Brief Definitive Report

THE SEQUENCE OF THE μ TRANSMEMBRANE SEGMENT DETERMINES THE TISSUE SPECIFICITY OF THE TRANSPORT OF IMMUNOGLOBULIN M TO THE CELL SURFACE

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IgM is expressed on the surface of B lymphocytes but not on the surface of IgM-secreting plasma cells. This shift from the expression of surface IgM to that of the secreted form is partly achieved by control at the level of RNA processing. However, post-translational regulation also plays a critical role. Thus, even when the membrane form of IgM is synthesized in plasmacytoma cells, it fails to reach the cell surface; rather, it is retained within the endoplasmic reticulum (1, 2). The molecular basis of this differential handling of surface IgM in B cells and plasma cells is not understood, although a B cell-specific protein that associates with membrane IgM has been proposed to play a role (3). Here, we present experiments that suggest that the proteins that potentiate the surface expression of membrane IgM in B cells recognize the COOH-terminal end of μ_m chains and work through interaction with the transmembrane segment.

Materials and Methods

All plasmids directing μ expression contain the μ transcription unit cloned between the Eco RI and Xho I sites of a pSV2gpt derivative that includes an Xho I linker in the Bam HI site. Plasmid pV_H-μ_m directs the synthesis of μ_m (but not μ_s) chains under the control of the V_H promoter/IgH enhancer, and is identical to pSV-Vμ_m (plasmid No. 463) described previously (1). Plasmid pβG-μ_m (No. 773) directs μ_m synthesis under the control of the β-globin promoter and was constructed by transferring the Bgl II fragment of No. 463 (which extends from C_μ1 through the rest of C_μ into gpt) into a Bgl II-digested pSV-pβGVμ[In1] vector (4). Plasmids Nos. 938 and 940, which direct the expression of μ_m *1 and μ_m*2 mutant H chains, were constructed by site-directed mutagenesis and are identical to pβG-μ_m, except for the changes in the μ_m transmembrane (Fig. 4). For plasmid pβG-μ/H2 (No. 860), a Pst I-Bgl I fragment that spans exons 5-8 of the mouse H-2K^b gene (5), was inserted between the Bcl I and Xho I sites of pβG-μ_m. The λ L chain transcription unit consists of an Mlu I-Bam HI fragment (gift of S. Emtage, Celltech, Slough, U.K.) in which the cytomegalovirus (HCMV) promoter and SV40 poly A site potentiate expression of a mouse λ1 cDNA. Derivatives of pV_H-μ_m, pβG-μ_m, and pβG-μ/H2, which direct expression of both H and λ chains (Nos. 784, 785, and 903, respectively), were created by inserting the λ1 transcription

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unit into the unique Eco RI site of the parental plasmid. An expression vector for human CD8 (plasmid No. 782) was constructed by linking a CD8 cDNA Eco RI fragment (6) to the HCMV promoter and cloning into pSV2neo with the vector providing the poly A site (Fig. 1). The hybrid CD8/C_μ4-C_μM gene (CD8/$\mu 4_m$; plasmid No. 808) was constructed by cutting the CD8 cDNA fragment at the Eco RV site just preceding its transmembrane region (6) and fusing to the ApaLI-XhoI C_μ4-C_μM fragment of plasmid No. 463, in which the Apa LI site had been blunted by use of T4 polymerase and dGTP + dTTP. The reading frame across the fusion junction was verified by sequencing; the gene was linked to the HCMV promoter and cloned into pSV2gpt.

Plasmacytoma J558L secretes λ1 L chains. An Ig^- derivative of mouse B cell lymphoma M12 (7) was obtained from B. Chain (University College, London). Transfection of lymphoid cell lines and COS-7 cells was carried out as previously described (4, 8). The M12 and COS-7 cells were transfected with plasmids that included both the μ and λ chain transcription units, whereas pSV2gpt derivatives containing only the μ chain unit were used for J558L. COS-7 cells were trypsinized 24 h after transfection and distributed onto microscope slides; immunofluorescence was performed 24 h later using a confocal microscope (1) on surface-stained cells or after fixation for 10 min in 4% paraformaldehyde in PBS and permeabilization with NP-40. Analysis in the FACS with FITC-conjugated anti-μ or anti-CD8 (OKT8; Ortho Diagnostic Systems Inc., Westwood, MA) reagents was performed on surface-labeled cells or on cells labeled after fixation for 5 min in 3.8% formaldehyde in PBS and permeabilization for 3 min in 0.05% NP-40 in PBS.

Results

We have previously shown that transfectants of the J558L plasmacytoma that make μ_m and λ chains retain the membrane IgM in the endoplasmic reticulum (1). However, surface IgM expression is achieved in transfectants of the B cell lymphoma M12 harboring a plasmid directing the synthesis of membrane IgM (Figs. 1 and

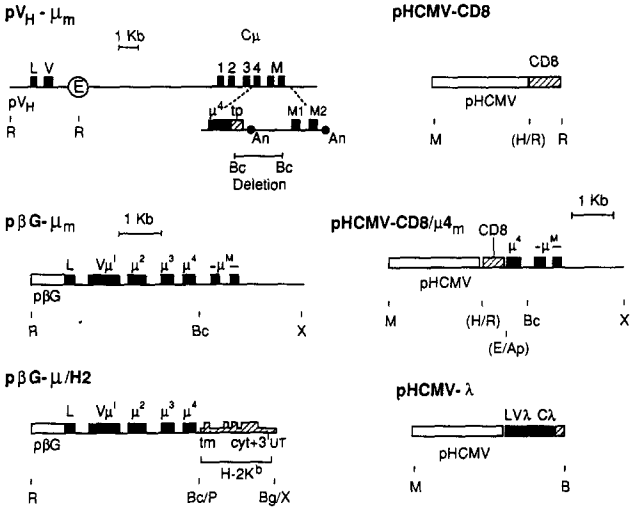

FIGURE 1. Structure of plasmids. The transcription units are shown without vector sequences. Ig sequences are in solid lines; H-2K^b, SV40, and CD8 sequences are hatched, and the human β-globin and cytomegalovirus promoters (*p*) are in open boxes. The IgH enhancer is depicted (*E*) and restriction sites are abbreviated B, Bam HI; Bc, Bcl I; Bg, Bgl I; M, Mlu I; P, Pst I; R, Eco RI; X, Xho I. H/R and E/Ap denote the junction of Hind III and Eco RI or Eco RV and Apa LI sites, respectively. The Bcl I deletion that takes out the μ_s tailpiece (*tp*) and poly A site (A_n) in all μ plasmids is depicted below pV$_H$-μ_m. All μ and CD8/$\mu 4_m$ plasmids encode only the membrane form of the H chain; analysis by Northern blot and SDS/PAGE revealed that they encode mRNAs and polypeptides of the expected sizes (1, 4, and unpublished data).

2 *A*). Does this distinction between B cell lymphoma and plasmacytoma reflect a feature specific to plasma cells that inhibits surface expression of IgM, or is it that B cells contain an activity that facilitates transport of IgM to the cell surface? To analyze this, we transfected a plasmid directing the synthesis of μ_m and λ chains into the monkey fibroblast line COS-7. Immunofluorescence analysis (Fig. 3 *A*) revealed that IgM was made within the cell but was not transported to the surface. This suggests that B cells contain factor(s) that facilitate the transport of membrane IgM to the cell surface; in their absence, membrane IgM would be retained within the cell.

The COOH-terminal Portion of μ_m Is Sufficient to Confer B Cell Specificity of Surface Expression. In contrast to the membrane form, the secretory form of IgM proceeds through the Golgi of plasmacytoma and out of the cell. We therefore suspected that the intracellular retention of membrane IgM might be mediated through the COOH-terminal end of μ_m chains, as this is where μ_m and μ_s differ. To test this, we constructed a hybrid gene between C_μ and the human cell surface antigen CD8 (which has a single extracellular domain and is a member of the Ig superfamily). The CD8/$\mu 4_m$ hybrid gene (Fig. 1) has the single extracellular CD8 domain linked to the μ C_H4, transmembrane, and cytoplasmic portions, and encodes a glycoprotein of the predicted molecular weight (not shown). When the CD8/$\mu 4_m$ chimeric protein is made in M12 cells, it is transported to the cell surface; in COS-7 cells, however, it is retained within the cell (Figs. 2 *B* and 3 *B*). This contrasts with CD8 itself, which is transported to the surface in both cell types (Fig. 3 *B*, and data not shown). Thus, provision of the μ C_H4, transmembrane, and cytoplasmic portions is sufficient to confer on CD8 the B cell–specific transport phenotype of membrane IgM. These results not only indicate that the sequence responsible for the intracellular retention of membrane IgM in non-B cells is located at the COOH-terminal end of μ_m, but also that this part of μ_m is sufficient for recognition by those factors that facilitate surface IgM expression in B cells.

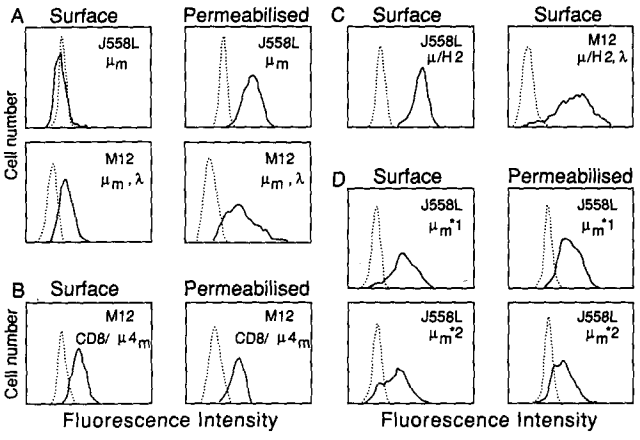

FIGURE 2. FACS analysis of lymphoid transfectants stained on the surface or after permeabilization for μ or CD8/$\mu 4_m$ expression. Fluorescence (on a logarithmic scale) is plotted against cell number. As a control, staining of untransfected cells (*dotted line*) is shown with each profile. (*A*) J558L cells transfected with pSV2gpt(pV_H-μ_m) and M12 cells transfected with pSV2gpt(pβG-μ_m + pHCMV-λ) were stained with FITC-conjugated anti-μ. (*B*) M12 cells transfected with pSV2gpt(pHCMV-CD8/$\mu 4_m$) were stained with FITC-conjugated anti-human CD8. (*C*) J558L cells transfected with pSV2gpt(pβG-μ/H2) and M12 cells transfected with pSV2gpt (pβG-μ/H2 + pHCMV-λ) were stained with FITC-conjugated anti-μ. (*D*) J558L cells transfected with pSV2gpt(pβG-μ_m*1) or pSV2gpt(pβG-μ_m*2) were stained with FITC-conjugated anti-μ.

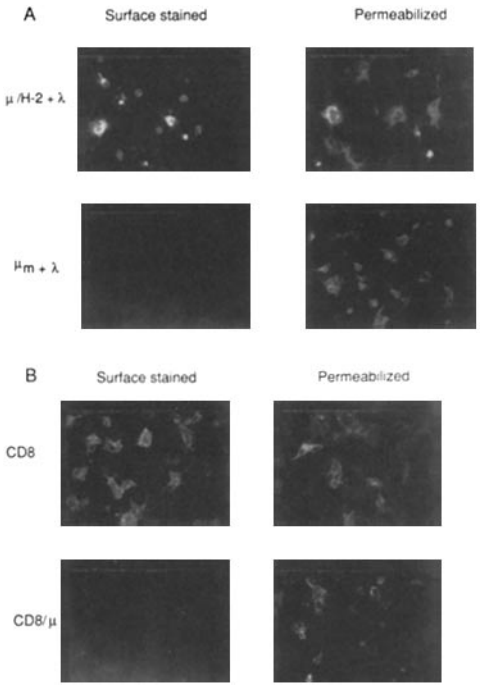

FIGURE 3. Immunofluorescence analysis of (*A*) COS-7 cells transfected with either pSV2gpt(pβG-μ_m + pHCMV-λ) or with pSV2gpt(pβG-μ/-H2 + pHCMV-λ); (*B*) COS-7 cells transfected with either pSV2gpt(pHCMV-CD8/$\mu 4_m$) or with pSV2neo(pHCMV-CD8).

Mutating the Transmembrane Segment Permits Surface IgM Expression in Non-B Cells. The results with the CD8/$\mu 4_m$ protein suggest that alteration of the COOH-terminal end of μ_m might overcome the retention of membrane IgM by non-B cells. We therefore constructed a plasmid that directs the synthesis of a hybrid H chain that is composed of V_H, $C_\mu 1$, $C_\mu 2$, $C_\mu 3$, and $C_\mu 4$ domains linked to the transmembrane and cytoplasmic segments of the MHC class I molecule H-2K^b (Fig. 1); MHC class I molecules are expressed on the surface of a wide variety of cells. The hybrid μ/H2 molecules associated with λ L chains and were efficiently transported to the surface of J558L and M12 cells (Fig. 2 *C*); they were also transported to the surface of COS-7 cells (Fig. 3 *A*).

The fact that the IgM comprising the μ/H2 H chains reached the cell surface must be due to the change either in the transmembrane segment or in the cytoplasmic tail. We felt that, given the short length of the μ_m cytoplasmic tail, the alteration of the transmembrane segment was the more likely cause. Inspection of the sequence of the segment of μ_m chains that spans the membrane (Fig. 4) reveals that while it contains no charged residues, it comprises two regions (TTAST and YSTTVT) that include polar amino acids. We therefore tested whether the mutant H chain μ_m*1 (Fig. 4), in which these two regions of the μ transmembrane segment have been mutated so as to increase its hydrophobicity, would be transported to the cell surface. This was indeed the case (Fig. 2 *D*). In fact, mutation of just the NH_2-terminal portion of the transmembrane segment (mutant μ_m*2) was sufficient to allow surface IgM expression in plasmacytoma (Fig. 2 *D*).

	EXTRACELLULAR	TRANSMEMBRANE	CYTOPLASMIC
μ_m	Cμ - KSTEGEVNAEEEGFE	NLWTTASTFIVLFLLSLFYSTTVTLF	KVK*
μ_m*2	Cμ - ----------	----VV-AV----------	---*
μ_m*1	Cμ - ----------	----VV-AV----------FAVV-V--	---*
μ/H2	Cμ - KSTEPPPSTVS	NMATVAVLVVLGAAIVTGAVVAFVM	KMRR-36aa-*

FIGURE 4. Transmembrane sequences. An asterisk denotes a translation stop.

Discussion

Membrane IgM is only able to proceed onto the cell surface in B lymphocytes. In contrast, it is retained within the endoplasmic reticulum in plasmacytoma and nonlymphoid cells. However, mutation of the transmembrane segment overcomes this retention. This raises several questions. What retains membrane IgM within the endoplasmic reticulum of non-B cells? How does mutating the transmembrane segment overcome the retention? How is the retention obviated in surface IgM-expressing B lymphocytes?

Our demonstration that specific amino acid substitutions in the transmembrane segment overcome the retention of membrane IgM in non-B cells means that this retention must be mediated by interactions involving the transmembrane segment. What proteins could mediate the retention? The H chain binding protein (BiP), which is implicated in the intracellular retention of secretory IgM by B cells, is unlikely to play a role, as no association between BiP and membrane IgM is found in μ_m transfectants of the J558L plasmacytoma (9). Rather, if a protein of the endoplasmic reticulum is involved, it is likely to be a resident transmembrane protein. However, the most striking feature of the mutant μ_m chains is that they are more hydrophobic than the wild type. Therefore, it may simply be that the transmembrane segment is too hydrophilic for stable incorporation into the lipid bilayer; membrane incorporation might require a protein sheath.

How then do B cells overcome the intracellular retention of membrane IgM? Hombach et al. (3) have described the isolation of a variant of the J558L plasmacytoma that transports IgM to the cell surface, and have shown that this variant now expresses an IgM-associated protein (B34) that is absent from the parental J558L. The authors suggest that B34 is the product of the *mb-1* gene isolated by Sakaguchi et al. (10), and propose that B34 mediates the transport of membrane IgM to the cell surface. From the data we present here, it is evident that the factor(s) that mediate the transport of membrane IgM to the B cell surface need no more than the μ C_H4, transmembrane, and cytoplasmic portions for recognition. They must act by either forming a sheath that allows stable incorporation of the μ transmembrane segment into the lipid bilayer, or they must displace an interaction that involves the transmembrane segment and that would otherwise cause retention.

Summary

Membrane IgM is expressed on the surface of B lymphocytes. It is not transported to the surface of transfected plasmacytoma or COS cells. Here, we show that mutation of four hydrophilic amino acids in the μ_m transmembrane is sufficient to overcome the intracellular retention of membrane IgM in non-B cells. This suggests that the B cell-specific IgM-associated proteins that have been postulated to assist the transport of membrane IgM to the cell surface (3) act either by forming a hydrophobic sheath that surrounds the μ_m transmembrane segment or by displacing an interaction with this segment that would otherwise cause retention. Experiments with a CD8/μ hybrid H chain indicate that the proteins that assist the transport of membrane IgM to the B cell surface at most need the μ C_H4 and transmembrane/cytoplasmic portion for interaction.

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