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THE ROLE OF THE MICROSOMAL TRIGLYGERIDE TRANSFER PROTEIN IN ABETALIPOPROTEINEMIA

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■ **Abstract** The microsomal triglyceride transfer protein (MTP) is a dimeric lipid transfer protein consisting of protein disulfide isomerase and a unique 97-kDa subunit. In vitro, MTP accelerates the transport of triglyceride, cholesteryl ester, and phospholipid between membranes. It was recently demonstrated that abetalipoproteinemia, a hereditary disease characterized as an inability to produce chylomicrons and very low-density lipoproteins in the intestine and liver, respectively, results from mutations in the gene encoding the 97-kDa subunit of the microsomal triglyceride transfer protein. Downstream effects resulting from this defect include malnutrition, very low plasma cholesterol and triglyceride levels, altered lipid and protein compositions of membranes and lipoprotein particles, and vitamin deficiencies. Unless treated, abetalipoproteinemic subjects develop gastrointestinal, neurological, ophthalmological, and hematological abnormalities.

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INTRODUCTION

The microsomal triglyceride transfer protein (MTP) is a member of a diverse group of proteins that are able to accelerate the transport of lipid molecules between membranes in in vitro assays. However, the in vivo role of these transfer proteins, in most cases, is not clear. The discovery that the absence of MTP causes the rare human disease abetalipoproteinemia has not only helped to elucidate the in vivo function of MTP at the molecular level, but has also shown the many diverse downstream physiological ramifications of its absence.

This review covers three main areas: (*a*) the biochemical and structural features of MTP, (*b*) the clinical features of abetalipoproteinemia (ABL), and (*c*) recent studies defining the molecular role of MTP in very low-density lipoprotein (VLDL) and chylomicron (CM) production. References related to MTP and its role in lipoprotein assembly are generally limited to the first or the most pertinent examples of what is discussed, and, where possible, references to previous reviews are used. These topics have been covered in detail in recent reviews. More extensive citations are used to support discussions of the clinical aspects of abetalipoproteinemia. A comprehensive review of published case reports of abetalipoproteinemic subjects and discussions of the clinical manifestations of the disease are available on the World Wide Web (*http://neuro.AnnualReviews.org*).

MICROSOMAL TRIGLYCERIDE TRANSFER PROTEIN

Heterodimeric Structure

MTP was originally defined as a protein found in the bovine liver microsomal fraction which catalyzes the transport of triglyceride (TG), cholesteryl ester (CE),

and phospholipid (PL) between membranes. A similar activity was subsequently detected in rat liver and intestine (210). The rat liver protein is isolated from microsomes by extraction procedures that are commonly used to release soluble proteins entrapped within the lumen of microsomes. This fact, combined with the protein's stability when intact microsomes are subjected to protease treatment, indicated that MTP is a soluble protein that is located within the lumen of microsomes.

After its purification, bovine liver MTP was found to be a 150,000-molecular-weight complex of two proteins with apparent molecular weights of 88,000 and 58,000 (209). The small subunit was identified as the previously described multifunctional protein, protein disulfide isomerase (PDI) (206). PDI is a member of the thioredoxin superfamily (reviewed in 58). It is an abundant protein found in high concentrations in the lumen of the endoplasmic reticulum (ER) of most tissues. In some tissues, it approaches millimolar concentrations within the ER. PDI plays an important role in the maturation of resident ER and secreted proteins. It promotes disulfide bond formation, isomerization, and reduction within the ER. PDI is composed of four thioredoxin domains, of which the first and fourth contain a copy of the active site sequence -Cys-Gly-His-Cys-. The unusually acidic pK_a and thus reactivity of the amino terminal Cys of the two active sites contributes to the redox and isomerase activity of PDI. By promoting disulfide bond formation and disrupting incorrect disulfide bonds, PDI allows proteins to avoid being trapped in undesirable folding intermediates or protein-protein aggregates. PDI also has peptide binding and chaperone activities that appear to contribute to its ability to promote the proper folding of newly synthesized proteins.

The presence of PDI in a multimeric protein complex is not unusual. PDI itself is typically isolated from microsomes as a dimer and has been found to be the β component of the tetrameric enzyme prolyl 4-hydroxylase ($\alpha 2 \beta 2$). There are some interesting parallels between the apparent roles of PDI in MTP and prolyl 4-hydroxylase (reviewed in 58). In both cases, PDI is required for the formation of a soluble, active complex. In the absence of PDI, the other subunit forms insoluble aggregates. In the prolyl 4-hydroxylase and MTP complexes, PDI has reduced disulfide isomerase activity, indicating that the active sites of PDI are either buried or inactive. In prolyl 4-hydroxylase, isomerase activity is 50% that of normal PDI, whereas, in MTP, it is $\sim$10% of normal. Coexpression of the large subunit of MTP with PDI or mutant PDI, in which the two active sites are mutated to inactive sequences, showed that the disulfide isomerase activity of PDI is not required for MTP transfer activity. Similar studies indicated that the disulfide isomerase activity of PDI is not required for prolyl 4-hydroxylase activity either. Coexpression studies have also shown that the carboxyl-terminal 27 or 30 amino acids of PDI are necessary but not sufficient for PDI binding to the α subunit of prolyl 4-hydroxylase or the MTP large subunit, respectively.

Tertiary Structure Model

The cDNA encoding the large subunit of MTP has been cloned from a variety of species and found to be highly conserved (reviewed in 208). The human protein is composed of 894 amino acids, including an 18-amino-acid signal peptide. The mature protein has a molecular mass of 97 kDa (172). Although the MTP large subunit is not highly homologous to other known proteins, Shoulders et al (175) predicted, based on sequence similarities, structural predictions, and a comparison of gene structures, that MTP is a member of the vitellogenin gene family. This was subsequently supported by X-ray crystallographic data indicating that MTP and lipovitellin have similar structural folds (189). Lamprey lipovitellin, the vitellogenin gene product, forms a homodimer. It contains a β-barrel amino-terminal region, followed by an α-helical structure and a carboxyl-terminal lipid-binding cavity containing two β-pleated sheets (5). The two monomers interact through contact between their β-barrel and α-helical structures. In a model by Mann et al (133), the amino-terminus of MTP (amino acid residues 34–263) has 13 β strands and corresponds to the barrel-like structure of lipovitellin. This is followed by an α-helical domain (amino acid residues 304–598). A yeast two-hybrid approach and co-expression studies were used to provide evidence that the large subunit of MTP interacts with PDI through its α-helical domain.

Lipid Transfer Activity

MTP catalyzes the transport of a wide variety of neutral and PL molecules in in vitro assays. A typical assay measures the transport of radiolabeled or fluorescently labeled lipid molecules between PL small unilamellar vesicles. In these assays, MTP has a distinct preference for transporting neutral lipid molecules, in particular TG and CE, when transfer rates are expressed as percentages of vesicle lipid transferred per time (209). Because of the low solubility of neutral lipid molecules within a PL bilayer, only a small fraction of the substrate vesicle is actually neutral lipid ($<$4 mol%). Thus, although the fractional transfer rate of PL is about 2%–3% that of TG, there are so many more PL molecules in the substrate membrane that there is actually much more PL transported than neutral lipid.

Kinetic and lipid-binding studies have demonstrated that MTP binds and shuttles individual lipid molecules between membranes. MTP appears to have one neutral lipid-binding site and two or three PL-binding sites (7). Not all of the PL-binding sites are equivalent in that there appear to be one or more sites that rapidly transport PL and another one or more sites that transport PL more slowly. The binding of a PL molecule to MTP probably does not involve specific contact between the protein and the headgroup of the PL molecule because MTP has little or no preference for the various PL molecules it transports (102). MTP transports acidic (phosphatidic acid, phosphatidylserine, or phosphatidylinositol), zwitterionic (phosphatidylcholine or phosphatidylethanolamine), and even basic (ethyl-phosphatidylcholine) PL molecules with approximately equal rates.

ABETALIPOPROTEINEMIA

Microsomal Triglyceride Transfer Protein and Apolipoprotein B–Containing Lipoproteins

Apolipoprotein B (ApoB)-containing lipoproteins are composed of a large TG core surrounded by more polar components, including PL, free cholesterol (C), and protein (reviewed in 79). Human liver produces a particle with a 30- to 80-nm diameter, VLDL, which contains ApoB100 (4563 amino acids). Human intestine produces even larger particles with diameters as large as 75–1200 nm, called chylomicrons (CM). Their primary protein component is ApoB48. In the intestine, an editing process introduces a stop codon post-transcriptionally into the mRNA encoding ApoB100, producing an mRNA encoding ApoB48, which is about 48% of the full length of ApoB100.

VLDL and CM are assembled within the lumen of the ER of hepatocytes and enterocytes, respectively; transported to the Golgi apparatus; and then secreted. CM and VLDL are converted to CM remnants and low-density lipoproteins (LDL) in the circulatory system and are subsequently cleared by receptor-mediated processes. Early studies characterizing MTP suggested that it plays an important role in the assembly of VLDL and CM. MTP is found at the sites of assembly, and it was speculated, based on its in vitro transfer activity, that MTP is transporting TG and possibly CE and PL from the ER membrane, where lipid molecules are synthesized, to developing lipoproteins within the lumen of the ER. The first direct evidence supporting this hypothesis came from studies that demonstrated that ABL, which is characterized by a defect in the production of CM and VLDL, is caused by defects in MTP.

Clinical Characteristics of Abetalipoproteinemia

The clinical manifestations of ABL was initially described by Bassen & Kornzweig (15). An association of the signs of lipid malabsorption, acanthocytosis, pigmentary degeneration of the retina, and ataxia with hypocholesterolemia (104, 109), suggested that the disease results from an inborn error of lipid metabolism that leads to deleterious effects on the erythrocytes and nerve cells. The characteristic absence of lipoproteins with beta-electrophoretic mobility (121, 131) led Salt et al to propose the name "abetalipoproteinemia" for the disease (168). About 100 cases of ABL have now been reported (see supplementary Table, available online only, through *http://www.AnnualReviews.org*, Electronic Materials). Approximately one-third of the cases result from consanguinous marriages, and family studies suggest an autosomal recessive mode of transmission. The sex ratio is 1/1. Most of the obligate heterozygotes (∼80%) have been reported to have normal lipid levels. However, there are at least 19 obligate heterozygotes with cholesterol >240 mg/dl (2, 16, 26, 36, 53, 61, 87, 88, 96, 115, 140, 145, 154). This suggests that there is an adaptation in the heterozygous state with an increase in the synthesis of MTP

by the normal allele or that MTP activity is present in excess in the normal state, with a single allele being able to provide a sufficient level of activity. However, Raabe et al (157) have noted that, for 8 cases of ABL for which the mutation in MTP has been established (145), 7 (two of which are young children) of 15 obligate heterozygotes have low C levels (130–165 mg/dl). We have noted four additional obligate heterozygotes with low C levels (88, 96, 154, 165). Three additional Japanese heterozygotes have C values between 122 and 159 mg/dl but are reported as being within normal limits for Japanese individuals (2, 90).

At birth, infants with ABL are asymptomatic. With a diet that is rich in lipids, digestive signs appear during the months after birth. The initial presentation is that of celiac disease with diarrhea, vomiting, and abdominal swelling. Later on, the digestive signs abate, in part because the patients themselves institute a lipid-poor diet because of their intolerance to lipids (2, 64, 110, 178, 212). The chronic malabsorption of lipids leads to lipid-soluble vitamin deficiency because the plasma transport and tissue distribution of these vitamins depend almost exclusively (vitamin E, beta-carotene) or in part (vitamins A, D, and K) on the lipoproteins containing ApoB. Plasma levels of vitamin E and beta-carotene are thus extremely low in cases of ABL. Because of alternate modes of transport which are present to various degrees, the levels of vitamin A, vitamin K, and vitamin D are diminished, but not to the extent of vitamin E or beta-carotene.

During the first 10 or 20 years of life, the vitamin deficiencies result in neuro-opthalmologic complications that dominate the clinical picture and determine the morbidity of ABL. Dietary vitamin supplementation may prevent these complications. However, there is a great degree of heterogeneity in the evolution of the disease. Except for one case (2), all reported ABL patients >20 years of age who have not received vitamin E supplementation exhibit neuro-retinal complications. Some patients are blind and bedridden. In $\sim$25% of the cases reported in the literature, the diagnosis was established after 20 years of age. This clinical heterogeneity remains unexplained.

As introduced above, there are numerous downstream consequences resulting from an absence of MTP. Abnormalities in lipid absorption and transport lead to the various pathologies observed in ABL patients. These are summarized in Figure 1 and described in detail below.

Intestinal Signs Steatorrhea, which reflects lipid malabsorption, is always present under a normo-lipidemic diet. Maternal milk, rich in lipids, and diets not diminished in their fat content are poorly tolerated. There is normal intraluminal digestion of lipids, micelle formation, and absorption of fatty acids (FA) by the enterocytes (11, 87, 100, 122). Furthermore, the FA are normally re-esterified into TG and PL (11, 100, 201). The lipid malabsorption leads to decreased gains in height and weight, and may lead to secondary malabsorption of other nutrients. These secondary malabsorptions disappear after installation of a fat-poor diet.

Endoscopic examination of the intestine reveals a "gelée blanche" or white hoar frosting appearance (2, 46, 171, 212). This coating of the duodenum and jejunum

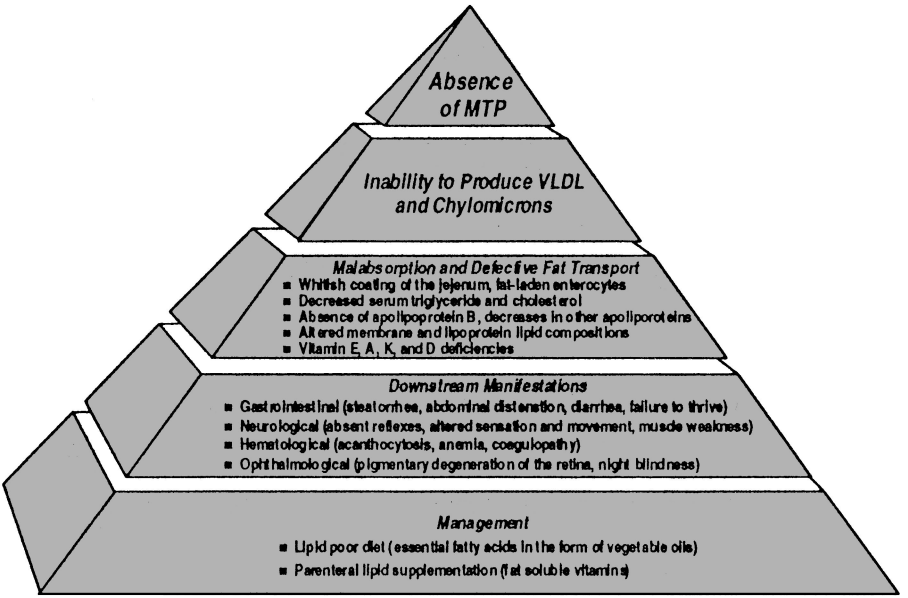

Figure 1 Pathophysiology of microsomal triglyceride transfer protein deficiency. The absence of MTP leads to the inability to produce ApoB-containing lipoproteins and numerous downstream consequences.

reflects the infiltration of the mucosa by lipids. Histologically, the mucosa of biopsies taken from the duodenal-jejunal region of fasting patients is of normal thickness with villi of normal height. The enterocytes are distended with a clarified cytoplasm owing to the presence of numerous vacuoles that predominate in the upper two-thirds of the villi. The strong staining with Oil Red indicates the presence of neutral lipids (mainly TG) in the vacuoles and also suggests that re-esterification of FA is probably normal. In patients with ABL, the lipid content of the jejunal mucosa is 1.5- to 3.5-fold that found in normal subjects (202). Neither intestinal fat loading nor steatorrhea exists in the parents of patients (202).

Electron microscopy shows lipid droplets that are variable in size, the majority of which are very large. In some cases the lipid droplets are free in the cytoplasm, not surrounded by a membrane (29, 100, 108), whereas in other cases they are membrane bound (48, 100). The extracellular spaces are devoid of CM-size particles. The results suggest a defect in TG-rich lipoprotein assembly (29, 48, 100, 178).

Hepatic Signs Hepatic biopsies consistently show an hepatic steatosis (8, 11, 22, 24, 36, 41, 53, 96,100, 182, 203, 212). Of the 12 cases of steatosis reported, 3 are moderate centro-lobular steatoses, which are not accompained by elevated transaminases nor by hepatomegaly (53, 100, 212). Three other cases are associated with a discrete elevation of transaminases either with (8) or without (36, 41) associated

hepatomegaly. Finally, in four cases, an evolution to fibrosis was noted (24, 96, 182, 203), one progressing to cirrhosis and requiring transplantation (24). Here the steatosis was more extensive, with the hepatocytes being distended and filled with voluminous lipid droplets. As in the intestine, the livers of most ABL subjects contain marked accumulations of lipid droplets (8, 11, 24, 41, 48, 53, 87, 100, 212). The lipid droplets are reported, in some cases, to be surrounded by a membrane (11, 41) and, in other cases (8), are cytoplasmic and not membrane bound. Although hepatomegaly and/or abnormal hepatic-function tests with elevated transaminases have been reported in seven other cases of ABL (47, 87, 96, 107, 153, 163, 171), reports of severe liver pathology are rare.

Neurological Signs The first neurological sign is often the diminution and then the loss of deep tendon reflexes followed by a progressive alteration of proprioception (loss of position and vibratory senses and a positive Romberg sign), a cerebellar syndrome (dysmetria, ataxia, and wide-based spastic gait), and muscular weakness (with development of a kyphoscoliosis, lordosis, and pes cavus) (see 15, 16, 31, 32, 40, 47, 56, 87, 96, 105, 117, 121, 130, 139, 140, 170, 177, 187, 201, 211; see also supplementary Table, available online only, through *http://www.AnnualReviews.org*, Electronic Materials). The myopathy is complex, resulting from both neural degeneration and an intrinsic myositis (10, 47, 82, 84, 119, 123, 211). Although the clinical course is variable without treatment, it leads progressively to impaired mobility, and some patients require a wheelchair, or they become effectively bedridden (32, 36, 64, 100, 135, 177, 195, 203, 212).

Ophthalmological Signs The first ophthalmological signs are alterations in night and color vision (see supplementary Table, available online only, through *http://www.AnnualReviews.org*, Electronic Materials). A decrease in visual acuity follows. The visual field shows a concentric contraction. Fundoscopic examination shows an atypical pigmentation of the retina, which is characterized by brilliant small, white spots (rounded or elongated) that are irregularly distributed and attain the macula in some cases. In some cases, angioid streaks have been reported (52, 70).

The electro-retinogram and angiofluorography show that the retina is affected even before the presence of clinical signs and anomalies in the fundoscopic examination. In more advanced stages, scotopic elements disappear with an evolution towards extinction. The rare histological examinations that have been performed show a disappearance of the photo-receptors and an invasion of the retina by pigmentary epithelium with an accumulation of lipofuscin (39, 196). Opthalmoplegia, ptosis, and anisocoria have also been described (109).

Hematological Signs Acanthocytes (erythrocytes with irregular cytoplasmic projections) are found in ABL subjects. Because acanthocytes don't form rouleaux, they have a very low sedimentation rate. Although initially described in ABL (15), acanthocytes are not specific for this disease. In patients with ABL, acanthocytes

often represent >50% of the erythrocyte population. The deformation of the erythrocyte membrane is irreversible and is produced after erythrocytes pass into the peripheral circulation (15).

The membrane deformation might be explained by the decrease in the erythrocyte membrane fluidity that is observed in patients with ABL. Changes in membrane fluidity could result from changes in lipid composition. Although the global amounts of PL and C in the acanthocyte membrane are little or not at all modified, the ratio of sphingomyelin to lecithin is always increased (11, 13, 42, 43, 90, 127, 134, 155, 201). There also exist anomalies in the FA composition of the PL (essentially the lecithins), which are deficient in linoleic and arachidonic acids and which also contain increased amounts of saturated FA (11, 13, 42–44, 87, 90, 155, 201). These anomalies, which are more marked in older erythrocytes than younger ones (200), may be responsible for the secondary deformation into acanthocytes observed in ABL.

Normal erythrocytes acquire an acanthocytic form after transfusion into abetalipoproteinemic patients and circulation in the plasma (63). Incubation of normal erythrocytes with plasma from patients with ABL, however, does not result in these modifications (43, 170). This suggests that the mechanisms of formation of the acanthocytes in ABL are complex and do not result simply from the passive exchange of lipids between the erythrocyte membrane and the plasma, but also may reflect exchange with vessel wall membranes.

In patients with ABL, a moderate to severe anemia may be observed (see supplementary Table, available online only, through *http://www.AnnualReviews.org*, Electronic Materials). The anemia is associated with hemolysis and shortening of the erythrocyte half-life (50, 137, 176, 201). The hemolysis is not related to an increase in osmotic fragility (11, 176). It might be related to a decreased deformability of the erythrocytes, which is related to a decrease in the membrane fluidity of the acanthocytes. It also could be caused by vitamin E deficiency. It has been shown that during vitamin E deficiency, there is peroxidation of the unsaturated FA of the membrane PL, leading to hemolysis (11, 112, 124, 176, 202).

Coagulation Abnormalities Abnormalities in coagulation (elevated prothrombin time) in patients with ABL are caused by deficits in vitamin K-dependent coagulation factors, arising from the lipid malabsorption (see supplementary Table, available online only, through *http://www.AnnualReviews.org*, Electronic Materials). This deficit may be symptomatic (3 cases) and lead to hemorrhagic signs (11, 34, 182).

Platelet aggregation functions are largely dependent on lipid factors, including platelet arachidonic acid, a precursor of prostaglandin (proaggregant), and lipoproteins. In ABL, there are modifications of both platelet and lipoprotein lipids [decrease in linoleic and arachidonic acids and increase in C (44, 185)], which may alter platelet aggregation functions. Nevertheless, in patients who show a perturbation of in vitro platelet aggregation tests, there is no increase in the bleeding time nor hemorrhagic diathesis (44, 185). Platelet activation in ABL patients may

occur via high-density lipoproteins (HDL) (9) compared with normal individuals, in whom HDL inhibit platelet reactivity (180). This suggests that the repercussions on platelet function in vivo are limited.

Cardiac Complications Five cases of rapidly evolving cardiac insufficiency leading to premature death have been reported (16, 36, 47, 177, 203). In one case, the post-mortem studies showed an interstitial myocardial and pericardial fibrosis and deposits of lipofuscin (47) that are reminiscent of vitamin E deficiency. However, the relatively low incidence of these myocardial effects suggests the presence of additional factors.

Recent studies have shown that, in humans, the heart may also express both ApoB100 and MTP and therefore has the capacity to secrete lipoproteins (27, 149). The physiological role of this cardiac secretion remains to be determined. In ABL, the lack of assembly of lipoproteins in the intestine and the liver leads to a tissue lipid overload. Nevertheless, post-mortem histological studies of these patients do not show any cardiac lipid overload (47, 177). The relationship of MTP expression in the heart to the myocardiopathies observed in some ABL patients is unknown.

Lipid Metabolism

Plasma Lipoproteins and Apolipoproteins The biological signature of ABL is the absence of ApoB-containing lipoproteins and plasma levels of ApoB that are virtually undetectable by classical methods. After a fat load, CM and ApoB48 do not appear in the plasma. In fasting ABL patients, the plasma C levels are very low (20–50 mg/dl). TG levels are often $<$10 mg/dl. Furthermore, after a fat-rich meal, the TG levels do not increase (see supplementary Table, available online only, through *http://www.AnnualReviews.org*, Electronic Materials). PL and free FA are also decreased (11, 17, 70, 87, 100, 106, 108, 138, 201).

Small amounts of circulating ApoB100 have been detected (1, 24, 51, 74, 136). In six ABL patients, there was a low amount of circulating ApoB100 as well as N-terminal ApoB degradation fragments, the majority of which were 85 kDa (51). These degradation fragments were also detected in normal individuals. The small amounts of ApoB100 present in the plasma (1) or in the medium of ABL hepatocytes cultured with ^{35}S-methionine (24) are in the form of lipoproteins of LDL density. These particles did not show anomalies of density or charge as compared with LDL from normal subjects. However, they were enriched in ApoAI, AII, CIII and E, and unesterified cholesterol. Despite the virtual absence of ApoB100 in ABL, there are low to normal levels of circulating Apo (a) [0.2–2.03 mg/dl (86, 136)], some of which is associated with ApoB.

Although HDL and ApoAI are decreased $\sim$50%, HDL is the major lipoprotein population present in the plasma of ABL subjects and contains virtually all of the plasma C and TG. Kinetic studies in vivo show that this decrease results from both a decrease in production and an increase in catabolism (38, 91). These results suggest that the absence of ApoB100 and ApoB48 secretion has an impact on HDL biosynthesis.

The HDL of ABL patients are enriched in total C (25, 29, 118), with an increased ratio of free to esterified C (38, 169), and are also relatively enriched in PL (38, 62), with an elevated ratio of sphingomyelin to lecithin (12, 45, 106, 169, 201). In parallel with the increased ratio of free to esterified C of the HDL, there is a decrease in the lecithin:cholesterol acyltransferase (LCAT) activity of ABL patients (43, 86, 118, 169, 183). This diminution in LCAT activity results, in part, from a decrease in LCAT protein mass, but also from the absence of acceptor lipoproteins (59), because the LCAT activity can be partially restored in vitro in the presence of normal LDL and VLDL (183). Furthermore, the post-heparin lipolytic activities of lipoprotein lipase and hepatic lipase are diminished to about one-half of normal in ABL (92).

The FA composition of the PL in HDL is modified with relatively less FA of type 18:2 (linoleic acid) and 20:4 (arachidonic acid) (12, 106, 169). Because lecithin is the substrate of the esterification reaction of C by LCAT, the FA composition of the CE is similarly modified, because there is less 18:2 and more 18:1 FA (12, 106, 169, 184).

The enrichment in lipids is manifested by an increased average diameter (25, 29, 38, 45, 62, 106, 169) and a decreased density of the HDL particles as compared with the HDL_2 of normal subjects. There is also a minor population of very small and very dense spherical HDL of the HDL_4 type (38, 45, 169). In general, the HDL that are rich in ApoE have diameters that are larger than those of HDL that are poor in ApoE and are catabolized more rapidly (25, 91).

Plasma levels of ApoAI and ApoAII, which are the major HDL apolipoproteins, are 50%–70% lower than normal (29, 38, 65, 88, 91, 118, 126, 154, 159). Kinetic studies in vivo show that the decrease in plasma ApoAI results both from a decrease in production and normal or increased catabolism (91, 173). A major characteristic of the HDL of ABL patients is their enrichment in ApoE [twice as much as normal (25, 45, 91, 94)], allowing the plasma levels of ApoE to remain normal or even slightly increased in ABL (25, 173). The in vivo rate of production is equivalent to that in normal subjects (91).

The fasting level of plasma ApoAIV is diminished by one-half as compared with that in normal subjects (65, 126) and does not increase post-prandially in ABL (126). ApoAIV mRNA levels and the synthesis of ApoAIV are $\sim$50% of normal in the enterocytes. The plasma levels of ApoCI, CII, and CIII are also low (94, 186). Only the hypersialylated forms of ApoE and ApoCIII (29, 37, 62, 71, 94, 118, 169) are present in the plasma, and the isoform pattern of ApoAII is altered (29).

Factors controlling the synthesis and secretion of other apolipoproteins (AI, AIV, C, and E), as well as the assembly of HDL, as related to the ApoB-containing lipoproteins, are poorly understood. In HepG2 cells, ApoE can be synthesized and secreted independently of the synthesis and secretion of ApoB-containing lipoproteins (57). Similarly, in hepatic cell lines, MTP inhibitors decrease the secretion of ApoB-containing lipoproteins but do not interfere with the secretion of ApoAI (68, 76, 103). Finally, in ABL patients, small apolipoproteins are secreted independently of ApoB-containing lipoproteins (B100 and/or B48).

Cholesterol Metabolism In ABL, despite the absence of ApoB-containing lipoproteins and, in particular, LDL which normally assures the distribution of C to cells via the ApoB/E receptor, the metabolism of C is not markedly disturbed. In most ABL patients, a state of C deficiency is not observed. The synthesis of C in subjects has been found to be normal or somewhat elevated, the elevation perhaps being caused by impaired reabsorption of biliary cholesterol (67, 95, 144). The synthesis of C in cells that are isolated from patients with ABL has, in general, been reported as normal or increased (3, 85, 161). Furthermore, in clinical studies, measurements and functional tests of steroid hormones are most often normal (53, 178, 202, 212), although corticotropin-stimulated maximal cortisol secretion may be less than normal (64, 97). At least seven female ABL patients have had normal pregnancies and deliveries at term of normal children (50, 55, 66, 110, 135, 173; M.-E. Samson-Bouma, unpublished data). One male subject has fathered a normal child (96).

Although, in normal adult subjects, HDL are principally engaged in reverse C transport to the liver, the HDL of the ABL patients may assure forward C transport and thus substitute functionally for LDL (25, 93, 94, 99). It has been estimated that ApoE-enriched HDL have the capacity to deliver to tissues, via the LDL receptor, an amount of C corresponding to a plasma LDL concentration of 50–150 mg/dl (25).

Vitamin Deficiency

In all ABL patients, the plasma levels of vitamin E are very low. This decrease is observed at birth (140) and is secondary to a defect in the transport of vitamin E (114). The metabolism of vitamin E is closely linked to the metabolism of lipoproteins that contain ApoB. Vitamin E is absorbed with alimentary lipids in the form of micelles. It is then secreted and transported in CM. In the course of CM lipolysis, a part of the vitamin E is distributed to tissues with the hydrolyzed FA. The other part is captured by the liver with the CM remnants and then re-secreted in VLDL. In a normal fasted subject, the plasma vitamin E is found mainly in the LDL fraction. It is then distributed to the other tissues via the LDL receptor (192). It should be noted that, like lipids, vitamin E exchanges among the different lipoprotein classes. Part of the plasma vitamin E, therefore, is found in HDL.

In 1965, Kayden & Silber were the first to propose that the neurological and ophthalmological complications of ABL could result from lack of vitamin E (112). They observed that the neuro-ophthalmological signs present in patients with ABL were identical to those observed in monkeys with vitamin E deficiency (60). Later, other arguments reinforced this hypothesis.

ABL patients show the same lesions in the nervous system as those observed in vitamin E-deficient experimental animals (80, 132, 147, 164, 190) and in malabsorption syndromes including tocopherol deficiency (78, 116, 181, 204). In addition, ABL patients have deposits of lipofuscin in several tissues [skeletal striated muscle, liver, myocardium, retina, smooth muscle of the intestinal wall, and spinal

cord (39, 41, 47, 84, 119, 123, 177, 196)], which suggests vitamin E deficiency. The formation of this pigment is an indication of lipoperoxidation (152) and can result from an attack of free radicals on the mitochondrial membrane, secondary to an absence of the anti-oxidant activity of vitamin E (21).

The role of vitamin A in retinal complications in ABL is not clear. The night blindness that is found in ABL is classically a sign of vitamin A deficiency. Nevertheless, other signs of vitamin A deficiency (xerophthalmia and keratomalacia) are rarely mentioned in ABL. Supplementation with vitamin A alone may sometimes improve the nightblindness and abnormalities of the electroretinogram (22, 23, 72, 82, 179) but do not prevent or correct the pigmentary degeneration observed in ABL (82, 213).

Treatment

Diagnosis and early treatment are essential to avoid growth retardation and neuroretinal complications secondary to chronic lipid malabsorption and in particular, to deficits in the lipid-soluble vitamins. What is known about the various treatments of ABL is discussed below.

Lipid Malabsorption The chronic diarrhea and vomiting caused by the lipid malabsorption leads to secondary deficiencies in carbohydrates and proteins. A lipid-poor diet eliminates signs of digestive intolerance and permits normal absorption of carbohydrates and proteins. The proportion of protein and carbohydrate in the diet must be increased to provide an adequate number of total calories. Under these conditions, the lipid-poor diet allows resumption of growth in height and weight. In general, lipids are initially limited to 5 g/day in children. Later, depending on individual digestive tolerances, larger quantities of lipids may be allowed. Studies of the effects of a lipid-poor diet on liver and intestinal lipid excess are contradictory (53, 171, 202). To combat malnutrition, medium-chain TGs may be used. However, the suspicion that they induced hepatic fibrosis in an ABL patient (96) has limited their use.

Deficiency in Essential Fatty Acids In ABL patients, there is a decrease in the plasma level of linoleic acid (18:2) (63, 106, 110, 169), which is accompanied by a decrease in linoleic acid levels in erythrocytes and platelets (12, 90, 110, 201), and in the adipose, hepatic, and intestinal tissues (100, 135, 201). In contrast, the plasma levels of arachidonic acid (20:4) have been found to be normal (110) or decreased (87, 100). A role for docosahexanoic acid, a metabolite of linolenic acid, in nerve and retinal function has been suggested (83). Plasma levels of this acid are decreased in some patients (159). However, deficits in nervous and retinal tissue remain to be shown.

Despite the decrease in the plasma and tissue levels, several arguments exist against an important risk of FA deficiency in ABL patients. The plasma levels of eicosatrienoic acid (20:3ω-9), which is a marker of essential FA deficiency in animals, are only slightly elevated in ABL patients (14, 53, 100, 106, 110).

Furthermore, the classical clinical signs of essential FA deficiency, anomalies of the skin and hair, are only exceptionally reported in ABL (53, 119, 142).

Although ABL subjects have an inability to form CM, the lipid malabsorption is relative. The coefficients of absorption of lipids are diminished (∼50%–75%), but not zero (see supplementary Table, available online only, through *http://www.AnnualReviews.org*, Electronic Materials). With an enriched diet, plasma levels of linoleic acid sometimes increase, although they do not normalize (14,106,110, 135,156). This suggests that, in the absence of ApoB, there are alternative mechanisms for the secretion of lipids from the enterocyte. The lipids could be secreted by the portal pathway in the form of FA bound to albumin and/or via the lymphatic pathway in the form of PL associated with ApoAI or AIV.

The recommendation at present is that the lipid-poor diet must provide the daily requirements in essential FA in the form of vegetable oils [the maximum tolerated (110)]. In addition, weekly or twice monthly parenteral treatment, consisting of an intravenous perfusion of lipid emulsions containing lipid-soluble vitamins, is sometimes added (53). The benefits of this parenteral treatment have not been throughly evaluated to date.

Deficiency in Lipid-Soluble Vitamins The principal function of vitamin E is to capture peroxide radicals and, thus, to interrupt the chain reaction of lipid peroxidation (33). Therefore, its deficit, as shown by the peroxidative hemolysis test, leads to an increase in the peroxidation of polyunsaturated FA in photoreceptor cells, myelin, and cell membranes in general. In the absence of lipoproteins containing ApoB, the plasma levels of tocopherol do not reflect the tissue bioavailability. Traber et al have proposed evaluating the vitamin status of these patients by measuring adipose tissue levels, because their studies show a correlation between the concentrations of α-tocopherol in adipose tissue and in peripheral nerve tissue (195).

After therapy with vitamin E, the plasma levels increase (10, 22–24, 41, 53, 82, 96, 110, 135, 143, 171, 212). However, other than in exceptional cases (41, 171), the plasma levels do not normalize and rarely exceed 10%–30% of the normal levels even after long-term therapy. Nevertheless, the levels in adipose tissue (41, 82, 111, 194), hepatic tissue (22), and erythrocytes (41, 110) may increase and even normalize under massive doses of vitamin E administered by oral and/or parenteral routes. Furthermore, the autohemolysis (112, 176) and the peroxidative hemolysis (49, 135, 143) in ABL may be corrected, in vitro as well as in vivo, by the addition of vitamin E. The bioavailability for the central nervous system and the retina remain to be shown.

It appears that, in the absence of lipoproteins containing ApoB, alternative paths for intestinal and hepatic secretion, and for tissue distribution of vitamin E are used. In the intestine, vitamin E may be secreted by the enterocytes either via the portal pathway, in a form bound to albumin, or after packaging in intestinal HDL. Similarly, in the liver, the vitamin E may be packaged in the HDL. An in vitro study with brefeldin A, which blocks hepatic secretion of VLDL, suggests that secretion

of tocopherol may occur independently of VLDL (6). From the results of Traber et al it seems that the tissue distribution occurs via the HDL, which contain almost all of the plasma vitamin E (191).

No evaluation has been made in ABL patients of the different modes of vitamin E administration (oral, intraveinous, and intramuscular). However, because supplementation is instituted for life, the oral route is currently the most commonly used mode of administration. The oral doses required are large, ~100–300 mg/kg per day (111, 143). Whatever the dose and the duration of supplementation, it appears that vitamin E is not toxic (18). There is no hepatic storage of tocopherol because it is excreted in the bile when excessive amounts are administered (22, 193).

Vitamin A plays a role in stabilizing the membrane structure of photoreceptor cells and the pigmented epithelium of the retina. The daily requirement in adults is 5000 IU/day. In ABL patients, vitamin A deficiency is easily compensated for by oral supplementation because, after intestinal absorption and transport to the liver, vitamin A has its own transport system, which is independent of lipoproteins. Generally, the levels of vitamin A normalize with daily doses two- to fourfold the normal recommended doses (22, 73). Supplementation with vitamin A requires close surveillance because the risk of toxicity exists (182).

Oral or parenteral administration of vitamin K rapidly corrects coagulation factors when they are decreased. Several studies have shown that the absorption of large doses of vitamin E may exacerbate the deficit in vitamin K (see 18 for a review). Thus, it is important to monitor the coagulation status and to prophylactically administer vitamin K when beginning vitamin E therapy.

In the normal individual, the recommended dose of vitamin D is 100–800 IU/day. Deficiency of vitamin D is not classically described in ABL because the metabolism of vitamin D depends relatively less on lipoproteins containing ApoB, in that there is partial absorption via the portal path and specific vitamin D transport proteins. Nevertheless, signs of rickets and osteomalacia are sometimes noted (56, 61, 123). Prophylaxis should be systematically instituted in infants during growth.

The precise evaluation of the effects of vitamin supplementation on the neuroretinal complications in these patients is difficult, owing to the rareness of the pathology; differences in the modes of administration, doses, and times at which supplementation begins; and clinical heterogeneity. Nevertheless, it seems that, in general, the treatment is beneficial for the evolution of the neuroretinal complications. The severity of the complications, which appears to be correlated with the duration of vitamin deficiency, makes it imperative to begin vitamin E supplementation as early as possible.

In fact, certain studies suggest that, when instituted early and over the long term in children, supplementation with large doses of vitamin E associated with vitamin A may prevent the appearance of neuroretinal complications (23, 24, 140–143). Furthermore, it seems that vitamin therapy permits the stabilization or the regression of signs (including myopathy) that are already present in patients prior to therapy (10, 22, 23, 82, 113, 139–143, 166). However, vitamin therapy does

not stabilize the evolution in certain patients who are treated too late and in whom the neurological and ophthalmological signs are already present (123, 135). This underlines the importance of instituting therapy early, before the appearance of neuroretinal signs. Furthermore, it appears that supplementation in large doses, even when initiated very early, does not suffice to prevent the appearance of neurological and ophthalmological signs in some cases (96, 140–143).

This heterogeneity in the evolution of the disease and the response to treatment might be related to differences in the metabolism of vitamin E. In particular, in ABL, Traber et al. have shown that, after oral treatment with the same doses of vitamin E, some patients normalize their adipose tissue levels whereas others do not, even when intramuscular injections are used (194). Furthermore, this same study showed that some ABL patients are capable of discriminating among the isomer of tocopherol, whereas others are not. A better understanding of the metabolism of vitamin E in ABL would help to optimize treatment. In addition, there is a lack of sufficiently long-term follow-up in the published studies. The preventive role of various vitamin therapies should be confirmed by prospective studies of much longer duration. It is also possible that the deficit of other factors, not taken into account at present, may be involved in the physiopathology of the complications.

Molecular Defect in Abetalipoproteinemia

The intracellular accumulation of lipid droplets without the formation of lipoprotein particles, shown by the intestinal and hepatic ultrastructural studies of ABL patients, suggested a defect in the assembly of ApoB-containing lipoproteins in ABL subjects. It was initially thought that this defect in assembly was linked to an absence of ApoB synthesis as suggested by immunolabeling studies (66), thus implicating the *ApoB* gene. Later on, other immunohistochemical (29, 53, 65, 120), molecular biological (24, 29, 65, 120), and genetic (88, 154, 188) studies showed that the defect did not directly involve the *ApoB* gene.

The first study to establish the link between MTP and ABL was that of Wetterau et al in 1992 (205). In intestinal biopsies from ABL patients, the large 97-kDa subunit of MTP was not detected, although PDI was readily detected. In parallel, in these same biopsies, the TG transfer activity in vitro was undetectable. These data thus suggested that the gene for the large MTP subunit was involved in ABL. This was rapidly confirmed in 1993 by two studies that showed anomalies of the MTP 97-kDa subunit gene from ABL subjects (172, 174). The gene was localized on chromosome 4q22-24. At present, only 17 patients have been studied at the molecular level (Table 1). Mutations in the MTP gene were demonstrated on both alleles in the majority of cases (13 homozygotes and 4 compound heterozygotes). There is one case of maternal isodisomy (215). Thus, 18 different mutations have been described at present (81, 133, 145, 150, 160, 162, 172, 174, 215). These mutations lead, for the most part, to the expression of truncated forms of the large MTP subunit. Two missense mutations have been reported to date (133, 150, 160).

TABLE 1 Mutations in the large subunit gene of microsomal triglyceride transfer protein in cases of abetalipoproteinemia

Case/genetics	Mutation	Site[a]	Protein encoded (amino acids[b])	Reference(s)
1/Homozygote	Frameshift	215, del 1	78	172
2/Homozygote	Frameshift	419, ins 1	140	145
3/Homozygote	Frameshift	1147, del 1	407	145
4/Homozygote	Donor splice site	1344 + 5 → 11, del 7	463, 858	145
5/Homozygote	Frameshift	1385–1389, del 1	476	150
6/Homozygote	Nonsense	1783, C → T	594	172
7/Homozygote	Donor splice site	1867 + 5, G → A	653, 590	145, 174
8/Homozygote	Frameshift	2212, del 1	746	145
9/Homozygote	Frameshift	2349, ins 4	789	81
10/Homozygote	Maternal isodisomy acceptor splice site	1237 − 1, G → A	858	215
11/Homozygote	Nonsense	2593, G → T	864	162
12/Homozygote	Missense	(Asn780 → Tyr)	894	150
13/Homozygote	Missense	1619, G → A (Arg540 → His)	894	133
14/Compound heterozygote	Frameshift Frameshift	419, ins 1 1401, ins 1	140 511	145
16/Compound heterozygote	Donor splice site Donor splice site	1867 + 1, G → A 1989, G → A	653, 590 680, 625	145
15/Compound heterozygote	Frameshift Donor splice site	419, ins 1 1867 + 5, G → A	140 653, 590	145
17/Compound heterozygote	In-frame deletion Missense	1237, del 108 1619, G → A (Arg540 → His)	858 894	160

[a]Abbreviations: del, deletion; ins, insertion.

[b]Including signal peptide.

ROLE OF MICROSOMAL TRIGLYCERIDE TRANSFER PROTEIN IN THE ASSEMBLY OF PLASMA LIPOPROTEINS

General Model for Lipoprotein Assembly

Having established that MTP is required for lipoprotein production, research efforts began to focus on determining the precise role of MTP in the assembly process. Although there is considerable controversy regarding the details of ApoB lipoprotein assembly, a general consensus for a major pathway has emerged (see Figure 2; reviewed in 151). As ApoB is synthesized, it is translocated through the ER membrane. Addition of the lipid to ApoB begins cotranslationally. A small

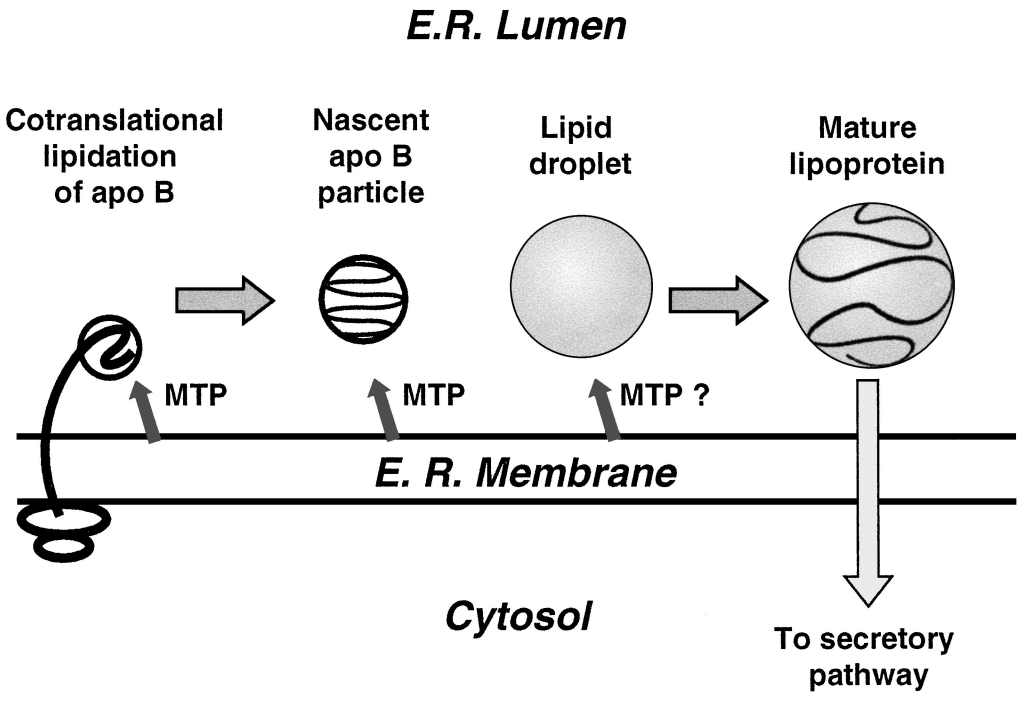

Figure 2 Role of microsomal triglyceride transfer protein in the assembly of ApoB-containing lipoproteins. Plasma lipoproteins are believed to be assembled in a two-step process. In the first step, small dense nascent ApoB lipoprotein particles are made. In the second step, lipid is added to the particle either by fusion with a preformed lipid droplet or the addition of individual lipid molecules. Although considerable data indicate that MTP is involved in the first step, the role of MTP in the latter stages of assembly is less clear. *MTP-labeled arrows* represent MTP-dependent lipid transport.

ApoB-containing lipoprotein particle is formed in what is referred to as the first step of lipoprotein assembly. Additional TG is added in the second step of assembly. This may involve the fusion of the nascent ApoB particle with a preformed TG droplet within the lumen of the ER.

When studied in cultured cells, the production of ApoB-containing lipoproteins is controlled by the availability of lipid and, in particular, TG. When FA are plentiful for TG synthesis, most ApoB is incorporated into lipoproteins. However, when FA are limiting, most of the ApoB is degraded within the ER or by the ubiquitinproteasome pathway after reverse translocation of the ApoB back to the cytosol.

Microsomal Triglyceride Transfer Protein Plays a Role in the Early Steps of Lipoprotein Assembly

Several observations indicate that MTP plays an important role in the early stages of lipoprotein assembly. Cell culture studies show that the products of the first step of lipoprotein assembly, small ApoB particles with a density of HDL and LDL, can be secreted from hepatocytes and enterocytes. Because these particles are not readily observed in individuals who lack MTP, the block in lipoprotein synthesis that occurs in the absence of MTP must occur at a point in the assembly pathway before the formation of these particles. Thus, one could conclude that MTP plays an important role in the first step of lipoprotein assembly in humans.

Additional evidence comes from studies in which lipoprotein assembly is reconstituted in cell lines that normally do not make lipoprotein particles. When ApoB and MTP are co-expressed in HeLa cells or COS cells, small dense lipoprotein particles are produced (69, 125). However, in the absence of MTP, lipoprotein particles are not produced. It is interesting that shorter forms of ApoB are less dependent on MTP for secretion. In a study by Wang et al (197), a short form of ApoB (ApoB18, which is 18% the length of ApoB100) was secreted from COS-7 cells in an MTP-independent manner, whereas the secretion of ApoB23 through ApoB42 was stimulated by MTP. Longer forms of ApoB required MTP for secretion.

MTP inhibitors have been used to further elucidate the role of MTP in lipoprotein assembly. The importance of the various lipid transfer activities of MTP was, in part, addressed in studies using MTP inhibitor BMS-200150 (103). In in vitro assays, BMS-200150 completely inhibits MTP-mediated TG and CE transfer with a 50% inhibitory concentration of $\sim 1\ \mu$M; however, it is able to inhibit only $\sim$30% of the phosphatidylcholine transfer. Similar concentrations of BMS-200150 also inhibit the secretion of ApoB100 from HepG2 cells. These results indicate that a neutral lipid-selective MTP inhibitor is able to inhibit lipoprotein production; thus the ability of MTP to transport neutral lipid plays a key role in the assembly process.

Amino-terminal proximal domains of ApoB are dependent on MTP for the formation of a lipoprotein complex. Unlike the majority of ApoB, which is predicted to consist of lipid-binding β sheets and α helices, the amino-terminal 18% of

ApoB (ApoB18) is globular in nature and is secreted without significant bound lipid. Ingram & Shelness (98) studied the assembly of ApoB28 (amino-terminal 28% of ApoB100). They found that proper folding of the globular amino-terminal domain of ApoB can occur independently of MTP, but that MTP was required for the formation of a lipoprotein particle. Proper folding of the globular domain, which contains six disulfide bonds, is required for the MTP-dependent addition of lipid to ApoB28.

A physical interaction between ApoB and MTP has been demonstrated by co-immunoprecipitation experiments. This interaction appears to be stronger in the early stages of the assembly process (214). Proper folding of the amino-terminal globular domain of ApoB may be a prerequisite for optimal MTP-ApoB interaction and subsequent lipidation. Consistent with this model, MTP-binding sites have been identified in the amino-terminal globular domain of ApoB (30, 89, 133). Two specific domains have been identified, one of which corresponds to ApoB residues 1–152, which interacts with MTP residues 22–297 (133), and the other of which corresponds to ApoB residues 512–592, which interacts with MTP residues 517–603 (30).

Benoist & Grand-Perret (20) used the MTP inhibitor 4′-bromo-3′-methyl-metaqualone to further elucidate the role of MTP in the early assembly of lipoproteins. MTP inhibition before or immediately after a ^{35}S-methionine pulse decreased secretion of ApoB100 from HepG2 cells. However, if the inhibitor was added 10 min into the chase period, ApoB secretion was not affected. Benoist & Grand-Perret then used puromycin to induce premature release of polypeptides from the ribosomes and synchronize translation before a 5-min pulse and a 10-min chase. When experiments were performed in the presence or absence of an MTP inhibitor, ApoB peptides of 100–200 kDa were labeled equivalently, indicating that initiation of ApoB100 translation was not affected by MTP inhibition. However, inactivation of MTP resulted in increased degradation of polypeptides that were longer than 65% of the length of ApoB100.

To understand why the degradation of longer forms of ApoB was accelerated in the absence of MTP, proteasome inhibitors were added to prevent ApoB degradation. This allows one to trace the ultimate fate of undegraded ApoB. Under these conditions, the additional full-length ApoB produced was not secreted. Apparently MTP is needed for the proper folding of ApoB and the formation of a secretion-competent particle, which in turn prevents ApoB degradation. Further studies showed that a domain in ApoB between ApoB51 and ApoB53 confers sensitivity to the presence of MTP (148). This region is predicted to be a flexible, lipid-binding α-helical domain between two extensive lipid-binding β sheets.

The Role of Microsomal Triglyceride Transfer Protein in Later Steps of Lipoprotein Assembly Is Not Clear

The role of MTP in the second step of lipoprotein assembly remains controversial. There are two proposed mechanisms by which the neutral lipid core may be added to the nascent lipoprotein product of the first step of lipoprotein assembly:

(*a*) by addition of individual lipid molecules, possibly mediated by MTP transfer or (*b*) by the fusion of the nascent particle with a lipid droplet. Evidence for a fusion mechanism comes from the identification of both ApoB particles within the rough ER and ApoB-free lipid droplets within the smooth ER of rat liver by electron microscopy (4, 28). Alexander et al (4) proposed that the two particles fused at the junction of the smooth and rough ER to form the mature lipoprotein particle. These early studies, in part, led to the current two-step hypothesis of lipoprotein assembly. More recently, lipid particles the size of CM particles were found in the ER lumen of enterocytes of mice in which intestinal ApoB expression was genetically oblated (77). These particles presumably represent the ApoB-free, lipid droplets, which fuse with the nascent ApoB B-containing particles in the second step of CM assembly.

MTP inhibitors have been used to test whether MTP is involved in the late stages of lipoprotein assembly, by two different groups. Although some details of the experimental design were different, both groups pulse-labeled ApoB and then chased under conditions that allowed the production of the nascent ApoB lipoprotein particles, but delayed the second step of assembly. The second step was then allowed to proceed in the presence or absence of an MTP inhibitor. Conflicting results were found for the production of VLDL from nascent ApoB48 and ApoB100 particles in McA-RH7777 cells. One group found that TG addition to the nascent ApoB48 and ApoB100 particles was MTP independent (68, 167), whereas a second group found that it was MTP dependent (198, 199).

It is interesting that, in an MTP knockout mouse (see below), Raabe et al (158) were unable to detect VLDL-sized, lipid-staining particles within the ER. It appears that the absence of MTP prevents the production of the TG droplets that fuse with the nascent ApoB particles in the second step in lipoprotein assembly. In the pulse-chase studies discussed above, it is possible that these lipid droplets are long lived and were formed before the addition of the MTP inhibitors. In this scenario, the nascent ApoB particles in the pulse-chase studies may have fused with preformed lipid particles. Thus, although the formation of the TG droplet would be MTP dependent, its fusion to nascent ApoB particles may be MTP independent, leading different investigators, using different experimental protocols, to reach different conclusions. This seems somewhat unlikely because there appears to be a tight temporal relationship between TG synthesis and the second step of lipoprotein assembly. Presumably the newly synthesized TG is rapidly added to the nascent particle. Additional investigation is required to more fully understand the role of MTP in the latter stages of lipoprotein assembly. Definitive determination of the presence or absence of membrane-bound lipid droplets in ABL cases would help to elucidate the role of MTP in these processes in humans.

Role of Microsomal Triglyceride Transfer Protein in Regulating Lipoprotein Production

MTP is expressed primarily in the liver and intestine, although its expression has been observed in various other tissues, including the heart (149), kidney, and testis

(174). In hamsters, there is ~25% as much MTP activity in the liver as there is in the proximal small intestine mucosa (duodenum and jejunum) (129). A similar difference was found between mouse liver and intestine, although the reported magnitude of the difference between the two tissues was not as large (157). MTP large subunit mRNA levels in hamster liver are only 10% that of the proximal small intestine. Within the intestine, there is a gradient of MTP expression from the proximal intestine through the colon, where mRNA levels are 5% of those found in the proximal intestine.

The promoter region of MTP has been shown to contain some elements that regulate the cell type-specific expression in humans and hamsters. The human promoter activity is positively regulated by C and negatively by insulin (75). In addition, this promoter contains the consensus recognition sequences for liver HNF1, HNF4, and activator protein AP1 and for the cytokine interleukin 1 (75, 146).

In animal models, MTP large-subunit gene expression is regulated in response to various diets (19, 129). A chronic, but not acute, high-fat diet increases MTP large-subunit gene expression by ~50% in the livers of hamsters. Message levels can be further increased another 30% by the addition of C to a high-fat diet (19). A high-fat diet acutely and chronically up-regulates intestinal message levels about two fold. An important question is whether the modest changes in MTP levels predicted by these changes in MTP gene expression could play a role in controlling ApoB secretion or the composition of the lipoproteins produced.

Most ABL-obligate heterozygotes do not show any abnormalities in plasma lipoprotein levels (see the section on clinical characteristics of ABL above), suggesting that, in humans, MTP is not rate limiting for lipoprotein production. However, ABL subjects represent a wide variety of genetic and environmental backgrounds, which may preclude the detection of moderate changes in lipoprotein patterns. In addition, there could be compensating regulation of MTP in ABL subjects. Cell culture models and transgenic mice have been used to study the role of MTP in regulating lipoprotein production under more defined conditions.

Jamil et al (101) used an irreversible-photoaffinity MTP inhibitor, BMS-192951, to inactivate MTP to various extents in HepG2 cells. After UV light exposure of inhibitor-treated cells, unbound inhibitor was removed by changing the culture medium. The level of ApoB secreted into the medium was then determined and correlated with MTP activity, which was measured with a standard lipid transfer assay in a cell homogenate. Decreased MTP activity resulted in a proportional decrease in ApoB100 secretion, with no change in ApoAI secretion, suggesting that MTP is rate limiting for ApoB secretion. Similar experiments showed that decreased MTP activity resulted in decreased ApoB100 and ApoB48 secretion in primary rat hepatocytes and ApoB100 secretion in McArdle RH-7777 cells. Thus, in several cell culture models, MTP activity appears to be limiting for the secretion of ApoB.

To determine whether overexpression of MTP in HepG2 cells results in increased ApoB secretion, Liao et al (128) overexpressed MTP by using an adenovirus vector containing cDNA that encoded the large subunit of MTP. MTP activity was increased about five fold with this approach. Although adenovirus

infection of HepG2 cells results in decreased secretion of ApoAI and albumin, ApoB secretion into the medium was increased about two- to three-fold. The density of the ApoB100 particles (LDL and HDL density) was not affected by the increased levels of MTP expression. ApoB secretion appeared to be increased owing to decreased intracellular ApoB degradation.

Although MTP inhibitors decrease lipoprotein production in various animal models, it is not possible to measure the extent of MTP inhibition in situ, thus preventing one from determining the relationship between MTP activity and lipoprotein production with MTP inhibitors. Initial genetic attempts to modify MTP activity in mice met with limited success. Raabe et al (157) used gene targeting to knockout the MTP large subunit in mice. In mice, a homozygous MTP knockout is embryonically lethal at midgestation. In contrast, no increase in the frequency of spontaneous abortions has been noted in ABL families. Although the reason for this difference is not clear, it is thought that the lethal phenotype in mice may be caused by an inability to produce lipoprotein particles in the visceral yolk sac endoderm, which is important for the delivery of nutrients to the embryo before the development of chorioallantoic placenta. The importance of this source of nutrition for the embryo appears to be different in rodents and humans. Another explanation for the differences between humans and rodents is that, in the absence of lipoproteins containing ApoB, adaptation occurs in humans that provides an alternative route for transport of nutrients.

In studies by Raabe et al (157), the heterozygote knockout mice also showed a somewhat surprising phenotype. MTP activity and mRNA levels were reduced 50% in both the liver and intestine. These reduced levels apparently did not support normal lipoprotein production because plasma IDL/LDL C was reduced whether animals were fed a normal or high-fat diet. ApoB was reduced ~28% in the heterozygotes on a low-fat diet, with no change in the ratio of ApoB100 to ApoB48. On a high-fat diet, total plasma C was decreased ~20% owing to decreased VLDL and LDL. ApoB100 appeared to be decreased ~15%–20%.

To circumvent the lethality of an MTP knockout in mice, two groups independently did a conditional knockout of hepatic MTP by inserting loxP sequences in the gene encoding MTP, then inducing Cre-mediated recombination by either infecting the animals with an adenovirus that expressed the Cre recombinase or inducing expression of Cre recombinase through induction of an inducible Cre transgene. The induced recombination inactivated the MTP gene. Both groups achieved an almost complete knockout (>95%) of hepatic MTP and observed a complete loss of plasma ApoB100. ApoB48 was either barely detectable (35) or minimally decreased [~20% (158)] after the loss of hepatic MTP. VLDL and LDL cholesterol were almost absent, and HDL cholesterol decreased in the homozygous mice. Although, in the study by Raabe et al (158), ApoB48 was minimally decreased, it was absent in the VLDL density range. Heterozygous mice generally showed an intermediate phenotype.

In rodents, the liver makes a significant contribution to plasma ApoB48 levels; thus, it is surprising that ApoB48 levels were minimally affected by a hepatic MTP knockout. This suggests, as has been observed in studies with MTP inhibitors (76),

that ApoB48 secretion is less sensitive to decreases in MTP activity than ApoB100. Raabe et al (158) confirmed this by isolating primary hepatocytes from normal and MTP-deficient mice and comparing ApoB secretion rates. They showed little difference in ApoB48 production, although ApoB100 secretion, as expected, was markedly lowered in the absence of MTP. The reason for the differences in plasma ApoB48 levels found by the two groups is unclear.

ApoB, lipid, and MTP are all required for the production of ApoB-containing lipoproteins. From the studies discussed above, it is clear that changes in MTP levels affect lipoprotein production. In some cases, the magnitude of the changes in MTP that affect lipoprotein production are similar to the MTP changes predicted from studies of MTP regulation. Previous studies have also shown that lipid availability and ApoB expression affect lipoprotein production. It appears that, under defined conditions, a change in any one of these three factors can affect lipoprotein production. To fully understand the regulation of lipoprotein production, the complex relationship between lipid availability, ApoB levels, and MTP levels must be more fully understood. Whether cell culture or rodent models are predictive for humans remains to be determined.

MICROSOMAL TRIGLYCERIDE TRANSFER PROTEIN INHIBITORS FOR THE TREATMENT OF HYPERLIPIDEMIA

The identification of MTP defects as the proximal cause of ABL suggests that MTP inhibition will inhibit the production of VLDL and CM and lower plasma lipid levels in animals. Although there have been several MTP inhibitors reported in the literature that inhibit lipoprotein production in cell culture models, there are few reports of the effects of MTP inhibitors in vivo. A potent MTP inhibitor was reported to inhibit VLDL and CM production in rats and dramatically lower plasma C and TG levels in hamsters and Watanabe-heritable hyperlipidemic rabbits (207). Watanabe-heritable hyperlipidemic rabbits have a genetic defect in the LDL receptor that results in plasma TG and C levels of $\geq$300 and $\sim$700 mg/dl, respectively. The MTP inhibitor was able to normalize plasma lipid levels in this model. More recently, it was reported that an MTP inhibitor lowers plasma C and TG levels in humans (54), raising the possibility that this approach will provide effective therapy for treatment of hyperlipidemia in humans.

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