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

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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.

The urokinase (uPA)1–uPA receptor (uPAR) system plays a 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...
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agons, 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 obtained 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) and >95% pure low molecular weight urokinase (uPAk) were purchased from American Diagnostics (Greenwich, CT). scuPA was provided by L. K. Ashman (University of Adelaide, Australia). The anti-uPAR antibody (mAb) YB5.B8 (40) (IgG1) was kindly provided by J. H. Butterfield (Mayo Clinic, Rochester, MN). 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 IgG1; anti-uPAR), 3B10 (IgG2b; anti-uPAR), and MR19 (IgG1; anti-LRP). Anti-tryptase mAb (IgG2b) 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-e-ist mAb YB5.B8 (40) (IgG1) was kindly provided by L. K. Ashman (University of Adelaide, Australia). Highly purified (>95%) high molecular weight urokinase (uPA) and >95% pure low molecular weight urokinase (uPAk) were purchased from American Diagnostics (Greenwich, CT). scuPA was provided by L. K. Ashman (University of Adelaide, Australia). The anti-uPAR antibody (mAb) YB5.B8 (40) (IgG1) was kindly provided by J. H. Butterfield (Mayo Clinic, Rochester, MN). 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.

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 nylon 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 contained 2.5–6.6% MC (by Giemsa staining). Specimens of uterus were washed in RPMI 1640 medium. The primary lung cell suspensions were dispersed from surgical specimens removed from patients (n = 2) suffering from chronic tonsillitis. Human skin MC were dispersed from circumcision of 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 chronic tonsillitis. Human skin MC were dispersed from surgical specimens removed from 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 circumcision of juvenile foreskin (n = 3). Lung, uterus, tonsil, and skin mast cells were isolated by use of collagenase without other enzymes.

Northern Blot Analysis

RNA Isolation and rtPCR

TABLE I

| Antigen         | Sequence of oligonucleotide probe     | Ref. |
|-----------------|---------------------------------------|------|
| uPAR            | 5′-GGTTCTGTGCCGTGTGAACCTTGCTATACGG-3′ | 16   |
|                 | 5′-CCGATTGCCATGTCGAGTTGCTCAAGGAG-3′  | 61   |
| LRP             | 5′-ATACCTGGTGACGTTACGATCTGGTTCC-3′   | 62   |
| c-kit           | 5′-CCCTATACCACTGGCTGAGCTTGTTGGAATC-3′| 63   |
| SCF             | 5′-GCCGTCGCAAACTGATCCTGATCTTCC-3′    | 64   |
| CD25            | 5′-TCCGGTGTAAGAGCCCGCTATCCGCG-3′    | 65   |
| c-fms           | 5′-GGT CTC ACT CNG CAG CAC GCT CGT CGC-3′ | 66   |
| GDPDH*          | 5′-CCATTGTTGTTAGCAAGGACCGTTGACCT-3′  | 67   |

* GDPDH, 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 cytosin 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 × 106 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 × 106 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 Stratalinker 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, 0.1% polyvinylpyrrolidone, 0.02% Ficoll), 10% dextran sulfate, 20 µM sodium phosphate, pH 7.0, 7% SDS, 100 µg/ml sonicated salmon sperm DNA, 100 µg/ml poly(A)3. 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)PdATP. 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.).
Urokinase Receptor on Human Mast Cells

Mat 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 × 10⁶ 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), and DFP-uPA were placed into the lower chamber of the wells. Then, microporous filter membranes (0.6 cm², pore size, 3.0 µm; 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 visualized by fluorescence microscopy (Olympus, Vienna, Austria). Camphotographic. In “blocking experiments,” MC or HMC-1 were preincubated with mAb VIM5 (recognizing the uPA binding domain on uPAR). After 30 min, cells were washed, exposed to the second step goat anti-mouse IgG/F/γ (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-

Labeling of uPA with ¹²⁵I and Radio Receptor Analysis

Human urokinase was labeled with ¹²⁵I using lactoperoxidase. For this purpose, 100 µg of uPA dissolved in 0.1 ml phosphate buffer (pH 7.0) was labeled with 1 mCi of [¹²⁵I]NaI (Cyclotron Research Center, Karlsruhe, Germany) by slowly mixing with 0.3 µg of H₂O₂ and 5 µg of lactoperoxidase (Sigma). The reaction mixture (50 µl) was injected into a reversed phase C18 high performance liquid chromatography column and eluted with a gradient of 25 to 50% 50 mM sodium citrate buffer. Radiochemical purity was determined by radio-TLC. The specific activity was 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 ¹²³I-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 ¹²³I-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 × 10⁶ 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 µm; 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 visualized with the fluorescent dye Calcein AM (5 mM; Molecular Probes, Eugene, OR) for 30 min at room temperature. Then, labeled HMC-1 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 (DPP-uPA). DPP-uPA showed less than 5% specific
Expression of uPAR mRNA in Human Mast Cells—Northern blot analysis. Enriched human lung MC (91% purity, 3 x 10^6) 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 uPAb) for 30–90 min at 37 °C in 5% CO_2. 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 uPAI (150 units/ml, 30 min), uPAb (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 μg/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, tryptase, 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 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 mAbs, 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 mAbs including VIM5 bound to HMC-1 cells at pH 7.4. Binding of VIM5 antibody was inhibitable by preincubation of HMC-1 with rhSCF.
cells with uPAh but not by uPAi (mean fluorescence intensity: control (2.9) versus VIM5 (37.4) versus VIM5 + uPAi (10.3) versus VIM5 + uPAh (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 uPA 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-uPA 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 cytoxin slides and mAb VIM5.

Characterization of 123I-uPA Binding Sites on HMC-1 Cells—In initial experiments, the time course of association and dissociation of 123I-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 $k_+ (ln 2t/L_0)$ (ligand concentration at time point “0”); $\tau = 234 s$ was $5.92 \times 10^{-5}$ M$^{-1}$ s$^{-1}$. Binding of 123I-uPA to HMC-1 membranes rapidly declined following addition of an excess (500 nM) of unlabeled uPA (Fig. 4). The dissociation rate constant $k_-$ (in 2t/ln $L_0$) was $3.87 \times 10^{-5}$ M$^{-1}$ s$^{-1}$. The $K_d$ value (1.3 ± 0.3 nM) of our saturation experiments thus fits quite well with the time rate constants ($K_d = k_- / k_+ = 3 \times nM$), both being in the lower nanomolar range.

Receptor binding experiments using 123I-uPA and HMC-1 cells revealed specific binding at 4 °C. To assess whether the binding behavior of unlabeled uPA differs from that of 123I-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 123I-uPA to HMC-1 cells was displaced by addition of unlabeled uPA, reaching an IC$_{50}$ value of 5.1 ± 0.9 nM (Fig. 5). Scatchard plot analysis of binding of 123I-uPA to HMC-1 cells revealed a single class of 271,000 ± 55,000 high affinity uPA binding sites with a calculated $K_d$ of 1.29 ± 0.3 nM (Fig. 6A 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 ± 18%; 20 nM uPA, 640.7 ± 42.3%) and skin MC (control, 100 ± 5.9%; 20 nM uPA, 1630 ± 97%) as well as in HMC-1 cells (control, 100 ± 18%; 300 nM uPA, 179 ± 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 µg/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-

| mAb | CD Antigen | LM C | SMC | UMC | CMC | ToMC | HMC-1 |
|-----|------------|------|------|------|------|-------|------|
| YB5.B5 | c-kit | + | + | + | + | + | + |
| VIM5 | uPAR | + | + | + | + | + | + |
| 3B10 | uPAR | + | + | + | + | + | + |
| L21 | uPAR | + | + | + | + | + | + |
| MR19 | LRP | - | - | - | - | - | - |
staining experiments, by indirect immunofluorescence and the human mast cell line HMC-1. Expression of the receptor was also demonstrable; in particular, this receptor apparently mediates MC chemotaxis.

DISCUSSION

A number of previous and more recent observations suggest that MC are involved in several microvascular processes such as activation of endothelial cells, vasodilatation, 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.

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 uPAR 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 binding to MC 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...
(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 HMC-1 cells. Binding constants and Scatchard plot analysis using 123I-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 × 10⁶/tube) were incubated with increasing concentrations (0.1–8 nM) of 123I-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 ± 55,000 high affinity uPA binding sites, with a $K_d$ of 1.29 ± 0.3 nM.

![Graph of 123I-uPA binding to HMC-1 cells](image)

### 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 µg/ml), and uPA (200 nM) + control mAb S14 (10 µg/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 ± S.D. from three independent experiments.

![Chemotaxis assay](image)

### 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 µg/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 chamber. Results for lung MC represent the mean ± S.D. of three independent experiments. In the case of skin MC (B), triplicate determinations (mean ± 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 123I-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
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 LRPPAI-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 (60). 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 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 fibroinosis.

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