review

High density lipoprotein receptors, binding proteins, and ligands

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Abstract **Several HDL binding proteins, quite disparate in structure, have recently been cloned and their role in HDL metabolism is currently being assessed. High density lipoprotein binding protein, HBP (vigilin), which lacks a transmembrane domain is responsive to cell cholesterol levels, but its physiological significance remains unknown. On the other hand much is known about SR-B1, a member of the class B scavenger receptors. The level of SR-B1 expression correlates with both the selective transfer of cholesteryl ester into cells and cholesterol efflux from cells, the transfers probably mediated after docking of HDL at the cell surface. SR-B1 exhibits broad ligand specificity and, in animal models, appears to be regulated by the action of pituitary hormones that stimulate steroidogenesis, suggesting an important role for steroid hormone production in supplying precursor cholesterol. Another candidate HDL receptor, HB_2, one of a pair of liver HDL binding proteins, shows high sequence homology with adhesion molecules, particularly activated leukocyte-cell adhesion molecule (ALCAM). When HB_2 is overexpressed in cells, HDL binding increases. After PMA-induced differentiation of monocytes into macrophages, HB_2 mRNA is strikingly elevated, which correlates with increased binding of HDL, but is down-regulated by cholesterol loading of macrophages. The ligand specificity of the HDL receptors, confounded by nonspecific lipid interactions, remains controversial. Their affinity for apoA-I versus apoA-I/A-II-rich HDL particles has clinical implications; both specific sequences in apoA-I and amphipathic α-helices may determine binding events. Post-receptor-mediated signalling events may regulate cell functions which, although not primarily related to lipid transport, nevertheless protect against coronary artery disease. Growing evidence for the involvement of lipid-poor apoA-I as a mediator of such pathways is also discussed.—**Fidge, N. H. **High density lipoprotein receptors, binding proteins, and ligands.** *J. Lipid Res.* **1999.** 40: **187–201.**

Supplementary key words HBP • SR-BI • HB_2 • ligands • biochemical signalling • activated leukocyte-cell adhesion molecule • cholesterol • apolipoprotein E

As a consequence of the complex structure and function of circulating high density lipoprotein (HDL), the search continues for cell surface receptors that recognize HDL. There is an expectation that the processing of this complex lipoprotein will be determined by more than one site, some interactions mediating lipid exchange, others possibly connected with apolipoprotein catabolism, while signalling events triggered by HDL binding may be regulated by distinct, but as yet unidentified receptors.

HDL comprises several subclasses varying in size and composition, and the existence of these various particles is inextricably related to an organized lipid transport system that features remodelling and probable recycling of the macromolecules in response to the action of lipolytic enzymes and lipid transfer proteins. That various plasma factors orchestrate exchange and transfers of both lipid and apolipoprotein moieties, producing a number of identifiable subclasses of HDL particles, apparently with different functions in the body, is well documented.

Implicit in the concept of a lipid transport system is the existence of biological systems that regulate the entry and exit of metabolites (that comprise the circulating lipoproteins) into and out of the circulation. Apart from the low density lipoprotein (LDL) receptor there is much less certainty about the nature of the cellular as opposed to the intraplasma processing of lipoproteins and with regard to HDL, continues to engage the attention of researchers seeking clues to the cell events that regulate HDL metabolism.

This review mostly concerns HDL receptors although such a discussion would be incomplete without briefly considering the involvement and nature of the ligands that bind to the putative receptors. It is also important to consider the range of biochemical events that are influenced by ligand (HDL)-receptor interactions and consequently the review is divided into 3 sections:

- Structure and properties of candidate HDL receptors
- Identification of ligands recognized by HDL receptors
- Biochemical events affected by the HDL receptor interaction

Abbreviations: HDL, high density lipoprotein; HBP, HDL binding protein (vigilin); SR-B1, scavenger receptor binding protein-1; apo, apolipoprotein; LBS, lipoprotein-binding site; PC, phosphatidylcholine; PI, phosphatidylinositol.

STRUCTURE AND PROPERTIES OF CANDIDATE HDL RECEPTORS

Many cell proteins that bind HDL have been advanced as putative HDL receptors, but in most cases such claims remain unsubstantiated. The search for cell surface receptors recognizing HDL followed earlier reports of specific HDL binding sites on different cultured cells including intestinal mucosal cells (1), fibroblasts (2), hepatocytes (3) adipocytes (4), and steroidogenic tissues (5). It was apparent from these studies that a substantial proportion of the total binding was attributable to nonspecific binding sites, a portent possibly of problems later encountered in attempts at purifying and cloning the elusive receptor(s). Subsequently, binding sites for HDL, or its major apolipoprotein A-I, with parameters more convincing of the features of a physiological receptor were identified in liver plasma membranes (6) and in hepatocytes (7). These sites, characterized by a rapid dissociation of the ligand, bind HDL with high affinity (3×10^{-9} m) and are only revealed by rapid removal of adsorbed HDL. Under these circumstances both the high affinity binding site and another of lower affinity (approx. 2×10^{-7} m), the latter possibly reflecting weaker (but not necessarily less physiologically significant) lipid–lipid or protein–lipid associations, are identified. Unfortunately, the existence of these nonspecific sites introduces false positives that confound attempts at purification of receptors and cloning strategies which depend on binding assays to identify only specific interactions, no doubt frustrating many previous attempts at identification and receptor characterization.

Nevertheless, several candidate HDL receptors, have now been isolated. Only those that have been cloned will be considered in detail in this review. Earliest identifications of candidate receptors were based on the use of ligand binding techniques to detect HDL binding proteins in tissues or cultured cells, the assumption being that electrophoretic separation of partially purified membrane proteins would facilitate the isolation of specific binding proteins and provide a useful assay to monitor the progress of their purification. A binding protein of M_r 78,000 was isolated from adrenal cortical membranes (8), and from sheep kidneys (9). Although further attempts at purification were hampered by loss of activity, the size of this protein, its presence in steroidogenic tissue, and the demonstration that binding activity was weakened after reduction, suggest in hindsight that this protein was SR-B1. Other HDL binding proteins of larger size, ranging between 100 and 120 kDa, were found in various cells including fibroblasts and endothelium (10), adipocytes (11), and hepatocytes (12). Some laboratories identified two binding proteins in the same cells (12, 13) raising the possibility that more than one class of HDL receptors exists.

That candidate receptors were identified in cells from tissues known to process HDL in the body was consistent with the theory of reverse cholesterol transport, in which it was anticipated that cholesterol transfer to HDL from peripheral cells may involve receptor recognition of HDL, a preferred acceptor of cell cholesterol, and the cholesterol associated with HDL would then be delivered to the liver through a mechanism that most probably also depended on specific recognition through the same or a different receptor.

Evidence was produced to support this suggestion. Graham and Oram (10) found that the "binding activity" of an HDL binding protein identified in fibroblasts, bovine aortic endothelial cells, and hepatocytes was enhanced several-fold when human arterial smooth muscle cells were pre-treated with cholesterol; the expression of this 110 kDa protein appeared to be stimulated in a dose-dependent manner by cholesterol loading. To test the hypothesis that this protein was a candidate HDL receptor, McKnight et al. (14) isolated a cDNA clone encoding the novel 110 kDa binding protein, designated high density lipoprotein binding protein (HBP), and demonstrated that HBP mRNA expression in cultured cells increased in response to cholesterol loading (**Table 1**). It was later shown to be identical to vigilin (see below). The predicted structure of HBP revealed neither the presence of a classic hydrophobic transmembrane spanning sequence nor clearly defined cytoplasmic or extracellular domains that characterize known receptors. Apparently it became attached to the cell

TABLE 1. Summary of the characteristics of HDL binding proteins that have been cloned

Receptor	Reference	Homologues	Structure	Distribution (Major)[b]	Ligands	Function / Properties
HBP	McKnight et al. (14), Chiu et al. (15)	Vigilin	150 kDa,[a] 110 kDa, no transmembrane domain, not glycosylated	Cytoplasmic, ubiquitous, endothelial cells, macrophages	HDL, apoA-I	Unknown, estrogen-inducible, cholesterol-sensitive
SR-B_1	Acton et al. (21)	CD36, CLA-I	82 kDa, glycosylated, acylated, colocalized with caveolae, two transmembrane domains	Plasma membrane, steroidogenic tissue, liver	HDL, LDL, modified LDL, apoA-I, apoA-II, apoC	Docking protein, CE uptake, cholesterol efflux
HB_2	Matsumoto et al. (46)	ALCAM, BEN	100 kDa, glycosylated, one transmembrane domain	Plasma membrane, liver, brain, intestine, lung, macrophages	HDL, apoA-I, apoA-II	Unknown, cholesterol,-statin sensitive, adhesion, signalling?

HBP, HDL binding protein; SR-B_1, scavenger receptor, class B_1; HB_2, HDL binding protein$_2$.
[a] Precursor form.
[b] Tissue distribution of immunoreactive proteins.

surface at some stage where it most likely bound HDL (**Fig. 1**). If anchored through glycosylphosphatidylinositol-enriched domains, there is a possibility for interaction with signalling molecules or caveolae, known to be sensitive to cell cholesterol content. Future investigations may reveal the existence of such a mechanism.

McKnight et al. (14) were also cautious about the interpretation of experiments testing the function of the protein when overexpressed in cells, because the HBP products lacked HDL binding activity, although cell processing may have produced different forms of HBP, not all of which are capable of binding HDL. In addition, HBP-specific antibodies were unable to block HDL binding to isolated cell proteins, or to immunoprecipitate binding activity from isolated membranes. They concluded that there was insufficient data to either support or exclude the possibility that HBP functions as an HDL receptor and that more studies were needed to assess its involvement in cell cholesterol metabolism. Chiu et al. (15) recently detected HBP (vigilin) in human atherosclerotic plaques but not in non-diseased coronary intima, consistent with earlier observations that HBP (vigilin) is up-regulated by cholesterol loading of cells. Although vigilin/HBP lacked properties of a typical cell surface receptor and McKnight et al. (15) concluded that it was unlikely to be an HDL receptor, vigilin nevertheless appears to respond to steroid hormones and cholesterol levels of cells. Vigilin, which binds to RNA, may act to stabilize message and influence RNA metabolism (16). As it associates with RNA and contains K homology domains as well as a nuclear localization sequence, it is conceivable that HBP-vigilin responds downstream of events initiated by HDL binding and signals pathways that lead to cholesterol mobilization. In the processed form (110 kDa), HBP/vigilin binds HDL and apoA-I on ligand blots (10); conceivably therefore apoA-I may interact with cytoplasmic vigilin promoting changes in cholesterol flux.

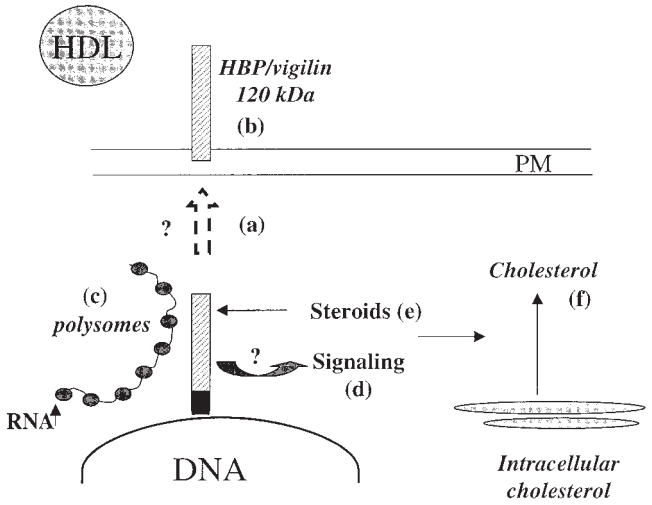

Fig. 1. HBP/vigilin, a ubiquitous protein, is cytoplasmic but may attach to the plasma membrane (a) and in the 120 kDa form (b) is known to bind HDL (10). HBP/vigilin binds RNA (c), associates with polysomes (16), is regulated by steroid hormones (e) (16), and contains a nuclear localization sequence. HBP/vigilin levels increase in cholesterol-loaded plaque macrophages and it colocalizes with apoE (15). Whether HBP/vigilin, through stablization of message, mediates signaling events that promote cholesterol efflux (f) remains unknown.

Their cautionary interpretation of these findings related to HBP transfected cells were understandable as Oram, Brinton, and Bierman (17) previously found strong evidence supporting a relationship between cholesterol loading of cells and a corresponding increase in HDL-binding sites that appeared to be "specific" as assessed by the customary binding experiments. Further, a specific metabolic response appeared to be triggered by the interaction because HDL binding was accompanied by translocation of intracellular cholesterol to the plasma membrane (18) and because the investigators had obtained preliminary data implicating the activation of protein kinase C (19) after receptor–ligand association. This proposal, not inconsistent with a physiological role for the HDL–cell interaction in reverse cholesterol transport, broadened prevailing opinion that HDL acted merely as a passive acceptor of cholesterol, independent of specific processes or of direct contact between the lipoprotein and the cell membrane.

Several other lipoprotein binding sites exhibiting broad lipoprotein specificity have been reported in the literature, some probably pre-empting identification of SR-B1. Bachorik et al. (3) identified a putative receptor in pig hepatocytes that mediated the uptake and degradation of both apoE-free HDL and LDL. As HDL levels are much higher than LDL in pig plasma, it is conceivable that this "lipoprotein binding site, LBS" acts as a functional HDL receptor despite its inability to distinguish the two lipoproteins. The affinity of the site(s) for binding either HDL or LDL was in the order of 2×10^{-8} m, similar to that observed for HDL binding sites in other tissues. Brisette and Noel, (20) also reported an unspecified lipoprotein binding site in rat liver membranes, which suggests that the 'LBS' is, or is closely related to, SR-B1.

Acton et al. (21), by expression cloning, isolated the cDNA for a new member of the CD36 family of membrane proteins, named SR-B1, while investigating the physiological properties of CD36 in a variant CHO cell line using acetylated LDL as ligand. SR-B1 belongs to the class B scavenger receptor family of proteins which are characterized by an immunodominant ligand binding domain (residues 155–183; Puente et al. (22)) as in CD36, compared to the charged collagenous structure that typifies the class A type I and II macrophage scavenger receptors. Class B-also differ from class A-scavenger receptors in terms of binding specificity, the main difference being that the large number of polyanionic ligand binding sites in class A proteins are not present in class B receptors. CD36 was isolated as a result of expression cloning of oxidized LDL receptors (23) but, like other of the scavenger receptors, exhibited broad specificity and was expressed in a variety of tissues. As well as binding native and modified LDL, SR-B1, after transfection into LDL receptor-negative CHO cells, also bound HDL with high affinity and mediated the selective transfer of cholesteryl esters into the cells (Table

1). Whereas approximately 20% of the HDL cholesterol added to the medium was taken up by SR-B1 transfected cells, less than 0.5% of the apolipoprotein was internalized, indicating that whole HDL particles were not endocytosed after receptor recognition. These findings are essentially similar to those first reported by Pittman et al. (24) who demonstrated that selective uptake of cholesteryl esters occurs in a variety of cultured cells, is not endocytotic, and represents a net mass transfer and not mere exchange of lipid components. Similarly, Brissette, Charest, and Falstrault (25) demonstrated that LBS (see above) appeared to be responsible for the selective uptake of LDL cholesteryl esters by HepG2 cells and, as 45% of the total cholesteryl ester taken up was hydrolyzed, LBS was clearly physiologically significant. Attempts to isolate LBS or estimate its molecular mass by ligand-blotting were unsuccessful, but the exceptional similarities in properties suggest that LBS and SR-B1 are identical or close members of the same family of scavenger receptors.

SR-B1 is similar in structure to CD36 and recent findings of Tao, Wagner, and Lublin (26), who observed two sites of palmitoylation at cysteine residues at the extreme of the N-terminus (residues 3 and 7) and the C-terminus (residues 464 and 466), are consistent with the proposal that both the N-terminus and the C-terminus are cytosolic. These termini flank the two hydrophobic domains of CD36, and it is well established that the bulk of the segment between these two hydrophobic (transmembrane) regions is extracellular. Palmitoylation may assist in targeting proteins to membranes. Structural studies have shown that SR-B1, like CD36, is also fatty acylated, most likely at Cys^{462} and Cys^{470} (26). The predicted amino acid sequence of $_{m}$SR-B1 suggests that Cys^{462} resides at and Cys^{470} is near the junction of a putative transmembrane domain (residues 444–464) and the cytoplasmic C terminus (465–509). SR-B1 colocalizes with plasma membrane caveolae and is copurified with caveolin (27). These membrane domains may play an important role in SR-B1-mediated lipid transfer between cells and lipoproteins. SR-B1, like CD36, is substantially glycosylated, involving N-linked oligosaccharide chains. After deglycosylation, the mass of SR-B1 reduced from 82 to 54 kDa (27).

Numerous reports have rapidly followed that establish its receptor status and providing clues to SR-B1's role in HDL metabolism. SR-B1 is highly expressed in liver, ovary, and adrenal gland with lower expression in the testis and mammary gland and only a trace amount in the heart (21) consistent with the proposal that SR-B1 mediates delivery of cholesterol to steroidogenic tissues (as a precursor of steroid hormones) and to the liver as a final process of reverse cholesterol transport. An increase in expression of the receptor in the adrenal gland and corpus luteum (but a decrease in the liver) after treatment of rats in vivo with estrogen supports this proposal (28). Independent confirmation for the presence of SR-B1 in the rat was reported by Mizutani et al. (29) who isolated a gonadotropin-inducible gene from rat ovaries that was homologous to SR-B1. Ovarian SR-B1 was also strongly induced by the corresponding pituitary hormone. In the study of Landschultz et al. (28), administration of human chorionic gonadotropin also induced a dramatic increase in SR-B1 in the Leydig cells of the testis, providing further evidence for an association between SR-B1 regulation and fluctuations in steroid hormone production. Rigotti et al. (30) localized SR-B1 in murine adrenal cortex to the surface of cortical cells and also demonstrated SR-B1 induction in vivo in the adrenal cortex after administration of ACTH to mice. These studies are consistent with the proposal that up-regulation in expression of SR-B1 by key steroid hormones makes provision for extra supplies of precursor cholesterol to tissues for synthesizing additional steroid hormones.

Sometimes overlooked is the fact that in rodents, HDL carries most of the circulating cholesterol, unlike humans where LDL is the chief sterol transporter and where most tissues synthesizing steroids obtain supplies additional to de novo synthesis through regulation of the LDL receptor. A number of laboratories have demonstrated in vitro and in vivo that LDL and not HDL is the preferred source of cholesterol by steroidogenic tissue. With cultured adrenal cells, LDL uptake and catabolism was stimulated 5- to 6-fold by the addition of ACTH to the medium (31) but cholesterol in HDL_3 (devoid of apoE) was not used for adrenal steroid hormone production. Illingworth, Kenny, and Orwoll (32) studied corticosteroid synthesis in patients with phenotypic abetalipoproteinemia, a condition characterized by extremely low or absent plasma LDL. Although cortisol production appeared normal in these patients, possibly as a result of increased de novo sterol synthesis, chronic stimulation of adrenal hormone production by ACTH resulted in subnormal production of corticosteroids, therefore providing little evidence for a pituitary-stimulated up-regulation of a receptor in humans that provides extra precursor sterol via HDL. During the midluteal phase of the menstrual cycle, the corpus luteum produces large quantities of hormone (approximately 30–40 mg progesterone/day from 1 g of tissue). Illingworth et al. (33) reported a marked reduction in progesterone levels in a female patient with hypobetalipoproteinemia despite normal levels of circulating HDL. Taken together, these studies provide little evidence for the utilization of HDL cholesterol by steroidogenic tissue in humans, or at least for the up-regulating of a receptor pathway that can adequately substitute for LDL in cases of LDL deficiency, especially for maintaining steroidogenesis under conditions of sustained stimulation by pituitary hormones such as ACTH. To evaluate the precise role of SR-B1 in delivering HDL cholesterol to tissues in human subjects will require further investigation.

There is also evidence that SR-B1 participates in reverse cholesterol transport at the initial phase of cholesterol entry into the pathway. Ji et al. (34) found that cholesterol efflux was significantly enhanced in CHO cells stably transfected with murine SR-B1, the efflux rates correlating with the degree of overexpression of the receptor and HDL concentration. That the rate of cholesterol efflux to HDL was positively correlated ($r = 0.859$) with SR-B1 expression in six different cell types, ranging from

peritoneal macrophages to fibroblasts, hepatoma, and adrenal cells, underscores the functional significance of this finding.

That SR-B1 apparently mediates both cholesterol efflux from cells and selective transfer of cholesteryl esters into cells endorses the proposal that this receptor protein alone facilitates all the cellular phases of reverse cholesterol transport, with the caveat that its action in humans mirrors its performance in rodents. The model proposed by Steinberg (35) of a docking action for SR-B1 that results in the anchoring of HDL to cells thus receives an experimental imprimatur (**Fig. 2**). By a mechanism yet to be identified, HDL docks onto SR-B1 where it either donates or accepts cholesterol from cells, the direction of movement presumably determined by a differential concentration gradient. It should be noted that the selective transfer of cholesteryl ester or efflux of cholesterol from the membrane to SR-B1 are most likely mediated by different mechanisms, the former possibly involving a more specific process than the latter. Other membrane properties, including cholesterol: phospholipid ratios or changes in fatty acid composition affecting spatial distribution of cholesterol in either cell membranes or HDL, may act as signals to change bi-directional diffusion to a more productive net movement of sterol between cells and HDL. The quantitative net movement of lipid will also depend on the level of SR-B1 expression which, in turn, is undoubtedly dependent on regulation of genes, some of which as already observed above, are involved with steroid hormone metabolism. However, the mechanism involved in the cellular uptake of HDL lipids mediated by $_{m}$SR-B1 is becoming clearer. Gu et al. (36) recently showed that whereas both SR-B1 and CD36 mediated surface binding

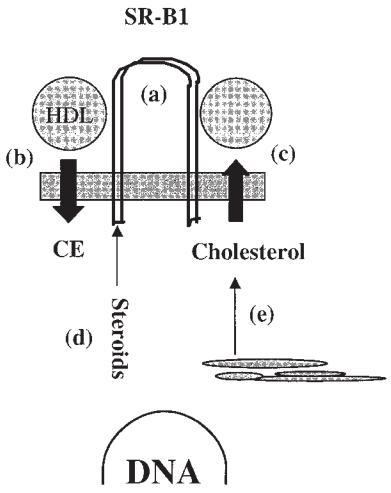

Fig. 2. SR-B1, an 80 kDa plasma membrane protein (a) traverses the membrane twice, the NH2- and COOH-termini both within the cell. It appears to act as a docking protein and mediates the selective transfer of cholesteryl ester into cells (b) independent of the uptake of the whole particle (21). It also mediates cholesterol efflux (c) from cells (34), but whether this involves mobilization of intracellular cholesterol) (e) is unknown. SR-B1 is found in many cell types (21) and increases during steroidogenesis; it is also regulated (d) by steroids and pituitary hormones (28–30).

of HDL, only COS cells transiently expressing SR-B1 efficiently mediated the transfer of lipid to the cells. By generating SR-B1/CD36 domain swap chimeras, the authors (36) also demonstrated that the extracellular domain differences accounted for the difference in lipid uptake. SR-B1 also mediates the transport of cholesterol through the intestinal mucosa providing further evidence of its role as a transport protein (37).

The ability of SR-B1 to promote both efflux of cholesterol and uptake of cholesteryl esters in a coordinated, regulated manner has been strengthened by recent in vivo studies of Fluiter, van der Westhuijzen, and van Berkel (38). The investigators showed that, whereas SR-B1 was down-regulated in liver parenchymal cells following ethinyl estradiol treatment, receptor expression in Kupffer cells was significantly increased. This contrast in regulation possibly reflected the opposing roles of SR-B1: on the one hand, parenchymal cells lowered SR-B1 expression in response to cholesterol loading whereas Kupffer cells (macrophages) increased expression of SR-B1, possibly related to its function as a mediator of cholesterol efflux.

This concept is supported by other literature. That SR-B1 is a functional receptor under feedback control was established using apoA-I or hepatic lipase knockout (KO) mice as models of cholesterol depletion. Wang et al. (39) found a substantial increase in expression of adrenal SR-B1 mRNA and protein levels in apoA-I KO mice but not in apoA-II, apoE, or LDL receptor KO mice, and found no changes in the liver, possibly because liver cholesterol is less depleted than adrenal cholesterol or else SR-B1 is less important as a determinant of liver cholesterol. SR-B1 expression was also up-regulated approximately 3- to 4-fold in female but not male hepatic lipase KO mice, in which adrenal stores of free and esterified cholesterol were significantly depressed. The increased expression of SR-B1 compensates for the loss of hepatic lipase, which also mediates the uptake of cholesterol and the selective transfer of cholesteryl esters into tissues, and its absence would deplete adrenal sterol stores. Further evidence that SR-B1 expression is under feedback control was obtained when mice were stressed by cold swimming, a challenge which is known to stimulate ACTH release and corticosteroid synthesis, with a substantial depletion of adrenal cholesterol stores. The challenged mice showed a 2-fold increase in SR-B1 mRNA over untreated mice.

Using an alternative approach, where SR-B1 was overexpressed in mice, Kozarsky et al. (40) found a dramatic decrease in both HDL cholesterol and apoA-I levels and a substantial increase in biliary cholesterol. These mice transiently overexpressed SR-B1 after infection with a recombinant, replication-defective adenovirus, in which >99% of transgene expression is localized to the liver. In view of this nonphysiological mode of expression (exclusive to the liver) and considering that cholesterol depletion in apoA-I KO mice had no effect on liver expression of SR-B1 (39), more information is needed to establish whether SR-B1 plays a significant role in human liver. Nevertheless, these studies suggest that SR-B1 possibly regulates cholesterol concentrations in the bile and may

therefore influence gallstone formation, if the events are applicable to humans. In this respect, CLA-1, a human homologue of rodent SR-B1, has recently been cloned and sequenced (41). Sharing 81% homology with SR-B1, CLA-1 also mediates uptake of cholesteryl esters and is abundant in the adrenal gland, liver, and testis. CLA-1 is also present in monocytes and recognizes apoptotic thymocytes, suggesting it may play an additional role in processing damaged cells.

Rodents carry most cholesterol in HDL and the developing fetus presumably depends on a supply of sterol during the development stage. This has recently been confirmed by Hatzopoulos et al. (42), who also followed the expression of SR-B1 in intra- and extra-embryonic tissues. Peaks of SR-B1 expression followed a pattern, commencing in the trophoblasts surrounding the embryo, to the placenta and yolk sac (E10), and appearing in the adrenal gland of the embryo proper at E14.5. In the liver it only appeared after birth.

A variant of SR-B1 was recently discovered. Termed SR-BII, it arises from alternative splicing of SR-BI precursor transcripts (43), such that the encoded C-terminal cytoplasmic domain is almost completely different. SR-BII also mediates selective lipid transfer between HDL and cells, albeit at lower efficiency than SR-BI.

Two other membrane proteins that bind HDL, named HB_1 and HB_2 with molecular mass of 120 and 100 kDa, respectively, have been isolated and purified from rat liver (12, 44). As the expression of both proteins was down-regulated by 50–60% after administration of simvastatin to rats (45), their identity was further pursued. HB_1, present in low amounts and possibly unstable to extensive purification procedures, has not been sequenced but HB_2, the more abundant of the pair, has now been cloned (46). HB_2 is not structurally related to any of the HDL receptors described above but belongs to the immunoglobulin superfamily and bears striking resemblance to the adhesion molecules ALCAM (93% homology) and BEN (70% homology) (Table 1). These proteins, originally identified as adhesion molecules, may have several functions. ALCAM was isolated by immunoabsorbance (47) from a breast carcinoma cell line (HBL-100) that expressed high levels of CD6 and an antigen showing significant homology with BEN, a chicken neural adhesion molecule (48). Bowen et al. (47) used BEN DNA fragments to screen a PHA-activated human T-cell cDNA library which resulted in the cloning and sequencing of ALCAM.

To determine whether HB_2 remained a candidate for an HDL receptor, COS, CHO, or HepG2 cells were transfected with HB2. Cells that expressed HB2 showed 80–100% increases in HDL binding and ligand blots confirmed the findings, revealing substantially enhanced binding of HDL with a band corresponding to HB_2. A more clinically interesting observation was that HB_2 mRNA, hardly detectable in undifferentiated THP-1 cells, was strikingly elevated after their transformation with PMA into macrophages (46). Ligand blots of membranes taken from these transformed cells also showed considerably higher association of HDL to both HB_1 and HB_2 than with untreated cells. Furthermore, after incubation with acetylated LDL, thereby increasing the macrophage cholesterol content, a dose-dependent decrease in HB_2 mRNA was observed (46), suggesting some association, either direct or indirect, between HB_2 expression and cholesterol metabolism. This finding was consistent with the previous observation (45) that simvastatin treatment caused down-regulation of HB1 and HB2 in rats and another more recent demonstration of a similar finding in simvastatin-treated rabbits (49). Further work is needed to clarify the connection between changes in HB_2 expression and cholesterol metabolism; however, preliminary studies with [^{3}H]cholesteryl ester-labeled HDL appear to exclude selective uptake of cholesteryl ester from HDL as a process mediated by HB_2 (46).

HB_2 like ALCAM, is widely distributed in the body (46), being detected in the lung, liver, intestine, and in smaller amounts in the kidney, ovaries, and testis. HB_1, on the other hand, differed slightly in tissue distribution, strongest signals (on Western blots) being observed in the spleen, but weaker although detectable quantities were seen in the liver, intestine, and lung (50). This, together with previous findings that HB1 and HB2 do not immunochemically cross-react (12), suggests that the two proteins may have different functions. The distribution of HB_2 mRNA, although similar, was not identical to HB_2 protein distribution, as in the rat it was strongly expressed in the brain, possibly representing a rat homologue of BEN, known to be present in neuronal tissue. In fact, human HB_2 mRNA was expressed most strongly in the brain, then prostate, small intestine, and liver, with low levels in the lung (46).

The structural features of HB_2, based on available structural information concerning this group of membrane proteins of the IgG superfamily, are consistent with a 'receptor' role for this protein. They are characterized by the presence of a 32-amino acid cytoplasmic tail (C-terminus), a 24-amino acid hydrophobic transmembrane domain, and approximately 500 residues of an extracellular domain terminating in the NH_2 residue. There are eight potential *N*-glycosylation sites, all extracellular, at residues 95, 167, 265, 306, 361, 457, 480, and 499. Although most of these sites in isolated HB2 appear to be glycosylated, their function remains unclear, as deglycosylation did not appear to affect HDL binding according to previous ligand blot studies (44). Provisional PKC phosphorylation sites were also found at residues 8, 73, 74, 209, and 421 but being extracellular, their signalling potential is unknown, although they may become active if intact or cleaved products of HB_2 are internalized.

The increased expression of HB_2 after PMA treatment of THP-1 (46) cells may be significant in the context of HDL's protective function against heart disease. HB_2/ALCAM is an adhesion molecule and appears to be up-regulated after cytokine-induced differentiation of monocytes into macrophages. Together with other known adhesion molecules, it possibly contributes to vascular remodelling and the initiation of atherosclerosis. HDL, particularly subclasses of anti-atherogenic apoA-I-rich par-

ticles, may bind to adhesion sites on HB_2, thereby reducing the migration of macrophages into the arterial wall (**Fig. 3**). The influence of HDL may be significant considering the tendency of these adhesion molecules to self-associate. HB_2/ALCAM forms multimers in solution (51) and may potentially form oligomers on cell surfaces, resulting in amplification of reactive sites and an increase in homo- or heterotypic cell adhesion. By disrupting oligomerization, HDL could decrease these interactions and thereby reduce migration of macrophages into the intima and consequent foam cell formation. Together with the observation that HDL inhibits the expression of soluble adhesion molecules such as VCAM and ICAM (52), a combined reduction in cell adhesion due to HDL actions may provide a formidable offensive against arterial wall disease.

Emerging evidence, discussed below, suggests that the interaction between HDL, or its apolipoprotein moiety, and candidate receptors may stimulate biological events through biochemical signalling. Investigators also search for a cell surface protein that mediates uptake of intact HDL, as biochemical evidence exists for this pathway (3). Some members of the adhesion molecule family apparently act as signalling receptors, and these proteins (such as HB_2, HB_1) may trigger transduction pathways leading to antiatherogenic effects, or reduce others that tend to produce atherogenic metabolites. Future research should unravel speculative from valid biological events involved in these pathways.

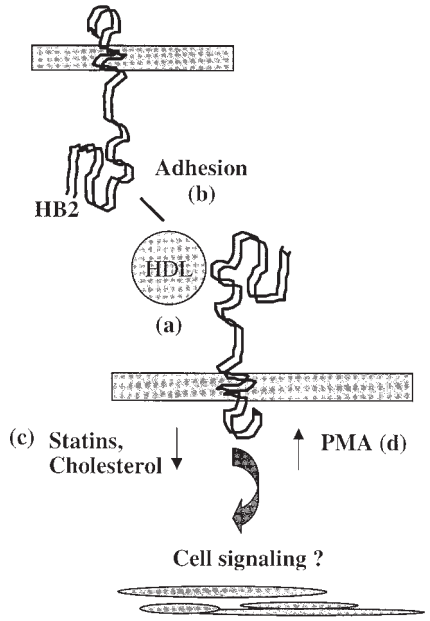

Fig. 3. HB_2 (ALCAM), 120 kDa, has one transmembrane region and a short cytoplasmic tail, the larger NH_2-terminal domain being extracellular. HB_2 binds HDL (a), (12, 44) and is down-regulated by statins (45, 49) or when cells (c) are cholesterol-loaded (46), and up-regulated after PMA treatment (d) of monocytes (46). Due to homo- or heterotypic interactions, this adhesion molecule (47, 51) possibly increases macrophage migration into the vessel wall (46) which, as suggested (b) in the model shown, is reduced as a result of binding HDL. Like other adhesion molecules, it may also activate cell signaling pathways.

IDENTIFICATION OF LIGANDS RECOGNIZED BY HDL RECEPTORS

This review would be incomplete without considering the nature of the molecules that bind to the receptors, a consideration particularly applicable to HDL due to its complex nature. While there is a consensus that apoA-I binds to candidate receptors, the extent to which other apolipoproteins participate in binding or share common receptor sites remains controversial. The biophysical influences of the shape and composition of HDL presumably affect conformation of receptor binding domains, and the fate of various HDL subpopulations is most likely determined partly by the cells and by the receptors to which they bind. Even the formation of some HDL particles may depend on cell processing acting through receptors or docking proteins. Information gained from early in vitro studies suggested that HDL_3, or subpopulations within this subclass which are smaller and depleted of lipid including cholesterol, are better acceptors of cell cholesterol and would participate in the reverse cholesterol process at a different level than HDL_2. After LCAT processing, these larger, cholesteryl ester-rich particles are less efficient in vitro acceptors of cholesterol and therefore are presumably destined for the final stages of reverse cholesterol transport that involves discharging their overloaded sterol cargo to the liver for eventual excretion from the body. Thus the roles of one as an acceptor and the other as a donor may have required different receptors, one mediating cholesterol efflux, but another involving CE transfer or endocytosis of the whole particle. However, early studies (53) did not support this concept as unlabeled HDL_2 and HDL_3 competed equally for binding of ^{125}I-labeled HDL_3 to cells despite differences in their properties as promoters of cholesterol efflux. It appeared that the moieties shared in common between the two particles, such as the apolipoproteins, were the key influence to binding events, (possibly to the same receptor sites) and that the differences in sterol content between the particles determined the direction of the movement and their capacity to act as acceptors or donors of cell cholesterol. The recent demonstration that SR-B1 can mediate both cholesterol efflux as well as selective transfer of cholesteryl esters into cells, together with the fact that this receptor exhibits broad ligand specificity (54), provides a plausible explanation for those earlier observations.

In an early study of apolipoprotein specificity, Fidge and Nestel (55), showed that both A-I and A-I1 apolipoproteins appeared to be involved, while other laboratories reported sites that specifically recognized only apoA-I (56–58), some finding evidence of broader specificity as apos A-I, A-I1, and C appeared to be recognized by HDL 'receptors' (59). Because of the instability of many ligand preparations, however, these data must be interpreted cautiously. A fairly rapid exchange of apolipoproteins or phospholipid is known to occur between lipid–protein complexes that are used as competitors which can result in the formation of mixed, undefined lipoproteins, precluding a definitive interpretation of the results. Probing

suspected ligand sites with antibodies has pitfalls also, including possible steric hindrance if IgG is used, and the uncertainty that epitopes associated with binding sites are represented in the antibody mixture.

Recognizing these constraints, Vadiveloo and Fidge (60) prepared HDL particles containing almost exclusively either apoA-I or A-II that in size, protein mass, and lipid composition resembled native HDL_3. It was reasoned that apolipoprotein conformational changes, known to be influenced by the above parameters, would be minimally affected. With this approach it was found that apoA-I-HDL_3 bound to bovine aortic endothelial cells with higher capacity (3- to 4-fold) than apoA-II-HDL_3, but with only half the affinity of apoA-II-HDL_3. Because ligand blots indicated that similar membrane binding sites were involved, a model consistent with these binding parameters (60) indicates that apoA-II-containing particles can occupy up to four times the number of receptors as apoA-I-only particles, and consequently fewer apoA-II-containing particles are required to saturate the receptors, as reflected in experiments which showed their lower binding capacity.

These biochemical findings supported the clinical/epidemiological observations of Puchois et al. (61) who observed that elevated levels of plasma HDL_3 containing mainly apoA-I protect against heart disease while apoA-II-richer HDL are less effective. They are also less effective at promoting cholesterol efflux than particles rich in apoA-I. As apoA-II appeared to act as an antagonist for apoA-I particles, and apoA-I-rich HDL are more efficient acceptors of cell cholesterol, the model described previously (60) may provide a clue to the problem. Not all investigators have demonstrated marked differences in the ability of apoA-I- or A-II-rich HDL particles to mediate cholesterol efflux however. Johnson et al. (62) and von Hodenburg et al. (63) reported that both were equally effective in transferring cholesterol from Fu5AH hepatoma cells or macrophages. In this context it is important to distinguish between efflux from plasma membrane or from intracellular sources (17, 64), one (plasma membrane) being less dependent on specific interactions than mobilization from intracellular pools which appears to require apolipoprotein binding to initiate the process. Whether or not cholesterol efflux depends on apolipoprotein–receptor interactions, work carried out in transgenic mice has shown that the animals overexpressing human apoA-I were protected (65), whereas apoA-II transgenic animals were not spared from the development of atherosclerosis (66). Amongst groups of animals in which LpA-I: LpA-II ratios varied, atherosclerosis was least evident in mice where LpA-I-rich HDL particles predominated (67). If both apolipoproteins equally promote cholesterol efflux from cells, then it follows that other biochemical pathways, which distinguish between the actions of A-I and A-II apolipoproteins, are regulated principally by apoA-I, achieving the antiatherogenic effects observed in the in vivo experiments described above.

However, attempts to assign specific ligand properties to a particular apolipoprotein have not yet been successful. A possible explanation is that all potential candidate HDL receptors exhibit a very broad specificity for apolipoproteins or that most of the physiological events involving lipid exchanges between HDL and cells do not involve 'classical' receptor interaction, or at least are not triggered by a specific amino acid sequence of a particular apolipoprotein, a concept proposed by Segrest et al. (68), Mendez et al. (69), Yancey et al. (70), and Leblond and Marcel (71). The view shared between most of these investigators is that cooperativity between class A amphipathic helices (possibly requiring at least four tandem repeat helices) provides the structural basis for binding and lipid efflux properties. At issue, however, is not whether the amphipathic repeated structures interact with membrane lipid or protein (or both) but whether the relatively low affinity (classified as "high affinity" in some reports) sites identified using the synthetic or native peptides (as models of helices) represent physiological sites capable of stimulating downstream cellular events.

Of some relevance to this proposition is the reported existence of two HDL binding sites on cell membranes. In one study (6), time course experiments were performed to measure association and dissociation rate constants, to determine whether the high affinity site displayed mass action kinetics, and which revealed the presence of two distinct sites, one with a K_d of 0.94 μg/ml (3.1×10^{-9} m) and another low affinity site with a K_d = 33 μg/ml, the latter being in the range of published values for HDL binding sites. Proteolysis of HDL with trypsin abolished the high affinity but not the low affinity site, suggesting the requirement for an intact peptide sequence. These studies were essentially confirmed by Barbaras et al. (7) who further showed that this high affinity site recognized apoA-I either in a lipid-poor form or when present in HDL, while another lower affinity site was also revealed for HDL. The nonspecific characteristics of amphipathic helices that are commonly shared among apolipoproteins are unlikely ligands for this putative apoA-I receptor.

Biological events following binding, including the processing of the ligand (either HDL or apoA-I) remain poorly understood and controversial. For every report claiming evidence of internalization by endocytosis, there is one denying the existence of such a pathway. A paucity of morphological data exists, presumably because HDL, less stable and smaller than LDL, is difficult to visualize and resists forming a complex with electron-opaque elements such as gold. Despite early claims to the contrary, no electronmicrographic evidence of gold-labeled HDL exists, whereas LDL–gold complexes, easy to prepare, have appeared in many reports (72, 73). Any postreceptor events that follow HDL binding must therefore be detected by biochemical assays, immunochemical identification, or attachment of fluorescent probes to trace movement of the ligand. Furthermore, the rapid dissociation kinetics characteristic of HDL high affinity binding and uptake require sensitive assays to follow its cellular localization. With these constraints in mind, Garcia et al. (74), using radiolabeled HDL, recently observed a high level of HDL internalization (100 ng/mg cell protein) by HepG2 cells, which corresponded to 45% of the total HDL associated with the cells within 15 min. Most of the radiolabeled

HDL was associated with clathrin-coated vesicles, with little evidence for the involvement of caveolae in the process. SR-B1 competitors did not inhibit HDL binding, suggesting that at least in hepatocytes, sites other than SR-B1 can mediate the uptake of HDL.

Although the specificity and identity of the ligand(s) that contribute to HDL receptor processing remain controversial, there is a consensus that apoA-I mediates some of the action and is therefore a justifiable target for investigating receptor domains in HDL. Morrison, Fidge, and Tozuka (75), studied direct binding of cyanogen bromide fragments of apoA-I incorporated into apolipoprotein–phospholipid complexes and found that the carboxyl terminal fragment bound with the highest affinity, the binding parameters resembling those of the intact apoA-I molecule. Importantly, only the C-terminal fragment bound to HDL binding proteins on ligand blots thus eliminating protein–lipid association from the interaction. These findings were supported by Dalton and Swaney (76) who fragmented apoA-I by proteolysis and found that carboxyl terminal residues 149–219 were essential for cell membrane interaction as well as lipid binding. Subsequent work showed that HDL binding could be inhibited by monoclonal antibodies that recognized epitopes between residues 150 and 243 (77) and the same antibodies inhibited binding to the putative HDL receptors HB_1 and HB_2 on ligand blots. Leblond and Marcel (71), however, were not able to identify a specific binding domain using their panel of nine monoclonal antibodies that reacted against epitopes widely distributed along the A-I apolipoprotein. They interpreted their results as an indication of a nonexclusive interaction of each of the amphipathic α-helical repeats of apoA-I. Their monoclonal antibodies similarly affected the association of cholesteryl ester-labeled HDL with cells. While clearly different from the results reported above (77), it is possible that the presence of whole cells presents a wider opportunity for less specific lipid–lipid or lipid–protein associations than occurs with purified plasma membranes. A study of the inhibitory properties of the antibodies using purified receptors was also not included in those HDL binding studies (71).

ApoA-I antibodies have also been used to gain information about sites that may regulate cholesterol efflux, the assumption being that HDL-receptor association may precede cholesterol acceptance by the lipoprotein. As we gain new knowledge about the events involved in the exchange or transfer of sterol between cells and plasma, it is clear that such an experimental approach can offer only a limited view of the process. As pointed out by Oram et al. (17) and Sviridov and Fidge (64), it is important to distinguish between cellular pools of cholesterol as not all are controlled by biochemical signalling pathways. The movement of sterol from the cell surface is a passive process, influenced by the rate of diffusion into the aqueous phase (Phillips et al. (78)) whereas cell regulatory factors appear to influence the mobilization of intracellular cholesterol, possibly to prevent uncontrolled fluctuations in sterol concentration and accompanying changes in composition of cell membranes. Therefore, when probing receptor binding domains of apoA-I in experiments related to activities like cholesterol flux, cellular pools should be distinguished so that nonspecific processes, that may outweigh specific events, do not confound the result, a point not always conceded in some reports.

Recognizing these differences, Sviridov, Pyle, and Fidge (79) probed, with monoclonal antibodies, sites of apoA-I that may specifically mediate mobilization of intracellular cholesterol, and found evidence that a central region involving residues 140–150, when masked by antibodies, inhibited the transfer of intracellular sterol to serum in a dose-dependent manner. None of the antibodies affected transfer of plasma membrane cholesterol. The region identified in these experiments was close to, or overlapped, sites identified in other reports, namely apoA-$I_{137–144}$ and apoA-$I_{141–148}$ (80) and apoA-$I_{135–148}$ (81) or apoA-$I_{96–111}$ (82). The inhibiting antibodies used by Sviridov et al. (79) recognized epitopes within one amphipathic α-helix, but it is unlikely that disruption of only one helical region would specifically affect this type of apoA-I–membrane association, as many lipid binding helices remain intact and dispersed throughout the entire molecule. According to Mendez et al. (69) or Davidson et al. (83), one or two amphipathic domains are sufficient to promote cholesterol efflux. Amphipathic α-helices, by virtue of their capacity to form apolipoprotein–phospholipid complexes, which are essential prerequisites for sequestering membrane cholesterol (Forte et al. (84)), will most likely have influenced plasma membrane cholesterol, whereas a biologically active motif within the central apoA-I region appears to influence intracellular cholesterol mobilization. The fact that both the carboxyl terminus and a central region of apoA-I could be involved in cholesterol efflux was confirmed by Sviridov, Pyle, and Fidge (85) using apoA-I that had been systematically truncated to eliminate, sequentially, C-terminal portions of the molecule. The residues between 222–243 of apoA-I were clearly important in phospholipid binding, but truncation to either residues 135 or 150 completely restored cholesterol and phospholipid efflux, suggesting that another cryptic binding domain had been exposed.

It is clear, as has been the case in searching for active motifs in other receptor systems, that a series of point mutations needs to be systematically produced to probe active domains in apoA-I. From the discussion above it is likely that domains of apoA-I involved with cholesterol efflux may not coincide with those that bind to receptors that signal biochemical pathways unrelated to cholesterol metabolism. Now that some HDL receptors have been cloned, sufficient quantities of these proteins can be expressed to allow structural studies of interactive sites and more precise identification of binding domains, particularly those present in the ligand, apoA-I.

BIOCHEMICAL EVENTS AFFECTED BY HDL RECEPTORS

The least understood area in the field of HDL receptors is the nature of the post-receptor events that occur after

HDL binding. Apart from the observation that the degree of expression of SR-B1 correlates positively with cholesterol efflux and its transient overexpression in mice depletes plasma of HDL cholesteryl ester, there is little other data showing cause and effect after interaction between HDL and candidate receptors. With SR-B1, the processes of sterol transfers are unresolved, even though the membrane protein putatively acts as a docking agent for HDL. As mentioned before, the direction of movement appears not to be regulated by receptor-driven events but by a gradient that is dependent on the relative concentrations of cholesterol in the ligand and cell membrane. HB_2, a member of the immunoglobulin superfamily of membrane proteins that binds HDL, appears to be regulated by a number of biochemical events, some associated with cholesterol synthetic pathways or others related to cell differentiation (46). Like many other adhesion molecules, HB2 (almost identical to ALCAM) may also trigger signalling pathways, but whether HDL binding initiates signal transduction is not yet known.

There is a growing body of evidence, however, for the activation of signalling pathways following the interaction between HDL and cells, and such observations inevitably focus attention on receptor activity. That the receptors that have been cloned and sequenced have, as yet, not been attributed with signalling properties, fuels speculation that signalling receptors (responding to HDL moieties) exist but have not yet been identified. In this context it is intriguing to briefly reconsider the association between putative receptors and apoA-I. As discussed before, a site in cell membranes exhibits high affinity for apoA-I, characterized by fast association–dissociation constants (6). Despite this binding site having all the hallmarks of a physiological receptor, and potentially being an important determinant of biochemical events, scepticism prevails because the circulating levels of HDL, even in some HDL deficiencies, would fully saturate the receptors, perpetually up- or down-regulating downstream events. However, if apoA-I existed in a form dissociated from HDL, it could satisfy the credentials of a ligand in many ways. First, it would circulate at variable concentrations depending on the metabolism of HDL which is influenced by the interactive processes of many factors (lipolytic enzymes, CETP, PLTP, and diet). In this context the concentration of lipid-poor apoA-I may reflect normal or dysfunctional lipoprotein states and, through negative feedback control, might influence pathways that restore (or maintain) balances in lipid metabolism.

Evidence is also emerging that apoA-I does exist at low concentrations in a lipid-"free" or at least lipid-poor form in human plasma (86). These concentrations could fluctuate widely, depending on lipase activities and fat absorption (87). At the lower levels of apoA-I in extracellular fluids than in blood, or in trace amounts if apoA-I is transiently dissociated and then rapidly reassociated with HDL, the high affinity sites recognizing apoA-I could be activated at a critical mass of the ligand or by conformation changes that accompany the removal or addition of lipid, exposing receptor binding domains (**Fig. 4**). In fact, changes in the net charge and structure of apoA-I do occur in response to increases or decreases in HDL cholesterol content, the magnitude of such changes being considered sufficient to affect the interaction between HDL and cell surfaces (88).

Interaction between free apolipoprotein and cells is considered to be one mechanism for the formation of HDL (89) although not all cells participate in this process (90). In our laboratory, we have demonstrated that ^{14}C-labeled apoA-I is internalized by HepG2 cells (N. H. Fidge, unpublished observations) in a manner suggestive of a regulated rather than a passive process. Holian, Kumar, and Attar (91) found that apoA-I, but not HDL-bound A-I, was phosphorylated by protein kinase C (PKC) in vitro, independent of calcium or lipids, suggesting that apoA-I is a novel cofactor of PKC (Fig. 4). The addition of HDL, which inhibited apoA-I phosphorylation, indicates that the presence of lipid masks the phosphorylation site on the apolipoprotein. Presumably then, the reaction only occurs after dissociation of apoA-I from HDL, possibly after receptor occupancy. Taken together with emerging evidence for signal transduction after HDL binding to cells, the likelihood for the existence of an apoA-I receptor is strengthened considerably.

How convincing is the evidence that biochemical signalling is activated by HDL? As argued above, the fact that HDL circulates in plasma at concentrations above those needed for receptor saturation does not negate a role for "active" HDL subclasses or moieties that reside at very low concentrations in the blood or extracellular fluids and which may have different functions. As discussed above, at least two biochemical processes have been identified that respond to HDL, or more probably apoA-I, the first being mobilization of intracellular cholesterol and the other related to its capacity to stimulate several mitogenic effects (92–94). ApoA-I-induced cholesterol efflux from cells is associated with activation of protein kinase C (19) and Garver et al. (95) recently identified three phosphoproteins that were phosphorylated after incubation of skin fibroblasts with HDL (**Fig. 5**). Two of these were phosphorylated only by HDL, not "free apoA-I, whereas one termed pp18 appeared to be responsive to apoA-I. Wu and Handwerger (96) found that both HDL and apoA-I stimulated phosphorylation of a PMA-inducible 80 kDa protein in human trophoblasts, concomitant with an increase in human placental lactogen release. That PKC is involved in apolipoprotein-mediated cellular cholesterol efflux was strengthened in a recent report (97), the authors using PKC inhibitors to selectively down-regulate PKC, which substantially reduced cholesterol efflux to extracellular apoA-I.

Whereas the signalling events related to cholesterol efflux probably respond to low concentrations of active HDL moieties (e.g., apoA-I), the strong mitogenic effect that has been observed with HDL in endothelial cells or smooth muscle cells increases up to 300–500 μg/ml HDL. An explanation for this phenomenon follows from recent observations by Walter et al. (98) that HDL stimulates multiple signalling pathways in fibroblasts and appears to

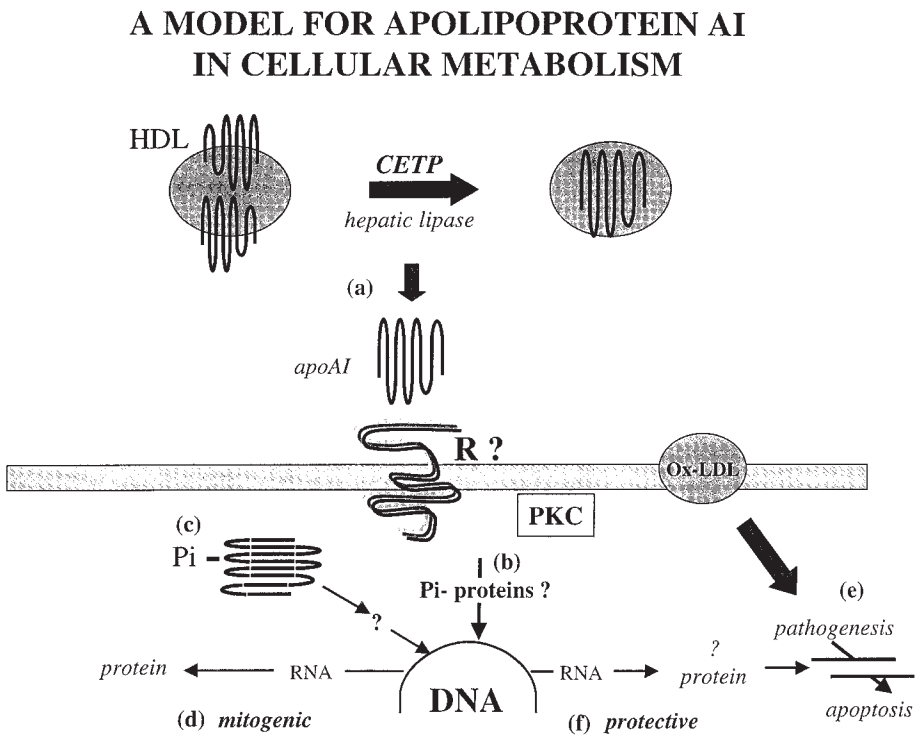

Fig. 4. A model for apolipoprotein A-I receptor influence in cellular metabolism based on experimental evidence. During plasma remodeling of HDL, apoA-I dissociates (a) in a 'free' or lipid-poor form (87) which may interact with a receptor (R) yet to be identified. A membrane site that binds apoA-I with high affinity, characterized by a rapid dissociation constant, has been identified (6, 7). Interaction between apoA-I and cells activates PKC (19), which may subsequently trigger phosphorylation of cell proteins (b) as described in Fig. 5. In the lipid-poor form, apoA-I can be phosphorylated (c) and appears to act as a cofactor independent substrate of PKC (91). Whether this occurs after uptake of apoA-I is yet to be determined. HDL or apoA-I induce mitogenic (d) actions in a variety of cells (92–94) that ultimately depend on gene regulation, while apoA-I protects endothelial cells (f) against the cytotoxicity of oxidized LDL (e). The protective events involve new protein synthesis, and it is suggested (100) that a gene product stimulated through apoA-I blocks a pathogenic rise in Ca^{2+} that is associated with apoptosis.

involve phosphatidylcholine (PC) turnover as well as phosphatidylinositol (PI) hydrolysis. The breakdown of PC occurring after HDL activation of phospholipases C and D, the former producing diacylglycerol and the latter phosphatidic acid, has important biochemical consequences as both products are known to activate second-messenger responses (Fig. 5). The authors point out that whereas mitogen-induced hydrolysis of PI is rapid and transient, PC hydrolysis is sustained and that both are induced by growth factor-like activity of the HDL particle; thus mitogenic effects of HDL could be observed over a wide concentration range of HDL.

In addition to the proposed down-regulation of the membrane adhesion molecule HB_2/ALCAM by HDL(discussed above), cytokine-induced expression of the soluble adhesion molecules VCAM and ICAM also appears to be regulated by HDL (52). The involvement of an HDL or apoA-I receptor in this process is being investigated based on the hypothesis that HDL binding, via signal transduction, influences gene regulation by altering the DNA binding of calcium-dependent transcription factors. Endothelial adhesion molecule expression induced by tumor necrosis factor is coupled to pathways that involve sphingosine kinase and NF-κB activation (99) and possibly HDL, by inhibiting these factors, reduces VCAM and ICAM synthesis.

There is more evidence for a link between apoA-I, gene regulation, and antiatherogenicity. Suc et al. (100) found that HDL and apoA-I (but not apoA-II) protected endothelial cells against death induced by oxidized LDL. The protective effect was independent of paraoxonase–linked HDL activity or contact between HDL and oxidized LDL. Preincubation with apoA-I, presumably by activating cell signalling, blocked a pathogenic pathway in the cells that was associated with a rise in Ca^{2+} levels. As protein synthesis was essential to the protective effect, it appears that a gene product was induced as a result of apoA-I interaction (Fig. 5).

In conclusion, it appears that several biochemical pathways are affected by HDL. Some, though not all of them, appear to be related to lipid metabolism, but most impinge in some way on atherogenesis, which will no doubt stimulate further attempts at unravelling the active receptor sites behind the pathways. This will involve detailed probing of sites present in receptors we already know about, and a continuing search for other membrane proteins

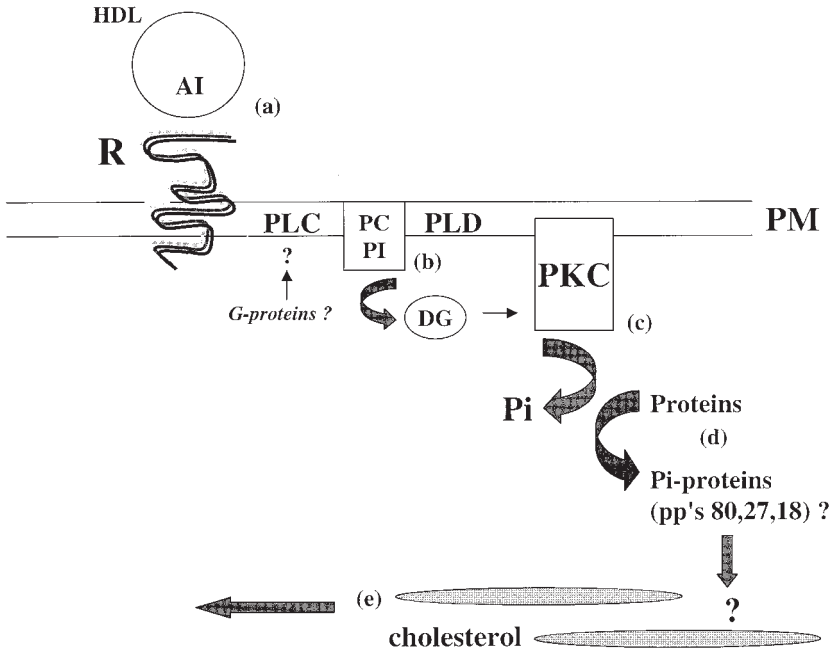

Fig. 5. HDL and cell signaling. The pathway shown is suggested by evidence, assembled from the literature, that strengthens a role for HDL, or its apolipoproteins, in biochemical signaling. The elements of a complete signal transduction system triggered by HDL are yet to be confirmed. After binding of HDL (a) (probably apoA-I) to a receptor (R), possibly distinct from the three binding proteins described in Figs. 1–3, phospholipases C and D are activated (98) with a concomitant increase (b) in diacylglycerol (DG). This pathway probably involves different cell proteins than G-proteins which principally bind to 7 transmembrane receptors, while HDL may interact with functionally distinct receptors, stimulating several pathways. Protein kinase C (PKC) has been identified (c) as a mediator of HDL-receptor-dependent efflux of cellular cholesterol (19), and HDL/apoA-I cell interaction was shown to stimulate (d) phosphorylation of three phosphoproteins 80, 27, and 18 (95, 96), the latter (pp18) (95) possibly involved in apoA-I-mediated cholesterol efflux (e).

which undoubtedly exist that play active roles in processing HDL.

The author gratefully acknowledges support from the National Health and Medical Research Council (Australia) and the National Heart Foundation.

Manuscript received 20 July 1998 and in revised form 14 October 1998.

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