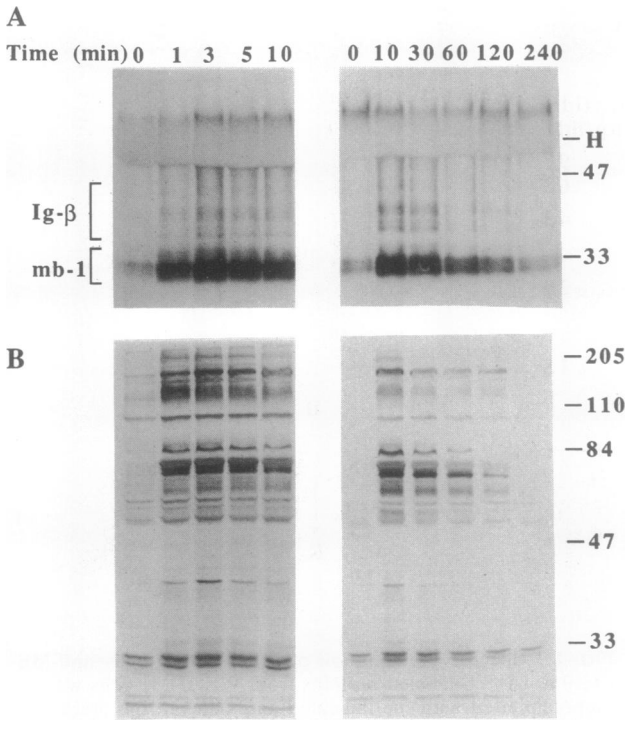

FIG. 4. Time course of mIgM-stimulated tyrosine phosphorylation for MB-1, Ig-β, and other proteins in Bal 17 cells. Anti-Tyr(*P*) immunoblots of anti-MB-1-immunoprecipitated proteins from Bal 17 cell lysates (0.7 mg of protein; 10^7 cell equivalents) (*A*) or Triton X-100-soluble proteins from Bal 17 cell lysates (40 μg of protein) (*B*) are shown. Bal 17 cells were incubated with 3 μg of goat anti-IgM antibodies per ml for the indicated times. Reprobing the blots in *A* with anti-MB-1 showed that similar amounts of MB-1 were present in each lane (not shown). Tyrosine-phosphorylated polypeptides that did not comigrate with MB-1 are labeled Ig-β as in Fig. 3. H indicates the heavy chain of the anti-MB-1 antibody. Sizes are shown in kDa.

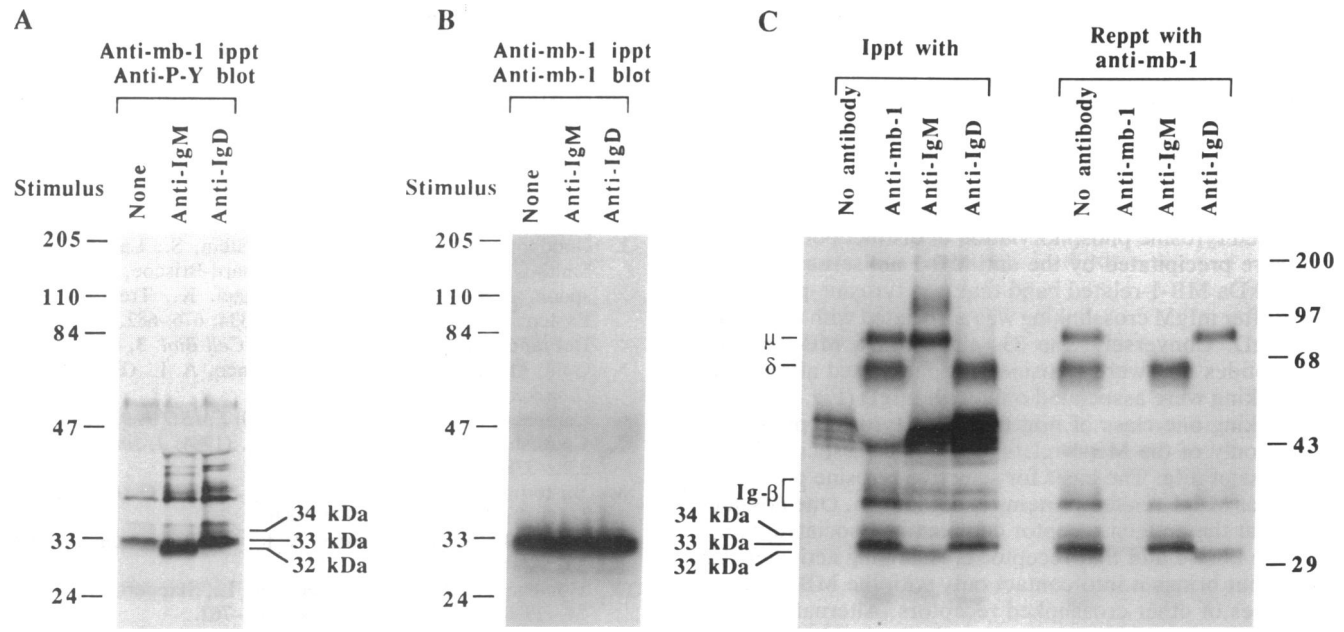

FIG. 5. Tyrosine phosphorylation of different forms of MB-1 after crosslinking mIgM or mIgD. (*A*) Splenic B cells were stimulated 10 min with 50 μg of goat anti-IgM (μ-chain specific) or 5 μg of goat anti-IgD (δ-chain specific) per ml. Anti-Tyr(*P*) blots (anti-P-Y blots) were performed on anti-MB-1 immunoprecipitated proteins (ippt) from 0.9 mg of cell lysate (1.5×10^8 cell equivalents). Phosphotyrosine-containing proteins were visualized by color development. Bands that comigrated with material that reacted with anti-MB-1 when the blot was reprobed are indicated to the right. (*B*) The blot in *A* was stripped of bound antibodies and reprobed with anti-MB-1. Anti-MB-1-reactive proteins were detected with ^{125}I-labeled protein A. This technique detects both cell surface and intracellular forms of these glycoproteins and does not resolve the different MB-1-related proteins. (*C*) Splenic B cells were surface-iodinated and lysed in 1% digitonin lysis buffer. Lysates from 8×10^7 cells were precipitated with anti-MB-1 or with antibodies specific for the heavy chains of IgM or IgD. The material that was not precipitated was then incubated with anti-MB-1 to precipitate MB-1-related molecules and proteins associated with them. Reppt, reprecipitate. Sizes are shown in kDa.

The specificity of this anti-Tyr(*P*) antibody has also been demonstrated elsewhere (5, 24).

Mature splenic B cells express mIgM and mIgD, which on a single cell have the same antigen-binding site. While mIgM is associated with Ig-β and MB-1, mIgD is associated with Ig-β and IgD-α. IgD-α is 1 kDa larger than MB-1 and may be a distinct gene product (13). We asked if tyrosine phosphorylation of MB-1 required crosslinking of mIgM or whether signaling by mIgD would also result in tyrosine phosphorylation of MB-1. Anti-Tyr(*P*) blots of anti-MB-1-immunoprecipitated proteins (Fig. 5*A*) showed that mIgM crosslinking stimulated tyrosine phosphorylation of a 32-kDa polypeptide that reacted with anti-MB-1 when the blot was reprobed (Fig. 5*B*). In contrast, crosslinking mIgD increased the tyrosine phosphorylation of two polypeptides of 33 kDa and 34 kDa that were immunoprecipitated by anti-MB-1 and that also reacted with anti-MB-1 when the blots were reprobed. The 33-kDa MB-1-related polypeptide was tyrosine phosphorylated to some extent in unstimulated cells. Crosslinking either mIgM or mIgD also stimulated tyrosine phosphorylation of a series of polypeptides of 36 kDa and 40–48 kDa that were immunoprecipitated by anti-MB-1 but did not react with anti-MB-1 when the blot was reprobed. These molecules are presumably Ig-β isoforms. Thus, crosslinking mIgM and mIgD in mature splenic B cells stimulated tyrosine phosphorylation of Ig-β as well as different MB-1-related molecules.

To see if the polypeptides reacting with the anti-MB-1 antiserum were preferentially associated with mIgM or mIgD, we solubilized surface-iodinated splenic B cells in digitonin to preserve the interaction of mIgM and mIgD with their associated proteins. Anti-MB-1 precipitated three polypeptides of 32–34 kDa as well as polypeptides with the molecular masses of Ig-β (36–40 kDa) and the heavy chains of IgM (78 kDa) and IgD (65 kDa) (Fig. 5*C*). Thus, both mIgM and mIgD were associated with molecules that were recognized by anti-MB-1. Anti-μ chain antibodies precipitated only μ chain, Ig-β, and the 32-kDa band. In contrast, anti-δ chain antibodies precipitated δ chain, Ig-β, and the 33- and 34-kDa bands. The 33- and 34-kDa bands were more strongly labeled than the 32-kDa band, which is consistent with observations that splenic B cells express more mIgD than mIgM (25). The material that was not precipitated by the various antibodies was then reprecipitated with anti-MB-1. Precipitation with anti-μ chain in the first round removed all mIgM-associated molecules, and now anti-MB-1 precipitated δ chain, Ig-β, and the 33- and 34-kDa bands. In contrast, precipitation with anti-δ chain had removed all of the mIgD-associated molecules, and now anti-MB-1 precipitated μ chain, Ig-β, and the 32-kDa band. Thus the 32-, 33-, and 34-kDa bands were all recognized by anti-MB-1: the 32-kDa band was specifically associated with mIgM, and the 33- and 34-kDa bands were specifically associated with mIgD and are likely to be IgD-α. These bands corresponded exactly with those seen in the phosphotyrosine blot (Fig. 5*A*), arguing that the 32-kDa band which was tyrosine-phosphorylated after mIgM crosslinking is MB-1, while the 33- and 34-kDa bands that were phosphorylated after mIgD crosslinking are IgD-α. Thus, only the MB-1-related molecules associated with the receptor that was crosslinked were tyrosine-phosphorylated.

DISCUSSION

We have shown that crosslinking mIg stimulates tyrosine phosphorylation of components of the B-cell antigen receptors. This may be analogous to the tyrosine phosphorylation of the T-cell receptor ζ chain after T-cell receptor activation (26). Crosslinking mIgM stimulated tyrosine phosphorylation of the two previously identified mIgM-associated proteins, MB-1 and Ig-β, as well as a novel 54-kDa disulfide-linked polypeptide associated with MB-1. The 54-kDa polypeptide

may be a component of the mIgM receptor complex that has not been identified in previous studies (10–13). Alternatively, it could be the putative mIg-associated tyrosine kinase or a substrate for the kinase that becomes associated with MB-1 or Ig-β after these proteins are phosphorylated—a phenomenon that has been observed with several tyrosine kinase growth factor receptors (27).

Crosslinking mIgM and mIgD on mature splenic B cells stimulated tyrosine phosphorylation of distinct polypeptides that were precipitated by the anti-MB-1 antiserum (Fig. 5). The 32-kDa MB-1-related band that was tyrosine-phosphorylated after mIgM crosslinking was associated with mIgM but not mIgD. Conversely, the 33- and 34-kDa MB-1-related polypeptides that were tyrosine-phosphorylated after mIgD crosslinking were associated only with mIgD (Fig. 5). Thus, crosslinking one class of mIg results in tyrosine phosphorylation only of the MB-1-related molecules associated with that class of mIg. The basis for selective tyrosine phosphorylation of MB-1-related proteins is not known. One possibility is that the antigen receptor complex is associated with a tyrosine kinase and that receptor crosslinking activates this kinase but brings it into contact only with the MB-1-related molecules of other crosslinked receptors. Alternatively, receptor crosslinkage may induce conformational changes in the MB-1-related proteins that reveal sites for tyrosine phosphorylation.

The mIgD-associated proteins recognized by anti-MB-1 are probably IgD-α. Consistent with the results of Wienands *et al.* (13), we found that mIgD is associated with polypeptides that are $\approx$1 kDa larger than the mIgM-associated MB-1 (Fig. 5). Although IgD-α and MB-1 may be products of different genes (13), our anti-MB-1 antiserum reacted with both, suggesting the presence of a crossreactive epitope.

Tyrosine phosphorylation of mIg-associated proteins could modulate signal transduction by mIg. Cambier *et al.* (28) reported that crosslinking mIgM inhibits subsequent calcium increases in response to mIgD crosslinking and vice versa. They propose that this phenomenon represents heterologous receptor desensitization and is mediated by phosphorylation of all mIg molecules following signaling by either mIgM or mIgD. We found that crosslinking either mIgM or mIgD on splenic B cells resulted in tyrosine phosphorylation only of the MB-1-related molecules associated with the crosslinked receptor (Fig. 5). Thus, tyrosine phosphorylation of MB-1-related molecules is unlikely to be involved in this desensitization process. Moreover, tyrosine phosphorylation of MB-1 and Ig-β occurred rapidly and with the same kinetics observed for tyrosine phosphorylation of other cellular proteins (Fig. 4). This is in contrast to phosphorylation associated with receptor desensitization, which usually lags behind receptor signaling (29).

Tyrosine phosphorylation of mIg-associated proteins may instead facilitate signaling by mIg. It may be required for activation of the guanine nucleotide-binding protein that regulates phospholipase C (30, 31). Alternatively, tyrosine phosphorylation of MB-1 and Ig-β could promote interaction of tyrosine kinase substrates with the mIg receptor complex, which presumably includes an as-yet-unidentified tyrosine kinase.

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