Cellular and Molecular Life Sciences

Visions & Reflections (Minireview)

Apolipoprotein M affecting lipid metabolism or just catching a ride with lipoproteins in the circulation?

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Abstract. Apolipoprotein M (apoM) is a novel apolipoprotein found mainly in high-density lipoproteins (HDL). Its function is yet to be defined. ApoM (25 kDa) has a typical lipocalin ß-barrel fold and a hydrophobic pocket. Retinoids bind apoM but with low affinity and may not be the natural ligands. ApoM retains its signal peptide, which serves as a hydrophobic anchor to the lipoproteins. This prevents apoM from being lost in the urine. Approximately 5% of HDL carries an apoM molecule. ApoM in plasma (1 μM) correlates strongly with both low-density lipoprotein (LDL) and HDL cholesterol, suggesting a link to cholesterol metabolism. However, in case-control studies, apoM levels in patients with coronary heart disease (CHD) and controls were similar, suggesting apoM levels not to affect the risk for CHD in humans. Experiments in transgenic mice suggested apoM to have antiatherogenic properties; possible mechanisms include increased formation of pre-ß HDL, enhanced cholesterol mobilization from foam cells, and increased antioxidant properties.

ApoM, a novel lipoprotein-associated protein

Apolipoprotein M (apoM) is a recent addition to the long list of lipoprotein-associated proteins. It is mainly found in high-density lipoproteins (HDL) but is also present in apoB-containing lipoproteins, i.e. chylomicrons, very low-, and low-density lipoproteins (VLDL and LDL) [1, 2]. The plasma concentration of apoM is close to 1 μM and ~5% of HDL and ~2% of LDL from human plasma are estimated to contain a single apoM molecule per particle. ApoM is expressed in the liver and in the kidney, apoM in plasma presumably being mainly derived from the liver [1]. Human apoM contains 188 amino acid residues, the sequence of which demonstrates no obvious similarity to other proteins. However, secondary structure prediction combined with three-dimensional database searches suggested apoM to be a lipocalin. The lipocalins form a large superfamily of proteins sharing a conserved folding, and many of them bind small lipophilic substances [3, 4]. To date, there is no experimentally determined structure of apoM, but computational analysis and homology modelling have predicted
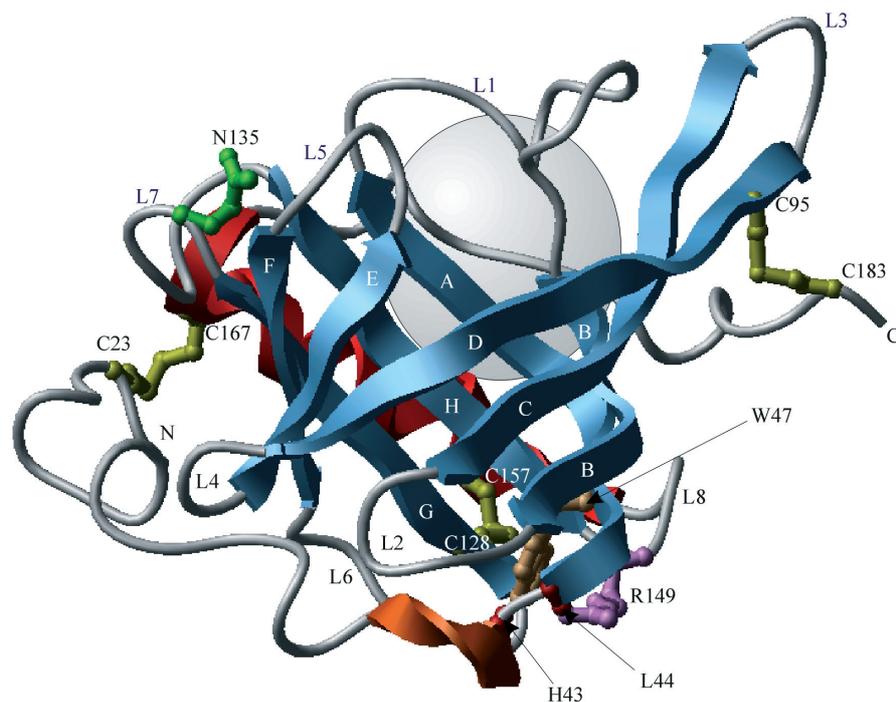
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Figure 1. Structural model of human apoM. Retinol binding protein and MUP1 (major urinary protein, a protein used to mark territories among rodents), i.e. two lipocalins with known three-dimensional structures, were used to create the homology model of apoM. The model highlights the typical lipocalin fold with eight β-strands (blue arrows) forming the β-barrel, and the α-helix (red). The model is shown with a circular molecule (white) in the proposed ligand bind pocket. Reproduced from Duan et al., with permission [5].

apoM to have a typical lipocalin fold (Fig. 1) with a hydrophobic binding pocket and a highly conserved tryptophan in its bottom [5]. This tryptophan has intrinsic fluorescence which is quenched when a lipophilic ligand binds in the pocket. ApoM is capable of binding retinol and its two metabolites, all-trans retinoic acid and 9-cis retinoic acid [6]. However, the physiological importance of this binding is not known and it is uncertain to what extent apoM may be involved in retinoid metabolism *in vivo*. Retinol binding protein binds retinoids with higher affinity than apoM and in addition has a higher plasma concentration (~3 μmol/l) [7] than apoM [6]. Thus, apoM is not a major transport protein of plasma retinol.

The retained signal peptide of apoM serves as a phospholid anchor

ApoM retains its signal peptide because the sequence lacks a signal peptidase cleavage site. This unusual feature is shared only with paraoxonase-1 (PON-I) and haptoglobin-related protein (HRP), both being HDL-associated proteins [8, 9]. The signal peptide mediates the import of the newly synthesized polypeptide chain into the endoplasmic reticulum during translation. Normally, it is cleaved off by a type 1 signal peptidase before secretion of the mature protein [10]. ApoM's retained signal peptide is experimentally proven to mediate the binding of

apoM to plasma lipoproteins [11, 12]. In recombinant apoM, replacement of glutamin at position 22 with alanin (apoM^{Q22A}) created a signal peptidase cleavage site and this apoM^{Q22A} was secreted from transfected cells without its signal peptide (Fig. 2A) [11, 12]. The apoM^{Q22A}, unlike wild-type apoM, did not associate with the lipoproteins in the cell medium (Fig. 2B) [11]. Transgenic mice expressing either human wild-type apoM or apoM^{Q22A} in the liver were created to determine the physiological importance of the retained signal peptide. In mice expressing the wild-type protein, human apoM was exclusively associated with plasma HDL. In contrast, the human mutant apoM^{Q22A} did not associate with lipoproteins and was rapidly lost from the plasma by glomerular filtration in the kidney [12]. Thus, it can be concluded that the preserved signal peptide is essential to preserve apoM in the circulation (Fig. 3).

Binding of apoM to megalin in proximal tubuli

Megalin is a receptor that binds many lipocalins including the retinol-binding protein. It is synthesized in the proximal tubular epithelium in the kidney. The high level of apoM expression in the kidney, taken together with its lipocalin structure, inspired us to test whether apoM is a ligand for megalin. Direct binding of apoM to immobilized megalin was demonstrated and furthermore, rat yolk sac cells expressing megalin bound and internalized apoM in a megalin-dependent
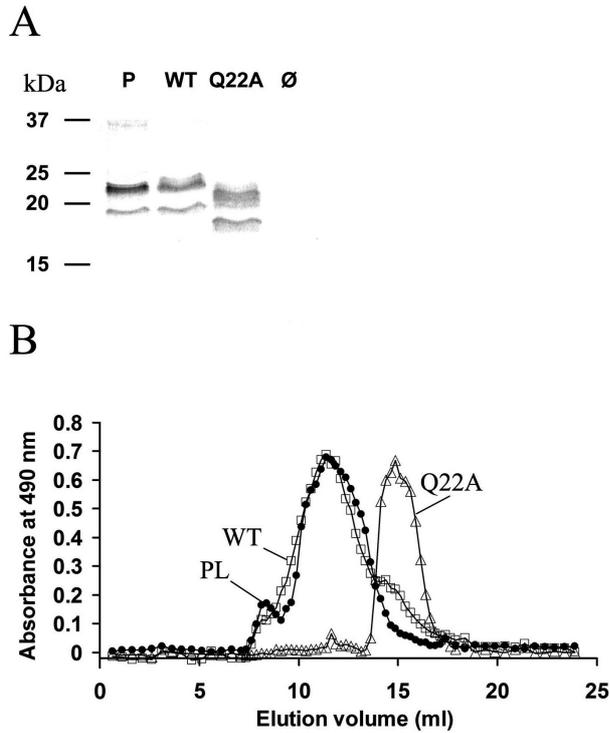
A

B

Figure 2. The retained signal peptide anchors apoM in lipoproteins. Stable cell lines expressing either full length apoM or the apoM^{Q22A} mutant were established. *(A)* The signal peptide is cleaved off the apoM^{Q22A} variant, as demonstrated by the enhanced migration on the western blotting. **P**, plasma apoM; **WT**, wild type apoM; **Ø**, mock-transfected cells. *(B)* The full-length WT apoM (open squares) elutes like apoM from plasma (closed circles) on gelfiltration chromatography, suggesting that WT apoM associates with HDL particles in the culture medium. In contrast, apoM^{Q22A} (open triangles) is recovered in later fractions, demonstrating that the apoM variant without the signal peptide is not associated with lipoproteins. Reproduced from Axler et al., with permission [11].
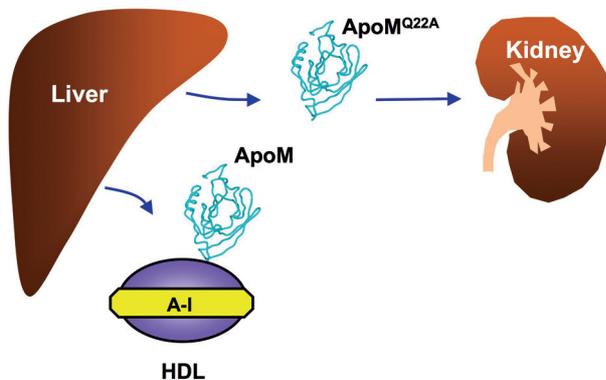

Figure 3. Metabolism of the full-length apoM and the apoM^{Q22A} variant *in vivo* in mice. Full-length apoM is synthesized in the liver and associates with lipoproteins (mainly HDL) in the circulation. In contrast, the apoM^{Q22A} variant is not associated with lipoproteins. Due to its small size, it is filtered in the glomerular membrane and lost in the kidney. Model based on data from Christoffersen et al. [12].

fashion [13]. ApoM is not found in the urine of normal mice or in human urine, even though it is expressed in kidney tubular epithelial cells [1, 14]. However, in mice lacking megalin in their kidneys, apoM is secreted in the urine [13]. This suggests the possibility that kidney-derived apoM is secreted into the pre-urine in the proximal tubules and, under normal conditions, subsequently taken up in a megalin-dependent fashion. It is tempting to speculate that the lipocalin structure of apoM enables it to bind a small lipophilic ligand in the pre-urine, thus preventing loss of the ligand in the urine.

APOM is located in a highly conserved inflammatory region of MHC class III

In the human genome, the apoM gene (*APOM)* is located on chromosome 6 on position p21.3 in the major histocompatibility complex (MHC) III. This is a highly conserved chromosomal region rich in genes involved in innate immunity and inflammation, e. g. tumor necrosis factor (TNF) and lymphotoxins A and B. This inflammatory gene cluster has been well preserved during 450 million years of evolution predating the divergence of mammals from fish [15]. In this context, it is interesting that inflammation and infections depress *APOM* expression and lower apoM protein secretion in cell cultures as well as in mice and humans [16]. It is not known to what extent apoM may be involved in inflammatory responses to infection or other pro-inflammatory stimuli.

APOM expression is controlled by several transcription factors that affect hepatic lipid metabolism, i. e. hepatic nuclear factor (HNF)-1α, liver receptor homolog (LRH)-1, and Foxa2 [17–19]. HNF-1α binds to the *APOM* promoter and mice with complete HNF-1α deficiency have no or little *APOM* expression as well as defective bile acid transport and increased bile acid and liver cholesterol synthesis [18, 20, 21]. LRH-1 is a nuclear hormone receptor that regulates genes involved in bile acid synthesis [22, 23], reverse cholesterol transport [24–26], and the hepatic acute-phase responses [17]. LRH-1 response elements are present in the *APOM* promoter regions in both human and mouse, and LRH-1 stimulates apoM gene transcription in cultured liver cells [27]. Farnesoid X receptor (FXR) ligands such as bile acids and the synthetic liver X receptor (LXR) agonist TO901317 decrease *APOM* expression *in vitro*. Also, *APOM* expression was decreased in bile acid-fed mice [27, 28]. This was in part caused by recruitment of a small heterodimer partner (SHP) to the *APOM* promoter [27]. The notion that SHP regulates *APOM* expression was further supported by studies

demonstrating that SHP-deficient mice have increased *APOM* expression [29]. Foxa2 is a transcription factor that affects the development of pancreas and liver and regulates glucose homeostasis in the liver and pancreatic β-cells [30, 31]. A binding site for Foxa2 was identified in the *APOM* promoter at position -474 and it was demonstrated that haploinsufficient Foxa2-/+ mice had decreased expression of *APOM* [19].

Circulating apoM-containing lipoproteins

Isolated apoM-containing HDL particles from human plasma are heterogeneous in size, charge, and density [2]. Whether apoM is present in pre-β HDL is a matter of debate. One study suggested that a large fraction of plasma apoM resides in small pre-β HDL particles [32], whereas other studies report the opposite [2, 33]. The lipid composition of apoM-containing human HDL was found to be similar to that of total HDL [2]. Human apoM-containing HDL particles contain many other apolipoproteins, including apoA-I, apoA-II, apoE, and apoCs. They were not enriched in or devoid of PON-1 or HRP, but were found to be enriched in apoJ [2]

In normal mouse plasma, apoM is predominantly found in the α-migrating HDL fraction [33]. In plasma from LDL-receptor-deficient and apoE-deficient mice, apoM is also found in LDL and VLDL-sized lipoproteins [20]. From this, it can be concluded that the retained signal peptide can anchor apoM in different lipoprotein classes. The presence of apoM in intestinally derived chylomicrons and the lack of apoM expression in the intestine indicate that apoM can be transferred from HDL or LDL particles to triglyceride-rich lipoproteins. This idea is supported by studies of healthy humans showing accumulation of apoM in the d<1.006 g/ml plasma fraction after ingestion of a fat-rich meal [1]. PON-1, which (like apoM) is anchored in lipoproteins via its signal peptide, demonstrates similar accumulation in chylomicrons after a fat-rich meal [34]. Thus, similar to amphipathic helix-containing apolipoproteins such as apoCI, apoCIII and apoE, signal peptide-anchored lipoproteins can be transferred from HDL to chylomicrons, but the mechanisms involved need further exploration.

ApoM, cholesterol and the development of atherosclerosis

In humans, the plasma concentration of apoM correlates strongly with total cholesterol (r=0.52). It is particularly noteworthy that the correlation to LDL cholesterol (r=0.43) is equally high as that to HDL cholesterol (r=0.36), despite apoM being mainly associated with HDL [35, 36]. This supports the notion that apoM is linked to plasma cholesterol metabolism. Animal experiments have suggested that apoM may have antiatherogenic properties. Thus, overexpression of both the human and mouse apoM genes in LDL-receptor knockout mice results in increased plasma apoM concentrations and protects against development of early atherosclerotic lesions. Possible mechanisms include involvement of apoM in the formation of pre-ß HDL [32, 33], enhanced cholesterol mobilization from macrophage-derived foam cells, and antioxidant properties of apoM-containing HDL [2, 33]. Despite a possible atheroprotective effect of apoM in mice, two independent case-control studies found almost identical plasma apoM concentrations in patients with coronary heart disease (CHD) and controls, and thus were unable to identify apoM plasma concentrations as a risk factor for CHD in humans [36].

HNF-1α deficiency in mice is associated with virtually abolished apoM expression [18, 20], making it interesting to measure apoM in patients with MODY3, a disease caused by haploinsufficiency of HNF-1α [37, 38]. Maturity onset diabetes in the young (MODY) encompasses monogenic forms of diabetes characterized by autosomal dominant inheritance, onset before 25 years of age, and pancreatic β-cell dysfunction. In an early study, MODY3 patients were suggested to have decreased plasma apoM levels [18]. More recently, apoM levels were assessed in 48 MODY3 patients, 55 patients with type 2 diabetes, and 55 controls using a plasma dot-blot technique. In this study, there was no difference in the plasma apoM levels between the three groups [39]. We have measured apoM levels in 71 Finnish MODY3 patients using our quantitative apoM ELISA and found apoM to be only ~5 % lower in MODY3 patients than in 75 non-carriers (Axler et al., to be published). Thus, according to those studies, apoM unfortunately cannot be generally used as biomarker for MODY3.

Future perspective

It is likely that the near future will bring insights into the three-dimensional structure of the lipocalin part of apoM. We have hypothesized that the hydrophobic binding pocket of apoM binds one or more lipophilic substances, and the future will hopefully bring insights into the nature of such substances. This might prove very helpful for the elucidation of the function of apoM. Hopefully the physiological function of apoM

will be elucidated in a not too distant future. The availability of genetically modified mice, both apoM knockout mice and apoM transgenic mice, will be instrumental for this research. Another interesting question relates to the expression of apoM from eukaryotic cells. Its signal peptide being retained, apoM needs to be transferred from the phospholipid layer of the cell to the phospholipids of the lipoprotein. Future studies will clarify the mechanism for this. Other research areas where the future presumably will bring new exciting findings are questions related to the interaction of apoM with other soluble or cell-bound proteins and the identification of structure-function relationships.

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