Apolipoprotein B-100: immunolocalization and synthesis in human intestinal mucosa

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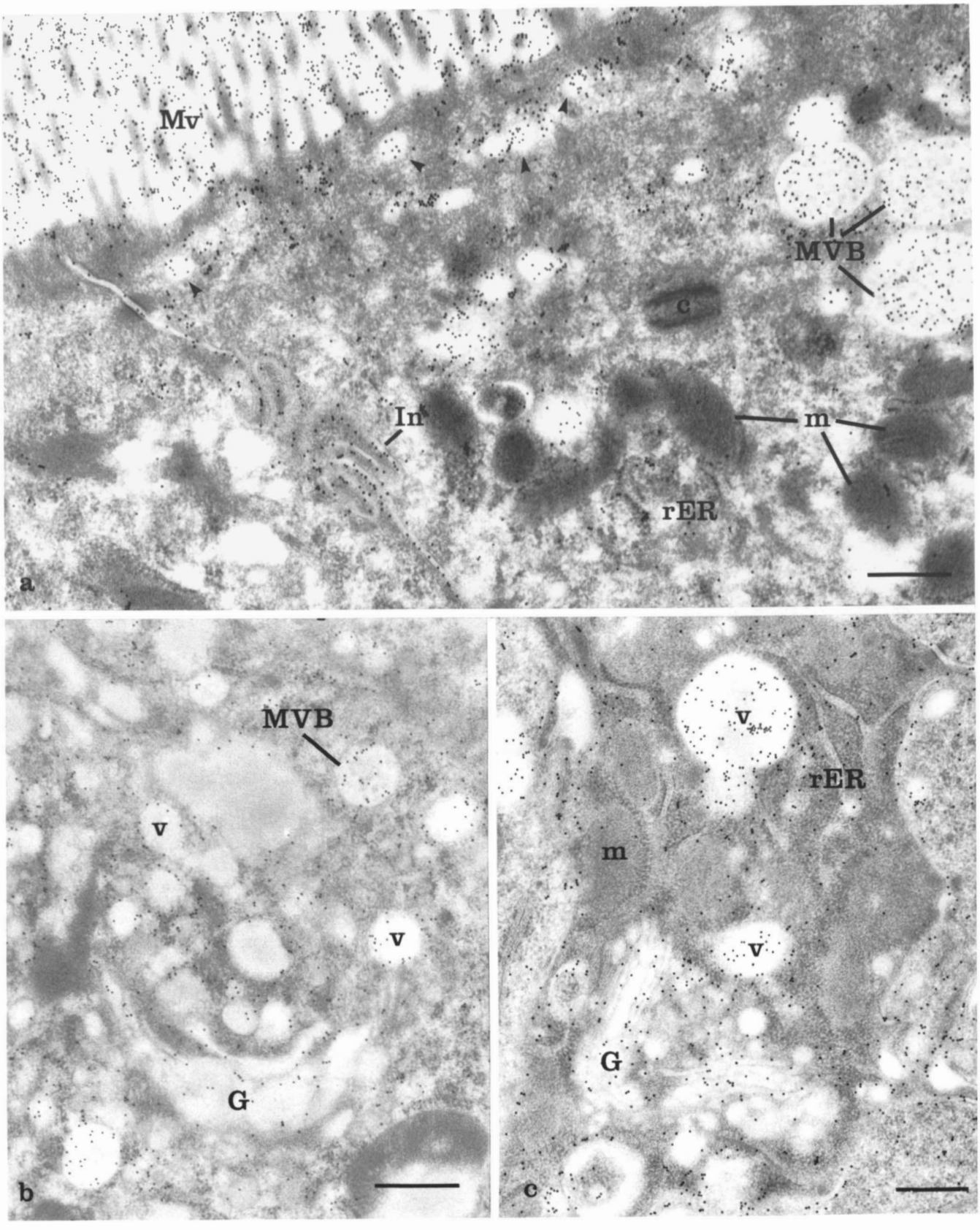
Abstract Despite the evidence that the human small intestine produces two separate species of apoB mRNA encoding for B-100 and B-48, there is a paucity of data concerning the expression of the latter form in this organ. Using a high resolution immunogold approach, with specific polyclonal antibodies and a panel of monoclonal antibodies (2D8, 3A10, 4G3), both forms of apoB (B-48 and B-100) were revealed over enterocytes of pediatric intestinal samples. Intense labeling was observed over microvilli, apical smooth membrane vesicles, multivesicular bodies, the basolateral membrane, as well as the *trans* Golgi region. Only low labeling was found over the rough endoplasmic reticulum (rER). Similar patterns of apoB distribution characterized both duodenal and jejunal regions. The presence of labeling over the Golgi apparatus and rER suggests a synthetic activity of both forms of apoB by the epithelial cells. To test this hypothesis, human intestine was incubated with [^{3}H]leucine, homogenized, and subjected to immunoprecipitation for apoB. Immunoprecipitates contained radioactivity mainly in apoB-48 with relatively small amounts in apoB-100 when examined by $NaDodSO_4$-polyacrylamide gel electrophoresis. These findings were further supported by the biochemical determination of apoB-100 and apoB-48 in chylomicron particles isolated from thoracic duct lymph of a human donor. Taken together, our data suggest that the human intestine is able to synthesize and to express the apoB-100. —**Levy, E., C. Rochette, I. Londono, C.C. Roy, R.W. Milne, Y.L. Marcel, and M. Bendayan.** Apolipoprotein B-100: immunolocalization and synthesis in human intestinal mucosa. *J. Lipid Res.* 1990. **31:** 1937-1946.

Supplementary key words apoB-48 • enterocyte • protein A-gold • protein G-gold • chylomicron • intestinal explants • lymph

Apolipoprotein (apo) B is a large hydrophobic glycoprotein found in plasma in heterogeneous sizes (1). ApoB-100, a 549-kDa protein, is an essential component of very low density lipoprotein (VLDL) and low density lipoprotein (LDL). ApoB-48, a smaller protein of 264 kDa, is a major surface constituent of chylomicrons (1, 2). ApoB-100 has received a great deal of attention since its recognition as the ligand responsible for the receptor-mediated uptake of low density lipoproteins, the principal human cholesterol-transporting lipoproteins in plasma (3). There is much interest in the study of inherited disorders of apoB deficiency, due either to a simple structural modification or to a defect in secretion, which results in major abnormalities in lipoprotein metabolism, such as those seen in abetalipoproteinemia, homozygous hypobetalipoproteinemia, and chylomicron retention disease (2, 4-7).

Though human B-100 and B-48 are considered to be metabolically distinct, they share some common features in structure and function. The apoB-100 is synthesized in the liver and is required for the intracellular assembly and secretion of pre-VLDL lipid components (2). Similarly, apoB-48, which is produced by the intestine, is essential for the packaging of pre-chylomicron dietary triglyceride and cholesterol (2, 4-7). Furthermore, evidence from protein and nucleotide sequencing, as well as peptide and monoclonal antibody mapping, has indicated that apoB-48 is homologous over its entire length with the amino-terminal portion of apoB-100 (8). Consideration of the selective deficiency of only one of the two apoB forms was suggestive of separate genetic control (5, 9). However, the concomitant deficiency of both hepatic and intestinal apoB found in the abetalipoproteinemia and homozygous hypobetalipoproteinemia on the one hand (2, 4, 7), and the presence of an in-frame stop codon into intestinal apoB-100 on the other hand (10-12), are in favor of a single gene coding for apoB-100 apoB-48. Transcriptional or post-transcriptional modification of a single nucleotide from C in apoB-100 for a T in apoB-48 converts codon 2153 in the human apoB-100 sequence from glutamine (CAA) to a stop codon (TAA). This novel RNA editing process raises the possibility that apoB-100 as well as apoB-48 may be synthesized in the human intestine. Indeed, Glickman, Rogers, and Glickman (13) have reported the synthesis of

Abbreviations: VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; Mabs, monoclonal antibodies; PBS, phosphate-buffered saline.



apoB-100 in fetal intestine that progressively decreased during maturation. Concomitantly, apoB-48 appears during this period and becomes the sole isoform present in the adult intestine. Nevertheless, the ontogeny of both the expression of the two apoB mRNAs and the synthesis of the apoB-48 and apoB-100 remains to be established.

In the present investigation, we took advantage of the specificity of various polyclonal and monoclonal antibodies and high resolution immunogold approach to examine the presence of apoB-100 and apoB-48 in human intestinal epithelial cells. Moreover, the de novo biosynthesis of apoB-100 was examined in human intestine using specific immunoprecipitation and $NaDodSO_4$-polyacrylamide gel electrophoresis. Finally, the presence of apoB-100 in human thoracic duct lymph was studied.

MATERIALS AND METHODS

Subjects

Histologically normal jejunal and duodenal biopsy specimens were obtained with a Carey capsule at the ligament of Treitz from six fasting children (boys and girls aged 3 months to 8 years) suspected of malabsorption. Informed consent was obtained from the parents and the study was approved by the Ethics Committee of Hôpital Sainte-Justine.

The human donor of thoracic duct lymph was a 5-year-old boy who was transferred to our institution for evaluation of severe hypertension. Investigations revealed bilateral renal artery stenosis with left renal atrophy, without uremia. He initially underwent unsuccessful left renal angioplasty. Postoperatively, he was noted to have increasing abdominal distension. Abdominal paracentesis confirmed the diagnosis of traumatic chylous ascites. The composition of fluid obtained was 95% chylomicrons, 1% VLDL, and 4% HDL while on a normal diet. Control of ascites was achieved using total parenteral nutrition over a period of 9 weeks, followed by oral nutrition with an enteral formula (Vivonex) and medium chain triglycerides. There was no relapse of the ascites subsequent to his returning to a normal diet. He eventually underwent succesful reconstitution of the right renal artery, and has had no recurrence of his hypertension on medical managements.

An additional segment of normal distal jejunum was obtained for apoB biosynthesis from a patient with Crohn disease undergoing elective bowel resection. Absence of disease in this segment was confirmed by light microscopy.

Tissue preparation for electron microscopy

Intestinal biopsies were fixed by immersion in 1% glutaraldehyde-0.1M phosphate-buffered (pH 7.4) for 2 h at 4°C and embedded in Lowicryl K4M at −20°C according to previously described procedures (14). Tissue blocks were thick-sectioned and examined by light microscopy to select well-oriented villous tips. Thin sections (60-80 nm) of the different tissue blocks were mounted on nickel grids with a carbon-coated Parlodion film and processed for immunocytochemistry.

Polyclonal and monoclonal antibodies

The polyclonal anti-human apoB antibody used in this study was an IgG fraction, raised in sheep, obtained from Boehringer Mannheim (Montreal, Canada). To test the specificity and the potential binding of this antibody, immunodiffusion was performed in a 2% agar gel (**Fig. 1**). The antibody gave a single precipitation arc against purified normal human LDL and against normal human serum, while none was detected against human high density lipoprotein (HDL). The monoclonal antibodies (Mabs), identified as 2D8, 3A10, 4G3, were the products of cell fusion between cells of the plasmacytoma cell line SP 2-0 and isolated spleen cells from BALB/c mice previously immunized with human LDL (15, 16). Mabs were found to be of the IgG class; 2D8 was an IgG_1, whereas 3A10 and 4G3 were IgG_2 (15). These Mabs were directed against different antigenic determinants of both forms of apolipoprotein B (15): 2D8 recognized the NH_2-terminal of the amino acid chain common to both apoB-100 and apoB-48, whereas 3A10 and 4G3 were directed against COOH-terminal of the amino acid chain of apolipoprotein B-100. Immunoblot experiments were performed demonstrating their specificity (16).

Immunocytochemical labeling

Protein A and/or protein G-gold immunocytochemical techniques were applied in order to detect the presence of apolipoprotein B in human intestinal tissues as described previously (14, 17, 18). Briefly, the tissue sections were initially incubated on a drop of saturated aqueous solution of sodium metaperiodate for 25 min, in order to increase the intensity of the labeling (19, 20). The grids were washed in distilled water, further incubated for 3 min on a drop of PBS containing 1% ovalbumin, and subsequently transferred to a drop of the PBS-diluted antibody (see below). After incubation for 90 min at room temperature, the grids were rinsed with PBS to remove unbound antibody; they were

Fig. 1. Labeling obtained with the polyclonal antibody and the protein A-gold complex. Biopsies were taken from children. (a) Apical part of a jejunal enterocyte. Intense labeling is present over microvilli (Mv), apical smooth membrane vesicles (arrow head), and multivesicular bodies (MVB). Gold particles are numerous over interdigitations (In) in the lateral membranes; Centriole (c); (× 30,000) bar: 0.5 μm. (b, c) Golgi regions (G) or jejunal (b) and duodenal (c) enterocytes. Gold particles are distributed mainly over the *trans* cisternae and associated vacuoles (v). Some gold particles are present over rough endoplasmic reticulum (rER). Mitochondria (m) are devoid of labeling. (b: × 30,000 and c: × 26,500) bars: 0.5 μm.

transferred to the PBS–ovalbumin (3 min), and incubated on a drop of protein A (pH 7.2) or protein G–gold complexes (pH 5.0) for 30 min at room temperature. The tissue sections were then thoroughly washed with PBS, rinsed with distilled water, and dried. Sections were stained with uranyl acetate and lead citrate before examination with a Siemens Elmiskop 101 electron microscope.

The polyclonal antibody was used at a 1:3000 dilution in combination with the protein A–gold complex, whereas the Mabs were used as such or diluted 1:3 followed by the protein G–gold complex. The protein A–gold as well as the protein G–gold complexes were prepared using 15-nm gold particles according to techniques previously described (14, 17).

Control experiments were performed to assess the specificity of the antibody interaction. Excess of LDL purified by ultracentrifugation (21) or purified apoB obtained after delipidation of LDL by ethanol–ether 1:1 was added to the antibody solution. The incubation with these solutions was followed by the protein A–gold complex. Preimmune rabbit serum (diluted 1:10) was used on tissue sections before their incubations with protein A–gold complex. An unrelated mouse monoclonal anti-DNA antibody (diluted 1:10) was used followed by the protein G–gold complex in order to determine the extent of nonspecific adsorption of mouse immunoglobulins to tissue sections. Incubations were also performed with the protein A or the protein G–gold complexes alone, omitting the antibody step, in order to test for nonspecific adsorption of the protein A or protein G–gold complexes to the tissue sections.

Pulse labeling of intestinal explants and immunoprecipitation procedure

The intestinal segments were cultured within 20 min of the surgical removal as described previously (6, 7). The mucosal layer was isolated, cut into small pieces, and placed in RPMI-1640 tissue culture medium containing 5% decomplemented fetal calf serum, penicillin (50 units/ml), gentamycin (25 μg/ml), Trasylol (100 units/ml), and [^{3}H]leucine (500 μCi/ml). The petri dishes were thereafter placed in anerobic jars, sealed, and gassed with 5% Co_2–95% 0_2. At the end of the 30-min incubation at 37 °C, explants were washed (3 ×) and homogenized in 1 ml of phosphate-buffered saline (20 mM sodium phosphate, 145 mM NaCl, pH 7.4) containing 1% (wt/vol) Triton X-100, leucine (2 mM), phenylmethylsulfonyl fluoride (1 mM), and benzamidine (1 mM). The homogenate was centrifuged (4 °C) at 105,000 *g* for 60 min in a 50-Ti rotor (Beckman, CA) and subsequently reacted with excess monoclonal anti-apoB (4G3 and 2D8) for 18 h at 4 °C. Pansorbin (Calbiochem, CA) was then added, and the mixture was reincubated at 4 °C for 30 min. The immunoprecipitate was washed extensively and analyzed by linear 3–20% acrylamide gradient and a 2% stacking gel. Gels were sectioned into 2-mm slices and counted after an overnight incubation at 40 °C with 1 ml of BTS-450 (Beckman) and 10 ml of liquid scintillation fluid (Ready-Solv NA, Beckman).

Chylomicron isolation

The separation of chylomicrons from collected human lymph was immediately carried out, after collection of the lymph, in the presence of a preservative mixture containing 500 units/ml penicillin-G, 50 μg/ml streptomycin sulfate, 20 μg/ml chloramphenicol, 10 mM ε-amino caproic acid, 3 mM EDTA, and 1.6 mM glutathione (GSH) + N_2 as described by Lee and Singh (22). Chylomicrons were floated by ultracentrifugation at 25,000 rpm, at 4 °C, for 30 min in a Ti-50 rotor in a Beckman model L5-65 ultracentrifuge. They were washed by two additional spins after layering with saline solution containing the preservatives and immediately used for electrophoresis.

SDS-polyacrylamide gel electrophoresis and immunoblotting

Chylomicron apolipoproteins were separated by SDS-polyacrylamide gel electrophoresis followed by a Western blot (16). The apolipoproteins were detected with the 2D8 monoclonal antibody and iodinated protein A. The autoradiography was performed on XAR-5 Kodak films with intensifier screen (Cronex, Dupont).

RESULTS

Immunocytochemistry

After applying the polyclonal anti-human apoB antibody with the protein a–gold technique on both duodenal and jejunal tissues, a specific pattern of labeling was found in enterocytes (Fig. 1). The apical portion of the cell revealed an intense labeling which was located over microvilli, smooth membrane vesicles, and multivesicular bodies (Fig. 1a). In Golgi areas (Figs. 1b and 1c), labeling was found over the *trans*-cisternae mostly on the lateral sides. Vacuoles associated with the *trans*-cisternae were also labeled (Figs. 1b and 1c) and, in some instances, the labeling was restricted to their limiting membrane. The basolateral plasma membrane was also labeled; this was particularly evident at the level of the interdigitations where the gold particles were aligned along the membranes (Fig. 1a). On the other hand, the rER was only weakly labeled. Very few particles were present over mitochondria and nuclei (Fig. 1). An identical pattern of labeling was observed in enterocytes from either jejunal (Fig. 1b) or duodenal (Fig. 1c) regions.

Although the three monoclonal antibodies used in this study specifically recognized different antigenic determinants of apolipoprotein B, their patterns of labeling obtained with the protein G–gold complex were similar (**Fig. 2, Fig. 3,** and **Fig. 4**). Indeed, the labeling with the 2D8 (Figs. 2a and 2b), the 4G3 (Figs. 3a and 3b), and the 3A10 (Figs.

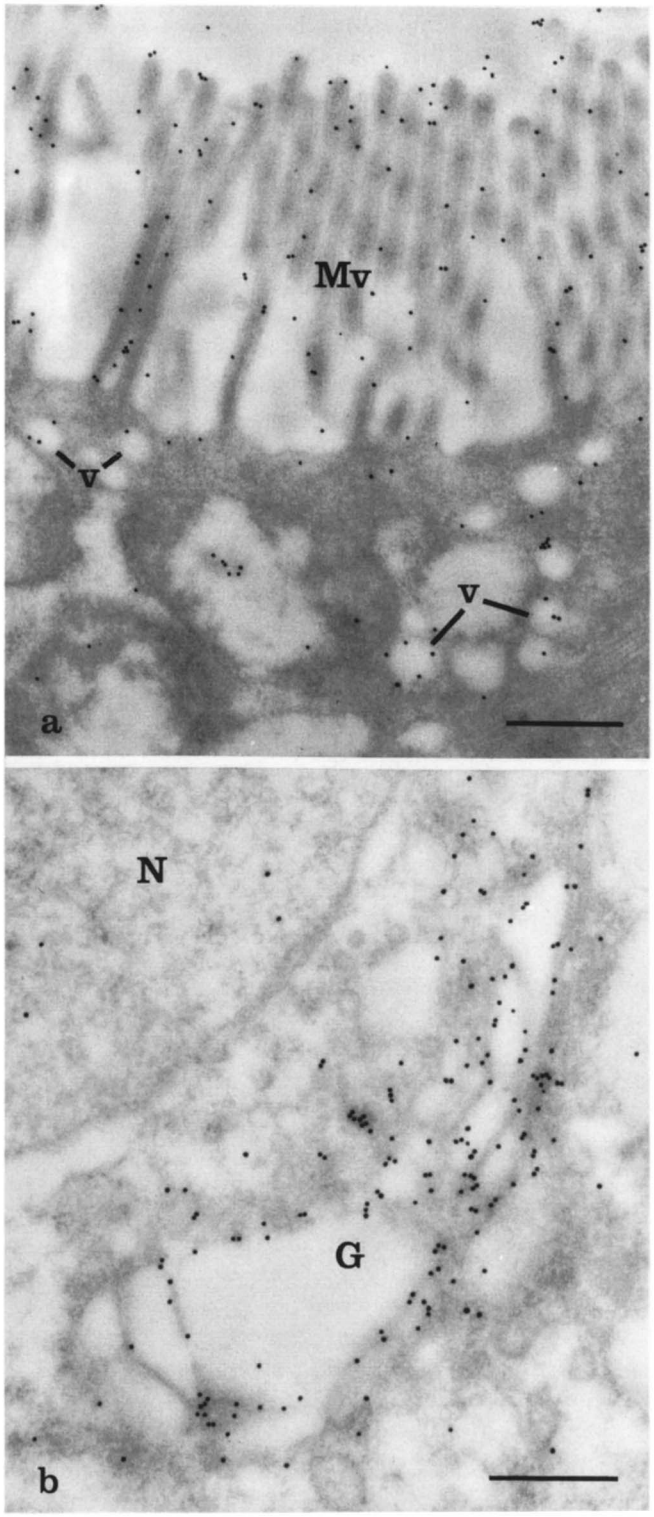


Fig. 2. Labeling obtained with the 2D8 monoclonal antibody and the protein G-gold complex. Biopsies were taken from children. (a, b) Microvilli (Mv), smooth membrane vesicles (v), and Golgi region (G) are labeled. Very few gold particles are found in the gut lumen and the nucleus (N). (a: × 31,000 and b: × 33,500) bars: 0.05 μm.

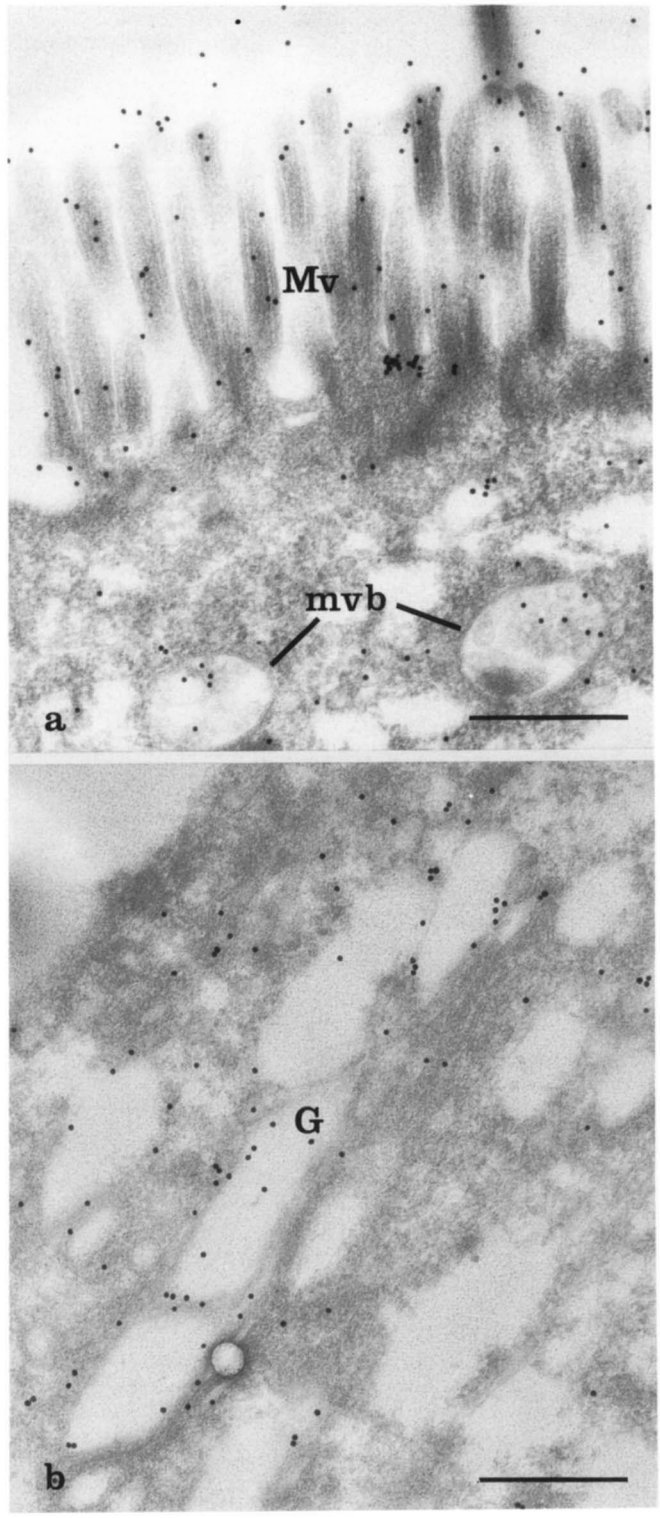


Fig. 3. Labeling obtained with the 4G3 monoclonal antibody and the protein G-gold complex. Biopsies were taken from children. (a, b) Gold particles are located over microvilli (Mv), multivesicular bodies (mvb), and Golgi are (G). (a: × 41,500 and b: × 39,000) bars = 0.5 μm.

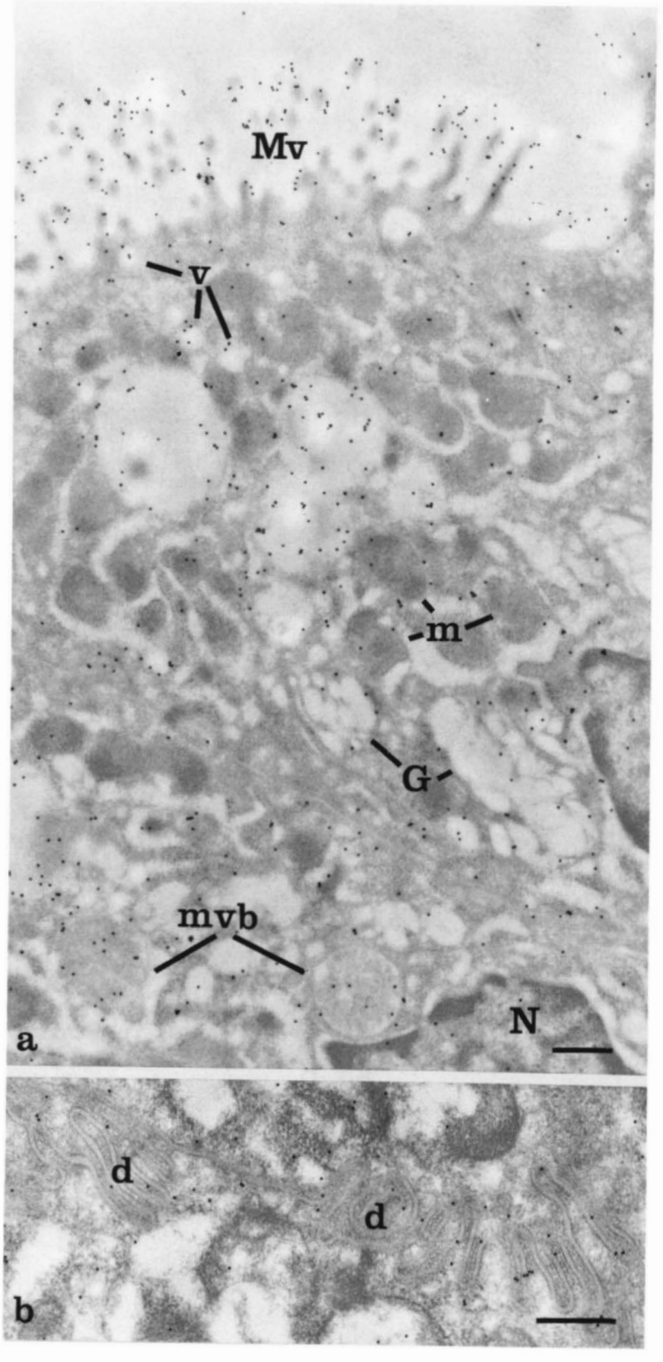


Fig. 4. Labeling obtained with the 3A10 monoclonal antibody and the protein G-gold complex. Biopsies were taken from children. (a) The labeling is present over microvilli (Mv), multivesicular bodies (mvb), and apical smooth membrane vesicles (v). Gold particles are distributed over the Golgi area (G). Few gold particles are associated with the rough endoplasmic reticulum; mitochondria (m) and nucleus (N). (b) The basolateral membrane and particularly the interdigitations (d) of enterocyte are labeled. (a: × 16,000 and b: × 24,000) bars = 0.5 μm.

4a and 4b) monoclonal antibodies was distributed mainly over the apical region of the cell and over the Golgi area. Furthermore, the pattern of labeling was similar to that obtained with the polyclonal antibody. Indeed, a labeling was observed over microvilli, multivesicular bodies, and smooth membrane vesicles in the apical part of the cell. The Golgi area was also labeled while fewer gold particles were found over the rER. The basolateral plasma membrane was labeled, though to a much lesser extent than the labeling obtained with the polyclonal antibody. This labeling observed over the basolateral membrane, however, was not consistent and varied from one cell to the other. As illustrated in Fig. 4, some basolateral membranes were more intensively labeled than others. The only difference found between results obtained with the polyclonal and monoclonal antibodies was in the levels of the intensity of the labeling: the monoclonal antibodies yield labeling of low intensity.

The control experiments did confirm the specificity of the results. Adsorption of the antibodies with LDL or apoB led to significant reduction of the labeling (**Figs. 5a and 5b**). Tissue sections incubated with normal rabbit serum were almost devoid of gold particles (Fig. 5c). The anti-DNA monoclonal antibody followed by the protein G-gold, specifically labeled the nucleoplasm but not the microvilli or the Golgi area (Fig. 5f). Also, no specific labeling was obtained on tissue sections incubated with protein A or protein G-gold complexes alone (Figs. 5d and 5e).

Biosynthesis of apoB in intestinal explants

The radioactivity profile of apolipoprotein B in human intestinal explant is shown in **Fig. 6**. The immunoprecipated apoB from homogenate supernatant revealed two peaks at the positions of B-100 and B-48. The predominant form appeared to be apoB-48.

Chylomicron analysis

To examine whether the apoB-100 was associated with freshly isolated lymphatic chylomicrons, we characterized the content of apolipoproteins by SDS electrophoresis and immunoblotting. After incubation of the nitrocellulose membrane with the 2D8 monoclonal antibody, followed by the iodinated protein A, apoB-100 and apoB-48 bands were clearly delineated by autoradiography (**Fig. 7**). Irrelevant antibodies against insulin or against phenyl-β-D-glucoside did not bind either apoB-100 or apoB-48. These results demonstrate that apoB-100 is present in lymphatic chylomicrons.

DISCUSSION

Controversial data concerning the presence of apoB-100 in intestinal cells have been reported in the literature. Previous morphological studies performed at the light microscopy level were of low resolution and unable to discriminate between apoB-100 and apoB-48 (22–28). In the present study, we have combined high resolution immunoelectron microscopy with polyclonal and monoclonal antibodies specific for intracellular localization of apoB-100 and apoB-48. The synthesis of apoB-100 in intestine and its presence in lymphatic circulating human chylomicrons were also examined in this investigation.

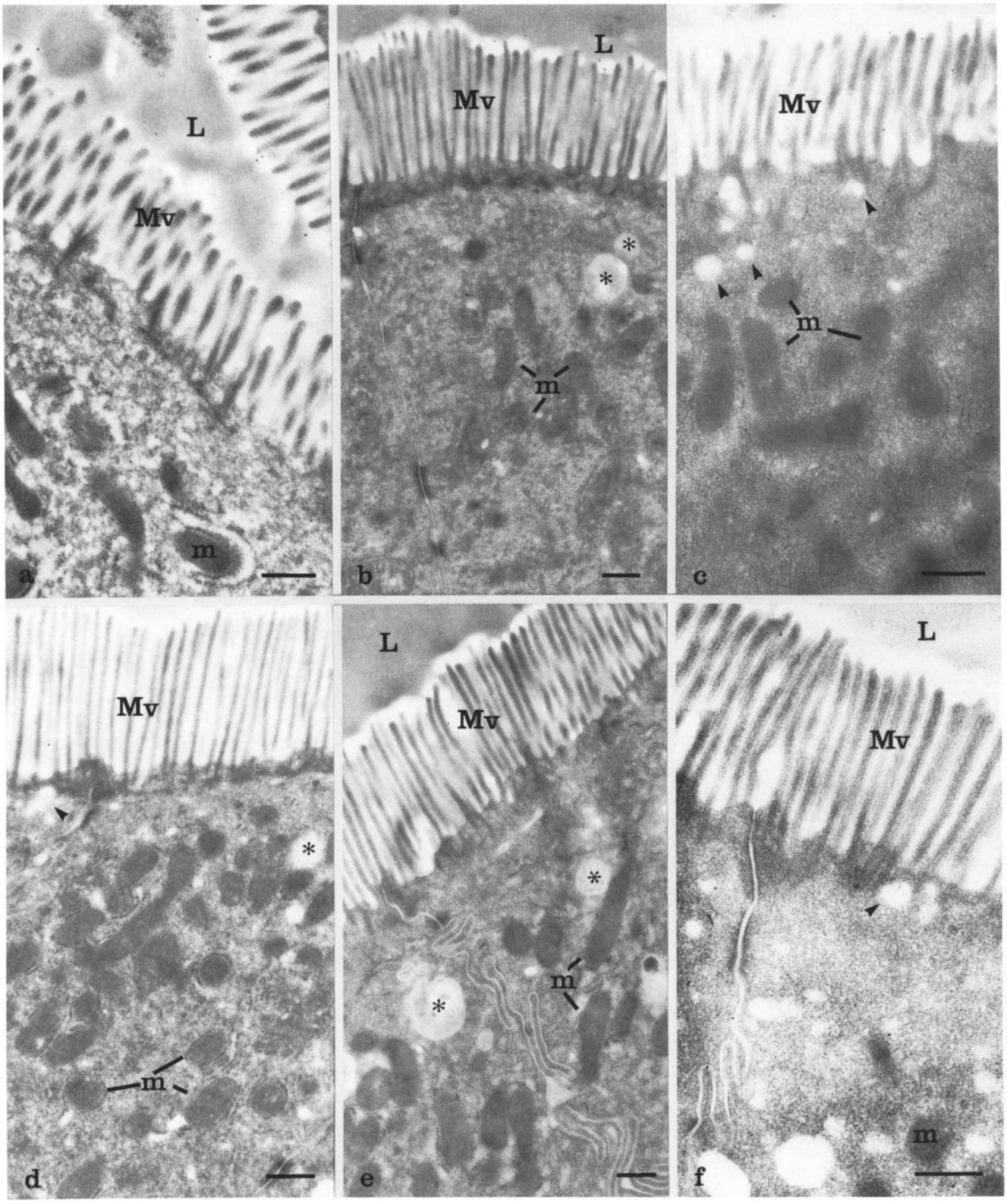


Fig. 5. Control experiments. Biopsies were taken from children. Adsorption of the polyclonal anti-apoB antibody with human LDL (Fig. 5a; × 19,000) or purified apoB (Fig. 5b; × 13,775) resulted in significant reduction of the labeling. Intestinal tissues incubated with normal rabbit serum (Fig. 5c; × 20,425), mouse monoclonal anti—DNA (Fig. 5f; × 23,750), protein A-gold (Fig. 5d; × 16,625), or protein G-gold complexes (Fig. 5e; × 13,775) were devoid of labeling. Microvilli (Mv); apical smooth membrane vesicles (arrow head); multivesicular bodies (*); mitochondria (m); gut lumen (L); bars = 0.5 μm.

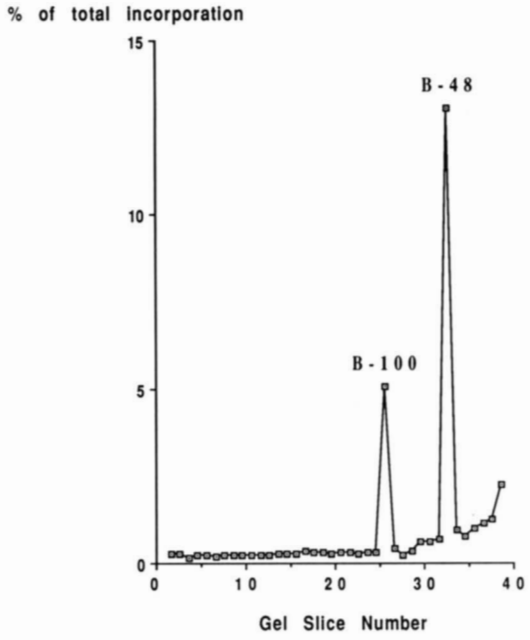


Fig. 6. Synthesis of apoB-100 in small intestine. Explants were incubated with [^{3}H]leucine and apoB was immunoprecipitated. Immunoprecipitates were electrophoresed in 3–20% $NaDodSO_4$ polyacrylamide gels, and the radioactivity in 2.0-mm gel slices was determined.

The ultrastructural localization of the two forms of apolipoprotein B was achieved by applying the protein A–gold (14) and protein G–gold (17) immunocytochemical techniques in combination with highly specific polyclonal and monoclonal anti-apoB antibodies. It has been demonstrated that the protein G–gold complex is more appropriate than the protein A–gold complex for binding mouse monoclonal antibodies (18). Our data reveal that, at the electron microscope level, both apolipoproteins, apoB-100 and apoB-48, are present in several subcelluar compartments of the human enterocytes. The polyclonal and the 2D8 monoclonal antibodies are directed against epitopes common to both forms of the apolipoprotein B, while 4G3 and 3A10 monoclonal antibodies recognize onle epitopes in the apoB-100. Thus, these antibodies constitute a valuable tool for specifically revealing the apoB-100 in the intestinal mucosa.

The combination of protein A–gold with the polyclonal antibody yielded a labeling over the *trans* Golgi region, as well as a low intensity labeling over the rER. The microvilli, apical smooth membrane vesicles, and basolateral membrane were intensively labeled. The labeling on the apical region of the cell could be assigned to an internalization phenomenon. The low labeling intensity over rER indicates a slow rate of de novo synthesis of apoB-100 during the fasting state. It has been previously reported that its rate of synthesis in fasting tissue is slower than under fed conditions (29). The preferential labeling of the Golgi membranes might be due to the high hydrophobicity of apoB. The intense labeling of the *trans* Golgi region obtained in this study might be interpreted as uptake of apoB-100 by the epithelial cells. After internalization, apoB-100 could be transported within smooth membrane vesicles or multivesicular bodies and then transported to the *trans* Golgi citernae. The labeling observed along the basolateral membrane may reflect the preformed pool previously described by Green and Glickman (30) and Sabesin (31) or to hydrophobic apoB preferentially linked to the membrane and associated with a secretory activity.

The intense labeling found over microvilli, multivesicular bodies, and smooth membrane vesicles in the apical area of the enterocyte, reflecting internalization activity, does not fit the classical theory of chylomicron formation (31–35). Microvilli, apical smooth membrane vesicles, multivesicular bodies, as well as the *trans* Golgi cisternae are subcellular compartments traditionally involved in endocytotic activity (36). Our preliminary results (Levy, E., C. Rochette, and M. Bendayan, unpublished results) concerning the internalization of apoB by the enterocyte suggest, in fact, that LDL or VLDL (containing apoB-100) might be incorporated into human epithelial cells. Thus, exogenous apoB might be internalized by absorptive cells and further transported to the *trans* Golgi region to be incorporated into the chylomicron pathway. The exogenous source of the apoB-100 might correspond to the liver, known to synthesize large amounts of apoB-100. Along this line, Sewell et. al. (37) have recently reported the presence of apoB immunoreactivity in bile samples. Thus, apoB-100, originating from liver, might reach the

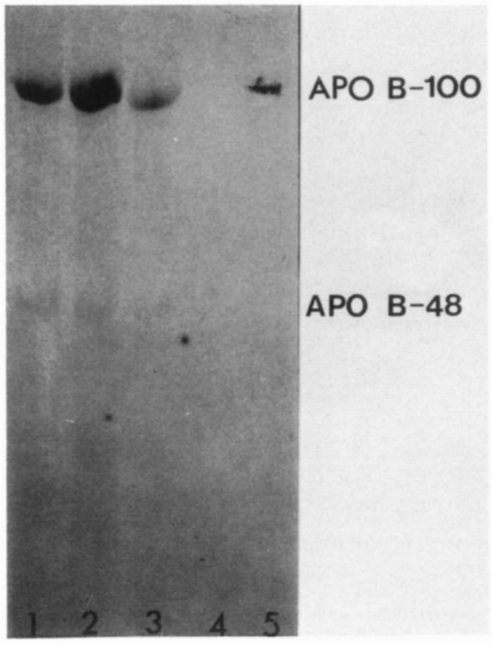


Fig. 7. Immunoblot with 2D8 monoclonal antibody of the proteins isolated from chylomicrons (CM) fractions and separated by SDS-polyacrylamide gel electrophoresis gradient (3–15%). The following samples were applied to the differenct lanes: lane 1, VLDL from type III hyperlipoproteinemia; lanes 2 and 3, two different concentrations of lymphatic CM; lane 4, lymphatic CM reacted with insulin antibodies; lane 5, plasma LDL.

gut lumen through the common duct and be absorbed through endocytotic activity by duodenal and jejunal enterocytes. Along this process, it is unlikely that apolipoproteins would remain as whole polypeptides. ApoB-100 might be partially degraded by luminal proteolytic intestinal enzyme, retaining sufficient antigenic determinants however to be recognized by polyclonal and monoclonal antibodies.

The use of three different Mabs and a polyclonal antibody yielded specific labelings over enterocytes regardless of the age of the patients involved. Although directed against distinct epitopes on the same molecule, they did generate a similar labeling pattern. The main difference between the results obtained with the monoclonal and polyclonal antibodies resides in the level of labeling intensities. Mabs, being directed against a single epitope, yielded lower intensities than the polyclonal antibody which recognizes various antigenic determinants on the apolipoprotein B. The specificity of these Mabs had previously been established and the epitopes to which they are directed have been well characterized (15, 16). On the one hand, 2D8 interacts with the NH_2-terminal portion of the peptide chain common to both forms of apolipoprotein B, while 3A10 and 4G3 interact with the COOH-terminal part of the peptide chain. The three Mabs yielded a similar pattern of labeling which demonstrates that apoB-100 is undoubtedly present in the intestinal epithelial cells. These results are supported by the biochemical analysis demonstrating apoB-100 in lymphatic chylomicrons; plasma chylomicrons also displayed apoB-100. The fact that apoB-100 in the intestine was difficult to detect up to now may be due to the apoB-100 degradation into apoB-48 by an intestinal proteinase during the secretion by epithelial cells (22). Furthermore, Lee and Singh (22) suggested the possible presence of a proteinase that copurifies with chylomicrons and converts apoB-100 from a large molecular form into a smaller one resembling apoB-48. The fact that both forms are present in the intestine is further supported by Higuchi et al. (12) who reported that both organs that synthesize apoB, human intestine and liver, contain two distinct apoB mRNAs, and mRNA that codes for apoB-100 and an apoB mRNA that contains the premature stop codon, coding for apoB-48. In addition, Hoeg et al. (38) have recently proposed that apoB-100 might also be secreted by adult human culture cells, and Dullaart et al. (39) have evidenced local synthesis of intestinal apoB-100 in recessive abetalipoproteinemia.

In view of the detection of apoB-100 in the rER and the Golgi apparatus, it was reasonable to consider that the intestine is able to produce apoB-100. To test this hypothesis, a segment of the small intestine was placed in culture in presence of [^{3}H]leucine. Newly synthesized apoB-100 was evident within 30 min of incubation. A substantial understanding of the regulation of apoB mRNA editing was gained from rat liver which produces both forms of apoB by the same mechanism as in humans (39, 40). Physiological studies using molecular biology techniques in hypothyroid, euthyroid, and hyperthyroid rats have demonstrated that the modulation of apoB-100 and B-48 production is mediated at the level of the RNA editing process (41). In accordance with the counterpart plasma apoB concentrations, hyperthyroid animals contain more than 80% apoB-48 mRNA in their liver, whereas hypothyroid animals contain more than 80% apoB-100 mRNA. Euthyroid animals were intermediate between these two positions, indicating a dose-response relationship for thyroxine. Thus, hormonal and intraluminal factors may determine the proportion of apoB-48 and apoB-100 mRNA. Whether or not a stop codon modulation takes place in the intestinal tissue of our donor patients remains to be clarified. Finally, the information presented in this report lays the basis for further studies that may better define the regulation of apoB mRNA editing.

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