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Macrophage proteases can modify low density lipoproteins to increase their uptake by macrophages

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When low density lipoprotein (LDL) was incubated with sonicated macrophages at acidic pH, its protein moiety was partially degraded by cathepsins B and D. The reisolated LDL was taken up by intact macrophages up to about 20 times as fast as control LDL. LDL proteolysis and its enhanced uptake could be inhibited almost entirely by the selective protease inhibitors leupeptin and pepstatin. If macrophages in atherosclerotic lesions were to release acidic proteases (either by exocytosis or following cell death) and these were to modify LDL, this may help to explain why so much cholesteryl ester accumulates in these cells.

Atherosclerosis; Low density lipoprotein; Apolipoprotein B-100; Proteolysis; Cathepsin B; Cathepsin D; Macrophage

1. INTRODUCTION

Large-scale cholesteryl ester accumulation within macrophages is a prominent feature of atherosclerotic lesions. This is believed to be derived from low density lipoproteins (LDL) in humans, but macrophages take up normal LDL only slowly. LDL oxidised by arterial endothelial [1] or smooth muscle cells [2] or by macrophages [3,4], however, is taken up more rapidly by macrophages by their scavenger receptors [5]. We report here another modification of LDL that can greatly increase its uptake by macrophages. When the protein moiety of LDL, apolipoprotein B-100, was partially degraded by acidic proteases from macrophages, the LDL was taken up by intact macrophages much faster than control LDL.

2. MATERIALS AND METHODS

Mouse resident peritoneal macrophages were isolated from female T.O. mice, 20–25 g, and cultured overnight [6]. They were washed 3 times and scraped off the dishes with a 'rubber policeman' in 300 mM sucrose/1 mM Tris/1 mM Na_2EDTA/gentamicin (50 μg/ml) (pH 7.4) and sonicated for 15 s [6]. The protein content of the sonicate was determined [7] and it was diluted to 200 μg protein/ml with the above buffer.

This was incubated at 37°C for 4–48 h with an equal volume of ^{125}I-labelled LDL (200 μg protein/ml) in 200 mM sodium acetate buffer, pH 4.5, containing 1 mM Na_2EDTA and 5 mM dithiothreitol. The LDL (1.019–1.063 g/ml) was isolated from normal human blood and radioiodinated [6] and discarded after about 1 month. For the control, ^{125}I-labelled LDL was incubated with the appropriate buffers without the sonicate. At the end of the incubation, small samples were taken for the determination of the noniodide trichloroacetic acid-soluble degradation products [6].

The LDL was reisolated by adjusting its density to 1.1 g/ml with solid KBr [8] and centrifuging it at $108\,000 \times g_{av}$ for 18 h at 10°C. It was removed from the top of the tube and dialysed against several changes of 154 mM NaCl/16.7 mM NaH_2PO_4/21.1 mM Na_2HPO_4/100 μM Na_2EDTA (pH 7.4). Its protein content and radioactivity were determined.

The LDL was incubated for 24 h at 20 μg protein/ml (unless otherwise specified) with intact macrophages plated at 10^6 peritoneal cells per 22.6 mm well (Costar) or with cell-free wells. The culture medium (500 μl per well) was Dulbecco's modified Eagle's medium containing foetal calf serum (10%, v/v) and gentamicin (50 μg/ml) [6]. The noniodide acid-soluble degradation products appearing in the medium were determined and expressed per mg of cell protein [4]. The determined specific activities of the reisolated ^{125}I-labelled LDL were used to calculate their rate of degradation by the intact macrophages.

The protease inhibitors pepstatin A and leupeptin were obtained from Sigma.

3. RESULTS

Mouse resident peritoneal macrophages in culture were sonicated and incubated at pH 4.5 for 4 or 24 h with human ^{125}I-labelled LDL. The LDL was then reisolated by ultracentrifugation and incubated with intact macrophages and its degradation by them measured as an estimate of its rate of uptake [9] (Fig. 1).

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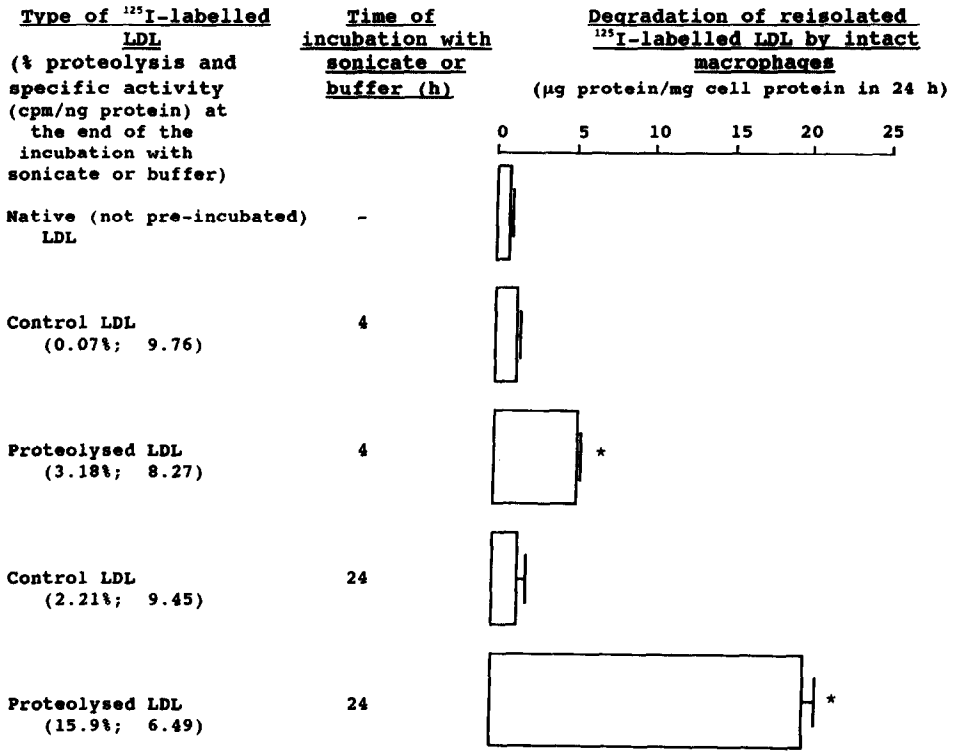

Fig. 1. Increased uptake of proteolysed LDL by macrophages. ^{125}I-Labelled LDL was incubated with a macrophage sonicate at pH 4.5, reisolated and its rate of degradation by intact macrophages determined as a measure of its rate of uptake. The mean ± SE for 4 wells of cells is shown. *$P<0.001$ compared to the relevant control (t-test).

There was a large increase in the rate of degradation of the partially proteolysed LDL by the intact macrophages and this depended on the time of incubation with the macrophage sonicate and therefore on its extent of proteolysis. The specific activity of the LDL declined progressively during its incubation with the sonicate, presumably due to the loss from the LDL particles of peptide fragments with a relatively high specific activity.

The proteolysis of LDL in the sonicates and its increased uptake by macrophages could be inhibited to a large extent by adding the protease inhibitors leupeptin and pepstatin A to the sonicate (Table I). It should be noted that EDTA was present during both the incubation of the LDL with the sonicates and its reisolation and this should have reduced any oxidation of the LDL [4,10].

Untreated ^{125}I-labelled LDL was added to the buffer

Table I

Effect of protease inhibitors on the modification of LDL by macrophage sonicates

Incubation mixture at pH 4.5		% proteolysis of ^{125}I-labelled LDL at pH 4.5	Specific activity of ^{125}I-labelled LDL (cpm/ng protein)	Degradation of reisolated ^{125}I-labelled LDL by intact macrophages (μg protein/mg cell protein in 24 h)	
Macrophage sonicate	Leupeptin plus pepstatin				
−	−	0.26	7.46	6.41 ± 0.04	
+	−	12.8	6.05	17.9 ± 0.35	$P<0.001$
−	+	0.16	7.52	4.24 ± 0.09	
+	+	0.74	6.82	6.79 ± 0.19	

^{125}I-labelled LDL was incubated for 24 h at 37°C with buffer or a macrophage sonicate, with or without leupeptin (35 μM) plus pepstatin A (5 μM). It was then reisolated and added to intact macrophages and its rate of degradation by them determined. The mean ± SE for 3 wells of macrophages is shown. There was a significant difference between the values indicated as assessed by t-test. In another experiment, not shown here, proteolysed LDL produced by 48 h incubation with a macrophage sonicate was degraded by intact macrophages 21 times as fast as control LDL. This increased degradation by intact macrophages was reduced by about 90% by adding leupeptin and pepstatin to the sonicates.

of pH 4.5 or to the macrophage sonicate in buffer, either with or without leupeptin plus pepstatin, and then immediately reisolated by ultracentrifugation followed by dialysis. The degradation of this reisolated LDL by intact macrophages was similar to that of native LDL. Another control that was carried out consisted of incubating at 37°C a macrophage sonicate in buffer of pH 4.5, in the absence of LDL, and then putting it through the reisolation procedure for LDL. The 'phantom' LDL fraction that was obtained did not affect the rate of degradation of native ^{125}I-labelled LDL by intact macrophages.

The degradation of proteolysed LDL by the intact cells was directly proportional to its concentration up to at least 50 μg protein/ml, the highest concentration tested (Fig. 2). The scavenger receptor did not appear to be involved in its uptake, as the degradation by intact macrophages of proteolysed ^{125}I-labelled LDL at 2.5 μg protein/ml was not reduced significantly by a large excess of nonlabelled acetylated LDL (100 μg protein/ml). The same batch of nonlabelled acetylated LDL, however, decreased the degradation of acetylated ^{125}I-labelled LDL by about 90% (data not shown). A large excess of nonlabelled native LDL also did not compete for the degradation by intact macrophages of proteolysed ^{125}I-labelled LDL.

Proteolysed LDL was degraded by intact cultured smooth muscle cells from pig aortas [11] somewhat

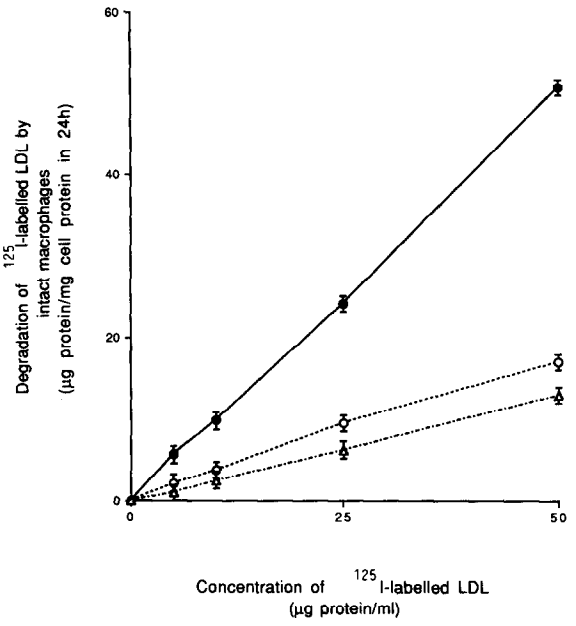

Fig. 2. Effect of proteolysed LDL concentration on its uptake by intact macrophages. ^{125}I-Labelled LDL was incubated for 48 h with (●) or without (○) a macrophage sonicate. After the incubation, 8.36% and 0.59%, respectively, of the radioactivity was present in a noniodide acid-soluble form. These LDLs were then reisolated and together with native (nonincubated) LDL (△) incubated for 24 h at various concentrations with intact macrophages and their rates of degradation determined. Each point is the mean ± SE for 3 wells of cells. Similar results were obtained in a separate experiment.

faster than control LDL but its rate of degradation per mg of cell protein was much less than that for the macrophages (results not shown).

4. DISCUSSION

We have shown that acidic proteases from macrophages can modify LDL so that intact macrophages will take it up much faster. The increased uptake of LDL was due to its modification by proteases, as its proteolysis in the sonicates and its increased uptake by macrophages could be almost entirely prevented by the protease inhibitors leupeptin and pepstatin. This agrees with our previous finding that these two inhibitors together almost entirely inhibit LDL proteolysis by macrophage sonicates [6], presumably due to the inhibition of lysosomal cathepsin B by leupeptin and cathepsin D by pepstatin.

The uptake of proteolysed LDL by intact macrophages did not show any evidence of saturation, at least up to 50 μg of protein/ml, the highest concentration tested. Macrophages contain active scavenger receptors, which have very recently been cloned and sequenced [5] and which recognise various ligands including oxidised LDL and acetylated LDL. These do not, however, appear to be involved in the uptake of proteolysed LDL as the degradation of labelled proteolysed LDL by intact macrophages was not competed for significantly by a large excess of nonlabelled acetylated LDL. The reason for the increased uptake of proteolysed LDL by macrophages is therefore unknown.

Previous studies have shown that LDL can be acted upon by pepsin to increase its uptake by mouse peritoneal macrophages [12] and trypsin-treated LDL has been shown to stimulate cholesteryl ester synthesis in these cells more than native LDL [13]. LDL treated with trypsin is taken up faster by fibroblasts deficient in LDL receptors [14] and binds more to heparin [15] and aortic glycosaminoglycans [16]. Mast cell granules have been shown to contain two neutral proteases, chymase and carboxypeptidase A, which partially degrade apolipoprotein B-100 and increase its binding to the granules [17]. When the granules are phagocytosed by macrophages, this leads to the increased uptake of LDL by these cells. Polymorphonuclear leukocytes release elastase which can partially degrade LDL, cause it to form dimers and increase its rate of uptake by human monocyte-derived macrophages by their LDL receptors [18]. In addition, mouse peritoneal macrophages have been shown to modify β-migrating very low density lipoproteins so that they induce increased cholesteryl ester accumulation in arterial smooth muscle cells, possibly by secreting a serine protease that acts on these lipoproteins [19].

The results presented here raise the possibility that LDL partially degraded by acidic proteases from macrophages in atherosclerotic lesions may be taken up

more rapidly in macrophages and contribute to their conversion into cholesterol-laden foam cells. The lysosomal proteases could either be exocytosed by the macrophages or released into the extracellular space when the macrophages die and lyse, which is common in the more advanced lesions. The lysosomal proteases would only be active at an acidic pH but atherosclerotic lesions may well be somewhat acidic as they are analogous in some ways to chronic inflammation and the pH is low in inflammatory sites [20]. In the deeper layers of larger lesions the cells may be in a somewhat hypoxic environment [21] and may release lactic acid. Also the pH near the surface of activated macrophages can drop to as low as 3.6 [22]. Another possibility is that LDL may be internalised by macrophages and delivered to the lysosomes or to an acidic prelysosomal compartment containing cathepsins [23], be partially degraded there and then released from the cells back into the extracellular space.

Thus it is possible that LDL may be modified locally in atherosclerotic lesions not only by oxidation but also by partial proteolysis by macrophage proteases so that it is taken up faster by macrophages.

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