Degradation of Chylomicron Remnants by Macrophages Occurs via Phagocytosis[†]

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ABSTRACT: Chylomicron remnants bound to rabbit alveolar macrophages with high-affinity ($K_d = 3.3 \pm 0.71$ μg of protein/mL). The binding of chylomicron remnants was competitively inhibited in the presence of unlabeled remnants and to a lesser extent by unlabeled low-density lipoproteins. Pretreatment of cells with either trypsin or pronase inhibited degradation in a dose and time dependent manner, suggesting involvement of a cell surface protein. Chylomicron remnants were degraded by alveolar macrophages from Watanabe heritable hyperlipidemic (WHHL) rabbits, which are devoid of LDL receptor activity. Moreover, colchicine and monensin which are endocytotic and lysozomal inhibitors, respectively, did not have any effect on the degradation of chylomicron remnants by macrophages from normal rabbits. The absence of divalent cations was found to enhance chylomicron remnant degradation by macrophages. Activated α2-macroglobulin and lactoferrin had no effect on chylomicron remnant degradation, indicating that the low-density lipoprotein receptor-related protein was not involved. In addition, the scavenger receptor inhibitors polyinosinic acid and fucoidan increased degradation of chylomicron remnant-ruling out uptake as a consequence of lipoprotein modification. Rather, the phagocytotic inhibitor cytochalasan D was found to significantly decrease chylomicron remnant degradation. Collectively, our data show that chylomicron remnants are metabolized by phagocytotic pathways initiated after binding to a cell surface protein which is distinct from the LDL receptor, LRP, or scavenger receptors.

Atherosclerotic lesions are first identified by the deposition of lipid beneath the subendothelial space. The early aetiology of the disease indicates the accumulation of cholesteryl esters in lipid-laden cells primarily of monocytic origin (Gown et al., 1986). The origin of cholesterol in foam cells is not unequivocally known, although plasma lipoproteins trapped within the subendothelial space are thought to be the primary source. Chylomicrons are lipoproteins synthesized by the intestine in response to a dietary fat intake. Chylomicrons or specifically their remnants (which are formed post hydrolysis) are delivered efficiently to arterial tissue, particularly in lesioned sites containing large numbers of macrophages (Mamo & Wheeler, 1994; Proctor & Mamo, 1996). Clearly then, chylomicron remnants might contribute to cholesterol accumulation in macrophages. However, presently, the metabolism of chylomicron remnants by macrophages is not clear. Van Lenten and colleagues (1985) demonstrated that chylomicron remnants are metabolized by monocyte-derived macrophages. The mechanism of degradation was not identified, although it was not thought to be via the low-density lipoprotein (LDL) receptor, because degradation persisted in LDL receptor negative cells (Van Lenten et al., 1983). However, in contrast to Van Lenten's studies, Koo et al. (1988) showed that chylomicron remnants bound exclusively to the LDL receptor of human macrophages and that binding of β-VLDL (which shares the same binding characteristics as chylomicron remnants) was significantly depressed in cells from subjects lacking the LDL receptor (Koo et al., 1986). Alternatively, a number of high-affinity processes other than the LDL receptor might contribute to chylomicron remnant metabolism by macrophages. The low-density lipoprotein receptor-related protein (LRP or the α2-macrogobulin receptor) can bind chylomicron remnants enriched with apolipoprotein E or containing lipoprotein lipase (Hussain et al., 1981; Biesiegel et al., 1994). The asialoglycoprotein receptor possesses broad specificity for a number of ligands, including chylomicron remnants (Windler et al., 1991), and macrophage scavenger proteins could be yet another degradative pathway (Brown & Goldstein, 1983). More recently, Gianturco and colleagues (1994) identified macrophage cell surface proteins which bound remnants of triglyceride rich lipoproteins.

In order to explore the mechanisms by which chylomicron remnants might induce macrophage cholesterol accumulation, this study was designed to assess the quantitative significance of alternate uptake processes.

MATERIALS AND METHODS

Preparation of Chylomicron Remnants. Lymph chylomicrons were collected from donor animals as previously described (Redgrave et al., 1975; Mamo & Wheeler, 1994; Proctor & Mamo, 1996). The source of lipid used as substrate for chylomicron synthesis was 2% Intralipid in 4% glucose. Chylomicrons were isolated from lymph by gradient ultracentrifugation (Redgrave et al., 1975). New Zealand white rabbits were anesthetized with ketamine/xylazine (30 and 5 mg/kg, respectively). Following a midline incision, the celiac and mesenteric arteries were ligated within 0.5 cm beyond their origin from the aorta. The esophagus was ligated above the stomach, followed by the rectum and

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associated blood vessels. Finally, the portal vein was ligated. Hepatectomized rabbits were given 1.5 g of chylomicron triglyceride by way of a femoral vein catheter over a 5 min period. A large dose of triglyceride was chosen to dilute residual very low-density lipoproteins. To prevent hypoglycemia, it was necessary to infuse glucose at 1.5 mL/min during the procedure. Complete anesthesia was maintained by intramuscular administration of ketamine and xylazine at 45−90 min intervals. Rabbits were bled from the abdominal aorta.

Chylomicron remnants were isolated from plasma by density gradient ultracentrifugation. Chylomicron remnants were considered "pure" on the basis of the absence of apolipoprotein B_{100} containing lipoproteins following sodium dodecyl sulfate−polyacrylamide gel electrophoresis (Proctor & Mamo, 1996). The apolipoprotein compliment was also indicative of a chylomicron remnant fraction with significant quantities of apolipoprotein E (52%) and lesser amounts of apolipoprotein C's (18%), B_{48} (16%), and AI (7%). Chylomicron remnant preparations had a constant diameter of around 40−47 nm compared to greater than 200 nm for nascent lymph chylomicrons (laser particle sizing, Brookhaven Instruments, New York and scanning electron microscopy). The chylomicron remnant fraction was substantially depleted of triglycerides as indicated by the triglyceride to cholesterol ratio of less than 1. The lipid composition of chylomicron remnants was 34.5 ± 3% triglycerides, 26.4 ± 2% cholesteryl ester, 16.6% ± 2% phospholipid, and 22.5 ± 2% free cholesterol (mean ± SEM, $n = 8$).

Low-density lipoproteins (1.019 g/mL < ρ < 1.063 g/mL) were isolated by serial centrifugation of human plasma. The purity of each lipoprotein fraction was confirmed by agarose gel electrophoresis (β mobility).

Radioiodination of Lipoproteins. Chylomicron remnants were radioiodinated as we have previously detailed (Mamo & Wheeler, 1994; Proctor & Mamo, 1996), utilizing the tyramine cellobiose procedure, to avoid exposure of lipoproteins to oxidizing agents. Briefly, tyramine cellobiose is radioiodinated, activated, and then linked to the lipoprotein. Removal of unbound iodine and the distribution of the label were achieved by standard techniques (Fidge & Poulis, 1974; Egusa et al., 1983; Mamo & Wheeler, 1994). The distribution was found to be 49.6 ± 2.1% (mean ± SEM) on apolipoprotein B, 32.0 ± 1.9% on non-B apolipoproteins, 9.2 ± 0.98% on lipids, and 8.9 ± 1.0% free iodine. Note that transfer of radiolabeled CM proteins to HDL in vitro does not occur even after prolonged incubation (24 h) at 37 °C (Mamo & Wheeler, 1994). There were no detectable lipid oxidation products (sensitivity greater than 1 μM, PeroXO-quant, Pierce Ltd.) or oxysterols (gas chromatography/mass spectrometry) following iodination.

Alveolar Macrophage Isolation. Rabbit alveolar macrophages were isolated from nonstimulated New Zealand white rabbits (Van Lenten et al., 1985). Briefly, lungs were lavaged with 1 L of RPMI 1640 media containing gentamycin (200 μg/mL) and amphotericin (2.5 μg/mL) at 25 °C. Cells were isolated by centrifugation at 1350*g* over 15 min at 4 °C. The yield of cells varied between 10×10^6 and 80×10^6 cells. Cells were placed in 75 mL culture flasks and incubated in RPMI containing 5% bovine fetal calf serum. A property of rabbit alveolar macrophages is that cells adhere strongly initially (<16 h) but are readily removed by gentle aspiration beyond 24 h. Cells were cultured for a maximum of 5 days. Two days prior to binding or degradation studies, cells were cultured in RPMI 1640 media alone. Immediately prior to experimentation, cell viability was assessed by trypan blue exclusion and was typically >98%. Alveolar macrophages were identified on the basis of morphology following staining with Harris's hematoxylin and chromotrope 2R. All cultures for binding and degradation studies contained greater than 99% macrophages.

Binding and Degradation Studies. Cells were harvested by aspiration and dispensed into siliconized polystyrene tubes at a density of approximately $0.25-1.0 \times 10^6$. Preliminary binding experiments at 4 °C were done with increasing concentrations of lipoproteins, to establish the submaximal specific binding concentrations. Alveolar macrophages were incubated with lipoproteins for 1 h in a shaking water bath (4 °C). Competitions with increasing amounts of unlabeled lipoproteins were done up to a 25 times greater protein content. The binding constant K_d and maximum bound B_{max} were determined following computer-aided nonlinear regression analysis (Prism, GraphPad Inc.). Note that this procedure is more accurate than analysis following linear transformation, for example Lineweaver−Burke plots or Scatchard analysis. Linear regression assumes that the variability among replicate Y values follows a Gaussian distribution with a standard deviation that does not depend on the value of X. This assumption is rarely true with transformed data.

Degradation studies were done as described for the binding studies except that the temperature of the water bath was 37 °C. Cells were incubated for 2 h and then placed on ice prior to centrifugation at 225*g* for 10 min at 4 °C. The supernatant was removed and discarded. To each tube was added, 3 mL of RPMI 1640 media; the cell pellet was displaced by bubbling nitrogen, and the cells were resedimented by centrifugation. The washing procedure was repeated five times, after which the macrophage pellet was resuspended in 1 mL of ice cold PBS. Aliqouts in duplicate were taken for determination of radioactivity. Cell protein was solubilized overnight in 0.1 M NaOH and then determined in duplicate using the enhanced BCA protocol (Pierce, Australia) described in the manufacturer's instruction sheet.

RESULTS

To assess whether cell surface proteins were involved in the degradation of chylomicron remnants, macrophages were pretreated with proteases. Macrophages incubated with either pronase or trypsin showed up to a 50% decreased rate of chylomicron remnant degradation (Figure 1).

The affinity and capacity of rabbit alveolar macrophages to bind chylomicron remnants was assessed by competition studies which are depicted in Figure 2. Macrophages were incubated with a constant concentration of radioiodinated chylomicron remnants found to be submaximal (20 μg of protein/mL). Unlabeled chylomicron remnants competed efficiently for binding with approximately 50% suppression of total binding in the presence of a 10-fold excess of unlabeled remnants. The K_d and B_{max} were determined following transformation of the competition data into a receptor/ligand binding curve. Macrophages displayed high-affinity binding of remnants with a K_d of 3.30 ± 0.71 μg of protein/mL (±SEM). However, the maximal binding capacity of macrophages varied between cultures with a mean of 85.2 ± 25.0 pg per microgram of cell protein.

Low-density lipoprotein competed for chylomicron remnant binding to macrophages, although less efficiently than

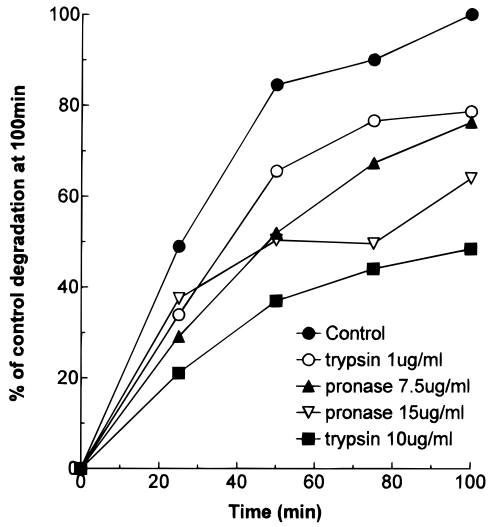

FIGURE 1: Effects of proteases on the binding and degradation (37 °C) of chylomicron remnants by rabbit alveolar macrophages. The binding and degradation by macrophages of chylomicron remnants were assessed in macrophages pretreated with proteases for 30 min at 37 °C at the concentrations indicated.

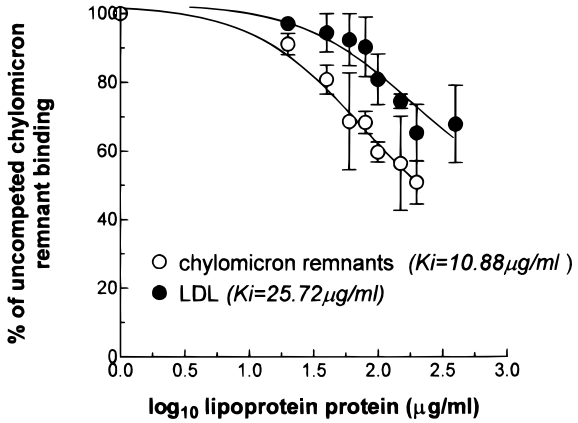

FIGURE 2: Competitive binding (4 °C)of radioiodinated chylomicron remnants to rabbit alveolar macrophages. Cells were incubated with 20 μg protein of radioiodinated chylomicron remnants and in the presence of increasing concentrations of unlabeled chylomicron remnants or LDL as indicated on the abscissa. Binding shown on the ordinate is expressed as a percentage of the amount bound in the absence of the competitor. The data indicate the mean ± SEM for $n = 5$ experiments. The curves are indicative of the regression equations from which the K_i values for chylomicron remnants and LDL were determined. The K_i represents the concentration at which 50% of binding was inhibited.

unlabeled chylomicron remnants (Figure 2). The inhibition constant K_i was calculated for chylomicron remnants and for LDL from the concentration of the two competitors producing 50% inhibition (EC_{50}) on the basis of the equation derived by Cheng and Prusoff (1973). Unlabeled chylomicron remnants were more than twice as effective in competing for the binding of 20 μg of labeled chylomicron remnants to macrophages, with K_i values of 10.88 ± 0.75 and 25.72 ± 3.08 μg/mL, respectively.

The degradation of chylomicron remnants and LDL by alveolar macrophages from normal and from WHHL rabbits is shown in Figure 3. Chylomicron remnants continued to be degraded in the absence of functioning LDL receptors, whereas there was no appreciable degradation of LDL. Chylomicron remnant degradation by macrophages was not affected by the absence of divalent cations (Figure 4), consistent with a nominal role of the LDL receptor in degradation. In fact, EDTA was stimulatory with increasing concentrations of up to 30 mM.

The potential role of LDL receptor-related protein in the catabolism of chylomicron remnants by macrophages was assessed by determining degradation in the presence of activated α2-macroglobulin (Vassiliou & Stanley, 1994) and lactoferrin. Table 1 shows that neither compound had any significant effect on the rate of chylomicron remnant degradation by macrophages.

To determine whether macrophage catabolism of chylomicron remnants occurred via receptor-mediated endocytosis, degradation was assessed in the presence of colchicine. Colchicine was stimulatory at concentrations of 0.1 μM, but a suppressive effect was observed at very high concentrations (Table 1). Following endocytosis, degradation of the ligand receptor complexes occurs in lysozomal compartments. Monensin, which is an inhibitor of lysozomal degradation, had no effect on remnant degradation (Table 1).

The uptake of chylomicron remnants via scavenger mechanisms was studied by comparing degradation in the presence of the inhibitors polyinosinic acid or fucoidan. Neither compound inhibited chylomicron remnant degradation by macrophages and in fact at concentrations beyond 20 μg/mL may have been stimulatory (Figure 5).

Macrophages are by nature phagocytic. To assess the potential contribution of this pathway to chylomicron remnant degradation, studies were done in the presence of cytochalasan D. Degradation of chylomicron remnants was found to be progressively inhibited by pre- and co-incubation of cells with cytochalasan D (Figure 6). The extent of inhibition varied for different cell isolates with inhibition between 35 and 60% at a cytochalasan D concentration of 2 mg/mL.

DISCUSSION

Chylomicron remnants bound with high-affinity to macrophages from normal rabbits. Unlabeled chylomicron remnants and LDL both competed with radiolabeled chylomicron remnants for binding, suggesting involvement of the LDL receptor. Chylomicron remnants competed more effectively than LDL which may have reflected the preferential binding of the LDL receptor for lipoproteins utilizing apolipoprotein E compared to apolipoprotein B_{100}. However, if binding of chylomicron remnants was to the LDL receptor, it would appear that degradation did not significantly occur via this route. Chylomicron remnants were degraded by alveolar macrophages isolated from WHHL rabbits. Furthermore, degradation of chylomicron remnants was not significantly altered in the absence of divalent cations (coincubation with EDTA) or by endocytotic (colchicine) and lysozomal (monensin) inhibitors. Collectively, the data indicate that endocytosis at the cell surface is not a requisite for uptake and therefore implies that degradation of chylomicron remnants did not occur via the LDL receptor or other higher-affinity processes utilizing endocytotic mechanisms.

The modification of LDL by chemical or physical means enables uptake by macrophages via alternate high-affinity mechanisms commonly known as scavenger pathways. We found no detectable lipid hydroperoxides, oxysterols or aggregation of remnant particles (laser particle analysis, Brookhaven Instruments) which might enable degradation via scavenger mechanisms. However, to assess the potential role of scavenger receptors in chylomicron remnant degrada-

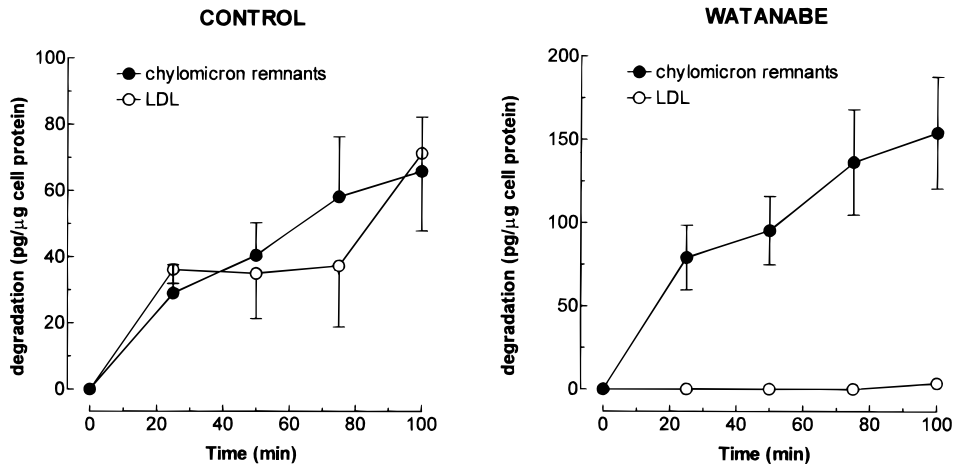

FIGURE 3: Binding and degradation (37 °C) of chylomicron remnants and low-density lipoproteins (LDL) by rabbit alveolar macrophages from New Zealand white and Watanabe heritable hyperlipidemic rabbits. Chylomicron remnants or LDL were incubated with alveolar macrophages at a concentration of 30 μg/mL for up to 100 min. The ordinate indicates the amount bound and degraded by macrophages per unit of cell protein. The data indicate the mean ± SEM for four experiments.

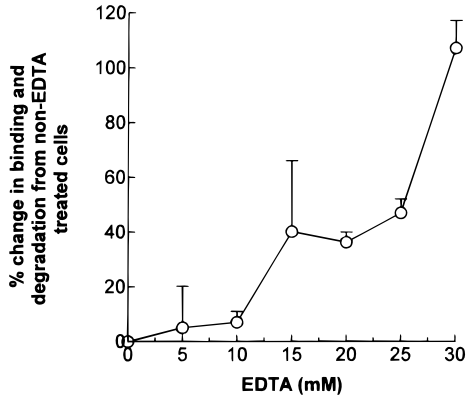

FIGURE 4: Effects of EDTA on the binding and degradation of chylomicron remnants by rabbit alveolar macrophages. The binding and degradation of radiolabeled remnants in the presence of increasing concentrations of EDTA were assessed at 37 °C. Cells and chylomicron remnants were co-incubated at the concentrations of EDTA indicated. The data represent the mean ± SEM for n = 3 experiments.

Table 1: Effect of activated α2-Macroglobulin, Lactoferrin, Colchicine, and Monensin on the Binding and Degradation of Chylomicron Remnants (20 μg/mL) by Rabbit Alvelar Macrophages[a]

	CM remnant degradation (% control)		CM remnant degradation (% control)
α2M (μg/mL)		colchicine (μM)	
0 (control)	100	0 (control)	100
10	126 ± 33	0.01	118 ± 11
25	148 ± 34	0.1	108 ± 4
60	120 ± 23	1.0	93 ± 9
100	115 ± 19	10.0	82 ± 1
lactoferrin (μg/mL)		monensin (μM)	
0 (control)	100	0 (control)	100
10	137 ± 32	0.01	107
25	111 ± 15	0.1	98
60	111 ± 34	1.0	87
100	132 ± 32	10.0	114

[a] Alveolar macrophages were co-incubated with the indicated concentrations of activated α2-macrogloblin or lactoferrin. Cells were pre- and co-incubated with colchicine or monensin for 2 h. Binding and degradation at 37 °C continued for 2 h, after which cellular radioactivity was determined. The data indicate the mean ± SEM of n = 3 experiments. Data without SEM are the mean of two experiments.

tion by macrophages, studies were done in the presence of inhibitors of ligand binding to scavenger receptors (Goldstein et al., 1979). Neither polyinosinic acid nor fucoidan inhibited chylomicron remnant metabolism by macrophages, and in fact, polyinosinic acid had a stimulatory effect on degradation.

Chylomicron remnants and β-VLDL can bind efficiently to LRP, particularly in the presence of exogenous apolipoprotein E or lipoprotein lipase. However, degradation in the presence of colchicine suggested that LRP was not involved in chylomicron remnant degradation by rabbit alveolar macrophages. More importantly, cross-competition studies with activated ligands for LRP, namely α2-macroglobulin and lactoferrin, established that LRP had only a nominal role if any in the degradation of chylomicron remnants by macrophages.

Macrophages are generally phagocytic, and so we investigated whether this property might explain chylomicron remnant degradation. We found that cytochalasan D progressively inhibited chylomicron remnant degradation with increasing concentrations. Cytochalasan interferes with the phagocytic cycle by blocking the formation of pseudopods (Hartwig & Stossel, 1976). It is likely that small molecules such as lipoproteins do not require substantial pseudopod formation which might explain why inhibition levels greater than 60% were not observed. The intracellular events following phagocytosis are unclear but are thought to include intercellular vesicular formation. Such a mechanism might explain why colchicine (an endocytotic inhibitor) became inhibitory only at higher concentrations. Furthermore, the stimulation of degradation in the presence of polyinosinic acid may reflect an enhancement of the "inflammatory" response to chylomicron remnants.

We propose that chylomicron remnants are degraded by macrophages primarily via phagocytosis. Chylomicron remnants appeared to require a cell surface protein (distinct from the LDL receptor) for phagocytosis to proceed because pretreatment of macrophages with trypsin/pronase inhibited degradation. Recently, Gianturco et al. (1994) reported two human monocyte/macrophage membrane binding proteins which bind triglyceride rich lipoproteins including chylomicron remnants. The molecular masses were estimated to be 200 and 235 kDa, respectively, but the identity of the proteins was not established. Phagocytosis via the alternate

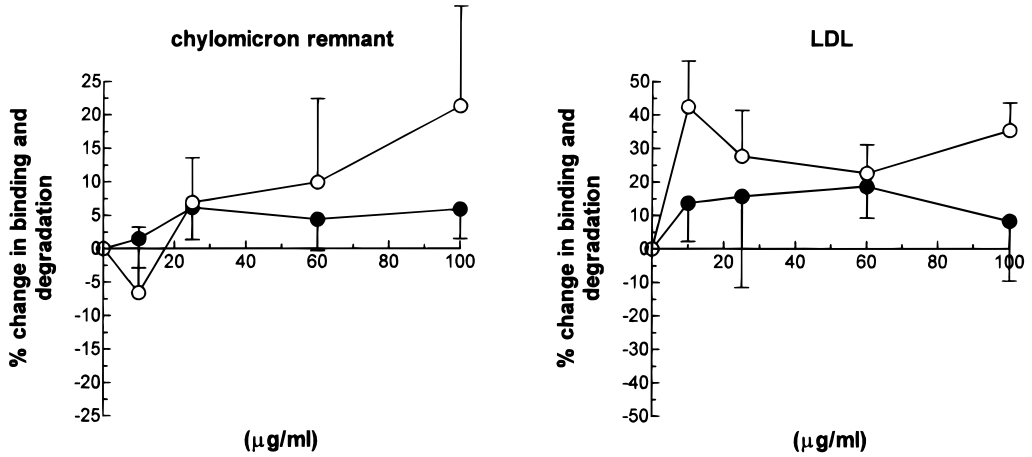

FIGURE 5: Effect of polyinosinic acid and fucoidan on the binding and degradation of chylomicron remnants by rabbit alveolar macrophages. The binding and degradation of radiolabeled chylomicron remnants in the presence of increasing polyinosinic acid (open symbols) or fucoidan (closed symbols) were determined at 37 °C. Cells and lipoproteins were co-incubated at the concentrations of the scavenger receptor inhibitors as indicated on the abscissa. The data represent the mean ± SEM for $n = 5$ experiments.

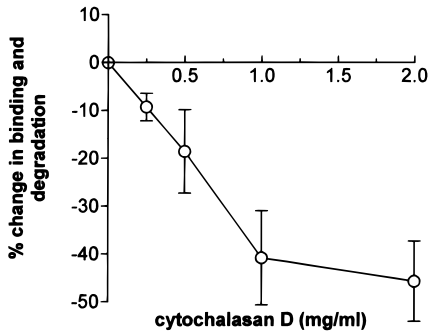

FIGURE 6: Effect of cytochalasan D on the binding and degradation (37 °C) of chylomicron remnants by rabbit alveolar macrophages. Cells and lipoproteins were co-incubated at the concentrations of the phagocytotic inhibitor as indicated on the abscissa. The data indicate the mean ± SEM of $n = 5$ experiments.

pathway involves binding to one of at least four complement receptor proteins. The most abundant of these is CR1, which has four allotypes with molecular masses between 160 and 250 kDa. The membrane binding proteins reported in Gianturco et al. (1994) might represent one of the complement binding receptors. Interestingly, CR1 binds with greater affinity in the absence of divalent cations such as calcium (Silverstein et al., 1986), consistent with our observations of chylomicron remnant binding in the presence of EDTA. Furthermore, while CR1 receptors can bind ligands efficiently, internalization only occurs in activated cells. Expression of macrophage CR1 receptors in unactivated cells could also explain why cytochalasan D on some occasions only weakly inhibited chylomicron remnant binding and degradation.

This study provides a new model of how chylomicron remnants might be metabolized by macrophages. The binding and degradation data are consistent with the earlier studies of Koo et al. (1988) and Van Lenten and colleagues (1985). The phagocytic degradation of chylomicron remnants suggests that these particles might be proinflammatory. The consequence of these observations coupled with the fact that chylomicron remnants penetrate arterial tissue efficiently is indicative of the potent proatherogenic properties of these particles. Furthermore, this model suggests that unabated cholesterol accumulation could occur in macrophages as a consequence of excessive chylomicron remnant uptake.

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REFERENCES

Biesiegel, U., Krapp, A., Weber, W., & Olivecrona, G. (1994) *Ann. N. Y. Acad. Sci. 10*, 737−769.

Brown, M. S., & Goldstein, J. L. (1983) *Annu. Rev. Biochem. 52*, 223−261.

Cheng, Y., & Prusoff, W. H. (1973) *Biochem. Pharmacol. 22*, 3099−3108.

Egusa, G., Brady, D. W., Grundy, S. M., & Howard, B. V. (1983) *J. Lipid Res. 24*, 1261−1267.

Fidge, N. H., & Poulis, P. (1974) *Clin. Chim. Acta 52*, 15−26.

Gianturco, S. H., Ramprasad, M. P., Lin, A. H., Song, R., & Bradley, W. A. (1994) *J. Lipid Res. 35*, 1674−1687.

Goldstein, J. L., Ho, Y. K., Basu, S. K., & Brown, M. S. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 333−337.

Gown, A. M., Tsukada, T., & Ross, R. (1986) *Am. J. Pathol. 125*, 191−200.

Hartwig, J. H., & Stossel, T. P. (1976) *J. Cell. Biol. 71*, 295−303.

Hussain, M. M., Maxfield, F. R., Mas-Oliva, J., Tabas, I., Ji, Z. S., Innerarity, T. L., & Mahley, R. W. (1991) *J. Biol. Chem. 266*, 13936−13940.

Koo, C., Wernette-Hammond, M. E., & Innerarity, T. L. (1986) *J. Biol. Chem. 261*, 11194−11201

Koo, C., Wernette-Hammond, M. E., Garcia, Z., Malloy, M. J., Uauy, R., East, C., Bilheimer, D., Mahley, R. W., & Innerarity, T. L. (1988) *J. Clin. Invest. 81*, 1332−1340.

Mamo, J. C. L., & Wheeler, J. R. (1994) *Coron. Artery Dis. 5*, 695−705.

Proctor, S. D. P., & Mamo J. C. L., (1996) *Coron. Artery Dis.* (in press).

Redgrave, T. G., Roberts, D. C. K., & West, C. E (1975) *Anal. Biochem. 65*, 42−49.

Silverstein, S. C., Greenberg, S., Di Virgilio, & Steinberg, T. H. (1986) *Fundamental Immunology*, (Paul. W. E., Ed.) 2nd ed., pp 703−720, Raven Press Ltd., New York.

Van Lenten, B. J., Fogelman, A. M., Hokom, M. M., Benson, L., Haberland, M. E., & Edwards, P. A. (1983) *J. Biol. Chem. 258*, 5151−5157.

Van Lenten, B. J., Fogelman, A. M., Jackson, R. L., Shapiro, S., Haberland, M. E., & Edwards, P. A. (1985) *J. Biol. Chem. 260*, 8783−8788.

Vassiliou, G., Stanley, K. K. (1994) *J. Biol. Chem. 269*, 15172−15178.

Windler, E., Greeve, J., Levkau, B., Kolb-Bachofen, V., Daerr, W., & Greten, H. (1991) *Biochem. J. 276*, 79−87.

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