The induction of macrophage foam cell formation by chylomicron remnants

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Abstract

The accumulation of foam cells in the artery wall causes fatty streaks, the first lesions in atherosclerosis. LDL (low-density lipoprotein) plays a major role in foam cell formation, although prior oxidation of the particles is required. Recent studies, however, have provided considerable evidence to indicate that CMRs (chylomicron remnants), which carry dietary lipids in the blood, induce foam cell formation without oxidation. We have shown that CMRs are taken up by macrophages and induce accumulation of both triacylglycerol and cholesterol, and that the rate of uptake and amount of lipid accumulated is influenced by the type of dietary fat in the particles. Furthermore, oxidation of CMRs, in striking contrast with LDL, inhibits, rather than enhances, their uptake and induction of lipid accumulation. In addition, the lipid accumulated after exposure of macrophages to CMRs is resistant to efflux, and this may be due to its sequestration in lysosomes. These findings demonstrate that CMRs induce pro-atherogenic changes in macrophages, and that their effects may be modulated by dietary factors including oxidized fats, lipophilic antioxidants and the type of fat present.

Introduction

The composition of the diet is believed to play a significant role in atherosclerosis development, with the amount and type of fat present being one of the most important factors [1,2]. Dietary lipids, including fats and cholesterol, enter the blood in chylomicrons, large, TAG (triacylglycerol)-rich lipoproteins which then undergo rapid lipolysis by lipoprotein lipase, resulting in the loss of some of the TAG and leaving smaller CMRs (chylomicron remnants) which are removed from the circulation by the liver [3].

It is known that dietary fats modulate atherogenesis by influencing plasma levels of LDL (low-density lipoprotein) [4], which plays a major role in the development of the disease [5]. However, recent studies have provided strong evidence to suggest that CMRs are able to interact directly with cells of the artery wall to promote atherogenic events [6–8]. CMRs penetrate the artery wall as efficiently as LDL [9]; dyslipidaemias in which remnants accumulate in the blood cause the development of premature atherosclerosis [8]; and remnant-like lipoproteins have been isolated from atherosclerotic plaque [10].

Atherosclerosis is initiated when macrophages which have invaded the artery wall take up lipid from the plasma lipoproteins and become foam cells [11]. A large number of studies have implicated LDL in macrophage foam cell formation; however, it is also clear that modification of the particles either chemically or by oxidation is necessary before extensive lipid accumulation is induced [5]. In sharp contrast, studies in our laboratory and others have demonstrated that CMRs cause macrophage foam cell formation without prior oxidation [8,12–15]. The present paper will review recent studies on the mechanisms involved in the uptake and induction of lipid accumulation by macrophages and on the subsequent efflux of the lipid from the cells, and will discuss how these processes may be modulated by the type of dietary fat or oxidative modification of the particles.

The induction of lipid accumulation in macrophages by CMRs

Since it is difficult to obtain a homogeneous preparation of CMRs from the blood uncontaminated with other lipoproteins of a similar density which are present postprandially, such as chylomicrons and VLDL (very-low-density lipoprotein), studies on macrophage foam cell formation in our laboratory and others have used CMRs obtained from rat or rabbit chylomicrons after collection of lymph from the thoracic duct, or model CRLPs [CMR-like particles containing human apoE (apolipoprotein E)] [8]. We have shown that CMRs or CRLPs cause the accumulation of lipid droplets in the cytoplasm of the murine macrophage cell line J774 [14], the human monocyte cell line THP-1 and primary HMDMs (human monocyte-derived macrophages) [15], and similar results have been reported by Yu and Mamo (HMDMs) [12] and Fujioka et al. (mouse peritoneal macrophages) [13]. Our studies additionally demonstrated that CRLPs caused an increase in cholesterol content in THP-1 cells and HMDMs comparable with that observed with a similar level (in terms of the cholesterol content of the lipoproteins) of oxLDL (oxidized LDL), while as might be expected, the TAG content was raised to higher levels [15].

Key words: atherosclerosis, chylomicron remnant, dietary fat, macrophage foam cell, oxidized lipoprotein.

Abbreviations used: ABCA-1, ATP-binding cassette transporter A-1; apoA1, apolipoprotein A1; apoB48, apolipoprotein B48; apoB48r, apoB48 receptor; apoE, apolipoprotein E; CMR, chylomicron remnant; CRLP, CMR-like particle containing human apoE; HMDM, human monocyte-derived macrophage; LDL, low-density lipoprotein; LDLr, LDL receptor; LRP, LDLr-related protein; MUFA, mono-unsaturated fatty acid; oxCRLP, oxidized CRLP; oxLDL, oxidized LDL; PC, phosphatidylcholine; PUFA, polyunsaturated fatty acid; RAP, receptor-associated protein; SFA, saturated fatty acid; SR-A, scavenger receptor-A; TAG, triacylglycerol; VLDL, very-low-density lipoprotein.

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Uptake of CMRs by macrophages

A number of studies have demonstrated that CMRs or CRLPs are taken up and degraded by macrophages, including HMDMs [16–18], mouse peritoneal macrophages [13,19], THP-1 macrophages [15,20] and the murine cell lines J774 and P388D1 [14,19,21]. In the liver, the LDLr (LDL receptor) and the LRP (LDLr-related protein), which both recognize apoE, are responsible for the uptake of CMRs [22]. The LDLr has also been implicated in CMR uptake by macrophages [17,20–22] and studies in our laboratory with CRLPs and THP-1 macrophages support this view. However, we have also found that mRNA levels for the LDLr are down-regulated by CRLPs [23], as is known to happen with LDL. In addition, there is evidence to suggest that CMR uptake is only partially impaired in macrophages in which the LDLr is lacking or blocked [13,16,24]; thus it is likely that CMRs are able to enter macrophages by routes other than the LDLr.

In experiments with CRLPs, we have found that lactoferrin, a ligand for the LRP, blocks their uptake by THP-1 macrophages by approx. 90%. This is consistent with the findings of Fujioka et al. [13], who found a marked decrease in CMR uptake by mouse peritoneal macrophages in the presence of RAP (receptor associated protein), an inhibitor of the LRP, and our work has also shown that CRLPs up-regulate the expression of the LRP mRNA in the cells [23]. In contrast, we found no evidence for a role for SR-A (scavenger receptor A) or B1 (SR-B1) in the process, although a blocking antibody for the class B scavenger receptor CD36 and cytochalasin, which inhibits phagocytosis, had a modest inhibitory effect on CRLP uptake.

It has been suggested that the apoB48 (apolipoprotein B48) receptor (apoB48r) may be involved in the uptake of CMRs by macrophages [22]. Since apoB48 is an integral apoprotein, it is not possible to bind it to model CRLPs in a physiological way. However, it has been reported that antibodies to apoB48 do not inhibit the uptake of a TAG-rich lipoprotein fraction high in CMRs in rat macrophages [25]. Moreover, Elsegood et al. [20] were unable to detect binding of CMRs to a protein with a molecular mass corresponding to the apoB48r in THP-1 cells, and suggested that it may be specific for VLDL remnants rather than CMRs.

These findings indicate that the major routes of uptake of CMRs by macrophages occur via the apoE-dependent LDLrs and LRP receptors, with the LRP predominating, while CD36 and phagocytosis may play a minor role.

The effect of oxidation of CMRs on their uptake and induction of lipid accumulation in macrophages

The oxidative state of CMRs may be altered in the artery wall by the oxidative action of enzymes such as cell-associated lipoxygenase and myeloperoxidase which are believed to oxidize LDL, or by the presence in the particles of oxidized lipids, which are produced when fat is cooked at high temperatures, or lipophilic antioxidants such as vitamin E or plant carotenoids from the diet [5,8].

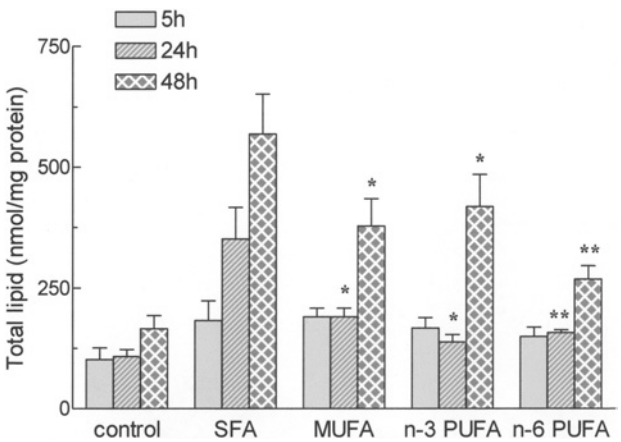
Although prior oxidation of CMRs is not required for their induction of foam cell formation [8], our studies have shown that oxCRLPs (oxidized CRLPs) as compared with CRLPs have different effects on the expression of key genes involved in the process. OxCRLPs were found to increase LRP mRNA expression nearly 5-fold, while CRLPs caused only a 2-fold rise, and expression of SR-B1, multidrug resistance-1 and apoE was increased only after oxidation of the particles. Moreover, mRNA for the ABCA-1 (ATP-binding cassette transporter A-1), which is involved in the efflux of cholesterol from macrophages, was decreased by CRLPs, but raised by oxCRLPs [23]. In addition, we have found that the incorporation of lipophilic antioxidants such as the tomato pigment, lycopene, or the drug, probucol, into CRLPs markedly increases lipid accumulation in THP-1 macrophages, and that this is due to more rapid uptake of the particles [26,27]. Further investigations in our laboratory using CRLPs, oxCRLPs (oxidized by exposure to $CuSO_4$) and CRLPs containing probucol (pCRLPs) have shown that the rate of uptake of the particles by THP-1 macrophages and the amount of lipid subsequently accumulated is inversely related to the oxidative state of the particles. Thus, in striking contrast with what happens with LDL, oxidation of CMRs inhibits their induction of macrophage foam cell formation, while protection of the particles from oxidation by the incorporation of antioxidants enhances the process. These unexpected findings may account for the apparently paradoxical effect that probucol, which has been found to retard atherosclerosis in many studies, consistently promotes lesion development in apoE-, or LDLr-, deficient mice, since CMR levels are raised and their clearance is delayed in these animals [28]. In addition, they may provide part of the explanation for the failure of large-scale intervention studies to show any cardiovascular benefit of dietary supplements of lipophilic antioxidants such as vitamin E and β-carotene [29].

The effect of the fatty acid composition of CMRs on their uptake and induction of lipid accumulation in macrophages

The type of fat in the diet is known to influence the development of atherosclerosis, with PUFAs (polyunsaturated fatty acids) or MUFAs (mono-unsaturated fatty acids), compared with SFAs (saturated fatty acids), decreasing the risk [2]. Our studies with CMRs from rats have demonstrated that the fatty acid composition of the particles reflects that of the diet [30], and this influences their removal from the blood by the liver, with CMRs enriched in $n-3$ or $n-6$ PUFAs being taken up more rapidly than those high in MUFAs or SFAs [31–33]. It is possible, therefore, that different dietary fats carried in CMRs may also influence the interaction of the particles with macrophages, and thus directly modulate foam cell formation. In experiments with CRLPs and THP-1 macrophages [34], we have found that particles containing SFAs cause increased accumulation of total lipid in the cells

Figure 1 | THP-1 macrophages were incubated with CRLPs enriched in SFAs, MUFAs, $n-6$ PUFAs or $n-3$ PUFAs (0.3 μmol of TAG/ml), and the total lipid (TAG+cholesterol) accumulated in the cells was measured after 5, 24 and 48 h

Results are means ± S.E.M. for three separate experiments. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with SFA CRLPs.



in comparison with those containing MUFAs or $n-6$ or $n-3$ PUFAs (Figure 1), and that this is mainly due to raised TAG levels. Investigation of the rate of uptake of the particles showed that CRLPs containing SFAs and MUFAs were taken up at a similar rate, which was significantly higher than that of CRLPs containing $n-6$ or $n-3$ PUFAs. The reason why the faster uptake rate results in greater lipid accumulation in the case of SFA-, but not MUFA-rich CRLPs, is not clear, but one explanation may be increased intracellular metabolism of TAG.

Since the oxidative state of CRLPs influences their uptake (see above), the differences observed with the particles of different fatty acid composition may be due to differences in their oxidation levels. In general, however, there was no correlation between the oxidative state of the various CRLP types either before or after exposure to $CuSO_4$ (6 h) and their rate of uptake by the cells. The MUFA-rich CRLPs, however, were less susceptible to oxidation that the other CRLP types, and this may partially explain their relatively rapid rate of uptake compared with PUFA-rich CRLPs [34]. These findings suggest that dietary SFAs carried in CMRs may enhance their ability to induce macrophage foam cell formation, thus increasing their atherogenicity.

The efflux of lipid from macrophages after exposure to CMRs

The efflux of lipid from macrophages after exposure to lipoproteins is an important factor in foam cell formation, since the amount of lipid accumulated will depend on the balance between the amounts entering and leaving the cells. Efficient cholesterol removal from macrophages in the presence of exogenous cholesterol acceptors has been demonstrated after incubation with acetylated LDL (acLDL), but cholesterol derived from oxLDL seems to be resistant to efflux [35,36]. The type of lipoprotein particle which delivers cholesterol to macrophages, therefore, may be important in determining the rate of its efflux from the cells.

After exposure of THP-1 macrophages to CRLPs (48 h) followed by incubation with or without cholesterol acceptors (24 h), we found that the mass of cholesterol was decreased only in the presence of apoA1 (apolipoprotein A1)–PC (phosphatidylcholine) vesicles, which resemble β-migrating HDL (high-density lipoprotein), a potent cholesterol acceptor, and the maximum decrease which could be achieved was 38% in 24 h. The mass of TAG was not decreased in any conditions [37]. Furthermore, investigation of the rate of efflux of lipid from THP-1 macrophages after loading the cells with CRLPs radiolabelled with [^{3}H]cholesterol or [^{3}H]triolein showed that less than 30% of the cholesterol label was removed from the cells in the presence of apoA1–PC in 24 h (Figure 2), less than 4% of the TAG label was effluxed in the same period, and there was little metabolism of the TAG taken up by the macrophages. Oxidation of the CRLPs had no effect on the mass of cholesterol and TAG found in the cells or effluxed into the medium (Figure 2) [37]. Thus the lipid accumulated in macrophages in response to CMRs is resistant to efflux, as has been found with oxLDL [35,36].

It has been suggested that the low level of efflux of cholesterol from macrophages loaded with oxLDL is due to the sequestration of the lipid in lysosomes after uptake of the particles [35,36]. In order to investigate whether this is also true for CMRs, we used fluorescence labelled CRLPs to evaluate the subcellular localization of the lipid taken up by the cells. The results showed that CRLP lipid is substantially co-localized with the lysosomal marker, lysosomal associated membrane protein-1 (Figure 3) [37], and similar results were obtained with oxCRLPs and CRLPs containing probucol.

These studies demonstrate that lipid taken up from CMRs, regardless of their oxidative state, is not readily cleared from the cells by efflux (cholesterol) or metabolism (TAG), and that this may be due to the sequestration of the lipid in lysosomes after their uptake by the cells. This resistance of the lipid to removal from macrophages, therefore, is another factor which is likely to contribute to the atherogenicity of CMRs.

Summary and conclusions

It is clear from studies to date that CMRs are taken up by macrophages and, in comparison to oxLDL, induce cholesterol accumulation to a comparable level and TAG accumulation to a greater level [8,12–22]. Although oxidation of CMRs is not required to bring about these effects, the oxidative state of the particles is inversely related to their rate of uptake and the amount of lipid accumulated. Thus, in striking contrast with what happens with LDL, foam cell formation is inhibited, rather than enhanced, with greater oxidation of CMRs. In addition, our work has demonstrated that the fatty acid composition of CMRs influences the rate of uptake and amount of lipid accumulated in macrophages, with particles enriched in SFAs causing greater accumulation

Figure 2 | **Efflux of radioactivity from THP-1 macrophages after incubation with (A) [^{3}H]cholesterol CRLPs or oxCRLPs (30 μg of cholesterol/ml, 48 h) followed by incubation without lipoproteins in the absence or presence of apoA1–PC (100 μg/ml); (B) [^{3}H]triolein CRLPs or oxCRLPs (30 μg of cholesterol/ml, 48 h)**

Data are expressed as a percentage of the total radioactivity in the cells at zero time (i.e. the end of the loading period) and are the means ± S.E.M. for three separate experiments.

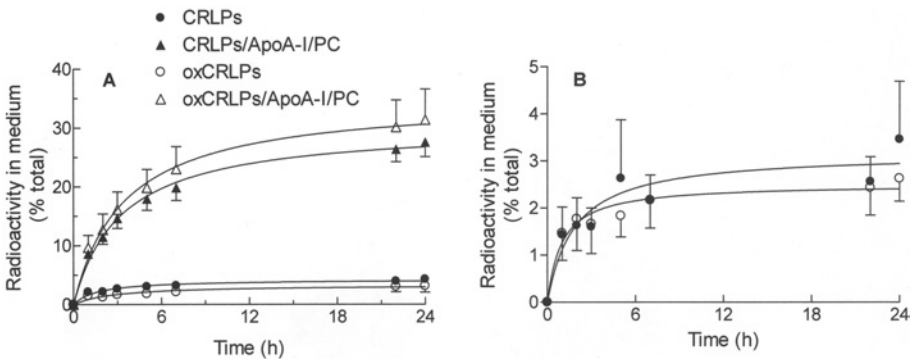
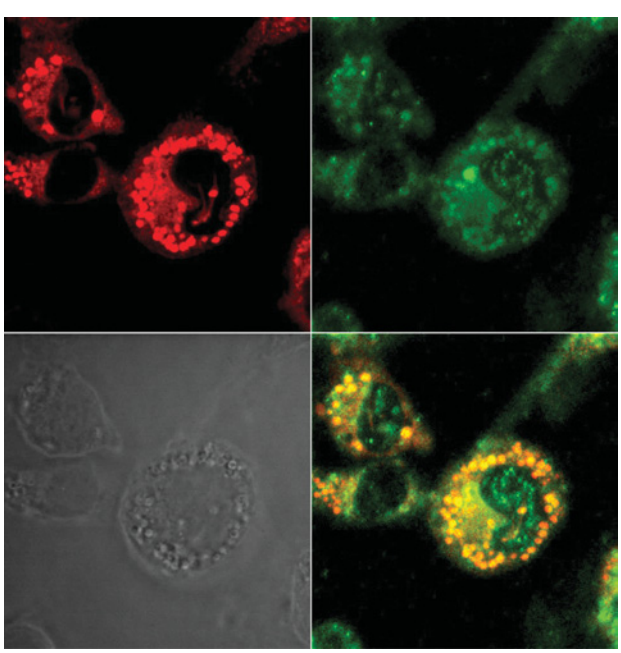


Figure 3 | **THP-1 macrophages were incubated with CRLPs labelled with 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate (DiI) (red fluorescence) (15 μg of cholesterol/ml) (48 h), then treated with mouse anti-LAMP-1 (lysosomal-associated membrane protein-1) followed by FITC-labelled goat anti-mouse immunoglobulin G (green fluorescence) and viewed using confocal microscopy**

The bottom left panel shows the cells only, the top left shows the DiI fluorescence, the top right the FITC fluorescence and the bottom right the DiI and FITC fluorescence merged. The yellow colour indicates co-localization of LAMP-1 and CRLP-derived lipid.



in comparison with those enriched with MUFAs or PUFAs [34]. Further investigations have shown that, after uptake by macrophages, lipid from CMRs, like that from oxLDL, is resistant to efflux, and that this may be due to its sequestration in lysosomes [37]. Overall, these findings demonstrate that CMRs induce pro-atherogenic changes in macrophages by mechanisms which differ markedly from those triggered by oxLDL, and suggest that these changes, and thus the direct atherogenic effects of CMRs, may be modulated by the type of fat as well as the content of oxidized lipids and antioxidants in the diet.

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