Brief Definitive Report

BIOGENESIS OF MEMBRANE-BOUND AND SECRETED IMMUNOGLOBULINS

I. Two Distinct Translation Products of Human μ-Chain, with Identical N-Termini and Different C-Termini*

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Two forms of IgM, functionally and topologically distinct, are produced during the course of B cell differentiation. On the plasma membrane of resting B cells, an 8S monomer serves as an antigen receptor, conveying the signal of antigen recognition across the lipid bilayer. As B cells mature to IgM plasma cells, IgM is, instead, secreted as a soluble, 19S pentameric structure. Notably, both forms derived from a single B cell clone have been shown to share similar V regions by idiotypic analysis (1, 2).

Differences in function and in topology between membrane-bound and secreted IgM have been attributed in the past to structural differences in their respective heavy chains, μ_m and μ_s, as evidenced by the following observations: μ_m exhibits hydrophobic properties not associated with μ_s (3–5); μ_m appears to be glycosylated to a different degree than μ_s (6–8), μ_m and μ_s generate similar, yet unique, peptide maps (8–10); and μ_m migrates more slowly than μ_s on sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis (PAGE) (7, 11), even after biosynthesis in the presence of tunicamycin (4). The results of amino acid sequence analysis have been ambiguous. Several groups have reported identical C-terminal sequences for the two (7, 8, 12), whereas one group has found that μ_m, from the cell line Daudi, differs in C-terminal sequence from μ_s, secreted by another cell line, Ram. (9).

The above results are all consistent with the notion that μ_m and μ_s differ in primary structure. However, because all of the data were generated in systems that analyze mature proteins, the relative contribution of co- and posttranslational modifications to the observed differences cannot be assessed. Differential glycosylation (e.g., of O-linked carbohydrates in the presence of tunicamycin) and proteolysis of otherwise identical primary translation products are alternative possibilities that have not been ruled out.

In the present studies, we have looked at the primary translation products of the μ-chain, with RNA extracted from human lymphoblastoid cell lines positive for both surface and secretory IgM. μ_m and μ_s are encoded as distinct polypeptide chains,

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identical in N-terminal sequence and in idiotype, yet with different molecular weights and different C-terminal tails.

Materials and Methods

Most of the procedures used in this study have been detailed previously. Among these are the maintenance of lymphoblastoid cell lines (13); the preparation and characterization of anti-μ and anti-idiotypic antisera (2), immunofluorescent staining for surface and intracellular immunoglobulins (13), pulse-labeling of cells in vivo with radioactive amino acids (14), extraction of total cellular RNA with SDS/phenol/chloroform/isoamylalcohol and proteinase K (14); the assay for cell-free protein synthesis with a staphylococcal nuclease-treated wheat-germ extract (15), and various posttranslational assays, including the preparation of immunoprecipitates for SDS-PAGE (16), fluorography of unstained gels (17), the preparation of samples for sequence analysis by automated Edman degradation (16), and the conditions for carboxypeptidase Y degradations (18). Details and modifications are included in the figure legends.

Tritiated amino acids and [^{35}S]methionine were purchased from New England Nuclear, Boston, Mass., at the highest available specific activity Carboxypeptidase Y (89 U/mg) was purchased from Millipore Corp , Bedford, Mass., Protein A Sepharose CL-4B from Pharmacia, Inc , Sweden, wheat germ from General Mills, Inc., Minneapolis, Minn , and Trasylol from FBA Pharmaceuticals, Inc , New York.

Results and Discussion

Two human lymphoblastoid lines were selected for their reciprocal expression of surface vs. intracellular (secretory) IgM. As judged by immunofluorescence, Daudi had a high percentage of cells staining brightly for surface IgM. In contrast, SeD (13) had a high percentage of IgM-secreting plasma cells. When either of these lines was pulsed with [^{35}S]methionine for 8 min (at which time most labeled intermediates are in the rough endoplasmic reticulum [RER]), two forms of the μ-chain could be resolved by SDS-PAGE (Fig. 1; lane 5). When, after a longer pulse, the labeled intermediates were chased in cold medium for 2 h, one form was found to be secreted, whereas the other remained associated with the cells (Fig. 1; lanes 7 and 9). In work that will be reported in detail elsewhere, these μ-chains are shown to differ also in their apparent association with the lipid bilayer. The upper band of Fig. 1 (lane 5) behaves as a membrane protein, resistant to extraction under conditions in which the lower band is not.

The above results permit a tentative assignment of the upper band (Fig. 1; lane 5) as μ_m and of the lower band as μ_s. Consistent with the results of others, these results document posttranslational distinctions between μ_m and μ_s in terms of mobility on SDS-PAGE, topology, and hydrophobicity. They do not, however, localize the distinction to the primary structure of the polypeptide chain. Indeed, the data serve better to underline the variations that occur as a result of posttranslational modifications: in the case of SeD, the mature, secreted μ_s is more slowly migrating than the mature, cell-associated μ_m; as RER intermediates, the converse is true.

To obtain polypeptide chains free of posttranslational modifications, messenger RNA (mRNA) from Daudi and SeD was translated in a wheat-germ cell-free system. Under these conditions, anti-μ antibodies specifically precipitated two distinct μ-chains from the translation products (Fig. 1; lanes 1–4). The relative proportion of these two products from Daudi was inversely related to that found in SeD (Fig 1; lane 1 vs 3), which correlates well with the relative abundance of membrane and secretory IgM as judged by immunofluorescence and by the pulse-labeling experi-

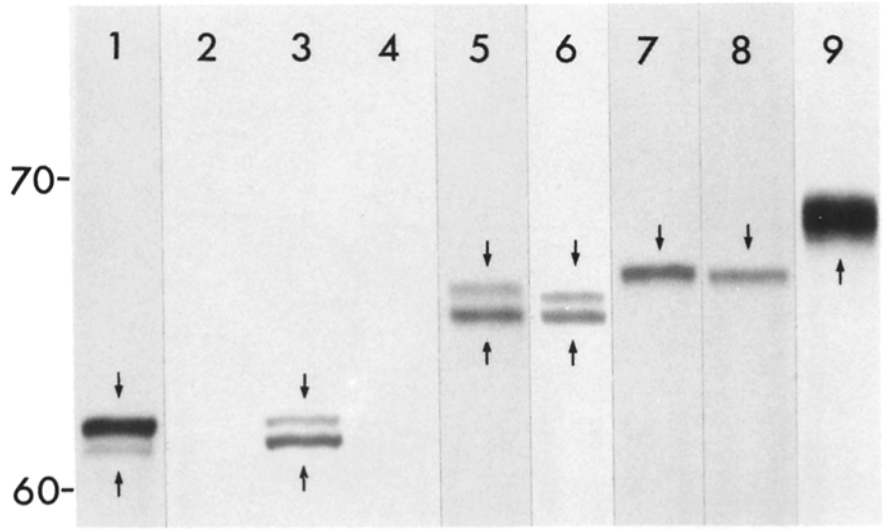

FIG 1 Resolution of two μ-chains, synthesized in vivo and in vitro, by SDS-PAGE Lane 1: translation products from Daudi, immunoprecipitated with rabbit anti-μ Lanes 2–4 translation products from SeD, immunoprecipitated with normal rabbit serum (lane 2), anti-μ (lane 3), and anti-μ in the presence of excess, unlabeled, human IgM (lane 4). Lanes 5 and 6 8-min in vivo pulse of SeD, immunoprecipitated with anti-μ (lane 5) and rabbit anti-SeD idiotype (lane 6) Lanes 7–9: in vivo pulse of SeD for 3 h, followed by a 2-h chase in cold medium. Labeled products that remained associated with cells were immunoprecipitated with anti-μ (lane 7) or anti-SeD idiotype (lane 8), products that were chased into the culture medium were immunoprecipitated with anti-μ (lane 9) Arrows pointing downward indicate μ_m, those pointing upward indicate μ_s [^{35}S]Methionine-labeled polypeptides were translated in a nuclease-treated wheat-germ extract (lanes 1–4) and adjusted to 1% Triton X-100 (Robin and Haas, Philadelphia, Pa.), 150 mM NaCl, 20 mM Tris-HCl (pH 7.4), 10 mM EDTA, and 1% Trasylol Cells pulsed in vivo (lanes 5–9) with [^{35}S]methionine were lysed in the same buffer that contained 1 mM phenylmethyl sulfonyl fluoride and 5 mM iodoacetamide for 10 min at 4°C; a postnuclear supernate was prepared by centrifugation at 1,000 *g* for 20 min After incubation with antisera for 2 h at room temperature, Protein A-Sepharose was added for 1–2 h at 4°C For immunoprecipitations with the rabbit anti-idiotype antiserum, Sepharose beads covalently coupled with a sheep anti-rabbit Ig fraction were used instead of Protein A-Sepharose After washing, the beads were resuspended in PAGE loading buffer (14) that contained 50 mM dithiothreitol, reduced for 30 min at 37°C, incubated for 3 min at 100°C, alkylated for 30 min at 37°C with 250 mM iodoacetamide, loaded onto 10% polyacrylamide slab gels, and electrophoresed for 36 h, constant current All tracks were aligned from the same gel. markers on the left represent apparent molecular weights

ments described above. The upper band, dominant in Daudi, is therefore likely to represent the primary translation product of μ_m (pre-μ_m), whereas the lower band, dominant in SeD, is likely to represent the primary translation product of μ_s (pre-μ_s). If so, the difference in molecular weight between these two forms of μ-chain must reside in the primary structure of their polypeptide chains. To localize this difference, amino acid sequences of pre-μ_m and pre-μ_s from SeD were compared.

Pre-μ_m and pre-μ_s from SeD were found to be homologous in N-terminal amino acid sequence (Fig. 2A and B). As determined by automated Edman degradation, they share a methionine in position 1; valines in positions 11 and 17, and leucines in positions 5, 8, 10, 14, and 23; the leucine shoulder in position 24 may also represent a residue common to both. This sequence is, as expected, not related to the known N-terminal sequence of μ_s (19), but is rather more like that of other signal peptides, including that predicted from the cDNA sequence of a murine μ-chain (20). This

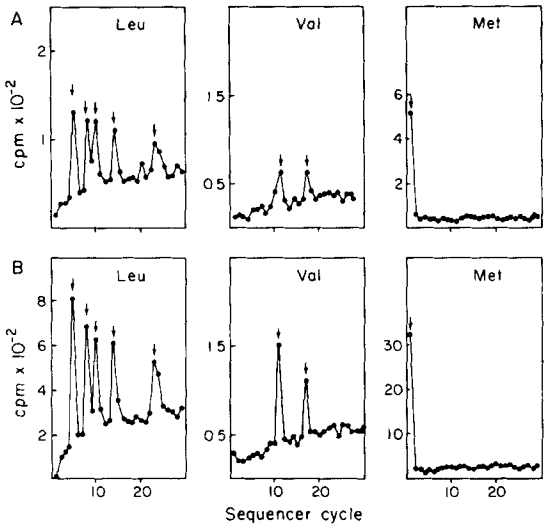

FIG 2 Partial N-terminal sequences of μ-chains synthesized in vitro (A) Partial N-terminal sequence of SeD upper band, μ_m (Fig 1, lane 3) (B) Partial N-terminal sequence of SeD lower band, μ_s (Fig 1, lane 3) Arrows indicate positions of the radioactive amino acid residue SeD RNA was translated in vitro in the presence of [^{3}H]leucine and [^{35}S]methionine or [^{3}H]valine and [^{35}S]methionine, and then processed for gel electrophoresis as per the legend of Fig 1 Gels were dried without fixing and autoradiographed The bands corresponding to pre-μ_m and pre-μ_s were excised from the gels, electroeluted, and prepared for automated Edman degradation as described (16) Radioactive residues released through 30 cycles were detected by double label counting

sequence homology suggests that the two pre-μ chains share common N-terminal signal peptides. Immunoprecipitation of the products labeled in vivo with an antiserum specific for IgM (SeD) idiotype (13) demonstrated that, both at the level of the RER (Fig. 1; lane 6) and as mature membrane-associated (Fig. 1, lane 8) or secreted (not shown) forms, SeD μ_m and μ_s shared similar idiotypic determinants. Thus, the N-terminal similarity between μ_m and μ_s extends further through the V region

Together with serologic evidence that μ_m and μ_s are antigenically similar throughout most of the remainder of the μ-chain, the above results indicate that the molecular weight difference between pre-μ_m and pre-μ_s must be the result of an amino acid sequence difference at or near the C-terminus. This difference was confirmed by carboxypeptidase Y (CPY) degradation of pre-μ_m and pre-μ_s from SeD (Fig. 3 C; U and L, respectively). [^{3}H]Lysine was released rapidly from pre-μ_m and not from pre-μ_s Parallel CPY degradations of the RER intermediates revealed two additional C-terminal amino acid differences. [^{3}H]Phenylalanine was released more rapidly from the upper band, μ_m (Fig., 3 B), whereas [^{35}S]methionine was released more rapidly from the lower band, μ_s (Fig. 3 A) Though the release kinetics of CPY degradation cannot assign a C-terminal sequence for μ_m and μ_s, these results do clearly demonstrate a divergence between the two at or near the C-terminus.

This divergence between μ_m and μ_s stands in striking contrast to the N-terminal identities of amino acid sequence and of idiotype. Of the four studies that have previously subjected μ_m to CPY degradation (7–9, 12), the results of one (9) are confirmed In contrast to the latter study, the data presented here are based on comparisons between μ_m and μ_s from the same cell line. That is, μ_m from SeD is

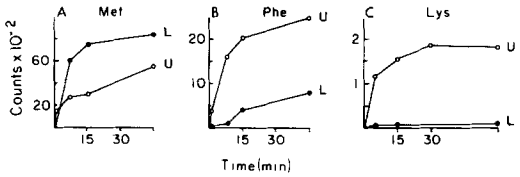

FIG 3 C-terminal analysis of μ-chains synthesized in vitro and in vivo, using CPY μ-Chains translated in vitro with [^{3}H]lysine (C) or synthesized in an 8-min pulse in vivo with [^{35}S]methionine (A) and [^{3}H]phenylalanine (B) were subjected to CPY degradation for varying times Indicated are the counts of each residue released from the upper bands (U) of Fig 1 (lanes 3 and 6) (pre-μ_m and μ_m, respectively), and from the lower bands (L) of the same lanes (pre-μ_s and μ_s, respectively) After detection by autoradiography, the μ-chains were electroeluted, with 100 μg of ovalbumin as carrier. The eluted samples were precipitated twice with 0 4 M KCl, 25% TCA, acetone extracted, boiled in 2 5% SDS, and then adjusted to 0 25% SDS in a 0 05 M Tris-acetate buffer (pH 5 8). For each time point, an equal amount (30 μg) of CPY was added to an equal amount of input counts, as follows: [^{35}S]methionine (12,000 cpm), [^{3}H]phenylalanine (4,000 cpm), and [^{3}H]lysine (5,000 cpm) Digestions, carried out at 30°C, were stopped by KCl/TCA precipitation, after which the released radioactive amino acids in the supernate were determined by double label counting The data plotted represent total counts in 10 min after subtraction for background [^{35}S]methionine (1,500 counts), [^{3}H]phenylalanine (600 counts), and [^{3}H]lysine (100 counts)

different in molecular weight and in C-terminal residues, relative to its internal control, μ_s from SeD. Because each is synthesized as a distinct translation product, the difference in molecular weight must reside, at least in part, in a different C-terminal primary structure.

The observation of two primary translation products in this study rules out the possibility that μ_m and μ_s have a precursor-product relationship posttranslationally. Instead, they appear to be encoded by two distinct mRNA, one for μ_m and the other for μ_s. The sequence data indicate that these two mRNA have identical 5′ and different 3′ coding regions, perhaps the result of a nuclear RNA splicing event or of a DNA rearrangement at the level of the single μ-structural gene.[1]

Summary

Structural differences between the heavy chain of membrane-bound IgM (μ_m) and the heavy chain of secreted IgM (μ_s) were investigated. The primary translation products of the μ-chain, free of posttranslational modifications, were synthesized in a wheat-germ cell-free system, programmed with messenger RNA derived from human lymphoblastoid cell lines positive for both membrane-bound and secreted IgM. Encoded in this system were two μ-chains, which shared N-terminal signal peptides and which differed both in molecular weight and in C-terminal amino acid sequence. In vivo pulse labeling of cells confirmed that, as intermediates in the rough endoplasmic reticulum, these two forms expressed the same idiotype and maintained their difference in molecular weight and in C-terminal sequence. By correlation with pulse-chase kinetics and with immunofluorescence, one form of μ-chain represents μ_m, and the other, μ_s. Because the molecular weight difference between the two is manifest at the level of their primary translation products, these studies demonstrate that μ_m is distinguished from μ_s by a difference in primary structure, at least in part at the C-terminus.

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[1] After completion of these studies, preprints of the studies of Hood and associates (*Cell* In press) were received Their studies, based on the nucleotide sequencing of genomic and cDNA clones of the murine μ-chain, predict the C-terminal differences reported here and demonstrate that μ_m and μ_s are encoded by two separate mRNA

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