Interaction of Native and Cell-modified Low Density Lipoprotein with Collagen Gel

Gayle A. Hoover, Suzanne McCormick, and Norman Kalant

We have examined the binding of native and cell-modified low density lipoprotein (LDL) to gels of Type I collagen. Diffusion of native 125I-LDL into the collagen gel was slow, reaching equilibrium after 24 to 48 hours, while L-3H-glucose, a low molecular weight marker, equilibrated in 6 hours. Binding of 125I-LDL was measured at 48 hours as the amount associated with the collagen after extensive washing. Binding was saturable with an increasing concentration of LDL. Prior incubation with cell-free culture medium resulted in modest, but progressive, increases in electrophoretic mobility and binding to collagen. Incubation with cells produced a marked increase in electrophoretic mobility and a 5- to 10-fold increase in collagen binding; the presence of butylated hydroxytoluene during incubation prevented both effects. These changes in LDL were induced by porcine aortic endothelial cells, smooth muscle cells, human skin fibroblasts, and a variety of cell lines, as well as by acetylation. There was a curvilinear relationship between the amount of LDL protein bound and the net negative charge of the LDL; increasing net charge was associated with progressively greater increases in binding. These results suggest a potential role for collagen in trapping lipid in the extracellular matrix of arterial intima by slowing the diffusion of and by binding LDL. The data also demonstrate that binding of LDL to collagen is enhanced by modifications that increase its net negative charge. (Arteriosclerosis 8:525–534, September/October 1988)

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The present study was designed to examine the in vitro interaction of native and cellularly modified LDL with type I collagen. We have used a collagen gel system to evaluate LDL diffusion into a collagen matrix and to measure its binding to the collagen fibers.

Methods

Cell Culture

The procedure of Gimbrone et al.\(^4\) for releasing EC from umbilical cord veins was adapted for obtaining EC from descending thoracic aortas of young pigs. Porcine aortic smooth muscle cells (SMC) were grown from explants of the middle layers of media from the arch region.\(^47\) Cultures of human skin fibroblasts (HSF) were established from single cell suspensions obtained by enzymatic dispersion of normal foreskin dermis\(^48\) of patients ages 7 months to 30 years. Stock cultures of all cell types were grown in 100 mm tissue culture dishes in Dulbecco's modified Eagle's medium (DMEM) (Flow Laboratories, Incorporated, Mississauga, Ontario) supplemented with 1 mM nonessential amino acids (GIBCO Canada, Burlington, Ontario) and sodium bicarbonate (2.25 g/l). For EC and HSF, the growth medium contained 10% (vol/vol) defined calf bovine serum (supplemented) (HyClone Laboratories, Incorporated, Logan, Utah) heat inactivated at 56°C for 30 minutes. SMC received 10% (vol/vol) fetal bovine serum (FBS) instead of the special calf serum. In addition, after the first day in primary culture, EC received EC growth factor in the form of a crude extract of bovine brain\(^48\) at 150 µg protein/ml medium. For EC and SMC, culture dishes were coated with human plasma fibronectin as previously described.\(^50\) All cell types were passaged at confluence once weekly by exposure to 0.05% trypsin/0.02% disodium EDTA in Earle's salt solution (GIBCO), followed by a wash in serum-containing medium. They were redistributed at 1:10 (SMC) or 1:20 (HSF, EC) split ratio and given fresh medium every 2 days.

To prepare cultures for incubating with LDL, cells between the second and fifteenth passages were seeded in 60 mm tissue culture dishes 1 week before use and grown to confluence under conditions described above for stock cultures.

Madin-Darby canine kidney cells (MDCK) and rat hepatoma cells (H4-II-E-C3) were from the American Type Culture Collection (ATCC, Rockville, Maryland) and were maintained for other purposes in this laboratory. They were cultured under conditions recommended by the ATCC. Human nongenital skin fibroblasts (from a 13-month-old donor) grown in Eagle's Minimal Essential Medium (GIBCO) with 10% FBS and Chinese hamster lung fibroblasts grown in DMEM with 5% FBS were gifts from Morris Kaufman and Ralph Germinario of this institute. Rabbit VX2 carcinoma cells donated by Hanoch Alpern-EIlan of this institute were a line grown in DMEM with 10% FBS from primary cultures of an enzyme-digested VX2 tumor.\(^51\) These cells were seeded 1 week before experiments. All cell cultures were maintained at 37°C in an atmosphere of 95% air/5% CO\(_2\) with a relative humidity of 95%.

Preparation of Low Density Lipoprotein

Samples of normolipemic serum from the diagnostic laboratories of the Jewish General Hospital were pooled. LDL was isolated from this serum after addition of 0.01% disodium EDTA by precipitation with heparin and MnCl\(_2\).\(^52\) To remove the very low density lipoprotein (VLDL) that coprecipitated, the pellet was redissolved in cold NaCl at density 1.084 g/ml and was pipetted into ultracentrifuge tubes under a layer of warm NaCl at density 1.006 g/ml; after centrifugation in an SW40 rotor at 35 000 rpm for 24 hours at 15°C, the VLDL formed a layer at the top and the golden LDL layer was removed from the center of each tube by puncture with a needle and syringe. The pooled LDL layers were dialyzed exhaustively against 0.9% NaCl:0.01% EDTA (pH 8.6). LDL isolated by this process retained its normal density range of 1.019 to 1.063 g/ml. For certain experiments as noted, LDL was prepared by centrifugation in KBr solutions as the fraction of density between 1.019 and 1.063 g/ml. Protein concentration was determined by the method of Lowry et al.\(^53\) and the purity of the LDL preparation was confirmed by electrophoresis on agarose gel stained for lipid\(^4\) and by electrophoresis on cellulose acetate stained for protein and quantitated by scanning densitometry.\(^55\)

Freshly prepared LDL was iodinated (\(^125\)I-LDL) with carrier-free Na\(^125\)I (Amersham Canada Limited, Oakville, Ontario) according to the iodine monochloride procedure of McFarlane\(^56\) as modified by Shepherd et al.\(^57\) After dialysis against the NaCl: EDTA buffer, more than 98% of the radioactivity was precipitable by cold 10% (wt/vol) trichloroacetic acid (TCA) and less than 3% was extractable in chloroform/methanol (2:1).\(^58\) The final specific activities ranged from 90 to 150 cpm/ng LDL protein.

Acetylated LDL was prepared as described by Basu et al.\(^59\) All LDL preparations were sterilized by filtration through a 0.22 µm membrane, were stored in the dark at 4°C, and were used within 3 weeks.

Incubation of Low Density Lipoprotein with Cells

Confluent cell cultures in 60 mm dishes were rinsed twice with Dulbecco's phosphate-buffered saline (PBS, GIBCO). They were incubated with 2 ml of serum-free Ham's F-12 medium (Flow), or Ham's F-10 medium (GIBCO) as specified, containing 200 µg \(^125\)I-LDL protein/ml. Parallel dishes without cells were incubated in the same fashion to provide what is referred to in this paper as cell-free modified (CF-modified) LDL. After 24 hours, the conditioned medium was collected sterilely and centrifuged for 10 minutes at 1000 g to remove cell debris. An aliquot was taken to measure the amount of radioactivity precipitable in cold 10% TCA; this was routinely in the range of 92% to 96%. The conditioned medium was diluted in DMEM to the desired concentration of \(^125\)I-LDL protein to be added to collagen gels prepared as described below.

For purposes of electrophoresis, cold LDL (200 µg protein/ml) was incubated for 24 hours with or without cells, and the conditioned medium was concentrated to
approximately 2 mg LDL protein/ml in a dialysis bag (MW retention limit of 3500) immersed in a thick slurry of polyethylene glycol (MW 8000, BDH Chemicals, Toronto) in water. Agarose gel electrophoresis was done at pH 8.6 in barbital buffer with a Panagel Electrophoresis Reagent Set (Princeton Separations Incorporated, Adelphia, NJ) as specified by the manufacturer for lipoprotein electrophoresis. Of the concentrated medium containing modified LDL, 20 μl was applied to the agarose gel slide. Native (unincubated) LDL was diluted to the same concentration and was applied as a control in each electrophoretic run. Dried gels were stained with Sudan Black B dye according to Johansson. Diffusion traveled by each sample was measured in millimeters from the origin to the front of the band. Relative mobility was expressed as a ratio of the migration distance of sample to that of native LDL.

For most experiments, EC were used to modify LDL. 125I-LDL incubated with EC was modified as verified by its increased buoyant density on a sucrose gradient to a peak density of 1.0754 g/ml and by its increased electrophoretic mobility on agarose gel to an average of 2.5 times the distance travelled by native LDL. This will be referred to as EC-modified LDL. The peak buoyant density of CF-modified LDL was slightly increased (1.0537 g/ml); its electrophoretic mobility also increased slightly to an average of 1.5 times that of native LDL.

Preparation of Collagen Gels

Type I collagen was extracted from rat-tail tendon as described by Elsdale and Bard but with dialysis against water adjusted to pH 4.0 instead of against dilute medium. All reagents and equipment for the extraction were sterilized. The final collagen concentration of individual preparations as determined by dry weight was 2 to 2.5 mg/ml.

Collagen gels were prepared by mixing rapidly on ice the collagen solution, the concentrated DMEM, and water to achieve the desired final concentration of collagen (generally 1 mg/ml) in 1 × DMEM. One milliliter aliquots of this solution were pipetted into 35 mm culture dishes and incubated at 37°C 5% CO2/95% air to allow the collagen to gel completely. After 1 hour, the gels were covered to gel the overlying medium with radioactive markers. The percent-concentration of CF-modified LDL was slightly increased (1.0537 g/ml); its electrophoretic mobility also increased slightly to an average of 1.5 times that of native LDL.

Measurement of 125I-Low Density Lipoprotein Binding to Collagen Gel

To measure the amount of 125I-LDL bound to the collagen gel matrix under various conditions, gels were prepared as described above. Preparations of 125I-LDL were added to the overlying medium at the desired concentrations and dishes were incubated for 48 hours. The overlying medium was aspirated and the gel was rinsed with 1 ml of PBS, all volumes were brought to 10 ml with PBS, and 0.5 ml was mixed with 5.0 ml scintillation fluid for counting in a scintillation spectrophotometer. For samples containing 125I-LDL, final volumes were adjusted to 4 ml; protein was precipitated by 10% (wt/vol) cold TCA by using a collagen standard.

Results

Diffusion of Low Density Lipoprotein Into Collagen Gel

The collagen gel system consisted of two compartments: the gel, containing 1 ml total volume of collagen fibers and interstitial medium and a 1 ml volume of overlying medium with radioactive markers. The percentage of total radioactivity that was contained within the gel compartment was determined at intervals up to 48 hours after addition of markers to the overlying medium (Figure 1). Although the diffusion of L-3H-glucose from the overlying medium into the interstitial medium of the gel matrix had reached equilibrium by 6 hours, native 125I-LDL diffused into the gel more slowly, reaching equilibrium only after 24 to 48 hours. The presence of 500 μg/ml of mixed GAG had no additional effect on the rate of 125I-LDL diffusion.

EC-modified 125I-LDL initially entered the gel at the same rate as native 125I-LDL up to 4 hours but was retained within the gel matrix; after 24 hours, 60% was in the gel compartment (Figure 2). CF-modified 125I-LDL behaved similarly to native LDL.
Binding of Low Density Lipoprotein to Collagen Gel

The time course of binding of $^{125}$I-LDL to collagen (Figure 3) indicates that binding was not complete by 48 hours; however, binding was routinely measured at 48 hours because at this time the amount bound was directly proportional to the concentration of the collagen gel over the range of 0.1 to 2 mg/ml of collagen (Figure 4). The amount of native or modified $^{125}$I-LDL bound increased with increasing concentration of $^{125}$I-LDL in the medium and in each case appeared to approach a saturation level (Figure 5). The amount of modified LDL bound was considerably greater than that of native LDL.

To determine if the increased capacity of EC-modified LDL to bind to collagen depended on the prior exposure of LDL to EC themselves or to factors secreted by EC into the medium, native $^{125}$I-LDL was incubated with F-12 medium previously exposed to an EC monolayer for 24 hours; when incubated with collagen gel (1 mg/ml), LDL (33 μg LDL protein/ml) bound to the same extent as $^{125}$I-LDL previously incubated in fresh medium (0.68 μg vs. 0.62 μg of LDL protein/mg collagen, respectively).

The amount of CF-modified $^{125}$I-LDL bound to collagen could be increased by longer incubation times in F-12
medium (Table 1). This increase was accompanied by a progressive increase in LDL mobility on agarose gel. Nevertheless, the electrophoretic mobility and amount bound to collagen of 125I-LDL incubated with EC for 24 hours in the same medium were greater than those of 125I-LDL incubated without cells for up to 72 hours. Changes in binding and in electrophoretic mobility by EC modification were similar for LDL prepared by heparin-manganese precipitation and by ultracentrifugation in a density gradient (Table 1). These effects on LDL of exposure to EC or to cell-free medium could be prevented by the addition of butylated hydroxytoluene (BHT) to the medium during incubation (Table 2) and, therefore, were probably due to oxidation.

To establish whether the cell-induced changes in binding of LDL to collagen were specific to EC, we compared binding of 125I-LDL previously incubated with EC, SMC, or HSF. All cell types modified LDL as indicated by LDL's increased electrophoretic mobility on agarose gel (Figure 6) and by increased binding to collagen gel matrix. Because others have reported that skin fibroblasts incubated in F-10 medium do not modify LDL, we compared LDL modification by the three cell types in F-10 and F-12 media. Although the effects were smaller in F-10 than in F-12, both media permitted modification of LDL by all cell types, as indicated by electrophoretic mobility. Furthermore, collagen binding of HSF-modified 125I-LDL in both media was greater than that of SMC- and EC-modified 125I-LDL (Figure 7). We tested several strains of human foreskin fibroblasts, as well as human nongenital skin fibroblasts, with similar results. We also measured the electrophoretic mobility and collagen binding of 125I-LDL incubated with several cell lines unrelated to the vascular system. In all cases, incubation of 125I-LDL with these cells resulted in an increase in its electrophoretic migration on agarose gel and in its binding to collagen gel matrix to a degree similar to or greater than EC (Table 3).

The changes in oxidatively modified LDL are similar to those in acetylated LDL in terms of uptake by specific cell receptors on macrophages. To determine if acetylation of LDL also changed its capacity to bind to collagen in a manner similar to oxidative modification, we measured the binding of various concentrations of acetylated 125I-LDL in that the amount of acetylated 125I-LDL bound per mg collagen was dependent on its concentration. The modification by acetylation was greater than that induced by incubation with EC for both binding to collagen and relative electrophoretic mobility (3.8 × native for acetylated LDL, 2.9 × native for EC-modified LDL in this experiment).

For all types of modified LDL combined, the relationship between the electrophoretic migration relative to that of

![Figure 5](image_url)  
**Figure 5.** Effect of 125I-LDL concentration on binding of native and modified 125I-LDL to collagen. Collagen gels (1 mg/ml/dish) were prepared with 1 ml of overlying DMEM containing native A), CF-modified (C), or EC-modified (g) 125I-LDL at various concentrations. The amount of radioactivity associated with collagen gels washed with PBS was measured after a 48-hour incubation at 37°C as described in Methods. The combined results of three experiments are shown, each point representing the mean of three to four replicates (coefficient of variation, 10% or less).

### Table 1. Effect of Incubation Time on Relative Electrophoretic Mobility and Binding to Collagen Gel of Modified Low Density Lipoprotein

<table>
<thead>
<tr>
<th>LDL preparation</th>
<th>Incubation time (hrs)</th>
<th>Relative electrophoretic mobility</th>
<th>125I-LDL bound (µg protein/mg gel protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Native</td>
<td>0</td>
<td>1.0</td>
<td>0.90 ± 0.06</td>
</tr>
<tr>
<td>CF-modified</td>
<td>6</td>
<td>1.1</td>
<td>0.84 ± 0.10</td>
</tr>
<tr>
<td>CF-modified</td>
<td>12</td>
<td>1.1</td>
<td>0.82 ± 0.05</td>
</tr>
<tr>
<td>CF-modified</td>
<td>24</td>
<td>1.3</td>
<td>1.09 ± 0.12</td>
</tr>
<tr>
<td>CF-modified</td>
<td>48</td>
<td>1.7</td>
<td>2.17 ± 0.14</td>
</tr>
<tr>
<td>CF-modified</td>
<td>72</td>
<td>2.0</td>
<td>4.95 ± 0.27</td>
</tr>
<tr>
<td>EC-modified</td>
<td>24</td>
<td>2.4</td>
<td>10.47 ± 0.55</td>
</tr>
<tr>
<td>Native (KBr)</td>
<td>0</td>
<td>1.0</td>
<td>0.63 ± 0.13</td>
</tr>
<tr>
<td>CF-modified (KBr)</td>
<td>24</td>
<td>1.2</td>
<td>0.86 ± 0.05</td>
</tr>
<tr>
<td>EC-modified (KBr)</td>
<td>24</td>
<td>2.5</td>
<td>14.81 ± 1.24</td>
</tr>
</tbody>
</table>

LDL (200 µg protein/ml) was incubated in 2 ml F-12 medium for various lengths of time without cells (CF) or for 24 hours with endothelial cells (EC). Mobility of modified LDL relative to native LDL on agarose gel electrophoresis and the amount of radioactivity associated with washed collagen gels (1 mg/ml) 48 hours after the addition of 125I-LDL (50 µg protein) to the overlying medium were assessed as described in Methods. Binding data are the means ± SD of four replicates. (KBr) indicates that the LDL was prepared by ultracentrifugation in KBr, density 1.019 to 1.063 g/ml.
Table 2. Effect of BHT on Relative Electrophoretic Mobility and Binding to Collagen Gel of Low Density Lipoprotein Preparations

<table>
<thead>
<tr>
<th>LDL preparation</th>
<th>BHT during incubation</th>
<th>BHT added after incubation</th>
<th>Relative electrophoretic mobility</th>
<th>^125I-LDL bound (µg protein/mg gel protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Native</td>
<td>-</td>
<td>-</td>
<td>1.0</td>
<td>0.82 ± 0.06</td>
</tr>
<tr>
<td>CF-incubated</td>
<td>-</td>
<td>-</td>
<td>1.4</td>
<td>1.08 ± 0.13</td>
</tr>
<tr>
<td>CF-incubated</td>
<td>+</td>
<td>-</td>
<td>1.2</td>
<td>0.76 ± 0.09</td>
</tr>
<tr>
<td>CF-incubated</td>
<td>-</td>
<td>+</td>
<td>1.3</td>
<td>1.18 ± 0.02</td>
</tr>
<tr>
<td>EC-incubated</td>
<td>-</td>
<td>-</td>
<td>2.4</td>
<td>9.92 ± 0.11</td>
</tr>
<tr>
<td>EC-incubated</td>
<td>+</td>
<td>-</td>
<td>1.4</td>
<td>1.07 ± 0.08</td>
</tr>
<tr>
<td>EC-incubated</td>
<td>-</td>
<td>+</td>
<td>2.5</td>
<td>9.80 ± 0.08</td>
</tr>
</tbody>
</table>

LDL (200 µg protein/ml) was incubated for 24 hours in 2 ml F-12 medium, with endothelial cells (EC) or without cells (CF); butylated hydroxytoluene (BHT) was added to a concentration of 20 µM during or after the incubation. Mobility of modified LDL relative to native LDL on agarose gel electrophoresis and the amount of radioactivity associated with washed collagen gels (1 mg/ml) 48 hours after the addition of ^125I-LDL (50 µg protein) to the overlying medium were assessed as described in Methods. Binding data are the means ± SD of four replicates.

Figure 6. Agarose gel electrophoresis of native and modified LDL. LDL (200 µg protein/ml) was incubated for 24 hours in F-12 medium with porcine aortic endothelial cells (EC), porcine aortic smooth muscle cells (SMC), human foreskin fibroblasts (HSF), or without cells (CF) and prepared for electrophoresis as described in Methods. Native, unincubated LDL (N) served as the control. The arrow indicates the origin. The amount of modified ^125I-LDL bound to collagen gel protein in this experiment (50 µg ^125I-LDL protein added/dish) was for CF, 2.00 ± 0.07 µg/mg, for HSF, 15.58 ± 0.35, for SMC, 11.25 ± 0.27, for EC, 12.02 ± 0.44 (means ± SD, n = 4).

Figure 7. Binding to collagen gel of ^125I-LDL modified by incubation with various cell types in Ham's F-10 and F-12 media. ^125I-LDL (200 µg protein/ml) was incubated in 2 ml of F-10 or F-12 medium without cells (CF) or with human foreskin fibroblasts (HSF), porcine aortic smooth muscle cells (SMC), or porcine aortic endothelial cells (EC) for 24 hours. Binding to collagen gels (1 mg/ml) was measured 48 hours after addition of modified ^125I-LDL (50 µg protein/dish). Means ± SD of four replicates are shown. Numbers above bars are the migration distance of modified LDL on agarose gel electrophoresis relative to native LDL.

native LDL and the amount bound per milligram of collagen gel was expressed most accurately by a curve of the type:

\[ y = ax^b \]

where \( y \) is the amount bound (µg LDL protein/mg collagen) and \( x \) is the mobility relative to that of native LDL under our standard conditions of electrophoresis (Figure 9). The curve was the same whether or not acetylated LDL was included in the data. Thus, the slope of the curve (the change in amount of LDL bound/mg collagen gel for a given change in relative migration) increased as relative migration increased.

Discussion

The concentration of LDL in arterial intima, especially in fibrous plaques, is higher than in plasma.22 One of the proposed mechanisms by which LDL may accumulate in the extracellular space of arterial intimas during atherosclerosis is that GAG may act as a molecular sieve, thus increasing the intimal LDL concentration relative to plasma LDL.30,31 This hypothesis is based on the observation that hyaluronic acid at concentrations of 0.3% (wt/vol) or less slows the flotation rates of serum lipoproteins during CsCl ultracentrifugation.30 In the present study, the diffusion of ^125I-LDL, both native and oxidatively modified, from overlying medium into the interstitial medium of a 0.1% (wt/vol) native collagen gel was retarded compared with that of small molecules measured by the diffusion of L-3H-glucose. Thus, the collagen matrix exerted a molecular sieving effect. The effective "pore" size of the gel was large enough that the diffusion of albumin was not retarded.81 We found that 0.05% (wt/vol) mixed GAG added to the collagen gel and overlying medium had no further effect on the diffusion rate of ^125I-LDL. This ratio of GAG/collagen (0.5:1) was greater than that found by Smith37 in normal intima and fatty streaks (0.11), fibrous plaques (0.06), or calcified plaques (0.02). Therefore,
Table 3. Relative Electrophoretic Mobility and Binding to Collagen Gel of Low Density Lipoprotein Incubated with Various Cell Types

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Relative electrophoretic mobility</th>
<th>125I-LDL bound (µg protein/mg gel protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell-free</td>
<td>1.3</td>
<td>0.64 ± 0.09</td>
</tr>
<tr>
<td>Porcine aortic</td>
<td>2.5</td>
<td>6.87 ± 0.45</td>
</tr>
<tr>
<td>endothelial cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat hepatoma</td>
<td>2.6</td>
<td>6.56 ± 0.62</td>
</tr>
<tr>
<td>Rabbit VX2 carcinoma</td>
<td>2.8</td>
<td>9.47 ± 0.73</td>
</tr>
<tr>
<td>Chinese hamster lung fibroblasts</td>
<td>2.9</td>
<td>11.40 ± 0.87</td>
</tr>
<tr>
<td>Medin-Darby canine kidney</td>
<td>2.9</td>
<td>(not tested)</td>
</tr>
</tbody>
</table>

LDL (200 µg protein/ml) was incubated in 2 ml F-12 medium for 24 hours without cells or with the type of cell indicated. Mobility of modified LDL relative to native LDL on agarose gel electrophoresis and the amount of radioactivity associated with washed collagen gels (1 mg/ml) 48 hours after the addition of 125I-LDL (50 µg protein) to overlying medium was assessed as described in Methods. Binding data are the means ± SD of four replicates.

Because of the relative abundance of collagen in vivo, our data imply that collagen may contribute much more than GAG to any potential molecular sieving effect by the extracellular matrix. However, it is not known what effect incorporation into proteoglycan complexes has on the effectiveness of GAG.

Another means by which LDL may be retained in the arterial intima is by binding to elements of the extracellular matrix. Amenta and Waters suggested such a role for collagen by demonstrating the ability of gelatin solutions to precipitate lipoproteins from plasma. Our data clearly illustrate the capacity of type I collagen to bind LDL apoprotein in vitro under physiological conditions of pH, temperature, and ionic strength. Binding was saturable and the amount bound depended on the concentration of collagen. Other workers have shown a transfer of phospholipid and of free and esterified cholesterol from LDL to other workers have shown a transfer of phospholipid and of free and esterified cholesterol from LDL to collagen by demonstrating the ability of gelatin solutions to precipitate lipoproteins from plasma. Our data imply that collagen may contribute much more than GAG to any potential molecular sieving effect by the extracellular matrix. However, it is not known what effect incorporation into proteoglycan complexes has on the effectiveness of GAG.

Figure 8. Binding to collagen gel of acetylated 125I-LDL (x), 125I-LDL incubated with EC (b) or without cells (c), and native 125I-LDL (a). Radioactivity remaining with washed collagen gels (1 mg/ml/dish) was measured 48 hours after addition to 1 ml of overlying medium of modified or native 125I-LDL at the indicated concentrations. Points represent the means ± SD of four replicates.

Figure 9. Relationship of relative electrophoretic mobility on agarose gel to collagen binding of all types of modified LDL. Data are from Tables 1 and 2, Figures 6, 7, and 8, as well as other experiments in which 125I-LDL (50 µg protein/dish) was added to medium overlying collagen gels (1 mg/ml). Radioactivity associated with washed collagen gels was measured 48 hours after addition of LDL. The curve was fitted by the method of least squares. For all points combined, y = 0.6655x + 0.0864, r² = 0.92. where y = 125I-LDL bound, x = relative migration, for all points except that for acetylated LDL, y = 0.6656x + 0.084, r² = 0.91.

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Another means by which LDL may be retained in the arterial intima is by binding to elements of the extracellular matrix. Amenta and Waters first suggested such a role for collagen by demonstrating the ability of gelatin solutions to precipitate lipoproteins from plasma. Our data clearly illustrate the capacity of type I collagen to bind LDL apoprotein in vitro under physiological conditions of pH, temperature, and ionic strength. Binding was saturable and the amount bound depended on the concentration of collagen. Other workers have shown a transfer of phospholipid and of free and esterified cholesterol from LDL to
increased ability to bind to type I collagen. The precise mechanistic relationships between the structural changes and the various consequences are not known. A significant degree of lipid peroxidation may occur in LDL incubated with EC in F-10 medium containing EDTA without a change in LDL degradation by macrophages, while an increase in degradation by macrophages may occur without lipid peroxidation. At the present time, we have no explanation for the mechanism of the increase in binding to collagen by modified LDL. Haberland et al. have shown that the increase in LDL uptake by macrophages can be induced by malondialdehyde modification of a specific group of lysine residues, which constitute only 16% of the total lysine of the LDL. This is associated with a minimal change in electrophoretic mobility. Further modification of lysines causes a progressive linear increase in LDL mobility with no further change in macrophage uptake. The relationship between electrophoretic mobility and binding to collagen was quite different, as increasing mobility was associated with progressively greater changes in binding. Thus, the sites involved in collagen binding are apparently unrelated to the binding sites for the macrophage receptor. The nature of the curve relating collagen binding to mobility suggests positive cooperativity of binding; this might be due to progressive binding-induced changes in collagen conformation that expose an increasing number of reactive sites or, more likely, to progressive modification of lysine residues that leads to an increasing change in conformation and exposure of reactive sites on the LDL.

Henriksen et al. reported that human skin fibroblasts were unable to increase the density of LDL (presumably the result of removal of lipid) or to increase its rate of degradation by macrophages. Morel et al. found no difference in electrophoretic mobility of LDL incubated with fibroblasts. However, we observed that a wide variety of cell types, including HSF, were capable of altering the electrophoretic mobility of LDL and its binding to collagen. The discrepancies may be related to the culture media used during exposure of LDL to the cells. Transition metals which facilitate free radical reactions play an important role in cell-mediated modification of LDL: copper and iron have been shown to promote modification by EC and SMC, and, when the concentration is high enough, copper alone, in the absence of cells, can bring about the same modification. Morel et al. used medium 199, which contains no copper or zinc and a lower concentration of iron than F-10 and F-12; Henriksen et al. used F-10, which has a much lower zinc content than F-12 and which we found to be less effective than F-12 even with EC and SMC. Thus it appears that different cell types have different requirements for metal ions "promoters" but that in the appropriate milieu many cell types can modify LDL.

Our results suggest another way in which modified LDL may contribute to atherogenesis, in addition to its proposed role in injuring cells, recruiting monocytes, inducing foam cell formation, and inhibiting their clearance from the arterial wall. Oxidative changes in the LDL molecule, which may occur upon its exposure to vascular cells as it passes through the intima, could render it highly susceptible to entrapment in the extracellular matrix by its binding to collagen. Furthermore, this study illustrates the potential importance of type I collagen in contributing to the retention of lipid in the arterial wall. By slowing the passage of LDL, collagen may increase LDL concentration, thus favoring its binding to collagen, as suggested here, and possibly to other extracellular elements. The binding of LDL, in turn, would increase the amount of extracellular lipid, contributing to the development of atherosclerotic plaques.

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