

The Products of Pre-B Cell-specific Genes (λ_5 and V_{preB}) and the Immunoglobulin μ Chain Form a Complex That Is Transported onto the Cell Surface

By Takeshi Tsubata and Michael Reth

*From the Max-Planck Institute for Immunobiology, D-7800 Freiburg,
Federal Republic of Germany*

Summary

We constructed expression vectors coding for the two pre-B-specific genes, V_{preB} and λ_5 , and transfected them together with a μ vector (μtm) into Ig^- myeloma cells. In a transfectant expressing all three introduced genes, the μtm chain is transported on the cell surface. A biochemical analysis demonstrated that, in these cells, the μtm chain is associated noncovalently with an 18-kD protein and covalently with a 22-kD protein, which are most likely the products of V_{preB} and λ_5 , respectively. Our results, thus, strongly suggest that the products of λ_5 and V_{preB} bind to μ chains and have the same capacity as conventional Ig L chains to allow surface expression of μ chains.

Fully assembled Ig H and L chains are transported onto the surface of B cells, while free H chains are retained in the endoplasmic reticulum (ER) in association with BiP (1). However, in pre-B lines that produce only μ H chains, small amounts of μ chains are expressed on the cell surface, suggesting that some other proteins substitute for the missing L chain in these cells. In fact, proteins of 14–22 kD have been shown to bind to the cytoplasmic and surface μ chains in murine and human pre-B lines (2–4). The λ_5 and V_{preB} genes, which are specifically expressed in pre-B cells, have strong homology to the C and the V regions of the λ L chain genes, respectively (5, 6), and thus, are likely to encode the μ -binding proteins. Antibodies specific for the human λ_5 and V_{preB} peptides react against μ -binding molecules of 22 and 18 kD, respectively, in human pre-B lines (7, 8). However, whether these proteins are expressed in association with the μ chain on the cell surface has not yet been shown.

We introduced expression vectors coding for the murine λ_5 and V_{preB} genes, together with a μ vector (pSV μtm), into Ig^- myeloma cells and we demonstrate that the products of the two genes are required and sufficient for μ expression on the cell surface. This result suggests that λ_5 and V_{preB} are essential components of sIg in pre-B cells.

Materials and Methods

The expression vectors coding for λ_5 and V_{preB1} , pEP λ_5 gpt and pEPVpBneo, are shown in Fig. 1, B and C. Genomic fragments of λ_5 and V_{preB1} genes were provided by Dr. A. Kudo, Basel Institute for Immunology, Basel, Switzerland. The BglII-PstI fragment of λ_5 was cloned into a pSV2gpt vector containing the VH promoter and the IgH enhancer. The BglII fragment of V_{preB1} gene

was similarly cloned into a pSV2neo vector. pSV μtm (9) is an expression vector for a chimeric μ chain where the membrane exon of μtm was replaced by the transmembrane and cytoplasmic part of the H-2K^k gene (Fig. 1 A). The vector for the λ_1 L chain, pSV λ_1 neo, was described previously (10). The vectors were transfected into myeloma cells by electroporation. Cell surface expression of μ chains in the transfectants was analyzed by flow cytometry as described previously (9).

For biochemical analysis, biosynthetic labeling, and surface iodination were done as described previously (9, 10). Cells were then lysed in 0.5% NP40 lysis buffer and immunoprecipitated with rabbit anti-murine μ chain antibody by using protein G-Sepharose (Pharmacia, Uppsala, Sweden). The purified Ig chains were analyzed by SDS-PAGE as described previously (9, 10).

Results

To analyze requirements for cell surface expression of μ chains, we introduced the pSV μtm vector encoding for the chimeric μtm molecule into cells of the Ig^- myeloma, X63Ag8.653. The μtm chain can be expressed on the cell surface in the absence of the IgM-associated heterodimer, IgM- α and Ig- β (9). The X63 μtm transfectants were tested for cytoplasmic μ production by a Western dot assay (data not shown), and for surface μ expression by flow cytometry. None of the cytoplasmic μtm^+ transfectants express μ chains on the surface (Fig. 2, A and B). If, however, a vector coding for the λ_1 L chain is transfected into one of the μtm transfectants, IgM molecules are found on the cell surface (Fig. 2 C). These results indicate that, as is the case for the μtm chain, binding to the L chains is necessary for surface expression of the μtm chain.

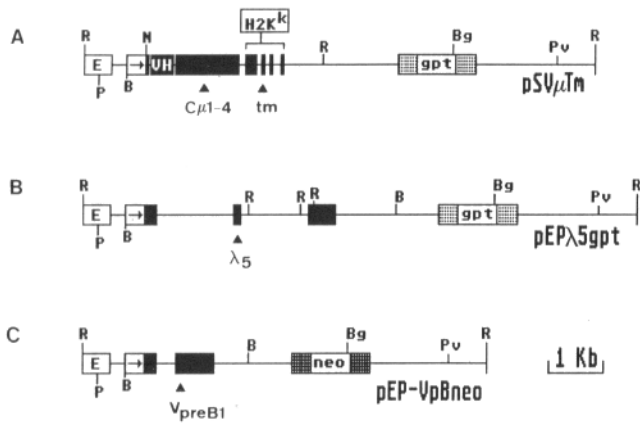


Figure 1. Structure of the (A) pSV μ tm, (B) pEP λ_5 gpt, and (C) pEPVpreBneo vectors. Solid black segments represent exons for the chimeric μ tm chain where the membrane exon of the μ chain is replaced by the transmembrane and cytoplasmic part of H-2K^k (A), λ_5 (B), and VpreB1 (C). These three vectors contain the IgH enhancer (E) and a 350-bp fragment from a VH promoter (indicated by arrows). B, BamHI; Bg, BglII; N, NcoI; P, PstI; Pv, PvuI; R, EcoRI.

To determine whether the products of the λ_5 and VpreB genes also allow the μ chain to be expressed on the cell surface, we introduced the pEPVpreBneo and pEP λ_5 gpt vectors together with the μ tm vector into X63Ag8.653 cells. The transfectants were first screened for cytoplasmic μ production. All μ tm⁺ lines were, then, tested for λ_5 and VpreB expression by Northern blotting (data not shown). In 12 μ tm⁺ lines, only one line (X63T14) expressed both the λ_5 and the VpreB genes, and the same line also expressed μ chains on the surface (Fig. 2 D). The other μ tm transfectants were sIg⁻ (data not shown). Thus, λ_5 and VpreB genes are likely to encode μ -binding proteins that are able to substi-

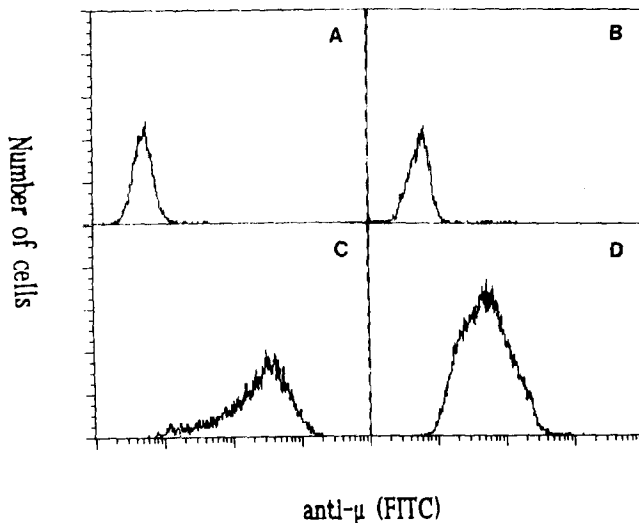


Figure 2. Surface μ expression of (A) X63Ag8.653, (B) X63 μ tm, (C) X63 μ tm λ , and (D) X63T14 cells. Cells were stained with FITC-conjugated goat anti-IgM antibodies. Cells (5,000) were analyzed on a FACScan.

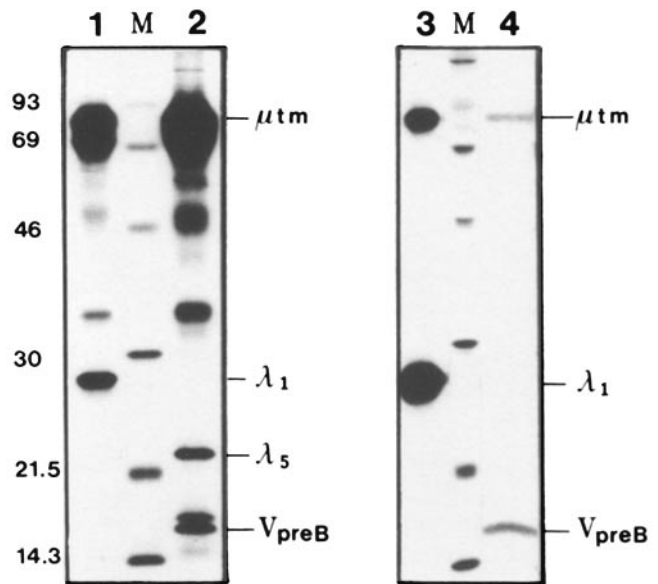


Figure 3. SDS-PAGE analysis of the μ tm-binding molecules in lysates of [³⁵S]methionine/cysteine metabolically labeled (lanes 1, 2) or surface iodinated (lanes 3, 4) cells. Cell lysates were immunoprecipitated with anti-IgM, and analyzed on a 12% acrylamide gel under reducing conditions. Lanes 1 and 3, X63 μ tm λ ; lanes 2 and 4, X63T14. M, size marker (Amersham Corp., Rainbow Markers).

tute for the L chain. This conclusion is supported by a biochemical analysis of metabolically labeled and anti- μ immunoprecipitated material from X63 μ tm λ and X63T14 cells. In X63 μ tm λ cells, the μ tm chain is bound to the 28-kD λ_1 light chain (Fig. 3, lane 1), while in X63T14 cells, the μ tm is associated with proteins of 22 and 18 kD, presumably the products of the λ_5 and VpreB genes, respectively, and with an 18.5-kD protein (Fig. 3, lane 2).

To determine how λ_5 and VpreB proteins associate with the μ tm molecule, biosynthetically labeled material from the X63 transfectants was analyzed by nonreducing/reducing two-dimensional SDS-PAGE. In such a gel, monomeric proteins are found on a diagonal, whereas subunits of disulfide-bound proteins appear below the diagonal. In the control line, X63 μ tm λ , μ tm, and λ_1 L chain molecules derived from H₂L₂ and HL complexes were observed below the diagonal (Fig. 4 A). When X63T14 cells were examined, the 22-kD protein (λ_5) was observed below the spots for the μ dimer and the μ monomer, while the 18-kD (VpreB) and 18.5-kD proteins were observed on the diagonal (Fig. 4 B), indicating that the 22-kD protein forms a disulfide-linked complex with the μ chain, while the 18.5- and 18-kD proteins bind noncovalently to this complex.

Finally, to test whether these proteins associate with the μ chain on the cell surface, X63T14 cells and X63 μ tm λ cells were labeled by surface iodination, immunoprecipitated, and examined by SDS-PAGE under reducing conditions. Surface-labeled μ tm and λ_1 L chain were precipitated from X63 μ tm λ cells (Fig. 3, lane 3). On the surface of X63T14 cells, however, only the 18-kD VpreB protein and the μ tm molecules were labeled (Fig. 3, lane 4).

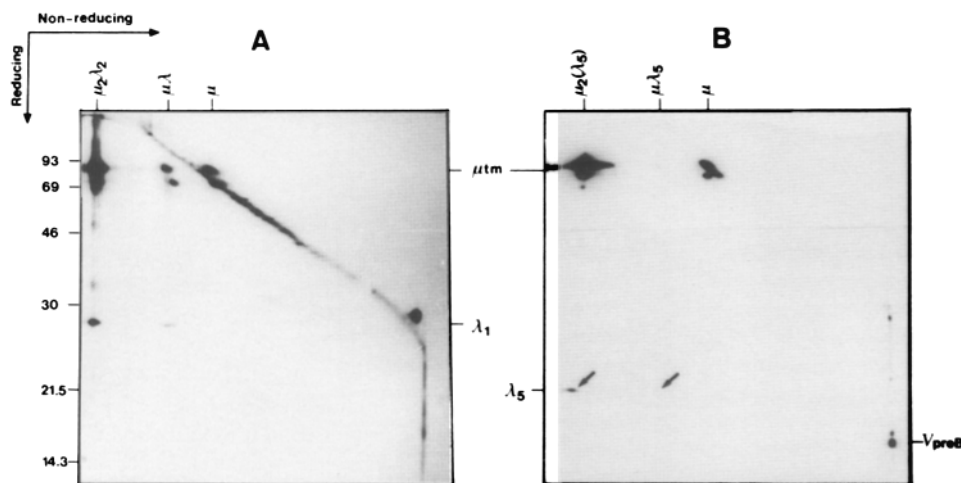


Figure 4. Two-dimensional SDS-PAGE analysis of the μ tm-binding proteins in X63 μ tm λ (A) and X63T14 cells (B). Proteins were metabolically labeled, size separated in the first dimension on an 8% acrylamide gel under nonreducing conditions, and subsequently run in the second dimension on a 12% acrylamide gel under reducing conditions.

Discussion

The transport of H chains from the ER to the cell surface is dependent on whether the H chains are assembled with L chains (1). BiP seems to be involved in this process by trapping free H chains in the ER. Assembled HL complexes no longer bind to BiP and are transported to the Golgi apparatus. In the present study, we demonstrated that a μ tm-producing myeloma transfectant expressed μ chains on the surface, provided it co-expressed λ_5 and V_{preB} . Thus, the products of λ_5 and V_{preB} seem to form a L chain-like structure that binds to μ chains, resulting in the transport of the μ chains to the cell surface.

In the X63T14 transfectants, the μ tm chain was specifically associated with proteins of 22, 18.5, and 18 kD. The 22-kD protein is likely to be the product of the λ_5 gene. It is covalently bound by the μ chain, presumably through a cysteine residue which is found at the COOH-terminal penultimate position of the λ_5 sequence (5). A cysteine residue at this position in the L chain is involved in the interchain disulfide bond. Since the 22-kD protein binds to the μ chain covalently, this protein is likely to be expressed together with the μ chain on the cell surface. The failure to label the 22-kD protein on the cell surface of X63T14 cells may indicate that it does not contain solvent-exposed tyrosines. This possibility is supported by the analysis of the λ_5 sequence. The 18-kD protein is very well labeled on the cell surface and nonco-

valently associated with the μ chain. It seems to be the product of the V_{preB} gene, whose sequence does not contain an interchain cysteine, but several exposed tyrosines. The above conclusions are supported by experiments showing that antibodies for human λ_5 and V_{preB} peptides recognize 22- and 18-kD proteins, respectively (7, 8). The 18.5-kD protein seems to be a derivative of either λ_5 or V_{preB} proteins, because it is found only in X63T14 cells. It may be a degradation product of λ_5 or a modified form of V_{preB} that is not expressed on the cell surface.

Pillai and Baltimore (2, 3) have demonstrated the existence of two μ -binding proteins, ι and ω in murine pre-B cells. The ι chain is a noncovalently binding, 14-kD molecule, and the 18kD ω molecule binds covalently. While ι is readily surface iodinated, ω is only poorly labeled. Thus, although the calculated molecular weights are slightly different, it is likely that what we see as a 22-kD molecule is the ω chain and as an 18-kD molecule the ι chain.

Our present results strongly suggest that the products of λ_5 and V_{preB} are essential components of sIg in pre-B cells. Although the function of the λ_5 - V_{preB} - μ tm complex on the pre-B cell surface is still unclear, this complex, upon cross-linking, may generate a signal that is involved in the feedback regulation of Ig gene rearrangement (discussed in reference 11).

We thank Dr. A. Kudo for providing us with plasmids, and Dr. L. Leclercq for critically reading the manuscript.

T. T. is an Alexander von Humboldt fellow.

Address correspondence to Dr. Michael Reth, MPI für Immunbiologie, Stübeweg 51, Postfach 1169, D-7800 Freiburg, FRG.

Received for publication 16 April 1990 and in revised form 29 May 1990.

Note added in proof: After submitting our manuscript, it was brought to our attention that Karasuyama et al., in a paper also published in this issue of the Journal, show that the transfection of the μ H chain gene and the genes coding for λ_5 and V_{preB} into a fibroblast line leads to the secretion of μ half-monomers associated with λ_5 and V_{preB} proteins.

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