Molecular components of the B cell antigen receptor complex of class IgD differ partly from those of IgM

Jürgen Wienands, Joachim Hombach¹,
Andreas Radbruch¹, Christa Riesterer and
Michael Reth

Max-Planck-Institut für Immunbiologie, Stübeweg 51, D-7800 Freiburg
and ¹Institut für Genetik, Universität zu Köln, Weyertal 121, D-5000
Köln 41, FRG

Communicated by K. Rajewsky

Two classes of immunoglobulin, IgM and IgD, are present as antigen receptors on the surface of mature B lymphocytes. We show here that IgD molecules are non-covalently associated in the B cell membrane with a heterodimer consisting of two proteins of 35 kd (IgD- α) and 39 kd (IgD- β), respectively. The two novel proteins are not found in the IgD-expressing myeloma J558L δ m, which fails to bring IgD antigen receptor onto the cell surface. In a surface IgD positive variant line of this myeloma, however, membrane-bound IgD molecules are associated with the heterodimer, suggesting that the formation of an antigen receptor complex is required for surface IgD expression. We further demonstrate that the IgD-associated heterodimer differs partly from that of the IgM antigen receptor and that its binding to the heavy chain only requires the presence of the last constant domain and the transmembrane part of the δ m chain. Key words: antigen receptor/B lymphocytes/heterodimer/immunoglobulins/myeloma

Introduction

Depending on their developmental stage, B cells express different classes of antigen receptors (Vitetta *et al.*, 1980). Immature B cells carry only the IgM antigen receptor (Kearney *et al.*, 1977). Cross-linking of this receptor seems to inhibit the growth of the early B cells, a response which may play a role in the establishment of B cell tolerance (Pike *et al.*, 1982; Nossal *et al.*, 1983). Mature resting B cells co-express IgM and IgD antigen receptors, with identical antigen binding sites (Goding and Layton, 1976; Stern and McConnell, 1976; Vitetta and Uhr, 1976; Yuan and Vitetta, 1978). A similar signal is generated upon cross-linking of either IgM or IgD antigen receptor and results in B cell activation (Cooper *et al.*, 1980; Monroe and Cambier, 1983a,b). After class switching, B cells are generated which express either IgG, IgA or IgE antigen receptors (Coffmann and Cohn, 1977). The reason why mature B cells carry two classes of antigen receptor, IgM and IgD, is poorly understood, and a detailed structural and functional analysis of these receptors may be helpful to elucidate whether they have different biological roles.

We have previously shown that IgM molecules are specifically associated in the B cell membrane with the 34 kd glycoprotein B34 (Hombach *et al.*, 1988b) which we now

call IgM- α (see Discussion). IgM- α seems to be the product of the B-cell specific gene *mb-1* (Sakaguchi *et al.*, 1988), encoding a transmembrane protein with sequence homology to CD3 proteins of the T cell receptor (Reth, 1989). The *mb-1* gene and the IgM- α protein are not expressed in a μ m transfectant of the J558L myeloma line (J558L μ m), which fails to bring membrane-bound IgM molecules onto the cell surface. In J558L μ m3, a surface IgM (sIgM) positive myeloma variant of J558L μ m, however, both IgM- α and *mb-1* are expressed (Hombach *et al.*, 1988b). Furthermore, after transfection of the J558L μ m myeloma with a vector encoding *mb-1*, the obtained transfectants express IgM- α and bring the IgM antigen receptor onto the cell surface (Hombach *et al.*, 1990). A biochemical analysis of purified IgM antigen receptor revealed that IgM- α forms a covalently linked heterodimer together with a 39 kd protein called IgD- β which has not been detected in the original analysis (Hombach *et al.*, 1988b). These data suggested to us that the B cell antigen receptor of class IgM comes on the cell surface only as a complex, including the IgM molecule and the IgM- α /IgD- β heterodimer. We show here that the IgD molecules are also associated in the B cell membrane with a heterodimer, which partly differs from that of the IgM antigen receptor. The finding that IgD and IgM antigen receptors have different associated proteins may bear upon their different biological role during B cell development.

Results

Lack of sIgD expression in δ m-transfected myeloma lines

To analyze the requirements for sIgD expression we constructed the two delta vectors pSV δ s and pSV δ m (Figure 1E). Both vectors carry the *gpt* gene of the pSV2-*gpt* vector and the mouse C δ gene (Lui *et al.*, 1980) which was placed 3' of the B1-8 V_H gene. The two vectors differ only in their 3' part: pSV δ s carries the exon specific for the secreted form of IgD whereas pSV δ m carries the exons specific for the membrane-bound form of IgD. These vectors were introduced either into the λ 1-producing myeloma line J558L or into the pSV λ 1-neo transfected B-lymphoma line K46 λ . From each experiment one transfectant producing high amounts of delta chains (Figure 2) was selected for further analysis.

The two pSV δ s transfectants, J558L δ s and K46 λ δ s, produced an NP (4-hydroxy-3-nitro-phenylacetyl) binding IgD antibody which was secreted (Figure 2, lanes 3 and 6), but not expressed, on the cell surface (Figure 1A and C). As expected no IgD molecules were secreted from the two pSV δ m transfectants, J558L δ m and K46 λ δ m, which produced similar amounts of δ m heavy chain (Figure 2, lanes 2 and 5). Although both δ m transfectants assembled membrane-bound IgD molecules intracellularly (see Figure 7), an IgD antigen receptor is brought onto the cell

Fig. 1. Lack of sIgD expression in the δ m transfected myeloma cells J558 L δ m. The δ transfectants J558L δ s (A), J558L δ m (B), K46 λ δ s (C) and K46 λ δ m (D) were stained with fluoresceinated goat anti-mouse IgD antiserum (Biogenzia Lemanja, Bochum, FRG) and analyzed for surface expression by using a FACScan (Becton, Dickinson, Mountain View, CA). Ten thousand cells were analyzed per histogram. (E) shows the vectors used in different transfection experiments: VH = variable gene of the anti-NP and antibody B1-8; E = heavy chain enhancer; H = hinge region; δ 1, δ 3 and C1, C2 are constant exons of the δ and μ chain respectively. Restriction sites: B = *Bam*HI; Pv = *Pvu*I; R = *Eco*RI; S = *Sal*I; S = *Xba*I.

surface only in the B lymphoma K46 λ δ m but not in the myeloma J558L δ m (Figure 1D and B). Thus, membrane-bound IgD behaves similarly to the membrane-bound IgM which is also not expressed on the cell surface of myeloma cells (Sitia *et al.*, 1987; Hombach *et al.*, 1988a). These data suggest that in order to be expressed on the cell surface, membrane-bound IgD molecules also require the binding to associated proteins.

IgD and IgM differ in their requirements for cell surface expression

Using a fluorescent activated cell sorter (FACS) we have previously isolated from the μ m transfectant J558L μ m the sIgM⁺ variant line J558L μ m3, and demonstrated that in the variant line the IgM molecule was non-covalently associated with the 34 kD glycoprotein IgM- α (Hombach *et al.*, 1988b). In a similar protocol we stained the J558L δ m transfectant (Figure 3A) with fluoresceinated anti- δ antibodies and sorted sIgD⁺ cells. Indeed, after two cycles of sorting, a sIgD⁺ population of J558L δ m was obtained, and a subclone J558L δ m2.6 was isolated whose cells stably expressed IgD molecules on their surface (Figure 3B).

Fig. 2. Analysis of δ chain expression in δ transfectants of the K46 λ B lymphoma and the J558L myeloma. Lysates (Lys.) and supernatants (Sup.) from 10^7 cells of each transfectant (lane 2, 3, 5 and 6) were tested for cytoplasmic and secreted δ heavy chain in a protein dot assay using radioactively labeled anti- δ antibodies. As negative control the recipient cell lines K46 λ (lane 1) and J558L (lane 4) were tested in parallel.

Fig. 3. Isolation of the sIgD positive variant J558L δ m2.6 by cell sorting. The δ m transfectant J558L δ m (A) and the variant J558L δ m2.6 (B) were analyzed for sIgD by double fluorescence analysis with biotinylated goat anti-mouse λ 1 antiserum/streptavidin-phycoerythrin (PE) (ordinate) and fluoresceinated goat anti-mouse IgD antiserum (abscissa). The cell populations are shown as 5% probability contour plots on logarithmic scales. The J558L δ m2.6 variant was obtained after two cycles of sorting of sIgD⁺ cells among the J558L δ m transfectants.

The J558L δ m2.6 variant allows us to ask whether IgM and IgD require the same or different associated proteins for cell surface expression. For this purpose we transfected the pSV μ m vector into the sIgD⁺ variant J558L δ m2.6. The resulting double transfectants J558L δ m2.6/ μ m were tested for sIgM and sIgD expression. Although complete IgM molecules were assembled intracellularly (data not shown) they were not brought onto the surface of the J558L δ m2.6/ μ m cells (Figure 4C). Identical results were obtained with four independent J558L δ m2.6/ μ m transfectants. The sIgD expression in the J558L δ m2.6/ μ m transfectants remained the same as in the parental line

Fig. 4. The sIgD positive variant J558L δ m2.6 does not express IgM on the cell surface. The sIgD positive variant J558L δ m2.6 (A and B) and its transfectant J558L δ m2.6/ μ m (C and D) and J558L δ m2.6/ μ m/ μ 1,2 δ 3m (E and F) were stained with either fluoresceinated goat anti-mouse IgM antiserum (A, C and E) or with fluoresceinated IgD antiserum (B, D and F). Ten thousand cells were analyzed per histogram.

J558L δ m2.6 (Figure 4B and D). The fact that the J558L δ m2.6 variant brings IgD but not IgM molecules to its cell surface thus demonstrates that the two classes of antigen receptor have different molecular requirements for surface expression.

To elucidate what part of the IgD molecule is responsible for δ -specific surface expression we constructed the chimeric μ - δ vector pSV μ 1,2 δ 3m (Figure 1E). This vector is a derivative of pSV δ m, in which the C δ 1 and the hinge region (H) exons were replaced by the first two constant exons (C μ 1 and C μ 2) of the μ chain. After transfection of the pSV μ 1,2 δ 3m vector into the J558L δ m2.6 variant, the obtained transfectant J558L δ m2.6/ μ 1,2 δ 3m expressed a chimeric μ - δ molecule (see below) which was detected on the cell surface with anti- μ antibodies (Figure 4E). This result demonstrated that the presence of the last constant domain (C δ 3) and the transmembrane part (δ m) of the δ chain is sufficient for δ -specific surface expression. A control experiment showed that when J558L cells were transfected with pSV μ 1,2 δ 3m the chimeric μ - δ molecules were not placed on the cell surface (data not shown).

The *mb-1* gene is not expressed in the sIgD⁺ myeloma variant

RNA of the different J558L transfectants were tested for expression of the *mb-1* gene which presumably encodes the IgM associated protein IgM- α . The *mb-1* gene (Sakaguchi *et al.*, 1988) is expressed in the sIgM⁺ variant J558L μ m3, as well as in an *mb-1* transfectant of J558L μ m (Figure 5A, lanes 2 and 4), but not in the myeloma J558L nor in

Fig. 5. *mb-1* transcripts are not found in the sIgD positive variant J558L δ m2.6. Total RNA from the indicated cell lines was fractionated on a 1% agarose formaldehyde gel, transferred to nylon filters and hybridized to a ³²P-labeled cDNA probe of *mb-1* (A) or of the ribosomal gene S12 (B).

the sIgD⁺ variant J558L δ m2.6 (Figure 5A, lanes 1 and 3). Each lane contained roughly the same amount of RNA (Figure 5B), as tested by stripping and rehybridizing the Northern blot with a probe for the ribosomal gene S12 (Ayane *et al.*, 1989). The absence of *mb-1* expression in the J558L δ m2.6 variant demonstrates that the IgD molecule does not require IgM- α for its surface expression and explains why IgM molecules were not transported to the cell surface in the J558L δ m2.6 variant.

Associated proteins of the IgD antigen receptor

In the different δ m transfectants of J558L and K46 λ the expressed δ chains, together with the λ 1 light chain, formed NP binding antigen receptor molecules which could be efficiently purified by affinity chromatography. To search for IgD-associated proteins we purified antigen receptor molecules from a digitonin lysate of biosynthetically labeled K46 λ δ m and K46 λ μ 1,2 δ 3m cells and analyzed them by two dimensional SDS-PAGE (Figure 6). In such an analysis, monomeric proteins are found on the gel diagonal, whereas subunits of disulfide-linked proteins appear below the diagonal. The purified receptors of the K46 λ δ m transfectant contained several disulfide-linked proteins (Figure 6A). In addition to the δ m chain which, together with the λ 1 chain, forms H₂L₂ and HL complexes, two novel proteins of 35 (IgD- α) and 39 kd (IgD- β) were visible. These two proteins seem to form a heterodimer of ~72 kd. The two heterodimeric proteins were also found together with the chimeric μ - δ chain when the receptor of biosynthetically labeled K46 λ μ 1,2 δ 3m cells were purified (Figure 6B). Different size forms of the heterodimer seem to be present in the biosynthetically labeled receptor material because the two components of the heterodimer are seen in two dimensional gels (Figure 6) as a string of protein spots trailing towards the upper left. This may indicate a heterogeneous glycosyl-

Fig. 6. Two dimensional (non-reducing versus reducing) SDS-PAGE of biosynthetically or surface labeled IgD antigen receptors. Affinity purified IgD antigen receptors of the transfectants K46λδm and K46λμ1,2δ3m were either metabolically labeled with [³⁵S]methionine/cysteine (A and B) or surface labeled with [¹²⁵I]iodine (C and D). The receptor molecules were size separated in the first dimension under non-reducing conditions (horizontal axis) and then after reduction of the gel separated under reducing conditions in the second dimension (vertical axis).

Fig. 7. Comparison of affinity purified antigen receptors of IgM and IgD classes. Indicated cell lines were either biosynthetically labeled with [³⁵S]-methionine/cysteine (lanes 1–6) or ¹²⁵I surface labeled (lanes 7 and 8). Affinity purified antigen receptor complexes were analyzed by 10% SDS-PAGE under reducing conditions. Protein bands which correspond to the components of the heterodimers are indicated by an arrow. Due to mixed molecules the endogenous α light chain is found in part of the biosynthetically labeled receptors of the K46λ transfectants. M = size marker (Amersham, Rainbow Markers).

ation of these proteins in the different cellular compartments, or some other kind of protein modification.

To test whether the heterodimer was expressed on the cell surface we labeled the surface of K46λδm and K46λμ1,2δ3m cells with iodine and analyzed their antigen receptors in the same way as described above. Indeed both proteins of the heterodimer were labeled by this method, although Ig-β was more strongly labeled than IgD-α (Figure 6C and D). These

results indicate that the heterodimer is expressed on the outer B cell membrane. Note that the surface labeled proteins of the heterodimers did not show as much heterogeneity as the biosynthetically labeled ones (Figure 6, compare A and C).

We next compared associated proteins of IgD and IgM antigen receptors with each other. Different μm and δm transfectants of J558L or K46λ were biosynthetically labeled and their purified receptor analyzed in parallel by

SDS-PAGE (Figure 7). The 34 kD IgM- α protein, which we have analyzed previously, was purified only with the IgM but not with the IgD receptor, which instead contained the 35 kD IgD- α protein (Figure 7, cf. lanes 1 and 2, 5 and 6). In comparison with IgM- α , the IgD- α protein is not always easy to identify on a one dimensional SDS gel, because it is sometimes only weakly labeled and because it consists of several bands of 35–36 kD, presumably due to heterogeneous glycosylation (see Figure 6A and B). The same is true for the Ig- β protein which also appears as several bands of 38–40 kD in this gel analysis.

The identification of these bands becomes easier, however, when comparing the different δ m transfectants. IgD- α and Ig- β were present in purified receptors of the sIgD positive cells K46 λ δ m and J558L δ m2.6 (Figure 7, lanes 2, 4 and 5), but were not co-purified with the IgD molecule from J558L δ m myeloma cells which fail to bring IgD to the cell surface (Figure 7, lane 3). The two proteins of the heterodimer were also not co-purified with the secreted form of IgD from the K46 λ δ s transfectant (data not shown). Together these data suggest that the heterodimer of IgD- α and Ig- β is specifically associated with the membrane-bound form of IgD in cells expressing sIgD.

Protein bands of an identical size (39–40 kD) are present in purified IgM and IgD antigen receptors of biosynthetically labeled J558L and K46 λ cells (Figure 7, lanes 5 and 6). This is seen even more clearly in a comparison of surface labeled IgM and IgD antigen receptors of the different K46 λ transfectants. Beside the Ig heavy and light chains, both receptors contained a dominantly labeled protein of the size of Ig- β (Figure 7, lanes 7 and 8). The heterodimers of the IgD and IgM antigen receptors may therefore share their Ig- β component.

Discussion

We have shown here that IgD molecules are specifically associated in the B cell plasma membrane with a heterodimer consisting of two proteins of 35 (IgD- α) and 39 kD (Ig- β). These proteins co-purified with the IgD molecules from digitonin lysates of the K46 λ δ m B lymphoma and the J558L δ m2.6/ myeloma variant both of which express IgD on the cell surface. In the J558L δ m transfectant, which failed to transport IgD onto the cell surface, the two proteins of the heterodimer were not found associated with IgD. These data suggest that the heterodimer is absent in the original J558L myeloma and that its binding to the IgD molecule is required for sIgD expression. The two novel proteins may be part of a transmembrane protein complex which, together with the IgD molecule, forms the functional IgD antigen receptor. The B cell receptor of the IgD class would thus be similar to the T cell receptor which is also expressed on the cell surface only as a complex with the CD3 molecules (Samelson *et al.*, 1985; Clevers *et al.*, 1986).

We have previously analyzed the IgM antigen receptor and showed that membrane-bound IgM molecules are associated with a heterodimer consisting of a 34 and a 39 kD protein (Hombach *et al.*, 1990). IgM and IgD antigen receptors thus seem to have similar structural features. However, several lines of evidence suggest that the two receptors differ from each other in at least one of their components. The J558L δ m2.6 variant can express IgD but not IgM molecules on its cell surface; thus, all components

of the IgD but not all those of the IgM antigen receptor seem to be present in this variant line. Indeed, *mb-1* transcripts which are correlated with sIgM expression and which presumably encode the IgM- α protein are not found in J558L δ m2.6 cells.

A biochemical analysis of the IgM and IgD antigen receptors expressed in μ m or δ m transfectants of either J558L or K46 λ support the notion of their different composition. The lower component of the heterodimer of each receptor differs in size, namely 34 kD for IgM and 35 kD for IgD. This, together with the functional data (see above), indicates that the lower component is isotype specific and we therefore suggest that these proteins be named IgM- α and IgD- α . The upper component, however, seems to be of identical size and we therefore suggest that this component be named Ig- β , as long as it is not clear whether or not it is isotype specific.

If it turns out that Ig- β is indeed the same molecule in both receptors, its presence may account for the identical calcium mobilization pattern seen when either IgM or IgD are cross linked on mature B cells (Monroe and Cambier, 1983a; Cambier and Monroe, 1984). Likewise, the differences in the α component may be responsible for the different biological response of these receptors (Ales-Martinez *et al.*, 1988; Goodnow *et al.*, 1988; Tisch *et al.*, 1988). As judged from the *mb-1* sequence the protein IgM- α has a long cytoplasmic tail carrying tyrosines in a sequence motif conserved between B and T cell receptor molecules (Reth, 1989). Protein phosphorylation may thus play a role in inducing or regulating signal transduction from the receptor. This assumption is supported by data of Campbell and Cambier (1990) who found that in normal splenic B cells the two components of the heterodimer of IgM and IgD can be phosphorylated.

There are several reasons why the IgD-associated proteins have not been found in previous studies. IgD and its heterodimer are non-covalently associated and are thus co-purified only from lysates of sIgD-expressing B cells made with a mild detergent, e.g. digitonin. Furthermore, the heterodimer proteins IgD- α and Ig- β are poorly labeled biosynthetically, suggesting that they have a low turnover rate. Indeed, it was found that the IgD antigen receptor has a lower turnover rate than the IgM antigen receptor (Yuan and Tucker, 1984; Yuan, 1984). The Ig- β component in the IgM antigen receptor is also only very poorly labeled biosynthetically, explaining why we missed it in our first analysis (Hombach *et al.*, 1988b). Inside the cell, the IgD molecules are heterogeneously glycosylated (Vasilov and Ploegh, 1982) and the same seems to be true for the heterodimers, explaining why each component appears as more than one band. Upon cell surface iodination the β component of the heterodimer was efficiently labeled, but not the α component. This may indicate that either the α component is more shielded inside the receptor or that it does not contain many exposed tyrosines. For the IgM- α component the latter seems to be true.

After transfection of the K46 λ B lymphoma with either the μ m or the δ m vectors, the IgM or IgD antigen receptor is expressed immediately on the cell surface. This suggests that the K46 λ B lymphoma is simultaneously expressing both types of heterodimer, IgM- α /Ig- β and IgD- α /Ig- β , in contrast to the J558L myeloma, which does not express any. However, each heterodimer was co-purified from K46 λ cells only with its respective antigen receptor, indicating that

binding to the Ig molecules occurs in an isotype-specific manner. Experiments with the μ/δ chimeric expression vector revealed that the binding site for the δ specific heterodimer is located within the last C domain (C δ 3) and/or transmembrane part (δ m) of the IgD molecule. The strong sequence conservation (Kabat *et al.*, 1987) between the C δ 3 domains of different species (70% at the amino acid level) may indicate that this domain is involved in binding to another protein. The last C domain of the μ m chain (C μ 4) is also highly conserved (80%) but markedly differs from that of the δ m chain. The conserved C domain may be in contact with the isotype specific α components of the heterodimer. Indeed a structural analysis (M. Reth, unpublished data) of the mb-1-encoded IgM- α component suggests that it forms an Ig-like domain at the cell surface which may mediate the specific contact.

Although antigen receptors of B and T cells are becoming more similar to each other with the finding that they both have associated proteins, they also show differences. The two types of T cell receptor (α , β and γ , δ) are both associated with the same CD3 molecules (Krangel *et al.*, 1987; Marusic-Galesic *et al.*, 1988), while the heterodimer found in the B cell receptor seems to be isotype specific. The reason for this difference may lie in their different biological roles. The TCR chains function only as receptor molecules, while immunoglobulin molecules have a dual function as membrane-bound antigen receptors and soluble antibodies with isotype-specific effector functions (Winkelhake, 1978). To carry out their diverse roles in the serum, antibodies had to evolve diverse C domains, which may require a diverse set of heterodimers for their proper assembly into their respective antigen receptor. Whether Ig classes, other than IgM and IgD, are also associated with a specific heterodimer has to await a further analysis. Our preliminary study of the IgG antigen receptor suggests that this is indeed the case (Weiser and Reth, unpublished data). A structural description of the different antigen receptors may lead to a better understanding of their diverse functions in the immune system and help to explain such important phenomena as B cell tolerance, B cell activation and B cell memory.

Materials and methods

Vector construction

For the construction of the pSV δ m and pSV δ s vectors a 6 kb *Bam*HI–*Eco*RI fragment containing the C δ exons (C δ 1–hinge–C δ 3) was cloned into the eukaryotic expression vector pSV2-*gpt* (Mulligan and Berg, 1981). The plasmid pSVC δ obtained thus was linearized with *Bam*HI and used for insertion of either the δ m region (pSVC δ m) or the δ s region (pSVC δ s) (Cheng *et al.*, 1982). All gene fragments for the δ heavy chain were derived from the λ phage Charon 28-257.3 (Liu *et al.*, 1980), which contains the whole C μ and C δ gene cluster of the BALB/c allotype. Finally the precursors, pSVC δ m and pSVC δ s, were linearized with *Eco*RI and a 3.8 kb fragment containing the IgH' enhancer (Neuberger, 1983) and the productively rearranged V_H gene 186.2 of the anti-NP hybridoma B1-8 was inserted to obtain pSV δ m and pSV δ s respectively. The δ chains, expressed from the two vectors, will form together with the λ light chain a binding site for the hapten 4-hydroxy-3-nitro-phenylacetyl (NP) (Reth *et al.*, 1978).

For the construction of the μ – δ chimeric expression vector, pSV μ 1,2 δ 3m, the C μ 1 and C μ 2 exons were prepared as a 4.0 kb *Eco*RI–*Sall* fragment, while the C δ 3 exon was prepared as a 0.42 kb *Sall*–*Kpn*I fragment. Both fragments were ligated together into *Eco*RI–*Kpn*I linearized pUC18, resulting in pC μ 1,2 δ 3. From pC μ 1,2 δ 3 the region carrying the three C exons C μ 1–C μ 2–C δ 3 was isolated as a 1.7 kb *Xba*I fragment and subsequently cloned into *Xba*I linearized pSV δ m. The plasmid thus obtained (pSV μ 1,2 δ 3m-

Δ E) was linearized with *Eco*RI and a 1.6 kb fragment, containing the IgH enhancer, was inserted to obtain pSV μ 1,2 δ 3m (see Figure 1E).

The expression vector for the λ 1 light chain, pSV λ 1-neo, was constructed by cloning the 7.5 kb *Eco*RI fragment of pIXE (Picard and Schaffner, 1983) into pSV2-neo (Southern and Berg, 1982).

Culture conditions and cell lines

J558L is a heavy chain loss variant of the myeloma J558 producing an IgA, λ 1 antibody (Lundblad *et al.*, 1972; Oi *et al.*, 1983). The B lymphoma K46 line used in this experiment produces an endogenous IgG2a, κ antibody of an unknown specificity. K46 λ is a K46 derivative which was transfected with the pSV λ 1-neo vector. All cell lines were cultured in RPMI 1640 medium containing 10% fetal calf serum, 2 mM L-glutamine, 50 U/ml penicillin, 50 mg/ml streptomycin and 2×10^{-5} M β -mercaptoethanol.

Transfection experiments

All expression vectors were linearized at the *Pvu*II site (boxed in Figure 1E) and introduced into the recipient cell lines by electroporation as described previously (Potter *et al.*, 1984; Reth *et al.*, 1987). δ Transfectants of K46 λ and J558L were selected in a culture medium containing 250 μ g/ml xanthine, 15 μ g/ml hypoxanthine and 0.1–1 μ g mycophenolic acid.

The μ transfectants of the J558L δ m2.6 variant were obtained by cotransfection (Perucho *et al.*, 1980) of either pSV μ m (Reth *et al.*, 1987) or pSV μ 1,2 δ 3m, together with the linearized pSV2-neo vector (Southern and Berg, 1982), into J558L δ m2.6 cells and selecting with G418 (GIBCO).

Dot blot and Northern blot analysis, staining, sorting, labeling procedures and affinity purification were carried out according to procedures already published (Reth *et al.*, 1987; Hombach *et al.*, 1988b).

Acknowledgements

We thank J. Roes and Dr P.W. Tucker for providing the λ phage 28-257.3, Drs L. Leclercq, G. Köhler and P. Nielsen for helpful discussion and critical reading of this manuscript, L. Lay and S. Kleinhans for the excellent graphic work and N. Novak for typing the manuscript. This work was supported by the Bundesministerium für Forschung und Technologie grant BCT-390-2.08.

References

- Ales-Martinez, J.E., Warner, G.L. and Scott, D.W. (1988) *Proc. Natl. Acad. Sci. USA*, **85**, 6919–6923.
- Ayane, M., Nielsen, P. and Köhler, G. (1989) *Nucleic Acids Res.*, **17**, 6722.
- Cambier, J.C. and Monroe, J.G. (1984) *J. Immunol.*, **133**, 576–581.
- Campbell, K.S. and Cambier, J.C. (1990) *EMBO J.*, **9**, 441–448.
- Cheng, H.L., Blattner, F.R., Fitzmaurice, L., Mushinski, J.F. and Tucker, P.W. (1982) *Nature*, **296**, 410–415.
- Clevers, H., Alarcon, B., Wileman, T. and Terhorst, C. (1986) *Annu. Rev. Immunol.*, **6**, 629–662.
- Coffman, R.L. and Cohn, M. (1977) *J. Immunol.*, **118**, 1806–1815.
- Cooper, M.D., Kearney, J.F., Gathings, W.E. and Lawton, A.R. (1980) *Immunol. Rev.*, **52**, 29–53.
- Goodnow, C.C., Crosbie, J., Adelstein, S., Lavoie, T.B., Smith-Gill, S.J., Brink, R.A., Pritchard-Briscoe, H., Wotherspoon, J.S., Loblay, R.H., Raphael, K., Trent, R.J. and Basten, A. (1988) *Nature*, **334**, 676–682.
- Goding, J.W. and Layton, J.E. (1976) *J. Exp. Med.*, **144**, 852–857.
- Hombach, J., Sablitzky, F., Rajewsky, K. and Reth, M. (1988a) *J. Exp. Med.*, **167**, 652–657.
- Hombach, J., Leclercq, L., Radbruch, A., Rajewsky, K. and Reth, M. (1988b) *EMBO J.*, **7**, 3451–3456.
- Hombach, J., Tsubata, T., Leclercq, L., Stappert, H. and Reth, M. (1990) *Nature*, in press.
- Kabat, E.A., Wu, T.T., Reid-Miller, M., Perry, H.M. and Gottesman, K.S. (1987) *Sequences of Proteins of Immunological Interest*. US Department of Health and Human Service.
- Kearney, J.F., Cooper, M.D., Klein, J., Abney, E.R., Parkhouse, R.M.E. and Lawton, A.R. (1977) *J. Exp. Med.*, **146**, 297–301.
- Krangel, M.S., Bierer, B.E., Delvin, P., Clabby, M., Stominger, J.L., McLean, J. and Brenner, M.B. (1987) *Proc. Natl. Acad. Sci. USA*, **84**, 3817–3821.
- Liu, C., Tucker, P.W., Mushinski, J.F. and Blattner, F.R. (1980) *Science*, **209**, 1348–1352.
- Lundblad, A., Steller, R., Kabar, E.A., Hirst, J.W., Weigert, M. and Cohn, M. (1972) *Immunochemistry*, **9**, 535–544.

- Marusic-Galesic, S., Pardoll, D.M., Saito, T., Fowlkes, B.J., Coligan, J., Germain, R.N., Schwartz, R.H. and Kruisbeek, A.M. (1988) *J. Immunol.*, **140**, 411–418.
- Monroe, J.G. and Cambier, J.C. (1983a) *J. Exp. Med.*, **157**, 2073–2086.
- Monroe, J.G. and Cambier, J.C. (1983b) *J. Immunol.*, **131**, 2641–2644.
- Mulligan, R.C. and Berg, P. (1981) *Proc. Natl. Acad. Sci. USA*, **78**, 2072–2076.
- Neuberger, M.S. (1983) *EMBO J.*, **2**, 1373–1378.
- Nossal, G.J.V. (1983) *Annu. Rev. Immunol.*, **1**, 33–62.
- Oi, V.T., Morrison, S., Herzenberg, L.A. and Berg, P. (1983) *Proc. Natl. Acad. Sci. USA*, **80**, 825–834.
- Perucho, M., Hanahan, D. and Wigler, M. (1980) *Cell*, **22**, 309–317.
- Picard, D. and Schaffner, W. (1983) *Proc. Natl. Acad. Sci. USA*, **80**, 417–421.
- Pike, B.L., Boyd, A.W. and Nossal, G.J.V. (1982) *Proc. Natl. Acad. Sci. USA*, **79**, 2013–2017.
- Potter, H., Weir, L. and Leder, P. (1984) *Proc. Natl. Acad. Sci. USA*, **81**, 7161–7165.
- Reth, M. (1989) *Nature*, **338**, 383.
- Reth, M., Hammerling, G.J. and Rajewsky, K. (1978) *Eur. J. Immunol.*, **8**, 393–400.
- Reth, M., Petrac, E., Wiese, P., Lobel, L. and Alt, F.W. (1987) *EMBO J.*, **6**, 3299–3305.
- Sakaguchi, N., Kashiwamura, S., Kimoto, M., Thalmann, P. and Melchers, F. (1988) *EMBO J.*, **7**, 3457–3464.
- Samelson, L.E., Harford, J.B. and Klausner, R.D. (1985) *Cell*, **43**, 223–231.
- Sitia, R., Neuberger, M.S. and Milstein, C. (1987) *EMBO J.*, **6**, 3969–3977.
- Southern, P.J. and Berg, P. (1982) *J. Mol. Appl. Genet.*, **1**, 309.
- Stern, C. and McConnell, I. (1976) *Eur. J. Immunol.*, **6**, 225–227.
- Tisch, R., Roifman, C.F. and Hozumi, N. (1988) *Proc. Natl. Acad. Sci. USA*, **85**, 6914–6918.
- Vasilov, R.G. and Ploegh, H.L. (1982) *Eur. J. Immunol.*, **12**, 804–813.
- Vitetta, E.S. and Uhr, J.W. (1976) *Eur. J. Immunol.*, **6**, 140–143.
- Vitetta, E., Pure, E., Isakson, P., Buck, L. and Uhr, J. (1980) *Immunol. Rev.*, **52**, 211–231.
- Winkelhake, J.L. (1978) *Immunochemistry*, **15**, 659.
- Yuan, D. (1984) *J. Immunol.*, **132**, 1566–1570.
- Yuan, D. and Tucker, P.W. (1984) *J. Immunol.*, **132**, 1561–1565.
- Yuan, D. and Vitetta, E.S. (1978) *J. Immunol.*, **120**, 353–356.

Received on November 16, 1989