Platelet LDL Receptor Recognizes With the Same Apparent Affinity Both Oxidized and Native LDL

Evidence That the Receptor-Ligand Complexes Are Not Internalized

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Abstract We have recently demonstrated that the platelet low-density lipoprotein (LDL) receptor is immunologically different from the "classic" receptor of nucleated cells. We undertook the current studies to investigate the interaction of this receptor with oxidized LDL and to determine whether an endocytosis-mediated response is involved in the binding of LDL to platelets. The platelet LDL receptor recognized with the same affinity both native and oxidized LDL particles (IC_{50}, 0.045 and 0.054 g/L; K_{d}, 45.8 and 65.9 nmol/L, respectively). The Hill coefficients of the displacement of ^{125}I-LDL binding were -1.10 and -1.05 for unlabeled native and oxidized LDL, respectively, thereby suggesting a single set of binding sites. To ascertain whether human platelets bind oxidized LDL, we performed ligand binding assays with ^{125}I-oxidized LDL. Saturation curves of ^{125}I-oxidized LDL binding at 22°C showed that human platelets bound these modified particles to a class of saturable binding sites, numbering approximately 3895±241 per platelet with a dissociation constant (K_{d}) of 96.2±10.3 nmol/L. Displacement experiments showed that ^{125}I-oxidized LDL binding was inhibited with the same affinity by both oxidized and native LDL (IC_{50}, 0.055 and 0.065 g/L; K_{d}, 88 and 64 nmol/L, respectively). The Hill coefficients of the displacement of the ^{125}I-oxidized LDL binding were -1.02 and -1.07 for unlabeled oxidized and native LDL, respectively, suggesting that a single set of binding sites is implicated. Moreover, oxidized LDL at a protein concentration of 0.5 g/L enhanced ADP- and collagen-induced platelet aggregation in a manner similar to native LDL. We present ample evidence to show that LDL binding to platelets is not mediated by an endocytotic process. First, dissociation studies showed that at 4°C, 22°C, and 37°C approximately 93% of the surface-bound LDL was dissociated and that in the absence and presence of an ATPase inhibitor such as sodium azide, similar values for the reverse rate constant (K_{r}, 6±2 and 8.9±3x10^{-4} s^{-1} mol/L^{-1}) and half-time (25±5 and 30±4 minutes) were found. Second, the lack of a blocking effect on LDL binding to platelets of microtubule inhibitors (colchicine), acidotropic agents (ammonium chloride and chloroquine), and Golgi apparatus disruptors (monensin) was found. Third, the inability of platelets to degrade ^{125}I-LDL was shown; finally, imipramine and dopamine, which fully prevented platelet [^14]C]serotonin uptake, were unable to alter the binding characteristics of ^{125}I-LDL to platelets. Overall, these results indicate that the platelet LDL receptor does not mediate an endocytotic response. Given that both native and oxidized LDL are recognized by the platelet LDL binding sites, we suggest that the receptor may play a role in the thrombogenicity of atheromatous plaques of hypercholesterolemic patients. (Arterioscler Thromb. 1994;14:401-408.)

Key Words • LDL • oxidized LDL • platelet aggregation • binding sites • metabolic inhibitors

It is widely accepted that platelets are implicated in the initiation of atherosclerotic lesions and that they play a major role in the later thrombotic complications that are expressed as clinical evidence of the disease process. Moreover, the evidence, both clinical and experimental, leaves no doubt that lipoproteins are important in the development of atherosclerotic lesions. Recently, different authors have demonstrated that low-density lipoprotein (LDL) enhances platelet reactivity. Moreover, LDL has been considered a thrombogenic factor involved in coronary artery thrombosis. The interaction between LDL and platelets may explain the increased thrombogenicity described in atheromatous plaques of hypercholesterolemic rabbits. It has also been postulated that the initial step in the modulation of the platelet reactivity by LDL is the binding of this particle to specific binding sites. Since first descriptions of the existence of specific, high-affinity, and saturable binding sites for LDL in platelets were given, some evidence has indicated that they could be different from the "classic" LDL receptor of nucleated cells. Recently, Koller et al have purified an LDL binding protein from human platelet membranes with an apparent molecular mass of 140 kD, suggesting that this protein could be related to or perhaps the same as the fibrinogen receptor (glycoprotein IIb/IIIa complex). Furthermore, we have recently reported that the human platelet LDL binding sites are immunologically different from the classic LDL recep-
tor of nucleated cells. A striking result arising from our studies was that when platelets were loaded with cholesterol by a receptor-independent process, receptor regulation (upregulation and downregulation) did not occur. Other authors had already reported that guinea pig platelets incubated with plasma from hypercholesterolemic animals did not accumulate excess cholesterol. Several groups have noted that platelets are unable to degrade the apolipoprotein of LDL. All these results suggest that the LDL receptor-mediated endocytosis described in nucleated cells is not involved in the interaction between LDL and platelets. In contrast, it has been reported recently that platelets take up and degrade LDL in a manner reminiscent of phagocytic cell types, albeit differently from nucleated cells. Moreover, it is well known that modification of LDL principally by cellular oxidation (oxidized LDL) could play an important role in the pathogenesis of the atherosclerotic process. It has been postulated that oxidized LDL in the intima is removed through the scavenger receptor, thus enhancing cholesteryl ester accumulation in macrophages, leading to foam cell formation. It has been demonstrated that oxidized LDL also enhances platelet reactivity. Furthermore, there are no data concerning the possible interaction between oxidized LDL and the platelet LDL receptor, nor is there any evidence that platelets bind oxidized LDL. In the present study we investigated the interaction of oxidized LDL with the platelet LDL receptor and the possible existence of an endocytotic response mediated by this receptor.

Methods

Materials

Bovine serum albumin (BSA) was purchased from Fluka Chemie AG; sucrose, EDTA, and ammonium chloride were supplied by E Merck; 1,3,4,6-tetrachloro-3a,6a-diphenylglycouril (Iodo-Gen), chloroquine, imipramine, and dopamine were supplied by Sigma Chemical Co; colchicine was obtained from Calbiochem Co; culture medium (minimal essential Medium [MEM]), fetal calf serum (FCS), penicillin, and streptomycin were provided by Flow Labs; plastic culture dishes and flasks were obtained from Costar; and [125I]NaI and [14C]serotonin were obtained from New England Nuclear.

Lipoproteins

Human LDL (d=1.019 to 1.063 g/mL) and lipoprotein-deficient serum (LPDS, d=1.210 g/mL) were obtained from pooled sera of normocholesterolemic volunteers and isolated by sequential ultracentrifugation for the competing ligands (nanomoles) were

Modification of LDL

LDL was oxidized by incubation of 1 mg protein with 25 μmol/L CuSO4 in phosphate-buffered saline for 24 hours at 37°C. To stop the oxidation reaction, LDL was dialyzed against buffer A containing 20 μmol/L butylated hydroxytoluene (2,6-di-tert-butyl-p-cresol-4-methyl-2,6-di-tert-butylphenol) for 24 hours at 4°C. Oxidation of LDL preparations was assessed by the following procedures: (1) thiobarbituric acid-reactive substances, (2) determination of the capacity for displacement of the binding of 125I-LDL to human fibroblasts, and (3) agarose-acrylamide gel electrophoresis mobility. Oxidized LDL was labeled as described for native LDL.

Final specific radioactivity was 1.2±0.1 Bq/ng protein; labeling efficiencies of greater than 60% were achieved; and less than 15% and 25% of the total radioactivity in the final preparation was due to the chloroform/methanol–extractable lipids and free iodide, respectively. The purity of labeled oxidized LDL was assessed by agarose-acrylamide gel electrophoresis.

Cells

Cultured human skin fibroblasts were grown and maintained in a humidified 5% CO2 incubator at 37°C as previously described. Briefly, fibroblasts were seeded at 5×10^4 in 35-mm culture wells and grown in MEM containing 10% (vol/vol) FCS, 100 U/mL penicillin, 100 μg/mL streptomycin, 24 mmol/L NaHCO3, 1 mmol/L pyruvate, and 2 mmol/L L-glutamine. The growth medium was replaced every 3 days. At confluence, the medium was removed, and the cells were incubated in LPDS (2 mL fresh medium containing 5% [vol/vol] LPDS instead of FCS) for 24 hours before the experiment.

Human platelets were isolated from freshly drawn citrated (sodium citrate 3.8%) blood of healthy volunteers. The samples were centrifuged at 300g for 10 minutes at room temperature. Platelets were sedimented from the resultant platelet-rich plasma (PRP) by centrifugation at 300g for 6 minutes. Then the platelet pellet was resuspended with incubation buffer (20 mmol/L Tris-HCl, pH 7.45, containing 0.15 mol/L NaCl and 5 g/L BSA) to give a platelet count of 10^8 platelets per liter.

Labeled Oxidized LDL Binding Assays and Competition Experiments

Washed platelets (10^9) were incubated at room temperature for 25 minutes with varying protein concentrations of 125I-oxidized LDL (up to 0.5 g/L) in a total volume of 0.25 mL incubation buffer. 125I-oxidized LDL binding to platelets was determined as previously described for 123I-native LDL. Non-specific binding was defined as binding that was not displaced by a 20-fold excess of unlabeled oxidized LDL. The displacement of 125I-oxidized LDL (0.05 g/L) in the presence of different protein concentrations of unlabeled oxidized and native LDL was measured as described above. Dissociation constants (Kd) for the competing ligands (nanomoles) were determined according to the method of Cheng and Prusoff. Displacement curves were also analyzed by Hill plots following the method reported by Bennett and Yamamura. Calculation of the bound oxidized lipoprotein was based on the specific activity of the labeled oxidized LDL, and the results were expressed as nanograms of protein bound per 10^9 platelets. The specific binding was evaluated mathematically by Scatchard analysis to determine the number of binding sites, and the dissociation constant was determined by using the KINETIC/EBDA/LIGAND program.

Aggregation Studies

Blood with 3.8% sodium citrate was collected from healthy volunteers who had not taken any drugs for the previous 10 days. PRP was obtained by centrifugation at 200g for 10 minutes at room temperature. EDTA (5.6 mmol/L) and
Fibroblasts and platelets were preincubated for 1 hour at 37°C, but internalize them at a very slow rate. In contrast, at 37°C it is possible that some receptor ligands such as LDL become internalized by cells. If platelets internalized 125I-LDL, this particle should not be able to dissociate at 37°C, and the presence of an ATPase mitochondrial inhibitor such as sodium azide could block this endocytotic process. In the present study we investigated the dissociation of 123I-LDL at 4°C and 37°C.

Platelet aggregation was performed as previously described. Platelets treated with serotonin uptake inhibitors was determined following a standard procedure. Briefly, washed platelets (4x10^6/mL) in a final volume of 0.25 mL Tyrode's buffer were incubated for 30 minutes at room temperature in the presence of adenine 0.5 μmol/L (imipramine and 10 μmol/L dopamine with [14C]serotonin (0.1 μCi/mL of platelet suspension). Then, the platelet pellet was recovered by centrifugation, and the radioactivity of the supernatant and platelet pellet was counted in a beta counter. 123I-LDL binding to both control platelets and platelets treated with serotonin uptake inhibitors was determined as indicated previously.

Fate of Platelet Surface-Bound 125I-LDL and Proteolytic Degradation Studies

To determine whether a fraction of bound labeled LDL was internalized by platelets, we used different approaches: (1) the study of binding dissociation at several temperatures, (2) the effect of microtubule inhibitors such as colchicine, (3) the effect of carboxylic ionophores such as monensin, (4) the effect of acidotropic agents such as ammonium chloride and chloroquine, (5) the effect of microtubule inhibitors such as colchicine, (3) the effect of carboxylic ionophores such as monensin, (4) the effect of acidotropic agents such as ammonium chloride and chloroquine, (5) the effect of ATPase and pinocytosis inhibitors such as ammonium chloride, colchicine, chloroquine, monensin, and sodium azide on the LDL endocytotic process in fibroblasts has been reported. There are no studies on platelets. Fibroblasts and platelets were preincubated for 1 hour at 37°C with and without the indicated concentrations of all metabolic inhibitors. Thereafter, the specific 125I-LDL binding to platelets and fibroblasts was measured as previously described, and the proteolytic degradation by these cells was measured according to Bilheimer et al. In short, platelets (4x10^5/mL) were incubated for 4 hours at 37°C with varying protein concentrations of 123I-LDL. Next, platelets were sedimented by centrifugation and the supernatant was removed. The 123I-LDL degradation by platelets was determined from the capacity for degradation of 123I-LDL to noniodide trichloroacetic acid-soluble material. The content of 131I-labeled soluble (noniodide) material formed by platelets and released into the supernatant was determined by the method of Bierman et al. Nonspecific proteolytic degradation was obtained in the presence of a 50-fold excess of unlabeled LDL. Confluent fibroblasts were treated similarly, and the specific proteolytic degradation of 123I-LDL was determined as indicated for platelets.

Platelet serotonin uptake was determined following a standard procedure. Briefly, washed platelets (4x10^6/mL) in a final volume of 0.25 mL Tyrode's buffer were incubated for 30 minutes at room temperature in the presence and absence of 5 μmol/L imipramine and 10 μmol/L dopamine with [14C]serotonin (0.1 μCi/mL of platelet suspension). Then, the platelet pellet was recovered by centrifugation, and the radioactivity of the supernatant and platelet pellet was counted in a beta counter. 123I-LDL binding to both control platelets and platelets treated with serotonin uptake inhibitors was determined as indicated previously. Furthermore, typical displacement experiments of 123I-LDL binding (0.5 g protein/L) by imipramine (up to 50 μmol/L) and dopamine (up to 100 μmol/L) were conducted.

Statistical Analysis

Results are expressed as mean±SEM. Differences between means were compared by Student's t test, with a value of P<.05 considered significant.

Results

Interaction Between Oxidized LDL and the Platelet LDL Receptor

LDL Oxidation

Fig 1A shows a typical dose-response curve of copper sulfate–oxidized LDL (in malondialdehyde equivalents). Production of thiobarbituric acid–reactive substances in oxidized LDL was 149±37 nmol/mg LDL protein (mean±SEM, n=6) versus 1.6±0.9 nmol/mg LDL protein in native LDL. Fig 1B shows the increase in relative...
electrophoretic mobility (expressed as $R_p$) of oxidized LDL (native LDL $R_p=1$, oxidized LDL $=1.6±0.2$).

**Binding of $^{125}$I-Oxidized LDL to Human Platelets**

Fig 2 shows saturation curves of $^{125}$I-oxidized LDL binding to platelets at 22°C. The binding derived from three independent experiments was saturable at a protein concentration of 0.2±0.05 g/L. A Scatchard plot of the data gave a linear correlation coefficient of $r=−.94$ (Fig 2, inset). With an assumed molecular weight for apolipoprotein B-100 of 550 kDa, the number of binding sites per platelet (mean±SEM, $n=3$) was 3895±241, with a $K_d$ of 96.2±10.3 nmol/L.

**Displacement Studies of $^{125}$I-Oxidized LDL by Oxidized and Native LDL**

A line plot showing inhibition of $^{125}$I-oxidized LDL binding to platelets by unlabeled oxidized and native LDL is shown in Fig 3A. Binding of $^{125}$I-oxidized LDL (0.04 g/L) to platelets was inhibited by both unlabeled oxidized and native LDL with the same apparent affinity. Similar IC₅₀ and $K_a$ values for unlabeled oxidized and native LDL were obtained (IC₅₀, 0.055 and 0.065 g/L; $K_a$, 88 and 64 nmol/L, respectively). The Hill coefficients for unlabeled oxidized and native LDL calculated from the slope of displacement curves of $^{125}$I-LDL binding were −1.02 and −1.07, respectively.

**Displacement Studies of $^{125}$I-LDL Binding by Native and Oxidized LDL**

Fig 3C shows a typical displacement experiment of $^{125}$I-native LDL by native and oxidized LDL in fibroblasts. Unlabeled native LDL competed with $^{125}$I-native LDL (0.01 g/L) for fibroblast LDL receptors with an IC₅₀ value of 0.008 g/L and a $K_a$ value of 2.03 nmol/L. In contrast to the inability of oxidized LDL to compete with native LDL for the occupancy of LDL receptor in fibroblasts (see Fig 3C), oxidized LDL completely inhibited the binding of $^{125}$I-native LDL to human platelets. Fig 3B shows the displacement curves of $^{125}$I-LDL binding to platelets. Similar IC₅₀ and $K_a$ values for oxidized and unlabeled LDL were obtained (0.054 g/L and 65.9 nmol/L versus 0.045 g/L and 45.8 nmol/L, respectively). The Hill coefficients for unlabeled oxidized and native LDL calculated from the slope of displacement curves of $^{125}$I-LDL binding were −1.05 and −1.10, respectively.

**Aggregation Studies**

Both native and oxidized LDL at a protein concentration of 0.5 g/L sensitized the platelets in response to low concentrations of ADP (0.5 μmol/L) (percent aggregation: saline, 21.3±3.7%; native LDL, 50.1±11.7%; and oxidized LDL, 55.3±6.2%) and collagen (0.5 μg/mL) (percent aggregation: saline, 27.5±8.9%; native LDL, 46.5±12.7%; and oxidized LDL, 56.4±6.3%). However, no differences between native and oxidized LDL were obtained. The effect was rapid and significant after 2 minutes (not shown) and 5 minutes following the addition of LDL and the inducers.

**Fate of Platelet Surface-Bound $^{125}$I-LDL**

**Dissociation of $^{125}$I-LDL From Surface Receptors at Different Temperatures**

In our conditions approximately 93% of surface-bound LDL is dissociated at 4°C, 22°C, and 37°C directly in the medium, whereas only 7% of the platelet-associated radioactivity was inaccessible to an extracellular excess of unlabeled LDL. We used an energy inhibitor such as sodium azide, which completely blocked endocytosis of LDL in fibroblasts, to measure the rate of dissociation of LDL from surface platelet receptors. Fig 4 shows the binding dissociation of $^{125}$I-LDL at 37°C in the absence and presence of 10 mmol/L NaNO₃. At 37°C (and also at 4°C, not shown) the dissociation of platelet-associated radioactivity was temperature independent, and in the absence and presence of sodium azide, similar half-life values (25±5 and 30±4 minutes) and reverse rate constants ($K_{-1}=6$ and $8.9×10^{-4} s^{-1} mol/L^{-1}$) were obtained.
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Effect of Microtubule Inhibitors on the Metabolism of 125I-LDL by Human Platelets

At 10 μmol/L colchicine reduced 125I-LDL degradation by human fibroblasts to 32±8% (mean±SEM, n=4) of control, and the binding of 125I-LDL was increased to 122±13% (mean±SEM, n=4). Colchicine at the indicated concentration did not alter 125I-LDL binding to platelets. The proteolytic degradation of 125I-LDL by platelets (see below) measured as the content of 125I-LDL-soluble material was similar in the presence and absence of platelets (93.6±7.4 and 104.5±7.6 ng, respectively, mean±SEM, n=4). Thus, the nonspecific proteolytic degradation detected was not modified by colchicine in platelets.

Effect of Lysosomotropic Agents on the Metabolism of 125I-LDL by Human Platelets

Under our conditions, when human fibroblasts were preincubated for 1 hour at 37°C in the presence of 10 mmol/L ammonium chloride, proteolytic degradation and binding of 125I-LDL were inhibited to 21±2% and 64±5% of control, respectively (mean±SEM, n=3). However, with 25 μmol/L chloroquine the proteolytic degradation of 125I-LDL was completely inhibited, whereas the specific binding increased to 144±16% (mean±SEM, n=3). In contrast, 125I-LDL platelet metabolism in the presence and absence of these metabolic inhibitors showed similar values for specific binding and nonspecific proteolytic degradation.
Effect of Golgi Apparatus Disruptors on the Metabolism of 125I-LDL by Human Platelets

Monensin (25 μmol/L) reduced the specific proteolytic degradation and binding of 125I-LDL by fibroblasts to 61±7% and 42±3%, respectively (mean±SEM, n=4). In contrast, this metabolic inhibitor had no effect on the binding of 125I-LDL to platelets.

Proteolytic Degradation of 125I-LDL by Platelets

Trichloroacetic acid-soluble 125I-LDL degradation products were determined according to the procedure described for lymphocytes with minor modifications. 125I-LDL degradation products after incubation of 0.1 g/L of 125I-LDL with 4×10⁸ platelets for 4 hours were not found. The content of 125I-labeled soluble material in the incubation medium of samples with and without platelets was similar (93.6±7.4 and 104.5±7.6 ng, respectively, mean±SEM, n=3). Thus, only nonspecific proteolytic degradation can be measured in platelets.

Platelet [14C]Serotonin Uptake

When platelets were incubated in the presence of [14C]serotonin uptake inhibitors (5 μmol/L imipramine and 10 μmol/L dopamine), only 14±5% (mean±SEM, n=3) of the total uptake was incorporated into the platelets. However, 125I-LDL binding to platelets treated with serotonin uptake inhibitors was similar compared with that obtained with control platelets. Furthermore, no displacement of 125I-LDL binding by varying concentrations of imipramine and dopamine was found.

Discussion

In previous studies we demonstrated that human platelets bind LDL by a receptor that is immunologically different from the classic LDL receptor of nucleated cells. In the present study we investigated the interrelatation of oxidized LDL with the platelet LDL receptor and extended the characterization of this receptor to ascertain whether a fraction of platelet surface-bound 125I-LDL was internalized and degraded by platelets.

We showed that the displacement of 125I-LDL (0.05 g/L) binding to human fibroblasts by oxidized LDL was insignificant, so this modified LDL was unable to compete with native LDL. In contrast, in human platelets we demonstrated that both unlabeled native and oxidized LDL interacted with the same high affinity (IC₅₀, 0.045 and 0.054 g/L; Kₛ, 45.8 and 65.9 nmol/L for native and oxidized LDL, respectively). The Hill coefficient for competition curves of 125I-LDL binding by oxidized LDL was −1.05, suggesting that the displacement involved a single set of binding sites.

To assess whether washed human platelets bound oxidized LDL, we studied the properties of 125I-oxidized LDL and the capacity of unlabeled native LDL to compete for the binding of 125I-oxidized LDL. Saturation studies showed that human platelets bound 125I-oxidized LDL to a class of saturable binding sites, numbering approximately 3895±241 per platelet, and the Kₛ was 96.2±10.3 nmol/L. These values are similar to those previously reported for native LDL. Moreover, displacement experiments showed that the 125I-oxidized LDL binding to platelets was inhibited with the same affinity by both unlabeled oxidized and native LDL. The Hill coefficient for displacement curves of 125I-oxidized LDL binding by native LDL was −1.07, suggesting, as in the displacement of 125I-LDL binding, that a single set of binding sites is involved. In conclusion, our saturation and competition data suggest that the platelet LDL receptor interacts with the same apparent affinity with both native and oxidized LDL.

The interaction between LDL and its receptor appears to facilitate platelet activation. In agreement with other studies reporting that oxidized LDL rapidly modulates the reactivity of human platelets to ADP, we observed under the same experimental conditions that oxidized LDL at a protein concentration of 0.5 g/L enhanced platelet reactivity to ADP. Similar results were obtained when other platelet inducers such as collagen were used. However, we did not find that oxidized LDL (0.5 g protein/L) was more reactive than native LDL. Furthermore, recently published studies also suggest that oxidized LDL, apart from its role as a source of lipid for macrophage foam cell formation, may have effects on other cells that are not related to foam cells and thus may have an impact on coagulation and thrombosis. Accordingly, we demonstrated that oxidized LDL is recognized as native LDL by the platelet aggregate receptor and that both particles similarly increased ADP- and collagen-induced platelet aggregation in vitro. These findings may explain in part the increased thrombogenicity described in atheromatous plaques of hypercholesterolemic rabbits and also provide new evidence of the non-foam-cell effect of oxidized LDL in platelets, as occurs in other cells.

Recently, it has been suggested that the interaction between LDL and its receptor is mediated by a saturable-specific process and that platelets are capable of internalizing and metabolizing LDL, albeit in a manner different from nucleated cells. In contrast, several groups have reported that platelets are unable to degrade the apolipoprotein of LDL. It is well accepted that platelets can take up particles (such as collagen fibrils, viruses, antigen-antibody complexes) by simple phagocytosis and also by active transport mechanisms but not via the surface-connected canalicular system. Moreover, metabolic inhibitors such as ammonium chloride and chloroquine completely block the uptake. Interestingly, although platelets are the richest source of lysosomal enzymes, they have only primary lysosomes that are capable of a fusion reaction with vesicles derived from plasma membranes. This might be relevant not only for the possible digestion of endocytosed materials but also for the receptor-mediated endocytosis of proteins to be stored in platelet granules.

In the present study, to ascertain whether LDL binding to platelets led to an endocytotic and degradation process, we used several approaches: (1) the study of binding dissociation at different temperatures, (2) the effect of different metabolic inhibitors on the endocytotic process, and (3) the study of the capacity of platelets to degrade 125I-LDL (platelet LDL receptor activity). We have presented ample evidence to show that the specific LDL binding to platelets is not mediated by an endocytotic process. First, dissociation studies showed that at 4°C, 22°C, and 37°C, approximately 93% of the surface-bound LDL was dissociated and an ATPase inhibitor such as sodium azide was unable to alter the reverse rate constant and half-time of the

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dissociation process. The second piece of evidence was that the microtubule inhibitor (colchicine), lysosomal agents (ammonium chloride and chloroquine), and Golgi apparatus disruptors (monensin), which affect the binding of [125I]-LDL in fibroblasts, were unable to alter the specific binding of [125I]-LDL to human platelets. The third piece of evidence indicating that the mediated response of the platelet LDL receptor was not an endocytotic process was provided by the determination of trichloroacetic acid-soluble [125I]-LDL degradation products. We found similar values for noniodide material in the incubation medium in samples with and without platelets and in the presence and absence of all tested inhibitors. These results indicate that platelets cannot degrade specifically [125I]-LDL and that this degradation is similar in the presence and absence of specific metabolic inhibitors. Finally, the lack of effect of imipramine and dopamine suggests that an endocytotic response similar to serotonin uptake is not involved in [125I]-LDL binding to platelets. Overall, these results show that the binding of LDL to platelets is completely reversible at several temperatures and not modified by specific metabolic inhibitors; also, specific degradation of [125I]-LDL by platelets is absent. The detected absence of degradation of [125I]-LDL by platelets was in agreement with the results previously reported except for one study. These authors observed accumulation of LDL cholesterol in the open canalicular system and on the plasma membrane of platelets by electron photomicrographs. They suggest that the content of the secretory granules and lysosomes of platelets (hydrolytic and proteolytic enzymes) is discharged into the open canalicular system on activation by LDL and is involved in the degradation process, albeit in a manner different from nucleated cells. Furthermore, it has recently been reported that platelets contain apolipoprotein B in their α-granules and that this can be released in response to several agonists.24 Because protein syntheses do not occur in platelets, this apolipoprotein B could be taken up from plasma lipoproteins or from the megakaryocyte. Schick and Schick3 demonstrated the absence of the uptake of [3H]-LDL cholesterol in platelets. This result and our findings that the platelet LDL receptor does not mediate an endocytotic response suggest that the apolipoprotein B contained in platelets is not taken up from circulating lipoproteins and that it probably originates in the megakaryocyte.

In conclusion, the platelet LDL receptor recognizes both native and oxidized LDL with the same affinity as previously reported for native LDL,3 and the binding of [125I]-oxidized LDL to washed human platelets is similar to that previously reported for native LDL.13 Furthermore, we have shown that the binding of LDL to human platelets is completely reversible at different temperatures and not mediated by an endocytotic process that involves proteolytic degradation of [125I]-LDL. Therefore, the platelet LDL receptor that recognizes both native and oxidized LDL may play an important role in the increased thrombogenicity of atheromatous plaques of hypercholesterolemic patients or in clinical conditions in which oxidized and native LDL are locally present at higher concentrations.

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