

Molecular and Functional Characterization of the Urokinase Receptor on Human Mast Cells*

(Received for publication, November 25, 1996, and in revised form, January 6, 1997)

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The urokinase receptor system is involved in several biological processes including extracellular proteolysis, cell invasion, and chemotaxis. Mast cells are multifunctional perivascular cells that play an important role in the regulation of microenvironmental events. We report that primary human mast cells and the human mast cell line HMC-1 express the receptor for urokinase. As assessed by Northern blotting and reverse transcription polymerase chain reaction technique, purified human lung mast cells and HMC-1 cells expressed urokinase receptor mRNA in a constitutive manner. Using a toluidine blue/immunofluorescence double staining technique and monoclonal antibodies, surface expression of urokinase receptor was demonstrable in lung, skin, uterus, heart, and tonsil mast cells, whereas the low density lipoprotein receptor-related protein was not detectable. Binding of monoclonal antibody VIM5 (recognizing the urokinase binding domain of urokinase receptor) to HMC-1 could be blocked by high molecular weight but not low molecular weight urokinase. Binding analyses performed with ¹²⁵I-urokinase revealed expression of 271,000 ± 55,000 high affinity urokinase binding sites per HMC-1 cell, with a calculated dissociation constant of 1.29 ± 0.3 nM. Purified urokinase induced dose-dependent migration of primary mast cells and HMC-1 in a chemotaxis assay without inducing release of histamine. The mast cell agonist stem cell factor also induced migration of HMC-1 and caused up-regulation of expression of urokinase receptor mRNA. Together, our data show that human mast cells express functional receptors for urokinase. Expression of urokinase receptors on mast cells may have implications for mast cell-dependent microvascular processes associated with fibrinolysis, migration, or local tissue repair.

crucial role in a number of microvascular processes including local fibrinolysis, cell migration, and tissue repair (1–5). In contrast to tissue-type plasminogen activator, uPA can induce plasmin generation in the absence of fibrin. The inactive precursor form of uPA, single-chain urokinase (scuPA), can be cleaved into the active molecular form, two-chain urokinase (1–3). A number of serine proteases including plasmin, kallikrein, trypsin, and mast cell tryptase are able to convert scuPA into the two-chain form of the molecule (1–3, 6). This activation process takes place primarily at uPA binding sites expressed on the cell surface membrane of local cells in the tissues (7, 8). Binding of urokinase to uPAR is important for the generation of enzymatic activity, since receptor-bound urokinase is protected from inhibition by plasminogen activator inhibitors (PAIs) (1–3, 7, 8).

The uPAR is broadly distributed throughout the mesenchymal cell system (7–13). Surface expression of uPAR has been described for endothelial cells (10), granulocytes (11), fibroblasts (12), mesenchymal tumor cells, and macrophages (12, 13). The receptor molecule has been characterized in detail and has been cloned (14–16). The mature protein is linked to the cell interior via a glycosylphosphatidylinositol anchor (9, 14, 15). Surface uPAR can bind uncomplexed uPA for long periods of time (17–19). However, when complexed with PAI-1, uPA may be internalized together with uPAR (17–19). Apparently, expression of the PAI-1 binding low density lipoprotein receptor-related protein (LRP, CD91) plays an important role for uPA-uPAR complex internalization (17–19). Thus, receptor-ligand complexes composed of LRP, PAI-1, uPA, and uPAR can be co-internalized (17, 18). The internalization is then followed by receptor-ligand disruption in endosomes and recycling of both LRP and uPAR to the cell membrane by vesicular transport (17). The signal-transduction cascade following activation of uPAR is complex and may involve protein tyrosine kinases (20, 21). Some of the uPAR-dependent signals (e.g. chemotaxis signal) may be delivered independent of receptor-complex internalization or presence of LRP.

Mast cells (MC) are multifunctional effector cells of the immune system (22, 23). These cells produce and store vasoactive and proinflammatory mediators (24–26). In response to diverse

The urokinase (uPA)¹-uPA receptor (uPAR) system plays a

* This work was supported by Fonds für Förderung der Wissenschaftlichen Forschung Grants F-005/01, F-005/09, and S6707, The Kommission Onkologie, and The Ministry of Sciences of Austria. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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¹ The abbreviations used are: uPA, urinary-type plasminogen activa-

tor (urokinase); uPAR, urokinase receptor; MC, mast cell(s); rh, recombinant human; SCF, stem cell factor; LRP, low density lipoprotein receptor-related protein; FCS, fetal calf serum; DFP, diisopropyl fluorophosphate; rt, reverse transcription; PCR, polymerase chain reaction; uPAh, high molecular weight urokinase; uPAL, low molecular weight urokinase; scuPA, single-chain urokinase; PAI(s), plasminogen activator inhibitor(s); mAb, monoclonal antibody(y)(ies); CD, cluster of differentiation.

agonists, MC can release their mediators into the extracellular space (25–27). In contrast to other hemopoietic cells, MC are extravascular cells usually located in the vicinity of small vessels and postcapillary venules in connective tissues (22, 23). A number of previous and more recent studies suggest that MC are involved in local microvascular processes such as endothelial cell activation (28), transmigration of blood cells into tissues (29, 30), and metabolism and turnover of various tissue hormones and matrix molecules (31, 32). MC also produce heparin (33) and can accumulate in areas of ongoing inflammation, tumor invasion, angiogenesis, fibrosis, or thrombosis (22, 23, 29, 30, 34–36). More recently, among other cells, MC have been implicated in the regulation of endogenous fibrinolysis (37, 38). The aims of the present study were to elucidate whether human MC express uPAR (CD87) or LRP (CD91) and whether uPAR expression is associated with a specific functional response of MC to urokinase.

MATERIALS AND METHODS

Reagents and Buffers

Recombinant human (rh) stem cell factor (SCF) was purchased from Genzyme (Cambridge, MA). Collagenase type II was purchased from Sebak (Suben, Austria). Iscove's modified Dulbecco's medium, glutamine, penicillin, and streptomycin were from Life Technologies, Inc., and gentamycin, amphotericin B, and fetal calf serum (FCS) were from Sera-Lab (Crawley Down, United Kingdom). RPMI 1640 medium was from PAA Laboratories Co. (Linz, Austria). Highly purified human uPA (two-chain type; 90% high molecular weight, 10% low molecular weight type) was purchased from Laboratories Serono (Aubonne, Switzerland). Highly purified (>95%) high molecular weight urokinase (uPA_h) and >95% pure low molecular weight urokinase (uPA_l) were purchased from American Diagnostics (Greenwich, CT). scuPA was provided by Technoclon (Vienna, Austria). One liter of Ca²⁺/Mg²⁺-free Tyrode's buffer contained 0.2 g of KCl, 0.05 g of NaH₂PO₄·H₂O, 0.8 g of NaCl, and 1 g of glucose.

Monoclonal Antibodies (mAb)

Antibodies against uPAR and LRP were obtained from the Fifth International Workshop and Conference on Human Leukocyte Differentiation Antigens (Boston, 1993) (39): these were the mAb L21 (subclass IgG_{2a}; anti-uPAR), 3B10 (IgG_{2a}; anti-uPAR), and MR19 (IgG₁; anti-LRP). Anti-tryptase mAb (IgG₁) was purchased from Chemicon (Temecula, CA). The mAb VIM5 (IgG₁) directed against the uPA binding domain of uPAR was produced at the Institute of Immunology, University of Vienna. The anti-*c-kit* mAb YB5.B8 (40) (IgG₁) was kindly provided by L. K. Ashman (University of Adelaide, Australia). The anti-monocyte chemotactic and activating factor mAb S14 (IgG₁) served as control and was purchased from Anogen (Mississauga, Ontario, Canada).

Preparation and Culture of Mast Cells

Primary MC were prepared from surgical tissue specimens according to published techniques (41–43). Informed consent was obtained from patients in each case. Lung tissue was obtained at surgery (lobectomy or pneumectomy) from 11 patients suffering from bronchiogenic carcinoma. The tissue (5–9 g) was cut into small pieces and washed extensively in Tyrode's buffer (41). Then, tissue fragments were exposed to collagenase type II (2 mg/g of tissue) at 37 °C for 2 h. Dispersed cells were recovered by filtration through nytex cloth, incubated in FCS, and washed in RPMI 1640 medium. The primary lung cell suspensions contained 2.5–6.6% MC (by Giemsa staining). Specimens of uterus were obtained from two patients with uterine myomata. Tonsil MC were dispersed from surgical specimens removed from patients (*n* = 2) suffering from chronic tonsillitis. Human skin MC were dispersed from circumcised juvenile foreskin (*n* = 3). Lung, uterus, tonsil, and skin mast cells were isolated by use of collagenase without other enzymes. Human cardiac MC were isolated from atrial appendages of two patients suffering from cardiomyopathy (heart transplantation) as described (43). Cardiac MC were dispersed by collagenase, followed by exposure to DNase (0.5 mg/ml), hyaluronidase (0.5 mg/ml), and Pronase E (2 mg/ml). To determine the percentage and numbers of MC, cells were stained with Giemsa or toluidine blue and counted in a hemacytometer. MC were cultured at 37 °C in RPMI 1640 medium with 10% FCS, glutamine, and antibiotics.

TABLE I
Oligonucleotide probes used in this study

Antigen	Sequence of oligonucleotide probe	Ref.
uPAR	5'-GGTTCTGTGCTGTGTAACCTTGCCATCAGG-3'	16
uPA	5'-CGATGGAACCTTGATGAAGTTCATTGCTGCC-3'	61
LRP	5'-ATACCTGTGGAGCACCTGGAACCTGTCC-3'	62
<i>c-kit</i>	5'-CCTTACATTCACCCGTGCCATTGTGCTTGAATGC-3'	63
SCF	5'-GCTGTGTGACAATTGTACTACCATCTCGCTTATCC-3'	64
CD25	5'-CCTCTGTGTAGAGCCCTGTATCCCTGG-3'	65
<i>c-fms</i>	5'-GGT CTC AAC AGT CAG CAG GCT CTG CAC CG-3'	66
GAPDH ^a	5'-CCATGGTGGTGAAGACGCCAGTGGACTCC-3'	67

^a GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

In a series of experiments, lung MC were purified to apparent homogeneity. For this purpose, dispersed lung cells (four donors) were subjected to counter flow centrifugation (elutriation) (42). The elutriated cell fractions (*n* = 10) contained varying amounts of MC. In one donor, a fraction contained 91% MC and was used for Northern blotting. Fractions containing 10–55% MC were used for fluorescence staining analyses and sorting. In three donors, elutriated lung MC were further enriched by sorting with mAb YB5.B8 as described (42). After sorting, MC were >99% pure and used for rtPCR analysis or immunostaining after cytospin preparation. Enriched or highly purified MC were cultured in RPMI 1640 medium supplemented with 10% FCS and antibiotics.

The human mast cell line HMC-1 was established from a patient suffering from mast cell leukemia (44) and kindly provided by J. H. Butterfield (Mayo Clinic, Rochester, MN). HMC-1 cells were cultured in Iscove's modified Dulbecco's medium containing 10% FCS, glutamine, and antibiotics at 37 °C and 5% CO₂.

Northern Blot Analysis

Primary lung MC (91% purity, 3 × 10⁷ cells in each sample, four samples in total) were incubated in RPMI 1640 medium plus 10% heat-inactivated FCS in the presence (*n* = 2 points) or absence (*n* = 2) of rhSCF (100 ng/ml) at 37 °C and 5% CO₂ for 2 h. HMC-1 cells (3 × 10⁷ cells for each point) were incubated in Iscove's modified Dulbecco's medium plus 10% FCS in the absence or presence of rhSCF (100 ng/ml) for 2, 6, or 12 h. RNA extraction and Northern blot analysis were performed essentially as described (45). Total cellular RNA was extracted from cells by the guanidinium isothiocyanate/cesium chloride method (46). Ten μg of RNA were size-fractionated on 1.2% agarose gels and then transferred to synthetic membranes (Hybond N, Amersham Corp.) with 20 × SSC (1 × SSC = 150 mM NaCl, 15 mM sodium citrate, pH 7.0) overnight. Then, RNA was cross-linked to membranes by UV irradiation (UV Stratilinker 1800, Stratagene). Prehybridization was performed at 65 °C for 4 h in 5 × SSC, 10 × Denhardt's solution (1 × Denhardt's solution = 0.02% bovine serum albumin, 0.02% polyvinylpyrrolidone, 0.02% Ficoll), 10% dextran sulfate, 20 mM sodium phosphate, pH 7.0, 7% SDS, 100 μg/ml sonicated salmon sperm DNA, 100 μg/ml poly(A)⁺. Hybridization was done using ³²P-labeled synthetic oligonucleotide probes (Table I) for 16 h at 65 °C in prehybridization buffer. Probes were labeled by terminal nucleotidyl transferase and [α -³²P]dATP. Blots were washed once in 5% SDS, 3 × SSC, 10 × Denhardt's solution, 20 mM sodium phosphate, pH 7.0, for 30 min at 65 °C and once in 1 × SSC, 1% SDS for 30 min at 65 °C. Bound radioactivity was visualized by exposure to XAR-5 film at -70 °C using intensifying screens (Eastman Kodak Co.).

RNA Isolation and rtPCR

For rtPCR, total RNA was isolated from >99% pure lung MC (2 × 10⁴ cells each point, *n* = 2 donors) or purified CD19⁺ B-cells (47) using the guanidinium isothiocyanate acid phenol extraction procedure (RNAzol™ B method, Biotecx Laboratories, Houston, TX) as reported (42). In brief, MC were centrifuged (400 × *g*, 10 min) and lysed in 0.8 ml of RNAzol™ B. Then, 80 μl of chloroform were added. Samples were shaken vigorously and stored at 4 °C for 5 min. After centrifugation at 12,000 × *g* (4 °C, 15 min) the upper aqueous phase was collected. Four μg of carrier RNA (yeast tRNA, stored as 4 μg/μl of solution in RNase-free water) were added before precipitating RNA with 0.4 ml of isopropanol overnight at -20 °C. Precipitated RNA was centrifuged for 15 min at 12,000 × *g* (4 °C). Then, the supernatant was removed and the RNA pellet washed once with 75% ethanol. Finally, the pellet was dissolved in 20 μl of RNase-free water and stored in liquid nitrogen. cDNA synthesis was performed using the first strand cDNA synthesis kit (Pharmacia Biotech Inc.) according to the manufacturer's instruc-

tions. Briefly, total RNA was dissolved in 20 μ l of RNase-free water, heated to 65 °C for 10 min, quick-chilled on ice, and then incubated with 11 μ l of bulk first-strand reaction mix (cloned, FPLC Pure^R reverse transcriptase, RNAGuard, RNase/DNase-free bovine serum albumin, 1.8 mM each dATP, dCTP, dGTP, and dTTP in aqueous buffer), 1 μ l of dithiothreitol solution (200 mM aqueous solution), 1 μ l of pd(N)₆ primer (random hexadeoxynucleotides at 0.2 μ g/ml in aqueous solution) at 37 °C for 1 h. The reaction was terminated by heating to 90 °C for 5 min. Samples were chilled on ice immediately and stored at -20 °C for subsequent analysis. Aliquots of the cDNA product, *i.e.* 3 μ l for the constitutively expressed β -actin gene as a positive internal control of rtPCR efficiency and 15 μ l for the uPAR gene (16), were used for rtPCR. The reaction mixture contained 10 μ l of 10-fold PCR buffer (100 mM Tris-HCl, 500 mM KCl, 15 mM MgCl₂, and 0.01% (w/v) gelatin (Perkin-Elmer)), 200 μ mol of dNTP-Mix (Pharmacia), and 2.5 units of Taq DNA polymerase (Perkin-Elmer). One hundred pmol of each primer pair (5'-uPAR, 23-mer, gene position 587-609 (16): 5'-TTCCACAACAACGACACCTTCCA-3'; 3'-uPAR, 23-mer, gene position 986-1008: 5'-AGGGTGATGGTGAGGCTGAGATG-3'; 5' β -actin, 20-mer, gene position 969-988: 5'-AGGCCGGCTTCGCGGGCGAC-3'; 3' β -actin, 21-mer, gene position 1327-1347: 5'-CTCGGGAGCCACACGCAGCTC-3') were added to a final volume of 100 μ l. A master mix of PCR components was made up for each set of reactions prior to addition of the cDNA templates. Primer sequences were oriented on separate exons of the genes so that the PCR product of the cDNA could readily be distinguished from the PCR product amplified from any contaminating genomic DNA. Samples were then subjected to PCR to amplify the 422-base pair DNA fragment between nucleotides 587 and 1008 of the uPAR cDNA (16) by 35 cycles at 94 °C for 1 min and 72 °C for 1 min after initial denaturation at 95 °C for 1 min. An aliquot of each reaction mixture was subjected to electrophoresis on a 2% agarose gel in 1 \times Tris acetate-EDTA buffer. The PCR products were visualized by ethidium bromide staining and photographed.

Immunostaining of Surface uPAR

Combined Toluidine Blue/Immunofluorescence Staining Technique—Expression of cell surface markers on primary MC was analyzed by a combined toluidine blue/immunofluorescence staining technique as described previously (48). In brief, cells were incubated with mAb for 30 min at 4 °C and washed twice in phosphate buffered saline. Cells were then conjugated with a "second step" fluorescein-labeled goat F(ab)₂ IgG + IgM anti-mouse antibody (30 min, 4 °C). Thereafter, cells were fixed in 0.025% glutaraldehyde solution for 1 min and incubated with toluidine blue (0.0125%) for 8 min at room temperature. After washing, cells were analyzed under bright field and fluorescent light with a fluorescence microscope (Olympus, Vienna).

Flow Cytometry and Epitope Analysis—After exposure of HMC-1 cells to first step and second step antibody (see above), flow cytometry was performed on a FACScan (Becton Dickinson, San Jose, CA) as described (42). Standard beads, provided by the Fifth International Workshop on Human Leukocyte Typing (39), were used to calibrate the FACScan in all measurements. In each staining experiment, isotype-matched control antibodies were used. A 5% cutoff channel was set as negative/positive gate to discriminate between positive and negative cells. To confirm expression of uPA binding sites detected by the mAb, blocking experiments were performed using uPAI and uPAh. In these experiments, HMC-1 cells were preincubated with uPAI (300 units/ml), uPAh (300 units/ml), or control medium at 4 °C for 3 h. Then, cells were washed in phosphate buffered saline and incubated at 4 °C with anti-uPAR mAb VIM5 (recognizing the uPA binding domain on uPAR). After 30 min, cells were washed, exposed to the second step goat anti-mouse IgG/IgM (4 °C, 30 min), washed again, and then subjected to fluorescence-activated cell sorter analysis. Epitope blocking was quantified as the difference in mean fluorescence intensities observed between cells exposed to ligands *versus* cells exposed to control medium.

In Situ Staining Experiments

Lung tissue was obtained from one patient suffering from encephalomalacia (autopsy), cardiac tissue from one patient with auricular thrombosis (autopsy), and small intestine tissue from one patient with cardiac infarction (autopsy). Autopsies were part of a study approved by the local ethical committee (36). Skin tissue was obtained from juvenile foreskin (circumcision, $n = 1$) after informed consent was obtained. Tissue was snap-frozen in precooled isopentane and prepared for cryostat sections. Sequential double immunohistochemistry was performed using mAb to uPAR and MC tryptase (second antigen) essentially as described (36). Endogenous peroxidase was blocked by 5% H₂O₂/meth-

anol. Sections were first incubated with mAb VIM5, then with biotinylated horse anti-mouse IgG, and then with streptavidin-biotin-peroxidase complexes and aminoethyl carbazole (Vector, Burlingame, CA) as chromogen, giving a reddish brown reaction product with horseradish peroxidase. Slides were then photographed. Thereafter, alkaline phosphatase-conjugated anti-tryptase mAb was applied and the reaction visualized by fast blue salt. Then, the same regions (as for VIM5) were photographed again. In control experiments, an isotype-matched control antibody was used. As a further control, staining results were verified on serial sections (heart and skin). The immunoalkaline phosphatase staining technique was applied on cytospin preparations of enriched human lung mast cells and HMC-1 cells. In these experiments the cells on cytospin slides were incubated with mAb VIM5 for 60 min, washed, and incubated with a biotinylated horse anti-mouse IgG for 30 min. Then, slides were exposed to streptavidin-alkaline phosphatase complexes. Neofuchsin was used as chromogen, giving a red reaction. Slides were counterstained in Gill's hematoxylin. Control slides were similarly treated either with the primary antibody omitted or using isotype-matched control mAb.

Labeling of uPA with ¹²⁵I and Radio Receptor Analysis

Human urokinase was labeled with ¹²⁵I using lactoperoxidase. For this purpose, 100 μ g of uPA dissolved in 0.1 M phosphate buffer (pH 7.0) was labeled with 1 mCi of [¹²⁵I]NaI (Cyclotron Research Center, Karlsruhe, Germany) by slowly mixing with 0.3 μ g of H₂O₂ and 5 μ g of lactoperoxidase (Sigma). The reaction mixture (50 μ l) was injected into a reversed phase C18 high performance liquid chromatography column and eluted with a gradient of 25 to 50% MeCN in 0.1% aqueous trifluoroacetic acid. The effluent was monitored by UV (280 nm) and radioactivity detectors. The ¹²⁵I-uPA peak was isolated, evaporated under vacuum, and redissolved in phosphate buffered saline. Radiochemical purity was analyzed by horizontal zone electrophoresis run on cellulose acetate stationary phase in 0.1 M barbital buffer (pH 8.6) using a field of 300 V for 10 min. Under these conditions, free [¹²⁵I]iodide migrated about 45 mm as verified by an appropriate standard. ¹²⁵I-uPA was obtained in 70% isolated radiochemical yield at a specific activity of about 4 mCi/mg. Radiochemical purity was more than 97% and remained stable for at least 20 h.

The receptor assay was performed using HMC-1 cells essentially as described (49). In a first set of experiments, specific binding of ¹²⁵I-uPA to intact HMC-1 cells was analyzed as a function of time. In saturation experiments, HMC-1 cells were incubated with increasing concentrations (0.01–8.0 nM) of ¹²⁵I-uPA in the presence or absence of unlabeled ligand (500 nM). Experiments were done in duplicate and performed six times. In saturation experiments, cells were incubated with 15 nM ¹²⁵I-uPA at 4 °C for 45 min in the presence or absence of increasing concentrations (0.01–500 nM) of unlabeled ligand. The binding data were analyzed according to Scatchard.

Chemotaxis Assay

Mast cell migration was quantified using a 24-well double chamber chemotaxis assay as described recently (50). Briefly, lung MC ($n = 5$), skin MC ($n = 3$), or HMC-1 cells were resuspended in RPMI 1640 medium and adjusted to a final cell concentration of 3×10^6 cells/ml. In initial experiments, the agonists, *i.e.* various concentrations of rhSCF (1, 10, and 100 ng/ml), uPA (0.2–400 nM; 1 nM corresponds to 5 units/ml), or control medium (RPMI 1640) were placed into the lower chamber of the wells. Then, microporous filter membranes (0.6 cm²; pore size, 3.0 μ m; CycloPore, Aalst, Belgium) were inserted. Thereafter, MC were placed in the upper chambers and incubated at 37 °C in 5% CO₂ for 3 h. The membranes were then detached and removed together with non-transmigrated cells. The migrated HMC-1 cells in the lower chamber were incubated with the fluorescence dye Calcein AM (5 mM; Molecular Probes, Eugene, OR) for 30 min at room temperature. Then, labeled HMC-1 cells were measured in a multiplate reader (Biosearch, Hamburg, Germany), and the number of migrated cells was calculated by the calculation program delivered by the manufacturer (Biosearch). In "blocking experiments," MC or HMC-1 were preincubated with mAb VIM5 (recognizing the uPA binding domain of uPAR) or an isotype-matched control mAb before starting chemotaxis experiments. In the case of primary MC, the cellular histamine values in transmigrated cells were measured as an objective parameter of MC migration (uPA did not induce histamine secretion). To delineate the enzymatic effect of uPA from its migration-inducing effect, chemotaxis experiments were performed on HMC-1 cells using natural, active uPA, enzymatically inactive scuPA, and diisopropyl fluorophosphate (Hoechst, Vienna, Austria) -treated uPA (DFP-uPA). DFP-uPA showed less than 5% specific

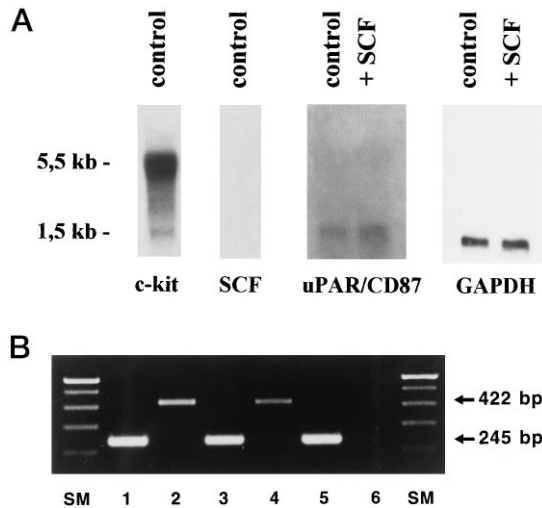


FIG. 1. Expression of uPAR mRNA in human lung mast cells. *A*, Northern blot analysis. Enriched human lung MC (91% purity, 3×10^7) were exposed to rhSCF (100 ng/ml) or control medium at 37 °C for 2 h. Then, RNA was extracted and prepared from MC as described in the text. Northern blot analysis using an oligonucleotide probe specific for uPAR was applied. After stripping the blots, oligonucleotide probes specific for other antigens (as indicated) were applied. The figure shows expression of uPAR mRNA in lung MC and the increased expression of uPAR mRNA after incubation of MC with rhSCF. The same cells expressed glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) as well as *c-kit*, whereas SCF was not expressed. *B*, reverse transcription PCR examination of total RNA derived from HMC-1, pure (>99%) lung MC, and purified (>99%, using CD19 beads; see text) peripheral blood B-cells. The figure shows expression of uPAR mRNA in HMC-1 (lane 2) and lung MC (lane 4). B-cells were found to lack uPAR (lane 6). The β -actin controls are shown in lanes 1 (HMC-1), 3 (lung MC), and 5 (B-cells).

enzymatic activity compared with untreated uPA. To differentiate between directed migration (chemotaxis) and nondirected migration (chemokinesis) of cells, checkerboard analyses were performed. In these experiments, the agonist uPA was placed into either the lower or upper wells, or both, of the chamber assay before cells were added.

Histamine-release Experiments

Histamine-release experiments were carried out on lung MC ($n = 7$). Experimental conditions were essentially as described earlier (51). MC were exposed to various concentrations of uPA (uPAI or uPAh) for 30–90 min at 37 °C in 5% CO₂. For IgE-dependent release, MC were preincubated with myeloma IgE (myeloma cell line U266) for 3 h at 4 °C, washed, and resuspended in histamine-release buffer (Immunotech, Marseille, France). In selected experiments ($n = 2$), lung MC were preincubated with uPAh (150 units/ml, 30 nM), uPAI (150 units/ml), rhSCF (1 ng/ml), or control medium for 15 min prior to anti-IgE activation. After preincubation, MC were exposed to various concentrations of the anti-IgE mAb E-124-2-8 (0.1–10 μ g/ml) for 30 min in 96-well microtiter plates (Costar, Cambridge, MA) at 37 °C for 30 min. Thereafter, cells were centrifuged at 4 °C and the cell-free supernatants recovered and analyzed for the amount of (released) histamine. Total histamine (extracellular plus intracellular) was quantified in whole cell suspensions. Histamine release was calculated and expressed as percentage of total histamine. Histamine was measured in supernatants and cell lysates by a radioimmunoassay (Immunotech) as described (48, 51). This assay showed a detection limit of 0.2 nM and no cross-reactivity with heparin, trypsin, rhSCF, tumor necrosis factor α , or other cytokines.

Statistical Analysis

Standard tests including Student's paired *t* test were applied to evaluate the significance of differences in the results. Results were considered significantly different when the *p* value was <0.05.

RESULTS

Expression of uPAR mRNA in Human Mast Cells—Northern blot analysis and rtPCR revealed expression of uPAR mRNA in primary human lung MC and HMC-1 cells. In Northern blot

TABLE II
Expression of mRNA in purified human lung mast cells and HMC-1 mast cells

Enriched human lung MC (91% pure) or HMC-1 cells were exposed to rhSCF (100 ng/ml) at 37 °C. RNA extraction and Northern blots using oligonucleotide-probes were carried out as described under "Materials and Methods." MC and HMC-1 expressed uPAR mRNA as well as *GAPDH* and *c-kit* mRNA. Increased expression of uPAR mRNA and decreased expression of *c-kit* mRNA in MC or HMC-1 was found after preincubation of cells with rhSCF (100 ng/ml; 2 h, 37 °C). Negative control genes including SCF, CD25, and *c-fms* were not detectable, excluding a significant contamination with macrophages or fibroblasts or CD25⁺ lymphocytes. *GAPDH*, glyceraldehyde-3-phosphate dehydrogenase.

Gene product	Expression in			
	Lung mast cells		HMC-1 cells	
	+SCF	-SCF	+SCF	-SCF
uPAR	+	±	+	±
uPA	-	-	-	-
LRP	-	-	-	-
<i>c-kit</i>	±	+	±	+
SCF	-	-	-	-
CD25	-	-	-	-
<i>c-fms</i>	-	-	-	-
<i>GAPDH</i>	+	+	+	+

experiments, primary lung MC (91% pure) and HMC-1 cells were found to express uPAR mRNA in a constitutive manner. Resting HMC-1 cells expressed significant amounts of uPAR mRNA. In contrast, the level of constitutively expressed uPAR mRNA in unstimulated primary MC was rather low (Fig. 1A). To exclude a signal delivered by contaminating cells, rtPCR analysis was applied using highly enriched (>99% pure) lung MC. These highly purified unstimulated MC expressed uPAR mRNA as determined by rtPCR (Fig. 1B). In Northern blot experiments, a small increase in expression of uPAR mRNA in lung MC was found after incubation of cells with the MC agonist rhSCF (100 ng/ml, 2 h) (Fig. 1A). Similar results were obtained using the HMC-1 cell line; again, exposure of HMC-1 cells to rhSCF (100 ng/ml, 2–12 h) resulted in an increased expression of uPAR mRNA (Table II). Urokinase or LRP mRNA were not expressed in unstimulated MC or in SCF-stimulated MC (Table II). In addition to uPAR and LRP, several control genes were examined by Northern blotting. "Positive control genes" (glyceraldehyde-3-phosphate dehydrogenase, *c-kit*) were found to be transcribed in primary MC and HMC-1, whereas "negative control genes" (*c-fms*, CD25, SCF) were not (Table II). rtPCR was controlled by using primers specific for T-cell receptor α chain and *bcl-2*, giving negative results for pure MC (see Ref. 42), thereby excluding the presence of significant levels of RNA from contaminating cells.

Detection of Surface uPAR on Human Mast Cells—In a first set of experiments, the reactivity of primary human MC obtained from lung, skin, uterus, heart, and tonsils with mAb clustered as CD87 (uPAR) was assessed by staining of cells with both toluidine blue and indirect immunofluorescence. MC from all organs tested were recognized by the anti-uPAR mAb L21 and 3B10 (Table III). More than 80% of the MC were stained by these mAb, irrespective of the origin of MC. In contrast, the tissue MC showed little or no surface reactivity (<10% of MC) with mAb VIM5 directed against the uPA binding domain of uPAR (Table III). Exposure of primary MC to pH 3.8 (30 min) resulted in an increased reactivity of MC with VIM5. However, the cells also showed an increased uptake of trypan blue compared with untreated cells.

Binding of anti-uPAR mAb could also be demonstrated for the human MC line HMC-1 (Table III). All three anti-uPAR mAb including VIM5 bound to HMC-1 cells at pH 7.4. Binding of VIM5 antibody was inhibitable by preincubation of HMC-1

TABLE III
Surface expression of various antigens on human
MC and HMC-1 cells

Surface expression of CD antigens on MC was determined by use of mAb and the indirect immunofluorescence staining technique. In the case of HMC-1, fluorescence-activated cell sorter analysis was performed. In the case of primary lung MC, a combined toluidine blue/immunofluorescence staining technique was applied. Technical details are described in the text. LMC, lung mast cells; SMC, skin mast cells; UMC, uterus mast cells; CMC, cardiac mast cells; ToMC, tonsil mast cells. +, more than 80% of cells reactive; \pm , less than 10% of cells reactive.

mAb	CD	Antigen	Surface expression on					
			LMC	SMC	UMC	CMC	ToMC	HMC-1
YB5.B8	117	<i>c-kit</i>	+	+	+	+	+	+
VIM5	87	uPAR	\pm	\pm	\pm	\pm	\pm	+
3B10	87	uPAR	+	+	+	+	+	+
L21	87	uPAR	+	+	+	+	+	+
MR19	91	LRP	-	-	-	-	-	-

cells with uPAh but not by uPAI (mean fluorescence intensity: control (2.9) versus VIM5 (37.4) versus VIM5 + uPAh (10.3) versus VIM5 + uPAI (31.8)) (Fig. 2). Incubation of MC or HMC-1 with rhSCF (100 ng/ml, 37 °C, 2–12 h) resulted in an increased expression of uPAR in fluorescence-activated cell sorter analyses (VIM5) compared with control (more than 2-fold increase in mean fluorescence intensity) (not shown). LRP was not detectable on either the surface of primary MC or on HMC-1 (Table III).

In Situ Detection of uPAR in Human Mast Cells—To confirm uPAR expression in MC, *in situ* staining experiments on tissue sections were performed. The VIM5 mAb was used in these studies, since the mAb was found to recognize the uPAR in the cytoplasm of HMC-1 cells. As assessed by double immunoperoxidase staining using VIM5 and anti-tryptase mAb, MC in all organs tested (lung, skin, gastrointestinal tract, and heart) were found to express uPAR. VIM5 labeling was found in cytoplasmic compartments of MC and showed a granular pattern. Almost all MC were labeled by the anti-uPAR mAb VIM5. Other cells in the tissues, including vascular cells, were also found to react with VIM5. Fig. 3 shows *in situ* double immunoperoxidase staining of one skin MC for uPAR (Fig. 3A) and tryptase (Fig. 3B). The uPAR could also be detected in purified lung MC (Fig. 3C) and HMC-1 (Fig. 3E) by immunostaining using cytospin slides and mAb VIM5.

Characterization of ^{125}I -uPA Binding Sites on HMC-1 Cells—In initial experiments, the time course of association and dissociation of ^{125}I -uPA binding to HMC-1 cells was analyzed. Association of binding showed a rapid increase and reached an apparent equilibrium within 20 min of incubation (Fig. 4). The calculated association rate constant k_1 ($\ln 2/\tau \times L_0$ (ligand concentration at time point "0"); $\tau = 234$ s) was $5.92 \times 10^{-5} \text{ M}^{-1} \text{ s}^{-1}$. Binding of ^{125}I -uPA to HMC-1 membranes rapidly declined following addition of an excess (500 nM) of unlabeled uPA (Fig. 4). The dissociation rate constant k_{-1} ($\ln 2/\tau$; $\tau = 378$ s) was $1.8 \times 10^{-3} \text{ s}^{-1}$. The K_d value ($1.3 \times \text{nM}$) of our saturation experiments thus fits quite well with the time rate constants ($K_d = k_{-1}/k_1 = 3 \times \text{nM}$), both being in the lower nanomolar range.

Receptor binding experiments using ^{125}I -uPA and HMC-1 cells revealed specific binding at 4 °C. To assess whether the binding behavior of unlabeled uPA differs from that of ^{125}I -labeled uPA, binding experiments with a constant uPA concentration (5 nM) but different proportions of unlabeled to labeled uPA were performed. In these experiments, no significant difference in the binding behaviors between unlabeled and labeled ligand was found (not shown). Binding of ^{125}I -uPA to HMC-1 cells was displaced by addition of unlabeled uPA, reaching an

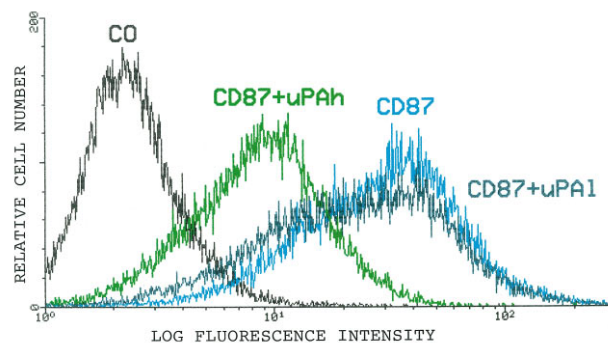


Fig. 2. **Flow cytometry analysis of uPAR.** HMC-1 cells were preincubated with either control medium (CO), uPAI, or uPAh at 4 °C for 30 min. The cells were then washed and incubated (4 °C, 30 min) with mAb VIM5 directed against the uPA binding domain of the uPAR. After washing, cells were exposed to a second step fluorescein-conjugated IgG + IgM goat anti-mouse antibody. The figure shows expression of uPAR on HMC-1 cells and specific competition between VIM5 and uPAh on the cell surface of HMC-1.

IC₅₀ value of 5.1 ± 0.9 nM (Fig. 5). Scatchard plot analysis of binding of ^{125}I -uPA to HMC-1 cells revealed a single class of $271,000 \pm 55,000$ high affinity uPA binding sites with a calculated K_d of 1.29 ± 0.3 nM (Fig. 6, A and B).

uPAR-mediated Migration of Human Mast Cells—To demonstrate a specific function for the uPAR on MC, a chemotaxis assay was applied. uPA induced a chemotactic response both in primary lung MC (control, $100 \pm 18.4\%$; 20 nM uPA, $640.7 \pm 42.3\%$) and skin MC (control, $100 \pm 5.9\%$; 20 nM uPA, $1630 \pm 97\%$) as well as in HMC-1 cells (control, $100 \pm 18\%$; 300 nM uPA, $179 \pm 44\%$) (Figs. 7–9). Figs. 7 and 9 show the migration-inducing effect of uPA on HMC-1 cells, and Fig. 8 shows the effect of uPA on lung (Fig. 8A) and skin (Fig. 8B) MC. The migration-inducing effect of uPA on MC was dose-dependent with optimal concentrations ranging between 0.2 and 20 nM (1 and 100 units/ml) for primary MC ($p < 0.05$) and between 150 and 300 nM (750 and 1500 units/ml) ($p < 0.05$) for HMC-1 cells (Figs. 7 and 9). SCF also induced MC chemotaxis in these experiments (optimal concentration, 10–100 ng/ml) and cooperated with uPA in the induction of chemotaxis in HMC-1 (Fig. 7). To provide evidence that the migration-inducing effect of uPA was mediated via uPAR, antibodies against the uPA binding domain of the uPAR (VIM5) were used in blocking experiments. In these experiments, lung MC or HMC-1 were preincubated with mAb VIM5 (10 $\mu\text{g}/\text{ml}$) or an isotype-matched control antibody for 30 min at 4 °C. Then, cells were washed and added to the chamber system. Preincubation with mAb VIM5 resulted in an almost complete inhibition of uPA-induced migration ($p < 0.01$) of HMC-1 cells (Fig. 7) and lung MC (Fig. 8A), whereas a control antibody did not block uPA-dependent migration (Fig. 7).

To determine whether the migration-inducing effect of uPA on MC is dependent on the enzymatic activity of the ligand (uPA), chemotaxis experiments were performed with the enzymatically inactive single-chain precursor of uPA (scuPA) and with DFP-uPA that exhibited less than 5% of the specific enzymatic activity when compared with untreated uPA. In these experiments, both scuPA and DFP-uPA induced chemotaxis of HMC-1 cells similar to natural purified active uPA (Fig. 9).

To discriminate between chemokinesis (undirected migration) and chemotaxis (directed migration) of MC against uPA, checkerboard analyses were performed using HMC-1 cells. These experiments revealed a (directed) chemotactic response of human MC against urokinase (Table IV).

Histamine-release Experiments—According to previous ob-

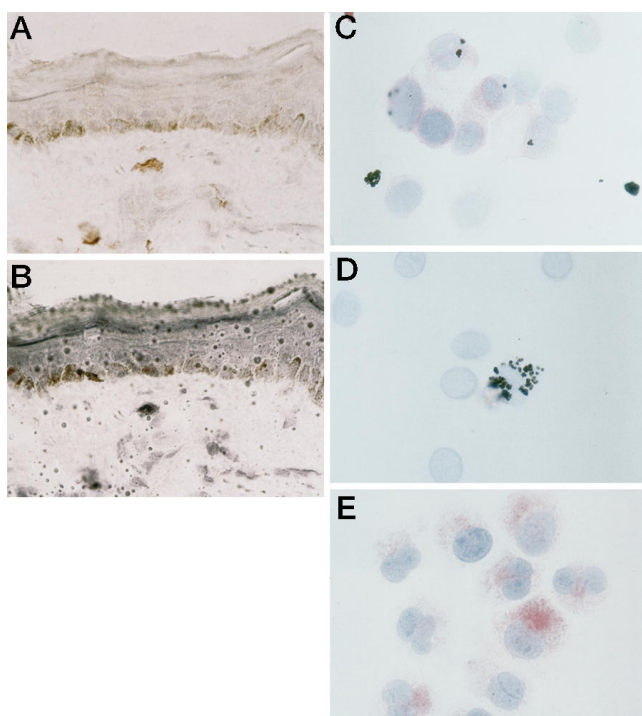


FIG. 3. Detection of uPAR in mast cells by *in situ* staining. *A* and *B*, double immunostaining of a skin MC with mAb to uPAR and tryptase. Labeling was performed as described in the text. The skin section was first incubated with anti-uPAR mAb VIM5 (*A*). The identity of the labeled MC was then demonstrated by sequential double immunostaining for mast cell tryptase (*B*). *C*, indirect immunalkaline phosphatase staining of purified human lung MC for uPAR using the mAb VIM5. Mast cells were purified to homogeneity, put onto a cytospin slide, and immunostained as described in the text. An isotype-matched control antibody gave a negative staining result with MC (*D*). HMC-1 cells were also found to stain positive for uPAR in the immunoalkaline phosphatase staining protocol (cytospin slides) using mAb VIM5 (*E*).

servations, anti-IgE (after preincubation of cells with IgE) induced histamine release from lung MC (Fig. 10), and preincubation of MC with rhSCF resulted in an increased response to anti-IgE (Fig. 10*B*). By contrast, between 1.5 and 150 units/ml (lower nanomolar range, *i.e.* concentrations inducing chemotaxis in lung MC) uPA (uPAI and uPAh) failed to induce histamine secretion in MC (Fig. 10*A*). Furthermore, in contrast to rhSCF, uPAI and uPAh did not promote (or inhibit) IgE-dependent release of histamine from MC (Fig. 10*B*). The anti-uPAR mAb VIM5 (10 $\mu\text{g/ml}$), L21 (10 $\mu\text{g/ml}$), and 3B10 (10 $\mu\text{g/ml}$) were also tested (cross-linking of uPAR) but did not induce histamine release (Fig. 10*A*).

DISCUSSION

A number of previous and more recent observations suggest that MC are involved in several microvascular processes such as activation of endothelial cells, vasodilation, capillary leak formation, or transmigration of blood-derived cells into tissues (22, 28–30). Moreover, MC and their products have been implicated in the process of extracellular proteolysis and fibrinolysis (24, 31, 37, 38). The receptor for urokinase is an important cellular antigen that mediates fibrinolysis, cell migration, and tissue repair in general (7–11, 52, 53). The results of this study demonstrate expression of uPAR on primary tissue MC and the human mast cell line HMC-1. Expression of the receptor for uPA was demonstrable by indirect immunofluorescence staining experiments, by *in situ* staining, by Northern blot analysis, and by rtPCR. The functional significance of this MC receptor was also demonstrable; in particular, this receptor apparently mediates MC chemotaxis.

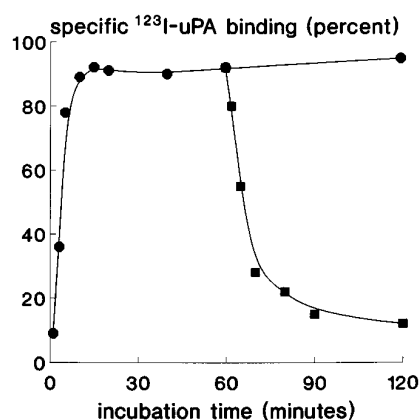


FIG. 4. Time course of specific binding of ^{125}I -uPA to HMC-1 cells. Association of specific ligand binding (\bullet) was analyzed by incubating HMC-1 cells (5×10^5 /test tube) with ^{125}I -uPA (5 nM) in the absence or the presence of unlabeled uPA (500 nM) for various times as indicated. Specific binding was calculated as the difference between total and nonspecific binding and reached $>90\%$ at the applied concentration of ligand (uPA). The binding equilibrium was reached within 15 min, with a calculated association rate constant of $5.92 \times 10^{-5} \text{ M}^{-1} \text{ s}^{-1}$. Displacement of specific binding (dissociation, \blacksquare) was then induced by adding an excess of unlabeled uPA (500 nM) at equilibrium (60 min). A rapid displacement of labeled ^{125}I -uPA by unlabeled uPA was observed, with a calculated dissociation rate constant of $1.8 \times 10^{-3} \text{ s}^{-1}$. Each time point represents the mean of three independent experiments.

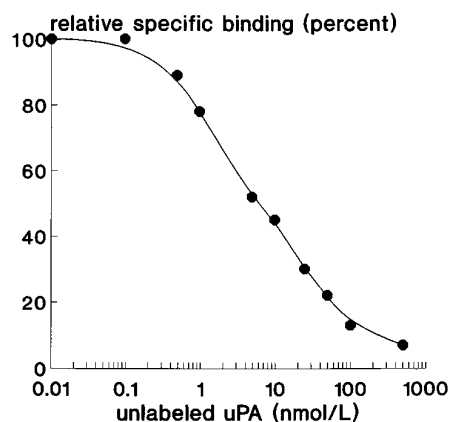


FIG. 5. Concentration-dependent displacement of ^{125}I -uPA binding to HMC-1 cells by uPA. HMC-1 cells (5×10^5 cells in each tube) were incubated with ^{125}I -uPA (5 nM) in the presence or absence of increasing concentrations of unlabeled ligand (0.01–500 nM) for 30 min at 4 $^{\circ}\text{C}$. Results represent the mean of four independent experiments. The IC_{50} value amounted to $5.1 \pm 0.9 \text{ nM}$.

The uPAR has recently been clustered as CD87 (39). In this study, three different mAb clustered as CD87 were found to bind to MC. One of these antibodies, VIM5, is directed against the uPA binding domain of the uPAR (39). Correspondingly, preincubation of HMC-1 cells with high molecular weight (but not low molecular weight) uPA resulted in a significant loss of reactivity with mAb VIM5, whereas the binding of other mAb against uPAR was not altered. The VIM5 domain of the uPAR was detectable on the surface of intact HMC-1 cells as well as by *in situ* (cytoplasmic) staining of primary tissue MC or HMC-1 cells. However, almost no surface reactivity of primary MC with mAb VIM5 was found, although the other anti-uPAR mAb showed significant reactivity. The most likely explanation for this phenomenon is receptor coverage by endogenous ligand (uPA) (4) or by other surface molecules. Alternatively, the VIM5 epitope is constantly shed from the MC surface. The fact that mAb VIM5 bound more effectively to MC at pH 3.8 than at pH 7.4 would be in line with the “coverage hypothesis.” However, since MC at pH 3.8 show increased trypan blue uptake

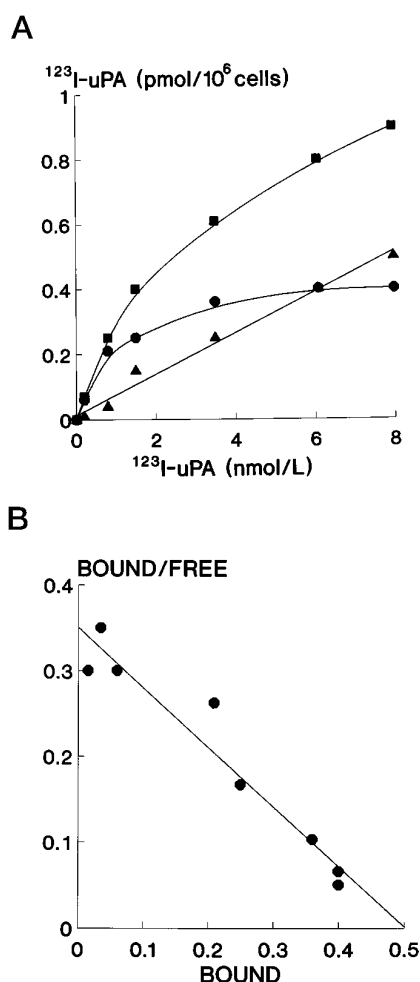


FIG. 6. Saturation and Scatchard plot analysis of $^{123}\text{I-uPA}$ binding to HMC-1 cells. Binding constants and Scatchard plot analysis using $^{123}\text{I-uPA}$ were performed using HMC-1. Cells were prepared and used for receptor analysis as described in the text. *A*, specific binding of labeled uPA to HMC-1 (●). HMC-1 ($5 \times 10^5/\text{tube}$) were incubated with increasing concentrations (0.1–8 nM) of $^{123}\text{I-uPA}$ in the presence (total binding, ■) or absence (nonspecific binding, ▲) of unlabeled ligand (500 nM) at 4 °C for 30 min. Specific binding of uPA was saturable at concentrations > 4 nM uPA. *B*, Scatchard plot transformation of data presented in *A*, suggesting the existence of a single class of uPA binding sites. As assessed by Scatchard plot analysis, HMC-1 cells express $271,000 \pm 55,000$ high affinity uPA binding sites, with a K_d of 1.29 ± 0.3 nM.

(due to disrupted membranes), the reactivity of VIM5 with MC at low pH (3.8) may also be due to binding to intracellular uPAR. The possibility that MC do not synthesize the VIM5 epitope seems rather unlikely, since the *in situ* staining experiments showed a clear reactivity of mAb VIM5 with the cytoplasm of MC and since uPA-induced chemotaxis of MC was inhibitable by the mAb VIM5.

So far, little is known about the regulation of expression of uPAR in MC. In this study, human MC expressed uPAR mRNA and surface uPAR in a constitutive manner, although the amount of expressed uPAR mRNA in unstimulated primary MC was rather low. However, an increase in expression of uPAR mRNA was found after stimulation of primary MC (and HMC-1 cells) with the MC agonist SCF. This cytokine, SCF, is a well recognized stimulator of MC differentiation, survival, and activation (54–57). The observation that SCF augments expression of uPAR in MC further supports the concept that this cytokine is a major regulator of MC.

The binding behavior of uPA to MC membranes was analyzed by a receptor assay using radiolabeled uPA and intact

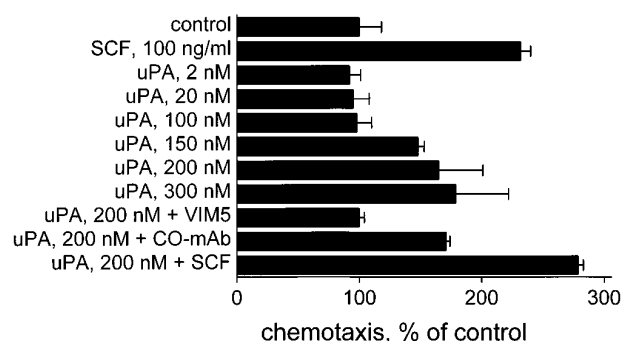


FIG. 7. Chemotactic effect of uPA on HMC-1 cells. HMC-1 cells were exposed to various concentrations of uPA, as indicated, as well as to SCF (100 ng/ml), uPA (200 nM) + SCF (100 ng/ml), uPA (200 nM) + VIM5 (10 $\mu\text{g}/\text{ml}$), and uPA (200 nM) + control mAb S14 (10 $\mu\text{g}/\text{ml}$) in a chemotaxis chamber. After 3 h, the migrated cells were harvested and counted by dye staining (see text). The results represent the mean \pm S.D. from three independent experiments.

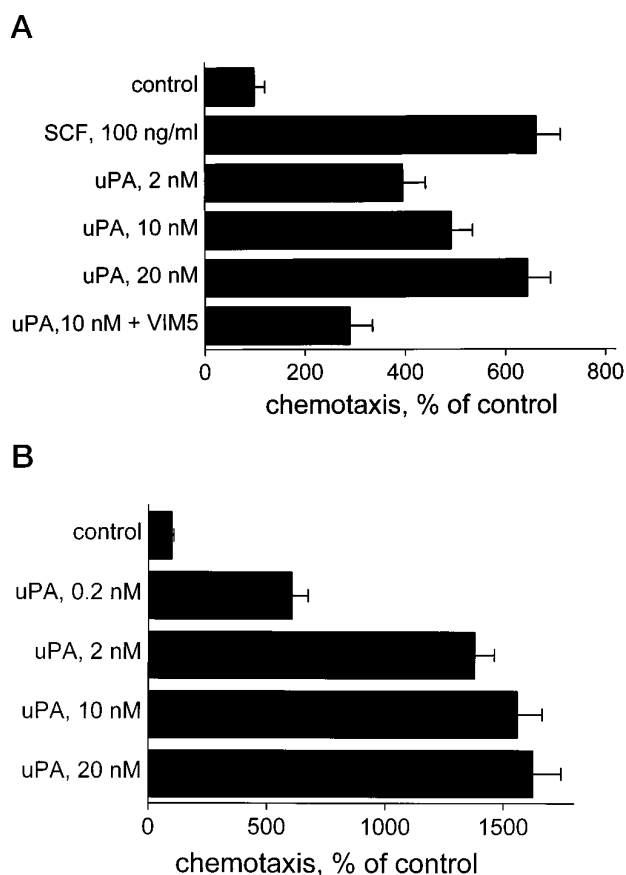


FIG. 8. Chemotactic effect of uPA on primary lung and skin MC. Primary MC from human lung (*A*) and skin (*B*) were incubated with various concentrations of uPA (3 h). In the case of lung MC (*A*), part of the cells were preincubated with the blocking anti-uPAR mAb VIM5 (10 $\mu\text{g}/\text{ml}$) as indicated. After exposure to uPA, the migrated cells in the lower chamber (equal volume) were washed, lysed, and examined for their histamine content by radioimmunoassay. The relative amount of migrated MC (compared with nonspecific migration) was calculated by comparing the amount of total cellular histamine in the lower chambers. Results for lung MC represent the mean \pm S.D. of three independent experiments. In the case of skin MC (*B*), triplicate determinations (mean \pm S.D.) of one typical experiment are shown (almost identical results were obtained in a second donor).

HMC-1 cells. In these experiments, HMC-1 cells expressed approximately 200,000–300,000 high affinity $^{123}\text{I-uPA}$ binding sites with a calculated K_d of 1.29 ± 0.3 nM. A similar range of uPAR has recently been described for blood monocytes, vascular endothelial cells, and tumor cells (4, 7, 12, 13, 15, 58). The

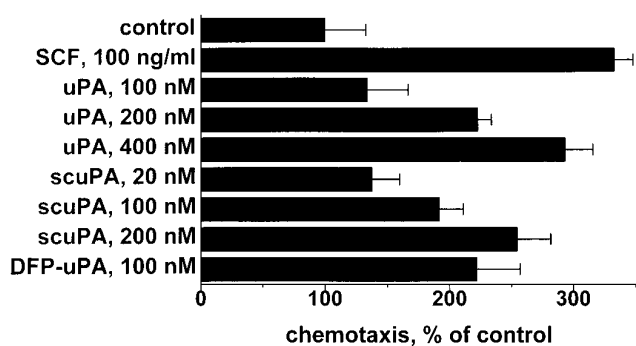


FIG. 9. Comparison of the migration-inducing effects of uPA, scuPA, and DFP-treated uPA on HMC-1 cells. HMC-1 cells were exposed to various concentrations (as indicated) of uPA (isolated from human urine; >90% two-chain uPA) and scuPA (single-chain uPA) in a chemotaxis chamber. HMC-1 cells were also incubated with 100 nM enzymatically inactive DFP-uPA. The chemotaxis assay was done as described in the text. Results represent the mean \pm S.D. of triplicate determinations.

TABLE IV

Checkerboard analysis of uPA-induced migration of HMC-1 cells

The checkerboard analysis of uPA-induced migration of HMC-1 cells was done as described in the text. Cells were loaded in the upper chamber, and after induction by uPA, the number of migrated cells was counted in the lower chamber by dye staining. Various concentrations of uPA (as indicated) were applied in the lower and/or upper chamber of the system. Selective migration against uPA suggests a chemotactic response.

[uPA] (nM, lower chamber)	[uPA] (nM, upper chamber)			
	0	100	200	400
0	2147	1912	1718	1310
100	2871	2431	2134	1906
200	3400	2968	2686	2495
400	3816	3469	3113	2572

number and binding constants of uPAR expressed on primary tissue MC could not be determined in this study because of the difficulty of purifying enough cells. However, when comparing fluorescence intensities for anti-uPAR mAb (L21 and 3B10), the numbers of uPAR expressed on primary MC might be in a lower range compared with HMC-1.

The fate of receptor-bound uPA depends on the cell type, the mobility of the receptor, and the presence of additional molecules. Thus, uncomplexed (free of PAIs) uPA may be expressed in association with uPAR on the cell membrane for prolonged periods of time without significant receptor turnover, internalization, or shedding (7, 17–19). However, in the presence of PAI-1 and LRP, uPAR may be internalized (17, 18). In this study, human MC were found to express uPAR but not uPA or LRP. Thus, endogenous receptor-bound uPA on MC may derive from neighboring cells (but not mast cells) and usually not be internalized by a LRP/PAI-1-dependent mechanism. These observations would favor the hypothesis that active uPA is expressed on MC in tissues for prolonged time periods. In this respect it is also noteworthy that MC are a unique source of tryptase (59), an enzyme that effectively activates uPA (6). Additionally, since MC are a source of uncomplexed tissue-type plasminogen activator (37, 38) but not PAIs, the currently favored concept is that the mast cell is a primary site of tissue fibrinolysis.

Recent data suggest that uPAR is not only a cellular substrate of endogenous fibrinolysis but also a "chemotaxis receptor" (4, 9, 39, 52, 53). We therefore asked whether uPA could be a MC chemoattractant. The results of this study show that uPA induces a significant chemotactic response in both primary human tissue MC and the human mast cell line HMC-1. The reason for the differences between HMC-1 cells and primary

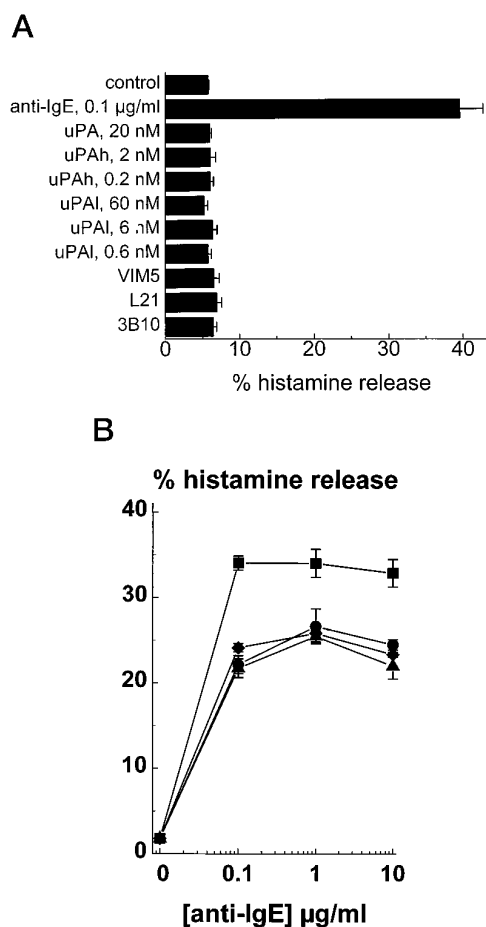


FIG. 10. Effect of urokinase on histamine release from human mast cells. Primary human lung MC were isolated and cultured according to published techniques (48). A, direct effect of uPA on MC. Mast cells were exposed to various concentrations of uPAh or uPAI, anti-IgE (0.1 μ g/ml, after preincubation of cells in IgE for 3 h), anti-uPAR mAb VIM5 (10 μ g/ml), L21 (10 μ g/ml), 3B10 (10 μ g/ml), or control buffer at 37 $^{\circ}$ C for 30 min. Thereafter, the cells were centrifuged (4 $^{\circ}$ C, 10 min) and the supernatants recovered and analyzed for the presence (amount) of secreted histamine by radioimmunoassay. Results are given in percentage of total (intracellular plus extracellular) histamine and represent the mean \pm S.D. from triplicate determinations. B, effect of uPA and SCF on the anti-IgE-induced histamine release from lung MC. In this experiment, lung MC were first exposed to IgE, then to either control buffer (\blacklozenge), uPAh (20 nM, 150 units/ml) (\blacktriangle), uPAI (60 nM, 150 units/ml) (\bullet), or SCF (100 ng/ml) (\blacksquare) for 30 min, and then challenged with the anti-IgE mAb E-124-2-8 as indicated. Histamine release is expressed as percentage of total histamine. The results represent the mean \pm S.D. of triplicate determinations in one typical experiment. Almost the same results were obtained in a second experiment.

MC regarding their responsiveness to uPA (different effective concentrations) are at present unknown. The range of the dissociation constant of the uPAR on HMC-1 (lower nanomolar range) fits quite well with the concentrations of uPA that induced chemotaxis in primary MC but fits less well with concentrations of uPA that could induce migration of HMC-1. One possibility could be that HMC-1 cells lack a potent signal transducer (such as a co-expressed surface signal-transducer molecule) required for induction of chemotaxis. The fact that preincubation of MC with mAb VIM5 (against the uPA binding domain of the uPAR) was followed by a significant blockage of uPA-induced chemotaxis strongly suggests that the effect of uPA was mediated via the uPAR in both types of cells.

We also asked whether uPA can influence mast cell functions other than chemotaxis. However, in the present study, uPA did not induce or promote release of histamine from human MC.

This is in contrast to SCF, another product of activated endothelial cells. Thus, SCF, unlike uPA, was able to augment both chemotaxis and histamine release in MC, confirming earlier observations (57, 60). An interesting aspect is that SCF promotes expression of uPAR on MC. Thus, SCF and uPA may cooperate through multiple mechanisms in the induction of MC chemotaxis and MC accumulation in tissues.

Together, our data show that human mast cells express functional uPA receptors. These receptors may be involved in the accumulation of MC and in mast cell-dependent processes associated with fibrinolysis.

Acknowledgment—We thank Doris Gludovacz, Petra Buchinger, Dieter Printz, and Hans Semper for skillful technical assistance.

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