Accumulation of low density lipoproteins in stimulated rat serosal mast cells during recovery from degranulation

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Abstract Stimulation of rat serosal mast cells in vitro with compound 48/80, a degranulating agent, resulted in an immediate increase in binding of low density lipoproteins (LDL) to the stimulated mast cells. The increase in binding was dose-dependent and closely followed the increase in histamine release, i.e., the exocytosis of mast cell granules. It could be demonstrated that the LDL were bound to exocytosed secretory granules which remained cell-associated. During the recovery period the granule-bound LDL were internalized by the mast cells along with the granules. A single stimulation of mast cells rendered their cytoplasm to be filled with granular material showing positive staining for both apoB and neutral lipid. This change was accompanied by a 30-fold increase in the cellular content of cholesteryl esters. Thus, rat serosal mast cells possess a specific mechanism for uptake of LDL that is activated by stimuli that lead to degranulation, the result being massive uptake of LDL by stimulated mast cells during recovery from degranulation. — **Kokkonen, J. O., and P. T. Kovanen.** Accumulation of low density lipoproteins in stimulated rat serosal mast cells during recovery from degranulation. *J. Lipid Res.* 1989. **30:** 1341–1348.

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The cytoplasm of mast cells is filled with specific organelles, the secretory granules. On antigen challenge, the mast cells are activated, their cytoplasmic granules become swollen, and their membranes fuse to produce chains of granules lying in so-called cytoplasmic degranulation channels (1). The degranulation channels eventually open to the exterior, whereupon the soluble components of the granules (e.g., histamine) are released and diffuse away to exert their mediator functions in allergic reactions. The remaining components of the granules, consisting of neutral proteases and heparin proteoglycan, remain tightly bound to each other, thus forming granule remnants (2).

The opening of the channels at the cell surface permits extrusion of some of the granule remnants into the extracellular fluid. The ultimate fate of the extruded granules is to be phagocytosed by macrophages located in the microenvironment of the stimulated mast cells (3). The granules that remain in the channels are internalized by the mast cells during recovery from degranulation (4). During this recovery period, the channel openings close, and the granule remnants become bounded by their original membranes and appear again as secretory granules in the cytoplasm of the stimulated mast cells.

We recently observed that the exocytosed granules that are extruded from their parent cells bind LDL and carry them into the macrophages in vitro (5). In the present study we tested whether the granules that remain in the degranulation channels and are internalized by the mast cells could bind LDL and transport LDL into the recovering mast cells. We found that binding of LDL to the recycling granules leads to massive uptake of LDL after a single stimulation of the mast cells.

MATERIALS AND METHODS

Materials and animals

$Na^{125}I$ (13–17 mCi/μg; 1 Ci = 37 GBq) was purchased from Amersham International. Avidin, bovine serum albumin, soybean trypsin inhibitor, cytochalasin B, heparin, compound 48/80, n-benzoyl-L-tyrosine ethyl ester (BTEE), and 3-amino-9-ethylcarbazole were obtained from Sigma. Human serum albumin was purchased from Kabi Diagnostica. Dulbecco's phosphate-buffered saline (PBS) and RPMI 1640 culture medium with 25 mM HEPES were obtained from Gibco. Rabbit antiserum to human apoB was purchased from Behringwerke AG. Normal swine serum, swine immunoglobulins to rabbit immunoglobulins, and soluble complexes of horseradish

Abbreviations: LDL, low density lipoproteins; BTEE, n-benzoyl-L-tyrosine ethyl ester; PBS, Dulbecco's phosphate-buffered saline; apoB, apolipoprotein B.

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peroxidase and rabbit anti-peroxidase (PAP-complexes) were obtained from Dakopatts. Male Wistar rats (300–500 g) were purchased from Orion (Espoo, Finland).

Preparation and iodination of lipoproteins

Human LDL (d 1.019–1.050 g/ml) were fractionated by sequential ultracentrifugation and iodinated by the iodine monochloride method, as described (6). The range of specific activities of ^{125}I-labeled LDL preparations was 175 to 400 cpm/ng of protein. At least 98% of the radioactivity was precipitable with 10% trichloroacetic acid. Acetyl-LDL were prepared by treatment of LDL with acetic anhydride (7). The concentration of each LDL preparation is expressed in terms of its protein concentration. For experiments, the labeled LDL was diluted with unlabeled LDL to give the specific activities indicated in the figure legends.

Isolation of rat mast cells

For preparation of rat serosal mast cells capable of full recovery, a mild method was introduced for isolation of the cells. The cells of peritoneal and pleural cavities of male Wistar rats were collected by lavage with 10 ml of buffer A (PBS supplemented with 0.5 mg/ml of bovine serum albumin and 5.6 mM glucose, pH 7.3). The pooled cells were washed once with buffer A by centrifuging at 150 *g* for 5 min at room temperature and resuspended in medium A. Medium A contained RPMI 1640 (with 25 mM HEPES) and was supplemented with 10 mg/ml human serum albumin, 5% fresh rat serum, 25 mM NaCl, 100 international units/ml of penicillin, and 2 mM L-glutamine, as suggested by Slutsky et al. (4) for studies with recovering rat serosal mast cells. Aliquots of the cells (5×10^6) were seeded into plastic petri dishes (60 × 15 mm) and incubated in a humidified CO_2 (5% CO_2 in air) incubator at 37°C for 1 h. Nonadherent cells, chiefly mast cells, were removed, washed twice with buffer A, and resuspended for experiments in buffer B (PBS supplemented with 10 mg/ml of human serum albumin, 1 mg/ml of soybean trypsin inhibitor, and 5.6 mM glucose, pH 7.3). The purity of the mast cell suspension was judged from cytocentrifuge slides stained with May-Grünwald-Giemsa. Of the cells examined, 90–95% were found to be mast cells, the contaminating cells being lymphocytes, eosinophils, and occasional macrophages.

Stimulation of mast cells

The standard incubation was conducted in 200 μl of buffer B containing $2.5–5.0 \times 10^5$ mast cells. After preincubation at 37°C for 15 min, the cells were stimulated by addition of the indicated amounts of compound 48/80, and incubation was continued for 1 min. Then 4 volumes of ice-cold buffer A were added and the cells were sedimented at 150 *g* for 5 min at 4°C. The supernatant containing granules detached from the stimulated mast cells was removed. To remove any extracellular granules loosely associated with the stimulated mast cells, the sediment was resuspended in 200 μl of buffer A and gently delivered 5 times through the tip of a micropipette (40–200 μl Finnpipette). During the removal of the loosely cell-associated granules, the mast cells remained intact, as shown by the absence of the intracytoplasmic enzyme lactate dehydrogenase in the surrounding medium. The mast cells were then sedimented as before, and resuspended in 200 μl of ice-cold buffer A. Finally, the quantity of extracellular granules tightly bound to the cells (i.e., in the degranulation channels) was determined by measuring the activity of the granule enzyme chymase. The assay was performed using intact mast cells (1×10^5) in 3 ml of buffer containing 100 mM NaCl, 50 mM Tris-HCl, pH 7.4, and 0.5 mM n-benzoyl-L-tyrosine ethyl ester (BTEE). The change in absorbance at 256 nm was recorded for 2 min at room temperature (8). The change in absorbance was linear during the first 2 min indicating that no cell-associated granules were internalized at room temperature during this time interval.

Binding of ^{125}I-labeled LDL to mast cells

Mast cells were stimulated and washed to remove the extruded granules and loosely cell-associated granules as described above. The standard binding assay (9) was conducted at 4°C in 200 μl of buffer B containing 250 μg/ml of ^{125}I-labeled LDL or ^{125}I-labeled acetyl-LDL, the competing molecules, and $1.5–5.0 \times 10^5$ mast cells. After incubation for 5 min, the reaction mixture was layered onto 340 μl of separation medium (0.25 M sucrose, 10 mg/ml of bovine serum albumin, 5 mM Tris-HCl, pH 7.4). The tubes were then centrifuged at 450 *g* for 15 min at 4°C. The supernatant was removed and ^{125}I radioactivity was determined in the area of the tube containing the cell pellet. The results are expressed as μg of LDL protein bound per 10^6 mast cells.

Uptake of LDL by recovering mast cells

Mast cells were stimulated and washed to remove the extruded granules and loosely cell-associated granules, as described above. To initiate the uptake process, the cell pellet was resuspended in 200 μl of warm (37°C) buffer B containing 250 μg/ml of ^{125}I-labeled LDL. After incubation at 37°C for the indicated times, the reactions were stopped by adding 4 volumes of ice-cold buffer A, and the cells were sedimented. The supernatant was removed and the cells were resuspended in 200 μl of ice-cold buffer B supplemented with 5 mg/ml of heparin for removal of any extracellular granule-bound LDL. After incubation for 10 min at 4°C, the reaction mixture was centrifuged through the separation medium as described above. The cell pellet containing the LDL resistant to heparin treatment was re-

suspended in 100 μl of PBS and removed for analysis. An aliquot was counted for its ^{125}I radioactivity. The total cholesterol content and free cholesterol content of the samples were determined after lipid extraction with chloroform–methanol 2:1 by fluorometry with commercial cholesteryl oleate or cholesterol as standards (10). The quantity of heparin-resistant cholesteryl esters was obtained by subtracting the amount of free cholesterol from that of total cholesterol. The results are expressed either as μg of heparin-resistant LDL protein or as μg of heparin-resistant cholesteryl esters per 10^6 mast cells. In control experiments performed in the cold, it could be shown that heparin treatment released more than 95% of the LDL bound to the mast cell-associated granules. The mast cells remained intact during the heparin treatment, as shown by the absence of the intracytoplasmic enzyme lactate dehydrogenase in the surrounding medium.

Immunoperoxidase staining of intracellular LDL in recovering mast cells

Mast cells (2×10^5) in 200 μl of buffer B were stimulated with compound 48/80 (1 μg/ml). After incubation for 1 min at 37°C, unlabeled LDL were added to make a final concentration of 250 μg/ml. Incubation was continued for 30 min at 37°C to allow internalization of the LDL-containing granules. After incubation the cells were sedimented, the supernatant was removed, and the cell sediment was resuspended in 1 ml of medium A. The mast cells were transferred to plastic petri dishes (35 × 10 mm) and incubated in a humidified CO_2 (5% CO_2 in air) incubator at 37°C for 18 h. All subsequent steps were performed at room temperature. The mast cells were removed from the dishes, washed once by centrifugation with buffer A, and then resuspended in buffer B. Cytocentrifuge slides were prepared by centrifuging 1.5×10^4 mast cells at 800 rpm for 5 min in a Shandon Cytospin 2 cytocentrifuge. In May-Grünwald-Giemsa stained preparations, 90–95% of the cells were found to be mast cells, the contaminating cells being lymphocytes, eosinophils, and occasional macrophages. The slides were air-dried overnight, after which the cells were fixed with 4% (v/v) paraformaldehyde for 30 min, treated with 0.8% (v/v) hydrogen peroxide in methanol for 20 min, and rendered permeable with 0.1% (w/v) saponin (6 times, each for 2 min). All subsequent treatments (until carbazole) were carried out in the presence of 0.1% (w/v) saponin in buffer B. The cells were treated for 20 min with normal swine serum (final dilution; 1:5), for 40 min with rabbit antiserum to human apoB (final dilution; 1:200), for 20 min with swine immunoglobulins to rabbit immunoglobulins (final dilution; 1:50), for 20 min with soluble complexes of horseradish peroxidase and rabbit anti-peroxidase (PAP complexes) (final dilution; 1:100), and for 20 min with 0.27 mg/ml of 3-amino-9-ethylcarbazole in 100 mM sodium acetate buffer, pH 5.2, containing 0.03% (v/v) hydrogen peroxide. Finally, the cells were counterstained with Harris hematoxylin. In control experiments, rabbit antiserum to apoB was replaced with rabbit nonimmune serum (final dilution; 1:200) or buffer B–saponin. No positive staining was observed. The cell-associated extracellular apoB was stained as described above, except that saponin was omitted from the treatments and treatment with hydrogen peroxide was performed after incubation of the cells with the primary antibody.

Oil Red O staining of recovering mast cells

Cytocentrifuge slides of mast cells incubated with LDL were prepared and the cells were fixed with paraformaldehyde as described above. The cells were postfixed for 60 min with 2.5% (w/v) potassium dichromate and 1% (v/v) osmium tetroxide in water. After staining with Oil Red O (11), the cells were counterstained with Harris hematoxylin.

Other assays

Histamine was determined by fluorometry (6). Protein was determined by the procedure of Lowry et al. (12), with bovine serum albumin as standard.

RESULTS

To confirm that a fraction of exocytosed mast cell granules remains associated with the mast cells, we performed an experiment in which the activity of cell-surface-associated chymase was determined in stimulated mast cells. Chymase, the major proteolytic enzyme of the granules, remains tightly bound to exocytosed granules, and can therefore be used as a granule marker (2). The mast cells were stimulated with compound 48/80, an agent known to cause specific and noncytotoxic degranulation of mast cells. After stimulation, the mast cells were sedimented and the supernatant containing granules detached from the cells into the free extracellular medium was removed. The stimulated mast cells were then washed to remove any loosely cell-associated granules and the activity of chymase was measured on intact cells and in the extracellular medium (see Materials and Methods). We could show that, irrespective of the degree of mast cell stimulation, approximately 25% of the total quantity of exocytosed granules was extruded into the free extracellular medium by the exocytotic process itself. Another 25% of the exocytosed granules could be removed by the washing procedure (loosely cell-associated granules). After washing, about 50% of the exocytosed granules remained cell-associated (tightly cell-associated granules). In all experiments mast cells containing only tightly cell-associated granules were used.

As shown in **Fig. 1A** (inset), addition of increasing amounts of 48/80 to the incubation medium caused release of increasing amounts of histamine into the medium, reflecting a dose-dependent exocytosis of mast cell granules. The activity of cell-surface-associated chymase, i.e., the quantity of tightly cell-associated granules, also increased in a dose-dependent fashion (Fig. 1A). Next, mast cells were stimulated, washed, and incubated in the cold in an LDL-containing medium to determine the binding of LDL to the

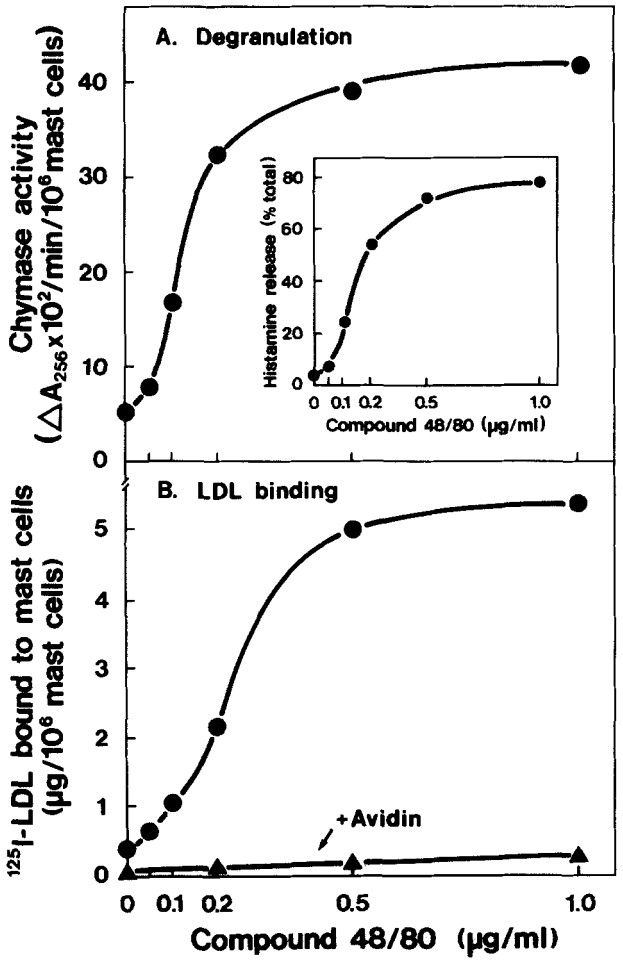

Fig. 1. Degranulation of mast cells by compound 48/80 (A) and concomitant binding of LDL (B) to mast cells. (A) Mast cells (5.0×10^5) were stimulated for 1 min at 37°C in 200 μl of buffer B containing the indicated concentrations of compound 48/80. Incubations were stopped by adding ice-cold buffer A, after which the cells were sedimented and washed to remove any loosely cell-associated granules (see Materials and Methods). The quantity of mast cell granules located in the degranulation channels was determined by measuring the chymase activity with BTEE as substrate, as described under Materials and Methods. The inset shows the amounts of histamine released into the incubation medium, expressed as percentages of the amount of histamine contained in the unstimulated mast cells. (B) Mast cells (2.0×10^5) were stimulated and washed as above. The binding assay was conducted at 4°C in 200 μl of buffer B containing 250 μg/ml of ^{125}I-labeled LDL (53,900 cpm/μg) in the absence (●) or presence (▲) of 1 mg/ml of avidin. After incubation for 5 min, the amount of ^{125}I-labeled LDL bound to the mast cells was determined as described under Materials and Methods.

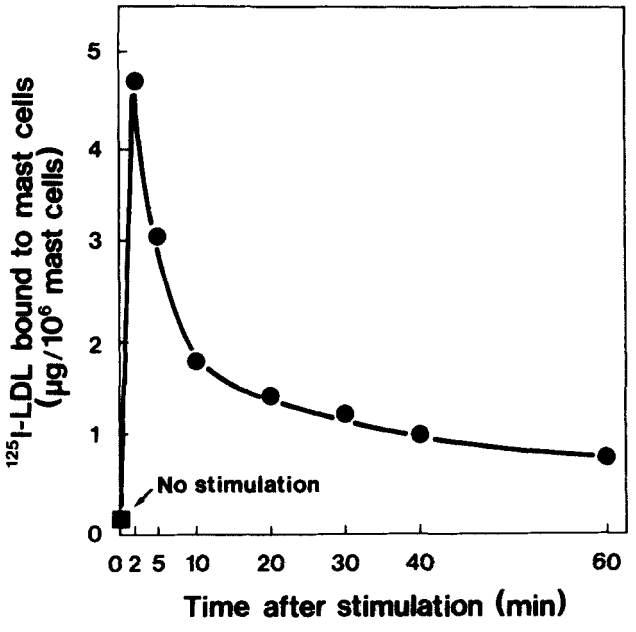

Fig. 2. Binding of LDL to stimulated mast cells as a function of time of recovery from stimulation. Mast cells (2.0×10^5) in 200 μl of buffer B were stimulated with compound 48/80 (1 μg/ml), and incubated at 37°C for the indicated times. Incubations were stopped by adding ice-cold buffer A, after which the cells were sedimented and washed to remove the loosely cell-associated granules. The binding assay was conducted at 4°C in 200 μl of buffer B containing the mast cells and 250 μg/ml of ^{125}I-labeled LDL (27,000 cpm/μg). After incubation for 5 min, the amount of ^{125}I-labeled LDL bound to the mast cells was determined as described under Materials and Methods.

stimulated mast cells. Binding of LDL to mast cells closely paralleled the increase in cell-surface-associated chymase activity (Fig. 1B). This result strongly suggests that binding of LDL to the stimulated mast cells actually represents binding of LDL to exocytosed granules which remain cell-associated. This conclusion was confirmed by the finding that binding of LDL to the stimulated mast cells was fully inhibited by avidin, a compound that specifically binds to mast cell granules (13) and competitively inhibits binding of LDL to isolated mast cell granules (9) (Fig. 1B). Moreover, acetylation of LDL, which renders apoB unable to bind to mast cell granules (9), also fully inhibited binding of LDL to the stimulated mast cells (data not shown).

During recovery from degranulation, the cell-associated exocytosed granules are rapidly (within 40 min) taken up by the recovering mast cells (4). Consequently, after this period the granules should no longer be available for binding of LDL. **Fig. 2** shows the results of an experiment in which binding of LDL by recovering mast cells was measured at various time intervals after stimulation. It can be seen that after stimulation the quantity of LDL bound suddenly increased (by 25-fold at 2 min), and thereafter rapidly ceased. Accordingly, 1 h after stimulation, the ability of mast cells to bind LDL had declined to a value that was only 18% of the maximal binding ca-

pacity. The above results (Figs. 1 and 2) demonstrated that, upon stimulation of rat serosal mast cells, LDL binds to those cell-associated exocytosed granules that are destined to be taken up by the recovering mast cell.

If the LDL-bearing granules are taken up by the recovering mast cells, like the granules without bound material (see Fig. 2), then uptake of LDL should ensue. In the experiment shown in **Fig. 3A**, stimulated cells were incubated at 37°C in the presence of ^{125}I-labeled LDL to allow both binding of LDL and the recovery process. We have previously observed that binding of LDL to mast cell granules is rapid, being completed in less than 1 min, and

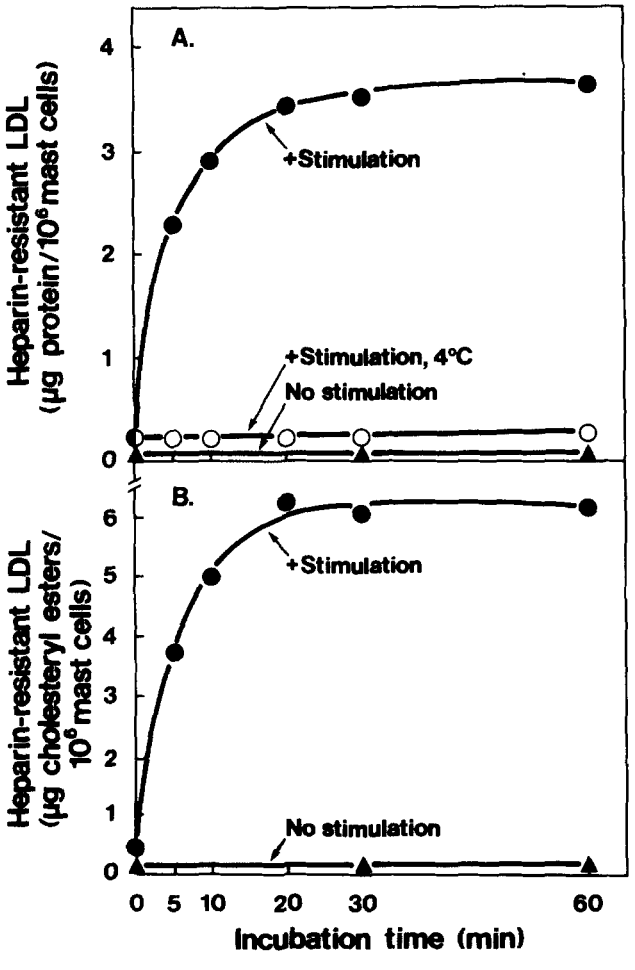

Fig. 3. Internalization of LDL by recovering mast cells. Mast cells (4.5×10^5) were stimulated with compound 48/80 (1 μg/ml), and subsequently washed to remove any loosely cell-associated granules as described in the legend to Fig. 1. The cells were then resuspended in 200 μl of buffer B containing 250 μg/ml of ^{125}I-labeled LDL (10,500 cpm/μg) and incubated either at 37°C (●) or at 4°C (○) for the indicated times. In the control experiment (▲) unstimulated mast cells were incubated with ^{125}I-labeled LDL at 37°C. After incubation, the amount of LDL protein (A) or cholesteryl esters (B) resistant to heparin treatment was determined as described under Materials and Methods. A value of cholesteryl esters (0.1 μg of cholesteryl esters/10^6 mast cells) in mast cells incubated without LDL has been subtracted from the values obtained with LDL and mast cells.

that the binding process proceeds equally well at 37°C and at 4°C (9). The amount of heparin-resistant LDL, i.e., intracellularly located LDL (see Materials and Methods), increased rapidly, reaching a maximum at 30 min. At that point the amount of intracellular LDL (3.6 μg LDL protein/10^6 mast cells) was 35-fold more than that observed in unstimulated mast cells, in which no increase of the heparin-resistant fraction of LDL was observed. Furthermore, when the stimulated cells were kept in the cold to prevent the recovery process, no increase in the amount of intracellular LDL was observed. On the assumption that the protein content of LDL (M_r 2.5×10^6) is 25%, it could be calculated that, after maximal stimulation, about 3.5×10^6 particles of LDL were internalized per mast cell. In a parallel experiment, we measured the content of cholesteryl esters in the mast cells. It appeared that stimulation led to an increase in the cholesteryl ester content of the recovering mast cells (Fig. 3B). The content of esterified cholesterol increased parallel to the uptake of ^{125}I-labeled LDL (see Fig. 3A). At its maximum, the content of cholesteryl esters was 30-fold more than that observed in unstimulated control mast cells.

The recovery of stimulated mast cells can be inhibited by agents known to interrupt the function of the cytoskeleton, such as cytochalasin B (4). In the experiment shown in **Fig. 4**, LDL was allowed to bind to recovering mast cells at 37°C and the initial rates of LDL uptake by mast cells at successive time intervals were measured in the absence and presence of cytochalasin B (10 μg/ml). In the absence of cytochalasin B, the intracellular content of LDL increased with time after an initial lag phase of 2 min. With cytochalasin B in the incubation medium, the increase in the cellular content of LDL was fully inhibited.[2]

To test the importance of binding of lipoproteins to granules in the process of lipoprotein uptake by mast cells, the following experiment was conducted. Mast cells were stimulated and allowed to recover in the presence of 250 μg/ml of LDL, or of 250 μg/ml of either acetyl-LDL or human HDL_3, two classes of lipoprotein particles unable to bind to mast cell granules (9). After completion of the recovery process (at 30 min), the amount of heparin-resistant LDL was 3.04 μg/10^6 mast cells, which was 34-fold that observed in unstimulated mast cells (0.09 μg/10^6 mast cells). In contrast, the amounts of heparin-resistant acetyl-LDL or HDL_3 were not influenced by stimulation of the mast cells and remained at low basal levels of 0.10 and 0.13 μg/10^6 mast cells, respectively. Accordingly, lipoproteins unable to bind to mast cell granules were not internalized by the recovering mast cells.

Finally, the accumulation of LDL by recovering mast cells was demonstrated by light microscopy. The cells were stimulated and incubated for 30 min in the presence

[2]Cytochalasin B did not affect the binding of LLL to the granules.

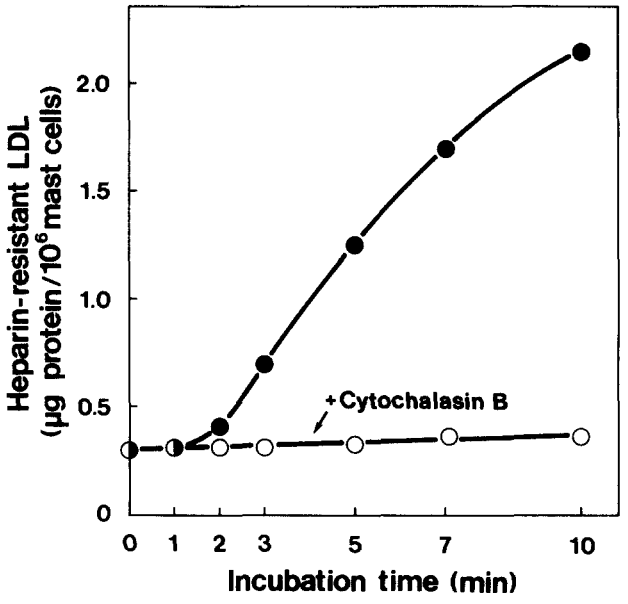

Fig. 4. Effect of cytochalasin B on internalization of LDL by recovering mast cells. Mast cells (3.5×10^5) were stimulated with compound 48/80 (1μg/ml), and subsequently washed to remove loosely cell-associated granules as described in the legend to Fig. 1. The mast cells were preincubated at 4°C for 30 min in 200 μl of buffer B in the absence or presence of 10 μg/ml of cytochalasin B. After preincubation the cells were sedimented and the supernatant was removed. The sediment was resuspended in buffer B containing 250 μg/ml of ^{125}I-labeled LDL (24,700 cpm/μg) and incubated for the indicated times at 37°C in the absence (●) or presence (○) of cytochalasin B. After incubation the amount of ^{125}I-labeled LDL resistant to heparin treatment was determined.

of LDL. The unbound LDL was removed and the recovery process was allowed to proceed for 18 h. In the control experiment the mast cells were left unstimulated. **Figs. 5A and B** show the immunoperoxidase staining of apoB in stimulated and unstimulated mast cells, respectively. During the staining procedure, the cells were rendered permeable to antibodies by saponin treatment. Intense positive staining for apoB was seen in the stimulated mast cells (Fig. 5A; in six experiments 92–94% of mast cells showed intracellular apoB - positive material). In the unstimulated cells no staining for apoB was apparent (Fig. 5B). In a control experiment with stimulated but unpermeabilized mast cells, only negligible amounts of cell-associated apoB-positive staining were seen as interrupted thin rims around the cells (not shown). Figs. 5C and D show mast cells stained with the lipid stain Oil Red O. Within the cytoplasm the stimulated mast cells contained numerous droplets that stained with Oil Red O (Fig. 5C; in three experiments 92–95% of mast cells showed intracellular staining for neutral lipid). In contrast, unstimulated mast cells were devoid of Oil Red O-positive droplets (Fig. 5D).

DISCUSSION

The present studies demonstrate that rat serosal mast cells take up LDL by a highly specific process that depends on binding of LDL to exocytosed granules of stimulated mast cells. A previous study has shown that binding of LDL to the mast cells granules depends on interaction between the apoB of LDL and the heparin proteoglycan component of the granules (9). Accordingly, the specificity of the uptake process is determined by the heparin component of the granules. A variety of proteins in the extracellular fluid show an affinity for heparin (14), and it is likely that, in addition to LDL, other molecules capable of interacting with heparin will be internalized by the granule-mediated pathway. One protein with high binding affinity for heparin is platelet factor 4. Indeed, the presence of this basic protein has been demonstrated in human mast cells in skin and in breast tissue (15). Using immunoelectronmicroscopy, these workers demonstrated that the platelet factor 4 was present in the cytoplasmic granules of human mast cells. This finding strongly suggests that the granules of mast cells mediate uptake of molecules with affinity for heparin, and that this process operates in vivo, as well.

The cytoplasmic secretory granules of mast cells are considered to be modified lysosomes (2). The mechanism by which extracellular molecules, such as LDL, gain access to these modified lysosomes differs fundamentally from those described for delivery of extracellular molecules to lysosomes in other cell types (16), in that no prelysosomal vacuolar compartment is involved. Indeed, with the opening of the degranulation channels to the exterior, the extracellular molecules are exposed directly to these modified lysosomes. Schwartz and Austen (2) have implied that rat serosal mast cells contain cytoplasmic granules of another type, which may function as typical lysosomes. However, these granules do not participate in the secretory response (17) and, consequently, cannot contribute to the uptake mechanism described.

In terms of the metabolic fate of LDL, a critical difference between typical lysosomes and modified lysosomes, i.e., the cytoplasmic secretory granules, is the presence of only one pair of proteolytic enzymes, consisting of chymase and carboxypeptidase A, in the granules (2). Although isolated, proteolytically active granules effectively degrade apoB of LDL, they can at most degrade only about 40% of the apoB (8). The current finding in light microscopic studies of apoB-positive material in the mast cells even 18 h after LDL had been internalized supports the notion that the intracellular granules, like their extruded counterparts, possess only limited proteolytic capacity. Thus, degradation of LDL particles taken up by stimulated rat serosal mast cells is only partial, a contri-

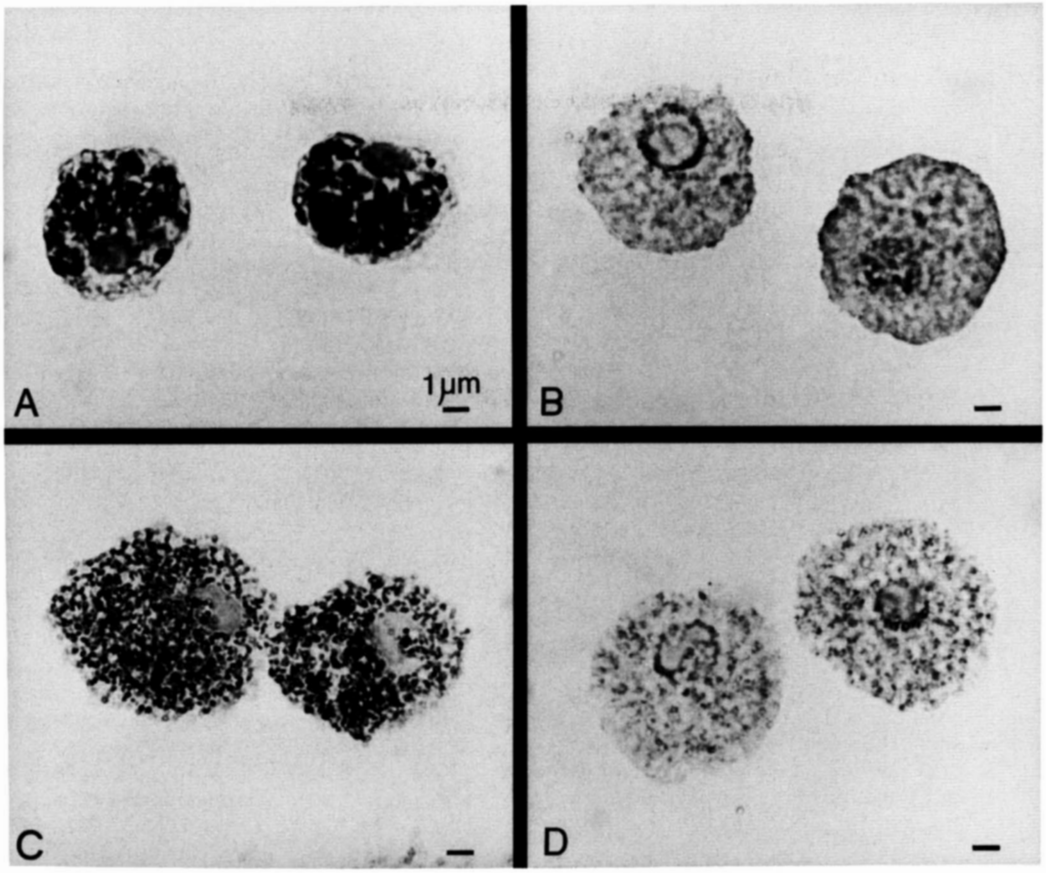

Fig. 5. Immunoperoxidase and Oil Red O staining of recovering mast cells incubated with LDL. Mast cells (2×10^5) were stimulated with compound 48/80 (1 μg/ml), and subsequently incubated with LDL (250 μg/ml) as described under Materials and Methods. In the control experiment unstimulated mast cells were incubated with LDL. Cytocentrifuge slides were prepared from stimulated (A and C) and unstimulated (B and D) mast cells, and subjected to immunoperoxidase staining (A and B) to show the distribution of the apoB-containing inclusions or to Oil Red O staining (C and D) to show the distribution of neutral lipid-containing structures, as described under Materials and Methods. Original magnification × 1250.

buting factor being the complete lack of cholesteryl ester hydrolase activity in the secretory granules of these cells (data not shown). Whether the apoB-positive material and the neutral lipid droplets found in the stimulated mast cells were surrounded by the granule membrane, i.e., were still granule-bound, cannot be determined from the present light microscopic observations. The visual image is that of relatively large cytoplasmic inclusions of apoB-positive material (Fig. 5A) and of much smaller and more numerous cytoplasmic globules of Oil Red O-staining neutral lipid (Fig. 5C), suggesting that the two major components of LDL are located in different mast cell compartments. However, a comparison between the sizes of the inclusions composed of apoB-positive material and of Oil Red O-positive material may be misleading. This is because the methods for apoB detection and for lipid detection are based on different principles, i.e., the former is an indirect staining method with amplification and formation of a large precipitate, whereas the latter stains the lipid structures directly, thus giving a more appropriate reflection of the true size and number of the structures under study.

If LDL remains bound to the granules, then partially proteolyzed LDL may reside in the granule compartment until the mast cells are again stimulated. During a second exposure to the extracellular fluid the granules can bind LDL again. Furthermore, upon repetitive stimulation of mast cells, previously unexposed secretory granules will be exposed to the extracellular fluid (4) and may bind LDL. Accordingly, recycling of cytoplasmic secretory granules in response to repeated stimulation of mast cells could lead to a progressive increase in the content of cholesteryl esters in the mast cells.

Mast cells are also present in the arterial intima, the site of atherogenesis. Indeed, both in experimentally induced and in human atherosclerotic lesions, lipid-filled mast cells have been found (18,19). Whether the lipid droplets contained in the intimal mast cells actually represent

sites of cholesteryl ester accumulation is not known. The studies described here identify a pathway which, if operating in vivo, could lead to retention of LDL in the intimal mast cells. Thus, stimulation of mast cells in atherosclerotic lesions, whether immunologic or nonimmunologic, could produce lipid-filled mast cells, and so contribute to the population of foam cells in these lesions.

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