Functional Reconstitution of an Immunoglobulin Antigen Receptor in T Cells

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Summary

Humoral immune responses are initiated by binding of antigen to the immunoglobulins (Igs) on the plasma membrane of B lymphocytes. On the cell surface, Ig forms a complex with several other proteins, two of which, MB-1 and B29, have been implicated in receptor assembly. We have reconstituted Ig receptor function in T lymphocytes by transfection of cloned receptor components. We found that efficient transport of IgM to the surface of T cells required coexpression of B29. Furthermore, IgM and B29 alone were sufficient to reconstitute antigen-specific signal transduction by Ig in the transfected T cells. Crosslinking of IgM with either antireceptor antibodies or antigen induced a calcium flux, phosphoinositol turnover, and interleukin secretion in T cells. These experiments establish a requirement for B29 in Ig receptor function, and suggest that the signaling apparatus of T and B cells is structurally homologous.

The antigen receptors of B and T lymphocytes are members of the Ig supergene family whose specificity is determined by a series of genomic rearrangements. In both cases, the ligand binding domains of the receptors are defined by the combination of two independently encoded polypeptides that are linked by disulfide bonds. In addition, both receptors are associated with a number of other proteins on the cell surface. The TCR is a complex of at least six different polypeptides, including the antigen-specific α/β or γ/δ chains, associated with the CD3 ϵ, γ, δ, and ζ or η chains, all of which are required for efficient assembly and surface transport (1–4). The homologues of the TCR-associated proteins in B cells are MB1 and B29, also known as IgMα and IGMβ (5–9). MB1 and B29 form a disulfide-linked heterodimer that is associated with IgM (10), and both proteins share an intracytoplasmic amino acid sequence motif with CD3 γ, δ, and ζ (11). In addition, MB1 and B29 appear to be required for transport of IgM to the plasma membrane of transfected fibroblasts and B cells (10, 12).

Experiments with mutant T cell lines and isolated TCR components have established that the CD3 ζ chain is both necessary and sufficient for signal transduction (13–16). In addition, the CD3 ϵ chain is also capable of inducing T cell activation, possibly by an alternative pathway (17). Crosslinking of the TCR, or the isolated ζ or ϵ chains, leads to CD45-dependent activation of protein kinases, increased phosphoinositol turnover, and calcium mobilization. A very similar set of events is induced by crosslinking IgM on the surface of B cells, but the functional role of the IgM-associated proteins is poorly defined.

As an initial step in studying the structural and functional requirements for signal transduction by Ig, we have reconstituted the Ig antigen receptor of B lymphocytes in the Jurkat T cell line by transfection. We found that transport of IgM to the surface of T cells required coexpression of the Ig heavy and light chains with B29. Furthermore, the transfected receptor was fully active in the presence of B29, and was able to couple to the downstream signaling apparatus in T cells. MB1, a second IgM-associated polypeptide, was not required for either transport or signal transduction.

Materials and Methods

Plasmid Construction. The spleen focus-forming virus (SFFV)[1] LTR from pFNeo (18) was combined with the BamHI to EcoRI fragment of the human growth hormone to produce an SFFV cDNA expression vector p463. The hGH sequences and polyadenylation signals were added to increase mRNA stability for cDNA expression. MB1 and B29 cDNAs obtained by PCR were sequenced and cloned into the polylinker of p463 to obtain the p466-B29 and the p467-MB1 expression vectors. The EcoRI fragment of the MB1 expression clone was then transferred to the EcoRI site of pSV2His (19) to produce a plasmid that carries both an MB1 expression vector and His resistance, p474-MB1. The IgM heavy chain minigene was composed of the V region of S107 (20) ligated to the HindIII to BamHI fragment of a human IgM constant region gene that had been modified to produce only the membrane-bound form of IgM protein (21). The IgM minigene was cloned just 3′ of the SFFV LTR in pFneo cut with SalI and BamHI to make plasmid p459. The κ minigene was composed of the S107 light chain V region

[1] *Abbreviations used in this paper:* IP, inositol phosphate; PC, phosphorylcholine; SFFV, spleen focus-forming virus.

(22) ligated to the EcoRI to BamHI fragment of human κ light chain constant region (23), and the promoter was the EcoRI to BamHI fragment of the SFFV LTR. The heavy and light chains were combined in a single plasmid by cloning the light chain minigene into the unique BamHI site of p459 to produce p468, a plasmid that carries the PC-specific heavy and light chains, and neomycin resistance genes.

Cell Lines. Jurkat cells were grown in RPMI 1640 supplemented with 10% bovine calf serum, 50 U/ml penicillin, 50 μg/ml streptomycin, and 2 mM L-glutamine (R10). Cells were transfected with linear plasmid DNA by electroporation (24), and selection was carried out in R10 with 0.7 mg/ml of G418 (Gibco Laboratories, Grand Island, NY), and/or 5 mM L-histidinol (Sigma Chemical Co., St. Louis, MO). Resistant cell lines were stained with 10 μg/ml fluorescein-labeled goat anti-human IgM (Southern Biotechnology Associates, Birmingham, AL) and positive cells sorted on a FACStar Plus® (Becton Dickinson & Co., Mountain View, CA). Analysis of surface staining was performed with the same antibody using a FACScan® (Becton Dickinson & Co.).

Antibodies. Monoclonal anti-human IgM, DA4.4 (25), and anti-human CD-3, OKT 3 (26), were purified from either ascites or tissue culture media by precipitation with ammonium sulfate and chromatography on protein A-Sepharose. Goat anti-human IgM, either labeled with fluorescein or unlabeled, IgG1 monoclonal isotype control antibody, and goat anti-mouse IgG, were from Southern Biotechnology Associates.

Northern Analysis. RNA was prepared as described (27). After electrophoresis and transfer, blots were hybridized with either B29 cDNA or MB1 antisense RNA as described (28).

Iodinations, Affinity Purification, and Protein Electrophoresis. 3–6 × 10^7 viable cells were labeled with 3–4 mCi of $Na^{125}I$ and lactoperoxidase/glucose oxidase as described (29), and solubilized in 1% digitonin, 100 mM NaCl, 50 mM Tris-HCl (pH 6.8), 1 mM PMSF at 4°C for 30 min. Insoluble material was separated by centrifugation and the supernatant fraction was incubated with phosphorylcholine (PC) beads for 4–5 h. The beads were washed three times with 0.1% digitonin, 100 mM NaCl, 50 mM Tris-HCl (pH 6.8) for 5 min. Elution of IgM was carried out by incubating the beads for 1 h with wash buffer supplemented with 20 mM PC. Protein samples were analyzed by SDS-PAGE on 7% gels under reducing conditions. The protein bands were visualized by silver staining. A Phosphor Imager (Molecular Dynamics) was used to visualize ^{125}I-labeled proteins.

Calcium Flux Measurements. Cells were resuspended at 5 × 10^6/ml in PBS supplemented with 5.6 mM glucose, 0.025% BSA, 20 mM Hepes (pH 7.0), 1 mM $CaCl_2$, 1 mM $MgCl_2$ (loading buffer), and 3 μg/ml Fura-2AM (Sigma Chemical Co.), and incubated at 37°C for 30 min followed by three washes with loading buffer. The Fura-2-loaded cells were then resuspended in loading buffer at a concentration of 10^6/ml, and fluorescence was measured in a spectrofluorimeter (SPF-500C; SLM Aminco Instruments Inc., Urbana, IL), where the excitation wavelength was 335 nm and emission was recorded at 510 nm. Calcium concentration was calculated as described (30).

PC-BSA and PC-Sepharose. BSA dissolved in PBS was incubated overnight with para-diazoniumphenylphosphorylcholine (DPPC) synthesized according to reference (31). Modified BSA (PC-BSA) was separated from unreacted DPPC by column chromatography on Sephadex G25 in PBS. For the synthesis of PC beads, the dipeptide alanyltyrosine was coupled to CNBr-activated Sepharose (Pharmacia Fine Chemicals, Piscataway, NJ). The beads were washed in PBS, and freshly synthesized DPPC was added, incubated overnight, washed with water, and stored at 4°C.

IL-2 Bioassay. Cells to be tested for IL-2 production were suspended at 1.2 × 10^6/ml in tissue culture media and used directly or pretreated with either, 10 μg/ml anti-CD3, 20 μg/ml of DA4.4 monoclonal anti-human IgM, or 10 μg/ml of an IgG1 monoclonal isotype control antibody for 30 min at 4°C. Unbound antibody was removed by extensive washing with PBS. 2 × 10^4 cells were then plated in the wells of a 96-well tissue culture plate precoated with either 10 μg/ml goat anti-mouse IgG (Southern Biotechnology Associates), 10 μg/ml PC-BSA, 10 μg/ml BSA, or nothing. PMA was added at a final concentration of 10 ng/ml. Supernatants were harvested after 24 h and bioassays were performed using the CTLL-2.20 IL-2-dependent cell line as described (32).

Inositol Phosphate Assay. Cells were incubated for 4 h with [^3H]myo-inositol at 15 μCi/ml, and 3–4 × 10^6 cells/ml in inositol-free RPMI 1640 supplemented with 10% dialyzed bovine calf serum. Excess [^3H]-myo-inositol was removed by washing in PBS. The loaded cells were then resuspended at 2 × 10^6/ml in Hepes-buffered saline supplemented with 10 mM LiCl, and equilibrated for 15 min at 37°C. The cells were sequentially stimulated with 1 μg/ml of either anti-CD3, DA4.4 monoclonal anti-human IgM, or the IgG1 isotype control followed after 10 s by 10 μg/ml of goat anti-mouse IgG crosslinking reagent. The incubation was terminated after 3 min by adding of 8 ml of 2:1 methanol/chloroform and the extracted material was subjected to chromatography on 1-ml columns of Dowex AG-1-8 (Bio-Rad Laboratories, Richmond, CA). Inositol phosphates were eluted with 0.1 M formic acid containing increasing concentrations of ammonium formate (0.2 M for inositol phosphate [IP], 0.4 M for IP2, 0.8 M for IP3, and 1 M for IP4). The eluted radioactivity was quantitated by liquid scintillation counting.

Results

Surface Expression of IgM in T Cells. PC-specific Ig receptor components were expressed in T cells by stable transfection of DNA constructs based on an SFFV-LTR promoter (18) (Fig. 1 *A*). A human IgM heavy chain minigene that directs the synthesis of membrane-anchored IgM (21) was combined with a PC-specific heavy chain variable region (20). The light chain was composed of the corresponding κ variable region (22), coupled to a human κ constant region gene (23). To insure coordinate expression of the Ig heavy and light chains, the two transcription units were combined in a single plasmid with a neomycin resistance gene. Human growth hormone polyadenylation and splice consensus sequences (18) were added to mouse MB1 and B29 cDNAs, and a histidinol resistance gene was included as a second drug resistance marker in the MB1 expression vector (19).

IgM expression was readily detected on the surface of Jurkat cells transfected with IgM and B29, or a combination of IgM, MB1, and B29. In contrast, surface IgM expression was difficult to detect on Jurkat cells transfected with either the heavy and light chain alone, or a combination of IgM and MB1. In all cases we enriched for surface IgM-positive cells by selection with a FACS® (Fig. 1 *B*). High levels of surface IgM expression were achieved after one or two rounds of selection of cell lines transfected with either IgM and B29, or IgM, B29, and MB1. However, eight rounds of sorting were required for cell lines transfected with IgM alone, while the combination of IgM and MB1 was always negative (Fig.

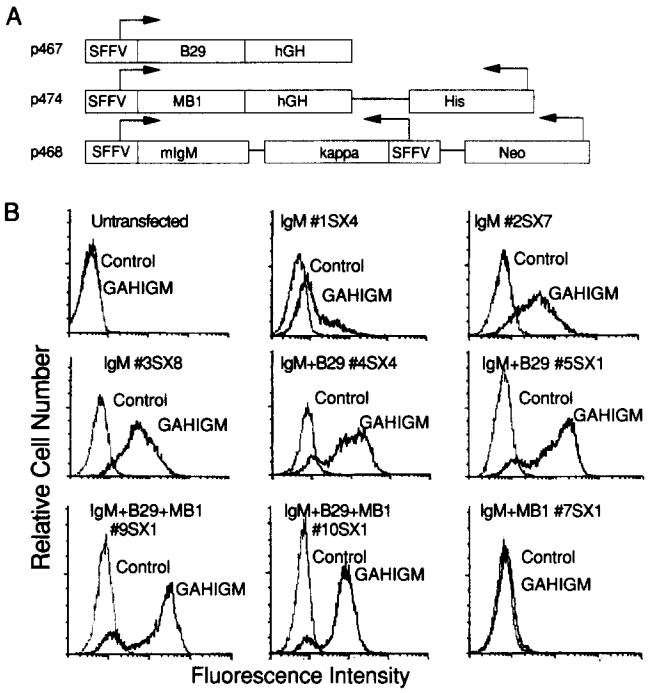

Figure 1. DNA constructs, and cell surface expression of human Ig in transfected Jurkat cells. (*A*) Maps of Ig, B29, and MB1 expression vectors. The SFFV-LTR promoter was used in all constructs for T cell expression. Human growth hormone introns and polyadenylation signals were added to both B29 and MB1 cDNAs, and a histidinol resistance gene was included in the MB1 plasmid. A PC-specific mouse variable region from S107 was combined with a human heavy chain constant region engineered to direct the synthesis of only the membrane bound form of IgM. The light chain was composed of an S107 κ variable region and the human κ constant region. The heavy chain and light chain were on the same plasmid as a neomycin resistance gene. (*B*) Flow cytometric analysis of surface expression of human IgM on transfected Jurkat cells. Relative cell number is plotted against fluorescence intensity on a logarithmic scale. The constructs transfected, cell line, and the number of times the cell line was sorted to enrich for surface expression (SX*n*) are indicated at the top of each panel. Control indicates unstained cells, and GAHIGM indicates staining with FITC-conjugated goat anti-human IgM. B29, B29 cDNA; MB1, MB1 cDNA; hGH, human growth hormone splice and polyadenylation signals; His, histidinol resistance gene; mIgM, PC-specific, membrane-bound form of human IgM heavy chain; kappa, PC-specific κ light chain; Neo, neomycin resistance gene; #, cell line number; SX*n*, the number of times a cell line was enriched for surface IgM by sorting; p467, B29 expression vector; p474, MB1 expression vector; p468, IgM expression vector.

1 *B*). We confirmed that the products of the transfected PC-specific Ig genes were appropriately assembled by affinity purification with PC coupled to Sepharose (PC-Sepharose). Equivalent amounts of both heavy and light chains were obtained from extracts of all cell lines and clonal derivatives regardless of the level of surface expression (Fig. 2 *A*). Thus, the level of surface IgM expression (Fig. 2 *A*). Thus, the level of surface IgM expression was not a simple function of the amount of IgM synthesis. In addition, the steady-state levels of transfected B29 and MB1 mRNA were comparable with a B cell control (Fig. 2 *B*). As expected, the mRNAs produced from the transfected genes were somewhat larger than their B cell counterparts since they contained additional human growth hormone sequences. We concluded that the combi-

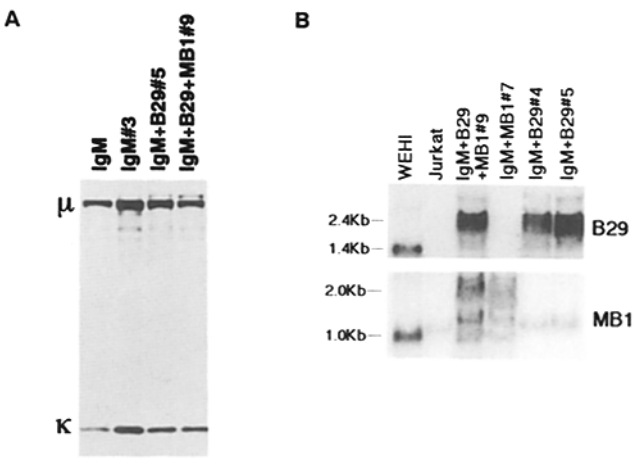

Figure 2. Expression of IgM protein, B29, and MB1 mRNAs. (*A*) Silver-stained SDS-polyacrylamide gel of affinity-purified PC-binding Ig from transfected Jurkat cells, and controls. (*B*) Northern analysis of B29 and MB1 expression in the transfected cell lines. The cell types, constructs transfected, and cell line number (Fig. 1 *B*) are indicated at the top of each lane: IgM, the starting cell line for production of IgM#3 had 1–2% surface IgM expression; μ and κ indicate the position of the heavy and light chains determined from purified standards.

nation of heavy chain, light chain, and B29 was sufficient for efficient transport of IgM to the surface of T cells. In contrast, powerful selection was required to obtain surface IgM–positive cells in absence of B29.

Transfected IgM Is Associated with B29 and MB1. To determine whether B29 and MB1 were associated with surface IgM, transfected T cell lines were iodinated, and PC-specific Ig and associated molecules were purified from digitonin extracts by adsorption with PC-Sepharose (Fig. 3). A polypeptide with the appropriate electrophoretic mobility for B29 (44 kD) was copurified with Ig heavy and light chains from Jurkat cells transfected with IgM and B29. The same polypeptide and three additional species were copurified from the IgM, B29, and MB1 transfectants (Fig. 3). The 32-kD band

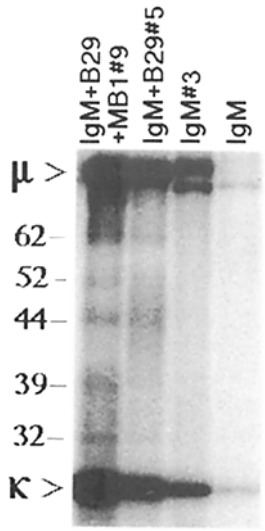

Figure 3. Affinity purification of surface iodinated proteins from transfected cell lines. PC-binding Igs and associated polypeptides were affinity purified from 1% digitonin extracts of surface iodinated cells. The cell types, constructs transfected, and cell line number are indicated at the top of each lane, the same as in Fig. 2 *A*; μ and κ indicate the position of the heavy and light chains determined from purified standards.

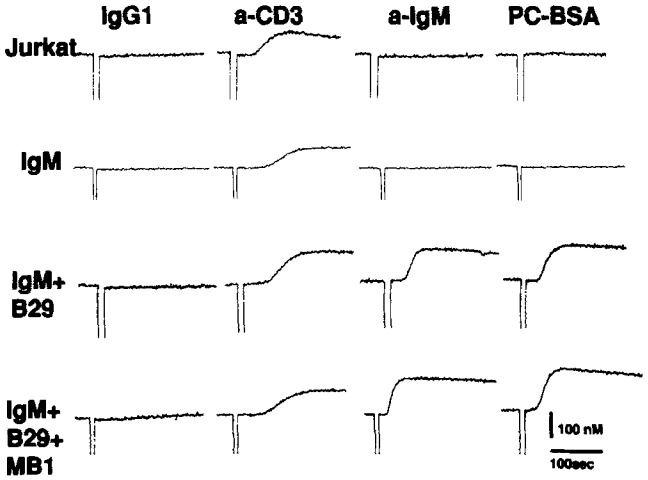

Figure 4. Calcium flux assays of transfected Jurkat cells. Cells loaded with Fura-2 were assayed fluorimetrically for calcium mobilization in response to either 1.5 μg/ml anti-CD3, 10 μg/ml anti-human IgM, or 10 μg/ml isotype control mAb or PC-BSA. The cell types and constructs transfected are indicated at left, and reagents added at the top. IgG1, isotype control mAb; a-CD3, anti-CD3 antibody; a-IgM, anti-IgM antibody; Jurkat, untransfected Jurkat cells; IgM, Jurkat cells transfected with IgM no. 3 (Fig. 1 *B*); IgM+B29, Jurkat cells transfected with IgM+B29 no. 4 (Fig. 1 *B*); IgM+B29+MB1, Jurkat cells transfected with IgM+B29+MB1 no. 9 (Fig. 1 *B*); standard scales for time and $[Ca^{2+}]$ are indicated at the bottom right.

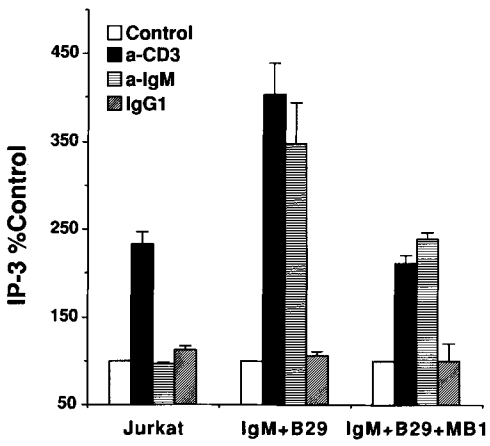

Figure 5. Generation of inositol triphosphate by transfected Jurkat cells. Cells metabolically labeled with [^{3}H]myo-inositol were stimulated with either anti-CD3, anti-human IgM, or an IgG1 isotype control antibody, and inositol triphosphate was measured by extraction and anion exchange chromatography. The percent of unstimulated IP3 production is plotted on the *y*-axis, error bars indicate the SD from the mean (n = 2). Control, no primary antibody; other symbols are as in Fig. 4.

was consistent with MB1, the additional bands at 52 and 39 kD may be alternate forms of B29 and MB1, or T cell–encoded proteins. As a control, the same experiments were performed with Jurkat cells that expressed surface IgM alone (cell line 3 Fig. 1 *B*). Although we were able to purify iodinated Ig heavy and light chains from the IgM control, there were no associated proteins (Fig. 3). Our interpretation of these experiments was that B29 was associated with IgM on the surface of T cells, and this interaction occurred in the absence of MB1. Furthermore, when both MB1 and B29 were present in the transfected T cells, both were associated with IgM.

Crosslinking of IgM with Antireceptor Antibodies Induces Calcium Mobilization, Phosphoinositol Turnover, and IL-2 Secretion. The function of the transfected Ig was initially assessed by measurement of calcium flux in response to receptor crosslinking. Fura-2-loaded cell lines were treated with an anti–human IgM mAb, monoclonal anti-CD3, or an isotype-matched mAb control. Jurkat cells transfected with either IgM and B29 or IgM, B29, and MB1 responded to crosslinking by anti-IgM with a rapid increase in free intracellular calcium (Fig. 4). Untransfected Jurkat cells, and Jurkat cells that expressed high levels of IgM alone, did not respond to anti-IgM, but were fully competent to respond to anti-CD3 (Fig. 4). In addition, the isotype control IgG1 antibody had no effect (Fig. 4), and polyclonal goat anti-IgM antibodies had the same effect as monoclonal anti-IgM (not shown).

IP turnover, triggered by the activation of phospholipase C, is another measure of signal transduction by Ig. To confirm and extend the results obtained in calcium flux assays, we measured inositol turnover in response to anti-IgM antibody (Fig. 5). Crosslinking Ig on the surface of T cell lines transfected with IgM and B29, or IgM, B29, and MB1, resulted in an increase in cellular IP3. In the same experiments, anti-IgM antibody had no effect on untransfected Jurkat cells (Fig. 5). Thus, the combination of Ig and B29 expressed on the surface of T cells was fully competent to activate intracellular calcium mobilization, and phosphoinositol turnover.

One biological effector function that is induced by activation of the TCR is secretion of IL-2. To determine whether Ig activates this downstream response in transfected T cells, we measured IL-2 production in response to anti-IgM (Fig. 6). Jurkat cells that expressed either IgM and B29, or IgM, B29, and MB1, secreted IL-2 in response to anti-IgM treatment. This response was specific, since untransfected Jurkat cells did not respond to anti-IgM but did produce IL-2 in

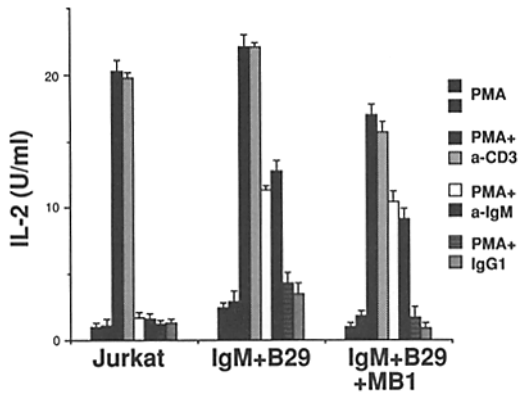

Figure 6. IL-2 secretion in response to anti-IgM by transfected Jurkat cells. Cell lines were stimulated with either anti-CD3, anti–human IgM, or an IgG1 isotype control antibody in the presence of PMA, or PMA alone. The mean and the SD (n = 3) of the number of U/ml of IL-2 secreted for two independent experiments is indicated. IgM+B29, Jurkat cells transfected with IgM+B29 no. 5 (Fig. 1 *B*); other symbols are as in Fig. 4.

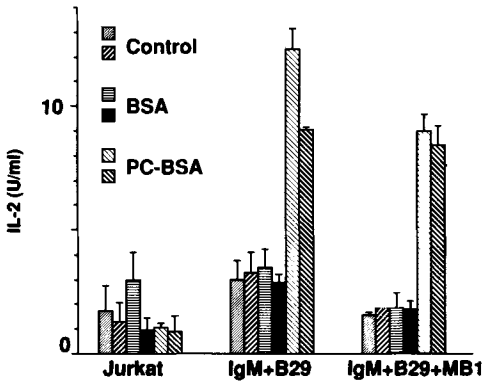

Figure 7. IL-2 secretion in response to PC by transfected Jurkat cells. Cell lines were stimulated with either BSA, PC-BSA, or nothing. The mean and the SD (n = 3) of the number of U/ml of IL-2 secreted for two independent experiments is indicated. IgM+B29, Jurkat cells transfected with IgM+B29 no. 5 (Fig. 1 *B*); other symbols are as in Fig. 4.

response to anti-CD3 stimulation. None of the lines were induced with the isotype control mAb (Fig. 6).

Signal Transduction Induced by Antigen. T lymphocytes respond to processed peptides associated with MHC on the surface of other cells, whereas B cells respond to native antigens. To determine whether Jurkat cells expressing anti-PC-specific IgM would respond to antigens in the absence of MHC, they were challenged with PC-BSA (Figs. 4 and 7). PC-BSA induced calcium flux and IL-2 secretion in T cell lines that expressed B29 in addition to IgM. Controls treated with albumin alone were always negative. Once again, in antigen stimulation experiments, T cells that expressed surface IgM alone failed to respond to receptor crosslinking (Fig. 4).

The potential additive or synergistic effects of MB1 were assessed in dose-response experiments (Fig. 8 *A*). We found that cell lines expressing equivalent amounts of surface IgM had similar responses to increasing concentrations of PC-BSA whether or not MB1 was present. In both cases, a response was obtained with as little as 0.5 ng/ml of PC-BSA, and the response reached a peak at 0.1–0.5 mg/ml of PC-BSA. The responses decreased at doses >1 mg/ml, consistent with the possibility that signaling was dependent on receptor crosslinking. The role of receptor crosslinking in signaling was further examined by treating cells with monomeric PC (Fig. 8 *B*). Monomeric antigen did not induce signaling. However, pretreatment of the cells with monomeric PC blocked the response to PC-BSA (Fig. 8 *B*). Thus, crosslinking of the transfected Ig appears to be an important feature of the signaling mechanism.

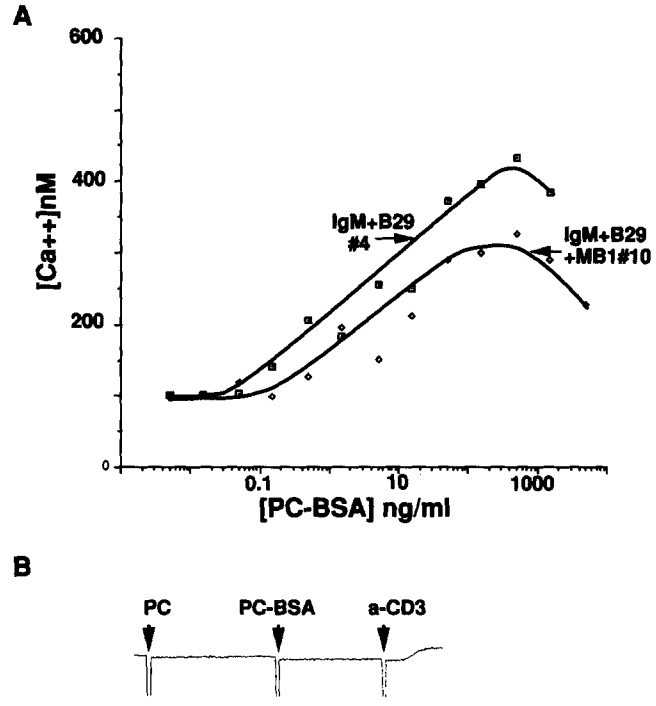

Figure 8. Dose-response to PC-BSA, and blocking by monomeric PC in transfected Jurkat cells. (*A*) Cells loaded with Fura-2 were assayed fluorimetrically for calcium mobilization in response to increasing doses of PC-BSA. Calcium concentration (y-axis) was calculated as indicated in Materials and Methods and plotted against PC-BSA concentration on a logarithmic scale (x-axis). The constructs transfected and cell line numbers are indicated. Diamonds represent IgM+B29+MB1-transfected Jurkat cells, and squares indicate IgM+B29-transfected Jurkat cells. (*B*) IgM+B29-transfected cell line no. 5 was stimulated with 2.5 mM PC followed by 500 ng/ml PC-BSA, and finally 5 μg/ml of anti-CD3 mAb as indicated by arrows. PC, monomeric phosphorylcholine; other symbols are as in Fig. 4.

Discussion

The finding that Ig is associated with several other proteins on the plasma membrane of B cells raises a number of interesting questions about the role of these accessory molecules in receptor assembly and signal transduction. In this study, we demonstrate that coexpression of IgM and B29 is sufficient to reconstitute both Ig surface expression and function in T cells.

There is persuasive evidence that two of the Ig-associated proteins, MB1 and B29, are important for transport of Ig to the surface of B cells and fibroblasts. B cell lines that lack MB1 fail to express surface IgM, and this phenotype can be restored by transfection of cloned MB1 (10). Thus, MB1 is required for receptor assembly in B cells. Similarly, in fibroblasts, both MB1 and B29 are required for surface expression of IgM, however, other Ig isotypes can be expressed on the surface of fibroblasts with B29 even in the absence of MB1 (12). One attractive model of IgM receptor structure proposes that IgM interacts with a pair of MB1 and B29 heterodimers. In this model, MB1 and B29 are disulfide linked and interact with IgM in part through polar amino acid side chains in the transmembrane domains. In view of this proposed quaternary structure, it was surprising to find that MB1 was not strictly required for either surface expression, or function of IgM antigen receptors on transfected T cells. Failure to obtain a functional antigen receptor with MB1 alone was not simply due to lack of transfected gene expression (Fig. 2 *B*) or to an inactive MB1 protein, since MB1 was associated with IgM and B29 in T cells transfected with all three components (Fig. 3). Furthermore, addition of MB1 to cell lines that also expressed B29 and IgM, but had low levels of sur-

face IgM, induced higher levels of surface Ig (not shown). One interpretation of our results is that one or more T cell components, not present in fibroblasts or B cells, can substitute for MB1.

Progress in understanding the mechanism of signaling by the Ig receptor has been hindered by the multisubunit nature of the receptor. In addition, transfected Ig expressed on the surface of fibroblasts does not appear to be functional in signal transduction, even in the presence of B29 and MB1 (12). The ability to produce a functional receptor by transfection in T cells establishes that there must be structural similarity between the T and B cell signal transduction pathways. This observation should greatly simplify the structural and functional analysis of IgM antigen receptor.

An intriguing question raised by our results is how the IgM receptor can interact with the T cell signal transduction apparatus. Although there are many similarities between the signaling pathways of B and T cells, there are several significant differences. For example, much experimental evidence points to activation of tyrosine kinases as one of the first steps in the signal transduction pathways of the TCR (reviewed in reference 33) and IgM (34, 35). Indeed, both B29 and MB1 are rapidly phosphorylated upon IgM crosslinking in B cells (9, 35, 36), and both ζ and ZAP-70 are phosphorylated upon TCR crosslinking in T cells (37, 38). However the specific kinases coimmunoprecipitated with IgM in B cells (p56*lyn*, *fyn*, and *blk*) (39, 40) differ from that associated with the TCR (*fyn*) (41). There are at least two mechanisms by which IgM and B29 could overcome the apparent differences between the signal transduction apparatus of T and B cells. First, IgM and B29 could interact directly with and activate one of the many T cell protein tyrosine kinases by forming a complex with structural similarity to CD3 ζ or ϵ. Crosslinking of the isolated ζ or ϵ chains is sufficient to induce signaling in transfected T cells (14, 15, 17), and there is sequence homology between ζ, ϵ, and B29 (11). Indeed, mutations in the shared intracytoplasmic consensus sequence destroy signal transduction mediated by ϵ. A second mechanism to explain signal transduction by IgM and B29 in T cells would involve direct association of IgM with T cell–encoded proteins that in turn make the appropriate cellular connections. The CD3 components are particularly appealing candidates for this function since they are both structurally and functionally related to B29 and MB1 (11). However, we have been unable to identify proteins other than a polypeptide with the apparent molecular weight of B29 associated with IgM in Jurkat cells transfected with IgM and B29. Additional polypeptides were detected in the IgM, B29, and MB1 transfectants, but, we noted no apparent effect on calcium flux, inositol metabolism, or IL-2 secretion (Figs. 4–8). Although the significance of these additional receptor-associated proteins remains unclear, we continue to pursue the possibility of a hybrid receptor complex. Experiments with mutant T cell lines that are deficient in specific TCR components should clarify this issue, and potentially elucidate the structural and functional relationship between the Ig- and TCR-associated proteins. In addition, our experiments suggest that it may be possible to use B cell lines to functionally reconstitute the TCR from cloned components.

One major difference between the TCR and Ig antigen receptors is the nature of the antigen recognized by the two receptors. Igs recognize antigens directly, whereas recognition of antigen by the TCR is restricted by MHC. The requirement for MHC recognition places severe limitations on the targets recognized by T cells, and makes transfer of cellular immunity MHC restricted. For this reason, there has been considerable interest in modifying T cell recognition to abrogate the MHC requirement. Several groups have shown that chimeric receptors composed of Ig variable regions and TCR constant regions can function in an MHC-independent fashion. However, this approach is limited by the formation of heterodimeric receptors composed of the endogenous TCR and the transfected V-Ig C-TCR chimeras, as well as formation of Vh-Cβ homodimers (42–45). Another approach has been to produce CD4 ζ chimeric proteins that were capable of directing T cells to targets that express HIV envelope proteins (15). In this system, antigen binding results in T cell activation via CD4 ζ crosslinking. Our reconstitution experiments offer an additional, more general solution to the difficult problem of MHC restriction in transfer of cellular immunity. T cells that express functional Ig antigen receptors would have the potential for recognizing any antigen recognized by antibodies in an MHC-independent fashion.

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