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Treatment of macrophages with oxidized low-density lipoprotein increases their intracellular glutathione content

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Macrophages derived from the human monocyte cell line THP-1 or isolated from the peritoneum of C3H/HEJ mice were incubated with oxidized low-density lipoprotein (LDL) and the total glutathione content (oxidized plus reduced) was measured. An initial depletion of glutathione was followed by an increase, such that after a period of 24 h the glutathione content had approximately doubled. This response required the oxidation of the lipid phase of the LDL molecule, since both native LDL and acetylated LDL had little effect on glutathione levels. The response of the cells to oxidized LDL was dependent on the extent of oxidative modification of the protein. It was also found that 4-hydroxynonenal had a similar effect on THP-1 cells, and we suggest that this or other aldehydes present in oxidized LDL causes the induction of glutathione synthesis in response to an initial oxidative stress and consequent glutathione depletion. In addition, we found that both cell types possess transferases and peroxidases capable of detoxifying aldehydes and peroxides. However, treatment of cells with oxidized LDL or 4-hydroxynonenal for a period of 24 h had no effect on the activities of these enzymes.

INTRODUCTION

The formation of macrophage-derived foam cells in the artery wall is associated with the pathogenesis of atherosclerosis [1,2]. Foam cells arise through lipid loading of macrophages, and the essential biochemical changes which underlie this process are reasonably well understood. The initial step is a chemical modification of low-density lipoprotein (LDL) to form a particle more electronegative than native LDL, so rendering the molecule an avid ligand for the macrophage scavenger receptor [3,4]. For this mechanism to be of relevance to the pathogenesis of atherosclerosis, it must be postulated that such a modification of LDL occurs in the artery wall. The present consensus of opinion is that this arises through oxidation of the lipids within the LDL particle [2,4]. The subsequent decomposition of lipid peroxidation products has been shown to result in the modification of the lysine residues of the apo B lipoprotein, so rendering the particle more electronegative and thus a ligand for the scavenger receptor [4–6]. In support of this hypothesis, it has been shown that antibodies raised to malondialdehyde and 4-hydroxynonenal (HNE), which are both produced during the oxidation of LDL, cross-react with material in the atherosclerotic lesions of both man and Watanabe rabbits [7]. In addition, some of the biological responses of various cell types to oxidized LDL have been established. For example, it has been demonstrated that oxidized LDL inhibits the expression of the genes coding for the cytokines interleukin-I and tumour necrosis factor in isolated macrophages [8]. It has also been shown that oxidized LDL is cytotoxic to fibroblasts and endothelial cells, and can induce smooth muscle cell proliferation [9,10]. These findings suggest that uptake by the macrophage scavenger receptor may provide a detoxification pathway for oxidized LDL and so protect other cells in the artery wall.

Oxidized LDL contains high concentrations of lipid peroxidation products such as peroxides and aldehydes, which are known to be cytotoxic [9,11]. Since the macrophage is able to bind and sequester this form of modified LDL, then it is likely that it is subjected to a severe oxidative stress. This form of toxic insult can result in cell death if the cells' antioxidant defences are overwhelmed [10]. It is therefore of some interest to know how the macrophage responds to the oxidative challenge presented by oxidized LDL.

The principal detoxification pathway for lipid peroxides is through the glutathione peroxidases. These enzymes require reduced glutathione (GSH) as a cofactor and produce oxidized glutathione, which can be re-reduced by the enzyme glutathione reductase at the expense of the oxidation of NADPH [12]. There are two forms of selenium-dependent glutathione peroxidases, which have different substrate specificities: GPX, which recognizes fatty acid peroxides, and PHGPX, which recognizes phospholipid hydroperoxides [12]. The latter form of the enzyme is particularly relevant to the metabolism of oxidized LDL, since unlike GPX it can detoxify cholesterol hydroperoxides [12]. In addition, oxidized LDL contains a number of different aldehydes which can be detoxified by glutathione *S*-transferases [11,13]. It is likely, therefore, that these glutathione-dependent pathways play a critical role in the metabolism of oxidized LDL. In fact, it has already been shown that endothelial cells, which are depleted of glutathione, are more sensitive to the toxic effects of oxidized LDL, and that glutathione oxidation precedes cell death [10].

In the present study we have investigated the effects of native LDL, oxidized LDL and acetylated LDL on the glutathione-dependent antioxidant defences of macrophages exposed to LDL. Of particular interest to us were the effects of modified forms of LDL on the glutathione content of the cells and on the activities of glutathione-dependent enzymes such as glutathione peroxidase and glutathione transferases. In addition, we have examined the effect of the specific aldehyde HNE, a lipid peroxidation product formed during the oxidation of LDL, on glutathione levels in macrophages.

Abbreviations used: LDL, low-density lipoprotein; PBS, phosphate-buffered saline; TBARS, thiobarbituric-acid-reactive substances; HNE, 4-hydroxynonenal; DMEM, Dulbecco's modified Eagle's medium.

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MATERIALS AND METHODS

Isolation, modification and characterization of LDL

Human LDL was isolated from plasma by differential centrifugation using the method described in [14]. After dialysis against Ca^{2+} free phosphate-buffered saline (PBS) containing 10 μM-EDTA the LDL was sterilized by filtration through a 0.22 μm pore-size filter and stored at 4 °C before use. The protein concentration was measured using the BCA protein assay reagent supplied by Pierce. Oxidation was initiated in 1 mg/ml solutions of LDL by the addition of 100 μM-$CuSO_4$ followed by incubation at 37 °C for various periods of time [11]. Acetylation of LDL was performed by diluting LDL to 2 mg/ml with sterile saline and then to 1 mg/ml with saturated sodium acetate. Acetic anhydride (10 μl of a 1 M solution) was added to the LDL solution with gentle stirring at 4 °C in 5 × 2 μl aliquots over a period of 1 h [15]. The sample was then dialysed extensively against the buffer used above for the isolation of LDL from plasma. The modified forms of LDL were characterized by the following procedures. (1) Measurement of the electrophoretic mobility of modified LDL relative to native LDL on agarose gels using the Beckman paragon electrophoresis system. (2) Measurement of thiobarbituric-acid-reactive substances (TBARS) using the spectrophotometric method described in [16]. We found that oxidized LDL was precipitated during this assay, and in order to correct for the consequent light scatter the absorbance spectrum between 500 and 600 nm was taken and the value for maximum absorbance at 532 nm was adjusted. The resulting TBARS values were converted to malondialdehyde equivalents by reference to a standard curve prepared by the hydrolysis of known amounts of tetraethoxypropane [16]. We have performed this calculation in order that our data may be compared with those from other laboratories, but we recognize that aldehydes other than malondialdehyde may give rise to a chromophore absorbing at 532 nm. (3) Measurement of the lipid hydroperoxide content using the lipid-peroxide-dependent oxidation of iodide to iodine as described in [17].

Isolation of macrophages and treatment with LDL or HNE

Peritoneal macrophages were isolated from the endotoxin-resistant strain of mice, C3H/HEJ by lavage with PBS [8]. After centrifugation (500 *g* for 5 min), cells were resuspended in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum, 2 mM-glutamine and 50 μg of gentamicin/ml to a concentration of approx. 2×10^6/ml and placed in 1 ml volumes in a 24 well plate. Cells were allowed to adhere for 2–3 h before the medium was changed and the incubation with LDL started. The human monocyte cell line THP-I was also prepared for incubation with LDL by seeding cells into wells at a concentration of 1×10^6 cells/ml in the presence of 10 ng of phorbol myristate acetate/ml and incubating at 37 °C for 3–5 days [18]. After this period, the cells had adhered to the plate and were treated in an identical fashion to the peritoneal macrophages isolated from the C3H/HEJ mice. Modified LDL was prepared as described above at a concentration of 1 mg/ml and diluted to give a final concentration of 100 μg/ml with medium before adding to the cells. Control medium contained PBS and, where appropriate, copper at the same concentration as that used to oxidize LDL. The cells were then incubated for 1–24 h with LDL before washing with PBS and lysing with 200–300 μl of 0.1% Triton X-100/PBS. The samples were kept on ice before assay. For the glutathione transferase assays, adherent cells were recovered from culture plates by scraping into PBS and then lysed by three cycles of freeze–thawing in liquid nitrogen.

HNE was generously provided by Professor H. Esterbauer, University of Graz, Austria, and was supplied as HNE-diethylacetal, from which HNE was prepared by incubation for 1 h in 1 mM-HCl. The final concentration of HNE was determined from its u.v. spectrum using a molar absorption coefficient of 13.75 mM·cm^{-1} at 223 nm. Typically, a stock solution of 10 mM was prepared which was then diluted into medium before adding it to the cells. Control solutions were prepared in identical fashion but without HNE.

Assay of glutathione, glutathione peroxidase and glutathione transferase

Glutathione was measured spectrophotometrically as the sum of both its oxidized and reduced forms as described in [19]. Glutathione peroxidase was assayed in the Triton X-100 cell lysates by monitoring the oxidation of NADPH with butyl hydroperoxide as a substrate in the presence of 2 mM-glutathione [12]. Glutathione transferases were assayed by measurement of the conjugation of glutathione (2 mM) with 1-chloro-2,4-dinitrobenzene as described in [13].

RESULTS

Preparation and characterization of native and modified forms of LDL

Human LDL was modified by acetic anhydride [15] and copper [11] to produce the acetylated and oxidized forms respectively of LDL. The different forms of LDL were then characterized by measuring their electrophoretic mobility on agarose gels relative to that of native LDL, lipid peroxide content [17] and levels of TBARS [16,17]. As shown in Table 1, native LDL contains very low levels of lipid hydroperoxides and TBARS, which are not significantly increased during the acetylation reaction. Furthermore, by incubating acetylated LDL with copper we could demonstrate that the maximal concentration of conjugated diene formed was similar to that found with native LDL (Table 1). In contrast, LDL incubated with copper showed evidence of both extensive lipid peroxidation and modification of the protein. The extent of these changes was dependent on the length of the incubation period with copper, as

Table 1. Characterization of native and modified LDL

LDL was used as prepared (native) or was modified by acetylation or by incubation for the times shown with $CuSO_4$. Aliquots were taken and the lipid peroxide content was measured, and TBARS and the mobility relative to native LDL on agarose gels (REM) were determined. Results are expressed as nmol of malondialdehyde or peroxide/mg of LDL protein. To determine the conjugated diene formation during the copper-initiated peroxidation of LDL, 10 μM-$CuSO_4$ was added to a solution of LDL (125 μg/ml) and the maximal absorbance at 234 nm was determined during the course of the peroxidation reaction. The conjugated diene content was determined as described in [17] and is expressed as nmol/mg of LDL protein. n.d., not determined. All results are shown as the means ± S.D., where numbers in parentheses represent the numbers of separate determinations on the same preparation of LDL.

Sample	Peroxides (nmol/mg)	TBARS (nmol/mg)	REM	Conjugated diene (nmol/mg)
Native	22 ± 4 (6)	None	1.0	446 ± 22 (4)
Acetylated	22 ± 5 (3)	None	4.9–5.0	480 ± 14 (3)
Oxidized				
2 h	395 ± 36 (3)	4.2 ± 0.2	1.6 ± 0.1	n.d.
4 h	236 ± 10 (3)	5.1	2.0 ± 0.1	n.d.
24 h	11 ± 9 (3)	2.2	3.0 ± 0.1	n.d.
48 h	8 ± 11 (3)	2.47 ± 0.08	3.2 ± 0.15	n.d.

Table 2. Glutathione content of mouse peritoneal macrophages and THP-1 cells after treatment with oxidized or native LDL

Adherent mouse peritoneal macrophages or THP-1 cells [(0.8–1) × 10^6/well] were incubated with 10 μM-$CuSO_4$ (control), native LDL (100 μg/ml) [relative electrophoretic mobility (REM) = 1], oxidized LDL (100 μg/ml) (REM = 5.0) or acetylated LDL (100 μg/ml) (REM = 4.9). After incubation at 37 °C for 24 h, cells were washed and then lysed in 0.1% Triton X-100. The total glutathione content was measured. The results are reported as means ± S.E.M. for three separate wells for each condition. * Significantly different from control, $P < 0.0005$, † significantly different from native LDL, $P < 0.0125$, †† $P < 0.0025$, as assessed by Student's t test. n.d., not determined.

	Total GSH (nmol/mg)	
Treatment	THP-1 cells	C3H/HEJ
Control	86 ± 4	28.3 ± 1.4
Native LDL	98 ± 17	36.7 ± 3.7
Oxidized LDL	171 ± 13††	52.7 ± 2.4*†
Acetylated LDL	n.d.	34 ± 4.0

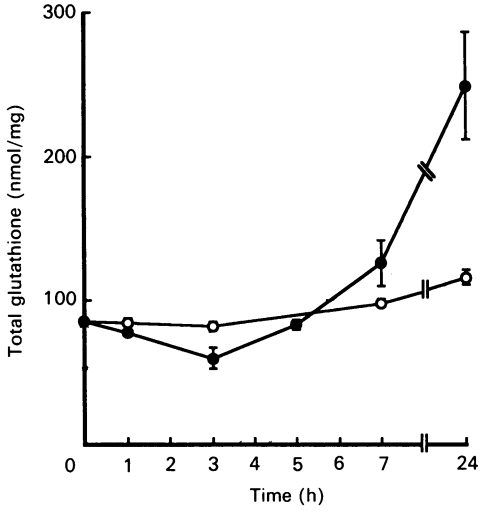

Fig. 1. Effect of oxidized LDL on the glutathione content of THP-1 cells

Adherent THP-1 cells were incubated for the time periods shown with either 10 μM-$CuSO_4$ (○) or 100 μg of oxidized LDL/ml (●; REM = 5.0) before washing and preparation of a cell lysate, after which the glutathione content was measured. The results are expressed as nmol of total glutathione/mg of protein in the cell lysate, and are shown as means ± S.D. for single determinations from three separate wells incubated under the same conditions.

reported in Table 1. For example, the concentration of lipid hydroperoxides increased rapidly within the first 2 h and then decreased, whereas the electrophoretic mobility showed a progressive and consistent increase with time. The data reported in Table 1 were derived from experiments with a single preparation of LDL, and we found that the extent of oxidative modification after a fixed period of incubation with copper varied from one donor to another. For example, after 48 h of oxidation with copper, under the conditions described in Table 1, we have found that the electrophoretic mobility varied from 3 to greater than 6. These results are in good agreement with those from other laboratories [11,20,21], and demonstrate that oxidized LDL is not a homogeneous chemical species, raising the possibility of heterogeneity in the biological responses it may elicit. In all of the experiments described in this study we have defined the extent of oxidative modification of LDL by the electrophoretic mobility relative to that of native LDL. Acetylated LDL, in contrast with oxidized LDL, showed no evidence of extensive lipid peroxidation, despite the fact it was distinctly more electronegative, as indicated by its high electrophoretic mobility relative to that of native LDL (Table 1). It is well established that this degree of modification of charge on the LDL molecule results in recognition by the macrophage scavenger receptor and the formation of foam cells [4,22].

Effect of modified LDL on the glutathione content of THP-1 cells and mouse peritoneal macrophages

Macrophages isolated either from the endotoxin-resistant mouse strain C3H/HEJ or from the human monocyte cell line THP-1 were allowed to adhere to the culture plates before being exposed to the modified or native forms of LDL at a concentration of 100 μg/ml for a period of 24 h. In these experiments the modified forms of LDL all had relative electrophoretic mobilities of between 4.9 and 5.1. After this period the cells were washed and then lysed with 0.1% Triton X-100 before measurement of their total glutathione (reduced plus oxidized) content. With both cell types we found that treatment with native LDL had little effect on cellular glutathione levels (Table 2). The glutathione contents in the two cell types were quite different, with the levels in the THP-1 cells being more than double those in cells isolated from the mouse peritoneum. We have no explanation for the differences in the basal level of this antioxidant. The response of macrophages to acetylated LDL was investigated only in the mouse peritoneal macrophages, and was found to be similar to the response to native LDL in that there was little effect on glutathione levels. In contrast, oxidized LDL elicited a large increase in glutathione content in both cell types (Table 2).

In the second series of experiments we investigated the time course of change in total glutathione content during exposure to oxidized LDL in THP-1 cells. The results shown in Fig. 1 demonstrate that, after 3 h of exposure, there was a significant decrease in the total cellular glutathione content, but this had almost recovered to control levels after a period of 4 h. After 24 h, glutathione levels in the cells treated with oxidized LDL were almost double those in the controls. Moreover, there was no decrease in the total protein content of the lysed cells after treatment with modified LDL nor any visible detachment of cells from the culture dish. We conclude that no significant cell death had occurred as a result of the exposure of cells to oxidized LDL.

Extent of the oxidative modification of LDL and its effects on glutathione levels

As we have discussed previously, it is clear that the chemical composition of oxidized LDL is highly variable depending on the extent of the peroxidation reaction (Table 1 and [11]). To determine whether the degree of oxidative modification of LDL had a significant effect on its ability to elicit an increase in the cellular glutathione content, we incubated mouse peritoneal macrophages with oxidized LDL of various electrophoretic mobilities (range 4.8–6.0) for a period of 24 h. As shown in Fig. 2, we found that the glutathione content increased as a linear function of the relative electrophoretic mobility.

Effect of HNE on cellular glutathione levels

It has been reported previously that HNE can induce the synthesis of glutathione in fibroblasts [23] and, since HNE is formed during the peroxidation of LDL, we examined its effects on glutathione levels in THP-1 cells. Cells were incubated with 50 μM-HNE for the periods of time shown in Fig. 3 before

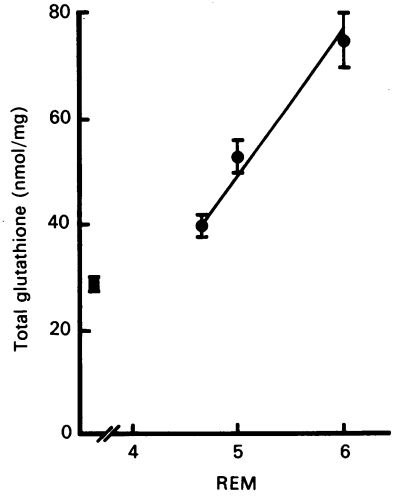

Fig. 2. Effect of oxidized LDL of different electrophoretic mobilities on the glutathione content of macrophages isolated from C3H/HEJ mice

Adherent peritoneal macrophages were incubated with 100 μg of oxidized LDL/ml of the electrophoretic mobilities shown for a period of 24 h. After washing and preparation of the cell lysate, the glutathione content was measured and is shown as a function of the electrophoretic mobility relative to that of native LDL (REM). Results are shown as means ± S.D. for single determinations of three separate wells incubated under the same conditions and are expressed as nmol of total glutathione/mg of protein in the cell lysate. The control value for incubation of cells in the absence of LDL is shown before the discontinuity in the REM axis.

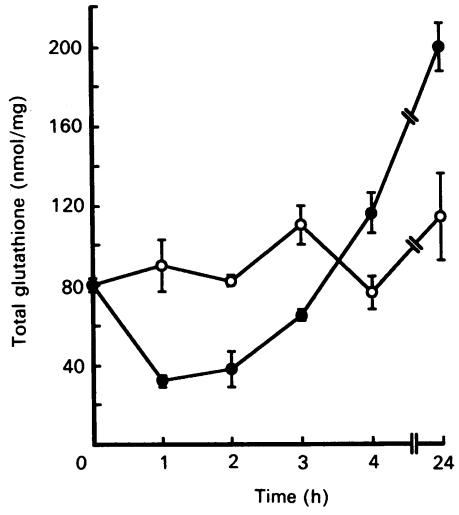

Fig. 3. Effect of HNE on the glutathione content of THP-1 cells

Adherent cells were incubated with either 50 μM-HNE (●) or the same concentration of HCl (○) for the time periods shown before washing, preparation of the cell lysate and determination of the glutathione content. Results are shown as means ± S.D. for separate determinations of three wells prepared under identical conditions and are expressed as nmol of total glutathione/mg of protein in the cell lysate.

isolation and measurement of their glutathione content. As can be seen from Fig. 3, exposure of the cells to this aldehyde resulted in a severe decrease in glutathione content over the first 2 h, after which levels began to recover. At 24 h after the start of the experiment the glutathione content had approximately doubled. However, in these experiments, and in contrast with the effects of oxidized LDL, some cells detached from the culture dish, and the yield of protein from the cell lysate was significantly decreased when compared with controls. Despite these overt toxic effects, the data in Fig. 3, which are normalized to the protein content of the cell lysate, suggest that HNE can induce the synthesis of glutathione in these cells, as was previously reported for fibroblasts [23].

Table 3. Activity of glutathione peroxidase and glutathione transferase in THP-1 cells and C3H/HEJ macrophages treated with LDL

Glutathione peroxidase (GPX) and glutathione transferase (GST) activities were measured in cell lysates prepared from both THP-1 cells and peritoneal macrophages isolated from C3H/HEJ mice. The results are expressed as units of activity/mg of protein in the cell lysate and are shown as means ± S.D. for single measurements from separate wells. n.d., not determined.

	Activity (units/mg)			
	THP-1 cells		C3H/HEJ	
Treatment	GPX	GST	GPX	GST
None	1.26±0.17	0.053±0.018	2.66±0.08	0.38±0.09
Native LDL	1.18±0.30	0.058±0.005	2.44±0.75	0.523±0.203
Oxidized LDL	1.08±0.07	0.051±0.009	2.32±0.08	0.426±0.117
Aceylated LDL	n.d.	n.d.	2.30±0.08	n.d.

Effect of oxidized, acetylated and native LDL on glutathione peroxidases and glutathione *S*-transferases

The activities of glutathione peroxidase in both cell types were high but were not affected by incubation with either modified or unmodified forms of LDL (Table 3). LDL oxidized for 1 h (200 μg/ml) and containing a high level of lipid hydroperoxides (200–300 nmol of peroxide/mg of LDL) could act as a substrate for the peroxidases present in the cell lysate, with an activity of between 0.26 and 0.42 units/mg of protein. This activity was not evident if LDL which had been oxidized for longer periods was used. Neither acetylated nor native LDL was able to support the glutathione-dependent oxidation of NADPH. In a similar fashion, no significant effect on glutathione *S*-transferase levels was found after incubation with either oxidized or native LDL (Table 3).

DISCUSSION

The formation of oxidized LDL in the artery wall is thought to be one of the key events in the pathogenesis of atherosclerosis [2,4]. Indeed, oxidized LDL appears to have a number of biological properties, such as toxicity to endothelial cells, which could contribute to the development of the atherosclerotic lesion [2,10]. The involvement of macrophages in atherosclerosis is one of continuing speculation and encompasses two contrasting aspects. It is clear that the macrophage has the potential to remove oxidized LDL from the artery wall through the scavenger receptor and through other uptake mechanisms which appear to operate specifically for this modified form of LDL [4,6]. In this sense the macrophage has a protective role. On the other hand, it is likely that macrophage-derived foam cells are important in the initial stages of atherosclerosis and that these cells may indeed initiate the oxidation of LDL [24,25]. Clearly in this case the macrophage may actively contribute to the progression of the disease. In the present study, we have examined the response of macrophages *in vitro* to treatment with oxidized LDL, with specific reference to the antioxidant status of the cells. Of key

importance in the detoxification of lipid-derived oxidation products is glutathione, which has the dual role of acting as a substrate for both peroxidases and transferases.

We have used two cell types in this study, the human monocyte cell line THP-1 and macrophages isolated by peritoneal lavage from the endotoxin-resistant C3H/HEJ strain of mice. Incubation of the THP-1 cells with phorbol myristate acetate induces the cells to adhere to the culture dish, express the scavenger receptor and adopt the morphology of the macrophage [18]. The initial response of the THP-1 cells on exposure to oxidized LDL was a decrease in the total glutathione content. As there is no evidence of cell lysis under these conditions, it is likely that this fall in glutathione occurs as a result of its oxidation or conjugation with aldehydes and export from the cell. This effect has been observed in many other cell types and organs which have been subjected to oxidative stress [23,25,26]. After a relatively short period of time, the glutathione levels began to recover and after a 24 h period they had approximately doubled. It is most likely that these elevated levels are the result of *de novo* synthesis, although the present experiments do not address this point. Since incubation of C3H/HEJ-derived macrophages with oxidized LDL also resulted in the elevation of cellular glutathione levels, we conclude that endotoxin, which can contaminate some LDL preparations [8], has little or no role to play in this process. Incubation of the cells with native LDL had little or no effect on glutathione levels in either cell type (Table 2). It is unlikely that the LDL has been oxidatively modified by incubation with macrophages, since for this to occur LDL must be incubated in serum-deficient medium containing trace amounts of transition metals. The medium used in this study, DMEM, was serum-replete, and when serum-deficient does not support the macrophage-dependent oxidation of LDL [11]. Native LDL is not recognized by the macrophage scavenger receptor and will not be taken up to any great extent by the cells. To test whether LDL with a normal lipid composition, but in a form that can act as a ligand for the scavenger receptor, could affect glutathione levels, experiments were performed with acetylated LDL. Acetylated LDL contains very low levels of oxidized lipid (Table 1) and has little effect on cellular glutathione levels, and so LDL uptake and lipid loading by the macrophage via this route is not sufficient in itself to affect glutathione levels.

We have attempted to determine the form of the oxidative stress which occurs when cells are exposed to oxidized LDL by characterizing the LDL particle after various periods of oxidation. We have found (Table 1), as have others [20,21], that oxidized LDL is a highly heterogeneous mixture of lipid and protein oxidation products. Only after extensive lipid peroxidation, accompanied by decomposition of lipid peroxides and a concomitant formation of aldehydes, has occurred, does uptake of oxidized LDL by the macrophage scavenger receptor commence [22]. Only if this highly electronegative form of oxidized LDL is used does an increase in glutathione occur on incubation with cells. This result suggests that the oxidant causing the initial depletion of glutathione is unlikely to be a lipid peroxide and raises the possibility that aldehydes, which occur as breakdown products of peroxide decomposition and whose levels increase during the peroxidation reaction, may play this role [27]. To test this hypothesis we incubated THP-1 cells with HNE, an aldehyde found in high concentrations in the oxidized LDL particle, and found that an initial severe depletion of glutathione was followed by enhanced levels after 24 h of exposure. This result is very similar to that reported previously for human fibroblast cells, in which the responses of the cells to HNE and cumene hydroperoxide were compared and found to be quite different [23]. In both cases depletion of glutathione occurs, but after treatment with peroxides oxidized glutathione is formed which is exported from the cell and is not followed by *de novo* synthesis. This is in marked contrast with the effects of treatment with HNE, where depletion of glutathione is followed by *de novo* synthesis. In the present study we did not measure the concentration of HNE in our samples of oxidized LDL, and in any event, since it is tightly bound to the LDL particle [22], it is not possible to compare the amounts in oxidized LDL with pure HNE in solution in any realistic way. The aldehyde concentration within the LDL particle has been calculated to be in the millimolar concentration range by Esterbauer and co-workers [11]. Since the macrophage actively sequesters oxidized LDL from the medium, it is not unreasonable to assume that these lipid peroxidation products may contribute a significant oxidative stress to the cell. It is interesting to note that, with both oxidized LDL and HNE, cellular glutathione levels are approximately doubled after 24 h of incubation, although the initial depletion of this antioxidant is far more severe with HNE than with oxidized LDL. Clearly there may be important differences in the response of the cell to aldehydes such as HNE when presented in solution compared with when they are bound to oxidized LDL and taken up by the macrophage scavenger receptor. The principal glutathione-dependent detoxification pathway for aldehydes in the cell is mediated by the glutathione transferases. Macrophages possess these enzymes, which can exist as a number of different isoenzymes. These cells also contain peroxidases capable of utilizing oxidized LDL as a substrate, the levels of which were not affected by treatment with oxidized LDL. This result suggests that the cells have responded to the oxidative stress caused by the uptake of oxidized LDL by synthesis of the substrate of these enzymes.

In summary, we have shown that, *in vitro*, exposure of macrophages to oxidized LDL results in an initial oxidative stress causing the depletion of glutathione, followed by re-synthesis and elevated levels of glutathione after 24 h. The component of oxidized LDL which is mediating these changes is unknown, but since HNE can elicit a similar response it is likely to be this aldehyde and others formed as a consequence of the breakdown of lipid peroxides in the LDL particle. It is possible that such processes may also occur in the artery wall, since it has recently been reported that in the atherosclerotic lesions of fat-fed rabbits, both the levels of glutathione and activity of glutathione-dependent enzymes are markedly elevated [28]. In addition, HNE and other aldehydes conjugated to proteins have been detected in the atherosclerotic lesions of Watanabe rabbits [29]. These results suggest that macrophages in the artery wall are capable of combating the oxidative challenge presented by oxidized LDL, although extended exposure to toxic agents such as HNE would be likely to result in cell death and so exacerbate the developing atherosclerotic lesion.

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