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Immunology

Biogenesis of membrane-bound and secreted immunoglobulins: Two primary translation products of the human δ chain, differentially *N*-glycosylated to four discrete forms *in vivo* and *in vitro**

(human B lymphoblastoid mRNA/coexpression of IgM and IgD with shared idiotypes/cell-free system and *in vivo* protein synthesis/endo-β-*N*-acetylglucosaminidase H/integration into dog pancreatic microsomal membranes)

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ABSTRACT **Structural differences between the heavy chain of membrane IgD (δ_m) and the heavy chain of secreted IgD (δ_s) were investigated by using a human lymphoblastoid cell line that expresses idiotypically identical IgM and IgD. In a wheat germ cell-free system, mRNA from this cell line was shown to encode two distinct δ chains that differed in molecular weight. When translated *in vitro* in the presence of dog pancreatic microsomal membranes or when synthesized *in vivo*, these two δ chains were processed to four discrete glycosylated forms, all of which shared idiotypic determinants, C region determinants, and light chain linkage. As shown by digestion with endo-β-*N*-acetylglucosaminidase H, these four δ forms represent two δ polypeptide chains that are differentially *N*-glycosylated. Pulse–chase experiments demonstrated that, after endo-β-*N*-acetylglucosaminidase H treatment, δ_m has a higher molecular weight than δ_s. After integration into dog pancreatic microsomal membranes *in vitro*, δ_m was found not to have a large cytoplasmic domain exposed to proteolytic digestion. The finding that δ_m and δ_s differ in primary structure is analogous to previous work with the corresponding heavy chains of IgM (μ_m and μ_s) from the same cell line. Thus, this cell line produces four Ig heavy chains (μ_m, μ_s, δ_m, and δ_s), with the same idiotype. The observation of differential *N*-glycosylation, apparently unique for the δ class, is discussed.**

IgD, first discovered as a minor class of circulating Ig (1), has since been identified as a major Ig receptor, usually coexpressed with IgM, on B cell membranes (2). When present on the same cell, both classes of membrane Ig carry the same idiotype (3, 4) and both are specific for the same antigenic determinants (5). Evidence has been obtained that immunologic interactions mediated via membrane IgD can lead to qualitatively different B cell responses than those mediated via membrane IgM (6). Accordingly, IgD serves not only as a model for a cell surface receptor but, in comparison with membrane IgM, as part of a system in which to study divergent modes of B cell activation.

Further analysis of these functional systems must eventually be accompanied by a detailed structural examination of the IgD heavy chain (δ). Until recently, the low incidence of IgD myeloma and the extreme lability of IgD to proteolysis hampered the determination of the Fc amino acid sequence of the heavy chain of secretory IgD (δ_s) (7). The study of the heavy chain of membrane IgD (δ_m) has been even more difficult. It is extremely susceptible to proteolysis and is present in low amounts, with a slow turnover rate on B cell membranes. Early structural studies on membrane IgD demonstrated that reduced δ chains migrate as a broad band on $NaDodSO_4$/polyacrylamide gel electrophoresis (8, 9). Subsequent analyses by two-dimensional gel electrophoresis indicated that the biosynthetic forms of δ exist as a heterogeneous group, reflective of modifications of N-linked oligosaccharides (10). As shown by charge-shift electrophoresis, these forms probably have a hydrophobic COOH terminus (11, 12). Studies using one-dimensional $NaDodSO_4$ gel electrophoresis systems have demonstrated that δ_m exists as two molecular entities (δ_{m1} and δ_{m2}), distinguished from one another by a difference in molecular weight (ranging from 2000 to 7000) (13–16). Likewise, δ_s has been resolved into two species (δ_{s1} and δ_{s2}) that differ in molecular weight (17–19). The relationship between these various forms of the δ chain is obscure at present. As a result, the structural relationships between δ_m and δ_s as well as those between δ_m and μ_m have yet to be resolved.

In this study, IgD biosynthesis was analyzed in a human B lymphoblastoid cell line (SeD) that coexpresses IgM. All Ig molecules synthesized by this cell line express the same idiotype. The δ chains of this line were found, by cell-free translation of extracted mRNA, to exist as two distinct primary translation products, most likely corresponding to δ_m and δ_s. When followed by pulse–chase experiments *in vivo*, these chains were found to be processed to four forms, differing in relative mobility as a result of differential *N*-glycosylation. δ_m appears to be a higher molecular weight than δ_s and does not have a large cytoplasmically exposed domain.

MATERIALS AND METHODS

Preparation of RNA and Cell-Free Protein Synthesis. Total cellular RNA was extracted with $NaDodSO_4$/phenol/chloroform/isoamyl alcohol and proteinase K as described (20) and translated in a staphylococcal nuclease-treated wheat germ system (21). [^{35}S]Methionine (New England Nuclear; 700 Ci/mmol; 1 Ci = 3.7×10^{10} becquerels) was used at a final concentration of 1 mCi/ml, and salts were adjusted to final concentrations of 150 mM KOAc and 3.8 mM $Mg(OAc)_2$. In indicated experiments, dog pancreatic microsomal membranes were added (5 A_{260} units/ml) to the translation mixtures; post-translationally, the mixtures were adjusted to 2 mM $CaCl_2$ and incubated for 1 hr at 0°C in the presence or absence of proteases or detergent as detailed in the figure legends.

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Abbreviations: δ_m and δ_s, the heavy chains of membrane-bound and secreted IgD, respectively; μ_m and μ_s, the heavy chains of membrane-bound and secreted IgM, respectively; endo H, endo-β-*N*-acetylglucosaminidase H; aM_r, apparent molecular weight on polyacrylamide gel electrophoresis.

* This is paper no. 3 in a series. Paper no. 2 is ref. 23.

Cell Culture and *in Vivo* Pulse-Labeling. Cell lines were maintained in suspension culture as described (22). Conditions for pulse-labeling with [^{35}S]methionine were essentially as described (23). Labeled cells, washed twice with ice-cold phosphate-buffered saline, were lysed and prepared for immunoprecipitation in 1% Triton X-100/150 mM NaCl/20 mM Tris·HCl, pH 7.6/10 mM EDTA/10 mM *N*-ethylmaleimide containing pepstatin at 0.5 μg/ml and 1% Trasylol (Mobay Chemicals, New York) as described (24). Modifications from this procedure are detailed in the text.

Immunoprecipitation and endo-β-*N*-Acetylglucosaminidase H (endo H) Treatment. Rabbit antibodies to human IgD and κ light chains, sheep antibodies to rabbit IgG, and rabbit antibodies specific for the IgM (SeD) idiotype were prepared as described (3, 22). Samples containing radiolabeled polypeptides, synthesized *in vitro* or *in vivo*, were adjusted in the 1% Triton X-100 buffer described above, incubated with antibody for 2 hr at room temperature or at 0°C (as indicated), and then incubated for 2 hr at 4°C with Sepharose CL-4B beads (Pharmacia) coated with sheep anti-rabbit IgG. The immunoprecipitates were washed (25) and, as indicated, divided for incubation in the presence or absence of endo H as described (23). This enzyme preparation was the kind gift of P. Robbins (Massachusetts Institute of Technology).

Gel Electrophoresis. All samples were adjusted to 2.5% in $NaDodSO_4$, 20% in sucrose, 0.008% in bromphenol blue, 80 mM in Tris·HCl (pH 7.0), and 50 mM in dithiothreitol and were incubated for 30 min at 37°C, for 3 min at 100°C, and then for 30 min at 37°C in the presence of 250 mM iodoacetamide. Discontinuous polyacrylamide gel electrophoresis in $NaDodSO_4$ was performed as described (24). Used throughout were 10% polyacrylamide slab gels run at 25 mA (constant current) for 24 hr. All gels were fluorographed (26).

RESULTS

Two Primary Translation Products of the δ Chain. The human B lymphoblastoid cell line SeD expresses two immunoglobulin classes, IgM and IgD, which share idiotypic determinants (22). In previous work with this cell line (24), structural differences were delineated between μ_m and μ_s (the heavy chains of membrane-bound and secreted IgM, respectively). In the present investigation, the δ chains of SeD were examined.

To obtain polypeptide chains presumably representing unmodified primary translation products, mRNA from SeD was translated in a wheat germ cell-free system. Under these conditions, rabbit anti-human δ antibodies specifically precipitated two distinct δ chains from the total translation products (Fig. 1, lane 1) with competition by unlabeled IgD (Fig. 1, lane 2). Two primary translation products of the δ chain were resolved not only when samples were reduced and alkylated and run on 10% $NaDodSO_4$ gel electrophoresis (as in Fig. 1) but also when immunoprecipitates were analyzed on different systems (10% with 6 M urea, 15%, or 7.5–15% gradients) or were subjected to different sample preparation conditions (no reduction or alkylation, reduction without alkylation, or alkylation alone). By analogy with μ_m and μ_s, one of these products probably represents the primary translation product of δ_m, whereas the other probably represents the primary translation product of δ_s. If so, the difference in apparent molecular weight (aM_r) between these two forms of the δ chain must reside in the primary structure of their polypeptide chains.

Four δ Chains Synthesized *in Vivo*. SeD cells were pulsed *in vivo* for 8 min with [^{35}S]methionine, lysed in 1% Triton X-100, and prepared for immunoprecipitation at room temperature with rabbit anti-human δ antibodies. As a carrier for these immunoprecipitations, Sepharose CL-4B beads were coated with sheep anti-rabbit Ig antibodies that had a weak but detectable crossreaction with native human IgM. Consequently, in all *in vivo* precipitations, the μ chains of SeD were coprecipitated and served as internal markers. Unexpectedly, after an 8-min pulse, four forms of the δ chain were resolved by 10% $NaDodSO_4$ gel electrophoresis (Fig. 1, lane 3) (the doublet migrating at a lower relative mobility represents the early biosynthetic forms of μ_m and μ_s). The precipitation of the four δ forms was quantitatively inhibited by the addition of unlabeled human IgD (Fig. 1, lane 4). With an anti-idiotypic antiserum specific for IgM (SeD) (22), the μ chains as well as the four δ forms were immunoprecipitated (Fig. 1, lane 5). In this case, unlabeled IgM (SeD idiotype) inhibited the precipitation of both μ and δ chains (Fig. 1, lane 6); as a control, an unrelated IgM macroglobulin (Cab) did not inhibit this precipitation (not shown). Each of the four δ forms, as well as μ_m and μ_s, were also precipitated by rabbit anti-κ antibodies (not shown).

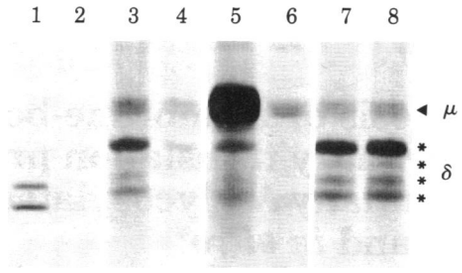

FIG. 1. Two primary translation products of the δ chain are synthesized *in vivo* as four discrete δ forms. Lanes 1 and 2: translation products from SeD, immunoprecipitated with rabbit anti-δ in the absence (lane 1) or presence (lane 2) of $NaDodSO_4$-denatured human IgD. Lanes 3–6: 8-min *in vivo* pulse of SeD, lysed in 1% Triton X-100, and immunoprecipitated at room temperature with rabbit anti-δ in the absence (lane 3) or presence (lane 4) of human IgD or with rabbit anti-IgM (SeD) idiotype in the absence (lane 5) or presence (lane 6) of IgM (SeD). Lanes 7 and 8: 8-min *in vivo* pulse of SeD, boiled in 1% $NaDodSO_4$, adjusted to 5% in Triton X-100, and immunoprecipitated at 0°C with rabbit anti-δ in the presence (lane 7) or absence (lane 8) of mannono-1,5-lactone (P-L Biochemicals) at 10 mg/ml. All precipitations of products labeled *in vivo* used Sepharose CL-4B beads coated with sheep anti-rabbit Ig antibodies (see text). The SeD μ chains coprecipitated by these antibodies are marked. Four δ bands, visible on the original in lanes 3, 5, 7, and 8, are marked with asterisks. All lanes were aligned from the same fluorographed gel.

To rule out proteolysis or mannosidase trimming of core oligosaccharides (27) as the basis for these results, cells pulsed for 8 min were instead boiled immediately in 1% $NaDodSO_4$, adjusted to 5% Triton X-100 to form mixed micelles, and then immunoprecipitated with rabbit anti-δ antibodies at 0°C, in the presence or absence (Fig. 1, lanes 7 and 8) of mannono-1,5-lactone (an inhibitor of mannosidase activity) at 10 mg/ml. Again, four intermediates of δ were resolved, comigrating with those immunoprecipitated from Triton X-100 lysates prepared at room temperature.

These data indicated that the two primary translation products of the δ chain were synthesized as four forms *in vivo*. These forms shared constant region and variable region determinants and were associated with light chain, but differed in mobility on $NaDodSO_4$ gel electrophoresis. Concomitantly, the two primary translation products of the μ chain were synthesized *in vivo*, as two discrete forms. This unexpected dichotomy in the processing of two classes of heavy chain in the same cell line prompted further investigation.

Differential *N*-Glycosylation of Two δ Polypeptide Chains. To investigate whether the mobility differences of the four δ chains were due to differential *N*-glycosylation, 8-min-pulse lysates were immunoprecipitated with rabbit anti-δ antibodies

and treated with endo H. This enzyme cleaves at the di-*N*-acetylchitobiose unit of high-mannose *N*-linked (core) oligosaccharides (28). After such treatment, the four δ forms were shifted to a doublet (Fig. 2*A*, lane 2); the μ chains were also shifted in mobility. The endo H-treated δ doublet had a lower aM_r than the untreated δ chains, consistent with the removal of N-linked oligosaccharides, as well as a lower aM_r than the two primary translation products (Fig. 2, lane 3), consistent with the removal *in vivo* of a signal peptide. The conversion of four bands into two bands after endo H treatment demonstrated that both primary translation products were synthesized *in vivo* in such a way as to yield four δ forms which differ in the number of attached N-linked oligosaccharides.

Differential processing of the δ chains, *vis-à-vis* the μ chains, of SeD might reflect features unique to the primary sequence of the δ chain or to the cell line itself. A test of the latter possibility was provided by the translation of SeD mRNA *in vitro* in the presence of dog pancreatic microsomal membranes. In this heterologous system, five δ chains were generated (Fig. 2*B*, lane 2), of which four were degraded by trypsin in the presence (Fig. 2*B*, lane 3) but not in the absence (Fig. 2*B*, lane 4) of detergent. The four protected δ chains comigrated with the four forms synthesized *in vivo* (Fig. 2*B*, lane 5). When treated with endo H (not shown), they were reduced, as were their *in vivo* synthesized counterparts, to a doublet. One difference between the *in vivo* and *in vitro* systems is that the relative stoichiometry of the four intermediates in each differed (compare lanes 4 and 5 in Fig. 2*B*). Nonetheless, in each system, different numbers of core oligosaccharide units were transferred to each of the two δ polypeptide chains.

δ_m Appears To Be Larger in aM_r than δ_s. The presence of two primary translation products for the δ chain (Fig. 1) suggested that δ_m and δ_s differ in polypeptide structure, but it remained to be determined which bands corresponded to δ_m and δ_s, respectively. Pulse-chase experiments and subsequent endo H treatment were carried out to resolve this (Fig. 3). Labeled products, pulsed *in vivo* for 8 min, were chased in medium containing unlabeled methionine for 1, 3, or 10 hr, immunoprecipitated with anti-δ antibodies, incubated in the presence or absence of endo H, and then resolved by 10% NaDodSO$_4$ gel electrophoresis. Four δ forms found after a 1-hr chase were chased into three forms after a 3-hr chase. At each time point, endo H treatment resulted in the generation of a doublet of lower aM_r. After a 1-hr chase, the μ chains of SeD were also shifted in mobility by endo H treatment; after a 3-hr chase, the oligosaccharide units of μ_s were resistant to endo H treatment, and only μ_m was shifted in mobility by endo H treatment. After a 10-hr chase, only one form of the δ chain remained associated with the cell pellet. The slow turnover of this chain would indicate that it represents δ_m. Treatment of this form with endo H demonstrated that, of the two endo H-treated chains present during earlier chase periods, only the higher aM_r form was present. This form is of a slightly faster mobility than the upper band of the two primary translation products (Fig. 3, lane 7) and is likely related to it by the removal, *in vivo*, of a signal peptide. If so, δ_m is of a higher aM_r than δ_s as a result of a difference in polypeptide structure.

δ_m Does Not Have a Large Cytoplasmically Exposed Domain. The difference in primary structure between δ_m and δ_s may include a hydrophobic COOH terminus on δ_m, necessary for membrane integration. Should δ_m be a transmembrane protein with a cytoplasmic domain, when translated *in vitro* in the presence of dog pancreatic microsomal membranes only that domain would be exposed to the action of proteases. The protection experiments in Fig. 2*B* carried out at 0°C with trypsin at 100 μg/ml, did not reveal the presence of such an exposed tail on any of the four δ forms. To test this possibility more stringently, SeD mRNA was translated in the absence or presence of dog pancreatic microsomal membranes and subsequently digested with proteinase K (100 μg/ml, 0°C, 1 hr) or chymotrypsin (250 μg/ml, 0°C, 1 hr) (Fig. 4). Five forms of the δ chain were resolved, as before, in the presence of membranes. In the presence of either protease, four dominant forms remained in the absence (Fig. 4, lanes 4 and 6) but not in the presence (Fig. 4, lanes 3 and 5) of detergent. In the absence of detergent, no

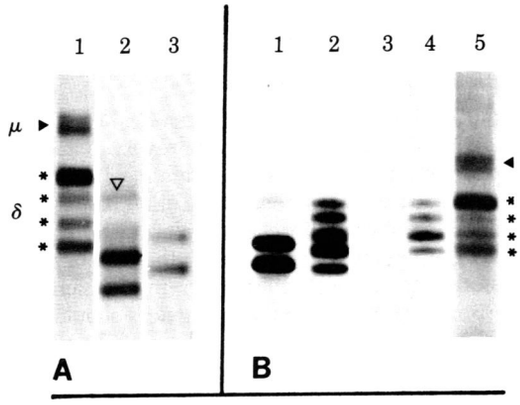

FIG. 2. (*A*) Differential *N*-glycosylation *in vivo* yields four δ forms from two primary translation products. Lanes 1 and 2: 8-min *in vivo* pulse of SeD, immunoprecipitated with rabbit anti-δ, and incubated for 16 hr at 37°C in the absence (lane 1) or presence (lane 2) of endo H at 60 ng/ml. Lane 3: translation products of SeD, immunoprecipitated with rabbit anti-δ. Open arrowhead, relative mobility of SeD μ chains after endo H treatment. (*B*) Four forms of δ are also generated by dog pancreatic microsomal membranes. Lanes 1–4: *in vitro* products of SeD mRNA translated in the absence (lane 1) or presence (lanes 2–4) of dog pancreatic microsomal membranes. Posttranslationally, samples in lanes 2–4 were incubated for 1 hr at 0°C in the absence (lane 2) or presence (lanes 3 and 4) of trypsin at 100 μg/ml; the sample in lane 3 additionally received 1% Triton X-100 during this incubation. After the addition of phenylmethylsulfonyl fluoride to 1 mM and Trasylol to 3%, samples were subjected to immunoprecipitation with rabbit anti-δ antibodies. Lane 5: 8-min *in vivo* pulse of SeD and immunoprecipitated with rabbit anti-δ antibodies. The lanes in *A* are from a different fluorographed gel than those in *B*. Solid arrowheads, μ chains; asterisks, δ chains.

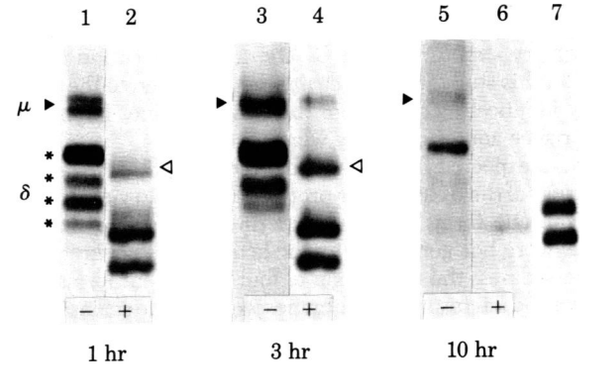

FIG. 3. δ_m appears to have a higher aM_r than δ_s. SeD cells were pulsed *in vivo* for 8 min with [^{35}S]methionine and then chased in medium containing unlabeled methionine for 1, 3, or 10 hr. Cell pellets were lysed in 1% Triton X-100, immunoprecipitated with rabbit anti-δ, and then incubated for 16 hr at 37°C in the absence (−) or presence (+) of endo H at 60 ng/ml. Lane 7: translation products of SeD immunoprecipiated with rabbit anti-δ. Solid arrowheads, μ chains; open arrowheads, μ_m chains after endo H treatment; asterisks, δ chains. The lanes shown were aligned from several different exposures of the same fluorographed gel.

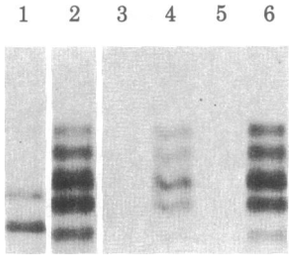

FIG. 4. δ_m does not have a large cytoplasmically exposed domain. SeD mRNA was translated *in vitro* in the absence (lane 1) or presence (lanes 2–6) of dog pancreatic microsomal membranes. Posttranslationally, samples were incubated at 0°C in the presence of proteinase K at 100 μg/ml (lanes 3 and 4) or chymotrypsin at 250 μg/ml (lanes 5 and 6), with (lanes 3 and 5) or without (lanes 4 and 6) 1% Triton X-100. After the addition of phenylmethylsulfonyl fluoride to 1 mM and Trasylol to 3%, all samples were immunoprecipitated with rabbit anti-δ. These tracks were aligned from the same fluorographed gel.

shift in the mobility of any form was discernible as a result of protease digestion. Therefore, δ_m appears not to possess a cytoplasmically exposed domain large enough to be detected by this assay.

DISCUSSION

These studies demonstrate that the δ heavy chains of the human lymphoblastoid cell line SeD are translated *in vitro* as two distinct primary translation products with different molecular weights. These two products probably represent pre-δ_m and pre-δ_s, and the difference in molecular weight must be the result of a difference in primary structure. *In vivo*, the two δ primary translation products are processed to yield four distinct forms which share δ determinants, SeD idiotypic determinants, and L chain linkage but which differ in aM_r as a result of differential *N*-glycosylation of two polypeptide chains. Endo H treatment of pulse–chased intermediates demonstrated that, of these two chains, the form with the slowest intracellular turnover had the highest aM_r. It is likely that this form represents δ_m. Finally, protection experiments *in vitro* with dog pancreatic microsomal membranes failed to reveal a large cytoplasmically exposed domain on δ_m.

Structural differences between μ_m and μ_s from SeD were previously investigated (24). They were found to share NH_2-terminal amino acid sequences and idiotype but to differ in aM_r, in primary structure at the COOH terminus, and in topology (24). In this study, these μ chains are compared to the idiotypically identical δ chains from the same cell line. Major points of variance and of similarity were observed.

An unexpected finding was that the two primary translation products of the δ chain were processed *in vivo* to yield four early biosynthetic forms. Control experiments indicate that these forms were not generated by the action of either proteases or mannosidases; the generation of four equivalent forms by dog pancreatic microsomal membranes would argue against a defect inherent to the cell line. Other, undetermined, artifacts appear to be ruled out by the fact that SeD μ_m and μ_s were uniformly processed to two early biosynthetic forms. Digestion with endo H demonstrated that the four δ forms were produced by differential *N*-glycosylation of two polypeptide chains. These results suggest that the transfer of N-linked oligosaccharides to the nascent δ_m and δ_s polypeptide chains is not 100% efficient in the cell line SeD. This phenomenon may refect unique structural features of the δ polypeptide chains that are incompatible with the uniform attachment of core oligosaccharides. A similar situation has been described for the bovine pancreatic RNases A and B; otherwise equivalent in structural and enzymatic properties, RNase B is a glycoprotein and RNase A is not (29, 30).

Previous studies analyzing δ chains from mixed populations of cells or immunoglobulins have identified heterogeneity in the $NaDodSO_4$ gel electrophoresis profiles of δ_m and δ_s (9, 10, 13–19). This heterogeneity may be related to the observation, in the present investigation, of differential *N*-glycosylation of the SeD δ chains. Four δ forms were derived from two primary translation products, and all are products of a single cell line. Two of these forms may correspond to the δ_{m1} and δ_{m2} membrane forms, and two to the δ_{s1} and δ_{s2} secretory forms. If so, these forms differ in two respects: δ_m and δ_s have different polypeptide chains and are differentially *N*-glycosylated.

The finding of two distinct primary translation products for the δ chain is directly analogous to previous findings with SeD μ_m and μ_s (24). The above results would indicate that, like μ_m, δ_m is integrated as a membrane protein with a slow turnover rate and a higher polypeptide aM_r than δ_s. As with μ_m and μ_s, the difference in aM_r between δ_m and δ_s, as well as the difference in topology, may be due to a difference in primary structure at the COOH terminus.

This investigation on δ_m, coupled with previous investigations on μ_m (24, 31, 32) and α_m (23), adds support to the existence of a new Ig domain. This membrane domain might have structural characteristics underlying several functions. Included among these would be information necessary for integration into the lipid bilayer. This information, carried in the primary sequence of the chain and referred to as a "stop transfer" sequence (33), may reside in or near the segment of the chain that becomes lodged in the membrane. The nucleotide sequence studies by Rogers *et al.* (31) have offered, in the case of μ_m, a predictive amino acid sequence for this domain, a sequence with highly hydrophobic properties consistent with membrane integration. Should δ_m carry a similar domain, the protection experiments reported in this study would indicate that, after integration, the membrane domain does not have a large cytoplasmically exposed region. Similar results have been obtained for μ_m (unpublished data).

It might be expected that all membrane Ig heavy chains will share a similar domain; it is likely, however, that meaningful differences exist. All should carry the minimal information requisite for membrane integration; on the other hand, each might carry distinct structural information relevant to the processes of B cell triggering (or inactivation). In the case of μ_m and δ_m, coexpressed on the same B cell and carrying the same idiotype, a comparison of COOH-terminal sequences should be of particular interest. The apparent dichotomy of B cell responses mediated through either membrane IgM or membrane IgD (6) may reflect unique COOH-terminal sequences for μ_m and δ_m, capable of initiating different intracellular cascades. These Ig heavy chains should serve as a useful model for the investigation of such structure–function relationships.

Finally, it should be emphasized that, in one cell line, primary translation products for μ_m, μ_s, δ_m, and δ_s are detectable. μ_m and μ_s have identical NH_2-terminal sequences (24). All four share the same idiotype. It would appear that the same variable region has been processed in such a way as to be contiguous to two different constant regions; these constant regions, in turn, have been processed in such a way as to yield two mRNAs, one for membrane Ig and one for secretory Ig. Given the close proximity of the δ structural gene to the 3′ side of the μ structural gene (34, 35), it is plausible that a single primary transcript could contain V_H, C_μ, and C_δ coding regions, along with the coding regions for the membrane domains of C_{μ_m} and C_{δ_m}. Differential processing of this primary transcript might yield mRNAs for μ_m, μ_s, δ_m, and δ_s, differing at the 3′ ends. The presence of these

four transcripts in cell line SeD might constitute an argument for such a model (34, 35) and certainly offers an experimental system in which to test it.

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