The EMBO Journal vol.7 no.11 pp.3451 – 3456, 1988

A novel 34-kd protein co-isolated with the IgM molecule in surface IgM-expressing cells

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Communicated by K.Rajewsky

Plasmacytoma cells, transfected with a vector encoding a membrane-bound IgM molecule, do not show cell surface IgM expression, although complete IgM molecules are assembled intracellularly. The isolation of a surface IgM-positive variant allowed us to analyse molecular requirements of surface IgM expression. Only in surface IgM-positive cells, a 34-kd protein (B34) was found to be associated with IgM. B34 is a glycoprotein which forms a disulphide-linked homodimer. The surface IgM-positive variant cell line expressing B34 also contains transcripts of the pre-B and B cell specific *mb-1* gene. The data are discussed in the context of a possible IgM–antigen receptor complex.

Key words: surface IgM expression/IgM-associated proteins/transmembrane signalling

Introduction

The antigen receptors on B cells are membrane-bound immunoglobulin (Ig) molecules, composed of two identical heavy (H) and two identical light (L) chains. Each Ig chain is structured into a variable N-terminal domain and one (L chain) or several (H chain) constant domains. The antigen binding sites of the receptors are formed by the variable domains of H and L chain. Membrane insertion of the molecules occurs via a conserved amino acid sequence at the C terminus of the H chain (Kehry *et al.*, 1980). On the cell surface of mature B cells two classes of antigen receptors are expressed, namely IgM and IgD. These receptors have identical variable domains, but differ in their constant regions (Vitetta and Uhr, 1976). In the presence of appropriate T cell help, binding of antigen to these receptors results in the activation of the B cell. The activated B cell proliferates and differentiates into Ig-secreting plasma cells or B memory cells (Rajewsky *et al.*, 1987).

In a previous paper we reported on transfection of expression vectors, coding for the membrane-bound form of IgM (μm), into lymphoma and plasmacytoma cells. In the presence of L chain, fully assembled IgM molecules were not transported to the cell surface of the transfected plasmacytomas (Hombach *et al.*, 1988). Similar results were obtained independently by Sitia *et al.* (1987). From these data we postulated that surface IgM (sIgM) expression is dependent on a further protein, whose expression is turned off at the plasma cell stage. We have now isolated a μm-transfected plasmacytoma variant, expressing sIgM. In this line a 34-kd IgM-associated protein (B34) was identified, which is only present in sIgM-expressing cell lines.

Results

Isolation of the surface IgM-expressing plasmacytoma line 558Lμm3

The pSVμm-transfected plasmacytoma line 558Lμm produces a vector-encoded μm chain and an endogenous λ_1 light chain (Reth *et al.*, 1987). Although both chains are assembled into a functional NP-binding IgM molecule, this NP-specific antigen receptor is not brought to the cell surface of this line (Figure 1A). If, however, the pSVμm vector is transfected into Abelson pre-B cells and B lymphoma cells, the IgM molecule is readily expressed on the cell surface. From these experiments we postulated the existence of proteins, whose presence is required for sIgM expression (Hombach *et al.*, 1988). Such proteins should be expressed in pre-B and B cells but not in sIgM-negative plasma cells.

To analyse the molecular requirements for sIgM expression, we tried to isolate a sIgM-positive variant of the 558Lμm line and to look for the presence of a novel μm chain-associated protein in this variant. A first analysis of sIgM expression of the original 558Lμm culture by fluorescence microscopy showed a low percentage ($\sim$0.05%) of sIgM-positive cells. This sIgM-positive population was enriched by the fluorescence-activated cell sorter (FACS). After two cycles of enrichment, 90% of the sorted population expressed sIgM (Figure 1B). This cell population was cloned, and one sIgM-positive clone (558Lμm3) was chosen for further studies (Figure 1C). The expression of sIgM on 558Lμm3 cells is stable over long periods of culture.

Identification of the 34-kd IgM-associated protein B34

To test whether sIgM expression of 558Lμm3 is correlated with the association of IgM to a further protein, the 558Lμm and 558Lμm3 cells were biosynthetically labelled and lysed by either Nonidet P-40 (NP-40) or digitonin. Digitonin is a mild detergent which lyses cells without disrupting non-covalently associated complexes of membrane proteins. It has recently been used to identify components of the murine T cell receptor/T3 complex (Oettgen *et al.*, 1986). NP-binding molecules, i.e. antigen receptors, in the NP-40 or digitonin lysates were bound to an NP–Sephadex affinity column. The adsorbed NP-binding molecules were subsequently eluted from the column with free hapten. In our hands this method of elution of membrane-bound IgM yields cleaner material than the conventional immunoprecipitation protocols. The hapten-eluted material of the 558Lμm and 558Lμm3 lines was analysed by SDS–PAGE under reducing conditions (Figure 2, lanes 1 and 2). In both lines and with both NP-40 and digitonin, μm and λ_1 chains are seen as predominant bands of $\sim$73 and 28 kd. Thus, no drastic physical alteration of the μm chain has happened in the sIgM-positive variant. However, the comparison of

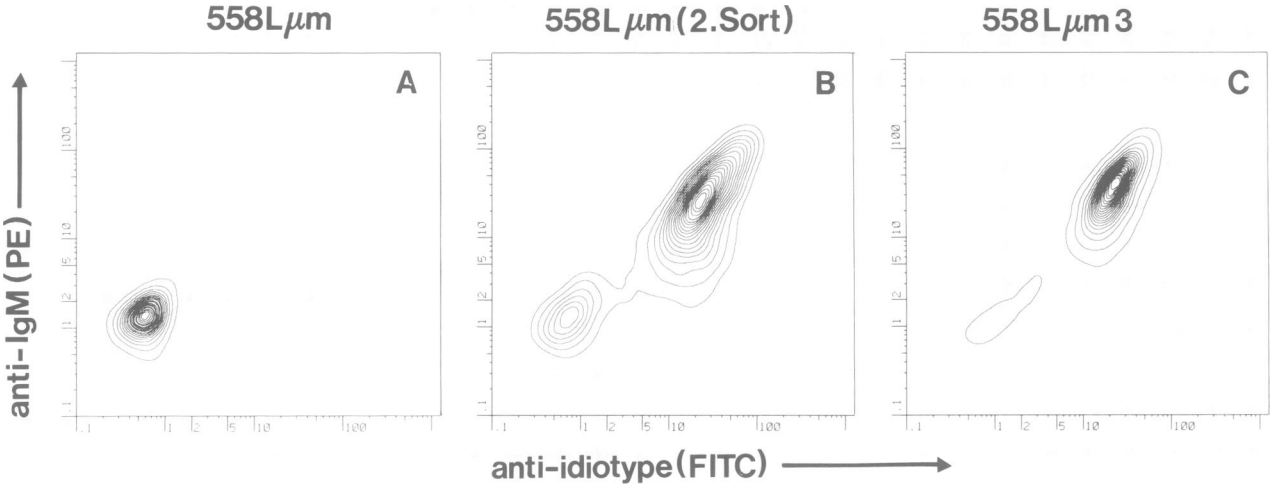

Fig. 1. Double fluorescence of the sIgM-negative line 558Lμm (**A**), the enriched sIgM-positive population of 558Lμm (**B**) and the 558Lμm3 variant line (**C**). The line 558Lμm3 was subcloned from the sorted cell population, seen in (**B**). Cells were stained with biotinylated goat anti-mouse IgM antiserum/streptavidin–phycoerythrin (PE) (ordinate) and the fluorescinated (FITC) anti-idiotypic antibody Ac146 (abscissa). The cell populations are shown as 5% probability contour plots on logarithmic scales.

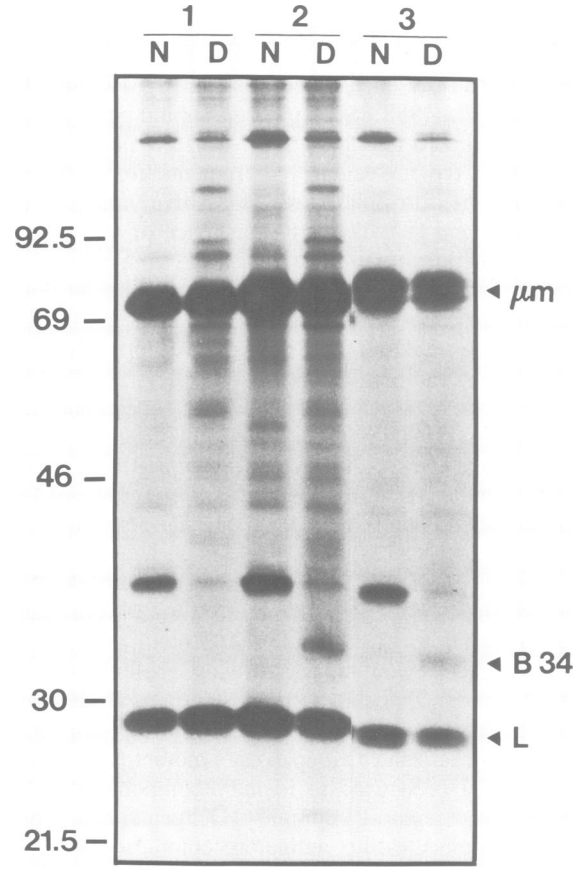

Fig. 2. SDS–PAGE analysis of NP-binding molecules in cell lysates of sIgM-negative and sIgM-positive cell lines. Cells were biosynthetically labelled with [^{35}S]methionine and lysed either with NP-40 (N) or digitonin (D). The proteins were size-fractionated on a 10% acrylamide gel under reducing conditions. **Lanes 1 N/D**: sIgM-negative plasmacytoma line 558Lμm. **Lanes 2 N/D**: sIgM-expressing variant 558Lμm3. **Lanes 3 N/D**: sIgM-expressing pre-B cell line 300-19μmλ36/8. Mol. wts in kd.

the material eluted from the digitonin lysates of 558Lμm and 558Lμm3 shows a 34-kd protein (B34), present only in the 558Lμm3 line (Figure 2, compare lanes 1D and 2D). In the NP-40 lysates of both lines the 34-kd protein is absent (Figure 2, lanes 1N and 2N).

In parallel to the plasmacytoma lines, the transfected Abelson pre-B cell line 300-19μmλ36/8 (Reth *et al.*, 1987) was analysed. This line produces μm and λ_1 chains (see Materials and methods) and thus carries the same NP-binding IgM molecule on the cell surface as 558Lμm3. As in the case of the 558Lμm3 cells, a 34-kd protein is found in the digitonin but not in the NP-40 lysate of this line (Figure 2, lanes 3D and 3N).

These results show that the B34 protein is only present in the digitonin lysates of sIgM-expressing lines.

B34 is a glycoprotein specifically associated with membrane-bound but not with secreted IgM

The above experiment shows that sIgM expression correlates with the appearance of the B34 protein. To test whether B34 is specifically associated with the membrane form of IgM, two additional anti-NP antibody-producing cell lines were analysed. These were 558Lμs, which only secretes IgM molecules, and B1-8.δ1, which secretes IgD and expresses membrane-bound IgD on the cell surface (see Materials and methods). The cells were biosynthetically labelled and lysed with digitonin; the affinity-purified molecules were analysed by SDS–PAGE under reducing conditions, and compared with those derived from the 558Lμm and 558Lμm3 lysates. As shown previously, the B34 protein is found in the digitonin lysates of 558Lμm3 but not of 558Lμm (Figure 3, lanes 2 and 3). However, B34 is not found in association with the Ig molecules of the 558Lμs or B1-8.δ1 line (Figure 3, lanes 4 and 5).

As a specificity control, the digitonin lysate of the untransfected λ_1-producing 558L plasmacytoma line was processed and analysed in parallel to the Ig-producing lines (Figure 3, lane 1). The 558L lysate shows that a few cellular proteins are non-specifically bound and eluted from the affinity columns. Besides these non-specifically bound proteins the lysates of the Ig-producing lines contain other proteins. Thus, a 41-kd molecule is found in the lysates of 558Lμm, 558Lμm3 and B1-8.δ1 but not of μs and may be

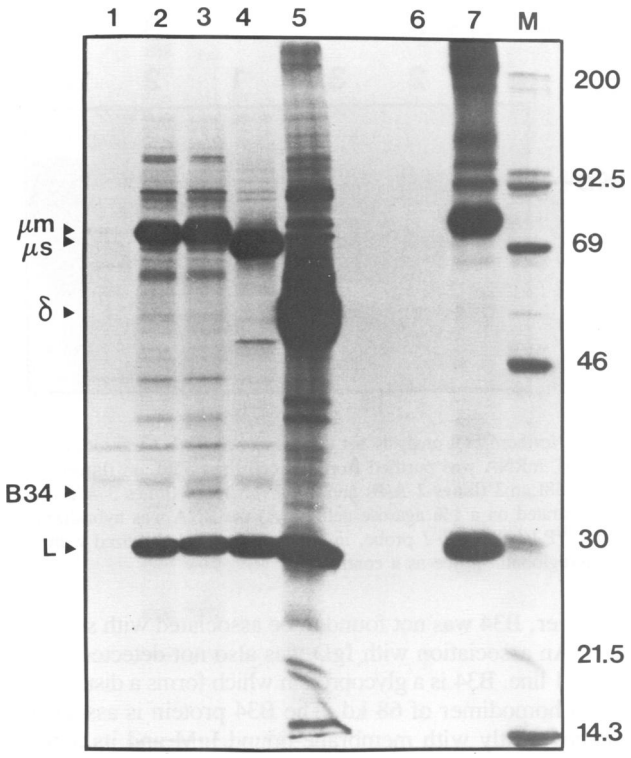

Fig. 3. SDS–PAGE analysis of NP-binding molecules of [^{35}S]methionine metabolically labelled (**lanes 1–5**) or surface iodinated (**lanes 6** and **7**) cells. Proteins were extracted from cell lysates with digitonin, affinity-purified and analysed on a 10% acrylamide gel under reducing conditions. The following cell lines were analysed: (**lane 1**) the untransfected plasmacytoma line 558L, (**lane 2**) the sIgM-negative transfectant 558Lμm, (**lane 3**) the sIgM-positive variant 558Lμm3, (**lane 4**) the IgM-secreting transfectant 558Lμs and (**lane 5**) the IgD-producing hybridoma line B1-8δ1. **Lanes 6** and **7** show the surface-iodinated NP-binding proteins of 558Lμm and 558Lμm3, respectively. Mol. wts in kd.

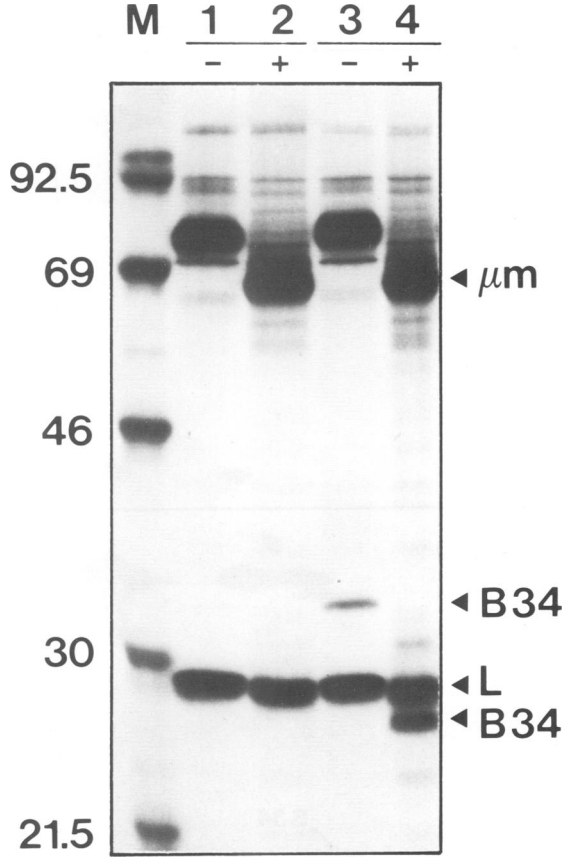

Fig. 4. Glycopeptide-N-glycosidase (PNGase F) treatment of NP-binding proteins of 558Lμm (**lanes 1** and **2**) and 558Lμm3 (**lanes 3** and **4**). (+) indicates PNGase F digestion. NP-binding proteins of [^{35}S]methionine metabolically labelled cells were affinity-purified, digested with PNGase F and size-separated under reducing conditions on a 10% acrylamide gel. Mol. wts in kd.

associated with both the membrane-bound IgM and IgD molecules. The μs chain, on the other hand, seems to be specifically associated with a 50-kd protein (Figure 3, lane 4).

To test for cell surface expression of B34, surface proteins of 558Lμm and 558Lμm3 were labeled, using the lactoperoxidase-catalysed iodination method. After labelling, the cells were lysed with digitonin and the affinity-purified proteins analysed on a reducing acrylamide gel. No labelled molecules are found in the lysate of the sIgM-negative 558Lμm line (Figure 3, lane 6), confirming the immunofluorescence analysis. In contrast, μm and λ_1 chains are easily detected in the lysate of 558Lμm3 (Figure 3, lane 7). The B34 protein is also visible, but is only very faintly labelled.

To test for N-linked glycosylation of B34, the metabolically labelled NP-binding molecules of 558Lμm and 558Lμm3 were digested with glycopeptide-N-glycosidase (PNGase F) and analysed on a reducing acrylamide gel (Figure 4). PNGase F is an enzyme that removes even very complex sugar trees. As expected, the mol. wt of the μm chains but not of the λ_1 chains was reduced after PNGase F treatment (Figure 4, lanes 2 and 4). In the enzyme-treated 558Lμm3 sample, the mol. wt of B34 was reduced to 26 kd. Thus, B34 is a glycoprotein.

The B34 protein forms a disulphide-linked homodimer

[^{35}S]methionine labelled NP-binding molecules of the digitonin lysates of 558Lμm and 558Lμm3 were analysed by two-dimensional SDS–PAGE. Proteins were first size-separated under non-reducing conditions (1st dimension), reduced and subsequently electrophoresed in the second dimension. In such a gel, monomeric proteins are found on a diagonal, whereas subunits of disulphide-bound proteins appear below the diagonal. In the control line 558Lμm several proteins are lying below the diagonal (Figure 5A). These are the μm and λ_1 chain, deriving either from HL or H_2L_2 complexes. These protein spots are also visible in the 558Lμm3 line (Figure 5B). However, in 558Lμm3 one additional protein of 34 kd is found. The correlation in size implies that this protein is the previously described B34. Its position on the gel suggests that B34 forms a homodimer of 68 kd.

Presence of a pre-B and B cell-specific transcript in the sIgM-expressing plasmacytoma line 558Lμm3

In the accompanying paper a gene (*mb-1*) is characterized, which is specifically expressed in pre-B and B lymphocytes, but not in plasma cells. The protein encoded by this gene seems to be a membrane-spanning glycoprotein. Judged from the deduced amino acid sequence the mol. wt of the protein is ~22 kd in the non-glycosylated form. We have analysed

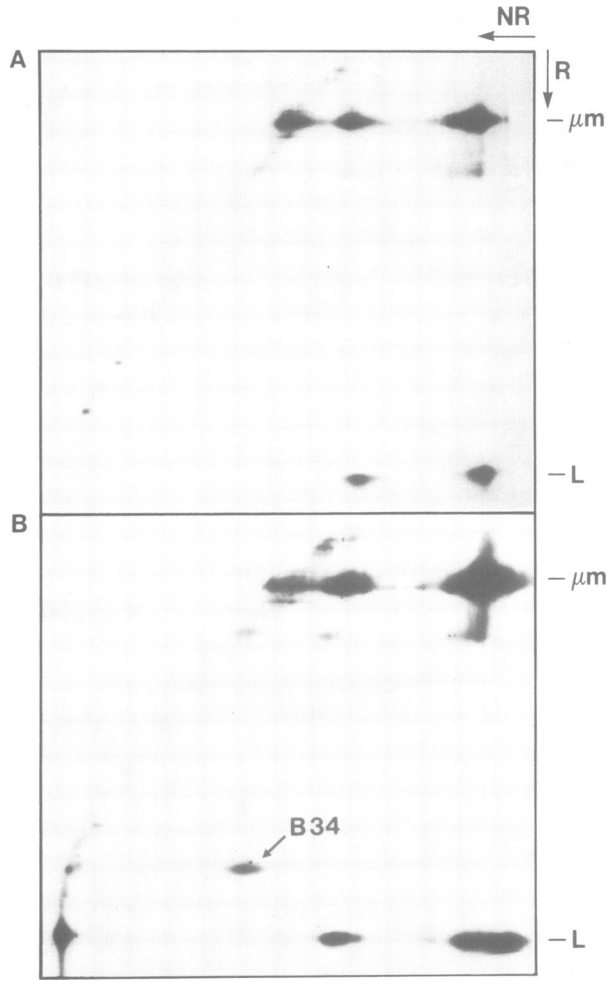

Fig. 5. Two-dimensional SDS–PAGE analysis of NB-binding proteins of the cell lines 558Lμm (**A**) and 558Lμm3 (**B**). NP-binding proteins of [^{35}S]methionine metabolically labelled cells were size-separated in the first dimension on an 8% acrylamide gel in their unreduced state, and subsequently run in the second dimension on a 10% acrylamide gel under reducing conditions. For arrows see text.

the lines 558Lμm, 558Lμm3 and the Abelson pre-B cell line 300-19μmλ36/8 for the presence of *mb-1* transcripts in a Northern blot (Figure 6). In accordance with the accompanying paper, a 1-kb *mb-1*-specific mRNA is produced in the pre-B cell line (Figure 6A, lane 3). No transcripts are found in the 558Lμm plasmacytoma (Figure 6A, lane 1). The 558Lμm3 line, however, produces *mb-1* transcripts, which thus seem to be correlated with sIgM expression (Figure 6A, lane 2).

Discussion

In plasmacytoma cells transfected with μm and L chain vectors, membrane-bound IgM molecules are assembled intracellularly but not transported to the cell surface (Sitia *et al.*, 1987; Hombach *et al.*, 1988). Transfection of the same vectors into pre-B and B cells readily resulted in sIgM expression. We have isolated a sIgM-positive variant (558Lμm3) from the sIgM-negative plasmacytoma line (558Lμm) and analysed both lines for the presence of IgM-associated proteins. A 34-kd protein (B34) was found in association with IgM in 558Lμm3 as well as in the transfected sIgM-expressing Abelson line 300-19μmλ36/8.

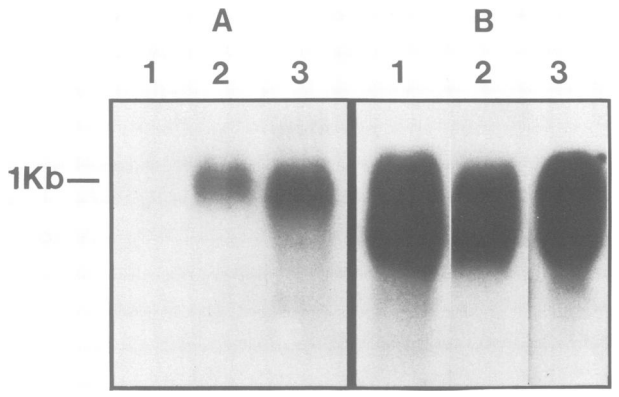

Fig. 6. Northern blot analysis for the presence of *mb-1* transcripts. Poly(A)$^+$ mRNA was purified from the cell lines 558Lμm (**lanes 1 A/B**), 558Lμm3 (**lanes 2 A/B**) and 300-19μmλ36/8 (**lanes 3 A/B**) and size-separated on a 1% agarose gel. In (**A**) the RNA was hybridized with a ^{32}P-labelled *mb-1* probe, in (**B**) the RNA is hybridized with a β_2-microglobulin probe as a control.

However, B34 was not found to be associated with secreted IgM. An association with IgD was also not detected in the B1-8.δ1 line. B34 is a glycoprotein which forms a disulphide-linked homodimer of 68 kd. The B34 protein is associated non-covalently with membrane-bound IgM and its association can only be shown using the detergent digitonin for protein extraction.

Previous papers have shown some evidence for IgM-associated proteins (Sidman *et al.*, 1980; Rosenspire and Choi, 1982; Haustein and von der Ahe, 1986). However, the different purification protocols used do not allow an easy comparison of the proteins identified (specifically in none of these experiments were mild detergents and affinity columns with subsequent hapten elution used). Furthermore, these experiments did not address the correlation of the appearance of those molecules with sIgM expression.

In the sIgM-expressing plasmacytoma line 558Lμm3 transcripts of a gene (*mb-1*) are found, which is specifically expressed at the pre-B and B cell stage, but not at the plasma cell stage (Sakaguchi *et al.*, 1988). Indeed, in the sIgM-negative plasmacytoma line 558Lμm, no *mb-1* transcripts are present. These data suggest that the product of the *mb-1* gene is involved in IgM cell surface expression. The product of the *mb-1* gene seems to be a B cell-specific membrane protein. Whether B34 is the gene product of *mb-1* remains to be established.

Our experiments do not exclude that B34 is an IgM-binding molecule (Fc receptor), which reacts with IgM after cell lysis. However, such an Fc receptor-type molecule would be expected to bind to intracellular IgM after lysis of 558Lμm as well as of 558Lμm3 cells. Because B34 could only be isolated from 558Lμm3 we regard the possibility of B34 associating with IgM after cell lysis as unlikely. For the same reason it seems also unlikely that B34 is a breakdown product of the μm chain.

What is the function of B34? B34 may be either a transport molecule which guides membrane-bound IgM through the Golgi stack to the cell surface and/or it may be a constitutive component of an IgM–receptor complex. The finding that B34 could be labelled on the cell surface, although only faintly, favours the latter possibility.

The antigen receptors in B and T cells show striking

similarities. The polypeptide chains from which they are assembled are members of the Ig superfamily and are structured into variable and constant domains. On the surface of T cells, the α/β heterodimer of the TCR is found in association with other transmembrane proteins (γ, δ, ϵ, ζ, P21), the so-called T3 complex (Clevers *et al.*, 1988). Activation of B and T cells upon binding of their receptors by multivalent antigens or MHC bound antigens, respectively, triggers cellular responses, which are initially similar and involve inositoltriphosphate degradation and Ca^{2+} mobilization (Cambier and Ransom, 1987; Weiss *et al.*, 1986). Although the molecular events during signal transduction have not yet been worked out in detail, the T3 complex is thought to take part in signal transduction in the case of the T cells.

The similarity of the antigen-induced signal in T and B cells suggests that the antigen receptors on both cells may have similar structural properties. An analogue for the T3 complex ('B3 complex'), however, has not previously been found on B cells. In particular, the virtual absence of a cytoplasmic tail in the μm chain calls for additional components of the IgM receptor, i.e. μm-associated membrane proteins. It was noticed that the μm transmembrane sequence is highly conserved between different species and Ig classes (Early *et al.*, 1980; Rabbitts *et al.*, 1981; Bernstein *et al.*, 1984). From these data an interaction of the IgM transmembrane part with other transmembrane molecules has been posulated (Tyler *et al.*, 1982; Yamawaki-Kataoka *et al.*, 1982). B34 and B29 may be such interactive transmembrane molecules and may be involved in signal transduction, in analogy to the components of the T3 complex. Indeed, B34 shows striking similarities to the proteins of the T3 complex. Both types of molecules are glycoproteins which can be co-purified with the antigen receptor chains only from digitonin but not from NP-40 cell lysates (Oettgen *et al.*, 1986). As is the case for the B cells suggests that the antigen receptors on both cells molecules has been postulated (Tyler *et al.*, 1982; Yamawaki-Kataoka *et al.*, 1982). B34 and others, e.g. the 41 kd protein in Figure 3, may be such interactive transmembrane molecules and may be involved in signal transduction, in analogy to the components of the T3 complex. Indeed, B34 shows striking similarities to the proteins of the T3 complex. Both types of molecules are glycoproteins which can be co-purified with the antigen receptor chains only from digitonin but not from NP-40 cell lysates (Oettgen *et al.*, 1986).

B34 is already expressed in pre-B cells, i.e. at a developmental stage where the cells produce μ chains but not yet L chains. The early expression of B34 may relate to the finding that the μm chain is playing a regulatory role in pre-B cells. Expression of μm chains in pre-B cells stops V_H to DJ_H rearrangements and activates $\varkappa$ L chain assembly (Reth *et al.*, 1987). V gene rearrangements in these cells may be controlled by a transmembrane signal, and B34 in association with the μm chain may be participating in this signalling. B34 may not be expressed at the plasma cell stage. Plasma cells are effector cells, secreting large amounts of antibodies during an immune response. Due to the alternative usage of two potential polyadenylation sites, virtually no membrane-bound antigen receptors are produced in such cells (Alt *et al.*, 1980; Early *et al.*, 1980). Down-regulation of B34 expression may represent an additional mechanism to block membrane expression of antigen receptors and thus antigenic triggering at the plasma cells stage.

In contrast to T cells, mature B cells carry two classes of antigen receptors on their surface, namely IgM and IgD. The biological difference between these two receptors is poorly understood (Blattner and Tucker, 1984). Although we could not isolate B34 from the B1-8.δ1 line, our data do not exclude the possibility of an association of B34 with surface IgD. High amounts of intracellular secretory IgD molecules would make it difficult to detect such an association. However, differences in the requirements of cell surface expression of IgM and IgD were indicated by an experiment where the μm expression vector was transfected into the B1-8.δ1 line. Such transfectants produce IgM and IgD molecules, but only IgD is displayed on the cell surface (J. Hombach, unpublished data). Whether this effect is due to the lack of B34 expression in these cells remains to be investigated.

Materials and methods

Cell lines and cell culture

The cell lines 558Lμm and 558Lμs are derived from the λ_1 chain-producing plasmacytoma line 558L (Oi *et al.*, 1983), transfected with the vector pSVμm or pSVμs, respectively. These vectors express either the membrane-bound or the secreted μ chain (Reth *et al.*, 1987). The Abelson pre-B cell line 300-19μmλ36/8 is transfected with the vector pSVμm and the λ_1 L chain-encoding vector pIXE (Picard and Schaffner, 1983; Reth *et al.*, 1987). In association with $V\lambda_1$, the vector-encoded V_H gene forms the NP-binding domain of the antibody B1-8 (Reth *et al.*, 1978). The hybridoma line B1-8.δ1 is a switch variant of the line B1-8μ (Neuberger and Rajewsky, 1981). Abelson and hybridoma cells were grown in RPMI culture medium supplemented with 10% FCS, 2 mM L-glutamine, 2×10^{-5} M β-mercaptoethanol, 50 U/ml penicillin and 50 mg/ml streptomycin. 558L plasmacytoma cells were cultured in Dulbecco's modified Eagle's medium (DMEM), supplemented as above. Transfection of cells was done by electroporation, as described previously (Potter *et al.*, 1984). All transfectants were grown in selective medium, containing 1–2 μg/ml mycophenolic acid.

FACS analysis and cell sorting

For FACS analysis, 10^6 cells were stained with biotinylated goat anti-mouse antibodies (Southern Biotechnology Associates, Birmingham, AL) and home-made fluoresceinated anti-idiotypic antibody Ac146 (Reth *et al.*, 1979). Bound biotinylated antibodies were detected by streptavidin–phycoerythrin (Becton Dickinson, Mountain View, CA). Cells were analysed on a FACS 440/electric desk in the presence of 1 μg/ml propidium iodide to exclude dead cells. 10 000 cells were analysed per diagram. Frequency determination of sIgM-positive cells was controlled by counting such cells within defined numbers of stained cells on cytocentrifuge smears.

For sorting sIgM-positive cells from the 558Lμm transfectants, such cells were stained with fluoresceinated goat anti-mouse IgM antiserum, and sorted on a modified FACS I (Weichel *et al.*, 1985). After the first sort, the cells were cultured for 2 weeks. After the second sort, some of the deflected cells were directly plated in 96-well plates together with macrophage feeder cells. From 1000 cells in two plates, 15 and 18 colonies were obtained. After testing for sIgM expression, one clone was selected for further studies (558Lμm3).

Biosynthetic labelling and affinity purification

For biosynthetic labelling, 10^7 cells were washed in phosphate-buffered saline (PBS) and resuspended in DMEM culture medium, containing 10% dialysed FCS, a 1/10 reduced methionine concentration and 250 μCi [^{35}S]methionine (1000 Ci/mM, Amersham, Braunschweig, FRG). Cells were incubated for 10–12 h, washed in PBS and lysed for 30 min on ice, either in 0.5 ml NP-40 lysis buffer [0.5% NP-40; 50 mM Tris, pH 7.5; 1 mM EDTA; 1 mM phenylmethylsulfonyl fluoride (PMSF)] or digitonin lysis buffer [1% digitonin (Aldrich), 10 mM triethanolamine; 150 mM NaCl; 1 mM EDTA; 1 mM PMSF; adjusted to pH 7.5 (Oettgen *et al.*, 1986)]. The lysates were centrifuged for 15 min at 10 000 *g* and the supernatants incubated with 20 μl packed NNP-cap-coupled Sepharose (kindly provided by S.Brands). After 1–3 h incubation at 4°C, the Sepharose was transferred into a column and washed 4 times in PBS/0.5% NP-40 or PBS/1% digitonin,

depending on the lysis buffer. The antibodies were eluted with ~100 μl of 10^{-3} M NNP-cap in lysis buffer. Aliquots were boiled in sample buffer and analysed by SDS–PAGE.

Iodinations and enzymatic treatments

3×10^7 cells were surface labelled with 1 mCi ^{125}I (Amersham Buchler, Braunschweig, FRG), using the lactoperoxidase-catalysed reaction according to Haustein (1975). After washing, cells were solubilized in 750 μl of a 1% digitonin extraction buffer and receptor molecules purified as described above.

For glycopeptide-N-glycosidase (PNGase F) digestion the eluted proteins were precipitated according to Wessel and Flügge (1984) and subsequently incubated for 14 h in 25 μl of the following buffer: 150 mM sodium phosphate, pH 7.7; 25 mM EDTA; 0.1% SDS; 1% β-mercaptoethanol; 0.2% NP-40 and 1 unit PNGase F, purchased from Boehringer Mannheim, FRG (Plummer *et al.*, 1984).

Two-dimensional SDS–PAGE

Proteins eluted from the column were boiled in non-reducing protein sample buffer and loaded onto 8% tube gels. Rainbow mol. wt markers were added (Amersham). After electrophoresis, the gels were pressed out of the tubes and reduced in 5% β-mercaptoethanol; 0.1% SDS; 100 mM Tris, pH 6.8 and bromophenol blue for 20 min. Subsequently the tube gels were placed on top of 10% acrylamide slab gels, fixed with agarose, and electrophoresed in the second dimension.

RNA analysis

Poly(A)$^+$ RNA was purified by oligo-deoxythymidylic acid–cellulose chromatography. 3 μg of RNA were incubated for 15 min at 55°C in 50% formamide, 2.2 M formaldehyde, 0.02 M morpholinopropansulfonic acid pH 7.0, 5 mM sodium acetate, 0.5 mM EDTA and electrophoresed through 1% formaldehyde agarose gels, transferred to Nytran (Schleicher and Schüll, FRG) and hybridized with a ^{32}P-labelled probe. The probe was a 600-bp *Kpn*I/*Eco*RI fragment from the W35-1 *mb-1* clone.

Acknowledgements

We thank Dr M.Cramer for performing the iodinations, him and Dr W.Weichel for discussion, Dr I.Haas for critically reading the manuscript, Drs F.Melchers and N.Sakaguchi for providing the *mb-1* probe and P.Butkeraitis for expert technical help. This work was supported by the Bundesministerium für Forschung und Technologie (Genzentrum Köln) and the Deutsche Forschungsgemeinschaft through SFB74. L.L. was supported by an Alexander von Humboldt fellowship.

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Received on July 6, 1988