Proteoglycans in macrophages: characterization and possible role in the cellular uptake of lipoproteins

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The murine macrophage cell line J774 was incubated with [^{35}S]sulphate. The cell-associated ^{35}S-labelled macromolecules were shown to be proteoglycans and glycosaminoglycans in similar amounts. The possible presence of cell-surface proteoglycans was investigated by incubating [^{35}S]sulphate-labelled cells with trypsin for 15 min. The released material contained approx. 70 % free glycosaminoglycan chains and 30 % proteoglycans. The latter component was demonstrated by HNO_2 treatment to contain heparan sulphate. In the total cell fraction not treated with trypsin a small but significant portion was shown to be chondroitin sulphate proteoglycan. The cell-associated glycosaminoglycans contained both chondroitin sulphate and heparan sulphate. To investigate possible biological functions of cell-surface proteoglycans in macrophages, cells were incubated with $NaClO_3$ to inhibit sulphation of proteoglycans and β-D-xyloside to abrogate proteoglycan expression. The uptake of oxidized ^{125}I-tyraminylcellobiose-labelled low-density lipoprotein (^{125}I-TC-LDL) was typically two to three times higher than that of native ^{125}I-TC-LDL in untreated J774 cells. The cellular uptake at 37 °C of native ^{125}I-TC-LDL was decreased 25 % after both $NaClO_3$ and xyloside treatment, whereas the uptake of oxidized ^{125}I-TC-LDL was decreased 35 % after both types of treatment. The mRNA levels for the scavenger receptor A-II and the LDL receptor were not affected by $NaClO_3$ or xyloside treatment. Furthermore, fluid-phase endocytosis, measured as uptake of horseradish peroxidase, and receptor-mediated endocytosis, measured as uptake of ^{125}I-TC-ovalbumin, were not affected by $NaClO_3$ treatment of J774 cells. Removal of cell-surface chondroitin sulphate with chondroitinase ABC decreased only the binding of native ^{125}I-TC-LDL, whereas removal of heparan sulphate with heparitinase decreased the binding of both oxidized and native ^{125}I-TC-LDL. Addition of lipoprotein lipase increased the uptake of oxidized ^{125}I-TC-LDL 1.7 times and the uptake of native ^{125}I-TC-LDL 2.1 times. The binding of the former was more sensitive to $NaClO_3$ treatment than the latter. The results presented support the notion that some of the uptake pathways for lipoproteins in the foam-cell-forming macrophages depend on the presence of cell-surface heparan sulphate and chondroitin sulphate.

INTRODUCTION

Proteoglycans are proteins with one or several glycosaminoglycan chains covalently attached. These highly acidic macromolecules are found in extracellular matrices, in intracellular granules and on cell surfaces. They are important for several cellular functions, such as binding of ligands to cellular receptors, anchoring cells to extracellular matrices, providing substrates for cell migration, and binding proteases and protease inhibitors [1]. Proteoglycans interact with several proteins and modulate their activities. Several of the proteins known to bind proteoglycans are important in lipid metabolism, such as lipoprotein lipase (LPL), apolipoproteins B and E [2–4].

Monocytes and macrophages belong to the mononuclear-phagocyte system. These cells have important functions in antigen presentation, as phagocytes clearing the body of debris and micro-organisms, and as secreting cells releasing cytokines, enzymes and growth factors. Monocytes and macrophages are important for, e.g., killing cancer cells, antimicrobial defence, inflammatory responses and wound healing. These cells also synthesize proteoglycans. Most of the proteoglycans synthesized by macrophages are of the chondroitin sulphate type, as is typical for most blood cells [5]. Serglycin has been shown to be a secretory product of human monocytes [6] and to interact with other secreted proteins from activated macrophages [7]. Both human monocytes and macrophages express almost exclusively chondroitin sulphate proteoglycans [8,9], and only negligible amounts of proteoglycans can be recovered from the cell surface. It has been demonstrated, however, that murine macrophages synthesize both chondroitin sulphate and heparan sulphate proteoglycans [10]. Peritoneal murine macrophages have also been shown to express syndecan 1 on the cell surface following activation [11]. In addition, invariant chain has been shown to be expressed on the cell surface carrying one chondroitin sulphate chain [12,13]. It has also been demonstrated that a chondroitin sulphate proteoglycan is associated with the cell surface of a murine macrophage cell line [14].

The murine macrophage cell line J774 has the ability to take up oxidized and native LDL, and the cells may serve as a model system for studying the generation of macrophage-derived foam cells [15]. Little information is available about the expression of proteoglycan in this particular cell line. The aim of the present investigation was, first, to characterize the proteoglycans in J774 cells with focus on the species present on the cell surface and, secondly; to analyse whether cell-surface proteoglycans are involved in the cellular uptake of oxidized LDL (oxLDL). For

Abbreviations used: FCS, foetal-calf serum; DMEM, Dulbecco's modified Eagle's medium; (ox)LDL, (oxidized) low-density lipoprotein; LPDS, lipoprotein-deficient serum; ^{125}I-TC-LDL, ^{125}I-tyraminylcellobiose-labelled LDL; C-ABC, chondroitinase ABC; HX-xyl, hexyl β-D-thioxyloside; LPO, lipid peroxide; R_m, relative electrophoretic mobility; LPL, lipoprotein lipase.

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this purpose we made use of $NaClO_3$, known to inhibit sulphation of proteoglycans [16], and hexyl β-D-thioxyloside (HX-xyl), shown to interfere with the expression of chondroitin sulphate proteoglycans [17]. Our results show that both heparan sulphate proteoglycans and free chondroitin sulphate and heparan sulphate can be recovered from the cell surface of J774 cells and that a small, but significant, portion of oxLDL as well as native LDL is taken up through pathways involving these particular cell-surface components.

MATERIALS AND METHODS

Materials

Chondroitinase ABC (C-ABC, EC 4.2.2.4) and heparitinase (EC 4.2.2.8) were obtained from Seikagaku Kogyo Co., Tokyo, Japan. The heparitinase will cleave glucosaminidic linkages in heparan sulphate with *N*-acetyl- or N-sulphated glucosamines and glucuronic or iduronic acid residues. Sephadex G-50 Fine, Sepharose CL-6B, Superose 6 and DEAE-Sephacel were all from Pharmacia LKB Biotechnology, Uppsala, Sweden. Blue Dextran and 2,4-dinitrophenylalanine were from Sigma Chemical Co., St. Louis, MO, U.S.A. $NaClO_3$ was obtained from Fluka, Buchs, Switzerland. HX-xyl was a gift from Dr. S. Suzuki, Aichi Medical University, Aichi, Japan. $Na_2{}^{35}SO_4$, [^{3}H]thymidine and ^{125}I were bought from Amersham. [^{3}H]Heparin was obtained from NEN Life Science Products, Boston, MA, U.S.A. LPL was provided to us by Dr. T. Olivecrona, Department of Medical Biochemistry, University of Umeå, Umeå, Sweden.

Cells

The murine macrophage cell line J774 A1 (referred to as J774) was from the American Type Culture Collection, Rockville, MD, U.S.A. The cells were cultured in Dulbecco's modified Eagles medium (DMEM; from Bio Whittaker, Walkersville, MD, U.S.A.) with 10% fetal-calf serum (FCS) from Sigma; L-glutamine and gentamycin were from Bio Whittaker. For passage, non-adherent cells were transferred to new culture flasks in the same medium.

Proteoglycans

For labelling of proteoglycans cells were seeded in 35-mm-diameter wells and, after reaching confluency, they were washed with sulphate-depleted RPMI 1640 medium (Gibco, Paisley, Renfrewshire, Scotland, U.K.) and incubated in the same medium with 2% FCS and L-glutamine, but without gentamycin (60 μg/ml). The cells were labelled with 100 μCi/ml of [^{35}S]sulphate for 20 h, after which the medium was collected and centrifuged to remove non-adherent cells and frozen until further analysis. The adherent and non-adherent cells were extracted in 4 M guanidinium chloride with 2% Triton X-100 in sodium acetate buffer, pH 6.0. To remove free [^{35}S]sulphate, the cell extracts were subjected to Sephadex G-50 Fine gel chromatography. Material eluting in the void volume was collected and used for further analyses.

To determine whether cell-surface proteoglycans were present in J774 murine macrophages, the cells were treated with 12.5 μg/ml of trypsin in PBS with 1 mM EDTA for 15 min at 37 °C after radiolabelling for 20 h. Prior to trypsin treatment the culture medium was harvested and the cells were washed three times with PBS to remove trypsin inhibitors in serum. The trypsin-released material was collected, treated with 2% FCS to inactivate trypsin, frozen, or subjected directly to further analyses. Controls were incubated for the same period of time with PBS without trypsin.

To define the structure of [^{35}S]proteoglycans from total cell extract and from material released from the cells with trypsin, the various fractions were subjected to treatment with C-ABC (0.01 unit per incubation) in 0.05 M Tris/HCl, pH 8.0, containing 25 μg/ml of BSA and 0.025 M sodium acetate for 15 h at 37 °C. Untreated and enzyme-treated samples were compared by Sepharose CL-6B gel chromatography using Dextran Blue and 2,4-dinitrophenylalanine as markers for the void and total volume of the gel respectively. Percentage chondroitin sulphate was calculated from the amount of radioactivity eluted in the retarded fractions after enzyme treatment. Running buffer was 0.05 M Tris/HCl, pH 8.0, with 0.15 M NaCl. Parallel samples were subjected to HNO_2 treatment at pH 1.5 [18], and analysed by Sepharose CL-6B gel chromatography. The amount of heparan sulphate was also established from the elution profiles after HNO_2 treatment. Some samples were also analysed by Superose 6 gel chromatography using a Pharmacia FPLC® pump system. This column was run in 0.05 M Tris/HCl, pH 8.0, with 0.15 M NaCl and 0.2% Triton X-100. Fractions (0.5 ml each) were collected and analysed for content of radioactivity.

Isolation and labelling of LDL

LDL was isolated from human plasma by sequential ultracentrifugation in the density range 1.019-1.063 g/ml [19] and thereafter dialysed against PBS at 4 °C. Lipid peroxide (LPO) levels in LDL were 1.1 nmol/mg and 1.8 nmol/mg before and after dialysis against EDTA-free PBS respectively. The relative electrophoretic mobility (R_m) of LDL was not changed after dialysis (for methods, see below).

LDL was labelled with [^{125}I]tyraminylcellobiose (TC) as described in [20], and finally dialysed extensively against PBS at 4 °C. ^{125}I-TC-labelled ligands will not escape the lysosomes after cellular uptake, but remain cell-associated. Accordingly, degradation products will not leak from the cells into the medium [20]. More than 97% of the incorporated radioactivity was precipitated with 10% trichloroacetic acid. The specific radioactivity was in the order of 200–500 c.p.m./ng of protein. For binding experiments the ^{125}I-TC-LDL was diluted with unlabelled LDL to a final specific radioactivity of 60 c.p.m./ng. It was stored under N_2 at 4 °C and used within 1–3 weeks. The concentration of protein was determined by bicinchoninic protein assay (Pierce) using BSA as standard [21].

Oxidative modification of LDL

LDL was oxidatively modified in a cell-free system [22]. To remove EDTA before oxidation, LDL was dialysed extensively against EDTA-free PBS (pH 7.4). Thereafter LDL (250 μg of protein/ml; specific radioactivity 60 c.p.m./ng) was incubated at 37 °C for 24 h in the presence of 10 μM $CuSO_4$. The oxidation was terminated by refrigeration and addition of EDTA (200 μM final concentration) and butylated hydroxytoluene (40 μM final concn.). Aliquots were assayed for LPOs and R_m (see below) to evaluate the extent of oxidation.

The content of LPOs was determined by a colorimetric-endpoint-kit method (Kamiya Biomedical Co., Thousand Oaks, CA, U.S.A.) with minor modifications (100 μl sample at a concentration of 250 μg of LDL protein/ml). The amount of LPOs was calculated using cumene hydroperoxides as standard.

The increase in the net negative surface charge of apolipoprotein B in LDL, a marker of oxidation of LDL, was measured by agarose-gel electrophoresis (Paragon Beckman Instruments, Inc., Fullerton, CA, U.S.A.).

The levels of LPO and the R_m were 223 ± 126 nmol/mg and

4.0 ± 0.4 ($n = 6$) respectively, measured in the oxidized LDL, prepared for use in all the experiments presented.

Binding and uptake of ^{125}I-labelled oxLDL and native LDL

J774 cells were preincubated with 20 mM $NaClO_3$ (see Figure 3 below) for 20 h in sulphate-depleted RPMI 1640 with 2% FCS without gentamycin before binding and uptake experiments. Control cells were incubated in normal RPMI 1640 medium with 2% FCS. The cells were washed three times with RPMI 1640 without serum and then incubated at 4 °C after ^{125}I-TC-oxLDL (10 μg/ml) and native ^{125}I-TC-LDL (5 μg/ml) (in RPMI 1640 without serum) had been added. After 2 h incubation the cells were washed six times with cold PBS and each well harvested in 1 ml of 0.1 M NaOH. The amount of cell-bound radioactivity was measured in a Packard γ-radiation counter. J774 cells used in xyloside experiments were incubated in the absence or presence of 1 mM HX-xyl in ordinary DMEM medium with 10% FCS for 20 h prior to binding or uptake studies of ^{125}I-TC-oxLDL and ^{125}I-TC-LDL. Dose–response experiments showed that 1 mM HX-xyl gave a maximum abrogation of proteoglycan expression in J774 cells. A different batch of J774 cells was used for the HX-xyl experiments. These cells had a higher uptake of oxLDL and native LDL on a ng/mg-of-protein basis than the cells used in the $NaClO_3$ experiments. Still, as expected, the uptake of oxLDL was at least three times higher than the uptake of native LDL in both cell batches.

Before binding experiments, some cells were also subjected to treatment with C-ABC or heparitinase. The cells were washed six times with PBS, whereafter they were treated with the enzymes separately. C-ABC was added at a concentration of 0.1 unit/ml in 0.05 M Tris/HCl, pH 7.4, with 25 μg/ml of BSA and 0.025 M sodium acetate. Heparitinase was used at a concentration of 2.4 units/ml in PBS, pH 7.4, with 3 mM $CaCl_2$ and 3 mM sodium acetate. After incubation at 37 °C for 2 h the cells were washed twice with cold DMEM, and used for binding experiments as described above.

For binding and uptake experiments with native ^{125}I-TC-LDL the cells were preincubated in RPMI 1640 with 5 mg/ml of lipoprotein-deficient serum (LPDS) for 20 h in the absence or presence of 20 mM $NaClO_3$ or 1 mM HX-xyl. The cells were treated with LPDS to induce up-regulation of the receptor for native LDL. During binding experiments, fresh medium without LPDS was added. Both $NaClO_3$ and xyloside were present during uptake experiments. Only $NaClO_3$ was present during binding experiments.

To measure the cellular uptake of both native ^{125}I-TC-LDL and ^{125}I-TC-oxLDL, J774 cells were incubated for 5 h at 37 °C with the two different ligands. After 5 h the cells were chilled on ice, washed six times with cold PBS and harvested in 0.1 M NaOH. Cell-associated radioactivity was measured in a Packard γ-radiation counter. LDL was labelled with ^{125}I-TC, which cannot escape the endosomal/lysosomal compartments. The cell-associated radioactivity does, accordingly, represent both intact and degraded forms of ^{125}I-TC-oxLDL or native ^{125}I-TC-LDL. In the text 'uptake of LDL' is used synonymously with 'cell-associated LDL'. The amount of ^{125}I-TC-LDL bound or taken up by the cells was related to the amount of protein in each individual well and expressed as ng of ^{125}I-TC-LDL/mg of cellular protein. The unspecific binding and uptake of ^{125}I-TC-oxLDL and native ^{125}I-TC-LDL was less than 20% in all experiments, as measured after addition of a 50–100-fold excess of cold ligand. No differences were observed in the unspecific binding between control-, $NaClO_3$- or HX-xyl-treated cells.

All binding and uptake experiments were done in triplicate, and the data are expressed as means ± S. D. The Mann–Whitney non-parametric test (U-test) was used for calculation of possible statistical difference between different types of experiments. The level of significance was set at $P < 0.05$.

Binding assay

Native and oxLDL were coated on 96-well plates at a concentration of 10 μg/well in PBS for 20 h at room temperature. The wells were washed three times with PBS and thereafter incubated with 1% BSA in PBS for 1 h at 37 °C. The wells were washed three times with PBS before binding experiments were started. Background binding was determined in wells only coated with BSA and was 15–20 c.p.m./well. J774 cells were labelled with 100 μCi/ml of [^{35}S]sulphate for 20 h, washed three times with PBS and then incubated with trypsin (12.5 μg/ml) for 15 min at 37 °C. The enzyme was inactivated by adding aprotinin at a ten times higher (molar) concentration. The released cell-surface ^{35}S-labelled proteoglycans were used to measure binding to immobilized native and oxLDL. Binding experiments were done in PBS with or without 1 M NaCl in PBS or heparin in PBS (final concn. 1 mg/ml) added. Control binding experiments were done with [^{3}H]heparin.

mRNA isolation and Northern-blot analyses

After confluency had been achieved, the cells were incubated for 20 h in the absence ('Control') or presence of either $NaClO_3$ ('Chlorate'; 10 and 20 mM) or HX-xyl ('HX-xyl'; 0.1 and 1 mM). Control cells used in the $NaClO_3$ and HX-xyl experiments were grown in RPMI 1640 without sulphate with 2% FCS and DMEM with 10% FCS respectively. Thereafter, the cells were washed once in cold PBS and harvested in lysis buffer. mRNA was isolated using the Dynabeads Direct kit (Dynal A/S, Oslo, Norway), separated on a 1%-agarose gel containing 6.7% formaldehyde and blotted on to Hybond N membrane (Amersham). cDNA probes for the human LDL receptor and β-actin probe were labelled with [^{32}P]dCTP as described elsewhere [23]. A 769-bp scavenger-receptor-A-II PCR product, amplified from c-DNA from the murine macrophage cell line RAW 264.7, was kindly given by Dr. Helga de Vries, Centre for Drug Research at Leiden University, Leiden, The Netherlands. This cDNA was labelled with [^{32}P]dCTP as described above. Hybridizations were carried out at 65 °C as described in [24]. mRNA signals of the scavenger receptor A-II, the LDL receptor, and β-actin were analysed by using a Phosphorimager (Molecular Dynamics, Sunnyvale, CA, U.S.A.).

Fluid-phase and receptor-mediated endocytosis

The effect of $NaClO_3$ on fluid-phase and receptor-mediated endocytosis was examined by using horseradish peroxidase and ^{125}I-TC-ovalbumin respectively as ligands. After confluency had been achieved, the cells were incubated for 20 h in the absence ('Control') or presence of 20 mM $NaClO_3$ ('Chlorate'). Thereafter, the cells were washed once and incubated further with either 500 μg/ml horseradish peroxidase (type VI, Sigma) or 10 μg/ml ^{125}I-TC-ovalbumin (grade VI, Sigma) up to 3 h at 37 °C. The horseradish peroxidase activity associated with the cell fractions was measured by using Pierce Immunopure TMB Substrate Kit. Absorbance was recorded at 450 nm. The cellular uptake (acid-precipitable) and degradation (acid-soluble) of ^{125}I-TC-ovalbumin was determined after precipitation with 10% trichloroacetic acid, followed by counting in a γ-radiation counter.

RESULTS

[^{35}S]Proteoglycans

J774 cells were labelled with [^{35}S]sulphate for time periods from 1 to 24 h. The incorporation into ^{35}S-labelled macromolecules was measured by Sephadex G-50 gel chromatography. A time-dependent increase in the synthesis of sulphated macromolecules in J774 cells could be demonstrated, both in the cell fraction and in those released into the culture medium. After 24 h the cell fraction contained 60 % and the medium 40 % ($n = 4$) of the sulphated macromolecules. To analyse the nature of ^{35}S-labelled macromolecules, material from both the cell fraction and the culture medium was subjected to DEAE-Sephacel ion-exchange chromatography. Most of the material (> 90 %) in both fractions bound to the column and was eluted off at high salt concentration, showing that these molecules were highly polyanionic (results not shown).

To determine whether the [^{35}S]macromolecules synthesized by J774 macrophages were of a proteoglycan nature, the cell and medium material was subjected to alkali treatment and Sepharose CL-6B gel chromatography (Figure 1). The cell fraction contained three [^{35}S]sulphated components: one peak was recovered in the void volume, the second part as a smaller peak/shoulder (k_{av} 0.43) and the third major component with a k_{av} value of 0.65 (Figure 1A). When this material was subjected to alkali treatment, which leads to the release of intact [^{35}S]glycosaminoglycan chains from [^{35}S]proteoglycans, the elution profile of the material changed such that more than 95 % was recovered with a peak k_{av} value of 0.65 (Figure 1A). This result shows that approx. 50 % of the ^{35}S-labelled macromolecules in the cell fraction of J774 cells contained proteoglycans. The third peak of the untreated material most probably represents glycosaminoglycan chains, as the alkali-liberated chains in the [^{35}S]proteoglycans were eluted with identical k_{av} values. When the medium fraction was subjected to the same treatment, a minor portion of the material was eluted in the void volume of the column, whereas the major part was eluted with a k_{av} value of 0.51 (Figure 1B). After alkali treatment the elution profile of all the material was shifted to higher k_{av} values, where the major peak was eluted at 0.65. Accordingly, the medium fraction contained high- and lower-molecular-mass components of a proteoglycan nature and a portion of free glycosaminoglycan chains. The medium fraction was not the subject of further detailed studies.

The cell fraction (Figure 1A) was subjected to C-ABC and HNO_2 treatment and Sepharose CL-6B gel chromatography. Approx. 30 % was degraded with the enzyme and approx. 70 % depolymerized after HNO_2 treatment at pH 1.5 (result not shown). The total cell fraction of J774 cells did, accordingly, contain approx. 70 % heparan sulphate and 30 % chondroitin sulphate (see also below).

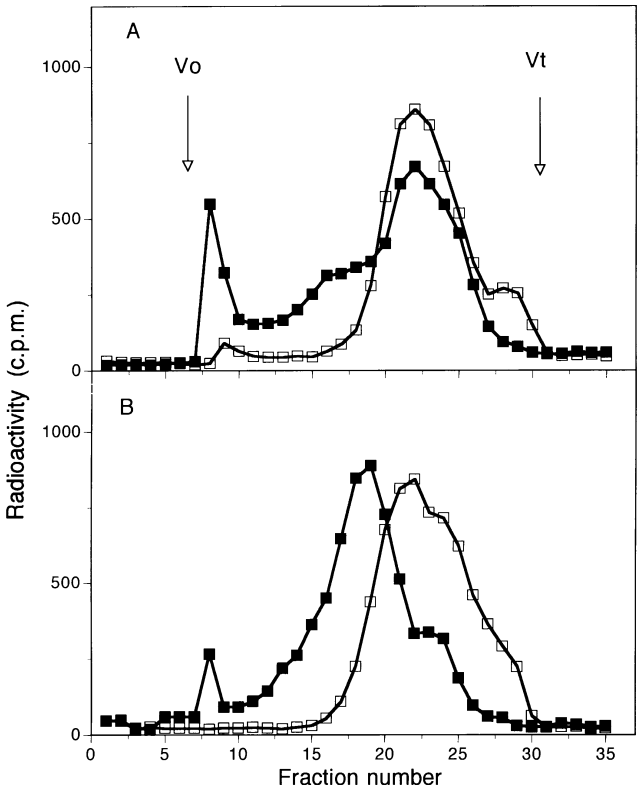

Figure 1 Sepharose CL-6B gel chromatography of medium and cell fractions from J774 cells

J774 cells were labelled with [^{35}S]sulphate (100 μCi/ml) for 20 h. Thereafter the medium and cell fractions from J774 cells were subjected to Sepharose CL-6B gel chromatography before (■) and after alkali treatment (□). The columns were eluted with 0.05 M Tris/HCl, pH 8.0, containing 0.15 M NaCl and protease inhibitors. V_0 ('Vo') and V_t('Vt') markers were Dextran Blue and dinitrophenylalanine respectively. (**A**) Cell fraction; (**B**) medium fraction.

Cell-surface proteoglycans

For further characterization of the cell-associated proteoglycans the cells were labelled with [^{35}S]sulphate for 20 h. The cells were subsequently washed and incubated further with trypsin to investigate whether [^{35}S]proteoglycans could be released from the cell surface. The medium was collected after trypsin treatment and analysed by Sepharose CL-6B gel chromatography. The cell-associated material released by trypsin contained two major parts, and only the first component, which contained about 30 % of the total material, was shifted after alkali treatment (Figure 2A). This portion of the ^{35}S-labelled material therefore represents the cell-surface proteoglycans in J774 macrophages. A major portion of labelled material released by trypsin did, however, contain glycosaminoglycan chains. When the whole-cell extract was subjected to gel chromatography, a higher-molecular-mass proteoglycan could also be observed (Figure 1A). This component was not released by trypsin treatment, but was only recovered in the medium and most probably represents proteoglycans secreted from the cells.

To investigate the nature of the material released from J774 cells with trypsin treatment, an aliquot was subjected to C-ABC treatment and thereafter analysed by Sepharose CL-6B gel chromatography. The proteoglycan component was resistant to this treatment, whereas a major portion (approx. 40 %) of the second peak was depolymerized after enzyme treatment and was eluted in a more retarded position (Figure 2B). A second aliquot was subjected to HNO_2 treatment at pH 1.5 and was also analysed by Sepharose CL-6B gel chromatography. From Figure 2(C) it is evident that the proteoglycan component is completely depolymerized by this treatment, demonstrating that it contains only heparan sulphate.

When the whole-cell extract was subjected to the same procedures, it was evident that a small but significant portion of the material eluted with a k_{av} of 0.43, contained chondroitin sulphate proteoglycan. J774 macrophages do accordingly also contain a small amount of chondroitin sulphate proteoglycan that is not released by trypsin treatment. These results show that the cell fraction of J774 cells contains chondroitin sulphate proteoglycan,

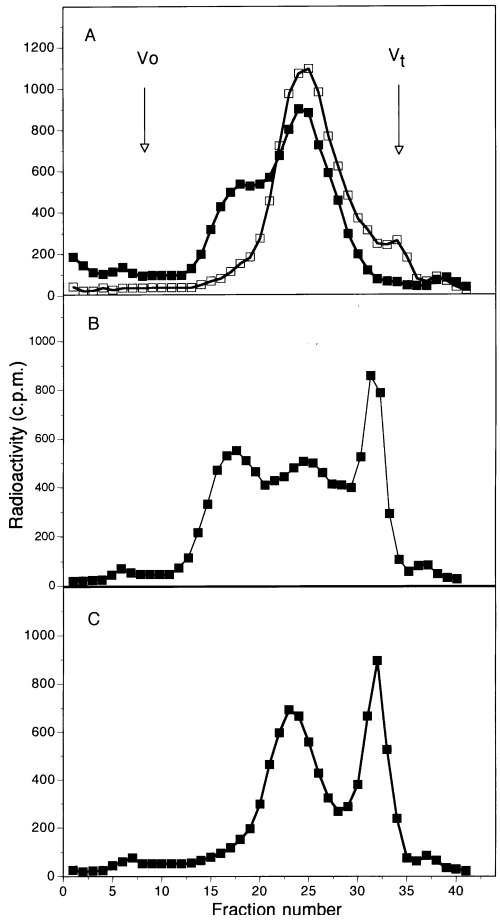

Figure 2 Sepharose CL-6B gel chromatography of trypsin-released material

J774 cells were labelled with [^{35}S]sulphate (100 μCi/ml) for 20 h. The cells were washed and incubated with trypsin (12.5 μg/ml) for 15 min. The material released into the medium was collected and subjected to gel chromatography before (■) and after alkali treatment (□), shown in (**A**). Aliquots of the same material were also applied to the column after C-ABC treatment (**B**) and after HNO_2 treatment (**C**). Running conditions were as described in the legend to Figure 1.

trypsin-sensitive heparan sulphate proteoglycan and free glycosaminoglycan chains of both types.

Preliminary analyses with immunoprecipitations with an anti-(syndecan 1) monoclonal antibody did not reveal the presence of such molecules in the cell fraction of J774 cells. However, using a cDNA probe for syndecan 4 showed that these cells have mRNA for this particular syndecan species (result not shown). This is in accordance with the results of previous studies [14].

Inhibition of sulphation

To investigate the possible significance of cell-associated proteoglycans for a defined cellular process, one experimental approach is to inhibit sulphation by treating cells with $NaClO_3$ [16]. To investigate the role of cellular proteoglycans for the binding and uptake of LDL, we first incubated the cells in the presence of $NaClO_3$ and measured the level of [^{35}S]sulphate incorporation into macromolecules. There was a dose-dependent inhibition of [^{35}S]sulphate incorporation into cell-associated ^{35}S-labelled macromolecules (Figure 3). At 3.5 mM $NaClO_3$ the incorporation was inhibited 50 %, whereas at 20 mM $NaClO_3$ the inhibition

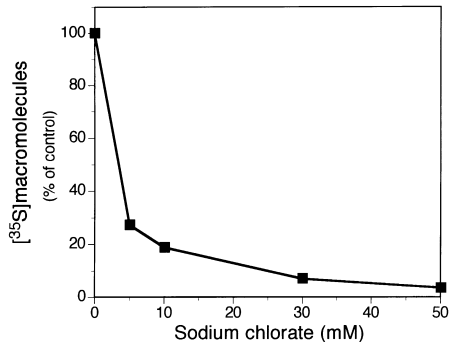

Figure 3 Incorporation of [^{35}S]sulphate into J774 cells

J774 cells were labelled with [^{35}S]sulphate (100 μCi/ml) in the absence or presence of increasing concentrations of $NaClO_3$ for 20 h. The cell fraction was harvested and analysed for content of ^{35}S-labelled macromolecules. The results are expressed as percentage of control. Three separate experiments gave similar results.

Table 1 Effect of $NaClO_3$ on the binding of native ^{125}I-TC-LDL and ^{125}I-TC-oxLDL to J774 cells

After confluency, the cells were incubated for 20 h in the absence ('Control') or presence ('Chlorate') of 20 mM $NaClO_3$ in RPMI 1640 (2% FCS) medium with or without sulphate respectively. The binding of native ^{125}I-TC-LDL and ^{125}I-TC-oxLDL was quantified in ng/mg of cell protein (control values 505 ± 310 and 98 ± 80 respectively). Results are means ± S.D. Six experiments in triplicate were performed with native LDL, and nine separate experiments with oxLDL. *Significant difference between control cells and $NaClO_3$-treated cells ($P < 0.05$; Mann–Whitney *U*-test).

		Cell-associated [^{125}I]TC-LDL (% of control)
LDL	Control	100
	Chlorate	77 ± 11*
oxLDL	Control	100
	Chlorate	83 ± 5*

was close to 85 %. The latter concentration was used for further studies. Cellular viability, as measured by [^{3}H]thymidine incorporation, was compared in untreated and $NaClO_3$-treated cells. The level of incorporation was the same in controls and cells treated with 20 mM $NaClO_3$ for 20 h (results not shown).

Binding and uptake of LDL after $NaClO_3$ treatment

J774 cells were preincubated for 20 h with and without 20 mM $NaClO_3$, washed and used for binding experiments at 4 °C. There was a small but significant decrease in the binding of both ^{125}I-TC-oxLDL and native ^{125}I-TC-LDL in cells preincubated with $NaClO_3$ compared with control cells, as can be seen in Table 1.

Furthermore, the uptake of both ligands was measured after incubation at 37 °C for 5 h. The uptake of oxLDL was typically two to three times higher than that of native LDL after 5 h. Furthermore, the uptake of oxLDL increased in a time-dependent manner, whereas the uptake of native LDL levelled off after 5 h incubation (results not shown). $NaClO_3$ had a small but significant effect on the uptake of both ligands. After 5 h the uptake was significantly decreased to 64 and 76 % of controls for ^{125}I-TC-oxLDL and native ^{125}I-TC-LDL respectively, as shown in Figure 4. To restore sulphation in $NaClO_3$-treated cells, $MgSO_4$ was added at different concentrations. The uptake of ^{125}I-TC-

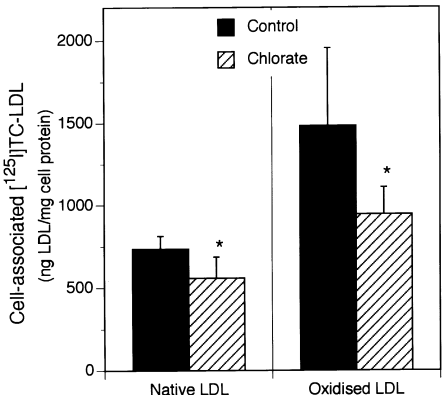

Figure 4 Effect of $NaClO_3$ on the uptake of oxidised and native ^{125}I-TC-LDL in J774 cells

J774 cells were incubated for 20 h with (solid bars) or without 20 mM $NaClO_3$ (hatched bars). Thereafter the cells were incubated with 5 μg/ml ^{125}I-TC-labelled native or 10 μg/ml ^{125}I-TC-labelled oxLDL for 5 h at 37 °C. The amount of cell-associated radiolabelled LDL is expressed as ng/mg of cell protein. *Significant difference between control and chlorate-treated cells ($P < 0.05$; Mann–Whitney *U*-test). Results are means ± S.D. for four separate experiments performed in triplicate.

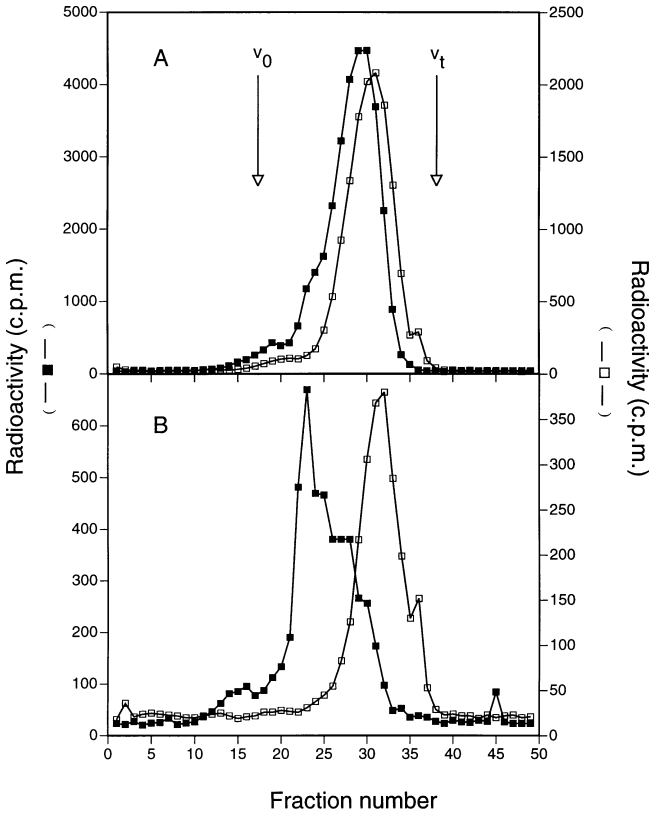

Figure 5 Superose 6 gel chromatography of medium fractions from J774 cells

J774 cells were cultured in the absence or presence of 1 mM HX-xyl for 20 h and labelled with 300 μCi/ml of [^{35}S]sulphate. Medium fractions were harvested and subjected to Superose 6 gel chromatography prior to (■) and after alkali treatment (□) after removal of [^{35}S]sulphate. (**A**) Medium from HX-xyl-treated cells; (**B**) medium from control cells.

oxLDL in these cells was similar to the level of control cells when 200 μM $MgSO_4$ was present during the 24 h $NaClO_3$ treatment. Hence, the effect of $NaClO_3$ is reversible and most likely due to the effect of $NaClO_3$ on the availability of sulphate to the biosynthetic machinery of the cells.

It is possible that the presence of $NaClO_3$ in the culture medium could interfere with the binding and uptake of LDL in macrophages. Control experiments were therefore performed with $NaClO_3$ present only during binding experiments and not in the preincubation. Neither the binding of ^{125}I-TC-oxLDL nor native ^{125}I-TC-LDL was affected by the presence of $NaClO_3$ in the culture medium (results not shown).

$NaClO_3$ treatment and uptake of other ligands

It is possible that the inhibition of lipoprotein uptake seen after $NaClO_3$ treatment could be due to unspecific effects on membrane turnover and lead to a general decrease in uptake of different types of ligands. To investigate this in further detail we measured the uptake of horseradish peroxidase (a marker for pinocytosis) and the uptake of ^{125}I-ovalbumin (a marker for receptor-mediated endocytosis) in untreated and $NaClO_3$-treated J774 cells. From Table 2 it is evident that uptake of both ligands was not affected by pretreating the cells with 20 mM $NaClO_3$. The degradation of ^{125}I-ovalbumin was also determined and not found to be affected, except at the latest time point.

Table 2 Effect of $NaClO_3$ (20 mM) on uptake and degradation of ^{125}I-TC-ovalbumin and uptake of horseradish peroxidase in J774 cells

After confluency, the cells were incubated for 20 h in the absence ('Control') or presence ('Chlorate') of $NaClO_3$ (20 mM) in RPMI 1640 (2% FCS) medium with or without sulphate respectively. Thereafter the cells were washed once and incubated further with 10 μg/ml ^{125}I-TC-ovalbumin or 500 μg/ml horseradish peroxidase for 3 h at 37 °C as described in the Materials and methods section. Results are means ± S.D. for three separate dishes.

	^{125}I-TC-ovalbumin (c.p.m./mg of cell protein)				Horseradish peroxidase [$10^{-3} \times (A_{450}$/mg of cell protein)]	
	Uptake		Degraded		Uptake	
Time (h)	Control	Chlorate	Control	Chlorate	Control	Chlorate
0	3434.7 ± 207.8	4713.3 ± 573.6	161.6 ± 56.8	219.0 ± 24.3	4.1 ± 1.0	5.6 ± 0.2
0.5	53 277.3 ± 418.5	55 236.3 ± 350.7	3855.7 ± 158.0	3779.0 ± 402.9	19.9 ± 2.9	26.3 ± 0.3
1	68 694.3 ± 213.0	76 796.0 ± 7923.2	10 295.0 ± 698.5	12 310.7 ± 2485.0	19.9 ± 2.6	22.6 ± 1.9
3	60 936.0 ± 6617.3	63 077.0 ± 7908.5	39 404.3 ± 4295.5	28 082.7 ± 5597.5	18.6 ± 0.3	19.3 ± 1.2

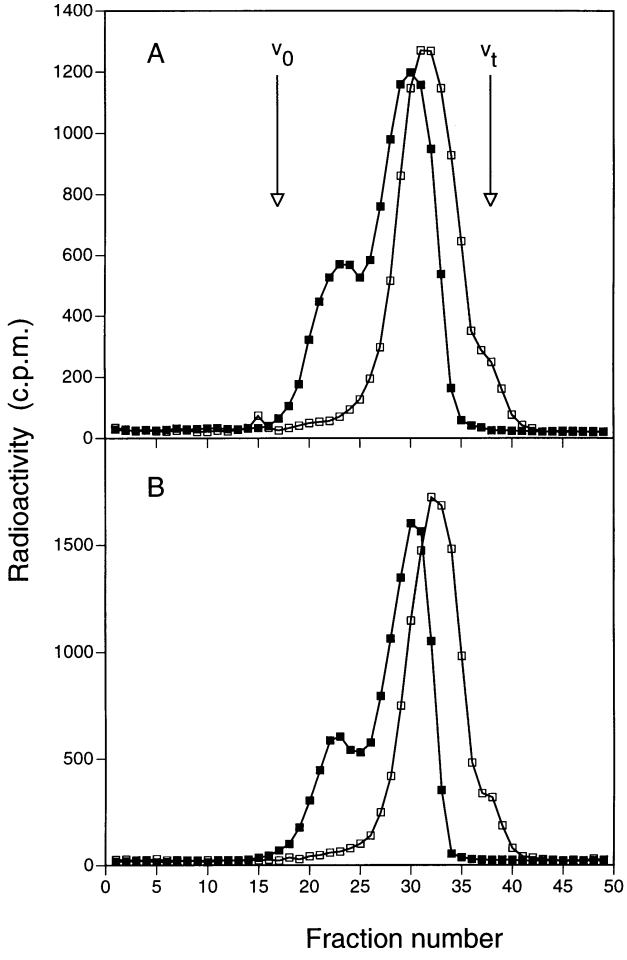

Figure 6 Superose 6 gel chromatography of cell fractions from J774 cells

J774 cells were cultured and labelled as described in the legend to Figure 5. Cell fractions were solubilized in 4 M guanidine with 2% Triton X-100 and subjected to gel chromatography after removal of [^{35}S]sulphate. The cell fractions were run before (■) and after alkali treatment (□). (**A**) HX-xyl-treated cells; (**B**) control cells.

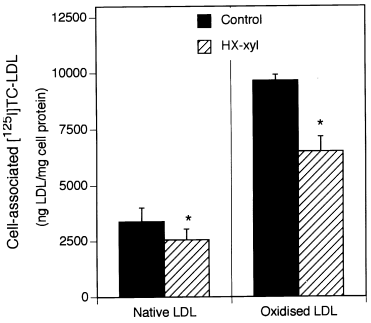

Figure 7 Effect of β-D-xyloside on the uptake of native ^{125}I-TC-LDL ('Native LDL') and ^{125}I-TC-oxLDL ('Oxidised LDL') in J774 cells

J774 cells were incubated for 20 h with (solid bars) or without 1 mM HX-xyl (hatched bars). Thereafter the cells were incubated with 5 μg/ml ^{125}I-TC-labelled native or 10 μg/ml ^{125}I-TC-labelled oxLDL for 5 h at 37 °C. The amount of cell-associated radiolabelled LDL is expressed as ng/mg of cell protein. *Significant difference between control and HX-xyl-treated cells ($P < 0.05$; Mann–Whitney *U*-test). Results are means ± S. D. of three separate experiments performed in triplicate.

Binding and uptake of LDL after β-D-xyloside treatment

The presence of significant amounts of chondroitin sulphate proteoglycan and chondroitin sulphate in the cell fraction of J774 cells prompted us to adopt another experimental strategy. β-D-Xylosides are potent abrogators of chondroitin sulphate expression [17], whereas the effect on heparan sulphate proteoglycan expression is minor, although there are exceptions to this general outline [25]. J774 cells were treated with 1 mM HX-xyl and labelled with [^{35}S]sulphate. Medium and cell fractions were analysed by Superose 6 gel chromatography. From Figure 5(A) it is evident that there is a large increase in the release of free glycosaminoglycan chains into the medium after HX-xyl treatment, as the elution pattern of this material is not significantly shifted after alkali treatment, which leads to the release of intact glycosaminoglycans from proteoglycans. Control cells release almost exclusively intact proteoglycans, as almost all the material is shifted in elution position following alkali treatment (Figure 5B). The level of ^{35}S-labelled macromolecules in the medium was found to be 6.2 times higher in HX-xyl-treated cells than in control cells. However, much smaller differences in elution patterns were observed in the cell fraction following HX-xyl treatment. As can be seen in Figures 6(A) and 6(B), the cell fractions contain proteoglycans/glycosaminoglycans similar to what has been shown (see Figures 1 and 2). The cell fraction of xyloside-treated cells contained intact proteoglycans and free glycosaminoglycan chains in approximately the same ratio as control cells. However, the level of ^{35}S-labelled macromolecules in the cell fraction of HX-xyl-treated cells was found to be 40% lower than in control cells. Cell fractions of HX-xyl-treated cells had a higher chondroitin sulphate/heparan sulphate ratio than control cells, as shown by C-ABC digestions and gel chromatography.

J774 cells were incubated with 1 mM HX-xyl for 20 h and the uptake of ^{125}I-TC-oxLDL and native ^{125}I-TC-LDL was measured after 5 h. As can be seen in Figure 7, the uptake of ^{125}I-TC-oxLDL in HX-xyl-incubated cells was 67% of control, whereas the uptake of native ^{125}I-TC-LDL was 76% of the control after HX-xyl incubation. It is possible that the presence of high levels of glycosaminoglycan chains released after HX-xyl treatment could interfere with the uptake of native as well as oxLDL. Binding of ^{125}I-TC-oxLDL and native ^{125}I-TC-LDL at 4 °C to J774 cells after washing was therefore measured in control and HX-xyl-treated cells. The inhibitory effect of HX-xyl treatment on the binding of both ligands was the same as the observed effect on uptake (result not shown).

Receptor mRNA levels

It can be argued that the lower uptake of native and oxLDL observed after xyloside and $NaClO_3$ treatment could be due to a lower level of receptor molecules after these treatments. To address this question, cells were treated with HX-xyl or $NaClO_3$ and mRNA was isolated. cDNA probes recognizing mRNA for either the scavenger receptor A-II or the LDL receptor were used and the expression levels were compared with those of mRNA for the housekeeping gene β-actin. From Figure 8 it is evident that the expression levels for the LDL receptor, which were consistently low, or for the scavenger receptor, were similar in untreated and treated cells. Exposing cells to either HX-xyl or $NaClO_3$ does not, accordingly, affect the mRNA levels for two

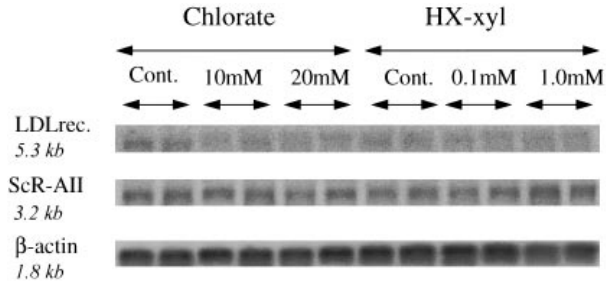

Figure 8 mRNA levels for the LDL and the scavenger A-II receptor

mRNA was isolated from control J774 cells ('Cont.') and from cells treated with 10 and 20 mM $NaClO_3$ ('Chlorate') or 0.1 and 1.0 mM HX-xyl for 20 h. mRNA was run on agarose gels, blotted and hybridized with probes for the LDL receptor ('LDLrec.'), scavenger receptor A-II ('ScR-AII') and the housekeeping gene β-actin. mRNA signals were recorded using a phosphoImager.

Table 3 Effect of C-ABC and heparitinase treatment on the binding of native ^{125}I-TC-LDL and ^{125}I-TC-oxLDL

J774 cells were pre-incubated for 2 h in the absence or presence of C-ABC (0.1 unit/ml) or heparitinase (2.4 units/ml). Thereafter the specific binding of radiolabelled LDL and radiolabelled oxLDL were quantified in ng/mg of cell protein. Control values were in the range 42–125 ng of LDL/mg of cell protein and 351–620 ng of LDL/mg of cell protein respectively. The data are given as percentages of control without enzyme and show means $\pm$ S.D. for four or five separate experiments. *Significant difference between control cells and enzyme treated cells ($P < 0.05$, Mann-Whitney *U*-test).

		Specific binding (% of control)
^{125}I-TC-LDL	Control	100
	C-ABC	77 ± 10*
	Heparitinase	59 ± 26*
^{125}I-TC-oxLDL	Control	100
	C-ABC	119 ± 25
	Heparitinase	67 ± 13*

receptors most likely to be important for the uptake of lipoproteins in J774 cells.

Binding of LDL and cell-surface proteoglycans

To analyse further the importance of cell-surface proteoglycans/glycosaminoglycans for the binding of native and oxLDL, cells were treated with C-ABC and heparitinase to remove chondroitin sulphate and heparan sulphate respectively. When the former enzyme was used there was a decrease only in the binding of native ^{125}I-TC-LDL. After heparitinase treatment, however, the binding of both native ^{125}I-TC-LDL and ^{125}I-TC-oxLDL was significantly decreased, as shown in Table 3. Clearly, cell-surface heparan sulphate is important for the binding of both LDL species to macrophages, whereas only binding of native LDL is partly dependent on chondroitin sulphate (see also below).

With the aim of showing a direct interaction between proteoglycans expressed on the cell surface of J774 cells and LDL, an *in vitro* binding assay was used. Native LDL and oxLDL were immobilized to microtitre plates, and the binding of [^{35}S]proteoglycans released from the cells after labelling and trypsin treatment was measured. The binding was consistently low (100–200 c.p.m./well of 100 000 c.p.m. added to both types of LDL coats). However, it was reproducibly completely abolished with 1M NaCl or 1mg/ml of heparin, as was also the case for the binding of commercially available [^{3}H]heparin to the same immoblized lipoproteins.

Binding experiments were furthermore done with J774 cells incubated in the presence of heparin and chondroitin sulphate. The binding of native LDL was significantly decreased in the presence of 0.1 mg/ml of chondroitin sulphate (81.2% of control). No further inhibition was seen at 1.0 mg/ml. At concentrations of 0.1 and 1mg/ml of heparin the binding of native LDL was 80 and 55% of the control respectively. Heparin has conventionally been used to block binding of LDL to the B/E receptor, a blocking that is not seen in B/E-receptor-negative cells [26]. Heparin inhibition of LDL binding may therefore occur both at the level of the LDL receptor and cell-surface heparan sulphate. In contrast, the binding of oxLDL was not inhibited by the presence of chondroitin sulphate in concentrations up to 1.0 mg/ml. When heparin was added at the same concentration, the binding was 53% of that in untreated control cells. [Three separate experiments gave similar results (not shown).]

Table 4 Effect of LPL on the uptake of ^{125}I-TC-LDL and ^{125}I-TC-oxLDL to J774 cells

After confluency, the cells were incubated for 20 h in the absence ('Control') or presence ('Chlorate') of 20 mM $NaClO_3$. Thereafter the 5 h uptake at 37° C of radiolabelled ligands was measured in the absence or presence of $NaClO_3$ (20 mM) and LPL (0.8 μg/ml) and cell association was measured as described in the Materials and methods section. Values are means $\pm$ S.D. for six to nine cultures. §$P < 0.05$ versus control; *$P < 0.05$ versus $NaClO_3$-treated cells using Mann–Whitney *U*-test.

		Cell-associated ^{125}I-TC-LDL (% of control)
^{125}I-TC-LDL	Control	100.0 ± 0.0
	Control + LPL (0.8 μg/ml)	209.2 ± 51.5§*
	Chlorate (20 mM)	66.4 ± 6.9§
	Chlorate + LPL (0.8 μg/ml)	126.4 ± 19.9§*
^{125}I-TC-oxLDL	Control	100.0 ± 0.0
	Control + LPL (0.8 μg/ml)	170.7 ± 67.0§*
	Chlorate (20 mM)	67.7 ± 14.5§
	Chlorate + LPL (0.8 μg/ml)	82.0 ± 13.0§*

LPL

In several cell systems it has been shown that cell-surface heparan sulphate binds LPL. It has furthermore been shown that J774 cells secrete LPL [27]. We wanted to investigate whether the effects of $NaClO_3$ on the uptake of native and oxLDL respectively could involve LPL-dependent mechanisms. The addition of LPL increased the uptake of both native and oxLDL to J774 cells, as can be seen in Table 4. When LPL was added to $NaClO_3$-treated cells the uptake of ^{125}I-TC-oxLDL did not increase significantly. The LPL-mediated increase in the uptake of oxLDL therefore seems to depend on the presence of cellular proteoglycans. The uptake of native LDL increased when LPL was added to $NaClO_3$-treated cells, but not the level seen in control cells treated with LPL. In terms of LPL-dependence it therefore seems as if native LDL depends less on cellular proteoglycans for its uptake into J774 cells than does oxLDL.

DISCUSSION

In the vascular wall, macrophages play a key role in the development of atherosclerotic plaques [28]. Pathological evidence shows that these cells are high in number and rich in

cholesterol in such lesions [29]. Only limited information is available on the possible role of proteoglycans expressed on macrophage cell surface in these complex processes. Our data show that, after inhibiting the sulphation of cellular proteoglycans in J774 cells with $NaClO_3$, the binding of both radiolabelled native and oxLDL was 20–25 % lower than in untreated cells. On a quantitative basis the uptake of oxLDL, and hence also the inhibition of such an uptake, is far more important than that of native LDL in J774 macrophages under *in vitro* conditions. Without chlorate treatment the binding of radiolabelled oxLDL was approx. three times higher than that of native LDL. Furthermore, we show that the uptake of radiolabelled native and oxLDL was 25–35 % lower in cells treated with HX-xyl. The effect of the latter treatment mainly affects the biosynthesis of chondroitin sulphate proteoglycans, leading to the expression and secretion of free chondroitin sulphate chains, suggesting that cell-associated chondroitin sulphate proteoglycans may play a role in the uptake of LDL in macrophages. Experiments using heparitinase further demonstrated that heparan sulphate is important for the association of native and oxLDL with the cell surface of J774 cells. The binding of oxLDL was reduced after xyloside treatment of the cells, but not by the enzymic removal of cell-surface chondroitin sulphate. However, with xyloside the level of proteoglycans/glycosaminoglycans was 40 % lower than in control cells, and the ratio of chondroitin sulphate/heparan sulphate increased. Furthermore, the binding of oxLDL decreased in the presence of excess heparin, but not when chondroitin sulphate was added. These results suggest that heparan sulphate is more important for the binding of oxLDL than is chondroitin sulphate.

LDL in its native form lacks the ability to generate macrophage foam cells [30]. However, although the level of oxLDL versus that of native LDL in the intima still remains to be determined, it has been shown to be present in atherosclerotic lesions [31], and accumulating evidence suggests that uptake of modified LDL in macrophages via different receptors is important for the generation of fatty streaks [32]. Several receptors have been shown to mediate the binding of oxLDL to macrophages, including the acetyl-LDL receptor, the FcγRII receptor, CD36 and a newly identified 94–97 kDa plasma membrane protein [33]. Also, LDL–proteoglycan complexes have been shown to be bound by a receptor in human macrophages different from the LDL receptor [34] and complexes of LDL and heparin-containing mast-cell granules are phagocytosed by macrophages [35]. With such a high number of possible ports of entry for oxLDL into macrophages it is not surprising that the interference with proteoglycan expression, either with $NaClO_3$ or β-D-xyloside, leads to only a partial decrease in the binding of oxLDL. The most probable scenario is that some of these receptors depend on the presence of cell-surface proteoglycans or glycosaminoglycans, either as mediators for receptor binding, as co-receptors, or as molecules collaborating with LPL to interact with LDL particles. Several previous studies on proteoglycans in human cells belonging to the monocytoid lineage have shown that these cells synthesize and secrete mostly chondroitin sulphate proteoglycans [5]. The presence of heparan sulphate proteoglycans in murine macrophages has, however, also been demonstrated [10]. Furthermore, syndecans 1 and 4 have been shown to be present in murine peritoneal macrophages and to increase in expression after stimulation of the cells [11]. Some subtypes of human monocytes must, however, contain heparan sulphate proteoglycans, as the human monocytic cell line THP-1 has the capacity to express this particular proteoglycan. Furthermore, the expression of heparan sulphate increased after stimulation with PMA [36]. These findings indicate that inflammatory human monocytes can indeed express heparan sulphate proteoglycans, which may have implications for the uptake of oxLDL and native LDL in these cells. In the murine macrophage cell line P388D1, the presence of both heparan sulphate and chondroitin sulphate proteoglycans on the cell surface could furthermore be demonstrated. The former proteoglycan was assumed to be syndecan 4 [14]. Here we show that the cell surface of J774 macrophages contain both heparan sulphate and chondroitin sulphate proteoglycans. The additional presence of free glycosaminoglycan chains from both types of proteoglycans may result from unspecific degradation or be the result of defined cellular processes. It has been shown in other cell systems that the cell surface may contain free glycosaminoglycan chains [37,38], and it has been suggested that these species may have functions independent of proteoglycan precursors [39]. Biological functions related to the presence of free glycosaminoglycan chains on cell surfaces have not been studied in great detail. From our experiments it is not possible to conclude whether it is the cellular proteoglycans or the glycosaminoglycans that play a role in the uptake of oxLDL and native LDL in J774 cells.

It is well established that heparan sulphate can bind apolipoproteins B and E and present LPL on the surface of endothelial cells [2–4]. It has further been demonstrated that cell-surface heparan sulphate is involved in the binding of very-low-density lipoprotein, chylomicron remnants and LDL in fibroblasts and HepG2 cells [40,41]. The binding of LDL to cell surfaces has also been shown to be stimulated by the presence of LPL, possibly through a bridge-forming mechanism by the enzyme between cell-surface heparan sulphate and LDL [42,43]. It has also been shown in the human monocytic cell line THP-1, induced to differentiate to macrophages, that the binding of LPL to the cells increased concomitantly with an increased expression of cell-associated heparan sulphate [36]. Furthermore, it has been demonstrated that the uptake of oxLDL by J774 macrophages was stimulated in the presence of LPL, an effect that was abolished after treatment with heparitinase [44]. Data presented here show that the LPL-dependent uptake of lipoproteins involves $NaClO_3$-sensitive pathways, further supporting the role of heparan sulphate proteoglycans for such uptake in macrophages [45]. LPL-mediated uptake of lipoproteins has previously been demonstrated in other cell types [46,47]. Our data clearly show that J774 cells express both heparan sulphate and chondroitin sulphate proteoglycans on the cell surface. Furthermore, removal of the former decreased the binding of both native and oxLDL and also affected the stimulatory effect of LPL addition, which suggests that both direct interactions between the lipoproteins and the cell-surface proteoglycans/glycosaminoglycans, and LPL-mediated interactions may be in operation at the cell surface. It is also possible that endogenously synthesized LPL [24] may contribute to the proteoglycan-dependent binding of oxLDL and native LDL in J774 cells. Our data using C-ABC and heparitinase also show that there is a difference between oxLDL and native LDL with regard to which type of glycosaminoglycan chains are important for cell binding. oxLDL depends primarily on heparan sulphate for binding, whereas native LDL depends on both chondroitin sulphate and heparan sulphate. A recent report has demonstrated that the syndecan family of proteoglycans can mediate a direct cellular internalization of lipoproteins [48]. Similar syndecan-dependent mechanisms may be operating in macrophages.

However, in macrophages there also exist other proteoglycan-dependent mechanisms for lipoprotein binding. In the P388D1 macrophage-like cell line, a secreted chondroitin sulphate proteoglycan was shown to bind to LDL, suggesting that such released proteoglycans may also be involved in the regulation of

LDL uptake in macrophages [49]. Interestingly, it has also been demonstrated that a proteoglycan form of macrophage colony stimulating factor, which carries chondroitin sulphate chains, formed complexes with LDL and such complexes could be detected in the arterial wall [50]. Secreted proteoglycans may, accordingly, play a role in regulating the access of both native and oxLDL to the cell surface of macrophages. In other macrophages, such as those from pigeon, it has been demonstrated that chondroitin sulphate proteoglycans released under *in vitro* conditions interact with LDL [51]. Versican synthesized by smooth-muscle cells also forms complexes with LDL. The formation of the latter complexes renders the lipoproteins more susceptible to oxidation, resulting in increased uptake in macrophages [3,4,52]. Such complexes have been isolated from atherosclerotic lesions in rabbits and have been shown to stimulate the accumulation of cholesteryl ester in macrophage-derived foam cells [53]. These findings, in addition to those reported here, suggest that both heparan sulphate and chondroitin sulphate glycosaminoglycans or proteoglycans may play a role in the uptake of lipoproteins in macrophages, and possibly participate in the generation of macrophage foam cells.

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