Chylomicron-remnant-induced foam cell formation and cytotoxicity: a possible mechanism of cell death in atherosclerosis

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ABSTRACT

The effects of chylomicron remnants on cytoplasmic lipid loading and cell viability were assessed in cultures of human monocyte-derived macrophages and rabbit arterial smooth muscle cells. At a cholesterol concentration of 150 µg/ml, chylomicron remnants induced substantial cytoplasmic lipid loading of macrophages, but not of smooth muscle cells, within 6 h of exposure. Chylomicron remnants were found to be cytotoxic to macrophages and smooth muscle cells, although the latter were generally more resistant. Chylomicron remnants contained no detectable oxysterols (> 1 ng) and contained less non-esterified (‘free’) fatty acids than non-lipolysed nascent chylomicrons. Chylomicron-remnant-induced cytotoxicity appeared to be time- and dose-dependent. Macrophage and smooth muscle cell viability were inversely related to the production of superoxide free radicals and were significantly improved in the combined presence of superoxide dismutase and catalase. Collectively, our data suggest that, in macrophages, cell viability is compromised as a consequence of superoxide free radical production following uptake of chylomicron remnants. We would suggest that, in arterial smooth muscle cells, chylomicron-remnant-induced cell death also occurs as a consequence of superoxide free radical production. Our observations in the present study suggest that macrophage foam cells in atherosclerotic plaques might be derived from the cellular uptake of chylomicron remnants. Furthermore, arterial accumulation of chylomicron remnants might contribute to plaque destabilization as a consequence of cell death following superoxide free radical production by macrophages and smooth muscle cells.

INTRODUCTION

Lipid-laden cells (foam cells), predominantly of monocytic origin, are a hallmark feature of atherosclerotic plaques [1]. In advanced plaques, necrotic lipid-filled cores are formed following the death of macrophages and smooth muscle cells. Cytotoxic substances released from the inflammatory cells are thought to contribute to plaque destabilization, which could then result in a clinical event such as a heart attack or stroke [1]. The source of sterols in foam cells and the subsequent cause of cell death are not unequivocally established. Lipid oxidation products are thought to be potentially involved in the pathogenesis of atherosclerosis, given the oxidative stress that occurs following activation of the macrophage myeloperoxidase system [2]. Oxidatively modified low-density lipoproteins have been shown to be avidly degraded by macrophages in vitro [3]. Lipoproteins of intestinal origin which transport dietary fats are also likely to contribute to arterial lipid

Key words: atherosclerosis, foam cells, macrophages, smooth muscle cells.
Abbreviations: apoB-100, apolipoprotein B-100; GC/MS, gas chromatography/mass spectrometry; HTG, hypertriglyceridaemic; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; MPO, myeloperoxide; NEFA, non-esterified (‘free’) fatty acids; SOD, superoxide dismutase.
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deposition, inflammation and atheroma formation. Several studies have shown that chylomicron remnants penetrate arterial tissue rapidly, efflux poorly and in vitro serve as a lipid substrate for macrophages [4–6]. Chung and colleagues [7] found that remnants of lipolysed triacylglycerol (triglyceride)-rich lipoproteins from hypertriglyceridaemic (HTG) human serum were cytotoxic to macrophages because of the high concentration of non-esterified (‘free’) fatty acids (NEFA) which became associated with the particles post-lipolysis. Along similar lines, Hennig et al. [8] found that lipolysed HTG serum altered endothelial ‘barrier’ function in vitro. However, based on the studies by Chung and Hennig, it is not clear whether chylomicron-remnant-derived NEFA contribute to arterial cytotoxicity in vivo, because remnants generated in vitro would contain unphysiological concentrations of NEFA.

In an earlier report, we found that chylomicron remnants produced in vivo decreased the cell viability of cultured arterial smooth muscle cells [9]. Smooth muscle cell death occurred in the absence of significant levels of NEFA. Our observations suggested that, while remnants of postprandial lipoproteins might be toxic to smooth muscle cells, the effects could be mediated by factors other than NEFA. The effect of chylomicron remnants on macrophage viability was not investigated.

The principal aim of the present study was to explore if and by what mechanisms chylomicron remnants induce cytoplasmic lipid loading and death of the two cell types (macrophages and smooth muscle cells) primarily found in atherosclerotic plaques. Based on our observations, we suggest that atherosclerotic plaques could be compromised by the presence of reactive-oxygen-containing molecules produced by macrophages and smooth muscle cells as a consequence of cellular interaction with chylomicron remnants.

METHODS

Preparation of chylomicron remnants

Chylomicron remnants were generated in functionally eviscerated rabbits following a bolus injection of native lymph chylomicrons. The procedure has been described in detail elsewhere [5]. Briefly, lymph chylomicrons were obtained from the thoracic lymph ducts of donor animals given 2% (v/v) intralipid solution (Baxter Healthcare Pty. Ltd., Sydney, NSW, Australia) in 4% (w/v) glucose. Hepatectomized rabbits were given 1.5 g of chylomicron-triacylglycerol per kg of body weight via the femoral vein. The large dose of chylomicrons dilutes the pool of liver-derived lipoproteins. Furthermore, residual very-low-density lipoprotein remnants attain a density which is sufficiently different to enable separation from chylomicron remnants by ultracentrifugation [10]. Chylomicron remnant isolates contain no detectable apolipoprotein B-100 (apoB-100) as determined by SDS/PAGE [5]. The apolipoprotein profile of the chylomicron remnants, as reported previously [5], consisted mainly of apoE. The sizes of the chylomicron remnants were determined by laser light scanning and electron microscopy [5]. Chylomicron remnants were sterilized by passing through 0.22 μm-pore-size filters (Gelman Sciences, Ann Arbor, MI, U.S.A.) and used within 2 days. Total cholesterol mass was determined colorimetrically by using an assay kit purchased from Trace Scientific (Sydney, NSW, Australia) (catalogue no. TR13015). Triacylglycerol mass was determined colorimetrically following corrections for free glycerol by using an assay kit purchased from Wako (Tokyo, Japan) (catalogue nos. 430-11291, 432-11491, 436-11391, 438-11591).

Cell cultures

Human monocyte-derived macrophages were obtained from blood by a modification of the procedure first described by Boyum [11]. Briefly, blood was collected into tubes containing 10 mM EDTA and cells were pelleted by centrifugation (1150 g, 20 min). Plasma was removed and the cell pellet was resuspended in calcium- and magnesium-free PBS (pH 7.4) (2:1, v/v). The cell suspension was layered on top of Ficoll-Haque solution (Pharmacia Biotech AB, Uppsala, Sweden) in proportions of 3:2 (v/v) and centrifuged at 400 g for 30 min. The buffy coat was removed by aspiration, resuspended in RPMI-1640 medium (Gibco BRL, Grand Island, NY, U.S.A.) and washed twice (~ 600 g, 10 min) in Ca²⁺/ Mg²⁺-free PBS. The cell pellet (containing mononuclear cells and free of erythrocytes) was resuspended in RPMI-1640 culture medium and seeded in 24-well (16 mm diameter) tissue culture trays (Falcon Labware; Becton Dickinson, Franklin Lakes, NJ, U.S.A.) at a density of (3–5) × 10⁶ cells per well. RPMI-1640 supplemented with 50 μg/ml gentamicin and 10% (v/v) foetal bovine serum (Gibco BRL) was used for culturing monocyte-derived macrophages in a humidified 95% air/5% CO₂ tissue culture chamber at 37 °C. Monocytes were isolated by adherence to culture plastic achieved by incubation for 1.5 h in the culture chamber. The purity of the monocyte cultures was judged to be > 95% by staining for non-specific esterase using a kit purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.; catalogue no. 91-A), and the yield was approx. 20–25% of the initial number of cells seeded. The number of monocytes usually ranged from 0.7 × 10⁶ to 1 × 10⁶ per well. Monocytes were maintained in culture for 7–10 days, and medium was changed at least twice a week.

Rabbit arterial smooth muscle cells were cultured from abdominal aortas by the explant method as previously described [12]. Smooth muscle cells were cultured in Dulbecco’s modified Eagle’s medium (Gibco BRL)
supplemented with 50 µg/ml gentamicin and 20% (v/v) foetal bovine serum in a humidified 95% air/5% CO₂ chamber at 37 °C for a further 3 weeks. Medium was changed every 3 days. Smooth muscle cells were subcultured using 0.05% trypsin/0.53 mM EDTA (Gibco BRL) into 24-well culture trays (Falcon Labware; Becton Dickinson) at a density of 20000 cells per well. At 10 days after passaging, the number of cells had increased to approx. 120000–160000 per well.

**Assay of cell viability using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide] method**

Chylomicron remnants were added to wells containing cells in the absence of serum, and incubations were carried out in a 95% air/5% CO₂ atmosphere at 37 °C. Cell viability was assayed by measuring cellular mitochondrial dehydrogenase activity using a tetrazolium salt, MTT, that is reduced to a Blue Formazan product as the superoxide dismutase (SOD)-inhibitable reduction of cytochrome c. Smooth muscle cells were subcultured as described above and seeded into 96-well trays (2 × 10⁵ cells/well) and monocytes were obtained out of a total of at least 100 cells counted in a single field in each slide, and expressed as a percentage of total cells. Assay of superoxide production

The method used was the assay described by McCord and Fridovich [14] to measure superoxide anion production as the superoxide dismutase (SOD)-inhibitable reduction of cytochrome c. Smooth muscle cells were subcultured as described above and seeded into 96-well culture trays (4 mm diameter; 2000 cells/well). Buffy coat mononuclear cells were seeded into 96-well culture trays (2 × 10⁶ cells/well) and monocytes were obtained using the same conditions as described above. Both cultures were maintained for 10 days and then assayed for superoxide in the presence of chylomicron remnants. Each well was filled with 200 µl of Hanks buffered saline solution containing chylomicron remnants and 1 mg/ml cytochrome c (Sigma Chemical Co.) with or without 25 µg/ml SOD (Sigma), and then incubated in the culture chamber. At the indicated time, the trays were read on a microplate ELISA reader at 550 nm against blanks (200 µl of Hanks buffered saline solution). Trays used for cell-free control experiments were assayed in parallel using identical conditions. Superoxide-specific reduction of cytochrome c was expressed as the difference in absorbance between cells incubated with and without SOD, calculated using a molar absorption coefficient of 21 mM⁻¹·cm⁻¹ [14]. Cell protein was measured using the enhanced bicinechonic acid kit from Pierce Chemical Co. (Rockford, IL, U.S.A.).

**Staining for cytoplasmic lipid droplets**

Macrophages and smooth muscle cells, grown on 16-mm glass coverslips, were incubated with chylomicron remnants in the humidified culture chamber using the same conditions described for assaying cellular viability. At termination, cells were washed once with PBS (pH 7.4) plus 1 mg/ml BSA, followed by two washes with PBS (pH 7.4). The cells were fixed in 60% (v/v) propan-2-ol for 20 s, stained with Oil Red O (Sigma) [2 mg/ml in 60% (v/v) propan-2-ol] for 8 min, and washed twice for 10 s each in 60% (v/v) propan-2-ol to remove unbound Oil Red O. Harris’ haematoxylin (1–2 min staining) was used as a counterstain, followed by washing once in PBS and once in tap water. Finally, the coverslips were mounted on to the glass slides using Aquamount (BDH Laboratories). Slide preparations were examined by light microscopy. The number of lipid-laden cells was scored out of a total of at least 100 cells counted in a single field in each slide, and expressed as a percentage of total cells.

**NEFA quantification**

Analysis of NEFA was by gas chromatography following methylation, as described by Lepage and Roy [15]. The internal standard used was 15 µg of heptadecanoic acid (C₁₇:₀), The methyl esters were extracted using hexane and were analysed by an HP5890 gas chromatograph equipped with a flame ionization detector, auto sampler and integrator (Hewlett-Packard, Palo Alto, CA, U.S.A.). The column used was an SGE 25 m × 0.32 mm (internal diameter) bonded-phase fused silica capillary column with helium as the carrier gas (SGE Pty. Ltd., Ringwood, Victoria, Australia).

**Assay for oxysterols in chylomicron remnants**

To assess oxysterols in chylomicron remnant preparations, isolates were analysed by using gas chromatography/mass spectrometry (GC/MS) [16]. Samples were saponified in 1 M KOH/methanol. 5α-Cholestane was used as the internal standard. The mixture was incubated for 1 h at 45 °C, after which it was diluted with water and the lipids extracted with hexane. The organic extracts were dried under nitrogen and derivatized with bis(trimethylsilyl)trifluoroacetamide and pyridine, after
which they were heated at 60 °C for 20 min under nitrogen and evaporated to dryness. The extracts were redissolved with heptane and analysed on an HP 5890 gas chromatograph coupled with an HP5970 mass-selective detector and an HP Chemstation using HP G1034C MS Chemstation software (Hewlett-Packard). The following oxysterols were determined: 6β-hydroxycholesterol, 7-lathosterol, 7β-hydroxycholesterol, 5α,6α-epoxide, 4β-hydroxycholesterol, 3β,5α,6β-triol, 7-oxocholesterol and 25-hydroxycholesterol. The minimum amount of oxysterols detectable by using this method is 1 ng.

**Statistics**

Results are presented as means ± S.E.M. unless otherwise stated. Statistical analysis was evaluated using Student’s t-test for paired or unpaired data; P ≤ 0.05 was accepted to be significant. The analysis was done using INSTAT* statistics software.

**RESULTS**

**Chylomicron remnants**

The procedure for isolating chylomicron remnants produced from hepatectomized rabbits was identical to that used in previous studies [5,17]. Each preparation was required to meet certain criteria. The triacylglycerol/cholesterol ratio had to be less than 5 (usually 2–4), representing at least 80% hydrolysis of triacylglycerol. Chylomicron remnants had a diameter of 40–50 nm and were free of apoB-100-containing lipoproteins (determined by Coomassie Blue staining of apolipoproteins separated by PAGE). Chylomicron remnants were not aggregated (as determined by laser light scattering and electron microscopy). There were no oxysterols detected by GC/MS (at a sensitivity of greater than 1 ng). Furthermore, the concentration of NEFA in chylomicron remnants (0.43 ± 0.205 μg of NEFA/μg of cholesterol; mean ± S.D.; n = 5) was significantly lower than that in non-lipolysed nascent lymph chylomicrons (1.97 ± 0.65 μg of NEFA/μg of cholesterol; mean ± S.D.; n = 5; P < 0.05).

**Cytoplasmic lipid loading**

Lipid-laden foam cells in arterial lesions are primarily of monocytic origin, although some may be derived from arterial smooth muscle cells that have migrated to the subendothelial space [1]. We determined whether incubation with chylomicron remnants induced cytoplasmic lipid loading of monocyte-derived macrophages and arterial smooth muscle cells *in vitro*. Figure 1(A) shows significant numbers of cytoplasmic lipid droplets in macrophages 6 h after incubation with chylomicron remnants.
remnants at a cholesterol concentration of 150 μg/ml, compared with macrophages incubated without chylomicron remnants (Figure 1B). However, some macrophages incubated without chylomicron remnants were lipid-laden, presumably due to culturing in medium containing foetal bovine serum. The number of lipid-laden macrophages after incubation with chylomicron remnants was found to represent 89.2 ± 2.2% (n = 5) of total cells, compared with 21.6 ± 1.7% (n = 5) after incubation without chylomicron remnants. Collectively, there was an approx. 4-fold increase in lipid-laden macrophages after incubation with chylomicron remnants (P < 0.05). Cytoplasmic lipid accumulation was homogeneous, in the sense that essentially almost all lipid-laden cells showed relatively grossly enlarged cytoplasmic volume, with numerous Oil Red O-stained droplets. In contrast with macrophages, incubation of smooth muscle cells with chylomicron remnants did not induce any noticeable level of lipid accumulation above that of cells incubated without chylomicron remnants (Figures 1C and 1D).

**Chylomicron-remnant-induced cytotoxicity**

We explored macrophage and smooth muscle cell viability when cells were incubated with chylomicron remnants at low (25 μg/ml) and high (150 μg/ml) concentrations of cholesterol. Cell viability was determined by monitoring mitochondrial dehydrogenase activity by the MTT method [13]. Figure 2(A) shows that there was a lag phase before macrophage cell viability became significantly compromised, the duration of which seemed dependent on the concentration of chylomicron remnant cholesterol. At 3 h, mitochondrial dehydrogenase activity of macrophages incubated with 150 μg of cholesterol/ml of chylomicron remnants had decreased to about 80% of controls, while those incubated with 25 μg of cholesterol/ml were unaffected. On the other hand, following 24 h of incubation with remnants, low (25 μg/ml) and high (150 μg/ml) concentrations of cholesterol had equal cytotoxic potency, with only 40% of the initial mitochondrial dehydrogenase activity remaining (Figure 2A). Mitochondrial dehydrogenase activity of macrophages in the absence of chylomicron remnants was not affected over a 24 h period.

The quantitative and qualitative impact of chylomicron remnants on smooth muscle cell survival was similar to that observed for macrophages at the higher concentration of cholesterol studied (150 μg/ml) (Figure 2B). However, at the lower concentration (25 μg/ml), smooth muscle cells appeared to be more resistant, with no significant change in viability for up to 24 h of exposure to chylomicron remnants.

The effect of chylomicron remnant concentration on cell survival at the end of a 24-h incubation was also explored (Figures 3A and 3B). Monocyte-derived macrophages and smooth muscle cells were incubated with increasing concentrations of chylomicron remnants (25, 50, 100 and 150 μg of cholesterol/ml). There was a clear ‘dose–response’ for smooth muscle cells, with less than half the number of cells surviving at concentrations of chylomicron remnant cholesterol greater than or equal to 100 μg/ml (Figure 3B). Macrophages, on the other hand, appeared to be less resistant to remnant-induced cytotoxicity, as remnants were equipotent at all concentrations studied (Figure 3A).
Figure 3  Effect of chylomicron remnant concentration on macrophage and smooth muscle cell viability

Cells were incubated without (0 µg of cholesterol/ml) or with chylomicron remnants (25, 50, 100 or 150 µg of cholesterol/ml) for 24 h. After the 24-h incubation, the cellular viability of (A) macrophages and (B) smooth muscle cells was determined using the MTT method (see the Methods section). The y-axis represents cell viability as a percentage of that of controls. Controls are cells that had their viability assayed before exposure to chylomicron remnants. Data are means ± S.E.M. of triplicate experiments. Significance of differences: *P < 0.05 compared with 0 µg of cholesterol/ml.

Superoxide production

Superoxide is a highly reactive oxygen free radical produced as a microbiocidal agent or for the generation of other deadly oxygen-containing molecules [18]. The production of superoxide by monocyte-derived macrophages is a feature of the respiratory burst, which commonly occurs following phagocytosis of extracellular particles [18]. Chylomicron remnant uptake by macrophages appears to be primarily dependent on micro-

Figure 4  Plots showing the time course of superoxide production by (A) macrophages and (B) smooth muscle cells (SMC) in the absence and presence of chylomicron remnants

Cells were incubated in a solution containing cytochrome c (1 mg/ml) without (0 µg of cholesterol/ml) or with chylomicron remnants (25, 50, 100 or 150 µg of cholesterol/ml). Parallel dishes had the same solutions but in addition contained SOD. At the indicated time points, absorbance was read in an ELISA microplate reader as described in the Methods section. The amount of SOD-inhibitable production of superoxide was calculated as the difference between values obtained in cells with SOD and in cells without SOD. Data are means of duplicate dishes and are expressed as nmol of superoxide produced per dish. Cells without chylomicron remnants acted as controls. The amount of macrophage protein per dish was 10.89 ± 6.65 µg (mean ± S.D.) and the amount of smooth muscle cell protein per dish was 53.8 ± 12.8 µg.
The morphology of smooth muscle cells and macrophages following incubation with chylomicron remnants for 24 h was assessed by phase-contrast microscopy (Figures 6A–6D). Macrophages incubated without chylomicron remnants displayed extensive pseudopod formation (cell membrane extensions) and were strongly adherent (Figure 6C). In contrast, macrophages incubated with chylomicron remnants (150 µg of cholesterol/ml; 24 h) rarely exhibited cell membrane extensions, and were fewer in number, comparatively smaller in size and easier to dislodge (Figure 6D).

Smooth muscle cells in the absence of chylomicron remnants were confluent (Figure 6A). However, we observed significant smooth muscle cell loss following incubation with chylomicron remnants (150 µg of cholesterol/ml), as well as decreased spreading, and the cells were less adherent (Figure 6B).
Figure 6 Photomicrographs showing the morphology of smooth muscle cells and macrophages after exposure to chylomicron remnants for 24 h

(A) Smooth muscle cells without chylomicron remnants (equivalent volume of 0.9% NaCl); (B) smooth muscle cells with chylomicron remnants (150 µg of cholesterol/ml); (C) macrophages without chylomicron remnants (equivalent volume of 0.9% NaCl); (D) macrophages with chylomicron remnants (150 µg of cholesterol/ml). The bar in (A) and (B) represents 83 µm, and that in (C) and (D) represents 40 µm.

DISCUSSION

Several studies have suggested that postprandial lipoproteins may cause intracellular lipid loading. Gianturco et al. [19] found that triacylglycerol-rich lipoproteins from HTG serum caused cholesterol accumulation in fibroblasts. Van Lenten et al. [20] demonstrated that cholesterol-rich remnant lipoproteins induced foam cell formation in macrophages, and Georgopoulos et al. [21] found that postprandial triacylglycerol-rich lipoproteins from diabetic subjects produced cholesterol accumulation in macrophages. Our present study provides specific evidence that remnants of postprandial lipoproteins, i.e. chylomicrons, are capable of inducing foam cell formation in human monocyte-derived macrophages, notably in the absence of significant oxidative modification. Our observations are consistent with an earlier report which found that chylomicron remnants were internalized by macrophages via a process that was insensitive to intracellular sterol levels [5].

Arterial foam cells may also be derived in part from smooth muscle cells whose phenotypic characteristics have become proliferative as opposed to contractile [22]. In contrast with human monocyte-derived macrophages, we found no evidence of cytoplasmic lipid loading following incubation of smooth muscle cells with chylomicron remnants. However, utilizing the same primary smooth muscle cell model as in the present study, we and others have demonstrated that the primary route of chylomicron remnant uptake by these cells occurred via the apoB-100/E (low-density-lipoprotein) receptor [23,24], a pathway whose regulation is determined by the intracellular sterol pool [25].

Sequential studies by Chung and colleagues [7] found that remnants of postprandial triacylglycerol-rich lipoproteins produced in vitro by lipolysis were cytotoxic to cultured human monocyte-derived macrophages. They later found that large quantities of NEFA were liberated post-lipolysis, associated with remnant lipoproteins, and concluded that the NEFA were the principle cytotoxic component of lipolysed chylomicrons [7]. However, in the present study, we found that the NEFA content of chylomicron remnants generated in vivo was substantially less than that of non-lipolysed nascent lymph chylomicrons. The production of chylomicron remnants in vivo is physiological and prevents excessive association with NEFA. Based on our findings that macrophage uptake of chylomicron remnants occurred via a ‘phagocytic-like’ process [5] and that a consequence of this pathway is the respiratory burst [18], we propose that chylomicron-remnant-induced cytotoxicity may have occurred following production of oxygen-containing free radicals.

Chylomicron remnants were found to be cytotoxic to both macrophages and smooth muscle cells. Decreased macrophage viability was generally observed sooner and at lower concentrations of chylomicron remnants than for smooth muscle cells. However, at concentrations greater than 100 µg/ml cholesterol, there was no difference between the two cell types. Differences in the rates at which cytotoxicity occurred may have been a consequence of different uptake mechanisms, i.e. principally a ‘phagocytic-like’ mechanism in macrophages and via the apoB-100/E receptor in smooth muscle cells. The two different pathways might lead to different rates of delivery and/or intracellular processing of chylomicron remnants.

Cultured human skin fibroblasts, like smooth muscle cells, internalize chylomicron remnants primarily via the apoB-100/E receptor [23]. However, in fibroblasts from apoB-100/E-receptor-deficient individuals, chylomicron remnants continue to be internalized (albeit at a much lower rate) by a ‘phagocytic-like’ process [17]. Therefore it is possible that chylomicron remnants compromise smooth muscle cell viability by a process not unlike that in macrophages, but that quantitatively this pathway is of less significance.

Chylomicron remnant uptake may not have been a
requisite for induction of the cytotoxic cascade. NADPH oxidase, which is a key enzyme involved in the respiratory burst, is plasma-membrane-bound and can be activated by receptor-mediated and receptor-independent means [18]. Continuous or prolonged contact between the cell and the stimulus, in this case chylomicron remnants, may have been sufficient to induce production of oxygen-containing free radicals (superoxide). However, receptor-independent activation of the respiratory burst would seem unlikely, at least in smooth muscle cells, because there was a distinct dose-dependent effect. Even at the lowest concentration of chylomicron remnants studied (25 µg of cholesterol/ml), the stimulus for this process would presumably be in excess.

Previously, Tabas and colleagues [26] showed that macrophage viability was directly dependent on the ratio of free cholesterol to phospholipid in the cell membrane. An increase in the cellular free cholesterol/phospholipid ratio (from approx. 0.6 upwards) resulted in cell death [26]. In a previous report, the ratio of free cholesterol to phospholipid in chylomicron remnant preparations identical to those used in the present study was found to be approx. 1.0 [5], which is considerably greater than that of plasma cell membranes and deemed highly toxic according to the proposal of Tabas et al. [26]. Hence an alternative explanation for chylomicron-remnant-induced cytotoxicity may be that uptake and/or prolonged exposure to chylomicron remnants altered plasma membrane stability by changing the proportions of amphiphilic lipids.

Incubation of cells with a combination of SOD and catalase was found to attenuate chylomicron-remnant-induced cell death, suggesting an association between free radical formation and cell viability. The respiratory burst in macrophages is part of an elaborate microbiocidal radical formation and cell viability. The respiratory burst induced cell death, suggesting an association between free catalase was found to attenuate chylomicron-remnant-philic lipids. The respiratory burst pathway remains to be clearly identified, the observation that chylomicron remnants can be internalized via a ‘phagocytosis-like’ process, particularly in macrophages [5], is consistent with diminished cell viability as a consequence of oxidative stress.

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REFERENCES

6 Fujioka, Y., Cooper, A. D. and Fong, L. G. (1998) Multiple processes are involved in the uptake of chylomicron remnants by mouse peritoneal macrophages. J. Lipid Res. 39, 2339–2349