Brief Definitive Report

TRANSFECTED PLASMACYTOMA CELLS DO NOT TRANSPORT THE MEMBRANE FORM OF IgM TO THE CELL SURFACE

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Igs can be expressed in two different ways depending on the developmental stage of B lymphocytes. Immunocompetent B cells carry Igs as receptors on the cell surface, while terminally differentiated plasma cells secrete the molecules in large amounts. The structural differences between membrane-bound and secreted antibodies are located in the COOH-terminal portion of the H chains (1).

Both types of H chains are encoded by the same gene. As it has been shown for the μ chain, two mRNAs differing in their 3′ end can be transcribed from the μ gene by alternative usage of two potential polyadenylation sites (2). Those transcripts (membrane-bound μ chain [μm] and secreted μ chain [μs] mRNA) are generated in B lymphocytes in a developmentally ordered fashion. In Abelson virus–transformed pre-B cells, equal amounts of μm and μs mRNA are transcribed (3); in B cell lymphomas the ratio is shifted to the μm form (2); while in plasmacytomas, μs mRNA is found nearly exclusively (2).

We have introduced a μm expression vector into B cell lines representing different developmental stages. In the presence of L chain, only in B lymphoma but not in myeloma cells, the membrane-bound IgM molecule was expressed on the cell surface, although fully assembled intracellular IgM antibodies could be identified in both cell lines.

Materials and Methods

The following vectors were used in cell transfection experiments: pSVμm38 encodes for a complete H chain, whose Cμ gene is cDNA derived, so that exclusively μm mRNA is transcribed (see also reference 4). The VHDJH gene is genomic and cloned from the hybridoma Ac38 (5). The plasmid pSVμmk38 carries the Ac38 χ chain gene in addition, and both H and L chains are under control of their natural promotor and enhancer sequences. The original vector is pSV2gpt (6) (both vectors will be described in detail elsewhere). The cells used for transfection were the myeloma cells J558L (7), a λ chain producer, X63-Ag8.653 (8), an Ig nonproducer, and A20 (9), a B lymphoma line that carries antibodies of the IgG2a/χ isotype on the surface. All cells were grown in DMEM culture medium supplemented with 10% FCS, 2 mM L-glutamine, 50 U/ml penicillin, 50

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mg/ml streptomycin, and 2×10^{-5} M 2-ME. The cells were transfected by electroporation (10), distributed into 48-well plates, and selected by mycophenolic acid. Fluorochrome conjugates were purchased from Southern Biotechnology Associates, Inc., Birmingham, AL. Cytoplasmic (8) and surface stainings (11) were analyzed by fluorescence microscopy; surface stainings were analyzed, in addition, in the FACS (model 440; Becton Dickinson & Co., Mountain View, CA). μ chains were detected with FITC-coupled goat anti-μ antiserum. The Ac38 V-region was identified by the antiidiotypic IgM/λ_1 antibody B1-8 (12), whose binding was detected by goat anti-λ coupled to FITC. For biosynthetic labeling, 5×10^6 cells were incubated with 125 μCi [^{35}S]methionine (1,000 Ci/mM; Amersham Corp., Braunschweig, FRG) for 3 h in culture medium after a preincubation over a period of 1.5 h in medium without methionine. Cell labeling, cell lysis, and precipitation of the Igs by Sepharose-bound antibodies were done according to reference 13. Endoglycosidase H (Endo H) treatment was done after precipitation in 50 mM citrate buffer, pH 5.5, 0.02% SDS, 0.1 M 2-ME, and 0.5 mM PMSF with 6 mU Endo H (Boehringer Mannheim, FRG) over 5 h. The proteins were separated by SDS-PAGE as described in reference 14 and two-dimensional gel analysis was performed according to reference 15.

Results and Discussion

To analyze the expression of membrane-bound Igs in myeloma cells, the plasmid pSVμmk38, encoding a μm chain and a χ chain, was stably introduced into the Ig-negative myeloma line X63-Ag8.653. Additionally, the μm chain encoding construct pSVμm38 was transfected into the λ chain producing myeloma J558L. The transfected cells, X63μmk38 and J558Lμm38, were subsequently analyzed for surface IgM expression. As a control we also analyzed the μm transfectant A20μm38 of the B lymphoma line A20 that carries surface IgG2a/χ.

While A20μm38 cells express high amounts of the transfected μm chain on their surface (Fig. 1 *A*), no IgM was detected on the surface of the X63μmk38 (Fig. 1 *C*) and J558Lμm38 transfectant (not shown). Indeed, X63μmk38 cells were as negative as the untransfected control lines A20 and X63-Ag8.653 (Fig. 1, *B* and *D*).

On the other hand, intracellular μm chain was identified in all transfected cell lines by cytoplasmic staining (data not shown). Furthermore, B1-8 (12), the antigen of Ac38 (5), was clearly bound as shown by intracellular staining of X63μmk38, suggesting that functional antibodies are assembled inside the myeloma but obviously do not appear on the surface. Another J558L transfectant, containing a μm vector encoding the B1-8 V$_H$ gene (4), gave identical staining results; i.e., no surface but intracellular μ chain was detected (data not shown). This indicates that lack of surface expression of the membrane-bound antibody is independent of its V region.

The correct assembly of the antibody was also shown in a biochemical analysis of the intracellular antibodies in X63μmk38 and A20μm38 (Fig. 2). The Igs were biosynthetically labeled, precipitated from cell lysate by Sepharose-coupled anti-χ antibody, and subsequently analyzed on an acrylamide gel under reducing and nonreducing conditions. From both transfectants, H chains were coprecipitated with the L chains in roughly equal amounts (Fig. 2, lanes *2* and *3*). In lane *1* the precipitate from the myeloma line was size fractionated under nonreducing conditions; apart from the free L chain, the HL dimer and also the complete antibody can be detected. These data prove that at least a significant fraction of

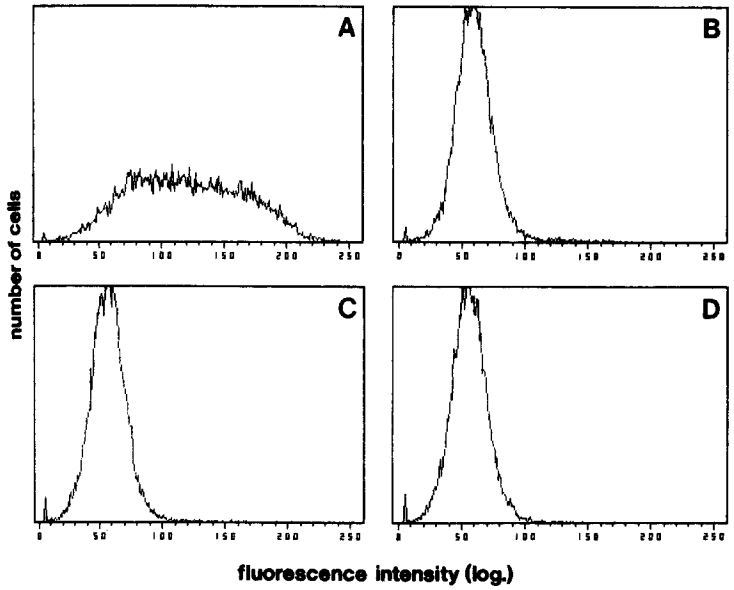

FIGURE 1. FACS histograms of surface-stained myeloma and B lymphoma cells. Cells were stained with FITC-coupled goat anti-μ antiserum. 10^4 cells were analyzed per histogram. (*A*) Transfected B cell lymphoma A20μm38; (*B*) untransfected lymphoma cells; (*C*) transfected myeloma cell X63μmk38; (*D*) untransfected myeloma cells.

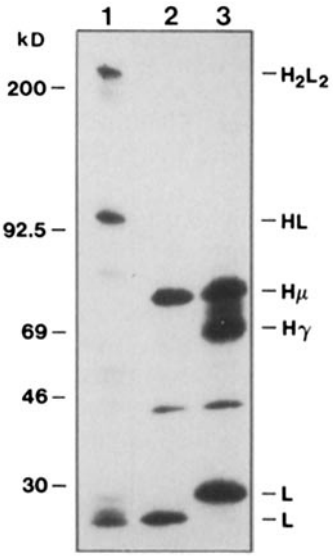

FIGURE 2. SDS-PAGE of biosynthetically labeled and immunoprecipitated antibodies. X63μmk38 and A20μm38 cells were biosynthetically labeled with [^{35}S]methionine and the Igs precipitated from the cell lysate by monoclonal anti χ antibody R33-18 (16), bound to Sepharose. Equivalent amounts were analyzed. Immunoprecipitated material from X63μmk38, under (lane *1*) nonreducing and (lane *2*) reducing conditions. (Lane *3*) Precipitated proteins from A20μm38 under reducing conditions; the proteins were size fractionated by SDS-PAGE on a gel with a linear acrylamide gradient from 5 to 15%.

the μm chains in the transfected myeloma cells is associated with L chain to full size antibodies.

Evidence for biochemical identity of the μm chains derived from the lymphoma and myeloma cells was obtained by two-dimensional gel analysis; the μm chains from the X63μmk38 (Fig. 3*A*) and the A20μm38 transfectant (Fig. 3*B*) were indistinguishable after IEF and subsequent size separation by SDS-PAGE. This

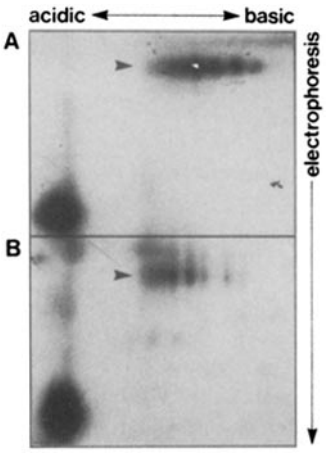

FIGURE 3. Two-dimensional gels of biosynthetically labeled μ chains. The cell lines (*A*) A20μm38 and (*B*) X63μmk38 were biosynthetically labeled with [^{35}S]methionine and the Igs precipitated from cell lysate by goat anti-μ–coupled Sepharose. First dimension: IEF gel; second dimension: 10% SDS-PAGE. For orientation, marker proteins were loaded together with the precipitates onto gel. Arrows indicate the μ chains.

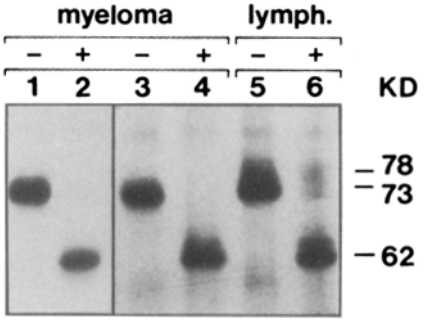

FIGURE 4. Endo H digest of μ chains from different cell lines. [^{35}S]Methionine biosynthetically labeled μ chains were precipitated from cell lysate by goat anti-μ–coupled Sepharose. The proteins were size separated on a 10% acrylamide gel after being reduced in the presence of 2-ME. (+) Indicates Endo H treatment. Lanes *1* and *2* show μm chains from J558Lμm38; lanes *3* and *4* from X63μmk38; and lanes *5* and *6* from A20μm38.

result was expected, since in both transfectants μm transcripts of the correct size (2.7 kb) could be detected with a probe specific for the sequence encoding the membrane portion of the μm chain (data not shown). We conclude that the μm proteins in the myeloma and B lymphoma are identical, despite their different behavior in the cells.

Where in the myeloma cells is IgM arrested? Treatment of glycoproteins with Endo H gives information about their intracellular location. Only the rough endoplasmic reticulum–specific high-mannose glycosylation type is sensitive to the enzyme. Glycoproteins that reach the Golgi apparatus become complexly glycosylated and their sugar chains are no longer cleaved by Endo H.

The analysis of the μm chains from X63μmk38, J558Lμm38, and A20μm38 is depicted in Fig. 4. The undigested H chains of the myeloma cells (Fig. 4, lanes *1* and *3*) show the molecular mass of the high-mannose glycosylated form (73 kD). Upon Endo H treatment all these chains are deglycosylated to a molecular mass of 62 kD (Fig. 4, lanes *2* and *4*). In contrast, in the B lymphoma, the μm protein is partly resistant to the enzyme. The undigestible material has a molecular mass of 78 kD, the size expected for μm chains carrying the complex sugar trees (Fig. 4, lane *6* [reference 17]). Thus, in the B lymphoma the membrane-bound antibodies reach the Golgi stack, in contrast to what is seen in the myeloma cells. A similar situation to the B lymphoma was observed in a μm/λ transfected Abelson pre-B cell line (see reference 4). The presence of only small amounts of

complexly glycosylated μm proteins in the B lymphoma may be due to different μm chain pool sizes in endoplasmic reticulum and Golgi complex.

Our data show that myeloma cells are unable to bring membrane-bound antibodies to the cell surface. However, these same cells can express on their surface the product of a transfected class I MHC gene (Weichel, W., personal communication), and thus do not seem to be generally deficient in transport and surface expression of membrane-bound proteins. The block of transport seems to be specific for the membrane-bound antibody molecule.

In principle, the selectivity of IgM transport can be due to two different kinds of control, depending on whether the proteins are targeted to the cell surface via a positively or negatively regulated mechanism. In the B lymphoma, surface expression of the antibody may occur only in concert with a second protein, whose expression is turned off in myeloma cells. This protein may play a role in the export of the antibody molecule and/or constitute with the antibody molecule part of an active antigen receptor complex on the surface of the B cell. Such a situation would be analogous to the antigen receptor on the T cell, where the T3 proteins are involved in function and transport of the $\alpha\beta$ heterodimer (18). This is in line with the finding that T cell receptor and surface Ig trigger a similar cellular response upon ligand crosslinking (19, 20).

The alternative, namely that transport of membrane-bound Ig is negatively regulated in myeloma cells, implies a specific factor that hinders membrane-bound antibodies from being transported to the cell surface. Recent studies on protein transport, especially on secreted molecules, give increasing evidence that protein sorting occurs by selective retention (21). Active retention of membrane-bound antibodies in myeloma cells would prohibit antigen triggering at this developmental stage.

Summary

Expression vectors coding for membrane-bound IgM antibodies were introduced into myeloma and B lymphoma cells. Only the lymphoma but not the myeloma cells were able to express the antibodies on the cell surface, although in both cases, complete antibodies were assembled intracellularly. In myeloma cells, the Ig molecules did not reach the Golgi compartment. Thus, the intracellular transport of membrane-bound antibodies is controlled in the B cell lineages in a developmentally ordered fashion.

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