Metabolism of LDL in Mast Cells Recovering From Degranulation

Description of a Novel Intracellular Pathway Leading to Proteolytic Modification of the Lipoprotein

Jorma O. Kokkonen, Ken A. Lindstedt, and Petri T. Kovanen

Rat serosal mast cells contain cytoplasmic secretory granules composed of a proteoglycan matrix in which histamine and neutral proteases are embedded. On stimulation, these granules are exocytosed, but some of them remain in the degranulation channels where on exposure to the extracellular fluid, they lose their histamine and a fraction of their proteoglycans. In vitro, such granule remnants efficiently bind low density lipoprotein (LDL) present in the incubation medium. After a lag period of about 10 minutes, the granule remnants, still within the channels and coated with LDL particles, are internalized by the parent mast cells. During subsequent recovery from degranulation, the apolipoprotein B of the intracellularly located remnant-bound LDL becomes efficiently (up to 70%) degraded by the proteolytic enzymes of the granule remnants. Since the granule remnants lack cholesteryl esterase activity, no LDL cholesterol is made available for cellular nutrition. Instead, selective proteolytic degradation of the bound LDL leads to formation of LDL particles enlarged by fusion on the granule remnant surface. In response to restimulation of the mast cells, about 50% of the fused LDL particles are exocytosed with the granule remnants. Of these, about one in five are expelled into the incubation medium. The granule remnants that again remain in the degranulation channels bind and internalize more LDL. This "round trip" of LDL in mast cells exposed to repeated stimulation constitutes a hitherto-unknown intracellular pathway for modification of LDL. (Arteriosclerosis and Thrombosis 1993;13:276–285)

KEY WORDS • chymase • endocytosis • exocytosis • granules

The cholesterol found in atherosclerotic lesions is mainly derived from low density lipoproteins (LDLs) that have penetrated into the intima, the inner layer of the arterial wall. Native LDLs are not effectively metabolized by macrophages, which are the precursors of most of the cholesteryl ester–filled foam cells found in atherosclerotic lesions. Therefore, it has been postulated that LDL must be modified in the intima to be recognized and taken up by macrophages. One cell type that is present in atherosclerotic lesions in both animals and humans and that is capable of modifying LDL and carrying it into macrophages is the mast cell. The most characteristic morphological feature of mast cells is their cytoplasmic secretory granules. These granules are thought to represent specialized primary lysosomes. The granules are composed of a proteoglycan matrix in which are embedded the other components, such as histamine and neutral proteases. The matrix is composed of heparin proteoglycans and oversulfated chondroitin sulfate proteoglycans in various proportions, depending on the particular subpopulation of mast cells. The neutral proteases of rat serosal mast cells, the model in our studies, consist of two proteases with complementary specificities: a chymotrypsin-like endopeptidase, chymase, and an exopeptidase, carboxypeptidase A.

The most important functional feature of mast cells is their ability, when stimulated, to exocytose some of their secretory granules, a process known as degranulation. Mast cells are stimulated by a wide variety of agents, including antigens, complement fragments, and cell-derived histamine-releasing factors. In this process, some of the secretory granules become swollen, and their individual membranes fuse to produce tubular degranulation channels in which the granules lie in chains. When the degranulation channels open to the extracellular space, the soluble mediators of mast cells, i.e., histamine, chondroitin sulfate proteoglycans, and some of the heparin proteoglycans, are released and diffuse away to exert their various functions. The insoluble components of the granules, i.e., the neutral proteases and the remainder of the heparin proteoglycans, remain tightly bound to each other and form granule remnants.

Degranulation of rat serosal mast cells leads to the formation of two pools of extracellular granule remnants, those that are expelled into the “free” extracellular medium (about 25%) and those that remain within the degranulation channels (about 75%). The granule remnants that are expelled into the free extracellular medium are ultimately phagocytosed and degraded by scavenger cells such as macrophages. The granule remnants that remain in the degranulation channels are...
reinternalized within a few minutes when the channel openings of the recovering mast cell close. During the ensuing "recovery period," each granule remnant acquires a membrane, and after several days when it has regained its lost constituents, it reappears as a secretary granule in the cytoplasm of the mast cell.

Previous studies have shown that stimulation of mast cells in the presence of LDL leads to binding of LDL to the extracellular granule remnants that are expelled from the mast cells.20 Furthermore, once LDL is bound to the granule remnants, the proteolytic enzymes of the remnants begin to degrade the apolipoprotein B (apo B) component of LDL.21 This proteolytic degradation of remnant-bound LDL leads to fusion and enlargement of LDL particles followed by the binding of more LDL to the granule surface.22,23 When macrophages phagocytose these LDL-laden granule remnants, the cholesteryl esters of LDL are converted into nonmembrane-bound cytoplasmic cholesteryl ester droplets, with formation of the macrophage-derived foam cells that are typical of atherosclerosis.18,22

In a recent series of experiments, we showed that LDL also binds to the granule remnants located in the degranulation channels of stimulated mast cells.16 Moreover, we demonstrated that such remnant-bound LDL was internalized by the mast cells, along with the granule remnants. Indeed, a single stimulation of mast cells caused them to be filled with material that stained positively (with oil red O) for neutral lipid. This change was accompanied by a 30-fold increase in the cellular content of cholesteryl esters. Thus, at the light microscopic level, stimulation of mast cells did produce lipid-filled mast cells that morphologically resembled macrophage-derived foam cells. In the present report, we describe the metabolic fate of the LDL internalized by the mast cells.

**Methods**

**Materials and Animals**

Sodium [125I]iodide (13-17 mCi/μg), [U-14C]sucrose (>350 Ci/mmol), and [1,2-3H]cholesteryl linoleate (66-77 mCi/mg) were purchased from Amersham International. Bovine serum albumin, chloroquine, compound 48/80, heparin, and soybean trypsin inhibitor were purchased from Kabi Diagnostica, and phenylmethylsulfonyl fluoride (PMSF) was obtained from Boehringer Mannheim. Dulbecco's phosphate-buffered saline (PBS) and RPMI 1640 culture medium with 25 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid were purchased from GIBCO. Male Wistar rats (200-500 g) were obtained from the Laboratory Animal Center of the University of Helsinki.

**Preparation and Labeling of Lipoproteins**

Human LDL (d = 1.019-1.050 g/ml) was isolated from plasma by sequential ultracentrifugation, i24 iodinated by the iodine monochloride method,25,26 and labeled with [3H]cholesteryl linoleate as described by Pittman et al.27 [3H]Cholesteryl linoleate ([3H]CL-LDL) by incubating human plasma with solid dispersions of [3H]cholesteryl linoleate on Celite as described.23 Colloidal gold particles (15 nm) were prepared and conjugated to LDL as described by Robenek et al.28 Gold-LDL preparations were examined by transmission electron microscopy using negative staining and were found to contain 3-5 LDL molecules per gold particle. In these experiments, the labeled lipoproteins were diluted with unlabeled LDL to give the specific activities indicated in the figure and table legends.

**Isolation of Mast Cells**

Mast cells were isolated from the peritoneal and pleural cavities of rats as described,16 resuspended in buffer A (PBS supplemented with 10 mg/ml human serum albumin and 5.6 mM glucose, pH 7.3), and immediately used for experiments.

**Uptake and Degradation of LDL by Recovering Mast Cells**

The standard incubation was conducted in 200 μl buffer A containing 0.3-1.0×10⁶ mast cells. After preincubation for 15 minutes at 37°C, the cells were incubated with 1 μg/ml of compound 48/80 for 2 minutes at 37°C. This time was sufficient for mast cell degranulation, i.e., for formation of degranulation channels and opening of the channels to the extracellular space. Then four volumes of ice-cold buffer A were added to the cells to cool them, thus leaving the degranulation channels open.16 The mast cells were then washed free of compound 48/80 and expelled granule remnants by sedimentation at 150g for 5 minutes at 4°C. Finally, the cells were resuspended in 200 μl of warm (37°C) buffer A containing 250 μg/ml of either [3H]sucrose-LDL or [3H]CL-LDL. After incubation at 37°C for the time intervals indicated in the figure legends, the reactions were stopped by adding four volumes of ice-cold buffer A. The cells were washed once by centrifugation at 150g for 5 minutes and resuspended in 200 μl of ice-cold buffer A supplemented with 5 mg/ml commercial heparin for removal of any extracellular cell-associated LDL (i.e., LDL bound to remnants remaining in the open degranulation channels). In a previous study, we found that heparin causes release of the LDL bound to the cell-associated granule remnants.16 Thus, treatment with heparin yielded a "heparin-resistant" fraction that represents intracellular LDL. After incubation for 10 hours at 4°C, the reaction mixture was layered onto 340 μl of separation medium (0.25 M sucrose, 10 mg/ml bovine serum albumin, and 5 mM tris(hydroxymethyl)aminomethane (Tris)-chloride, pH 7.4), and the tubes were centrifuged at 450g for 15 minutes at 4°C. The supernatant was discarded and the cell pellet resuspended in 200 μl PBS, and aliquots were taken for determination of radioactivity. An aliquot was treated with trichloroacetic acid (TCA) to determine the degree of intracellular proteolytic degradation of [3H]sucrose-LDL as described.24,29 The results are expressed as micrograms of LDL degradation products per 10⁶ mast cells. The contents of [3H]cholesteryl linoleate and free [3H]cholesterol were determined by thin-layer chromatography after lipid extraction with chloroform/methanol (2:1, vol/vol).30 The results are expressed as nanomoles of [3H]cholesterol per 10⁶ mast cells.

**Restimulation of LDL-Containing Mast Cells**

Mast cells were stimulated with compound 48/80 as described above. To induce uptake of LDL, the cells
were resuspended in 200 µl warm buffer A containing 250 µg/ml LDL. After incubation for 90 minutes at 37°C, the cells were sedimented and resuspended in 200 µl of fresh buffer A. The cells were then stimulated for a second time with compound 48/80 for 2 minutes, then cooled by addition of ice-cold buffer A, and sedimented as described above. The supernatant (free extracellular medium) was removed for analysis, and the cell pellets were treated with heparin as described above. In addition to the heparin-resistant fraction, the treatment yielded a "heparin-releasable" fraction, representing LDL bound to the granule remnants present in the degranulation channels that were open to the extracellular space. All fractions were then treated with TCA to determine the amount of TCA-precipitable LDL-associated apo B.

**LDL Uptake After the Second Stimulation of Mast Cells**

In experiments in which uptake of LDL was studied after a second stimulation, rats were given an intraperitoneal injection of 1 ml buffer A containing 1 mg protein of [3H]CL-LDL and 10 µg compound 48/80. After 18 hours, the cells of the peritoneal cavities were collected and the mast cells isolated. To determine the amount of internalized LDL, an aliquot of the mast cell suspension was counted for its ³H radioactivity. Mast cells were then stimulated for a second time with compound 48/80 (1 µg/ml) in 200 µl buffer A containing 250 µg/ml [14C]sucrose-LDL, and incubation was continued for 30 minutes at 37°C. After incubation the cells were sedimented at 150g for 5 minutes, and the 150g supernatants were removed. To sediment the granule remnants, the supernatants were centrifuged at 12,000g for 15 minutes. The cell pellets were fixed in 2.5% (vol/vol) glutaraldehyde for 60 minutes. To preserve the lipid membranes, the cells were incubated with native or gold-labeled LDL as described previously.31 The internalized LDL was degraded. The corresponding amounts of free and internalized LDL being converted into TCA-soluble degradation products of the cells.29 As shown in Figure 2B, the internalized [14C]sucrose-LDL became extensively degraded within 3 hours, with 43% (1.28 µg/10⁶ mast cells) of the LDL being converted into TCA-soluble degradation products. When the experiment was performed in the presence of chloroquine, a known inhibitor of lysosomal degradation of LDL,29 no inhibition of degradation was observed. In contrast, the presence of PMSF, an inhibitor of granule chymase,24 degradation of LDL was strongly inhibited; only 6% (0.13 µg/10⁶ mast cells) of the internalized LDL was degraded. The corresponding values for total uptake of LDL in the control cells and in the chloroquine- and PMSF-treated cells after 3 hours were 2.98, 2.68, and 2.14 µg/10⁶ mast cells, respectively.
indicating that internalization of LDL was not significantly affected by the inhibitors used. When the incubation time was extended from 3 to 18 hours, proteolytic degradation of the LDL was extensive; maximally 70% of the internalized labeled apo B was converted into degradation products. The aforementioned result strongly suggests that the observed proteolytic degradation of LDL was not a lysosomal process but rather occurred in the granule compartment of the mast cells.

To confirm the distinction between lysosomal and granule-mediated degradation of LDL, we studied the hydrolysis of the cholesteryl ester component of the intracellular LDL by using [3H]CL-LDL (Figure 3). During a 4-hour incubation, no cholesteryl ester hydrolysis occurred within the mast cells, a finding that accords with the above hypothesis that the observed degradation of LDL was effected solely by the cytoplasmic secretory granules.

We have previously shown that extensive proteolytic degradation of LDL by isolated secretory granules of mast cells leads to modification of the granule-bound LDL. The mast cell granule-modified LDL particles are characterized by selective loss of apo B with a concomitant increase in particle size up to 100 nm. We next studied whether such modified LDL particles were formed within recovering mast cells. For this purpose, mast cells were stimulated in the presence of LDL to induce internalization of LDL. As shown in Figures 4A and 4B, the granule remnants were surrounded by enlarged LDL particles with diameters of up to 100 nm.
These large LDL particles were identical in appearance to those formed when LDL was incubated with isolated granule remnants (Figure 4C). When exposed to successive treatments with osmium tetroxide, thiocarbonyldrazine, and osmium tetroxide, these modified LDL particles were encircled by the black rims typical of lipid droplets. The above results demonstrate that the LDL taken up from the extracellular space by the granule remnants is subsequently converted to lipid droplets within the granule compartment of the stimulated mast cells.

In the next series of experiments, we wanted to find out whether mast cells could be restimulated after their internalization of LDL. More specifically, we wanted to examine whether the LDL-carrying granule remnants would react to a second stimulation and whether the intracellularly modified LDL particles would be expelled from the degranulation channels into the free extracellular space. For this purpose, mast cells were stimulated with compound 48/80 in the presence of 125I-LDL. The medium was changed to remove any 125I-LDL that had not been internalized by the stimulated mast cells. After a 90-minute recovery period, the mast cells were again stimulated with compound 48/80 but this time in the absence of LDL. Two minutes after the addition of compound 48/80, the cells were cooled to 4°C to arrest exocytosis and to fix the degranulation channels in an open position. The mast cells were then sedimented by centrifugation at 4°C, and the supernatant, i.e., the free extracellular medium, was removed.

The cells were then treated with heparin to separate the LDL bound to the granule remnants present in the open degranulation channels (heparin-releasable fraction) from the LDL bound to the intracellularly located granule remnants (heparin-resistant fraction). Finally, all of the fractions were treated with TCA to measure the amount of LDL-associated apo B. As shown in Table 1, the second stimulation caused substantial secretion of LDL (0.39 μg LDL protein per 10⁶ cells), i.e., 19% of LDL was lost into the "free" extracellular medium. In addition, 0.79 μg LDL protein per 10⁶ cells was released by heparin from the mast cells, indicating that an additional 37% of the intracellular LDL had been involved in the second stimulation but had remained in the degranulation channels bound to the granule remnants. These results show that as much as 56% (19%+37%) of the LDL that was internalized after the first stimulation was involved in the second stimulation.

In a direct investigation of the secretion of LDL cholesterol by mast cells, the aforementioned experi-
FIGURE 4. Photomicrographs showing intracellular modification of low density lipoprotein (LDL) by secretory granules of recovering mast cells. Mast cells were stimulated with compound 48/80 as described in the legend to Figure 1. After incubation for 2 minutes at 37°C, the reaction was stopped by addition of ice-cold buffer A, and the cells were sedimented and resuspended in 200 μl buffer containing 250 μg/ml LDL. After incubation for 30 minutes at 37°C, the cells were sedimented and resuspended in culture medium. After incubation for 18 hours at 37°C, the cells were prepared for electron microscopy as described in “Methods.” In a control experiment (panel C), isolated native granules (40 μg) were incubated at 37°C in 400 μl buffer A containing 200 μg LDL. After incubation for 18 hours, the granules were prepared for electron microscopy as described in “Methods.” Panel A: Rat serosal mast cell showing several electron-transparent granule remnants (GR) containing enlarged LDL particles (arrow) within the granule membrane (open arrow). Bar=500 nm. Panel B: Magnification of an area in panel A showing a granule remnant (GR) covered with fused LDL particles (arrow). Bar=200 nm. Panel C: Isolated native granule remnant (GR) covered with enlarged LDL particles (arrow). Bar=200 nm.

ment was repeated by using LDL in which the cho-
esteryl ester component was radiolabeled ([3H]CL-LDL). After incubation for 18 hours, the mast cells were found to contain 4.5 μg cholesterol per 10^6 cells. In agreement with these results, at the second stimulation 15% of the LDL (recognizable by the label in the cholesteryl esters) was released into the incubation medium. When the released material was centrifuged at 12,000g for 15 minutes to sediment the exocytosed granule remnants, 70% of the cholesterol label was cosedimented, indicating that most of the released LDL remained bound to the granule remnants during sedimentation. This finding was verified by electron microscopy. The sedimented granule remnants that had been exocytosed from the mast cells were found to be covered with modified, enlarged LDL particles similar to those shown in Figure 4 (data not shown).

A further series of experiments was designed to test whether mast cells, at a second stimulation, could internalize previously unbound LDL. In preliminary experiments, we found that under in vitro conditions the mast cells did not internalize LDL after the second stimulation, thus reflecting their inability to close the degranulation channels a second time. To obtain cells that would be more viable during the second recovery period, we first stimulated mast cells in vivo in the
peritoneal cavity of the rats and then restimulated the cells in vitro. Both stimulations were carried out in the presence of labeled LDL, but different markers were used. For this purpose, 1 mg [3H]cholesteryl linolate-low density lipoprotein (LDL) (42,200 cpm/µg protein). After incubation for 90 minutes at 37°C, the cells containing 250 µg/ml [14C]sucrose-LDL (2,400 dpm/µg protein). Incubation was continued for 30 minutes at 37°C. After incubation, the cells were sedimented and the supernatants were removed and centrifuged at 12,000g for 15 minutes to sediment the granule remnants. The 3H radioactivity of the granule remnants was measured to determine the amount of intracellular LDL after the first stimulation. The mast cells (5 X 106) were stimulated in vitro with 1 µg/ml compound 48/80 in 200 µl buffer A containing 250 µg/ml [3H]sucrose-LDL (2,400 dpm/µg protein). Incubation and totally lack lipolytic activity. Accordingly, the LDL in these modified lysosomes is selectively degraded. After partial loss of the apo B component, the LDL particles on the granule surface fuse into large droplets with diameters as large as 100 nm. This contrasts with the fate of LDL particles in typical lysosomes, in which LDL disintegrates completely as a result of complete hydrolysis of all the components, i.e., not only apo B but also the various lipids.

Discussion

Figure 5 shows a model that we propose to account for the observations made in this and an earlier study with respect to uptake, modification, and secretion of LDL by rat peritoneal mast cells. The novel observation made in this study was that recovering mast cells, which have internalized LDL and modified it in the granule compartment, can secrete the modified particles, still bound to the granule remnants, back into the extracellular medium. The fate of such LDL-bearing exocytosed granule remnants is likely to be phagocytosis by scavenger cells, such as macrophages. This conclusion is based on our earlier observations of isolated granule remnants that were first allowed to bind and modify LDL and were then incubated with macrophages. Such incubations led to massive uptake of LDL, with ensuing formation of cytoplasmic cholesteryl ester droplets, such as are seen in macrophage-foam cells.

Rat serosal mast cells take up LDL by a highly specific process in which LDL is bound to the heparin proteoglycan component of the granules that are exocytosed by stimulated mast cells. Previously, we have shown that rat serosal mast cells do not express LDL receptors even under in vivo conditions that greatly enhance LDL receptor activity in other types of rat cells, such as those in the liver and adrenal gland. Accordingly, uptake of LDL by the exocytosed granules appears to be the only specific and regulated pathway that these cells possess for uptake of LDL.

The cytoplasmic secretory granules of the mast cell are considered to be modified lysosomes. In terms of the metabolic fate of LDL, a critical difference between typical lysosomes and the cytoplasmic secretory granules is that the granules contain only one major pair of proteolytic enzymes, chymase and carboxypeptidase A, and totally lack lipolytic activity. Accordingly, the LDL in these modified lysosomes is selectively degraded. After partial loss of the apo B component, the LDL particles on the granule surface fuse into large droplets with diameters as large as 100 nm. This contrasts with the fate of LDL particles in typical lysosomes, in which LDL disintegrates completely as a result of complete hydrolysis of all the components, i.e., not only apo B but also the various lipids.

Discussion

Figure 5 shows a model that we propose to account for the observations made in this and an earlier study with respect to uptake, modification, and secretion of LDL by rat peritoneal mast cells. The novel observation made in this study was that recovering mast cells, which have internalized LDL and modified it in the granule compartment, can secrete the modified particles, still bound to the granule remnants, back into the extracellular medium. The fate of such LDL-bearing exocytosed granule remnants is likely to be phagocytosis by scavenger cells, such as macrophages. This conclusion is based on our earlier observations of isolated granule remnants that were first allowed to bind and modify LDL and were then incubated with macrophages. Such incubations led to massive uptake of LDL, with ensuing formation of cytoplasmic cholesteryl ester droplets, such as are seen in macrophage-foam cells.

Rat serosal mast cells take up LDL by a highly specific process in which LDL is bound to the heparin proteoglycan component of the granules that are exocytosed by stimulated mast cells. Previously, we have shown that rat serosal mast cells do not express LDL receptors even under in vivo conditions that greatly enhance LDL receptor activity in other types of rat cells, such as those in the liver and adrenal gland. Accordingly, uptake of LDL by the exocytosed granules appears to be the only specific and regulated pathway that these cells possess for uptake of LDL.

The cytoplasmic secretory granules of the mast cell are considered to be modified lysosomes. In terms of the metabolic fate of LDL, a critical difference between typical lysosomes and the cytoplasmic secretory granules is that the granules contain only one major pair of proteolytic enzymes, chymase and carboxypeptidase A, and totally lack lipolytic activity. Accordingly, the LDL in these modified lysosomes is selectively degraded. After partial loss of the apo B component, the LDL particles on the granule surface fuse into large droplets with diameters as large as 100 nm. This contrasts with the fate of LDL particles in typical lysosomes, in which LDL disintegrates completely as a result of complete hydrolysis of all the components, i.e., not only apo B but also the various lipids.

Discussion

Figure 5 shows a model that we propose to account for the observations made in this and an earlier study with respect to uptake, modification, and secretion of LDL by rat peritoneal mast cells. The novel observation made in this study was that recovering mast cells, which have internalized LDL and modified it in the granule compartment, can secrete the modified particles, still bound to the granule remnants, back into the extracellular medium. The fate of such LDL-bearing exocytosed granule remnants is likely to be phagocytosis by scavenger cells, such as macrophages. This conclusion is based on our earlier observations of isolated granule remnants that were first allowed to bind and modify LDL and were then incubated with macrophages. Such incubations led to massive uptake of LDL, with ensuing formation of cytoplasmic cholesteryl ester droplets, such as are seen in macrophage-foam cells.

Rat serosal mast cells take up LDL by a highly specific process in which LDL is bound to the heparin proteoglycan component of the granules that are exocytosed by stimulated mast cells. Previously, we have shown that rat serosal mast cells do not express LDL receptors even under in vivo conditions that greatly enhance LDL receptor activity in other types of rat cells, such as those in the liver and adrenal gland. Accordingly, uptake of LDL by the exocytosed granules appears to be the only specific and regulated pathway that these cells possess for uptake of LDL.

The cytoplasmic secretory granules of the mast cell are considered to be modified lysosomes. In terms of the metabolic fate of LDL, a critical difference between typical lysosomes and the cytoplasmic secretory granules is that the granules contain only one major pair of proteolytic enzymes, chymase and carboxypeptidase A, and totally lack lipolytic activity. Accordingly, the LDL in these modified lysosomes is selectively degraded. After partial loss of the apo B component, the LDL particles on the granule surface fuse into large droplets with diameters as large as 100 nm. This contrasts with the fate of LDL particles in typical lysosomes, in which LDL disintegrates completely as a result of complete hydrolysis of all the components, i.e., not only apo B but also the various lipids.
The absence of cholesteryl ester hydrolase activity in the cytoplasmic secretory granules of rat serosal mast cells also prevents release of any cholesterol from the cholesteryl ester–filled core region of the internalized LDL particles. Accordingly, even massive uptake of LDL by stimulated mast cells will not result in transfer of cholesterol from the granule compartment into the cytosol. This again would imply that all of the LDL cholesterol present in the granule compartment is metabolically inert. In this respect, the metabolic fate of LDL cholesterol in the granule pathway would contrast sharply with that in the LDL receptor pathway. Indeed, we found that massive uptake of LDL (45-fold) after mast cell stimulation did not lead to suppression of the rate of acetate incorporation into cholesterol in the mast cells (data not shown). Moreover, under similar conditions, no oleate was incorporated into the cholesteryl esters, demonstrating that the granule-mediated uptake of LDL by stimulated mast cells did not lead to activation of acyl coenzyme A:cholesterol acyltransferase (data not shown).

The present study has extended our earlier observations on LDL modification by exocytosed and isolated granule remnants of rat serosal mast cells by demonstrating that a similar modification occurs within mast cells that are recovering after stimulation. Since the critical factor in the granule remnant–dependent modification of LDL is the proteolytic activity of chymase, the presence of inhibitors of proteolysis in the extracellular fluid may block LDL modification. When the experiment on LDL uptake by stimulated mast cells (Figure 2) was performed in the presence of a high concentration (1 mg/ml) of trypsin inhibitor, no inhibition of LDL degradation could be observed in the granule compartment of recovering mast cells. Furthermore, when the LDL-uptake experiment (Figure 2) was repeated in the presence of high concentrations (up to 20 mg/ml protein) of rat or human lipoprotein-deficient serum containing the natural, neutral protease inhibitors, no inhibition of intracellular LDL degradation was observed (data not shown), suggesting that even the natural proteolytic inhibitors are excluded from the granule compartment. Our previous finding has been that to be internalized by stimulated mast cells, LDL molecules must bind to granules, and trypsin inhibitor does not bind to granules. Accordingly, it seems conceivable that the neutral protease inhibitors present in the extracellular fluid also are unable to bind to granules and so are excluded from the granule compartment. Thus, in terms of proteolysis, the granule compartment of stimulated mast cells appears to be a "protected" environment.

The magnitude and efficiency of the granule-mediated uptake of LDL depend on whether other molecules with an affinity for heparin are present in the extracellular fluid. Such molecules include fibronectin, fibrinogen, and platelet factor 4. In the extracellular fluid of most tissues, the concentration of LDL, because of its large size, is much lower than that of the small-molecular-weight molecules listed above. Indeed, the concentration of LDL in lymphatic fluid is only one tenth of that in the circulating blood. Thus, the smaller
molecules with an affinity for heparin that are present in high concentrations in the extracellular microenvironment of the stimulated mast cell effectively compete for binding and may be taken up preferentially. For example, with immunoelectron microscopy, the presence of platelet factor 4 has been demonstrated in the cytoplasmic granules of human mast cells of the skin and breast tissue. In contrast, in the human arterial intima, which contains mast cells of connective tissue type (M. Kaartinen and P.T. Kovanen, unpublished results), the human counterparts of rat serosal mast cells, the concentration of LDL is high and equals or even exceeds that in the blood circulation. Hence, compared with mast cells at other tissue sites, the mast cells in the human arterial intima reside in an environment that favors binding of LDL to the extracellularly located mast cell granules. This conclusion is supported by the numerous findings that indicate that the extracellular proteoglycans present in the arterial intima also interact with LDL. Since heparin, among the glycosaminoglycans tested in vitro, shows the highest affinity for LDL, binding of LDL to granules with ensuing uptake and intracellular modification of LDL by stimulated mast cells is a likely event to also occur in vivo.

Acknowledgments

We thank Soile Sinisalo and Päivi Hiironen for excellent technical assistance and Yrjö Ritanen for help with photography.

References

8. Kokkonen JO, Kovanen PT: Stimulation of mast cells leads to cholesterol accumulation in macrophages in vitro by a mast cell granule-mediated uptake of low density lipoprotein. Proc Natl Acad Sci USA 1987;84:2287–2291
Metabolism of LDL in mast cells recovering from degranulation. Description of a novel intracellular pathway leading to proteolytic modification of the lipoprotein.

J O Kokkonen, K A Lindstedt and P T Kovanen

doi: 10.1161/01.ATV.13.2.276

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/13/2/276