値低下効果は約 20%であり、単剤で目標値に到達するのは難しい[31]。

B)HMGCoA 還元酵素阻害剤（スタチン）

スタチンは、コレステロール合成系の律速酵素である HMGCoA 還元酵素を競合的に阻害して細胞内のコレステロールプールを減少させる。結果として LDL 受容体を活性化することにより、血清 LDL-C 値を低下させる。LDL-C 値低下効果は 20～50%であり、スタチンの種類と容量による。小児に対するスタチンの効果および安全性に関する臨床試験の報告によると[32-38]、その脂質低下効果と発達、発育を含めた安全性に問題がなく、メタアナリシスでも一定の結論が出ている[39]。高コレステロール血症の小児に対して、スタチンが血管内皮機能の改善や IMT の減少に効果があるという報告もある[40,41]。小児に対してスタチンを使用する場合、長期の安全性が確立していないことを鑑みながら、将来の冠動脈疾患進展を予防する効果と、副作用などのリスクとを考慮した上で投薬開始を行うかどうかを判断し、副作用の出現には細心の注意をはらう必要がある。IMT などにより動脈硬化が進行していると評価される例については、スタチンの積極的な使用が必要である。

スタチンの小児への使用は最小容量から開始し、AST、ALT などの肝機能、CPK、血清脂質値、筋肉痛等の症状を最初の３ヶ月は毎月、その後は３ヶ月に１回はフォローして、肝機能障害、ミオパチー、稀ではあるが横紋筋融解症などの副作用の発症に留意する他、成長および性成熟についてもモニターする必要がある。スタチンは基本的には 10 歳未満の小児に使用すべきではない。国内では使用実例が少ないので安全性が確立していないとされている。FDA は複数のスタチンについて「小児及び青年期のヘテロ FH の治療のために食事および生活習慣の改善の補助として、十分な食事療法を施行後も LDL-C≧190mg/dl または LDL-C≧160mg/dl かつ早発性の冠動脈疾患家族歴または 2 つ以上の冠動脈疾患危険因子を有する場合に投与」を認可している[42]。また、認可されていないスタチンについても、現在、小児例について臨床試験が進行中である。女児に対してスタチンを使用する場合、催奇形性の強い薬剤であることを鑑み[43]（FDA 分類で X）[44]、妊娠の可能性には特に注意する必要がある。

C)エゼチミブ

エゼチミブは、小腸粘膜に存在する NPC1L1 と結合して、食事および胆汁由来のコレステロール吸収を阻害することにより、血中 LDL-C 値を低下させる作用を持つ薬物である。エゼチミブは、小児に対する安全性のデータはまだないが、スタチンでコントロールが困難な重症例に併用薬として用いられる。また、消化管などへの副作用のために良好なコンプライアンスを得るのが難しいレジンに代わる薬剤として、今後、注目に値する薬剤である。

Table 1 FH ヘテロ接合体の診断基準（小児：16 歳未満用）

1. 未治療時の LDL-C 値が 140 mg/dL（TC 値 220 mg/dL）以上。
2. 二親等までの家族が FH と診断されている。
3. アキレス腱黄色腫（注 1）または、皮膚結節性黄色腫の存在が確認できる場合。
4. 二親等までの家族が若年性冠動脈疾患（男性＜55 歳、女性＜65 歳）、未治療時 LDL-C 値＞160 mg/dL のいずれかを有する。
5. LDL レセプター遺伝子変異を有する。

注 1　アキレス腱黄色腫は、X 線軟線撮影またはゼロラジオグラフィーによるアキレス腱肥厚の判定で側面で最大径 9 mm 以上として診断する。

注 2　LDL レセプター活性低下は、診断の参考になり得る。

1 と 2 あるいは 3 があてはまれば確定診断。1 と 4 があてはまれば疑診。5 のみで確定診断。

Table 2 FH 接合体小児のリスク

1. 冠動脈疾患の家族歴（二親等以内）
2. 肥満（肥満度 20%以上）
3. 糖尿病（耐糖能異常を含む）
4. 高血圧（125/70）
5. 喫煙
6. 低 HDL 血症(<40 mg/dl)

Table 3　10 歳以上の小児に薬物療法開始を推奨される LDL-C 値 [45]

（米国小児科学会　2008 年発表）

カテゴリー	**推奨されるカットオフ値**
CAD のリスクなし	**食事療法を行っても LDL-C 値が常に 190mg/dL 以上**
CAD の家族歴、肥満、高血圧、喫煙、低 HDL-C 血症のうち 1 つ以上のリスクを有する	**食事療法を行っても LDL-C 値が常に 160mg/dL 以上**
糖尿病を有する	**LDL-C 値が 130mg/dL 以上**

Table 4 NCEP による小児の高脂血症への食事勧告 [30]

	第一段階	第二段階
総脂肪	総カロリーの 30%未満	総カロリーの 30%未満
飽和脂肪酸	総カロリーの 10%未満	総カロリーの 7%未満
多価不飽和脂肪酸	総カロリーの 10%未満	総カロリーの 10%未満
一価不飽和脂肪酸	残りの総脂肪カロリー%	
コレステロール	300mg 未満	200mg 未満
糖　質	総カロリーの約 55%	
タンパク質	総カロリーの 15～20%	
総カロリー	正常の成長発育を促し、かつ望ましい体重を達成・維持できるように	

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別紙４

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REVIEW ARTICLE

HDL biogenesis and cellular cholesterol homeostasis

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Abstract

Mammalian somatic cells do not catabolize cholesterol and therefore must export it to maintain sterol homeostasis at the levels of cells and whole body. This mechanism may reduce intracellular cholesterol accumulated in excess, and thereby contribute to prevention or cure of atherosclerotic vascular lesions. High-density lipoprotein (HDL) plays a central role in this reaction by removing cholesterol from cells and transporting it to the liver, the major cholesterol catabolic site to bile acids. Two independent mechanisms are identified for the cellular cholesterol release. One is non-specific diffusion-mediated 'efflux' of cell cholesterol that is trapped by various extracellular acceptors including lipoproteins. Cholesterol acyl esterification on HDL provides a driving force for net outflow of cell cholesterol in this pathway, and some cellular factors may also enhance this reaction. The other is apolipoprotein-mediated process to generate new HDL particles by removing cellular phospholipid and cholesterol. This reaction is mediated with a membrane protein, ATP binding cassette transporter (ABC) A1, and helical apolipoproteins recruit cellular phospholipid and cholesterol to assemble HDL particles. The reaction is composed of two elements: assembly of HDL particles with phospholipid by apolipoprotein, and cholesterol enrichment in this HDL. ABCA1 is essential for the former step, and apolipoproteins are dissociated from HDL or secreted from cells and interact with ABCA1 in their free form. The latter step requires other cellular factors, such that ABCA1 mediates production of cholesterol-rich and cholesterol-poor HDL while ABCA7 produces only cholesterol-poor HDL.

Key words: *ABC, apolipoprotein, caveolin, cholesterol efflux, HDL, membrane*

Introduction

Cholesterol constitutes a membrane domain 'raft' by forming a cluster with sphingolipid to provide an microenvironment for accumulation of specific membrane proteins related to intracellular signal transduction, and therefore plays essential key roles in the biological functions of the cell membrane especially for intercellular communication. Biosynthesis of cholesterol is therefore carried out in all the somatic cells in most animals requiring a complicated 37 steps in order to maintain such cellular functions. In contrast, catabolism of cholesterol is very limited in peripheral cells of vertebrates, and most of cholesterol molecules in the body are transported to the major organ for its catabolism, the liver, except for a small but important part in steroidogenic cells. In the liver, cholesterol is converted to bile acids that are heavily reused in an entero-hepatic circulation. It should be noted that cholesterol is never converted to energy. Bile acids still contain a sterol backbone, and it is biodegraded by bacteria mostly after excretion. Thus, we recognize it as an important and valuable molecule that should not be wasted at all. We are well prepared for crisis management of cholesterol shortage, but very poorly for its overload.

The regulation of cholesterol biosynthesis and receptor-mediated lipoprotein uptake have been extensively characterized for a long time (1), and the regulatory mechanism of cholesterol biosynthesis has been well established at the molecular levels such as sterol regulatory element binding protein system (2,3). On the other hand, release of cholesterol from somatic cells is equally important for cholesterol homeostasis both for cells and whole body, but understanding of this part has been substantially behind. However, knowledge has rapidly accumulated in this field in the last several years, and significant progress has been made for understanding the mechanism for cellular cholesterol release.

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Abbreviations

LDL	low-density lipoprotein
HDL	high-density lipoprotein
LCAT	lecithin: cholesterol acyltransferase
CETP	cholesteryl ester transfer protein
ACAT	acylCoA cholesterol acyltransferase
PLTP	phospholipid transfer protein
ABC	ATP-binding cassette transporter
HPLC	high-performance liquid chromatography

Key messages

- Cholesterol in extrahepatic cells, except for steroidogenic cells, must be released and transported to the liver for its conversion to bile acids mainly mediated by high-density lipoprotein (HDL), as its major catabolic pathway both for cellular and whole body levels.
- Cell cholesterol release is mediated by two independent mechanisms: a physicochemical diffusion-mediated pathway in which one of the driving forces for the net release is lecithin: cholesterol acyltransferase (LCAT) reaction on HDL, and an HDL biogenesis by the interaction of helical apolipoprotein and cellular lipid mediated by ATP-binding cassette transporter (ABC) A1.
- Helical apolipoprotein, represented by apoA-I, must be in a free form to interact with ABCA1-expressing cells to generate HDL, and it either dissociates from HDL or is secreted as a free form before the interaction for HDL biogenesis.
- Cholesterol enrichment of HDL in the ABCA1-mediated HDL biogenesis is independent of assembly of HDL particles with cellular phospholipid, and cholesterol-rich and cholesterol-poor HDL are generated by apoA-I in the presence of transfected-and-expressed ABCA1 and ABCA7, respectively.

Release of cellular cholesterol and its transport to the liver are both mediated by high-density lipoprotein (HDL). This pathway is under kinetic control and in a steady state with assembly and clearance of plasma lipoproteins and with extracellular cholesterol metabolism by lecithin: cholesterol acyltransferase (LCAT), cholesteryl ester transfer protein (CETP), and other active molecules (4). However, the most critical step for this pathway is the release of cholesterol from the cells, and it is also one of the key components of cellular cholesterol homeostasis. This pathway is often referred to as the concept of 'reverse cholesterol transport' and an anti-atherosclerosis nature of HDL, based on the two lines of evidence that plasma HDL level is negatively correlated to the risk of atherosclerotic vascular disease (5) and that incubation of the cells with HDL results in reduction of cellular cholesterol *in vitro* (6). Two major mechanisms are proposed for the cellular cholesterol release step (7–9): non-specific diffusion-mediated cell cholesterol 'efflux', and apolipoprotein/ATP-binding cassette transporter (ABC) A1-mediated biogenesis of HDL particles from cellular lipids.

Non-specific release of cell cholesterol

Non-specific cholesterol efflux from the cellular surface by physicochemical cholesterol exchange between the cell membrane and extracellular 'acceptors' is perhaps mediated by its diffusion in an aqueous phase. Net release of cellular cholesterol is driven by extracellular acyl-esterification of cholesterol by LCAT in this pathway. This concept was first proposed by Glomest in 1968 (10) as HDL is a major cholesterol acceptor in this reaction because of its capacity for cholesterol accommodation and because it provides a major and optimum site for the LCAT reaction. This is under kinetic control and the net release of cell cholesterol is in fact demonstrated only when outflow diffusion of cell cholesterol is not a rate-limiting factor (11,12) (Figure 1). Scavenger receptor B1 seems to expedite cholesterol exchange rate between cell membrane and HDL, perhaps through a specific mode of binding to HDL (13–16). ABCG1/ABCG4 alters intracellular cholesterol distribution to the direction to increase its release by this pathway (17).

Apolipoprotein-mediated HDL assembly

The other important mechanism is an assembly of new HDL particles with cellular phospholipid and cholesterol upon the direct interaction of helical apolipoproteins of HDL with cells. Many specific cellular functions are required for this reaction, including a cellular interaction site for apolipoprotein and specific intracellular cholesterol trafficking for the HDL assembly. This reaction seems to be a major source of plasma HDL, and ABCA1 is a key cellular factor.

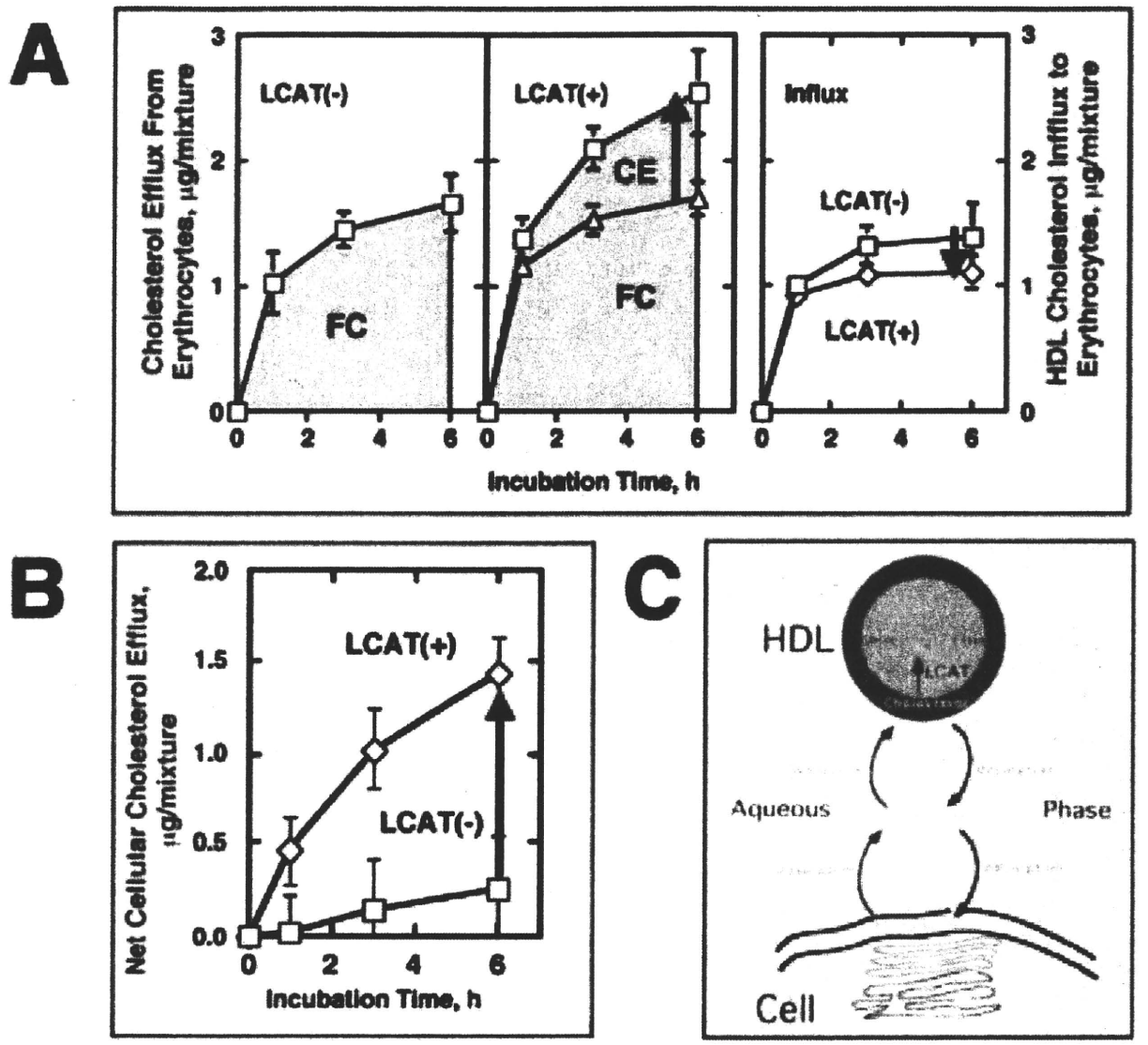

Figure 1. LCAT-mediated net cholesterol release from erythrocytes (12). Pig erythrocytes that lack apolipoprotein-mediated cell cholesterol release were used for increasing the cellular cholesterol pool in order to provide a high off-diffusion rate of cellular cholesterol and to make LCAT reaction a rate-limiting factor for net cholesterol efflux. Panel A shows cell cholesterol efflux to HDL in the medium in the absence and presence of LCAT measured by pre-labelling cell cholesterol. Cholesterol esterification by LCAT results in just as much increase of cell cholesterol efflux (an upward arrow). In contrast, influx of HDL cholesterol into erythrocytes measured by pre-labelling HDL cholesterol. Cholesterol influx was reduced in the presence of cholesterol esterification on HDL (a downward arrow). FC, free cholesterol; CE, cholesteryl ester. Panel B shows the net cholesterol efflux calculated from the results in the panel A. There is no net flux between erythrocytes and HDL without LCAT, and LCAT generates the net outflow of cell cholesterol to HDL (an upward arrow). Overall results indicated that acyl esterification of cholesterol on HDL is the driving force for its net release from cells by its diffusion between HDL and cell surface (Panel C).

The first finding of HDL assembly by cellular lipid and extracellular helical apolipoproteins was our observation that apolipoproteins of HDL, such as apoA-I, A-II, and E, remove phospholipid and cholesterol from mouse peritoneal macrophages and generate new HDL particles (18) (Figure 2). The lipoprotein thus generated meets the criteria of preβ-HDL with respect to physical and chemical properties (18) (Figure 2AB), morphological appearance (19,20), and biochemical characteristics such as reactivity to LCAT (11,21) (Figure 2C). Cholesterol in the cells reciprocally decreased mainly in the compartment accumulated as cholesteryl ester (18). The reaction can be carried out by various helical apolipoproteins having amphiphilic helices composed of some 20–22 amino acid residues, so that apoA-I, A-II, A-IV, E, and insect apoIII all generate HDL (18,22,23), and so do synthetic

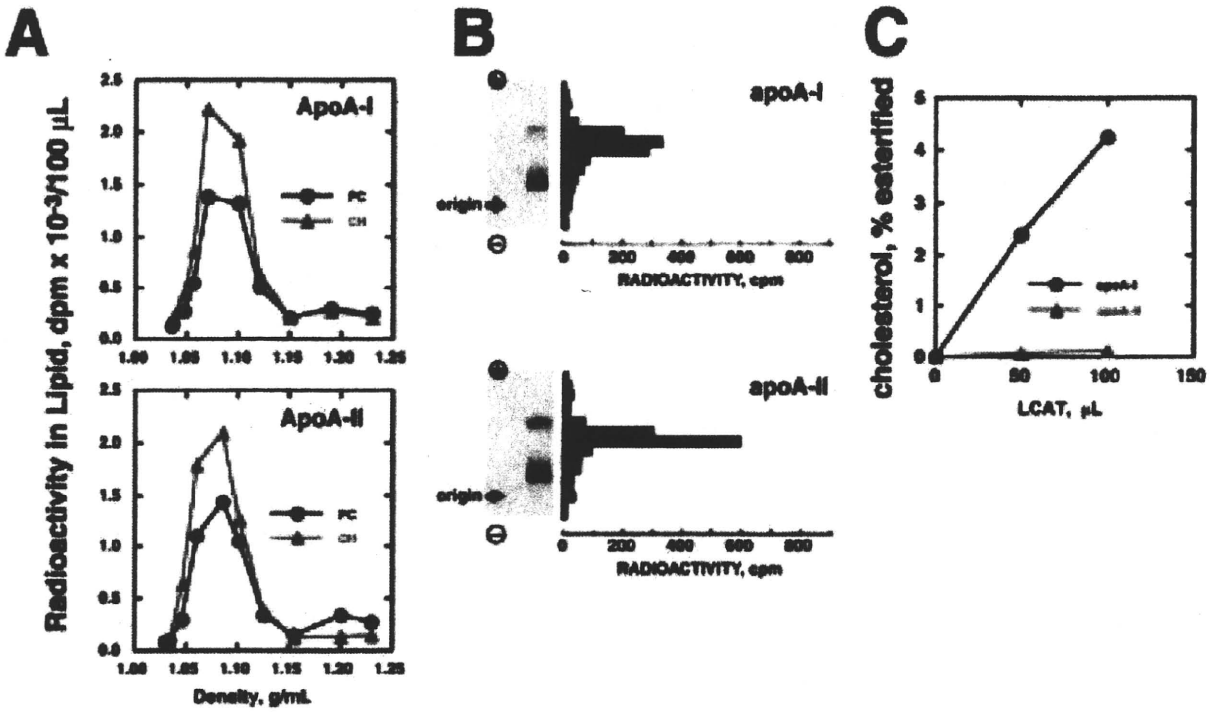

Figure 2. HDL biogenesis by apolipoproteins and cellular lipid. Panels A and B show the results of incubation of mouse peritoneal macrophages with apoA-I or apoA-II. The medium was analysed by ultracentrifugation (Panel A) and agarose gel electrophoresis (Panel B, bands of fast and slow mobility in each gel indicate HDL and LDL, respectively) (18). Panel C demonstrates the reactivity to LCAT (activity was standardized for plasma LCAT activity) of the HDL generated by human fibroblasts and apoA-I or apoA-II (11).

amphiphilic peptides as far as they meet such criteria (23,24). More recently the peptides were shown to be active whether composed of D- or L-amino acids (25). It seems that certain numbers of the helical segment are required to carry out the reaction.

The physiological relevance of this reaction became evident by the finding that the cells from patients with Tangier disease, familiar HDL deficiency, lack the interaction with apolipoprotein and the HDL assembly (26,27). Mutations were identified in the gene of ATP-binding cassette transporter A1 (ABCA1) in patients with this disease (28–33), and disruption of this gene resulted in the HDL deficiency in mice. Thus, ABCA1 was shown to be essential for production of plasma HDL (34,35). While apolipoproteins do not interact with the Tangier cells and generate no HDL (26,27), the cells are intact for the non-specific diffusion-based cholesterol release (26). This means that ABCA1 may act as or create a direct interaction site for apolipoproteins to generate HDL. To support this idea, induction of the HDL assembly reaction in RAW264 cells by cAMP is accompanied by induction of apoA-I binding and expression of ABCA1 (36,37). Thus, ABCA1 essentially functions as a mediator for apolipoprotein-cell binding and for subsequent assembly of nascent HDL particles from apolipoprotein and cellular phospholipid/cholesterol.

Helical apolipoproteins are in equilibrium between a lipid-bound form and a dissociated form from the lipid surface presumably free in solution. Although the dissociation constants of apolipoproteins are not known directly for the HDL surface, the constants measured for the LDL-size lipid particles are all in the order of 10^{-7} M, which may not be irrelevant to be extrapolated for the HDL surface (38,39). Assuming that the dissociation constant of apoA-I is in this range, and binding capacity of HDL is just enough to accommodate the total plasma apoA-I, a few percent of plasma apoA-I can be lipid-free in the aqueous phase in equilibrium. It should be noted that the K_m for the HDL assembly reaction is less than 1% of plasma apoA-I concentration (18) so that this protein in a free form can carry out the reaction at the V_{max}. Also, there are several reactions that reportedly liberate helical apolipoproteins from the HDL surface in plasma, such as CETP in the presence of free fatty acids (40–42). Phospholipid transfer protein (PLTP) (43) by itself also releases apolipoprotein from HDL, and transfer of cellular phospholipid and cholesterol to HDL was indeed enhanced by PLTP (44).

Apolipoproteins can be transferred from HDL to the cell surface simply due to the higher affinity of free apolipoproteins for the cells than for lipid surface (45).

We investigated the ABCA1-dependent interaction of HDL particles with cells (46) (Figure 3). ABCA1 mediates the interaction only of the protein moiety of HDL but not its lipid (Figure 3AB). It was also shown that a monoclonal antibody specific for lipid-free apoA-I selectively inhibited the ABCA1-dependent part of cell cholesterol release to HDL (46) (Figure 3CD). These findings were magnified when apoA-I was displaced by apoA-II to increase lipid-free apoA-I. In that paper, kinetic analysis of the data indicated that apoA-I has an affinity for HDL as high as that for cellular surface, and apoA-I could still be transferred from HDL to cell surface. It is thus not irrelevant to speculate that apolipoproteins dissociate from HDL and interact with the cells in their lipid-free form to generate new HDL particles.

Major sites for synthesis of helical apolipoproteins, especially for the main apolipoprotein of HDL, apoA-I, are believed to be the liver and intestine. In contrast to apoB-containing lipoproteins, however, no HDL particles, not even premature HDL, have been identified in the secretory pathways such as the endoplasmic reticulum and the Golgi apparatus in the cells of these organs. Nevertheless, HDL particles are found in the culture media of the hepatocytes (47,48) or in the perfusate of the liver (49,50), mostly as a so-called nascent HDL that is composed mostly of surface lipids, phospholipid, and cholesterol, not containing much core lipid, and

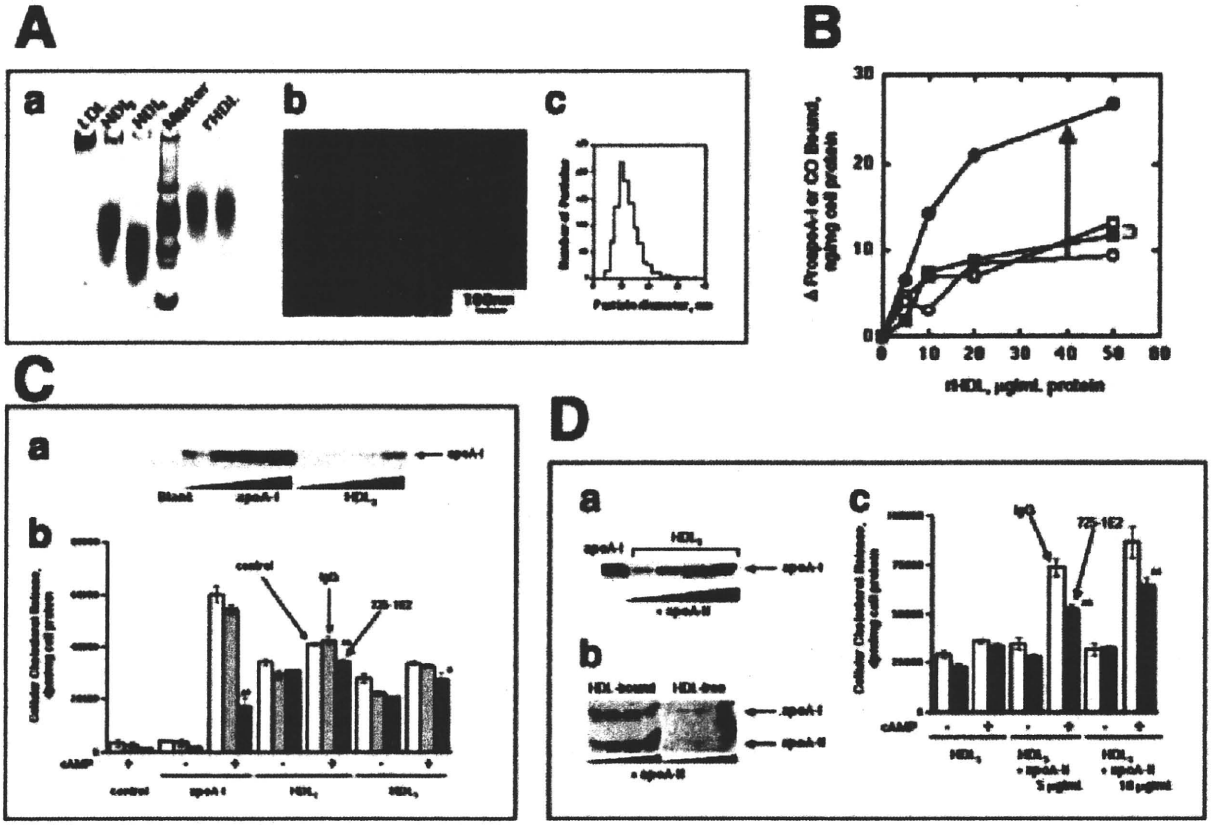

Figure 3. Binding of HDL components to RAW264 cells when ABCA1 expression is induced by cAMP (46). Panel A shows reconstituted HDL of apoA-I (proapoA-I), cholesteryl oleate and egg phospholipid. Panel B shows the results of binding of the particles labelled with uniformly labelled proapoA-I with ^{3}H and ^{14}C-cholesteryl oleate. An upward red arrow between open and closed circles indicates the increase of proapoA-I binding by induction of ABCA1 expression by cAMP. Red lines between open and closed squares indicate change of cholesteryl oleate (CO) binding by inducing ABCA1 expression. Binding takes places only with protein of HDL. Panels C and D demonstrate inhibition of the ABCA1/apoA-I-mediated cholesterol release by the monoclonal antibody specific for lipid-free apoA-I, 725-1E2. Panel C-a shows specificity of the antibody against lipid-free apoA-I, and Panel C-b shows inhibition by the antibody of the apoA-I- and HDL-mediated cell cholesterol release induced by cAMP. ApoA-I-mediated cholesterol release was inhibited by 75% of the cAMP-induced increment, and the increment of the HDL-mediated cholesterol release by cAMP was inhibited to the same extent as the apoA-I-mediated release was inhibited. Panel C shows the results of the similar experiments performed in the presence of apoA-II. ApoA-II displaces apoA-I from the HDL surface to make it a free form (Panel D-a and D-b), and therefore the increment of cell cholesterol release was larger in this condition (Panel D-c). The antibody inhibited so much as the apoA-I-mediated cholesterol release (Panel D-c).