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Immunology

B29 gene products complex with immunoglobulins on B lymphocytes

(immunoglobulin-associated proteins/B-cell development)

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ABSTRACT **B29 is a B-lineage-specific gene predicted from sequence information to be a transmembrane member of the immunoglobulin (Ig) superfamily, with a single extracellular Ig-like domain. Its presumptive cytoplasmic region contains a peptide motif present in CD3 and other molecules involved in lymphocyte activation. Affinity-purified goat antibodies were prepared to a TrpE fusion protein of B29 and used to study B29 expression on lymphoid cells. The antiserum precipitated surface-labeled heterodimers from B lymphoma cells. One was 65–88 kDa (unreduced) or 36–47 plus 32–34 kDa (reduced) by SDS/PAGE analysis, regardless of detergent. A smaller heterodimer was detected only with Triton detergent extraction. IgM molecules were coprecipitated by the B29 antiserum when the weak detergent digitonin was used. In addition, cocapping experiments revealed that most B29 molecules codistribute with Ig on the cell surface. Although early B-lineage cells and plasma cells contain B29 mRNA, surface expression was detectable only on B cells that had significant amounts of surface Ig. The surface expression was B-lineage-specific and included cells from mutant *xid* mice and B-cell lines representing μ, δ, γ, and α heavy-chain isotypes and both κ and λ light-chain types. The density of surface B29 protein correlated directly with surface μ heavy-chain density on subclones of a B-cell lymphoma and lipopolysaccharide-stimulated pre-B cells. These findings show that B29 is covalently linked in a heterodimer and are consistent with a recently proposed model of surface Ig complexes.**

Surface immunoglobulin (sIg) molecules on B lymphocytes function as specific receptors for antigen and to mediate B-cell activation. However, signal transduction by sIg is probably mediated by a complex of associated transmembrane and intracellular proteins, in a manner analogous to the CD3/T-cell receptor complex (1). The best defined Ig-associated protein is the product of the mb-1 gene (2, 3). It is expressed early in B-lineage development and the protein may be capable of signal transmission even at the pre-B-cell stage (2, 4, 5). On mature B cells, some mb-1 is present on the surface in monomeric form. Another fraction is in disulfide-linked heterodimers, which in turn form noncovalent complexes with sIg (4, 6, 7). These complexes were first revealed in anti-Ig immunoprecipitates of cellular extracts made with weak detergents (8, 9). They were subsequently detected as phosphorylated proteins by treatment of B cells with the G-protein agonist aluminum fluoride or sIg crosslinking (6, 10). Intracellular proteins, including the protein-tyrosine kinases encoded by the *lyn* and *blk* genes, also may be physically associated with the sIg complex (11, 12). Their action may be coupled in some way to CD45, a phosphatase that also interacts with membrane Ig (13). Thus, an understanding of the function of the sIg receptor depends on a detailed analysis of such associated proteins.

B29 was initially cloned as a B-cell-specific gene encoding a transmembrane protein of the Ig superfamily (14, 15). B29 mRNA is detected throughout B-lymphocyte development and its strict lineage specificity has been attributed to Ig-like regulatory sequences in the 5′ flanking region of the gene (16). Recently, partial amino acid sequences of Ig-associated complexes indicated that B29 may be covalently linked with mb-1-like proteins (7, 17). We now describe the preparation of an antibody specific for the B29 protein and its use in determining B29 expression on developing B cells.

MATERIALS AND METHODS

Cell Lines. Most of the lympho-myeloid cell lines were maintained in RPMI 1640 medium with 10% fetal bovine serum, 50 μM 2-mercaptoethanol, L-glutamine, penicillin, and streptomycin. The MPC11 line was propagated in Dulbecco's modified Eagle's medium with 20% heat-inactivated horse serum, L-glutamine, penicillin, and streptomycin. The Abelson virus-transformed pre-B lymphomas 2.6.2, 18.81, and 40E were provided by Naomi Rosenberg, Tufts University. Michael Kuehl of the Naval Medical Institute donated the BFO3, BAL17, A20-1.1, and A20-2J B lymphomas as well as the 39-7 erythroleukemia. The J558L plasmacytoma was obtained from Sherrie Morrison, University of California, Los Angeles. The interleukin 3-dependent multipotential FDCP1-Cl2 line was donated by Joel Greenberger, University of Massachusetts. Our stromal cell-dependent lymphocyte clones 1A9 and 2E8 have been described (18). All other cell lines were obtained from the American Type Culture Collection. Differentiation of the 70Z/3 pre-B lymphoma was induced by incubation with bacterial lipopolysaccharide (LPS; Difco). The isotypes and light-chain types expressed on Ig-bearing lines were as follows: 70Z/3 (μ, ω, ι); 1A9 (μ, ω, ι); 2E8 (μ, κ, ω, ι); W231 (μ, κ); W279 (μ, κ); BCL1 (μ, λ); BAL17 (μ, δ, κ, λ); BFO3 (α, λ); A20 (γ_{2a}, κ); CH1 (CD5, μ, κ); CH12 (CD5, μ, κ); and MPC11 (γ, κ).

B29-Specific Antibody. A fusion-protein vector for B29 was constructed by inserting a *Kpn* I–*Pvu* II fragment encoding amino acids 26–134 (corresponding to the extracellular Ig-like domain) into the TrpE vector pATH10. The fusion protein was expressed, salt-extracted (19), and then purified by preparative SDS/PAGE. A goat was immunized by repeated intradermal injections with ≈100 μg of protein in complete Freund's adjuvant. Serum was collected and used for initial experiments in comparison to preimmune goat serum. Affinity columns were then prepared with TrpE and B29–TrpE

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Abbreviations: BSA, bovine serum albumin; LPS, lipopolysaccharide; sIg, surface immunoglobulin.

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proteins coupled to Affi-Gel 10 (Bio-Rad). The proteins were first solubilized in 8 M urea/10 mM Tris·HCl, pH 8.3, and dialyzed against phosphate-buffered saline (PBS) before conjugation. After repeated cycles of passage through the TrpE affinity column, specific antibodies were absorbed to and eluted from the B29–TrpE column. Western blot analysis revealed that this affinity-purified antibody recognized a species of 47 kDa, the expected size of the fusion protein, but not the 37-kDa TrpE protein.

Northern Blots. Blot hybridization analysis of isolated cytoplasmic RNA was carried out as described (14, 15). Values for B29 and κ light-chain inductions were normalized to levels of mRNA from the housekeeping gene CHO-B, whose expression was not altered by LPS treatment.

Immunofluorescence. Cells (5×10^5) in suspension were preincubated for 20 min on ice with goat antibodies in PBS containing 2% bovine serum albumin (BSA) and 0.1% NaN_3. Antibodies to various Ig isotypes and light chains (Southern Biotechnology Associates, Birmingham, AL) were used to verify the characteristics of the B-lineage lymphoma cells. Fluorescein-labeled rabbit $F(ab')_2$ anti-goat antibodies were used as a second-step reagent. Propidium iodide was added after staining and used as a gating parameter to exclude nonviable cells. Labeled cells were then evaluated on a Coulter EPICS V flow cytometer and fluorescence intensity histograms were presented with a logarithmic scale. Comparisons of lymphoma subclones and induced pre-B cells (see Figs. 3 and 4*B*) were performed by conversion of logarithmic channel numbers to linear values.

Capping experiments were performed by treatment of W279 B lymphoma cells with affinity-purified goat anti-B29 antibody and Texas Red-labeled $F(ab')_2$ donkey anti-goat IgG (Jackson ImmunoResearch). Incubations with each antibody were for 20 min at 25°C in buffer without NaN_3. After the cells were washed with cold PBS containing 2% BSA and 0.1% NaN_3, they were stained for surface IgM with biotinylated rat anti-mouse IgM (Zymed Laboratories) followed by fluorescein-conjugated avidin at 4°C in buffer with NaN_3. Reciprocally, sIgM was first capped by treatment with biotin-labeled rat anti-mouse IgM and then fluorescein-conjugated avidin at 25°C in buffer without NaN_3. After the cells were washed with cold PBS containing 2% BSA and 0.1% NaN_3, they were stained for surface B29 as above. Controls established that the cold temperature and azide treatment were sufficient to prevent capping.

Cell Surface Labeling and Immunoprecipitation. Cells were surface labeled by a previously described biotinylation procedure (18), with minor modifications. Briefly, cells were treated with sulfo-NHS-biotin (Pierce). The cells were then lysed with either 1% (vol/vol) Triton X-100 or 1% (wt/vol) digitonin (Sigma) in 10 mM Tris, pH 7.5/0.15 M NaCl/1 mM phenylmethylsulfonyl fluoride/50 mM iodoacetamide/0.1% NaN_3/1 mM EDTA containing soybean trypsin inhibitor (10 μg/ml), leupeptin (1 μg/ml), and aprotinin (1 trypsin inhibitor unit/ml). Precleared lysates were added to 40–60 μl of Affi-Gel 10 conjugated with goat anti-B29 antiserum or rabbit anti-mouse IgM (Zymed Laboratories). After extensive washing, proteins were eluted from the beads by boiling and subjected to SDS/PAGE in a 7.5% polyacrylamide slab gel under nonreducing conditions. The lanes of the slab gel were cut out and equilibrated in reducing sample buffer for 1 hr at room temperature. Then they were overlaid on 10% polyacrylamide slab gels for electrophoresis in the second dimension. After electrophoresis, proteins were electrophoretically transferred to Trans-Blot membranes (Bio-Rad). These were blocked and incubated with 0.1% avidin-horseradish peroxidase in PBS containing 1% BSA and 0.05% Tween 20, washed again, and developed with 4-chloro-1-naphthol.

RESULTS

Preparation and Characterization of Anti-B29. The B29 gene was expressed as a TrpE fusion protein in *Escherichia coli* and injected into a goat to generate a polyclonal anti-B29 serum. Preliminary experiments revealed that this reagent specifically stained B lymphocytes, and immunochemical studies were done to characterize the protein(s) it recognized. Surface proteins of the W279 B lymphoma were labeled by biotinylation before extraction in various detergents. Solubilized proteins were subjected to immunoprecipitation and characterized by two-dimensional diagonal SDS/PAGE. With this technique, heterodimeric proteins are revealed as paired spots. One heterodimer, composed of 36- to 47-kDa and 32- to 34-kDa species, was consistently detected, regardless of detergent. When Triton X-100 was used for extraction, an additional heterodimer was detectable, consisting of the 36- to 47-kDa component and a 24-kDa species (Fig. 1*B*). Spots corresponding to Ig heavy and light chains were obvious when the relatively weak detergent digitonin was used for extraction and precipitations were done with either anti-B29 (Fig. 1*D*) or anti-μ (Fig. 1*C*). Specificity of the immunoprecipitation was established with irrelevant control antibodies (Fig. 1*A*). These results suggest that a noncovalent and detergent-sensitive association exists between at least one of the B29-containing heterodimers and sIg. This is consistent with recent reports of partial amino acid sequence identity between the larger component of the sIg-associated heterodimer and the sequence predicted from the B29 cDNA sequence (7, 17). Indeed, we found that the 36- to 47-kDa component of all heterodimers was detected by Western blot analysis with affinity-purified anti-B29 antibody (data not shown).

Codistribution of B29 and B-Cell sIg. The immunochemical analyses indicated that B29-containing heterodimers were noncovalently associated with sIg. It was important to rule out the possibility that this interaction occurred during pro-

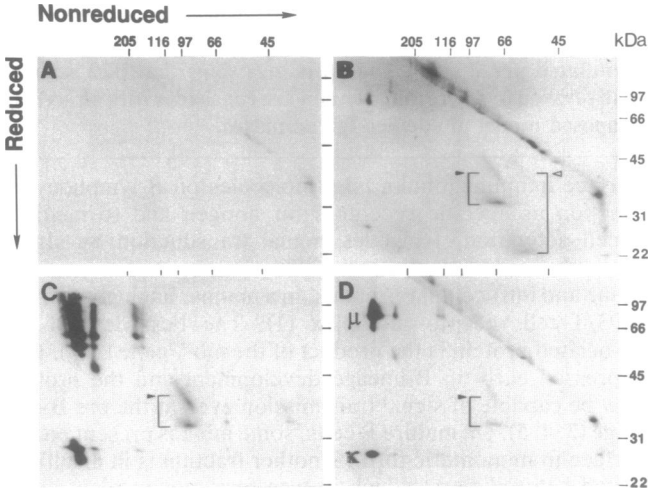

FIG. 1. B29 is exclusively in heterodimers and physically associates with sIgM on B cells. W279 B lymphoma cells ($2–7 \times 10^8$) were surface labeled with biotin and lysed with Triton X-100 (*A* and *B*) or digitonin (*C* and *D*). These extracts were precleared and immunoprecipitated with goat anti-B29 antiserum (*B* and *D*) or rabbit anti-mouse IgM (*C*). Control precipitations were done with preimmune goat serum (*A*) or normal rabbit IgG (data not shown). Immunoprecipitates were then subjected to two-dimensional nonreducing/reducing SDS/PAGE (7.5%/10%). Proteins were blotted to nitrocellulose membranes and visualized as described in *Materials and Methods*. Western blotting analysis (data not shown) was used to determine that the larger component of two heterodimers (indicated by brackets) is B29 (arrowheads). Positions of molecular size markers are indicated.

tein extraction, and we also wanted to investigate the stoichiometry of B29 complexes on intact cells. Redistribution of the B29 on viable B lymphoma cells was first achieved by treatment with affinity-purified anti-B29 and a labeled crosslinking second antibody. The cells were then chilled and treated with NaN_3 to prevent further capping and stained for surface IgM. This treatment resulted in colocalization of most of the surface IgM with caps of B29 (data not shown). Reciprocally, sIg was first capped by treatment with anti-IgM. Subsequent staining revealed that most B29 redistributed to the same location. These observations are consistent with preliminary modulation experiments and indicate that a majority of surface B29 is physically associated with sIg. However, they do not exclude the possibility that a minor fraction of sIg and/or B29 molecules are independently distributed on the cell membrane.

Stage- and Lineage-Specific Expression of B29. Affinity-purified antibodies were then used to determine the tissue distribution of the B29 gene product. A small percentage (average, 2%) of cells in bone marrow appeared to be positive, and a clearly resolved population of B29-bearing cells was always observed in spleen and lymph node cell suspensions (Fig. 2). This was true with cells from normal C57BL/6 and BALB/c mice as well as immunodeficient *xid* mice (data not shown). Thymocytes were consistently negative.

All B-lineage cell lines that have been examined by Northern blot analysis express B29 transcripts (refs. 14 and 18; see below). However, the protein was detectable only on cells that expressed significant amounts of sIg (Fig. 2 and *Materials and Methods*). Staining was negative with a series of Abelson virus-transformed pro-B and pre-B lymphoma lines (2.6.2, 40E, and 18.81) and only occasionally above background with the 70Z/3 pre-B lymphoma. The 70Z/3 line displays some surface μ in association with "surrogate" (ω and ι) light chains (20). B29 protein was demonstrable by immunoprecipitation or immunofluorescence on two stromal cell-dependent lymphocyte clones that express slightly more surface μ. These two clones (1A9 and 2E8) represent cells at the pre-B to B-cell transition stage (18). B lymphoma cells (W231, W279, BCL1, BAL17, BFO3, A20, CH1, and CH12) were all clearly positive for surface B29 protein (Fig. 2 and *Materials and Methods*). These lymphomas express κ and λ light chains and also represent several Ig heavy-chain isotypes (μ, δ, γ, and α). This indicates that multiple types of sIg may associate with the B29 protein. Similarly, positive lymphomas represented both conventional and CD5-bearing lymphocytes (CH1 and CH12). As with early B-lineage cells, plasma cells (J558L, SP2/0, and MPC11) generally did not have detectable surface B29 protein. A possible exception was MPC11, which bears some surface IgG and occasionally was just slightly above background for B29 staining. B29 was not found on thymomas (EL4 and R1.1), an erythroleukemia (39-7), an interleukin 3-dependent multipotential cell line (FDCP1), or a myelomonocytic leukemia (WEHI-3).

Coordinate Expression of B29 and sIg. Surface display of B29 protein appeared to be restricted to B lymphocytes that have substantial amounts of sIg, and the density of one protein seemed to correspond to the density of the other. This was investigated further by analysis of a series of subclones of the W279 B lymphoma, which differed in levels of sIgM expression and lacked other Ig isotypes (Fig. 3*A*). Linear fluorescence units determined by flow cytometry revealed good correlation between surface μ and B29 densities (r^2 = 0.99).

An inducible pre-B lymphoma line provided another opportunity to investigate the relationship between sIg and the B29 gene product. Treatment of 70Z/3 cells with LPS caused

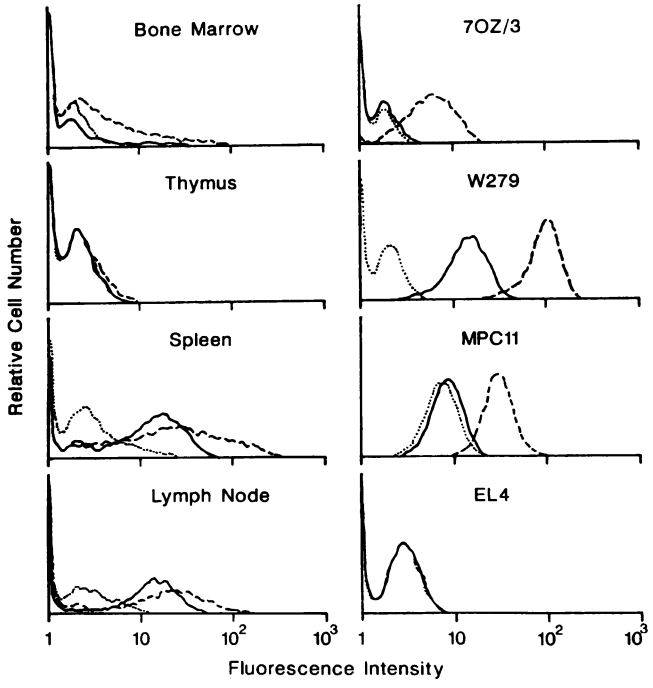

FIG. 2. B29-expressing lymphocytes and established cell lines. Cells from BALB/c mice were incubated with affinity-purified goat anti-B29 antibody or preimmune goat IgG. Cell-bound antibody was detected by fluorescein-conjugated rabbit $F(ab')_2$ anti-goat IgG and analyzed by flow cytometry. A similar analysis was performed with pre-B (70Z/3), B (W279), and T (EL4) lymphomas as well as a plasmacytoma (MPC11). Solid and dotted lines indicate staining profiles obtained with goat anti-B29 antibody and goat IgG, respectively. Broken lines depict Ig heavy-chain staining (μ except for MPC11, which was γ). Additional cell lines that were characterized are listed in *Materials and Methods*.

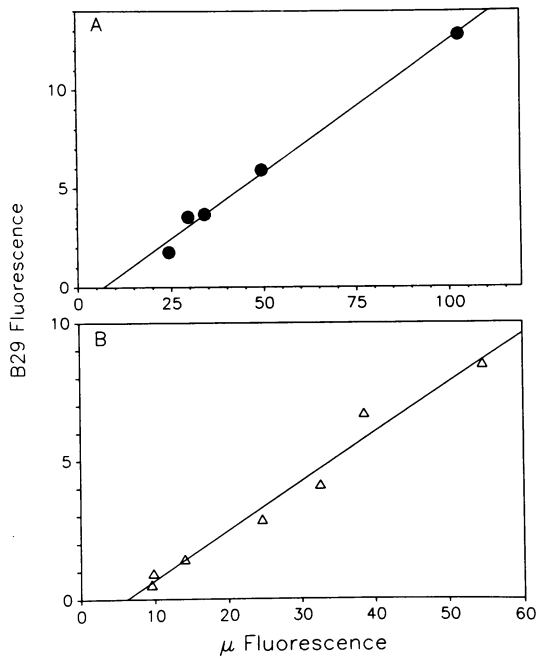

FIG. 3. Surface μ densities correlate with the B29 gene product. (*A*) Subclones of W279, which express different densities of sIgM, were prepared by cloning in semisolid agar. Flow cytometric analysis and conversion of the data to linear fluorescence units were performed as detailed in *Materials and Methods*. (*B*) Inducible pre-B lymphoma cells were exposed to LPS for the intervals illustrated in Fig. 4*B*. This plot illustrates the correlation of surface μ and B29 protein densities over the same time course.

a 2- to 3-fold increase in B29 mRNA within 24 hr and a 4- to 6-fold increase by 48 hr (Fig. 4*A*). For comparison, immunoglobulin κ light-chain mRNA was induced >20 fold by 48 hr of stimulation. The kinetics of surface acquisition of B29 generally paralleled that of IgM. Again, there was a very close correlation ($r^2 = 0.97$) between densities of surface μ and B29 protein (Fig. 3*B*). This suggests that the biosynthesis, intracellular transport, and/or display of these two molecules is coordinated.

DISCUSSION

The cDNA and upstream flanking genomic sequences of B29 were first used to predict its role as a B-cell-specific transmembrane glycoprotein (14, 16). Very recently, partial N-terminal amino acid sequencing suggested that B29 protein isoforms might function as Ig-associated molecules (7, 17). This has now been directly confirmed with a B29-specific antibody. In contrast to the mb-1 molecule, to which B29 is covalently attached (2, 4, 7, 17), no evidence was found for monomers of surface B29. The initial appearance and cell surface density of B29 directly correspond to the sIg molecules with which it associates. This pattern of expression suggests that the B29 gene product may be critical for the correct assembly, intracellular transport, and/or function of antigen receptor complexes on B lymphocytes.

Several laboratories have resolved disulfide-linked heterodimers that are noncovalently associated with B-cell surface IgM (9, 10, 21). With the IgM-bearing W279 lymphoma line, our B29-specific antibody immunoprecipitated a covalently bound complex of ≈41-kDa and ≈34-kDa proteins. Our studies, as well as other recent findings, indicate that isoforms of B29 comprise the larger protein species in these complexes. Partial N-terminal sequences suggested that the ≈41-kDa component of one heterodimer derives from the B29 gene (7, 17). Our Western blot analysis with affinity-purified anti-B29 positively identified that species, as well as a similarly sized protein that was part of a second, smaller heterodimer (data not shown). Anti-mb1 antibodies reportedly recognize the 34-kDa component of at least one heterodimer (4, 6, 7). Accordingly, B29 isoforms correspond to the murine glycoproteins designated Ig-β, pp37, Ig-γ, pp34, and M1 in previous reports (10, 21). Campbell *et al.* (7) found the same amino acid sequences in distinct Ig-β (pp37) and Ig-γ (pp34) bands prepared with internally labeled spleen cells. Distinct subspecies of B29 were usually not resolved with our surface labeling methods, and a smear was observed even with cloned cell lines.

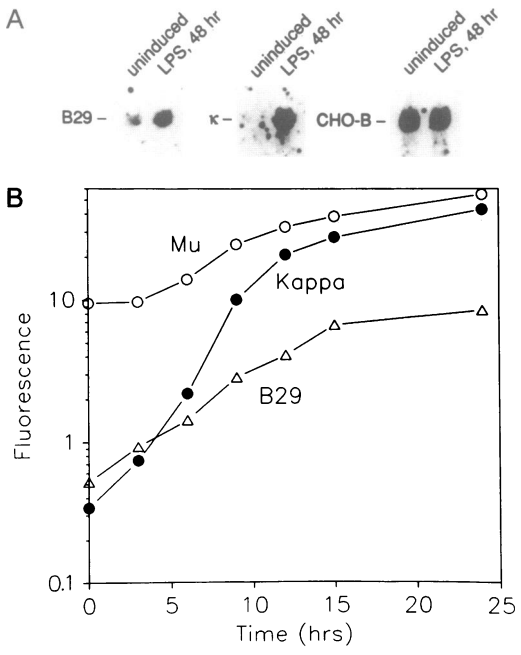

FIG. 4. Induction of Ig and B29 expression in 70Z/3 cells. Pre-B lymphoma cells were incubated with LPS (10 μg/ml) for the indicated times. (*A*) Total cytoplasmic RNA (20 μg per lane) was analyzed on Northern blots with ^{32}P-labeled probes for B29, κ, or the ubiquitously expressed gene, CHO-B. (*B*) Surface display of the B29 protein, κ light chains, or μ heavy chains was assessed by immunofluorescence and flow cytometry. The same data were used to construct the plot shown in Fig. 3*B*.

Ig-associated heterodimers have also been detected in studies with human B lymphocytes (22, 23). However, the size relationship between B29 and mb-1 may be reversed; antibody to human mb-1 recognized the larger component of the heterodimeric complexes. In humans, the deglycosylated forms of these proteins are nearly equivalent in size (23 kDa) and identical to that predicted from the murine B29 cDNA (23). Similarly, treatment of murine mb-1 with endoglycosidase F reduced the size from 34 to 25 kDa (4). Therefore, much of the heterogeneity and species differences between these proteins could result from glycosylation. This explanation is compatible with the lack of evidence for alternative splicing of B29 mRNA (14).

Our experiments directly demonstrate the association of B29 with IgM molecules. Previous studies suggest that it is also a component of an IgD-associated heterodimer (9). Indeed, in preliminary studies, modulation of B-cell surface IgD by prolonged anti-δ treatment diminished staining with our anti-B29 antibody. Lymphomas that expressed sIgA (BFO3) or sIgG (A20) had B29 detectable by immunofluorescence, and there could be associations of B29 with these Ig isotypes.

Our studies indicate that B29 may be displayed in covalent linkage with still another protein, of ≈24 kDa. This heterodimer was seen only when immunoprecipitations were performed with Triton X-100 extracts, and it is not clear whether it also associates with sIg (Fig. 1). While most B29 and sIgM molecules were colocalized on cells after antibody treatment, a minor fraction of each remained independently distributed. This again leaves open the possibility of some heterogeneity in B29-containing complexes. It has recently been demonstrated that receptor-associated complexes on T lymphocytes can vary in composition (24).

At the final stage of pre-B cell maturation, small amounts of μ heavy chains may appear on the cell surface with "surrogate" light chains instead of conventional κ or λ light chains (25). These unusual surface μ molecules may be capable of signal transduction (26). Surrogate light chains, which in the mouse have been named ω and ι, appear to be products of the λ_5 and $V_{pre\text{-}B}$ genes (27–29). Newly formed B cells, which express a higher density of surface μ, may still synthesize and display surrogate light chains (18, 26, 29). B29 protein-containing complexes may also be associated with these surface μ molecules. We examined two types of experimental models that represent the pre-B to B-cell transition. B29 was just at the limit of detection, or negative, on the 70Z/3 pre-B lymphoma. We caution that the B29 protein is much smaller than μ and may bear fewer immunogenic epitopes. For that reason, the sensitivity of detection is lower (Figs. 2 and 3) and it is difficult to document the appearance of a small number of B29 molecules. After exposure to LPS, increases in B29 mRNA and surface B29 protein expression closely paralleled induction of surface μ (Figs. 3*B* and 4). Stromal cell-dependent lymphocyte clones provide another model for studying events that correspond to this phase of B-lineage development (18). Two that bear significant amounts of surface μ also had detectable B29 protein. Thus, although B29 transcripts are detectable from an early stage of

B-lineage development (14), surface B29 was reproducibly observed by immunofluorescence only at the late pre-B or early B-cell stages. There are indications that mb-1 may appear on the surface slightly earlier, and possibly in monomeric form. Immunofluorescence detection of mb-1 protein on pre-B-cell clones and transmembrane signaling with mb-1 antibodies have been reported (2, 4, 5).

Chen *et al.* (21) found that splenic B cells from immunodeficient *xid* mice were deficient in, or had an altered form of, an Ig-associated protein. The properties of this molecule would be generally consistent with B29. However, our immunofluorescence analysis indicates that this mutation does not affect the density of B29 on B cells (data not shown). Further study might reveal that postsynthetic modifications of B29 protein are abnormal in these animals. Indeed, a unique 36-kDa species of Ig-associated protein was documented on *xid* B cells (21). This could conceivably be an underglycosylated form of B29 protein.

A strong correlation was found between surface densities of B29 protein and μ on a series of B lymphoma subclones (Fig. 3*A*). This suggests coordinate rates of synthesis, assembly, intracellular transport, and/or display of these molecules. Six similar promoter and enhancer sequences are shared by these genes, including an octamer (16). The B29 octamer binds Oct-1 and the B-cell-specific transcription factor Oct-2 (16). Binding of Oct-2 to the B29 octamer is inducible and induction of B29 mRNA was found to be comparable to the LPS-induced increase in Oct-2 binding activity (16, 30). Oct-2 could be the primary factor controlling B-cell specificity of B29 and its induction by LPS. The mb-1 gene has some, but not all, of the Ig-like regulatory sequences, and the 5′ regions of both B29 and mb-1 genes lack "TATA boxes" found in most promoters (16, 31).

Surface display of antigen-specific receptors on T lymphocytes is dependent on six separate gene products, which rescue these chains from intracellular degradation (1). Most of the B29 on B lymphocytes was complexed to mb-1-like proteins (Fig. 1). Experiments done with plasmacytoma variants and transfectants demonstrate that mb-1 is essential for sIgM expression (3, 8, 9). While strictly comparable studies have not been done with B29, its abundance may be rate-limiting to sIg expression (17).§ Certainly, this is compatible with our finding of concordance between surface densities of these molecules. The results presented here also support a recently proposed model for the topography of antigen receptor complexes on B lymphocytes (17, 32).

§After submission of this manuscript for review, a report appeared by Venkitaraman *et al.* (33) that is consistent with the conclusions of this study. Expressed IgM molecules were transferred to the surface of transfected fibroblasts only when products of the B29 and mb-1 genes were present.

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