Comparison of Human B Cell Antigen Receptor Complexes: Membrane-expressed Forms of Immunoglobulin (Ig)M, IgD, and IgG Are Associated with Structurally Related Heterodimers

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Summary

We have recently reported that on human B lymphocytes, membrane IgM (mIgM) associates with a heterodimer of 47- and 37-kD polypeptides, the 47-kD subunit being encoded by the *mb-1* gene. We show here that expression of *mb-1*, both at the mRNA and the protein level, is not restricted to IgM^+ B cells but can also be found in IgM^- pre-B cells and $mIgM^-IgG^+$ B cells. Membrane forms of IgD and IgG, isolated from freshly isolated human B cells and B cell lines, are expressed together with heterodimeric protein structures biochemically similar to the mIgM-associated polypeptides, and these were shown to comprise the products of the *mb-1* and B29 genes, or homologous genes. Finally, all three classes of antigen receptors are linked to protein kinases, capable of phosphorylating the Ig-associated heterodimers. Our findings provide insight in the structural organization of the different antigen receptors on human B cells and have implications for their function.

B cell ontogeny is characterized by the subsequent expression of different antigen receptors. Initially, maturation of committed progenitors to $CD19^+\mu^-$ pre-B cells is antigen independent, but as Ig molecules appear at the cell surface, differentiation is arrested unless ligand binds to these receptors (1–4). On pre-B cells that synthesize μ H chains but have not yet rearranged their L chain locus, a minority of the produced μ chains is expressed at the cell membrane in association with the λ5/VpreB pseudo-L chain complex (5–9). As maturation progresses, so-called virgin B cells can be discerned that have successfully rearranged a conventional L chain gene, which enables them to express membrane IgM (mIgM)[1] as antigen receptor. On mature B cells, mIgM is coexpressed with idiotypically identical mIgD molecules (10–14). Recognition of antigen elicits signals that lead to cellular proliferation and secretion of soluble forms of the receptors that can act, in concert with components of the nonspecific immunological system, as effector molecules. In these activated B cells, membrane expression of IgD is terminated. During the secondary immune response the majority of responding cells are memory B cells that express and secrete Ig of other isotypes than M and D, i.e., G, E, and A (2). Based on the qualities of their Fc portions and differences in valency, the secreted forms of the different Ig classes exhibit distinct effector functions. To date, however, it is unknown to what extent the membrane-expressed forms of the five classes of antigen receptors differ in their coupling to intracellular signaling pathways.

Recently, we have shown that mIgM on human B cells is noncovalently associated with a disulfide-linked dimer comprising glycoproteins of 47 and 37 kD (15, 16). These molecules were demonstrated to be newly defined B cell antigens (16) that are both membrane exposed and substrates for serine- and threonine-kinase activity in vivo (15). mAb raised against a synthetic peptide (17) identified the 47-kD component as the product of the human homologue of the *mb-1* gene (16, 18), and we have more recently found that the 37-kD subunit is encoded by the human homologue of the B29 gene (19, Mason et al., manuscript in preparation).

These findings were largely consistent with reports re-

[1] *Abbreviations used in this paper:* BCR, B cell receptor; B-LCL, B-lymphoblastic cell line; IPB, immunoprecipitation buffer; mIgM, membrane immunoglobulin M; RT, room temperature.

garding the murine B cell receptor complex (BCR). Membrane expression of transfected mIgM in murine plasmacytoma cells was shown to require the association with a heterodimer that contains products of the *mb-1* and B29 genes (20–26). The B29 component of the dimer associated with IgD was initially believed to be linked to a protein encoded by a gene other than *mb-1* (21, 22, 27), but recent studies by Venkitaraman et al. (28) show that δ, γ, α, and ϵ H chains can all assemble with transfected *mb-1* gene products at the cell surface of murine $B29^+$ plasmacytoma cells.

In this study we have analyzed the composition of the mIgM, mIgD, and mIgG receptor complexes as they naturally occur on normal human B cells and transformed human B cell lines. We show that all isotypes are associated with disulfide-linked heterodimers comprising products of the *mb-1* and B29 genes, or closely homologous genes. Moreover, together with the antigen receptors of μ, δ, or γ isotypes, protein kinases can be isolated that have specificity for the respective Ig-associated dimers.

Materials and Methods

Cells

The $\mu^-CD10^+CD19^+$ pre-B cell line NALM-16 (29) and the pre-B cell line NALM-6 (29, 30), which expresses μ H chain at the cell surface in association with the λ5/VpreB pseudo-L chain complex (7), the $mIgM^+$ Burkitt lymphoma cell lines Daudi and Ramos, the $mIgG^+$ lymphoblastic cell line EB4B, and the plasmacytoma cell line TH, recently generated at the author's institute (31), were analyzed. IgD^+ cells were sorted on a FACStar® (Becton Dickinson Immunocytometry Systems, San Jose, CA) out of a B cell population that had been transformed in vitro by EBV. This polyclonal B-lymphoblastic cell line (B-LCL) expresses high levels of mIgD and mIgM, and also secretes vast amounts of IgG. All cell lines were routinely cultured in IMDM supplemented with 5% FCS and antibiotics. Peripheral B cells were freshly isolated from tonsils of healthy donors.

Antibodies

mAbs specific for human μ chain (CLB-MH15) and γ chain (CLB-MH16) were obtained from the CLB (Amsterdam), and mAb and anti-δ chain was from Southern Biotechnology Associates, Inc. (Birmingham, AL). The mAb HM47, reactive with the human *mb-1* gene product, was raised as described in detail elsewhere (17). B29 antiserum was obtained by immunization of a rabbit with a synthetic peptide representing amino acids 215–228 of the predicted COOH terminus of the murine B29 polypeptide (19, Mason et al., manuscript in preparation). Purified mAbs were coupled covalently to CNBr-activated sepharose 4B beads (Pharmacia, Uppsala, Sweden). The B29 antiserum was coupled noncovalently to protein A–CL4B sepharose beads (Pharmacia).

Immunofluorescence Analyses

Cells were permeabilized by incubation in PBS/0.01% (wt/vol) digitonin for 5 min at room temperature (RT) and then fixed in 1% paraformaldehyde in PBS for 5 min on ice. Permeabilized or untreated cells were stained using the described mAb specific for human IgM, IgD, IgG, and *mb-1*, followed by FITC-conjugated goat anti-mouse Ig (G26M17; CLB). All antibodies were diluted in PBS supplemented with 0.5% BSA. Samples were analyzed on a FACScan® (Becton Dickinson Immunocytometry Systems).

RNA Isolation and Northern Blot Analysis

Total RNA was isolated from 10^7 cells per cell line using the guanidium-thiocyanate-phenol-chloroform extraction procedure described by Chomczynski and Sacchi (32). 15 μg RNA per sample was subjected to electrophoresis on a 0.8% agarose gel under denaturing conditions using formaldehyde and blotted to GeneScreenPlus (New England Nuclear, Boston, MA). The blot was prehybridized in hybridization buffer containing dextran sulphate for 1 h at 65°C, and then incubated with radioactive probe and ^{32}P-labeled by random priming (Boehringer Mannheim Biochemicals, Mannheim, Germany) for 16–20 h at 65°C. The blot was washed with 2× SSC/0.1% SDS once at RT and twice at 65°C, followed by a wash with 0.1× SSC/0.1% SDS at 65°C for 30 min, and exposed to Kodak X-Omat AR 5 film at −70°C. Radiolabeled probe was removed from the blot by incubation with 0.01× SSC/0.01% SDS at 90°C for 20 min. The same blot was hybridized with following probes in this order. (*a*) The complete 1.2-kb human *mb-1* cDNA, flanked by EcoRI sites (a kind gift of Dr. M. Reth, Max Planck Institut für Immunbiologie, Freiburg, Germany). (*b*) A 1.2-kb EcoRI germline genomic fragment containing human $C_{\mu 1}$, $C_{\mu 2}$, and $C_{\mu 3}$ exons (33) (from Dr. T. H. Rabbitts, Cambridge, UK). (*c*) A 1.3-kb NarI-NaeI cDNA fragment encoding human $C_{\gamma 1}$, $C_{\gamma 2}$, and $C_{\gamma 3}$ domains (from Dr. T. Honjo, Osaka University, Osaka, Japan).

Biochemical Analyses

Radiolabeling. For cell surface radioiodination, 20–100 × 10^6 viable cells were suspended in PBS and labeled with 1 mCi $Na^{125}I$ (Amersham Co., Amersham, UK) using lactoperoxidase as a catalyst.

Immunoprecipitation. Labeled or unlabeled cells were washed three times in serum-free medium and lysed in immunoprecipitation buffer (IPB), consisting of 0.01 M triethanolamine-HCl, pH 7.8, 0.15 M NaCl, 5 mM EDTA, 1 mM PMSF, 0.02 mg/ml ovomucoid trypsin inhibitor, 1 mM TLCK, 0.02 mg/ml leupeptin, supplemented with 1% digitonin as detergent. Nuclear debris was removed by centrifugation for 15 min at 13,000 *g*. Lysates were centrifuged for 30 min at 100,000 *g* and precleared by three incubations with 30 μl of a 10% (vol/vol) suspension of protein A–CL4B Sepharose beads (Pharmacia) coated with normal mouse Ig. Specific immunoprecipitation was carried out two subsequent times for 2 h. Immunoprecipitates were subjected to five washes in IPB with digitonin and resuspended in sample buffer.

In Vitro Phosphorylation. Immunoprecipitates from unlabeled cells were washed three times in EDTA-free IPB, and suspended in 25 μl kinase buffer containing 25 mM Hepes, 100 mM NaCl, 10 mM $MgCl_2$, and 10 mM $MnCl_2$ (pH 7.4). Next, 5–10 μCi ^{32}P γATP (Amersham Co.) was added and incubated for 10 min at RT. After the kinase reaction had been stopped with IPB containing 10 mM EDTA, the immunoprecipitates were washed twice and suspended in sample buffer.

Reprecipitation. The in vitro phosphorylated Ig-associated molecules were dissociated from the mIg chains by incubation of anti-Ig immunoprecipitation for 1 h on ice in 200 μl IPB, supplemented with 1% NP-40 (in the case of mIgM) or with 0.1% SDS, followed by addition of NP-40 to 2% (in the case of mIgD or mIgG). Proteins in the supernatants were reduced and alkylated before immunoprecipitation as follows: addition of 0.2% SDS, incubation for 5 min at 68°C, addition of 2 mM dithiothreitol, incubation

for 30 min at 45°C, and finally, addition of 20 mM iodoacetamide followed by incubation for 20 min at RT. The eluates were diluted with 5 vol of IPB with 1.5% NP-40, 100 μg myoglobin was added as carrier protein, and the eluates were precleared with Sepharose beads coated with normal mouse Ig, before immunoprecipitation with specific antibody.

Enzyme Treatment. Immunoprecipitates were resuspended in 50 μl of 0.05 M sodium acetate, pH 5.5, 0.9% NaCl, 0.1% $CaCl_2$, 1 mM PMSF, and 0.02 mg/ml trypsin inhibitor, and then incubated for 3 h at 37°C with Neuraminidase type VIII (Sigma Chemical Co., St. Louis, MO) at 0.1 U/sample.

Gel Electrophoresis of Proteins. Samples were analyzed by SDS-PAGE in 5–15% polyacrylamide gradient gels, using a modification of the Laemmli procedure, under reducing conditions (5% β-mercaptoethanol in SDS sample buffer) or nonreducing conditions (1 mM iodoacetamide in SDS sample buffer). IEF was done according to O'Farrell (34), using ampholytes (LKB, Bromma, Sweden) of pI 3.5–10, 4–6, 5–8, and 9–11 as 10:1:1:1. Samples were run in the first dimension from base to acid. IEF was followed by SDS-PAGE in the second dimension on 5–15% SDS-polyacrylamide gradient gels. Autoradiography took place at −70°C, using Kodak X-Omat AR5 film in combination with intensifier screens (Cronex; DuPont Co., Wilmington, DE).

KOH Treatment. The dried gel was rehydrated and incubated for 2 h at 60°C in 1 M KOH. After removal of the KOH by five subsequent wash steps within 2 h, the gel was dried and reexposed.

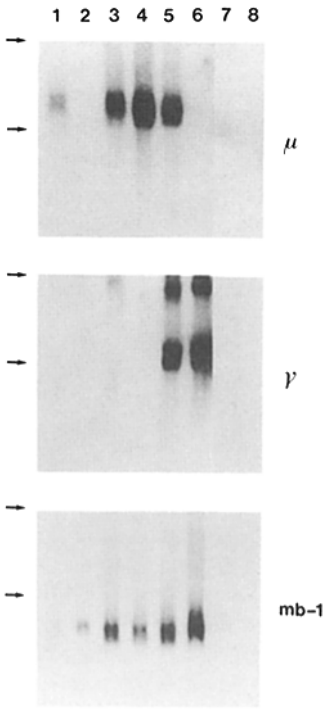

Figure 1. Expression of the *mb-1* gene is not restricted to mIgM$^+$ B cells. Total RNA (15 μg per lane), isolated from the B cell lines NALM-6 (lane *1*), NALM-16 (lane *2*), Daudi (lane *3*), Ramos (lane *4*), a mIgM$^+$IgD$^+$ LCL (lane *5*), EB4B (lane *6*), TH (lane *7*), and the T cell line HPB-ALL (lane *8*) was hybridized with ^{32}P-labeled probes specific for μ and γ H chains and *mb-1*. Arrows indicate the positions of the 28S (*top*) and 18S (*bottom*) ribosomal RNA subunits, respectively.

Results

Expression of the mb-1 Gene in B Cells at Different Maturation Stages. Evaluation of the *mb-1* mRNA gene in human B cell lines representing different stages of B cell development (Fig. 1) showed that this gene is transcribed in the pre-B cell lines NALM-16, which does not transcribe the μ H chain gene (Fig. 1, lane *2*), and NALM-6, known to synthesize μ H chains that appear at the cell surface in association with the pseudo-L chain complex (7) (Fig. 1, lane *1*). The more mature mIgM-expressing Burkitt lymphoma lines Daudi and Ramos, and a polyclonal lymphoblastic cell line (LCL) containing mIgM$^+$IgD$^+$ B cells, all had high levels of *mb-1* expression (Fig. 1, lanes *3–5*, respectively). The cell line EB4B, which contains γ but not μ mRNA, clearly expressed *mb-1* mRNA (Fig. 1, lane *6*). The human plasmacytoma cell line TH (31), which synthesizes Ig L chains (data not shown), showed very low detectable levels of *mb-1* mRNA (Fig. 1, lane 7), whereas the T cell line HPB-ALL was negative for μ, γ, and *mb-1* expression (Fig. 1, lane *8*).

Expression of the mb-1 Protein. Protein expression, analyzed by indirect immunofluorescence staining using mAbs specific for *mb-1*, μ, δ, and γ H chains was in agreement with mRNA expression. As the *mb-1* protein is a typical type I transmembrane molecule (18), the mAb does not react with intact B cells but only stains permeabilized cells (Fig. 2). The pre-B cell line NALM-16 did not express μ protein either at the cell surface (Fig. 2 *A*, *left*) or intracellularly (data not shown), but did react with the anti-*mb-1* mAb (Fig. 2 *A*, *right*). The pre-B cell line NALM-6 showed low membrane expression of μ protein, which is in agreement with a report by Kerr et al., (7), while *mb-1* protein was clearly present (Fig. 2 *B*). Strong reactivity of the anti-*mb-1* antibody could be demonstrated with the mIgM$^+$, mIgM$^+$IgD$^+$, and mIgM$^-$IgG$^+$ cell lines (Fig. 2, *C*, *D*, and *E*, respectively). On the mIgM$^+$mIgD$^+$LCL, IgG was not membrane expressed (Fig. 2 *D*), despite the fact that this line secretes vast amounts of this isotype (data not shown). Weak staining of the plasmacytoma line TH for *mb-1* was observed (Fig. 2 *F*) while T cells and T cell lines were negative (data not shown).

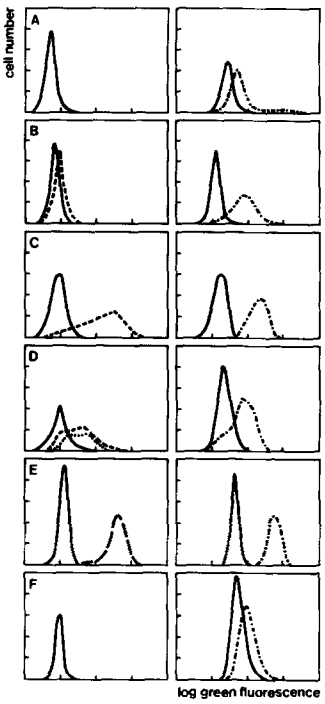

Figure 2. Expression of *mb-1* protein is not restricted to mIgM$^+$ B lymphocytes. FACS® analysis of B cell lines NALM-16 (*A*), NALM-6 (*B*), Daudi (*C*), mIgM$^+$IgD$^+$ LCL (*D*), EB4B (*E*), and TH (*F*), either untreated (*left*) or permeabilized (*right*). The cells were stained with mAbs specific for μ (- - - -), δ (· · · ·), γ H chains (— — —), *mb-1* (-·-·-), or medium alone (——).

Structural Comparison of mIgM, mIgD, and mIgG Antigen Receptor Complexes. The finding that expression of *mb-1* neither at mRNA nor protein level was restricted to mIgM-expressing B cells suggested that the *mb-1* gene product might participate in B cell antigen receptor complexes of different isotypes. Analysis of the mIgM complex (BCRμ), isolated from tonsil B cell populations, by IEF, followed by SDS-PAGE under reducing conditions, yielded a characteristic pattern, reminiscent of the one reported for the mIgM complex originating from B cell lines (16). The μ H chains (Fig. 3

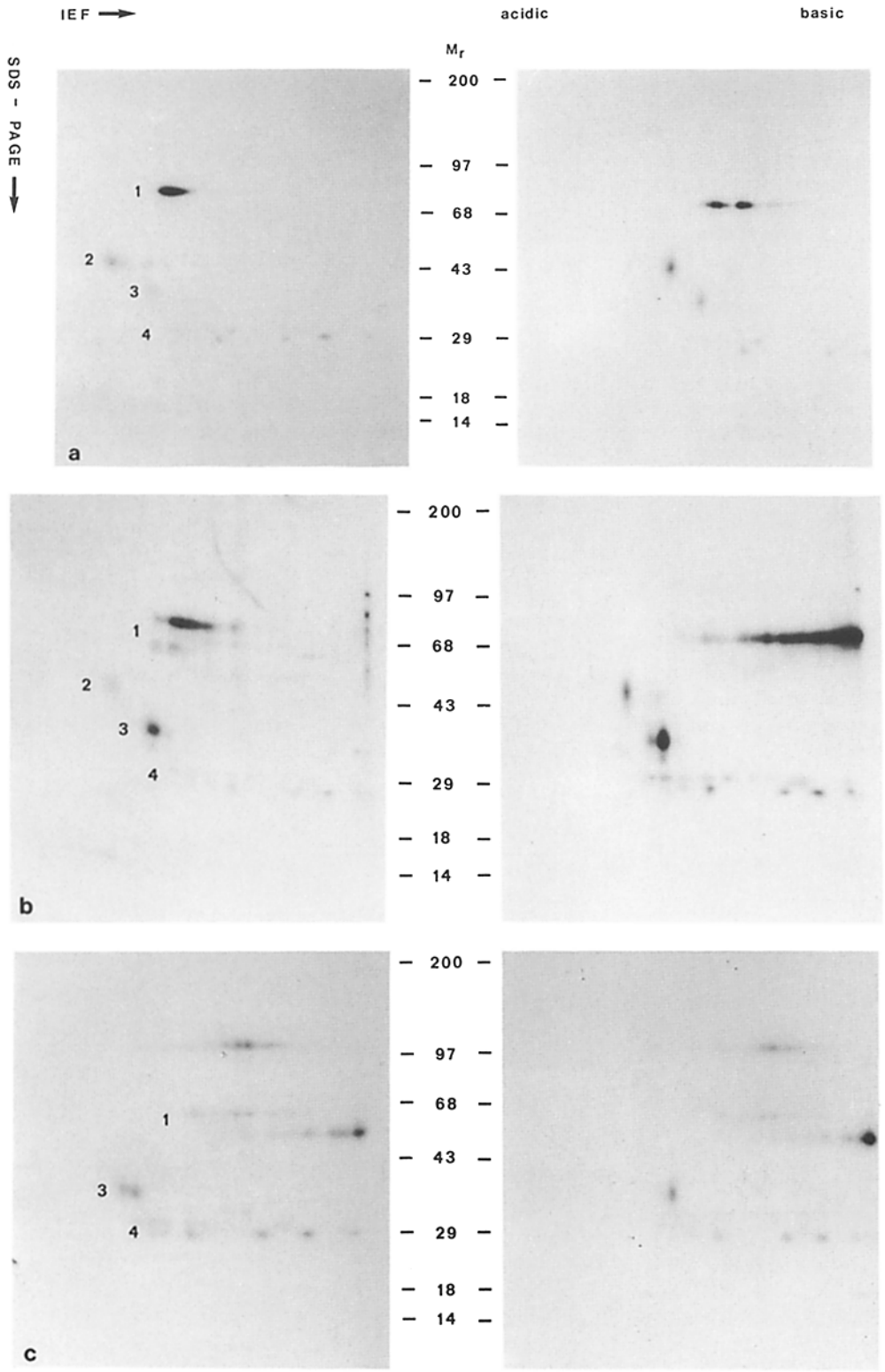

Figure 3. Membrane forms of IgM, IgD, and IgG on tonsil B cells are expressed in association with similar dimeric complexes. MIgM (*a*), mIgD (*b*), and mIgG complexes (*c*) were separately immunoprecipitated from a digitonin lysate of surface-iodinated tonsil B cells. The immunoprecipitates, either mock treated or neuraminidase treated (*left* and *right*, respectively), were analyzed by IEF followed by SDS-PAGE under reducing conditions. In all panels, the numbered spots represent the Ig H chains (*1*), Ig L chains (*4*), and the heterodimeric components (*2* and *3*). In *c*, the 45-kD dimeric component was only detectable on a longer exposed autoradiogram (not shown here).

a, no. *1*) run at ~80 kD, whereas the Ig K and λ L chain forms migrate as multiple spots ~30 kD (no. *4*). The mIgM-associated molecules of 47 and 37 kD (nos. *2* and *3*), being the products of *mb-1* (16) and B29 (Mason et al., manuscript in preparation), respectively, have both relatively acidic, but different, pIs. Removal of sialic acids by treatment with neuraminidase (Fig. 3 *a*, *right*) decreased their charge heterogeneity but did not eliminate the difference in pI.

Membrane IgD (Figs. 3 *b* and 4 *a*) and IgG (Figs. 3 *c* and 4 *b*) immunoprecipitates from tonsillar B cells and B cell lines contained, in addition to the various H and L chains (nos. *1* and *4*, respectively), pairs of relatively acidic polypeptides (nos. *2* and *3*) that are, as judged by M_r and pI, very similar between mIgM, mIgD, and mIgG complexes. The dimer isolated with mIgD consisted of subunits of ~49 and ~36 kD (Figs. 3 *b* and 4 *a*), whereas 45- and 35-kD molecules were precipitated in association with mIgG (Fig. 3 *c* and 4 *b*). A striking and consistent difference between the 47-kD mIgM-associated molecule (from both peripheral B cells and B cell lines) and the 49- and 45-kD components associated with mIgD or mIgG, respectively, is that the latter two polypeptides were weakly labeled by radioactive iodine. In the mIgG isolate from tonsil B cells, the 45-kD molecule was not detectable after normal exposure of the autoradiogram (Fig. 3 *c*), but was clearly discernable in IgG immunoprecipitates from the EB4B cell line. Moreover, in the mIgG immunoprecipitate from the cell line EB4B, an additional molecule of ~31 kD was detected (Fig. 4 *b*, *arrow*) that, to judge from its pI after neuraminidase treatment and since it complexed with mIgG under nonreducing conditions (data not shown), probably represents an alternative L chain species. Furthermore, two discrete proteins of low molecular mass were immunoprecipitated with mIgG from this cell line (Fig. 4 *b*, nos. *5* and *6*). These proteins have masses of 15 and 10 kD and are not sialylated since their pIs were not affected by neuraminidase treatment. So far these molecules could not be demonstrated on other B cells, either surface iodinated or metabolically labeled.

Molecules Associated with mIgM, mIgD, and mIgG Are Substrates for Protein Kinase Activity. We analyzed by an in vitro

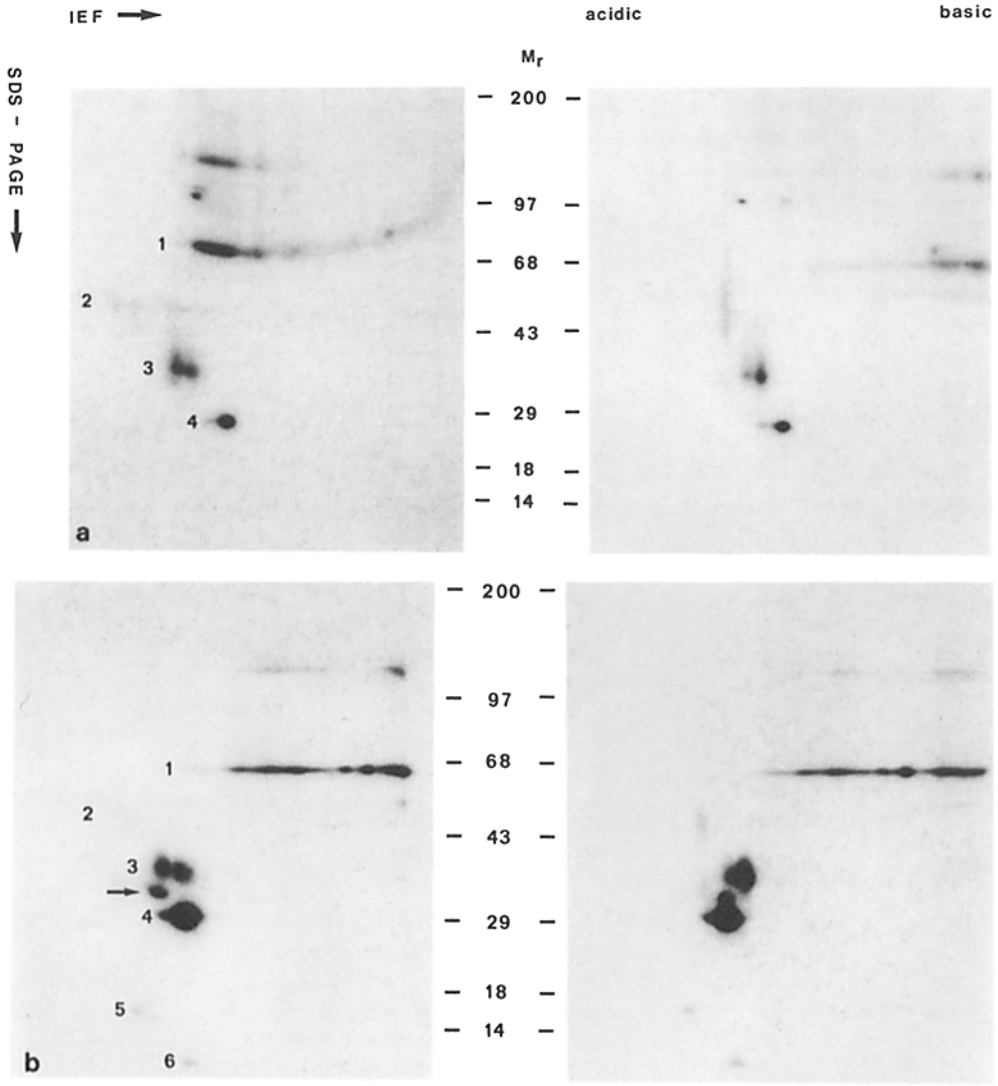

Figure 4. The 49- and 45-kD components of the mIgD- and mIgG-associated heterodimers are, compared with their disulfide-linked partners, labeled with poor intensity. The mIgD (*a*) and mIgG (*b*) antigen receptor complexes were isolated from digitonin lysates of surface-labeled $mIgM^+IgD^+$ LCL and EB4B cells, respectively. The immunoprecipitates, either mock or neuraminidase treated (*left* and *right*, respectively) were separated according to pI and M_r. Numbered spots represent Ig H chains (*1*), Ig L chains (*4*), and the heterodimeric components (*2* and *3*). The 31-kD protein (*b*, *arrow*) most likely depicts an alternative L chain species, whereas the 15- and 10-kD polypeptides (*b*, nos. *5* and *6*) are of unknown identity.

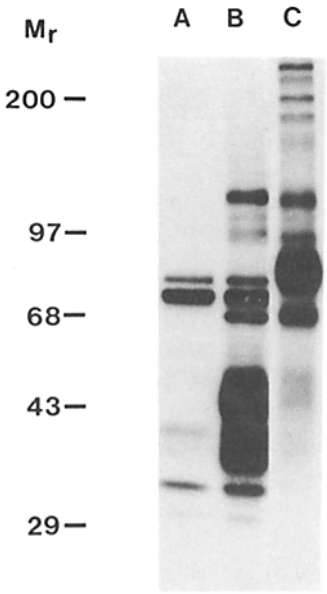

Figure 5. The mIgM complex contains a protein kinase with specificity for the 47- and 37-kD heterodimeric subunits. The mIgM receptor complex was isolated from a digitonin lysate of ^{35}S-methionine-labeled Daudi cells. The immunoprecipitate was phosphorylated by an in vitro kinase reaction using either unlabeled ATP (lane *A*) or γ[^{32}P]ATP (lanes *B* and *C*). The samples were separated by SDS-PAGE under reducing (lane *A* and *B*) or nonreducing (lane *C*) conditions.

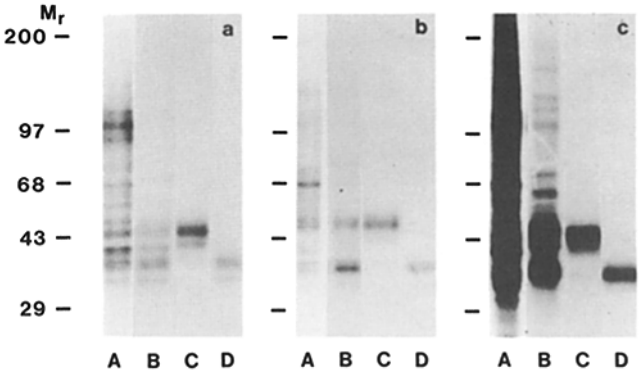

Figure 6. The mIgM-, mIgD-, and mIgG-associated heterodimers serve as substrates for associated kinases and react with *mb-1*- and B29-specific antibodies. From unlabeled, digitonin-solubilized, μ^+ Daudi (*a*), polyclonal tonsil (*b*) or γ^+ EB4B B cells (*c*), μ, δ, and γ H chains, respectively, were immunoprecipitated, phosphorylated in vitro, and analyzed under reducing conditions by SDS-PAGE. After exposure (lanes *A*), the gel was treated with KOH, dried, and reexposed (lanes *B*). From half of the samples, the Ig-associated heterodimers were eluted with IPB containing SDS and/or NP-40 (see Materials and Methods). Immunoprecipitation was performed out of these eluates, which had been reduced and alkylated, with the HM47 mAb (lanes *C*) and the B29 antiserum (lanes *D*).

kinase assay whether proteins contained within the human BCRs are substrates for kinase activity. BCRμ was isolated by immunoprecipitation from a digitonin lysate of Daudi cells that had been labeled biosynthetically with ^{35}S-methionine. In the presence of unlabeled ATP, mature and precursor forms of Ig H and L chains were detected (Fig. 5, lane *A*), whereas the Ig-associated heterodimer was only visible after longer exposure (not shown). However, after incubation with radioactive ATP the presence of previously undetected phosphoproteins of 37, 47, 70, 100, 113, and 133 kD was visualized (Fig. 5, lane *B*). Under nonreducing conditions, the 37- and 47-kD molecules proved to be the IgM-associated heterodimeric subunits, as they migrated as a single band of 80–85 kD (Fig. 5, lane *C*). The intensity of Ig H and L chain bands was not altered in case radioactively labeled ATP was used, which corroborates the specificity of the in vitro kinase reaction. Phosphorylation of both 47- and 37-kD molecules was resistent to potassium hydroxide treatment (see Fig. 6), implying that these polypeptides contain phosphotyrosine residues (35), which was confirmed by phospho–amino acid analysis (Brouns et al., manuscript in preparation).

To further elucidate the structural relationship between the different antigen receptor classes, we investigated whether the mIgD and mIgG complexes (designated as BCRδ and BCRγ) also contain kinase activity specific for the H chain–associated heterodimers. Indeed, the heterodimers associated with μ, δ, and γ H chains can all be phosphorylated by kinases apparently present in the immunoprecipitates (Fig. 6, *a*, *b*, and *c*, lanes *A*, respectively). KOH treatment of the gel significantly reduced the labeling of most of the immunoprecipitated phosphoproteins, whereas the intensity of the Ig-associated dimers was hardly diminished (Fig. 6, *a*, *b*, and *c*, lanes *B*), suggesting that they can all be phosphorylated on tyrosine residues.

Heterodimers Associated with mIgM, mIgD, and mIgG All Contain Components Reactive with mb-1 and B29 Antibodies. In agreement with the reprecipitation studies from surface-iodinated B cells, also from in vitro phosphorylated immunoprecipitates, the 47- and 37-kD components of the mIgM-associated heterodimer could be defined as *mb-1* and B29 gene products, respectively (Fig. 6 *a*, lanes *C* and *D*). Next to the 47-kD subunit, the *mb-1* mAb also reacted with a protein of 41-kD, previously shown to be a differentially glycosylated form of the same polypeptide (Fig. 6 *a*, lane *C*) (16). A similar explanation may hold for the B29 immunoprecipitate that contains a 35-kD protein in addition to the 37-kD band (Fig. 6 *a*, lane *D*). Analysis of the mIgD- and mIgG-associated dimers, originating from tonsil B (Fig. 6 *b*) and EB4B cells (Fig. 6 *c*), demonstrated that the anti-*mb-1* mAb and B29 antiserum were also reactive with the higher and lower molecular mass dimeric subunits, respectively (Fig. 6, *b* and *c*, lanes *C* and *D*, respectively).

Discussion

Expression of mIgM at the surface of murine plasmacytoma cells depends on its association with a disulfide-linked dimer of 34- and 39-kD proteins (20–22, 27, 36, 37) encoded by the *mb-1* (18) and B29 (19) genes (23–25). Wienands et al. (22) reported that the smaller 35-kD subunit in the mIgD-associated heterodimer is not equivalent to *mb-1* protein and that the murine mIgD receptor complex therefore differs from the mIgM complex. This report was based on their observation that *mb-1* mRNA could not be detected in mIgD-carrying myeloma cells. Later, it became evident that the mIgD molecules appearing on the surface of *mb-1*$^-$ plasmacytoma cells represent alternatively processed forms of mIgD, which do not depend on association with the *mb-1* gene product (38). Moreover, the 35-kD molecule of the heterodimer associated

with the complexed form of mIgD has now been shown to be the product of the *mb-1* gene, or a closely related gene (26, 38). These findings were recently extended by Venkitaraman et al. (28), who demonstrated by transfection studies that the membrane forms of μ, δ, γ, α, and ϵ H chains can all associate with heterodimers comprising the *mb-1* and B29 polypeptides. However, none of the reports so far exclude the possibility that on primary B cells the heterodimers associated with different Ig isotypes accommodate proteins encoded by alternative forms of the *mb-1* and or B29 genes.

In the present study we show that the membrane forms of human μ, δ, and γ H chains, on freshly isolated tonsillar B cells and leukemic B cell lines, are expressed together with membrane-spanning heterodimers that are very similar in terms of their biochemical properties (Figs. 3 and 4) and associated protein kinase activity (Fig. 6). The structural relationship between the mIgM-, mIgD-, and mIgG-associated heterodimers was confirmed by the reactivity of their components with the *mb-1* and B29 antibodies (Fig. 6). Nevertheless, differences were found in the susceptibility to radioiodination between the distinct isotype-associated *mb-1*-like molecules, and more stringent detergent conditions were required to dissociate the dimers from mIgD and mIgG than from mIgM. The variation in molecular mass between the Ig-associated dimers may, since carbohydrate moieties attribute substantially to the observed molecular mass of both dimeric subunits (15, 16), have less significance. Indeed, transfection experiments in the murine system have indicated that the nature of the associated mIg class determines the degree of glycosylation of the *mb-1* gene product (28, 38). Thus, we have shown a high degree of structural relatedness between the heterodimers associated with different H chain isotypes, but the identity of these molecules has yet to be proven.

The sequence of the human *mb-1* cDNA predicts a protein with cytoplasmatic, transmembrane, and proximal extracellular domains that are highly homologous to the same regions of the murine *mb-1* molecule (Reth et al., and van Noesel et al., unpublished data). However, a part of the extracellular domain, which spans the Ig-like protein loop, differs clearly between man and mouse. Transfection experiments by Hombach et al. (23) indicated that the C_H3 and C_H4 and transmembrane domains of the μ H chain are critical for interaction with the *mb-1* or B29 gene product. These findings suggest that if isotype-related differences between the *mb-1* (and B29) molecules exist, they may be found in the extracellular or transmembrane domains. Since both the anti-*mb-1* mAb and the B29 antiserum we used recognize epitopes at the COOH termini, they may not be able to detect these differences. The murine *mb-1* gene at the genomic level (39) includes all sequence information found in the previously cloned cDNA (18), but Southern blot analysis of mouse genomic DNA with the *mb-1* cDNA suggested the existence of an additional *mb-1*-related gene (39). Therefore, it cannot be ruled out that the *mb-1* proteins described here are derived from distinct, but closely related genes. Alternatively, different transcripts may be derived from the same gene by differential mRNA processing, although as yet only one type of human *mb-1* cDNA has been isolated and no obvious differences in mRNA size were detected in cell lines expressing different receptor isotypes.

When the BCR is compared with the TCR (40, 41) and the FcRIII (42–45), two receptors that are involved in immunological recognition processes, the most conspicuous difference is the lack of a ζ homologue. Compared with the ζ chains, the heterodimeric molecules have a different structural organization as they belong to the Ig supergene family (18, 19, 36, 39). On the other hand, they both contain, in common with CD3-γ, -δ, -ζ, and the γ and β chains of the rat mast cell Fc receptor, a conserved sequence motif of six identically spaced cytoplasmic amino acids (36, 46). The significance of the ζ protein was emphasized recently by Irving and Weiss (47), who showed that a number of signaling events that normally result from TCR ligation can be evoked by the cytoplasmic domain of the ζ chain only. In the in vitro kinase assay shown here, as well as in metabolic labeling experiments (data not shown), we have not found any evidence for the presence of ζ-like components in any of the three mIg complexes. In this respect, the isolation of the two additional low molecular mass molecules together with mIgG from EB4B cells (Fig. 4 *b*) was intriguing, but as yet we have not been able to demonstrate any reactivity with anti-ζ peptide sera (data not shown). Additionally, the 10- and 15-kD proteins are, contrary to the ζ chain, monomeric structures that are easily surface iodinated and have until now not been detected in phosphorylated form. The notion that a ζ homologue does not contribute to the BCR is supported by the reconstitution experiments by Ventikaraman et al. (28), which established that the presence of the *mb-1* and B29 gene products are sufficient for mIg surface expression in nonlymphoid cells.

We demonstrate that on mature peripheral human B cells, the BCRμ, BCRδ, and BCRγ are connected with protein kinases, which must at least be related as judged by the similarity of their substrates (Fig. 6). The murine mIgM- and mIgD-associated heterodimers have been shown to be phosphorylated on tyrosine residues upon antigen receptor ligation (48) or upon stimulation of permeabilized cells with aluminum fluoride (27). Moreover, murine mIgM was found to be closely linked to the src-family kinase p56 lyn (49). Since after KOH treatment the labeling intensity of the Ig-associated heterodimers is hardly diminished, the presence of protein tyrosine kinases in the human B cell antigen receptor complexes, comprising different H chain isotypes, is also suggested. The proposed composition of the BCR classes has implications for their function. The results obtained in this study, combined with the recent findings of Venkitaraman et al. (28), suggest that the membrane-expressed forms of the different isotypes may couple to the intracellular compartment in essentially the same way via identical *mb-1* and B29 molecules. However, the end result of antigen receptor triggering at different B cell maturational stages may very well be distinct, depending on the availability of intracellular substrates.

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