Role of Scavenger Receptors SR-BI and CD36 in Selective Sterol Uptake in the Small Intestine†

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ABSTRACT: The serum lipoprotein high-density lipoprotein (HDL), which is a ligand of scavenger receptors such as scavenger receptor class B type I (SR-BI) and cluster determinant 36 (CD36), can act as a donor particle for intestinal lipid uptake into the brush border membrane (BBM). Both cholesterol and phospholipids are taken up by the plasma membrane of BBM vesicles (BBMV) and Caco-2 cells in a facilitated (protein-mediated) process. The protein-mediated transfer of cholesterol from reconstituted HDL to BBMV depends on the lipid composition of the HDL. In the presence of sphingomyelin, the transfer of cholesterol is slowed by a factor of about 3 probably due to complex formation between cholesterol and the sphingolipid. It is shown that the mechanism of lipid transfer from reconstituted HDL to either BBMV or Caco-2 cells as the acceptor is consistent with selective lipid uptake: the lipid donor docks at the membrane-resident scavenger receptors which mediate the transfer of lipids between donor and acceptor. Selective lipid uptake implies that lipid, but no apoprotein is transferred from the donor to the BBM, thus excluding endocytotic processes. The two BBM models used here clearly indicate that fusion of donor particles with the BBM can be ruled out as a major mechanism contributing to intestinal lipid uptake. Here we demonstrate that CD36, another member of the family of scavenger receptors, is present in rabbit and human BBM vesicles. This receptor mediates the uptake of free cholesterol, but not of esterified cholesterol, the uptake of which is mediated exclusively by SR-BI. More than one scavenger receptor appears to be involved in the uptake of free cholesterol with SR-BI contributing about 25% and CD36 about 35%. There is another yet unidentified protein accounting for the remaining 30 to 40%.

Scavenger receptors present in the intestine (*1*, *2*) have been located mainly to the brush border membrane (BBM)[1] of the epithelial cells (*3*). Originally, the scavenger receptor class B type I (SR-BI) has been identified as a cell surface receptor in liver and steroidogenic tissue where it mediates selective lipid uptake of cholesteryl esters (*4*−*6*). The physiological ligand of SR-BI is high-density lipoprotein (HDL). In selective lipid uptake, HDL binds via apo A-I to SR-BI, and HDL-cholesteryl ester molecules are then transferred from the ligand to the acceptor membrane. The term selective implies that lipids of the donor particle are transferred selectively as contrasted to the endocytotic pathway where the entire lipoprotein particle is taken up. The term selective transport may, however, be misleading in the sense that SR-BI exhibits little lipid specificity. It functions as a port or docking receptor (*3*). Upon docking of the lipid donor particle, SR-BI mediates bidirectional flux of lipid molecules with little structural discrimination of the lipid molecules (*3*). SR-BI has also been reported to be involved in reverse cholesterol transport (*7*−*9*).

We showed that SR-BI and its isoforms are integral proteins of the small-intestinal BBM and presented in vitro evidence that these proteins are involved in the facilitated uptake of cholesterol and other dietary lipids (*3*). Very little is known about the mechanism of the lipid transfer facilitated by scavenger receptors. Here we address two mechanistic questions: (i) whether intestinal lipid uptake from donor particles to the small-intestinal BBM is consistent with the mechanism of selective lipid uptake observed in liver and steroidogenic tissue and (ii) whether other members of the family of scavenger receptors participate in intestinal lipid uptake.

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[1] Abbreviations: Apo, apolipoprotein; BBM, brush border membrane(s); BBMV, brush border membrane vesicle(s); CD36, cluster determinant 36; CE, cholesteryl ester; DMPC, dimyristoyl phosphatidylcholine; DTT, 1,4-dithio-rac-threitol; EPC, egg phosphatidylcholine; HDL, high-density lipoprotein(s); IgG (M), immunoglobulin class G (M); PAGE, polyacrylamide gel electrophoresis; PBS, phosphate-buffered saline; PC, phosphatidylcholine; rHDL, reconstituted high-density lipoprotein(s); SDS, sodium dodecyl sulphate; SR-BI, scavenger receptor class B type I; SUV, small unilamellar vesicle(s); Tris, Tris-(hydroxymethyl)aminomethane; TTBS, Tween 20 in Tris-buffered saline.

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EXPERIMENTAL PROCEDURES

Materials. Egg phosphatidylcholine (EPC) and dimyristoyl phosphatidylcholine (DMPC) were purchased from Lipid Products (Nutfield, Surrey, U.K.), bovine brain sphingomyelin (purity 99%), cholesterol (purity >99%), cholesteryl oleate (purity >98%), phosphate-buffered saline (PBS), the sodium salt of oleic acid, sodium taurocholate and monoclonal anti-human cluster determinant 36 (CD36) IgM from Sigma (Buchs, Switzerland), monoclonal anti-human CD36 IgM and polyclonal anti-human CD36 IgG raised in rabbit against amino acids 1−300 (pAb 300) from Santa Cruz Biotechnology (Santa Cruz, CA), polyclonal anti-SR-BI antibodies raised in rabbit against the extracellular domain (amino acids 230−380) of mouse SR-BI (pAb 150) from Novus Biologicals (AbCam, Cambridge, U.K.), [1α, 2α (N)-^{3}H] cholesterol (47 Ci/mmol) and [1α, 2α (N)-^{3}H] cholesteryl oleyl ether (37 Ci/mmol) from Amersham (U.K.), and the Chemiluminescent Kit (Immun-Star) from Bio-Rad (Glattbrugg, Switzerland).

Preparation of Human BBMV. Human small intestine was obtained by written consent from donors who underwent intestinal surgery in the Department of Surgery of the University Hospital Zurich. Pieces of small intestine of about 10 cm long were thoroughly rinsed with physiological saline, immediately frozen in liquid nitrogen, and stored at −80 °C prior to the preparation of BBMV. The preparation was carried out according to ref *10*, and some physicochemical properties of the brush border membrane vesicles (BBMV) were determined as a quality control as described previously (*11*).

Dose−Response Curves Describing the Inhibitory Effect of Anti-SR-BI IgG and Anti-CD36 IgM on Sterol Uptake. The kinetics of sterol uptake by BBMV were determined as described previously (*3*, *11*−*14*). The inhibitory effect of various antibodies raised against different scavenger receptors on sterol uptake was determined as described previously (*3*, *13*). Briefly, BBMV (5 mg of protein/mL) were incubated with egg PC small unilamellar vesicles (SUV) (50 μg/mL) containing 1 mol % of radiolabeled sterol (free or esterified cholesterol), and the transfer of radiolabeled sterol to the BBMV was determined after 20 min in the absence and presence of increasing amounts of antibodies. The loss in sterol uptake observed in the presence of antibody was expressed as % of the total sterol uptake observed in the absence of antibody (and equated with % inhibition). Dose−response curves were constructed showing % inhibition as a function of the antibody concentration. As a control, the inhibitory effect of human apoA-I on sterol uptake was determined as a dose−response curve. The experimental dose−response curves were either sigmoidal or hyperbolic and best fitted by a modified Hill equation (eq 1) (*15*) or a typical saturation curve (eq 2) (*13*), respectively:

$$\text{inhibition (\%)} = \frac{B_{max}}{[1 + (IC_{50}/x)^n]} \quad (1)$$

$$\text{inhibition (\%)} = \frac{B_{max}x}{(K_d + x)} \quad (2)$$

where B_{max} is the maximum effect (as %) observed, IC_{50} is the concentration of antibody (or inhibitor) at which 50% of B_{max} was observed, n is the Hill coefficient, and x is the concentration of antibody (inhibitor). The IC_{50} value is identical to the dissociation constant K_d of the interaction between the antibody and the scavenger receptor. For curve fitting, the programs MacCurveFit (Kevin Raner Software, Victoria, Australia) and Excel (Microsoft) were used on a Macintosh computer.

Miscellaneous. Published methods were used for the preparation of mixed bile salt micelles, small unilamellar vesicles (SUV) (*11*, *14*), rabbit BBMV (*10*, *11*, *14*), the digestion of BBMV by proteinase K (*16*), the preparation of human HDL fractions and human apolipoprotein (apo) A-I (*12*), the preparation of ^{14}C-labeled apo A-I (*17*) and ^{125}I-labeled apo A-I (*13*), the reconstitution of discoidal HDL particles from human apo A-I and an unsonicated DMPC dispersion (*18*), the kinetic measurements of lipid uptake using either bile salt micelles or small unilamellar lipid vesices as the donor and BBMV as the acceptor (*3*, *11*, *13*, *14*), the kinetic measurements of lipid uptake using Caco-2 cells as the acceptor (*19*), for SDS−PAGE and immunoblotting (*3*, *13*), the preparation and treatment of rabbit small-intestinal sections used for gold-labeling and immunoelectronmicroscopy (*20*), for the determination of phospholipid concentrations (*21*) and protein concentrations (*12*).

RESULTS

Lipid Uptake by Brush Border Membrane Vesicles using Reconstituted HDL as the Donor. We reported that SR-BI and the isoform SR-BII are present in the small-intestinal BBM mediating the uptake of sterols and other dietary lipids (*3*). Serum lipoproteins, apolipoproteins, and related compounds were shown to be competitive inhibitors of intestinal lipid uptake interacting directly with scavenger receptors (*12*, *13*). From this finding, it is anticipated that serum lipoproteins may function as donor particles in small-intestinal lipid uptake. This expectation is borne out by experiment. The kinetics of cholesterol and sphingomyelin transfer from reconstituted HDL as the donor to rabbit BBMV as the acceptor were measured at 37 °C and are shown in Figure 1. The experimental data were fitted adequately by a single-exponential function and values for the pseudo-first-order rate constant k_1 (half times $t_{1/2}$) derived from curve fitting are summarized in Table 1. The kinetic data points for cholesterol transfer exhibited considerable scatter; they resulted from five series of experiments using different preparations of rabbit BBMV. The cholesterol transfer from rHDL to BBMV was characterized by a half time of $t_{1/2}$ = 20 min. After proteinase K treatment of BBMV, the kinetics observed for cholesterol uptake from rHDL to BBMV were identical to those measured for cholesterol transfer from rHDL to protein-free EPC SUV (Figure 1A and Table 1), indicating that cholesterol transfer between rHDL and BBMV is protein-mediated.

Figure 1B shows that the k values depended sensitively on the lipid composition of the reconstituted HDL. If rHDL contained 1 mol % sphingomyelin, the cholesterol transfer was markedly slowed relative to that measured with rHDL particles containing no sphingomyelin (Figure 1B). Furthermore, with rHDL particles containing both cholesterol and sphingomyelin similar kinetic curves (Figure 1B) and hence similar half times were obtained for both cholesterol ($t_{1/2}$ =

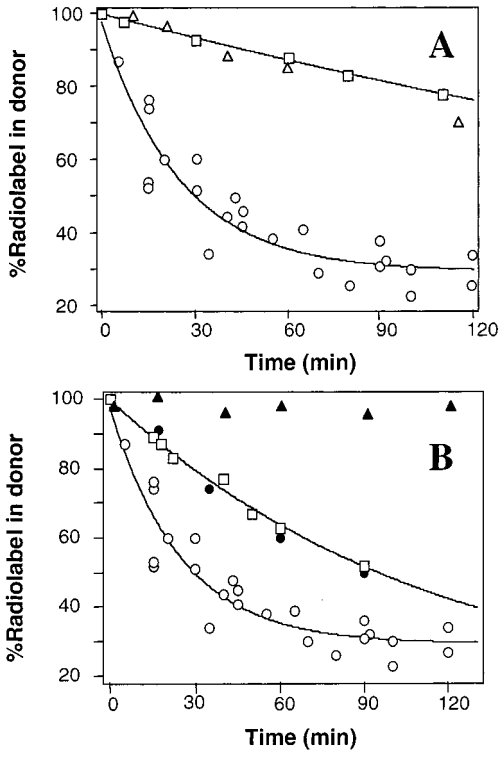

FIGURE 1: (A) Kinetic curves for the uptake of free cholesterol (○) from rHDL into the membrane of rabbit BBMV recorded at 37 °C. BBMV (2 mg of protein/mL) were mixed with reconstituted HDL (rHDL) (0.15 mg of human apo A-I/mL, 0.3 mg of total lipid/mL) at time zero. The lipid composition of rHDL was 95 mol % DMPC and 5 mol % radiolabeled cholesterol. The kinetic curves for cholesterol uptake from rHDL into proteinase K-treated BBMV (△) and into protein-free EPC SUV (□) are shown for comparison. (B) The kinetic curve for the uptake of cholesterol as described in panel A was compared to the kinetics of cholesterol and sphingomyelin uptake into membranes of rabbit BBMV using as a donor rHDL with a different lipid composition. These donor particles consisted of 95 mol % DMPC, 4 mol % cholesterol, and 1 mol % sphingomyelin. The lipid whose uptake was measured was radiolabeled. Cholesterol uptake and sphingomyelin uptake from sphingomyelin-containing rHDL are presented as full circles (●) and open squares (□), respectively. The uptake of [^{14}C]-apo A-I or [^{125}I]-apo A-I from rHDL to BBMV is represented by triangles (▲). The solid lines in both panels A and B represent single-exponential fits to the data points, and pseudo-first-order rate constants k_1 (half times $t_{1/2}$) and equilibrium values x_∞ derived from curve fitting were summarized in Table 1.

Table 1: Kinetic Parameters for Lipid Transport between rHDL and BBMV at 37 °C

lipid transported	donor particle	acceptor particle	k_1 (min^{-1})	$t_{1/2}$	x_∞ (%)
cholesterol	rHDL[a]	BBMV	0.041	20 ± 8	28 ± 2
cholesterol	rHDL[a]	digested BBMV	0.0061	114 ± 5	
cholesterol	rHDL[a]	EPC SUV	0.0058	120 ± 10	
cholesterol	EPC SUV	rHDL	0.011	66 ± 6	29 ± 7
cholesterol	EPC SUV	EPC SUV	0.011	64 ± 5	29 ± 4
sphingomyelin	rHDL[b]	BBMV	0.0121	57 ± 3	29 ± 7
cholesterol	rHDL[b]	BBMV	0.013	52 ± 8	29 ± 4

[a] Lipid composition of the rHDL: 95 mol % DMPC and 5 mol % radiolabeled cholesterol. [b] Lipid composition of the rHDL: 95 mol % DMPC, 4 mol % cholesterol and 1 mol % sphingomyelin. Either cholesterol or sphingomyelin was radiolabeled.

52 min) and sphingomyelin ($t_{1/2}$ = 57 min) (Table 1). We conclude that the rate of cholesterol transfer is reduced in the presence of sphingolipid and propose that this is due to complex formation between cholesterol and the sphingolipid (*22*). Furthermore, for both cholesterol transfer and sphingomyelin transfer identical equilibrium values x_∞ = 29% (Table 1) were derived from curve fitting, and this value agreed well with the x_∞ = 33% calculated from the lipid pools of rHDL particles and BBMV (*23*).

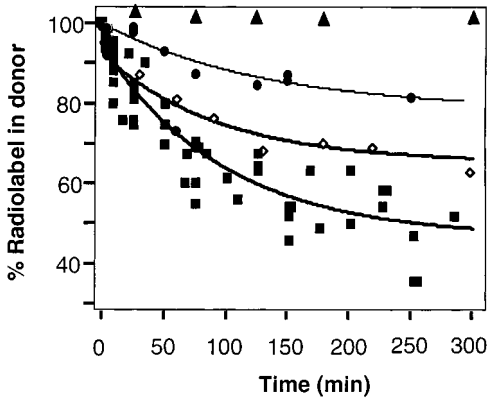

FIGURE 2: Kinetics of cholesterol uptake by Caco-2 cells from different donor particles measured at 37 °C. Caco-2 cells were plated on collagen-topped polycarbonate filters (Transwell-COL, Costar) and grown to a confluent monolayer of differentiated, polarized cells. Mixed bile salt micelles (5 mM sodium taurocholate, 0.6 mM sodium oleate, and 4 μM radiolabeled cholesterol) were added to the apical compartment at time zero and the radioactivity (%) remaining in the donor was plotted as a function of time: for cholesterol uptake in the absence of HDL_3 (■) and in the presence of HDL_3 (0.23 mg/mL) (●), and for the uptake of [^{125}I]-apo A-I (▲). For comparison, the cholesterol uptake by Caco-2 cells from rHDL as the donor (0.3 mg of total lipid/mL) (◇) was included. The solid lines represent the best fit to the data points using the sum of two exponentials. The kinetic parameters (pseudo-first-order rate constants k_1, half times $t_{1/2}$, and preexponential terms) derived from curve fitting were summarized in Table 2.

In a double-labeling experiment, [^{3}H]-cholesterol uptake was measured from HDL particles reconstituted with radiolabeled [^{14}C]-apo A-I. A kinetic curve was obtained for cholesterol transfer that closely resembled that shown in Figure 1A. Equilibrium was reached after about 2 h and the x_∞ value measured after 12 h was x_∞ = 20 ± 3% in good agreement with the calculated value of x_∞ = 23%. This result clearly shows that cholesterol equilibrated effectively between the lipid pools of donor and acceptor. In contrast to cholesterol no or very little apo A-I was transferred from rHDL to BBMV (Figure 1B).

Uptake of Cholesterol by Differentiated Caco-2 Cells Using Mixed Taurocholate Micelles and rHDL as the Donor. In Figure 2 kinetic curves are presented for the uptake of cholesterol by Caco-2 cells using mixed taurocholate micelles as the donor in the absence and presence of HDL_3. The uptake experiments have been carried out at 37 °C over a period of 2 years using Caco-2 cells differing in passage number. Both individual series as well as the total collection of data points were fitted best by the sum of two exponentials. There were large differences in kinetic parameters derived for individual experimental series (data not shown). The average pseudo-first-order rate constants (half-times $t_{1/2}$) derived from curve fitting of the total set of data points were $k_1 = 0.15 \pm 0.1$ min^{-1} ($t_{1/2}$ = 5 min) and $k_1' = 2.6 \pm 1.5 \times 10^{-3}$ (min^{-1}) ($t_{1/2}$ = 267 min) for the initial fast phase and the second slow phase of the kinetics, respectively (Table

Table 2: Uptake of Cholesterol by Caco-2 Cells from Different Donor Particles

donor particle	inhibitor	k_1 (min^{-1})	k_1' (min^{-1})	$(t_{1/2})$ (min)	$(t_{1/2})'$ (min)	x_∞ (%)	A_1	A_2
mixed taurocholate micelles		0.15 ± 0.1	$(2.6 \pm 1.5) \times 10^{-3}$	5	267	30 ± 10	25 ± 7	43 ± 12
mixed taurocholate micelles	HDL_3	0.0075 ± 0.002		92		80 ± 4	20 ± 4	
reconstituted HDL		0.30 ± 0.15	$(3.4 \pm 0.5) \times 10^{-3}$	2.3	207	44 ± 3	12 ± 3	42 ± 4

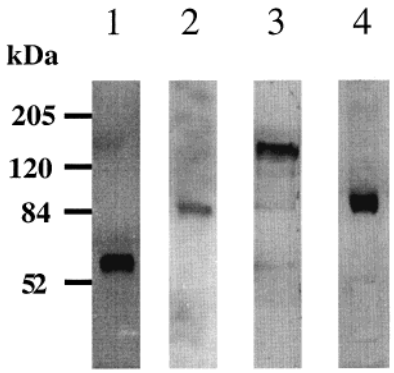

FIGURE 3: Immunoblot analysis of rabbit and human BBMV using commercial, monoclonal anti-human CD36 antibodies. BBMV were solubilized in SDS sample buffer containing 100 mM DTT, subjected to 7.5% SDS−PAGE, and transferred onto nitrocellulose membranes. Rabbit BBMV (lane 1) and human BBMV (lane 2) using monoclonal anti-human CD36 IgM from Santa Cruz Biotechnology and rabbit BBMV (lane 3) and human BBMV (lane 4) using anti-human CD36 IgM from Sigma. In each lane 50 μg of protein were applied. The secondary antibody was alkaline phosphatase-conjugated anti-mouse IgM. The signal detection was by chemiluminescence using the Immun-Star kit from Bio-Rad. The immunoblots shown in lanes 1−4 are representative of three reproducible experiments. The position of molecular mass markers (kDa) is given on the left.

2), the large standard deviation being an expression of the considerable scatter of the data (Figure 2). The two preexponential terms defining the extent (intensity) of the kinetic phases also varied widely ranging, respectively, between 14 and 35% and 30−60%. In the presence of HDL_3 (0.23 mg of protein/mL), the uptake of cholesterol was slowed significantly (Figure 2). The data points were adequately fitted by a single-exponential function yielding a half time of $t_{1/2} = 92$ min indicating that in the presence of sufficient HDL_3 the protein-mediated uptake of cholesterol by Caco-2 cells was abolished. The kinetics of cholesterol uptake by Caco-2 cells from rHDL as the donor was also biphasic (Figure 2). The pseudo-first-order rate constant k_1 for the initial fast phase of cholesterol uptake derived from curve fitting agreed within experimental error with the k_1 value for cholesterol uptake from mixed bile salt micelles (Table 2). The main difference between the two kinetic curves (Figure 2) appears to be in the amplitude of the first kinetic phase. Figure 2 shows that in the course of cholesterol uptake from rHDL no apo A-I was transferred to Caco-2 cells consistent with the results obtained with BBMV and with the concept of selective lipid uptake (*4*−*6*).

The Role of Different Scavenger Receptors in Sterol Uptake. The scavenger receptor CD36 which is structurally related to SR-BI (*4*) was shown to be present in the rat BBM where it was proposed to promote the uptake of dietary long-chain fatty acids (*24*). Immunoblotting of rabbit and human BBMV using different anti-CD36 antibodies is shown in Figure 3. Using a monoclonal anti-human CD36 antibody (from Santa Cruz Biotechnology), rabbit BBMV yielded a major band at 57 kDa and a minor one at 140 kDa (lane 1), while human BBMV gave a band at 88 kDa (lane 2) characteristic of fully glycosylated CD36 (*25*). The 57 kDa band is characteristic of a splice variant of CD36 lacking exons 4 and 5 (*26*). For comparison, the band pattern obtained with rabbit BBMV using another monoclonal anti-human CD36 antibody (from Sigma) consisted of a predominant band at 140 kDa and minor bands at 88 and 57 kDa (lane 3, Figure 3). Using the same antibody in immunoblotting of human BBMV, a predominant band at 88 kDa was observed and minor bands at 140 and 57 kDa (lane 4, Figure 3).

The results of the immunoblot analysis (Figure 3) are supported by immunoelectron microscopy. Immunogold labeling of rabbit small-intestinal sections using polyclonal rabbit anti-human CD36 IgG (pAb300) revealed the preferential location of CD36 in the BBM; in contrast no labeling of the basolateral membrane was observed (Figure 4).

From kinetic measurements of sterol uptake in the absence and presence of antibody or inhibitor, dose−response curves were constructed as detailed in previous reports (*3*, *13*). Such dose−response curves (% inhibition as a function of the antibody or inhibitor concentration) showing the effects of anti-SR-BI IgG and anti-CD36 IgM on the uptake of free and esterified cholesterol by rabbit BBMV are shown in Figure 5, panels A and B, respectively. The curve for the inhibition of cholesteryl ester uptake in the presence of anti-SR-BI IgG was sigmoidal, yielding a Hill coefficient of $n = 2.7$ (eq 1). The dose−response curve for cholesterol was probably also sigmoidal rather than hyperbolic (Figure 5A); however, a clear distinction was not possible due to the lack of precision of the isotherm. The plateau values of the dose−response curves (Figure 5A) were 22 ± 5 and 89 ± 3% for free and esterified cholesterol, respectively, indicating that in the presence of excess anti-SR-BI IgG (>100 μg of protein/mL) the uptake of free and esterified cholesterol was inhibited by ~20 and ~90%, respectively. The maximum inhibition B_{max} observed for free cholesterol uptake in the presence of anti-CD36 IgM (>100 μg of protein/mL) was 36% (Figure 5B). We note that for immunogold labeling polyclonal anti-human CD36 IgG (pAb300) was used for technical reasons because protein A-15 nm gold had little affinity for the monoclonal anti CD36 IgM used in the inhibition experiments (Figures 5−7). The maximum inhibition B_{max} observed for free cholesterol uptake into rabbit BBMV in the presence of polyclonal anti-human CD36 IgG (= 100 μg of protein/mL) was 30% similar to that observed with anti-human CD36 IgM. In contrast to the anti-SR BI antibody, the effect of anti-CD36 on the uptake of cholesteryl oleate was negligible (Figure 5B). The bar histograms (Figure 6) show that the effects of the two antibodies were additive. The combined effect on the uptake of free cholesterol observed in the presence of anti-SR-BI IgG and anti-CD36 IgM (both at 100 μg/mL) was about 50% and equal to the sum of the inhibitory effects observed separately (Figure 6A). The same is true for the combined

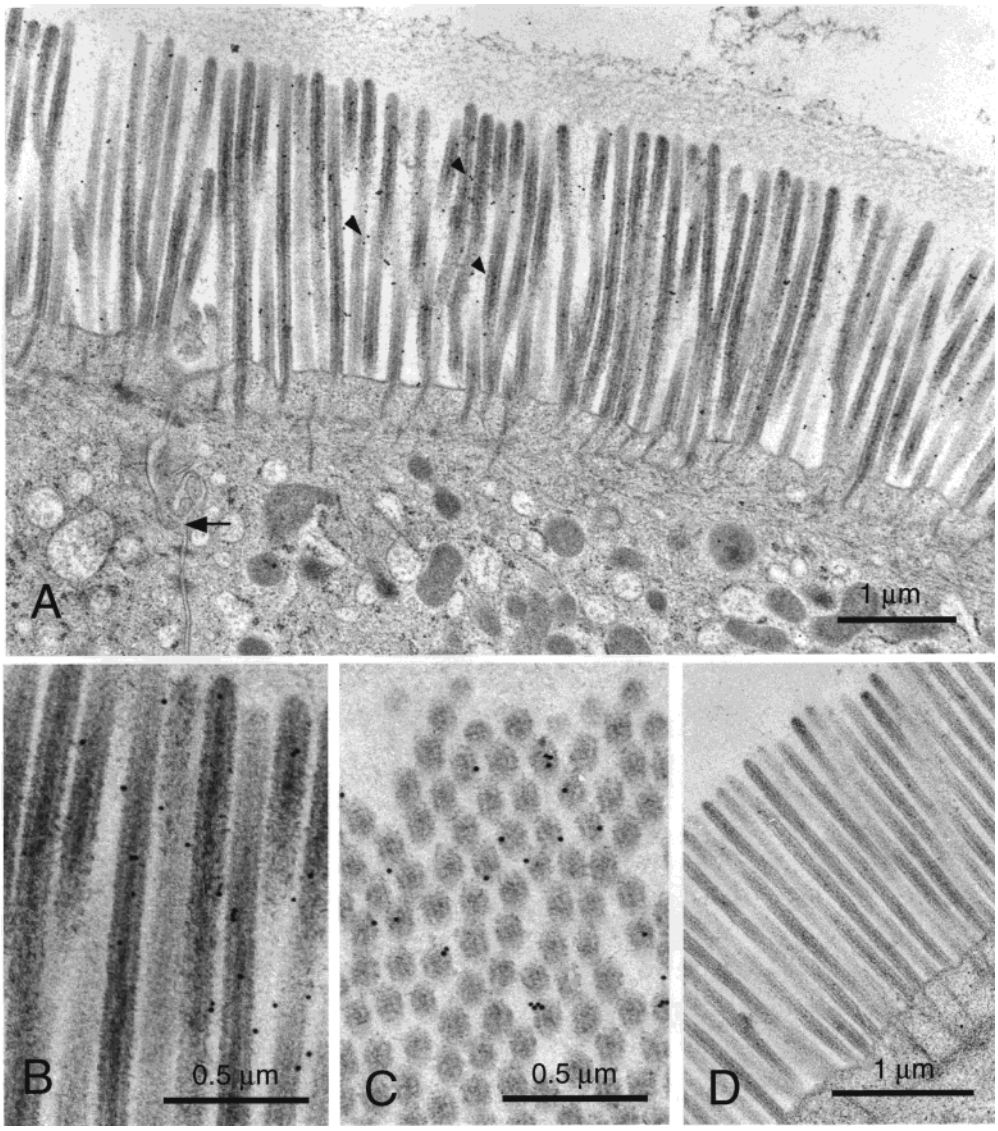

FIGURE 4: Immunoelectron microscopy showing sections of freeze-substituted and London resin-gold-embedded rabbit small intestine (*20*). Ultrathin sections were incubated with polyclonal rabbit anti-human CD36 antibody (pAb 300, diluted 1:100) for 60 min and labeled with protein A-15 nm gold (generously provided by Dr. H. Schwarz, Max-Plank-Institut für Entwicklungsbiologie, Tübingen, Germany). (A) Rabbit enterocyte with microvilli and glycocalix, cytosolic compartments. and basolateral membrane (see arrow). Arrowheads mark gold-labeled microvilli. (B) Enlargement showing microvilli immunogold-labeled. (C) Cross section of BBM microvilli immunogold-labeled. (D) In this control experiment, a similar section as in panel A is shown which was treated with nonimmune serum (diluted 1:100) and protein A-15 nm gold in exactly the same way as panels A to C except that the antibody was replaced by nonimmune serum.

effect of the two antibodies on the uptake of cholesteryl oleate (Figure 6B). It is interesting to note that the combined inhibitory effects measured in the presence of the two antibodies were identical within the experimental error to the inhibitory effect exerted by excess human apo A-I. The B_{max} values, i.e., the maximal inhibitory effect on cholesterol uptake observed in the presence of excess anti-SR-BI and anti-CD36 varied widely between different rabbit BBMV preparations and also depending on the specificity of the antibody.

The same kind of kinetic measurements of sterol uptake as discussed in Figure 5 for rabbit BBMV were carried out with human BBMV. Similar dose−response curves (data not shown) were obtained as shown for rabbit BBMV in Figure 5. The bar histograms (Figure 7) summarize the effects of the two antibodies (anti-SR-BI and anti-CD36) observed in isolation and in combination on the uptake of free and esterified cholesterol by human BBMV. The maximum inhibition of cholesterol uptake was about 25% in the presence of excess anti-SR-BI IgG, and this value increased to 40% in the presence of excess anti-CD36 IgM. The combined effects of the two antibodies were approximately additive for both free and esterified cholesterol uptake, and, as for rabbit BBMV, the combined effects of the two antibodies agreed within experimental error with the inhibitory effect observed in the presence of excess of human apo A-I (Figure 7). The uptake of esterified cholesterol was totally inhibited in the presence of anti-SR-BI and in the presence of apo A-I (Figure 7B). In contrast, the uptake of free cholesterol by human BBMV was inhibited to only ∼60% in the presence of both anti-SR-BI and anti-CD36 (Figure 7A).

DISCUSSION

We have shown previously that HDL as well as apo A-I, the major apoprotein of HDL, are inhibitors of intestinal

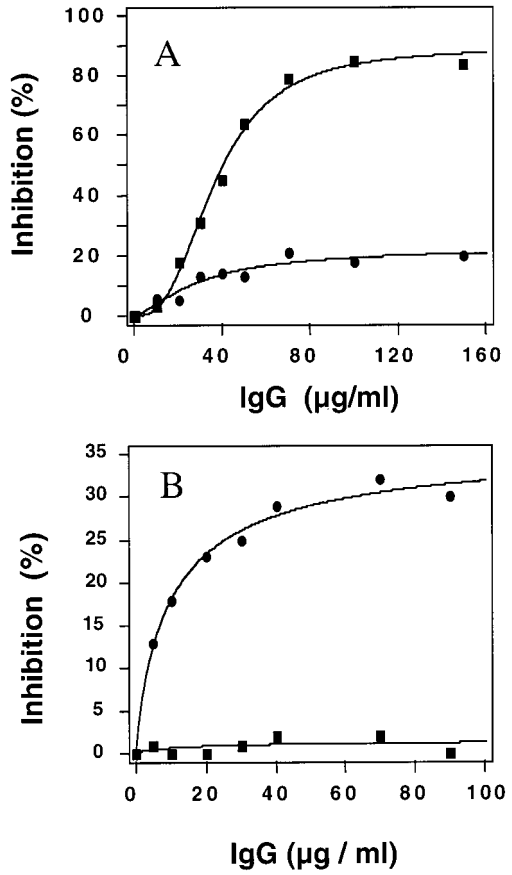

FIGURE 5: Dose−response curves showing the inhibition (%) of the uptake of free (●) and esterified cholesterol (■) in the presence of increasing amounts of anti-SR-BI IgG (panel A) and anti-CD36 IgM (panel B). All experiments were carried out with rabbit BBMV at room temperature. The solid lines were obtained by curve fitting using eq 1 for panel A and eq 2 for panel B.

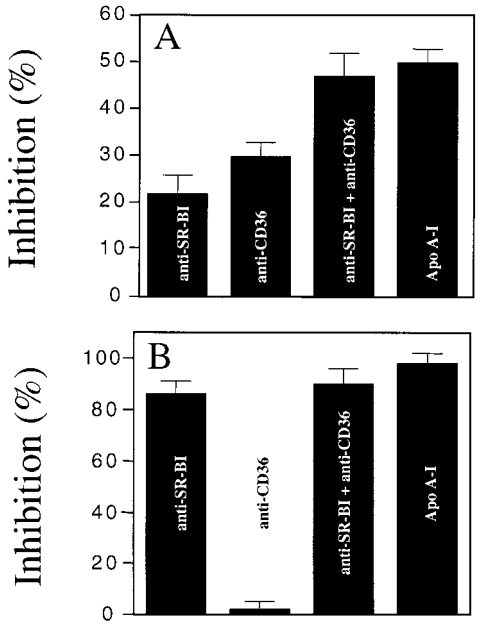

FIGURE 6: Bar histograms showing the maximum inhibitory effects (as %) of polyclonal anti-mouse SR-BI IgG (pAb150, 100 μg/mL) and monoclonal anti-human CD36 IgM (100 μg/mL) on the uptake of free (A) and esterified cholesterol (B) into the plasma membrane of rabbit BBMV (5 mg protein/mL). The combined effect of the two antibodies (each at 100 μg/mL) and the inhibitory effect of human apo A-I (at 150 μg of protein/mL) on free cholesterol uptake and on esterified cholesterol uptake into the plasma membrane of rabbit BBMV (5 mg of protein/mL) are shown in panels A and B, respectively.

cholesterol and lipid uptake (*12*, *13*). Serum lipoproteins and apoproteins were shown to be competitive inhibitors of intestinal lipid uptake, meaning that these inhibitors interact directly with BBM scavenger receptors responsible for facilitated lipid uptake (*12*, *13*, *27*). The mechanism of inhibition by serum lipoproteins involves specific binding to the receptors (*13*), and as a result of this interaction lipid uptake from the lipoprotein particle is inhibited. Such a mechanism is consistent with the notion that serum lipoproteins particles that are bound to scavenger receptors can act as donor particles for intestinal lipid uptake. This is borne out by experiment. Figure 1 shows that HDL is a good donor for lipid uptake into the BBM. All the different lipids present in the rHDL are transferred from the donor and incorporated into the plasma membrane of BBMV. With rHDL consisting of 95 mol % DMPC, 4 mol % cholesterol, and 1 mol % 1,2-dipalmitoyl-*sn*-phosphatidyl[*N*-methyl-^{3}H]choline (from Amersham, U.K.) and under otherwise identical conditions to those described for Figure 1A, the kinetics for the uptake of dipalmitoyl PC closely resembled those of cholesterol uptake (data not shown). The half time characteristic of dipalmitoyl PC uptake was $t_{1/2} = 17 \pm 9$ min agreeing within experimental error with the value measured for cholesterol uptake (cf. Table 1).

The uptake of cholesterol into the plasma membranes of the BBMV and Caco-2 cells is characterized by a large scatter of the data points (Figures 1 and 2) and in turn by large standard deviations of the average pseudo-first-order rate constants k_1 derived from these data (Tables 1 and 2). The scatter of the data very likely reflects variations in the transport properties of different BBMV preparations and different Caco-2 cells. On a molecular level, these variations are probably due to differences in concentration and composition of scavenger receptors present in the BBM. Differences in cholesterol absorption efficiency due to genetic variation have been shown to occur in both humans (*28*) and animals (*29*).

The observation (Figure 1A) that after proteolytic treatment of BBMV the rate of cholesterol transfer is greatly reduced and consistent with passive diffusion (Table 1) is interpreted to indicate that cholesterol uptake into native BBM (Figure 1A) is protein-mediated, entirely consistent with published data (*3*, *11*−*14*, *30*). This process is characterized by pseudo-first-order constants k_1 that increased linearly with the BBMV concentration (data not shown). Such a dependence suggests that the cholesterol uptake by the BBM from rHDL as the donor is probably a reaction of higher order than 1. In analogy with cholesterol uptake from other donor particles, it is very likely to be a second-order reaction involving collision-induced transfer of cholesterol from rHDL to the BBM (*14*).

For passive diffusion of cholesterol from rHDL to EPC SUV at 37 °C a half time of $t_{1/2} = 120 \pm 10$ min was obtained (Table 1). The rate constant k_1 for this process was independent of the EPC SUV concentration (data not shown), suggesting that this is a true first-order reaction. This is consistent with the desorption of cholesterol from rHDL being rate-limiting analogous to passive diffusion of cholesterol between two populations of SUV (*31*, *32*). The

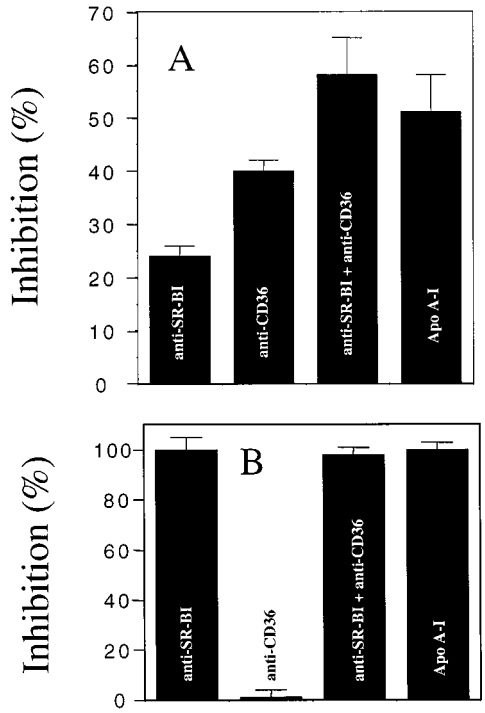

FIGURE 7: Bar histograms showing the maximum effect of inhibition (as %) on the uptake of free cholesterol (A) and esterified cholesterol (B) into the plasma membrane of human BBMV (5 mg of protein/mL) in the presence of polyclonal anti-mouse SR-BI IgG (pAb150, 100 μg/mL), monoclonal anti-human CD36 IgM (100 μg/mL), and the combined effect of the two antibodies (each at 100 μg/mL). For comparison, the inhibitory effects of apo A-I (at 150 μg of protein/mL) on free (A) and esterified cholesterol (B) are included.

passive transfer of cholesterol from rHDL containing mainly DMPC was significantly slower than that from EPC SUV (Table 1). First-order rate constants characteristic of cholesterol desorption were shown to depend on the nature and hence the fluidity of the lipid bilayer (*32*, *33*). For instance, Bar et al. (*33*) determined cholesterol transfer between two populations of SUV and obtained for the desorption of cholesterol from DMPC bilayers a half time $t_{1/2} = 121 \pm 30$ min and from 1-palmitoyl-2-oleoyl-phosphatidylcholine bilayers a half time $t_{1/2} = 59 \pm 15$ min (both values determined at 37 °C). These values representing the desorption rate of cholesterol from different lipid bilayers are in excellent agreement with our data (Table 1).

Table 1 clearly shows that the protein-mediated transfer of cholesterol from rHDL particles depends on the lipid composition of the rHDL particle. Adding 1 mol % sphingomyelin to the rHDL significantly slowed the transfer of cholesterol (Figure 1B). The half time $t_{1/2}$ increased from about 20 min in the absence of sphingomyelin to 60 min in the presence of sphingomyelin (Table 1). Cholesterol is known to interact specifically with sphingomyelin and glycosphingolipids (*22*), and the reduction in rate of cholesterol transfer observed in the presence of sphingolipid is probably due to complex formation between cholesterol and the sphingolipid.

Another question of interest is whether the process of lipid uptake from rHDL as the donor to BBMV (Figure 1) and Caco-2 cells (Figure 2) is consistent with selective lipid uptake reported for the SR-BI-mediated CE transfer from HDL particles (*4*−*6*). This process is fundamentally different from the endocytotic uptake of total lipoprotein particles. Figures 1 and 2 clearly show that only lipids present in the rHDL are taken up by BBMV and Caco-2 cells, but not the protein apo A-I consistent with the definition of selective lipid uptake. Hence, fusion of the lipoprotein particle with the BBM can be ruled out as a major mechanism contributing to intestinal lipid uptake. However, the term selective lipid uptake is misleading when applied to scavenger receptor-mediated intestinal lipid uptake: scavenger receptors lack lipid specificity and catalyze the transport of structurally different lipids such as free and esterified cholesterol, triacylglycerols, and phospholipids (*3*, *34*, *35*).

Immunoblotting and immunogold-labeling evidence is presented to show that CD36, another member of the scavenger receptor family, is present in both rabbit and human BBMV (Figures 3 and 4): it is located exclusively in the BBM but not in the basolateral membrane. Figure 3 shows that in rabbit and human BBMV the fully glycosylated form of CD36 with an apparent molecular mass of 88 kD (*25*) as well as a CD36 isoform of apparent molecular mass of 57 kD (*26*) are present with the isoform being more abundant in rabbit BBMV (Figure 3). In addition to bands at 57 and 88 kD, a band at 145 kD was observed with both anti-CD36 antibodies (Figure 3, lane 3). This observation is analogous to immunoblotting of BBMV using anti-SR-BI IgG (*13*). In the absence of reducing agents and depending on the type of antibody a predominant band at 145 kD was observed upon immunoblotting of rabbit BBMV using anti-SR-BI antibodies (*13*). One possible explanation of this result is that CD36 forms either homodimers or alternatively complexes with other proteins.

Figures 5 and 6 provide evidence that both scavenger receptors, SR-BI and CD36, contribute about equally to the uptake of free cholesterol. As evident from Figure 7, in human BBMV excess anti-human CD36 antibody (from Sigma) produced a bigger inhibition (40%) than the polyclonal anti-mouse SR-BI antibody (25%). For both rabbit BBMV (Figure 6) and human BBMV (Figure 7), the inhibitory effects of the two antibodies are additive. This is true for both free and esterified cholesterol. In the presence of excess anti-SR-BI and anti-CD36 antibodies, about 50 and 60% of the cholesterol uptake are inhibited in rabbit and human BBMV, respectively. These numbers agree within experimental error with the inhibition produced in the presence of excess apo A-I (cf. Figures 6 and 7). Since HDL and apo A-I are ligands of scavenger receptors (*27*), we conclude that 50 to 60% of the cholesterol uptake is mediated by these two scavenger receptors. Considering that maximally about 10% of the total uptake of free cholesterol is due to passive difffusion (*3*), 30 to 40% of the facilitated (protein-mediated) cholesterol uptake remains unaccounted for. A partial explanation is that the anti-mouse SR-BI antibody does not completely inhibit the SR-BI-mediated uptake into rabbit BBMV. That this cannot be the sole explanation is evident from the uptake of CE by rabbit BBMV in the presence of an excess of this antibody: The CE uptake was inhibited by 90% in rabbit BBMV and practically completely blocked in human BBMV (Figures 6B and 7B). Taking all facts together, we conclude that there are still other protein(s) contributing to the protein-mediated uptake of free cholesterol. Whether this is yet another

scavenger receptor or a protein of an entirely different class has to await future research.

In contrast to free cholesterol, the uptake of CE appears to be mediated solely by SR-BI with a negligible contribution from CD36 (Figures 5−7). The data in these figures suggest that SR-BI facilitates preferentially the uptake of lipid molecules more hydrophobic than cholesterol, such as CE, diacylglycerols, and triacylglycerols. It was shown previously (*30*) that native rabbit BBMV and proteoliposomes reconstituted from integral rabbit BBM proteins and egg PC exhibit approximately equal specific cholesterol, CE, and triacylglycerol uptake activity. On the basis of the data presented, we may assign the uptake of the very hydrophobic molecules to SR-BI and isoforms thereof. Also consistent with the above proposal is the notion that CD36 is responsible for the uptake of molecules that are significantly more hydrophilic than free cholesterol such as dietary long-chain fatty acids and monoacylglycerols.

In a previous paper, we reported that the uptake of free cholesterol into differentiated Caco-2 cells is completely inhibited by both excess rHDL and excess anti-SR-BI antibody. These results are seemingly at variance with the results of Figures 5−7. However, a possible explanation of this discrepancy is the finding that Caco-2 cells lack CD36 as evident from immunoblot analysis using different anti-CD36 antibodies (data not shown). Caco-2 cells may also lack the other yet unidentified protein involved in cholesterol uptake, and as a result, SR-BI and its isoform are the only proteins in the plasma membrane of Caco-2 cells mediating the uptake of free cholesterol.

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