Receptor-Mediated Binding and Internalization of Leukocyte Elastase by Alveolar Macrophages In Vitro

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ABSTRACT Radioiodinated leukocyte elastase was found to bind rapidly and specifically to alveolar macrophages in vitro. In contrast to the binding of pancreatic and bacterial proteases, leukocyte elastase binding did not require the presence of alpha$_2$ macroglobulin. The binding was inhibited by an excess of unlabeled enzyme and was saturable by increasing elastase concentrations. Leukocyte elastase binding thus met criteria for receptor-mediated binding, with an estimated association constant of $4.97 \times 10^5\ M^{-1}$ and an estimated total of 640×10^6 binding sites/cell. It differed from the previously described binding of lysosomal glycosidases to macrophages in that it was insensitive to trypsin pretreatment, did not require calcium ions, and was not inhibited by yeast mannan. High-resolution autoradiography indicated that the cell-associated radiolabeled leukocyte elastase was rapidly incorporated into phagolysosomes. Macrophage binding may have a role in clearance of leukocyte elastase from tissue sites where alpha$_2$ macroglobulin is absent or present in low concentration. Thus, enzyme uptake by alveolar macrophages may be an important factor in the amelioration of lung tissue injury by extracellular leukocyte elastase.

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INTRODUCTION

Tissue (1–6) and alveolar (7, 8) macrophages have surface receptors that facilitate the uptake and clearance of proteases from the extracellular space. Previous studies of pancreatic and bacterial proteases indicated that this receptor system is operative only when the proteases are complexed with alpha$_2$ macroglobulin. However, macrophages have been found to be responsible for uptake and clearance of certain lysosomal glycosidases (9–11) and lactoferrin, a neutrophil granule-associated protein (12, 13), by means of direct binding of these glycoproteins to cell surface receptors.

Recently, it was shown that immunoreactive human leukocyte elastase (HLE)[1] appears within alveolar macrophages within minutes after intratracheal instillation (14, 15). Because macroglobulin is absent or present in low concentrations in bronchoalveolar lining fluid (16), and because HLE is not immunoreactive when complexed with alpha$_2$ macroglobulin (17), finding HLE within alveolar machrophages by an immunologic technique suggested that these cells might bind neutrophil elastase without a requirement for prior association with macroglobulin. Thus, it appeared possible that macrophage uptake of this neutrophil enzyme might be different from that for the pancreatic and bacterial proteases previously studied.

[1] *Abbreviations used in this paper*: BAPNA, α-*N*-benzyl-DL-arginine-*p*-nitroanilide HCl; BSA, bovine serum albumin; HBSS, Hanks' balanced salts solution; HLE, human leukocyte elastase; HPE, human pancreatic elastase; NTM, serumless medium (Neuman and Tytell); PBS, phosphate-buffered saline; PMSF, phenylmethylsulfonylfluoride; PPE, porcine pancreatic elastase; PPT, porcine pancreatic trypsin; RBC, human erythrocytes; SLAPN, succinyl-L-alanyl-L-alanyl-L-alanine-*p*-nitroanilide; TLCK, *N*-α-*p*-tosyl-L-lysine chloromethyl ketone.

The present work provides evidence for a receptor-mediated clearance mechanism for HLE by human alveolar macrophages. This pathway for uptake of HLE may be particularly important in sites having low concentrations of alpha$_2$ macroglobulin, such as the bronchoalveolar lining and the interstitial space of the lung.

METHODS

Reagents. *N*-α-*p*-tosyl-L-lysine chloromethyl ketone (TLCK), α-*N*-benzyl-DL-arginine-*p*-nitroanilide HCl (BAPNA), soybean trypsin inhibitor, lima bean trypsin inhibitor, EDTA, phenylmethylsulfonylfluoride (PMSF), glucose oxidase, Sepharose 4B-200, yeast mannan, and CM-Sephadex were purchased from Sigma Chemical Company, St. Louis, Mo. Succinyl-L-alanyl-L-alanyl-L-alanine-*p*-nitroanilide (SLAPN) was purchased from Bachem, Inc., Torrance, Calif. The lactoperoxidase used was a product of Calbiochem-Behring Corp., American Hoechst Corp., San Diego, Calif. ^{125}I-NaI (11–17 mCi/μg) was purchased from Amersham Corp., Arlington Heights, Ill. Bio-Gel A-5m was obtained from Bio-Rad Laboratories, Richmond, Calif. Trasylol was obtained from Bayer AG, Wuppertal, West Germany.

Protease inhibitors. Purified alpha$_1$ antitrypsin was kindly supplied by Dr. J. A. Pierce, Washington University School of Medicine, St. Louis, Mo.

Alpha$_2$ macroglobulin was purified from human plasma supplied by the blood bank of The Jewish Hospital of St. Louis by a modification of the method described by Kaplan.[2]

Tissue culture supplies. Hanks' balanced salts solution (HBSS), trypan blue dye, and serumless medium (Neuman and Tytell) (NTM) were purchased from Grand Island Biological Co., Grand Island, N. Y. Conical centrifuge tubes (50 ml) were obtained from Falcon Labware, Div. of Becton, Dickinson & Co., Oxnard, Calif.

Proteins. Porcine pancreatic elastase (PPE), porcine pancreatic trypsin (PPT), and bovine serum albumin (BSA) were purchased from Sigma Chemical Co.

HLE was purified from human neutrophils obtained by leukapheresis of a single volunteer, using Trasylol-Sepharose chromatography (18). Both the amino acid composition as determined with a Beckman 119C amino acid analyzer (Beckman Instruments, Inc., Palo Alto, Calif.) and the electrophoretic properties by disc gel electrophoresis in 7.5% acrylamide at pH4 of the purified HLE corresponded closely to published reports for HLE (18, 19).

Human pancreatic elastase (HPE) was kindly purified by P. Heubner by ion exchange chromatography, followed by affinity chromatography with lima bean trypsin inhibitor as the binding ligand,[3] from pancreatic tissue obtained at autopsy.

Radiolabeling of proteins. Radioiodination of HLE, HPE, PPE, PPT, and BSA was achieved using a modification of the lactoperoxidase method described by Marchalonis (20). 200 μg of each purified protein was mixed with 10 μg of lactoperoxidase, 300 μCi ^{125}I-NaI, and 150 μg dextrose in a total volume of 150 μl phosphate-buffered saline (PBS: 50 mM phosphate, 0.9% wt/vol NaCl, pH 7.4). After 1 h at room temperature, 160 μg sodium metabisulfite, 250 μg potassium iodide, and 1.5 mg BSA were added, and the total volume was brought to 1 ml with PBS. This mixture was dialyzed against several changes of PBS at 4°C until the dialysate contained <500 cpm/ml. Typically, the radiolabeled proteins contained 750,000 cpm ^{125}I/mg. They were stored at 4°C and used within 4 wk of preparation.

Preparation of inactivated HLE. To inactivate HLE, the enzyme was exposed to the serine protease inhibitor PMSF, essentially as described by Polgar and Bender (21). PMSF completely inactivated the HLE, as measured by inability to hydrolyze SLAPN (22), even after prolonged incubation. The inactive HLE was dialyzed against PBS to remove unreacted PMSF. To prepare inactive ^{125}I-HLE, the enzyme was exposed to PMSF after iodination.

Alveolar macrophages. Alveolar macrophages were obtained from healthy volunteers by transbronchoscopic lavage of a basilar segment of the right lower lobe (23). To increase the yield of macrophages, only smokers (from one-half to three packs of cigarettes daily) were selected. After collection, the chilled cells were centrifuged at 500 *g* at 4°C for 10 min and then washed three times with cold NTM. The final suspension, at the desired cell concentration, was kept at 4°C for immediate use. Viability of the freshly collected cells, estimated by trypan blue dye exclusion, was >95%.

Cell binding of HLE and other proteins. 4 ml of cell suspension containing 5 × 10^6 macrophages/ml in NTM were transferred to conical centrifuge tubes, which were continuously shaken at 37°C or at 4°C. Radiolabeled protein (HLE, HPE, PPE, PPT, or BSA) was added, to a final concentration of 2 μg/ml. To determine the total radioactive counts per unit volume of suspension, three 100-μl aliquots were immediately pipetted into 400-μl microfuge tubes (Markson Science, Inc., Bliss & Laughlin Industries, Del Mar, Calif.) and counted. At intervals (typically 5, 10, 20, 30, 60, and 120 min), three 100-μl aliquots were transferred to microfuge tubes containing 200 μl of cold (4°C) PBS. In each instance, these were immediately centrifuged for 20 s (Beckman Microfuge B, Beckman Instruments). The cell pellet was washed twice with cold PBS and then counted.

In later experiments, to eliminate need for repeated washing, the aliquots of cell suspensions were layered over 50 μl of an inert oil (Versilube F$_{50}$, specific gravity 1.05, General Electric Co., Schenectady, N. Y.). Centrifugation of these tubes for 20 s resulted in sedimentation of the cells through the oil with a clean separation from the aqueous phase. The tips of the microfuge tubes, containing the cell pellets, were cut off with a razor blade and counted.

Nonspecific binding of the radiolabeled proteins to alveolar macrophages was esimated in each experiment from the binding to a parallel suspension of erythrocytes (RBC), as outlined by Debanne et al. (7, 8). The nonspecific binding of HLE averaged 6% at 37°C in the absence of serum and increased minimally with time. All data presented have been corrected for this nonspecific binding.

Effect of divalent cation. The macrophages and reference RBC were held in HBSS, (calcium and magnesium free), containing 1 mM EDTA, and washed twice more with HBSS. Triplicate binding assays were performed. In 400-μl microfuge tubes containing 50 μl of Versilube F$_{50}$, 5 × 10^5 cells were incubated with ^{125}I-HLE (2 μg/ml) in HBSS, HBSS with 200 mg/liter anhydrous $CaCl_2$, HBSS with 200 mg/liter $MgSO_4 \cdot 7\ H_2O$, or HBSS with both cations. After 30-min incubation at 37°C, the tubes were centrifuged and the cell-associated radiolabel was determined.

Trypsin sensitivity of binding of HLE. In microfuge tubes, crystalline PPT, 1.0 mg/ml, was incubated with 5 × 10^5

[2] Kaplan, J., and M. Nielsen. 1979. Analysis of macrophage surface receptors. I. Binding of α-macroglobulin-protease complexes to rabbit alveolar macrophages. *J. Biol. Chem.* In press.

[3] Heubner, P. Purification of human pancreatic elastase: the enzymatic activity with human elastin. Submitted for publication.

alveolar macrophages for 30 min at 37°C. TLCK, dissolved in NTM, was then added to achieve a final concentration of 3 mg/ml. This concentration of TLCK completely inhibited the activity of the trypsin against BAPNA, but did not affect binding of HLE to the cells. ^{125}I-HLE was then added, to a final concentration of 2 μg/ml. Triplicate assays were performed. After a 30-min incubation at 37°C, the cell-associated radioactivity was measured.

Effect of serum protease inhibitors, mannan, and bronchoalveolar lavage fluid on binding of HLE. Pooled human serum, 10% vol/vol, was added to the cell suspensions. An ^{125}I-HLE binding assay was then performed as previously described, and the effect of serum was compared to that produced by an equal volume of added PBS.

An additional experiment compared the effects of an excess of purified alpha$_1$ antitrypsin, alpha$_2$ macroglobulin, yeast mannan, or human bronchoalveolar lavage fluid (concentrated ×10 by positive pressure ultrafiltration) with that of whole pooled serum upon the binding of active and PMSF-inactivated ^{125}I-HLE.

Inhibition of HLE binding by unlabeled elastases. Various concentrations of HLE or HPE were mixed with 5×10^5 alveolar macrophages and ^{125}I-HLE (final concentration, 2 μg/ml) in microfuge tubes containing 50 μl of Versilube F_{50}. Binding of the radiolabeled HLE was determined as before, after an incubation for 30 min at 4°C. The adherence of label to the macrophages in the presence of 195 μg/ml of unlabeled HLE was only 0.9% greater than that to RBC in the absence of unlabeled enzyme. We considered this amount to be nonspecific binding. In each case, therefore, the inhibition of binding produced by 195 μg/ml of unlabeled HLE was defined as 100%.

Saturability, affinity, and receptor number. Binding assays were performed as described above, except that the macrophage number was reduced to 10^5 cells/assay, the incubation time was 10 min, and the ^{125}I-HLE concentration in the assay was varied.

High-resolution autoradiography. Macrophages incubated with ^{125}I-HLE in suspension for 5, 10, and 120 min were pelleted in microfuge tubes, washed by resuspension in PBS three times, and the final pellet fixed for 1 h with 3% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.3. The pellets were rinsed with PBS, fixed again for 1 h in 1% osmium tetroxide in cacodylate buffer, and embedded in epoxy resin. Thin (800 Å) sections were mounted on 300 mesh grids, coated with Ilford L-4 nuclear tract emulsion (Ilford Ltd., Essex, England) by the loop technique (24), and exposed in light-tight boxes for 2 mo at 4°C. The grids were developed for 5 min with Microdol-X (Eastman Kodak Co., Rochester, N. Y.), fixed in Kodak rapid fixer, and rinsed in five changes of distilled water. The emulsion was removed with 0.05N NaOH and the grids stained with uranyl acetate and lead citrate. Electron micrographs were made of 50 consecutive labeled cells for each time interval and printed at a final magnification of ×13,000.

To localize the enzyme within the cells, the center of each grain was marked using a transparent overlay on which a circle 1 cm in diameter was transcribed. A 1-mm hole was made in the overlay at the center of the circle. The circle was centered around each grain and a pinhole made in the micrograph through the hole in the overlay. This pinhole was taken as the center of the grain. The grains were tabulated according to the structures in which the pinhole occurred. The organelles used for the tabulation were nuclei, mitochondria, granules (lysosomes), vacuoles, and cytoplasm (which included endoplasmic reticulum). The vacuoles were found almost exclusively in the peripheral cytoplasm near the plasma membrane, and were presumed to be endocytic vacuoles.

To determine whether label was selectively concentrated in a given organelle, chi-square analysis was used to compare the number of grains observed over that organelle to the number of grains expected assuming a uniform concentration of label in all cellular structures. For each time period, the number of expected grains for each organelle was calculated by multiplying the total number of grains found over cells by the volume density (volume fraction) for that organelle. The volume density of each type of organelle was determined on 10 electron micrographs from each time interval by point counting, with a transplant overlay that provided 980 points/micrograph.

RESULTS

Radiolabeled proteins. Purified HLE migrated as a series of isozymes with slightly different electrophoretic mobility, as found by others (18, 19). The electrophoretic migration of HLE was unchanged by the labeling procedure, as shown in Fig.1. Radiolabeled HLE comigrated with freshly purified unlabeled HLE. Typically, 80% of the HLE activity against SLAPN remained after radiolabeling. Greater than 95% of the radioactivity of each radiolabeled protein was precipitable by 10% TCA.

Binding of HLE to human alveolar macrophages. When ^{125}I-HLE was incubated with alveolar macrophages at 37°C in NTM, a rapid initial phase of binding was observed (Fig. 2). In the experiment shown, 48% of the radiolabel was bound to the cells within 30 min at 37°C. After this time, a slow linear increase in cell-associated radiolabel was observed, which averaged ≅1.5%/h over many observations.

Similar binding occurred during the first 10 min of incubation (Fig. 3) at 4° as at 37°C. Thereafter, binding was less at 4°C.

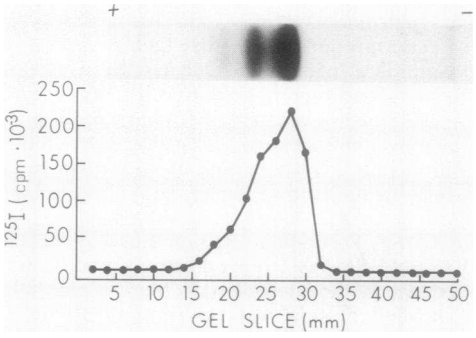

FIGURE 1 Top: Polyacrylamide gel, run at pH 4, of freshly purified HLE. In accordance with the findings of others, the purified HLE migrates as a series of isozymes with slightly different electrophoretic mobilities. Bottom: graph of results obtained when ^{125}I-radiolabeled HLE was chromatographed in identical fashion, after which the gel was cut into 2 mm slices and counted. Distance from top of gel is expressed on a scale to correspond with the gel photograph.

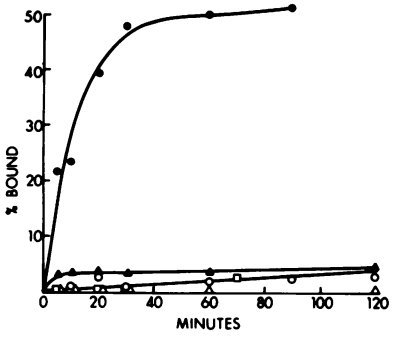

FIGURE 2 Binding of ^{125}I-HLE and various other ^{125}I-radiolabeled proteins to human alveolar macrophages at 37°C. Symbols: ●, HLE; ▲, PPE; ○, HPE; □, PPT; △, albumin. Percent bound = 100 × fraction of total counts per unit volume that were associated with the cell pellet. For details, see text. Each point represents the mean of three determinations.

Binding of proteins other than HLE. Fig. 2 also shows the binding of radiolabeled bovine serum albumin and several serine proteases, including human and porcine pancreatic elastase, when these proteins were incubated with alveolar macrophages under conditions identical to those employed for HLE. A small amount of binding was found, which displayed a progressive, linear increase with time.

Binding of inactivated HLE. The binding of ^{125}I-HLE that had been inactivated with PMSF was indistinguishable from that of active HLE (Fig. 4).

Effect of divalent ion. In comparison with a control of 32.9±1.4% (SEM), binding in the absence of calcium was 32.5±3.5%, binding in the absence of magnesium was 31.9±2.4%, and binding in the absence of both cations was 27.0±3.3%. No significant difference ($P > 0.05$) was found among the groups by t test.

Effect of trypsin pretreatment on binding of HLE. In comparison with binding by control cells of 20.0±2.4% (SEM), HLE binding of cells preincubated with 1.0 mg/ml trypsin was 18.0±1.6%. No significant difference ($P > 0.05$) was found between the two groups by t test.

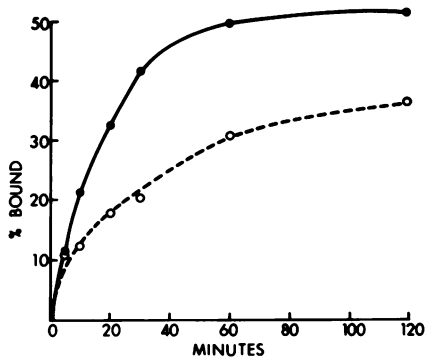

FIGURE 3 Effect of temperature upon binding of HLE by alveolar macrophages. ●, 37°C; ○, 4°C. Each point represents the mean of three determinations.

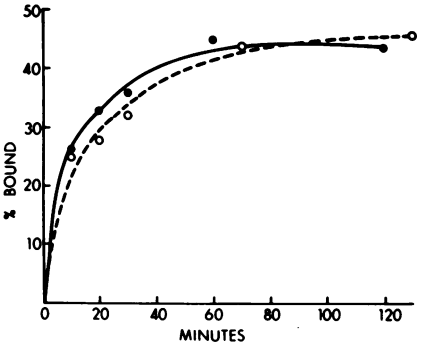

FIGURE 4 Effect of enzyme activity of HLE upon its binding to alveolar macrophages. ●, Active HLE; ○, PMSF-inactivated HLE. Each point represents the mean of three determinations.

Effect of serum, protease inhibitors, and yeast mannan on binding of HLE. Fig. 5 shows the effect of 10% added pooled human serum upon the binding of HLE by human alveolar macrophages. A small amount of binding occurred rapidly, but never exceeded 10% of the added radiolabel. As the incubation proceeded, additional binding occurred at a rate of ≅1.5%/h.

Further investigation of the effect of serum revealed that when active HLE was preincubated with alpha$_1$ antitrypsin, negligible amounts of enzyme bound to the cells (Table I). In contrast, the binding of PMSF-inactivated HLE was not changed by the presence of alpha$_1$ antitrypsin. Because inactivated proteases do not form complexes with serum inhibitors, these data indicate that alpha$_1$ antitrypsin affects binding of HLE by formation of complexes with HLE and that HLE-alpha$_1$ antitrypsin complexes do not bind to macrophages.

In other experiments, active or PMSF-inactivated HLE was incubated with macrophages in the presence of alpha$_2$ macroglobulin (Table I). Alpha$_2$ macroglobulin did not affect binding of PMSF-inactivated enzyme.

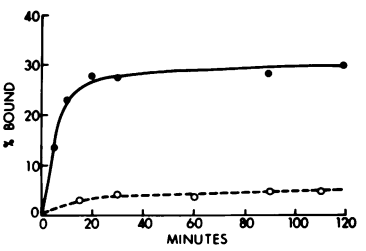

FIGURE 5 Effect of human serum upon binding of HLE by alveolar macrophages. ●, Standard binding assay; ○, 10% added pooled human serum.

TABLE I
Effect of Inhibitors upon Binding of Leukocyte Elastase by Alveolar Macrophages

Experimental group	Percent bound*	
	Active elastase	PMSF-inactivated elastase
	%	
Control‡	28.3±1.8	25.3±2.0
Pooled serum, 10%	1.1±0.2	6.1±2.0
α-1 antitrypsin, 40 μg/ml	2.8±0.1	26.7±2.0
Mannan, 1.25 mg/ml	23.3±0.9	22.3±2.4
Control‡	30.1±2.0	41.9±1.7
$Alpha_2$ macroglobulin	11.1±1.6	38.5±2.8
Lavage fluid supernate (concentrated ×10)	7.1±2.8	36.5±1.7

* Results are expressed as the mean of three determinations, ±SEM.

‡ Values for control groups represent binding of 2 μg/ml of ^{125}I-HLE in the absence of inhibitors. The upper and lower groups of experiments were performed separately, with different batches of cells and ^{125}I-HLE.

Complexes of active HLE with $alpha_2$ macroglobulin did bind, although to a lesser extent than free HLE.

Bronchoalveolar lavage fluid from a smoker, concentrated ×10, prevented binding of active, but not of PMSF-inactivated, HLE to macrophages. This demonstrated that the fluid contained inhibitory activity against HLE capable of complexing with active HLE and preventing its binding (Table I). The inhibitor was not identified.

Yeast mannan failed to alter the binding of active or PMSF-inactivated HLE (Table I).

Inhibition of binding by unlabeled elastases. Active, unlabeled HLE produced a concentration-dependent inhibition of binding of radiolabeled HLE (Fig. 6). In contrast, unlabeled HPE did not inhibit binding of labeled HLE. Additional experiments, not shown, demonstrated identical inhibition of binding of active and of inactivated radiolabeled HLE by active unlabeled HLE.

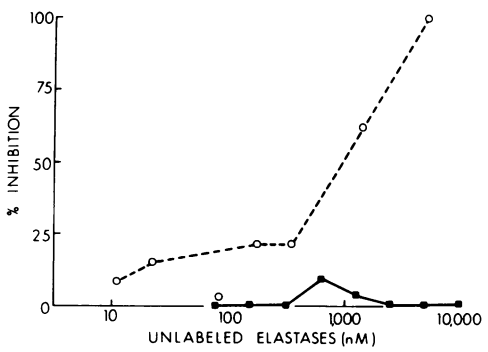

FIGURE 6 Inhibition of binding of ^{125}I-HLE by unlabeled elastases. Inhibition of binding of ^{125}I-HLE by unlabeled HLE (○) and by unlabeled HPE (■). The concentration of labeled elastase was 2 μg/ml (≅60 mM). Each point represents the mean of three determinations.

Saturability, affinity, and receptor number. The binding of ^{125}I-HLE to the control RBC was linear with respect to concentration. Fig. 7 shows the binding of ^{125}I-HLE to alveolar macrophages as a function of HLE concentration. The binding was clearly not linear with respect to concentration. Saturation of binding was evident with increasing concentrations of labeled HLE.

A Scatchard plot of the data (Fig. 8) allowed the total number of binding sites to be estimated as 640 × 10^6/macrophage. The association constant for the binding reaction was estimated from the slope of the Scatchard plot to be 4.97 × 10^5 M^{-1}.

Autoradiography. Background labeling (grains over empty resin) was negligible. After 5 and 10 min of incubation, grains tended to be at the periphery of the cell, whereas at 2 h the label was found throughout the cytoplasm. The nucleus and mitochondria were nearly free of grains at all time periods (Table II). At 5 min, the label was concentrated in the endocytic vesicles ($P < 0.001$), although some label was associated with lysosomal granules ($P < 0.05$). At 10 min both granules and vacuoles were labeled ($P < 0.001$), but at 2 h only the lysosomal granules (Fig. 9) were significantly labeled ($P < 0.001$). Calculation of grain density (grains/unit area) showed progressive accumulation of label in the lysosomal granules with time (Table II).

DISCUSSION

The work described in this report demonstrates that HLE binds rapidly and specifically to alveolar

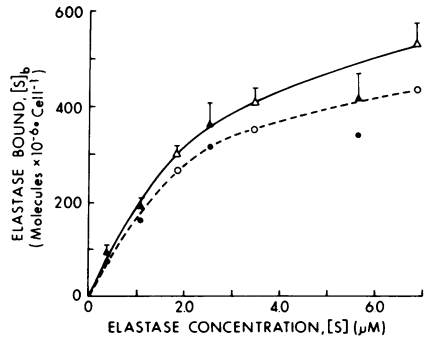

FIGURE 7 Binding of ^{125}I-HLE to alveolar macrophages as a function of concentration of labeled elastase. Solid line: total HLE binding to macrophages. Interrupted line: net binding (after subtraction of RBC control). Open (△, ○) and closed (▲, ●) symbols represent values obtained on two different days. All points are the mean of three determinations. Bars represent the SEM.

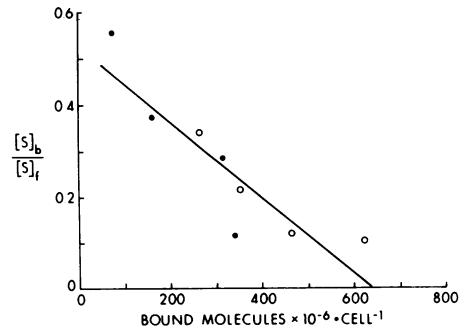

FIGURE 8 Scatchard plot of data obtained in HLE binding experiments in which the concentration of ^{125}I-HLE was varied, with linear regression line. Open and closed circles represent results obtained on two different days. $[S]_b$ = concentration of bound HLE; $[S]_f$ = concentration of free HLE. Intercept on abscissa (estimated total number of binding sites per macrophage) = $640 \times 10^6 \cdot cell^{-1}$. Association constant (calculated from slope of regression line) = $4.97 \times 10^5\ M^{-1}$. Data were obtained at 37°C and no correction has been made for receptor turnover or internalization.

macrophages. Trypsin and two other elastases tested showed no such binding. It is noteworthy that human pancreatic elastase did not behave like HLE, indicating that species differences alone do not explain the inability of the other enzymes to bind specifically to human alveolar macrophages. The specificity of binding, particularly in comparison with other elastases, and the concentration-dependent inhibition of binding of radiolabeled enzyme by unlabeled HLE, suggesting that the binding of HLE was mediated by cell surface receptors. Conclusive evidence for receptor-mediated binding was provided by experiments demonstrating that the binding was saturable by increasing concentrations of HLE. An association constant of $4.97 \times 10^5\ M^{-1}$ and a total of 640×10^6 binding sites/macrophage were estimated by Scatchard analysis of the binding reaction. Although these data were obtained using macrophages obtained from cigarette smokers, we have preliminary information indicating that alveolar macrophages from nonsmokers have similar binding capabilities to those obtained from smokers.

An important feature of the binding of HLE is that enzyme activity is not required for binding. This was demonstrated in two ways: (*a*) the binding of active HLE was indistinguishable from that of PMSF-inactivated HLE, and (*b*) unlabeled active HLE inhibited the binding of both the active and inactive forms of HLE identically.

The binding of HLE to macrophages is distinct from the receptor-mediated binding of mannose or *N*-acetyl glucosamine-terminal glycoprotein lysosomal enzymes to macrophages (10, 11), which are responsible for in vivo clearance of these substances from the extracellular space (25–30). This distinction is demonstrated by the trypsin insensitivity of binding of HLE, by the lack of requirement for divalent cation, and by the failure of yeast mannan to inhibit binding.

It is known that macrophages bind lactoferrin (12, 13), another neutrophil granule-associated glycoprotein (31, 32). Hepatocyte receptor recognition of lactoferrin (33), and macrophage recognition of lysosomal glycosidases (11), and probably also the Fc portion of immunoglobulin (Ig)G (34), are mediated by sugar residues on these ligands. HLE is a glycoprotein

TABLE II

Quantitation of the Autoradiographic Labeling of Organelles of Macrophages Exposed to ^{125}I-Leukocyte Elastase

Time	Grains	Organelles				
		Nucleus	Granules	Vacuoles	Mitochondria	Cytoplasm
min						
5	Observed	3*	39‡	30*	0*	66
	Expected§	20	28	13	9	73
	Density‖	0.003	0.028	0.045	0	0.018
10	Observed	2*	42*	55*	0*	79‡
	Expected	25	21	26	12	101
	Density	0.002	0.042	0.045	0	0.017
120	Observed	5*	139*	16	2*	102
	Expected	33	55	11	17	147
	Density	0.004	0.064	0.039	0.003	0.018

* Differs from expected number $P < 0.0001$.

‡ Differs from expected number $P < 0.05$.

§ The number of grains expected assuming that all cellular structures contained the same concentration of label per unit volume.

‖ Grains/μm^2.

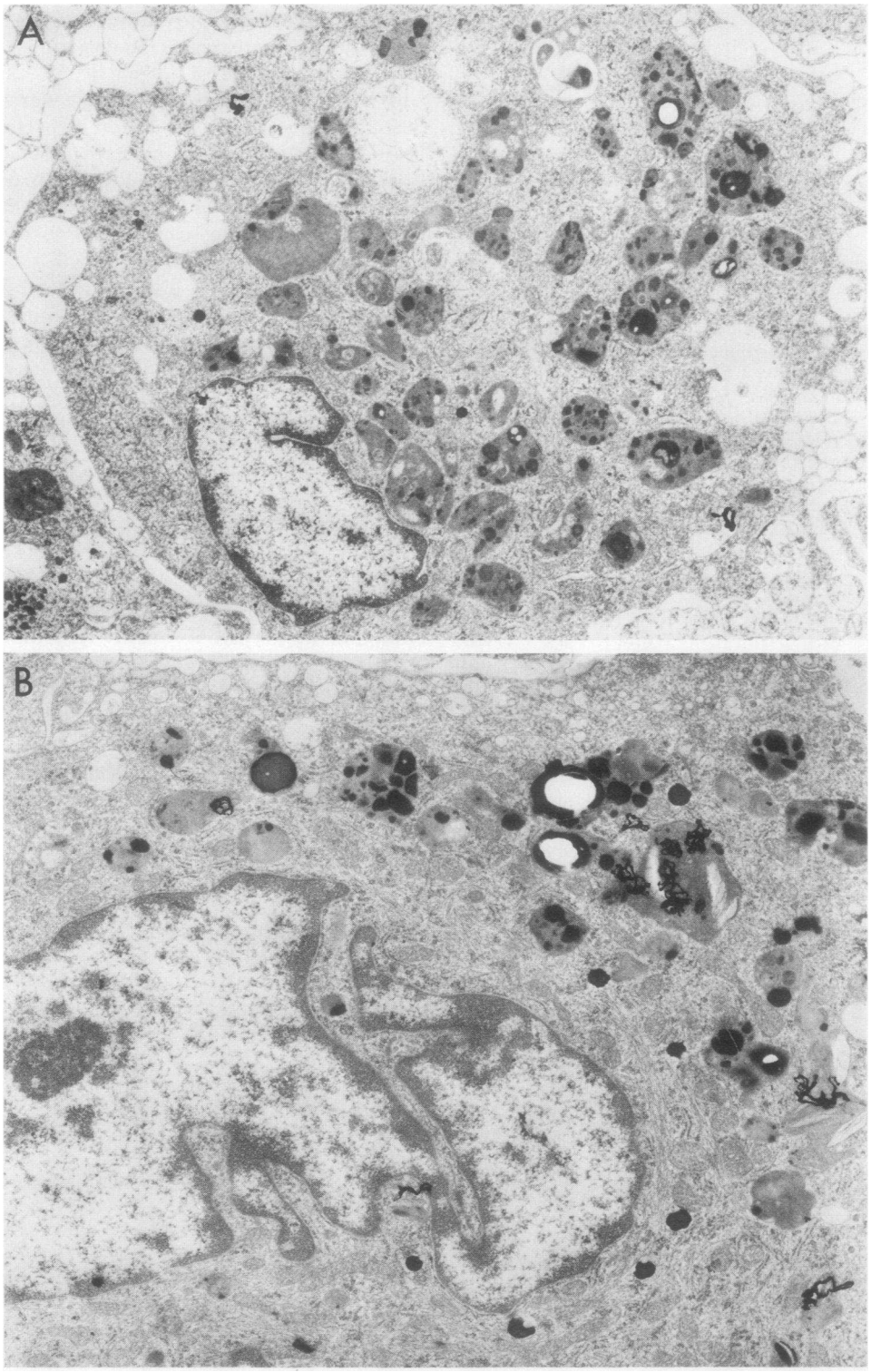

FIGURE 9 Autoradiographs of alveolar macrophages after exposure to ^{125}I-HLE. (A) After 5 min, two grains are visible at the periphery of the cell close to or overlying vacuoles. (B) After 2 h, most of the grains are seen overlying lysosomal granules. ×8,000.

with substantial carbohydrate content (18). Although the oligosaccharide residues of HLE have not been completely characterized, it seems likely that HLE binding to human alveolar macrophages is mediated by the sugar residues of the enzyme. Preliminary work in this laboratory suggests that the specific binding of HLE is mediated by fucose-terminal oligosaccharides on the enzyme, a mechanism which may be identical to that described for binding of lactoferrin to hepatocytes (33).

Neutrophils release a variety of substances from their cytoplasmic granules while engaging in phagocytosis in vitro (17, 35, 36), including 25–30% of their content of HLE (37, 38). Released HLE is potentially injurious to the surrounding tissues. Control of the activity of released HLE is accomplished by two interdependent mechanisms: inhibition by protease inhibitors and degradation by macrophages.

Active HLE in the extracellular space is susceptible to inhibition by a variety of inhibitors which include circulating $alpha_1$ antitrypsin and $alpha_2$ macroglobulin (39, 40), and also locally produced inhibitors such as those present in bronchoalveolar lining fluid (41, 42). $Alpha_1$ antitrypsin, however, is slowly hydrolyzed by the elastase with which it is complexed, releasing active enzyme (40, 43, 44). Proteases bound to $alpha_2$ macroglobulin retain activity against a range of substrates, particularly those of low molecular weight, while being protected from large molecular weight inhibitors (39, 45–48).

Although protease inhibition provides immediate partial or complete inactivation of extracellular HLE, ultimate disposal depends upon intracellular degradation. HLE (6), PPT (1, 2, 7, 8), PPE (5), alpha-chymotrypsin (3), subtilisin A (4), and presumably all endopeptidases that are inhibited by $alpha_2$ macroglobulin, bind to macrophages after they have formed complexes with macroglobulin. Binding of the complexes results in rapid clearance of the proteases from the extracellular space, and is followed in vivo by their degradation. It is particularly noteworthy that ^{125}I-HLE injected intravenously in man is cleared from the circulation in the form of enzyme-$alpha_2$ macroglobulin complexes, with localization of the radiolabel over the liver, then appearance in dialyzable form in the urine (6). No corresponding clearance mechanism for $alpha_1$ antitrypsin complexes exists.

These data have led to the view that $alpha_2$ macroglobulin binding is an important step leading to cellular degradation of proteases. In previous in vitro studies that established this mechanism, uncomplexed enzymes did not bind to macrophages; but all previous work looking for such binding utilized enzymes of bacterial or pancreatic, and not neutrophil, origin. This work agrees that the pancreatic proteases tested (PPT, PPE, and HPE) do not specifically bind to macrophages. It has already been established that differences in structure (18), immunoreactivity (18), and activity (6, 49) exist between pancreatic and neutrophil elastases. This study indicates yet another difference between the elastases from the two sources. The work described in this report also agrees that HLE complexed with $alpha_2$ macroglobulin, but not with $alpha_1$ antitrypsin, binds to macrophages. It is known that if a small amount of HLE is added to serum, 92% of the enzyme binds to $alpha_1$ antitrypsin, whereas only 8% binds to $alpha_2$ macroglobulin (40). This presumably explains the low binding of ^{125}I-HLE observed in this report when serum was present, although an additional unidentified factor(s) in serum also decreases binding, because the binding of PMSF-inactivated HLE is decreased by serum but not by purified $alpha_1$ antitrypsin.

The findings reported here provide evidence for an alternative pathway for cellular clearance of HLE, that of direct binding and internalization of the active enzyme. These data also permit estimation of the capability of macrophages to bind the HLE released by neutrophils in response to physiologic stimuli. Published data (38) show that 5×10^6 neutrophils release 22 μg of HLE (78×10^6 molecules/cell) in response to an immunologic stimulus in vitro. Based upon our estimated total number of binding sites, each macrophage has the capacity to bind the HLE released by eight neutrophils.

A previous report from this laboratory presented evidence that alveolar macrophages harvested from cigarette smokers released elastolytic activity into serum-free culture media (23). One hypothesis as to the origin of the elastase was that it represented leukocyte elastase that was associated with the harvested macrophages and that was then released in vitro, either spontaneously or as a consequence of macrophage death. This report lends credibility to that hypothesis by demonstrating that leukocyte elastase binds to alveolar macrophages.

High-resolution autoradiography in the present study showed that bound radiolabeled HLE was rapidly internalized and progressively incorporated into phagolysosomes. Although our analysis of the fate of internalized HLE is continuing, data thus far suggest that the majority of the internalized enzyme is degraded intracellularly.

The importance in vivo of direct macrophage binding of HLE depends upon the relative availability of circulating and tissue elastase inhibitors. Because the extracellular space contains an abundance of protease inhibitors, direct binding of active HLE to macro-

phages may be of limited importance in the immediate inactivation of HLE. However, in compartments where alpha$_2$ macroglobulin is likely to be absent or present in low concentrations, such as the interstitial space of the lung or the bronchoalveolar lining fluid (16, 50), no previously described mechanism other than bulk flow exists for ultimate clearance of HLE.

Several lines of evidence now link HLE to the pathogenesis of emphysema (15, 51). Thus, mechanisms for controlling the activity of extracellular HLE in the lung may be of critical importance to the pathogenesis of emphysema. It is noteworthy that whole cigarette smoke and cigarette smoke condensate are capable of inactivating alpha$_1$ antitrypsin (52, 53). Direct binding of HLE by lung macrophages may assume increased importance in cigarette smokers if inactivation of alpha$_1$ antitrypsin by cigarette smoke occurs in vivo.

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