Degranulation of cutaneous mast cells induces transendothelial transport and local accumulation of plasma LDL in rat skin in vivo

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Abstract In severe hypercholesterolemia and sometimes even in normolipemia, lipoprotein-derived cholesterol accumulates in the skin, forming xanthomas, but the local factors triggering this accumulation are not known. We therefore studied the effect of immunoglobulin E (IgE)-mediated stimulation of cutaneous mast cells on the transport of low density lipoprotein (LDL) from plasma to skin during the passive cutaneous anaphylaxis (PCA) reaction in rats. PCA sites were produced in rats by giving intracutaneous injections of rat serum containing high levels of IgE antibodies against ovalbumin. When ovalbumin was then injected intravenously into the rats, the skin histamine content at the sensitized skin sites decreased, reflecting degranulation of the passively sensitized (IgE-containing) mast cells at these sites, with ensuing release of histamine from the mast cells. Concomitant intravenous injection of increasing amounts of human [^{14}C]sucrose-LDL led to rapid and dose-dependent increases in the skin [^{14}C]sucrose-LDL content. These (maximally about 40-fold) increases were strictly localized to the PCA reaction sites: no such increase in [^{14}C]sucrose-LDL content was observed in nonsensitized skin areas or in any other tissue of the rats. The increase in skin [^{14}C]sucrose-LDL content could be blocked (by about 80%) with a combination of H_1 and H_2 receptor antagonists, and could be mimicked by intradermal injection of histamine. This study demonstrates an increase in plasma LDL influx into the skin at sites where mast cells are stimulated, and so suggests that mast cells play a role in the accumulation of LDL cholesterol that occurs in the skin when xanthomas form.—**Ma, H., and P. T. Kovanen.** Degranulation of cutaneous mast cells induces transendothelial transport and local accumulation of plasma LDL in rat skin in vivo. *J. Lipid Res.* 1997. **38:** 1877–1887.

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Lipid accumulation in the skin and tendons of patients with familial hypercholesterolemia leads to the formation of xanthomas, which resemble the early lesions of atherosclerosis, the fatty streaks. Both in fatty streaks and in xanthomas, cholesterol accumulates in macrophages, with ensuing formation of foam cells (1–4), suggesting that the two processes may have pathogenetic mechanisms in common. One factor that contributes to the accumulation of LDL cholesterol in the arterial intima is the transport of plasma lipoproteins through the endothelium with subsequent influx into the interstitial fluid (5). Indeed, the endothelium acts as a selective barrier between the bloodstream and the underlying tissues, and this ability depends upon its functional and structural integrity (6–9). The selective permeability of the endothelial barrier chiefly affects the macromolecular components of blood plasma, greatly restricting their transendothelial passage to the extravascular compartment. Accordingly, it is the largest components of plasma, the lipoproteins, that are restricted most, and, when the integrity of the endothelial barrier is broken down, the relative increase in the rate of transport should be greatest for the lipoprotein particles. Then, depending on the properties of the underlying tissue, the increased passage of lipoproteins, such as LDL, may contribute to local accumulation of cholesterol, such as is observed in the arterial wall during atherogenesis and in the skin during xanthoma formation. Indeed, the breakdown of the barrier function of the arterial endothelium has been considered a key factor in the pathogenesis of atherosclerosis (10), although this hypothesis has been challenged (11). In contrast, no information has been available about the possible role of increased endothelial permeability in the pathogenesis of xanthoma formation. In the skin, mast cells are especially numerous subepidermally, i.e.,

Abbreviations: ^{51}Cr-RBC, ^{51}Cr-labeled rat red blood cells; ELISA, enzyme-linked immunosorbent assay; $Fc_\epsilon RI$, high affinity receptor for IgE: IgE, immunoglobulin E; LDL, low density lipoprotein; LTC_4, leukotriene C_4; PBS, phosphate-buffered saline; PCA, passive cutaneous anaphylaxis; PGD_2, prostaglandin D_2; RBC, red blood cells; SEM, standard error of the mean; TMB, 3′,3′,5′,5′-tetramethylbenzidine.

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in the dermal layer (12). They typically cluster around small blood vessels, such as the postcapillary venules of the skin, where they are usually present in close proximity to endothelial cells (13, 14). Mast cells have the capacity to generate a wide variety of inflammatory mediators capable of increasing endothelial permeability (15, 16). Some of these mediators, such as histamine, are formed in resting mast cells and stored in their cytoplasmic secretory granules, and are called the "preformed mediators", whereas other mediators, such as the arachidonic acid-derived eicosanoids PGD_2 and LTC_4 are formed in the mast cell membrane when these cells are stimulated, and are called "newly generated mediators". For the mediators to be secreted, stimulation with ensuing degranulation of mast cells is necessary, the classic example being their immunoglobulin E (IgE)-mediated anaphylactic degranulation (17). In IgE-mediated degranulation, the mast cells are sensitized when relevant antigen (allergen) attaches to two or more IgE molecules bound to specific receptors ($Fc_\epsilon RI$) with high affinity for IgE that are located on the mast cell surface. It is this bridging of receptor-bound IgE that triggers mast cell degranulation, with ensuing release of the inflammatory mediators.

The objective of the present study was to develop an experimental system in which the IgE-dependent stimulation of skin mast cells could be used to measure transendothelial transport of plasma LDL and accumulation of LDL at the stimulation site in vivo. For this purpose, we used the passive cutaneous anaphylactic (PCA) reaction which is an established model for studying mast cell effects in rat skin. When antigen-specific IgE is injected into the skin of a rat, and the relevant antigen (e.g., ovalbumin) is then injected into the systemic circulation, mast cells degranulate and release histamine as the antigen molecules reach the sensitized mast cells at the reaction site (18). Indeed, several PCA studies have shown a local increase in endothelial permeability to plasma proteins such as albumin (19). Here we report that stimulation of cutaneous mast cells greatly enhances transendothelial transport and accumulation of plasma LDL at the reaction site.

METHODS

Animals, reagents and biologicals

Male Wistar rats 9–20 weeks old and weighing 200–350 g were used throughout the study. The rats were obtained from the Laboratory Animal Center of the University of Helsinki. Ovalbumin, leukotriene C_4 (LTC_4), prostaglandin D_2 (PGD_2), histamine, serotonin, and the H_1 antagonist diphenhydramine were from Sigma (St. Louis, MO); the H_2 antagonist ranitidine (Rantacid®) was from Leiras (Turku, Finland); aluminum hydroxide gel was from Merck (Darmstadt, Germany); medetomidin (Domitor®) was from Orion-Farmos (Espoo, Finland); mouse monoclonal antibody to rat IgE heavy chain (MARE-1), standard rat IgE myeloma IR162, and horseradish peroxidase-conjugated mouse monoclonal antibody to rat kappa light chain (MARK-1; MCA 187p) were from Serotec (Oxford, England); TMB (3′,3′,5′,5′-tetramethylbenzidine) was from Kirkegaard & Perry Laboratories Inc. (Gaithersburg, MD); ^{14}C-labeled sucrose [U-^{14}C]sucrose (200 Ci/mol) and chromium-51 (sodium chromate in aqueous solution; 1 mCi/ml) were from Amersham International (Buckinghamshire, England); pertussis vaccine was from the National Public Health Institute of Finland. ELISA dishes (Combiplate 8) were from Labsystems (Helsinki, Finland). Before subcutaneous or intravenous injections, the rats were anesthetized with an intramuscular injection of medetomidin (300 μg/kg)

Immunization of rats and preparation of IgE serum

Rats weighing 200–250 g were given a single intramuscular injection of 1 mg of ovalbumin as antigen in 1 ml of physiological saline containing 200 mg aluminum hydroxide gel (20). To enhance the production of IgE antibodies, inactivated *Bordetella pertussis* organisms (2×10^{10} in 0.5 ml physiological saline) were injected subcutaneously as adjuvant at the same time as the antigen (18). At 3 weeks after the start of immunization, the rats were killed to obtain immune serum. In the pooled (from 20 rats) immune serum used to passively sensitize cutaneous mast cells in the various experiments reported here, the concentration of IgE, as measured by ELISA, was 38 μg/ml. The IgE-containing serum was stored in aliquots at −70°C, thawed once, and used for the experiments.

Isolation of LDL and preparation of [^{14}C]sucrose-LDL

Human LDL (d 1.019–1.050 g/ml) was isolated from plasma by sequential ultracentrifugation in the presence of 3 mM Na_2EDTA, as described (21). The isolated LDL was labeled with [^{14}C]sucrose (22) to yield specific activities in the range of 8–24 dpm/ng (n = 6) of LDL protein.

Passive cutaneous anaphylaxis (PCA)

PCA was induced with IgE according to the method of Mota (18). Rats weighing 300–350 g were anesthetized with an intramuscular injection of medetomidin (300 μg/kg) and the dorsal skin was shaved. Then, two to sixteen dorsal skin sites per rat were sensitized with

intradermal injections of 50 µl of variously diluted IgE serum against ovalbumin. Control sites were injected with 50 µl of normal rat serum or PBS. In preliminary experiments, successful induction of the PCA reaction was tested by injecting Evans Blue intravenously with ovalbumin, and documenting the skin reactions (blue-colored skin) by photography. The size (diameters) and intensity (graded arbitrarily by eye) of the blue spots (i.e., the estimated quantity of Evans Blue in sensitized skin areas) correlated with the quantity of IgE injected, and showed a similar dose response to IgE as did [^{14}C]sucrose-LDL (not shown).

Determination of skin histamine content

The rats were killed and skin plugs (6 mm in diameter) were removed with a dermal biopsy punch. Subcutaneous fat was then removed, and the pieces of skin were weighed, and chopped up with a pair of scissors. The pieces were then placed in 500 µl of distilled water and subjected to five freeze-thawing cycles (liquid nitrogen–warm tap water). Trichloroacetic acid (final concentration 5%) was then added to the tubes, which were vortexed, incubated at 4°C for 30 min, and centrifuged at 10,000 *g* for 10 min. A 200-µl sample was then removed from each supernatant and the histamine content was determined fluorometrically (Beckman Ratio Fluorometer) according to the method of Shore, Burkhalter, and Cohn (23), and expressed as ng of histamine per mg skin (wet weight).

Determination of skin ^{14}C radioactivity

In each experiment, the stated quantity of IgE (in 50 µl of the appropriately diluted immune serum) or of a mast cell-derived mediator (histamine, serotonin, PGD_2, or LTC_4) was injected symmetrically into the left and right sides of the back skin of rats. Dorsal skin sites injected with 50 µl of normal rat serum or PBS served as controls. In one experiment (see Fig. 3), IgE was injected into the left side, and histamine into the right side of the skin of the back. For determination of [^{14}C]sucrose-LDL uptake by tissues, ^{14}C radioactivity was measured in the pieces of tissues after injection of the labeled LDL into the tail vein. The pieces of tissue were dissolved by incubating them overnight in 0.2–2.0 ml of 2 N KOH at 37°C. The measurements were conducted in 0.2-ml aliquots of the dissolved tissue, using a liquid scintillation counter (1215 Rackbeta). The results, calculated from the specific activities of the labeled LDL preparations and expressed in ng/mg (LDL protein/tissue wet weight), are means of the values obtained from the two contralateral, identically treated sites on the dorsal skin. All experiments were performed a minimum of three times.

Determination of blood volume in pieces of skin

The volume of the vascular bed in the PCA reaction sites was determined with the aid of ^{51}Cr-labeled rat red blood cells (^{51}Cr-RBC). The binding of ^{51}Cr to RBC (red blood cell) is tight, and persists after the labeled RBC are injected into the circulation (24). As release of histamine does not lead to extravasation of RBC, the ^{51}Cr radioactivity measured in the pieces of skin is present intravascularly. RBC isolated from rat blood were labeled by incubation for 30 min at room temperature with 500 µCi of ^{51}Cr, and then washed extensively. A dose of the ^{51}Cr-RBC was injected intravenously, and after 24 h, when the rapid phase of ^{51}Cr decay in the blood had leveled off, histamine or PBS was injected into various sites of the dorsal skin. The radioactivities of the blood (cpm/µl) and of the skin sites (cpm/piece of tissue; diameter 6 mm; mean weight 33 mg) were then measured, and the blood volumes in the skin sites were calculated.

Determination of IgE in the antiovalbumin sera

The concentration of total IgE in the serum pool was measured by ELISA with two monoclonal antibodies, anti-rat IgE (MARE-1), and labeled anti-rat immunoglobulin kappa light chain peroxidase (MARK-1), according to Holmes et al. (25).

Statistical analysis

The results were processed by SigmaPlot® or SAS. Descriptive statistics are presented as mean values of the experiments and their SEMs. The values for histamine and LDL (see Fig. 3) were analyzed by ANOVA, and model-based contrasts were formed for the effects of doses by comparing each dose with the baseline value. The difference between the skin sites injected with IgE (+ovalbumin vs. −ovalbumin was tested using Student's *t* test (see Table 1). Differences between groups were considered significant when $P < 0.05$.

RESULTS

IgE-mediated transport of plasma LDL to PCA reaction sites

To characterize the ability of IgE-mediated stimulation of mast cells of rat skin to induce transport of plasma LDL into the skin, the mast cells were sensitized by injection of antiovalbumin IgE-containing serum into rats intradermally. After 48 h, the sensitized mast cells at the injection sites were stimulated by injecting the rats with an intravenous bolus of ovalbumin, i.e., the specific IgE-binding antigen. With 5 mg of the oval-

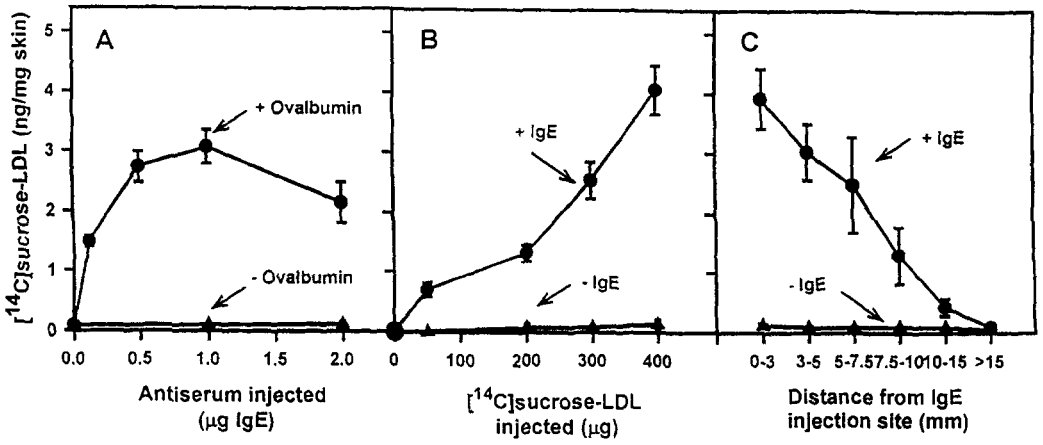

Fig. 1. IgE-mediated uptake of plasma LDL by skin as a function of the amount of IgE injected into the skin (A), of the amount of LDL injected intravenously (B), and of the distance from the IgE injection site (C). Sites of rat skin were sensitized by intradermal injections of immune serum containing the indicated quantities of IgE (A), or 1.0 μg of IgE (B and C). Injections without IgE (PBS buffer only) served as controls. After 48 h, the standard dose (5 mg) of ovalbumin was injected intravenously together with 300 μg (A and C) or the indicated quantities (B) of [^{14}C]sucrose-LDL. One hour later, the rats were killed, and the sensitized skin around the injection site (diameter 15 mm) was removed (A and B) with a biopsy punch, or cut into concentric rings 0–3, 3–5, 5–7.5, 7.5–10, 10–15, and > 15 mm (C) distant from the injection site. The contents of ^{14}C radioactivity in the skin samples were then measured. Data are expressed as mean ± SEM (n = 3–6).

bumin, 300 μg (protein) of [^{14}C]sucrose-LDL was also injected. One hour later, the rats were killed and at the sites into which IgE had been injected (=PCA reaction sites) pieces of skin were removed and their contents of ^{14}C radioactivity were measured. As shown in **Fig. 1A,** the content of LDL in the skin depended on the dose of IgE injected intradermally, the maximum content being achieved with 1.0 μg IgE. When LDL was injected without ovalbumin, no increase was observed, showing that it was not the injection of the IgE-containing serum into the skin that had triggered the transport of LDL. At the control sites, where normal rat serum devoid of antiovalbumin IgE had been injected, there was no increase in LDL content (not shown). The maximum content of LDL measured at the PCA reaction sites was about 40-fold higher than at the control sites. Figure 1B shows that the LDL content at the PCA reaction sites increased linearly as a function of the amount of LDL protein injected up to at least 400 μg of LDL. There was no increase in LDL content when buffer (PBS) had been injected into the skin, instead of IgE. To find out how strictly the effect of mast cell stimulation on the transport plasma LDL was localized in the skin at the PCA reaction sites, the content of LDL was measured at different distances round the IgE injection site. As shown in Fig. 1C, the amount of LDL taken up by the skin decreased as a function of the distance from the IgE injection site, being as low as at the control skin sites when the distance from the injection site was more than 15 mm.

The affinity of the receptors for IgE ($Fc_\epsilon RI$) is very high, and the rate of dissociation of IgE from the mast

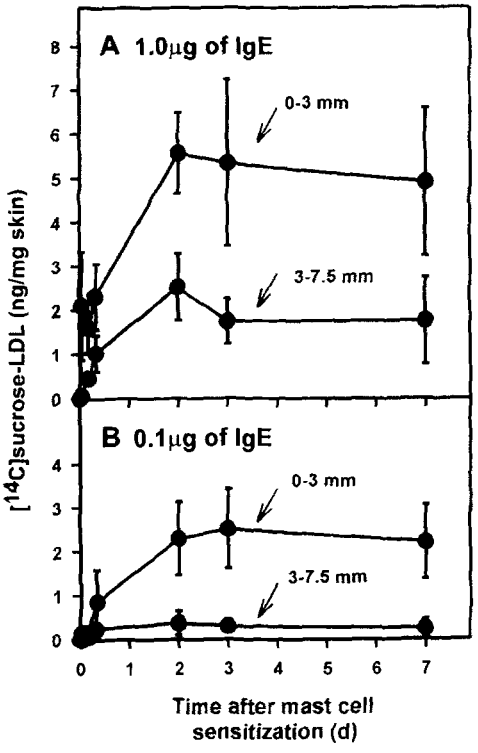

Fig. 2. Effect of distance from the IgE injection site and of the time after IgE injection on uptake of plasma LDL by skin. Sites of rat skin were sensitized with immune serum containing the indicated quantities of IgE (1.0 or 0.1 μg). At the indicated times after IgE injection, ovalbumin and 400 μg of [^{14}C]sucrose-LDL were injected intravenously, and 2 h later the rats were killed, the skin at the sensitized sites was cut into concentric rings 0–3 mm and 3–7.5 mm distant from the injection site, and the contents of ^{14}C radioactivity in the rings were measured. Data are expressed as mean ± SEM (n = 3).

TABLE 1. Uptake of plasma LDL by different tissues in the rat after passive cutaneous sensitization followed by intravenous antigen challenge

Tissue Site	Content of [^{14}C]Sucrose-LDL		−Fold Increase
	−Ovalbumin	+Ovalbumin	
	ng/mg tissue		
Skin(IgE)[a]	0.10 ± 0.02	2.51 ± 0.16[b]	25
Skin(PBS)	0.08 ± 0.03	0.12 ± 0.02	1.5
Skin(non injection)	0.07 ± 0.02	0.08 ± 0.01	1.2
Liver	2.69 ± 0.14	2.04 ± 0.09	0.8
Lung	1.96 ± 0.18	1.80 ± 0.11	0.9
Spleen	2.73 ± 0.01	2.00 ± 0.10	0.7
Heart	0.97 ± 0.08	0.79 ± 0.05	0.8
Kidney	0.99 ± 0.04	0.78 ± 0.04	0.8
Adrenal	3.10 ± 0.14	2.51 ± 0.28	0.8
	μg/ml		
Serum	19.8 ± 0.77	18.4 ± 1.38	0.9

Sites of rat skin were sensitized with intradermal injections of immune serum containing 1 μg of IgE. After 48 h, 300 μg of [^{14}C]sucrose-LDL without (n = 4) or with (n = 6) the standard dose of ovalbumin (5 mg) was injected intravenously. Two hours later, the content of ^{14}C-radioactivity in the indicated tissue sites was determined. From each tissue site, duplicate samples were taken (the diameter of each skin sample was 15 mm).

[a]Intradermal injection of IgE-containing (1 μg) immune serum.

[b]$P < 0.001$; the difference between the skin sites injected with IgE (+ovalbumin versus −ovalbumin) was tested using Student's *t* test.

cells is therefore very slow, the half-life of cell-bound IgE being estimated at over 100 h (26). In vivo, the effective half-life of IgE is probably even longer, because the IgE that dissociates from its receptors is functionally normal and can rebind to the same or other IgE receptors on the same or on other skin mast cells. To test the effect of this "tissue fixation of IgE" (18) on the ability of skin mast cells to induce uptake of plasma LDL by skin, we injected LDL and ovalbumin (to trigger the PCA reaction) 1 h, 8 h, 2 d, 3 d, and 7 d after intradermal injection of IgE, and, for each time point, measured the LDL content of the skin 2 h after triggering the PCA reaction. As shown in **Fig. 2,** the effect of IgE injection was found to last for at least 1 week, reflecting tissue fixation of IgE. With 1.0 μg of IgE, the content of LDL within 3 mm of the injection site was maximal at 2 d and then remained essentially the same (panel A). Similarly, an effect of tissue fixation of IgE on LDL uptake was observed at greater distances (3–7.5 mm) from the injection site, and at shorter distances (0–3 mm) when a smaller quantity of IgE (0.1 μg) was injected (panel B). Interestingly, in the two latter cases, the rates of uptake of LDL were closely similar. Finally, the smaller quantity of IgE did not sensitize the mast cells enough at the longer distance to allow induction of LDL uptake upon antigen injection. In summary, the finding of prolonged (up to 1 week) dependence of LDL uptake on the dose of IgE and on the distance from the injection site of IgE both accord with the known long-lasting binding of IgE to their receptors on skin mast cells. Moreover, these results are compatible with the notion that the amount of IgE available for sensitization of cutaneous mast cells is a critical determinant of the rate of uptake of plasma LDL at the PCA reaction sites.

The idea of the local character of mast cell-dependent uptake of LDL at tissue sites at which the mast cells had been sensitized was further strengthened by the results of an experiment in which we measured the content of LDL in several rat tissues after intravenous injections of LDL with or without ovalbumin. An ovalbumin-dependent increase in LDL uptake (25-fold) was present only at the sensitized skin site, but not at the unsensitized skin sites, or elsewhere in the body (**Table 1**).

Role of vasoactive compounds released by stimulated mast cells in the transport of plasma LDL to skin

To study the contribution of histamine, the major vasodilator contained in mast cells, to the observed mast cell-dependent uptake of the LDL by rat skin, the contents of histamine and LDL were related to each other in two experimental settings. IgE was injected intradermally into dorsal skin sites of rats in various quantities (**Fig. 3**), and 48 h later the rats received the standard intravenous injection of ovalbumin and [^{14}C]sucrose-LDL. Two hours later, the rats were killed, the skin sites were removed, and their contents of histamine (Fig. 3A) and ^{14}C radioactivity (Fig. 3B) were measured. A dose-dependent decrease in histamine content and a dose-dependent increase in LDL content were ob-

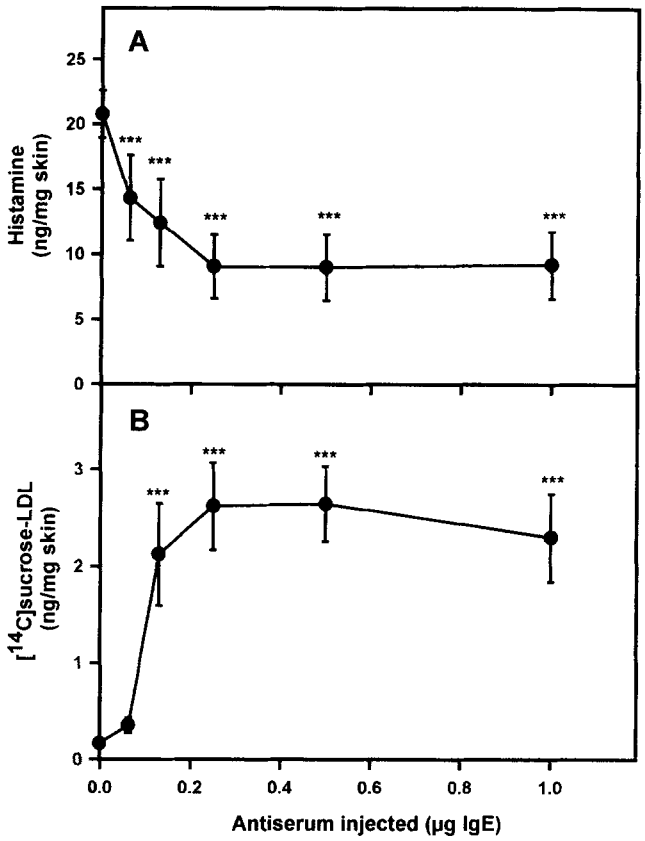

Fig. 3. Effect of intradermal injection of IgE on skin histamine content (A) and on uptake of plasma LDL by skin (B). Sites of rat skin were sensitized by intradermal injections of immune serum containing the indicated quantities of IgE. Forty-eight hours later, each rat received an intravenous injection of ovalbumin and 300 μg of [^{14}C]sucrose-LDL. After 2 h, the skin at the sensitized sites (15 mm diameter) was removed and the contents of histamine and ^{14}C radioactivity were measured. Data are expressed as mean ± SEM (n = 5). ***$P < 0.005$ vs. baseline value (no IgE injected).

served at the PCA reaction sites. The maximal decrease in histamine content was one-third, signifying that the mast cells at the PCA reaction sites had degranulated and had lost, on average, one-third of their histamine content. This degree of histamine loss was sufficient to induce the maximal uptake of labeled LDL (a 25-fold increase).

To obtain further evidence regarding the role of histamine in the transendothelial transport of LDL from the plasma to the PCA sites, we measured the effects of histamine receptor antagonists on the rates of LDL uptake. For this purpose, rats injected intradermally with IgE received, together with ovalbumin and LDL, intravenous injections of either an H_1 receptor antagonist (diphenhydramine) or an H_2 receptor antagonist (ranitidine). As shown in **Fig. 4,** both antagonists inhibited the rate of LDL transport to the skin at the PCA reaction site, the H_1 antagonist being somewhat more effective at the lower concentrations used. Inhibition

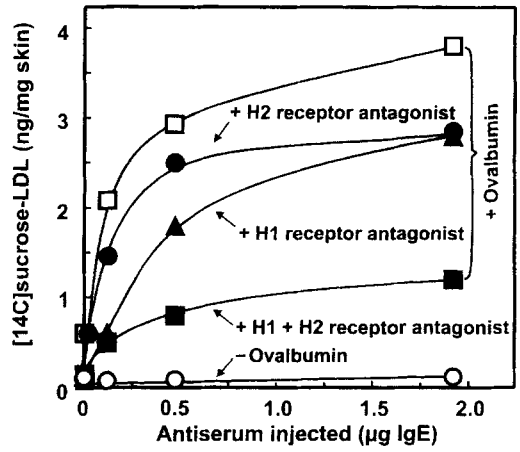

Fig. 4. Inhibition by H_1 and H_2 receptor antagonists of IgE-mediated uptake of plasma LDL by skin. Sites on rat skin were sensitized with intradermal injections of immune serum containing the indicated quantities of IgE. Forty-eight hours later, ovalbumin and 300 μg of [^{14}C]sucrose-LDL were injected intravenously with or without an H_1 receptor antagonist (10 mg diphenhydramine) or/and H_2 receptor antagonist (10 mg ranitidine). One hour later, the rats were killed, and the contents of ^{14}C radioactivity in the skin at the treated sites (15 mm diameter) were measured. The control rats received [^{14}C]sucrose-LDL without ovalbumin.

was even stronger (on average 70%) when the two inhibitors were injected together.

In the above experiment, about 30% of the PCA-induced transport of LDL was not inhibited by the two histamine receptor antagonists combined, suggesting the simultaneous action of other vasoactive compounds. After IgE-mediated stimulation, rat skin mast cells are known to release vasoactive compounds such as serotonin, LTC_4 and PGD_2. As shown in **Table 2,** these compounds, when injected intracutaneously into rat skin, also induced transport of LDL into the skin.

Local extravascular LDL accumulation at PCA reaction sites

Release of histamine from mast cells in the skin induces local vasodilation, which may have contributed to

TABLE 2. Effects of various vasoactive compounds on plasma LDL transport into rat skin

Compound	Content of [^{14}C]Sucrose-LDL
	ng/mg skin
Histamine (10 μg)	3.07 (2.86; 3.28)
Serotonin (10 μg)	2.68 (2.39; 2.92)
LTC_4 (1 μg)	1.59 (1.90; 1.28)
PGD_2 (1 μg)	1.30 (1.07; 1.52)
PBS (50 μl)	0.28 (0.29; 0.27)
No injection	0.04 (0.05; 0.04)

Two rats each received an intravenous injection of 400 μg of [^{14}C]sucrose-LDL and, immediately after the injection, separate skin sites received the indicated quantities of the indicated mediators (known to be contained in rat mast cells). The content of ^{14}C-radioactivity in the skin sites (diameter 6 mm) was determined 2 h later. Individual values for the two rats are shown in parentheses.

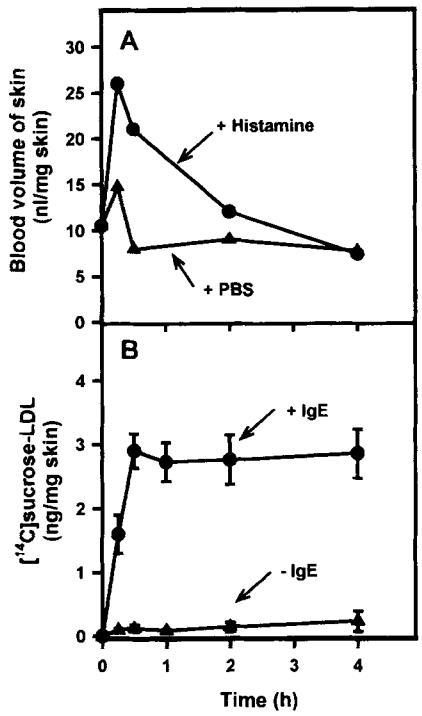

Fig. 5. The volume of blood at the intracutaneous histamine injection site (A) and the content of LDL at the PCA reaction site (B) as a function of time. A: A dose of the ^{51}Cr-RBC was injected intravenously into each rat. After 24 h, when the rapid phase of ^{51}Cr decay in the blood had leveled off, histamine or PBS was injected into the dorsal skin at various sites. After the indicated time interval, the rats were killed, and the radioactivities of the blood (cpm/μl), and in the skin at the injection sites (cpm/piece of tissue; diameter 6 mm; mean weight 33 mg) were measured, and the blood volumes in the skin sites were calculated (nl/mg skin). B: Sites of rat skin were sensitized by intradermal injections of immune serum containing 1.0 μg of IgE. Injections without IgE (buffer only) served as controls. After 48 h, the standard dose (5 mg) of ovalbumin was injected intravenously together with 300 μg of [^{14}C]sucrose-LDL. After the indicated time interval, the rats were killed, and the contents of ^{14}C radioactivity in the skin at the treated sites (15 mm diameter) were measured. Data (in panel B) are expressed as mean ± SEM (n = 4).

the observed uptake of LDL by the skin. To estimate the extent of the contribution of this vasodilation to the uptake of LDL, we related the blood volumes at the PCA reaction sites to the increases in LDL content at various time points after injection of histamine. As shown in **Fig. 5A,** histamine injection induced a rapid increase in local blood volume, vasodilation being maximal (an increase of about 2-fold) 15 to 30 min after the injection. Injection of PBS also caused a small transient increase of the blood volume, probably owing to nonspecific stimulation of mast cells. The blood volume in the skin at the treated site gradually decreased and returned to its original level 2 h after the histamine injection. When 300 μg of LDL was injected intravenously and uptake was measured as a function of time after the PCA reaction, the [^{14}C]sucrose-LDL content at the PCA reaction sites was observed to increase rapidly, the level reaching a maximum within 30 min of antigen injection; the level then remained unchanged over the 4-h observation period (Fig. 5B). When the skin sites had been sensitized with buffer only (PBS instead of IgE), the skin content of LDL did not increase significantly. From the experiments shown in Fig. 5 A and B, we conclude that the far greater increase in the content of [^{14}C]sucrose-LDL at the PCA reaction site, which was always more than 25-fold higher than at the control sites, was not due to the local vasodilation caused by histamine.

We then calculated of the intravascular content of [^{14}C]sucrose-LDL per mg skin, and related them to the total content of [^{14}C]sucrose-LDL per mg in the variously treated pieces of skin. The results of these calculations revealed that, in untreated skin sites, all of the labeled LDL was located intravascularly, i.e., was contained in the about 10 nl of blood (about 5 nl of serum) present in 1 mg of untreated skin, or in 1 mg of treated skin 2 h or more after the treatment (Fig. 5A). Accordingly, the control values for skin LDL in Table 1 (0.07 to 0.12 ng of LDL/mg skin; mean, 0.10 ng/mg skin) reflect the intravascular contents of LDL in the skin. Then, of the total content of LDL at the PCA reaction site (2.51 ng LDL/mg skin), 96% (2.41 ng) is located extravascularly and 4% (0.10 ng) intravascularly. Similarly, in Table 2, the 0.04 ng of the LDL present in the untreated skin (no injection) resides intravascularly. By subtracting this quantity of LDL from the other values in Table 2, we find that after injection of histamine, serotonin, LTC_4, PGD_2, and PBS, the percentages of LDL located extravascularly were 99%, 98%, 97%, 95%, and 86%, respectively. The value for PBS results from the nonspecific stimulation of skin mast cells (see also Fig. 5A). Accordingly, the increase in skin LDL content (i.e., LDL uptake) in the experimental system used in this study does, in fact, measure increased transendothelial transport of plasma LDL into the extravascular pools, as has been found for other plasma constituents at the sites of the PCA reaction (27). In a further experiment, we extended the period of observation and measured the skin LDL content for up to 24 h (**Fig. 6**). Again, there was a rapid increase in the LDL content at the PCA reaction sites, the level reaching a maximum within 4 h of antigen injection, then decreasing slightly, and subsequently remaining unchanged during the observation period.

DISCUSSION

The studies described here demonstrate, for the first time, that stimulation of mast cells in vivo leads to an increase in the transendothelial transport of LDL from

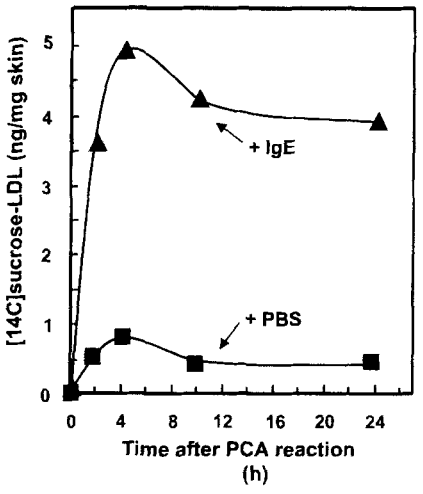

Fig. 6. Accumulation of LDL in sensitized skin during 24 h after PCA reaction. Sites of rat skin were sensitized by intradermal injections of immune serum containing 1.0 μg of IgE. Injections without IgE (PBS buffer only) served as controls. After 48 h, ovalbumin was injected intravenously together with 400 μg of [^{14}C]sucrose-LDL. After the indicated time intervals, the rats were killed, and the contents of ^{14}C radioactivity in the skin at the treated sites (6 mm diameter) were measured.

the plasma to a tissue site. We chose the PCA reaction in rats as a model because it has been widely used in studies of the local actions of mast cells. The increased transport of LDL was strictly localized to the PCA reaction site. This spatially restricted effect of mast cells can be attributed to the "tissue fixation" of IgE antibodies (18), i.e., their tight binding to the high-affinity IgE receptors on the mast cells, which also explains the greater accumulation of LDL in the central areas of the PCA site than in the peripheral areas: the mast cells in the immediate vicinity of the injection site had been exposed to higher concentrations of IgE than those further away. Consequently, the IgE receptors of the mast cells at the center were more likely to become occupied by IgE and so, when stimulated to release, on average, more histamine than their peripheral counterparts (20). However, within the 2-day sensitization period, some of the IgE molecules must have been released from their high-affinity receptors, diffused away, and bound to receptors on other mast cells. Despite such local redistribution of IgE, the spatial gradient in LDL uptake was maintained.

The highly localized effect of the PCA reaction on LDL transport revealed that the histamine (and other vasoactive compounds) released from the stimulated mast cells acted only in the vicinity of the parent cells. Indeed, we found no effect of the PCA reaction at the nonsensitized skin sites, or elsewhere in the body. Moreover, with mast cells as the source of histamine, the histamine reached the abluminal surface of the endothelial cells in concentrations within the (upper) physiological range when skin mast cells are stimulated to degranulate. In these respects, the PCA model complements previous studies of the effect of histamine on transendothelial transport of LDL, in which unphysiologically high (pharmacological) dose of histamine have been injected intraluminally into vessels (28, 29). However, other in vivo techniques must be devised if targeted stimulation of mast cells is to be measured in other tissues, such as the arterial wall, of experimental animals.

In addition to histamine, the mast cells of rat connective tissues are known to release other vasoactive substances such as serotonin, LTC_4, and PGD_2 (30), which also increase the microvascular permeability of animal and human skin, causing plasma protein and fluid leakage into the extravascular tissues (16, 27, 31, 32,). We confirmed that intracutaneous injection of these compounds increased the rate of LDL transport into the skin. As we did not perform dose–response studies with these agents, their relative effectiveness in increasing LDL transport in the rat skin cannot be assessed here. However, with regard to their actual contribution to the mast cell-mediated increase in LDL transport to the rat skin, the ability of histamine receptor blockers to suppress the increase in LDL transport by 70% seems to leave only a minor role to other agents. This suggestion agrees with the predicted responses of human mast cells on postcapillary venules: because histamine is more effective as an inducer of vascular leakage than PGD_2 or LTC_4, and human mast cells release histamine, PGD_2, and LTC_4 in approximate molar ratios of 1000:25:2 during allergic stimulation, the major mast cell mediator increasing venular permeability in man is also likely to be histamine (33).

In the skin, as in other tissues, mast cells are located around the vessels of the microvascular tree, notably the postcapillary venules (13). It is in these areas of the microvasculature, where the endothelium has been shown to be especially sensitive to histamine action (34), that investigations have been made into the structural basis for the increased permeability to plasma proteins, such as albumin, during histamine action. These studies have revealed that the basal permeability is governed by transendothelial transport of the plasma proteins in transcytotic vesicles (35), and that the high-permeability state induced by histamine results from rapid formation of transient gaps between the endothelial cells (36–38). Similarly, LDL particles also find their way into the extravascular space in transcytotic vesicles under normal circumstances (35) but, after exposure of the endothelial barrier to histamine, extravasation of LDL particles results from ultrafiltration coupled to water flow by a solvent drag mechanism at the sites where gaps have formed between the endothelial cells (28, 29). The onset and duration of the histamine-induced increase

in permeability to LDL were studied in an in situ preparation of hamster mesentery by Rutledge et al. (28). In their system, the peak in gap-dependent permeability occurred between 1 and 6 min after exposure of the vessels to histamine. In our model with living rats, the mast cell-mediated transendothelial transport of LDL started about 15 min after intravenous injection of the antigen, a delay that can be attributed to the time required *i*) for the ovalbumin to reach the sensitized skin mast cells, *ii*) for the mast cells to release histamine, and *iii*) for the histamine to reach the endothelial cells. In the mesenteric in situ preparation, the histamine-induced increase in transport of LDL was found to return toward basal level within about 15 min even when high concentrations (100 μM) of histamine prevailed (28). In the skin, uptake of LDL reached maximum only at 60 minutes, a delay conceivably owing to variation in the start of the LDL influx within the cutaneous microvasculature at the PCA site. However, even a rapid transient opening of the gaps should allow significant transport of LDL to the extravascular fluid of skin because, in the skin as in other extrahepatic tissues, there is a steep LDL concentration gradient from the plasma to the extracellular fluid (39).

Increased transendothelial transport of LDL in the skin could contribute to local accumulation of cholesterol. For cholesterol to accumulate in the skin, i.e., for xanthomas to form, a long-lasting and usually great increase in the concentration of lipoproteins in the blood plasma is required, the classic example being the homozygous form of familial hypercholesterolemia, in which the level of plasma LDL cholesterol is 5- to 10-fold higher than normal from the time of birth (40). However, despite such elevation, xanthomas develop in only a few highly selected skin sites, such as the skin of the elbows, knees, and buttocks. One feature common to all the xanthoma-prone skin sites is their exposure to mechanical strain. As mechanical strain (vibration) can also induce stimulation of skin mast cells with ensuing histamine release (41), it is possible that the degree of mast cell stimulation is higher in the xanthoma-prone skin sites than in the sites where xanthomas do not form. Moreover, in patients with various types of hyperlipidemia, xanthomas have been reported to form in traumatized skin areas, such as after a cat scratch or a bee sting. Thus, eruptive and tubero-eruptive xanthomas formed at sites of skin injury in patients with type III (42, 43), type IV (42, 44, 45), or type V hyperlipidemia (46). Interestingly, papular xanthomatosis was reported in a normolipidemic patient with erythrodermic atopic dermatitis (47). Finally, disseminated xanthomas were reported in systemic mast cell disease in a patient with type IV hyperlipidemia, lending support to the hypothesis of mast cell involvement in skin xanthoma formation (48).

For xanthomas to form, it is necessary for the plasma LDL particles not only to enter the subendothelial space of tissues, but also to enter local phagocytes, such as macrophages. Previously, we described a mechanism for mast cell-dependent foam cell formation in vitro. In this multistep pathway, exocytosed granules of rat serosal mast cells first lose their histamine, then the histamine-depleted granule remnants bind LDL (49), and finally are phagocytosed by cocultured macrophages, carrying LDL with them into the macrophages, with eventual foam cell formation (50). We recently obtained support for the view that this mechanism also operates in vivo, when we observed, with the aid of immunoelectronmicroscopy, that subendothelially located exocytosed mast cell granules bind LDL (apolipoprotein B-100) and so may carry it into the smooth muscle cells of the human arterial intima (51). In the intimal fluid, the concentration of LDL is high, normally equaling or even exceeding that in the corresponding plasma (52), and so rendering conditions optimal for binding of LDL to the granule remnants. In the skin, as in other extrahepatic tissues (with the notable exception of the arterial intima), the concentration of LDL is much lower (only 1/10) than in the corresponding blood plasma (39). As release of histamine from mast cells leads to a rapid increase in LDL concentration in the extracellular fluid of the skin, suitable conditions for binding of LDL to exocytosed mast cell granules with ensuing foam cell formation exist in the skin, as in the intima, provided the mast cells are first stimulated to degranulate.

In the present study, skin mast cells were stimulated only once, and after the LDL skin content reached a plateau (at 30 min), no further increase in LDL content was noticed within the observation period (up to 24 h); rather, there was a slight decline in skin LDL content. As we used sucrose-labeled LDL (which accumulates in lysosomes), we can interpret the above result to mean that, after the initial rapid influx, no new LDL entered the skin. Whether the LDL particles were anchored in the extracellular matrix of the skin or had been taken up by local cells cannot be deduced from the current experimentation. However, the results clearly indicate that repetitive stimulation of skin mast cells is required for progressive accumulation of LDL cholesterol in the skin, which is one of the prerequisites for xanthomas to form.

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