Degranulating Mast Cells Secrete an Endoglycosidase That Degrades Heparan Sulfate in Subendothelial Extracellular Matrix

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Mast cells are widely distributed in connective and mucosal tissues and are regarded as essential components of perivascular connective tissues.1 They have been recognized in association with tumors,2 especially in areas of active tumor growth3,4 and vascular reactivity.5,6 Tumor and capillary growth are both accompanied by effective dissolution of extracellular matrices and basement membranes associated with cell proliferation and cell migration. Previous studies indicated that mast cells might play a role in tumor angiogenesis, as these cells were observed to accumulate at tumor sites before ingrowth of new capillaries,5,9 and to stimulate locomotion of capillary endothelial cells in vitro.10 Nonneoplastic cells, other than mast cells, could stimulate endothelial cell locomotion.11 Protracted neovascularization, associated with a local increase in mast cell density, was also observed in several nonneoplastic pathologic conditions, such as rheumatoid arthritis,11 psoriasis,12 and chronic inflammatory diseases.13

Mast cells release various enzymes and mediators by degranulation (ie, proteinases, heparin, histamine, serotonin, prostaglandins, chemotactic factors),14 that might participate in processes involving endothelial cell proliferation and extracellular matrix dissolution, thus augmenting angiogenesis and tumor cell invasion. In this study we investigate the ability of cultured mouse bone marrow-derived mast cells (BMMC) to degrade the subendothelial extracellular matrix (ECM). These mast cells differ from connective tissue mast cells in that they synthesize chondroitin sulphate E, rather than heparin.13 In addition, BMMC lack Forssman glycolipid,16 contain low levels of histamine, express fewer surface receptors for immunoglobulin E (IgE), and appear immature by ultrastructure, as compared with connective tissue mast cells.17

The naturally produced ECM substrate used in this study has a composition close to that seen in vivo in capillary endothelial cell basement membrane (collagen types I, III, and IV; fibronectin; laminin; heparan sulfate; dermatan sulfate; and chondroitin sulfate – proteoglycans).18,19 Therefore, it provides an appropriate model system to study cell invasion, matrix degradation, and other cellular responses to contact with ECM. Using this ECM, it was found that invasive potentials of tumor cells20,21 and activated cells of the immune system22 correlate with the activity of heparan sulfate (HS)-degrading endoglycosidase (heparanase). We have also demonstrated that heparanase-mediated degradation of the ECM-HS is associated with release of HS-bound endothelial cell growth factors23 that are stored in basement membranes and extracellular matrices.24

In this study we report on the expression of heparanase by cultured bone marrow-derived mast cell lines, and characterize the requirements for release of the enzyme and degradation of HS in the subendothelial ECM. We suggest that heparanase activity in mast cells may provide an additional facet to the proposed role of these cells in angiogenesis and tumor growth.

MATERIALS AND METHODS

Materials. Dulbecco’s modified Eagle’s medium (DMEM), Roswell Park Memorial Institute (RPMI) medium 1640, calf serum, fetal calf serum (FCS), penicillin, streptomycin, and trypsin-EDTA solution were obtained from Gibco (Grand Island, NY). Tissue culture dishes were obtained from Falcon Labware Division.
Becton Dickinson (Oxnard, CA). Trypsin type III, chondroitinase ABC, chondroitin sulfate A and C, Ca²⁺-ionophore A23187, Triton X-100, and dextran T-40 were from Sigma Chemicals (St Louis, MO). Sodium heparin was from Diosynth (Oss, Holland). Heparan sulfate was from Seikagaku Kogyo Co (Tokyo, Japan). N/O-de-sulfated heparin, N-desulfated, N-resulfated heparin, N-acetylated heparin, and N-acetylated, O-desulfated heparin were prepared and kindly provided by Dr Lina Wasserman (Rogoff-Wellcome Medical Research Institute, Petah-Tikva, Israel). In brief, pyridinium heparin underwent exhaustive desulfation with dimethyl sulfoxide (Merck, Rahway, NJ) containing 10% water to yield totally desulfated heparin. N-desulfated heparin was obtained from the respective pyridinium salt with dimethyl sulfoxide containing 5% water. Totally desulfated heparin and N-desulfated heparin were N-acetylated with acetic anhydride, while resulfation of the free amino residues of the totally desulfated or N-desulfated heparin was performed with sulfur trioxide trimethylamine complex (Aldrich Chemical Co, Milwau-kee, WI) as described. Chondroitin sulfate E, derived from shark cartilage, was a kind gift from Dr N Seno, Ochanomizu University, Tokyo, Japan. Sepharose 6B was from Pharmacia Fine Chemicals (Upplusa, Sweden). Na₃[³⁵S]O₃ and Biofluor scintillation fluid were from New England Nuclear (Boston, MA). All other chemicals were of reagent grade furnished from Sigma.

**Cell cultures.** Bone marrow-derived mouse mast cells were obtained from femurs of 2-month-old male Balb/c mice, as described. Cells were cultured for 14 days at 37°C, at a starting density of 1 x 10⁵ cells/mL in 50% RPMI 1640 medium and 50% WEHI (mouse myelomonocytic cell line, American tissue culture collection T1B68) conditioned medium supplemented with 10% FCS, 2 mmol/L L-glutamine, 0.1 mmol/L nonessential amino acids, 100 U/mL penicillin, 100 µg/mL streptomycin, and 50 µmol/L 2-mercaptopethanol, pH 7.2. Cultures were maintained at 37°C in a humidified incubator with 5% CO₂. Medium was changed at the seventh day of culture. After 14 days in culture, cells were stained with toluidine blue at pH 3.5. Approximately 98% of the cells were identified as mast cells by the presence of metachromatic granules. Abelson-MuLV transformed bone marrow derived mast cells were cultured in RPMI 1640 medium supplemented with 10% FCS and 50 µmol/L 2-mercaptopethanol. Cultures of bovine corneal endothelial cells were established from steer eyes as described, and maintained in DMEM (1 g glucose/L) supplemented with 10% bovine calf serum, 5% fetal calf serum, penicillin (50 U/mL), and streptomycin (50 µg/mL) at 37°C in a 10% CO₂ humidified incubator. Partially purified bovine brain fibroblast growth factor (FGF, 100 ng/mL) was added every other day during the phase of active cell growth. Cloned populations of adult bovine aortic endothelial cells were cultured as described above, except that FCS was not included in the growth medium.

**Ca²⁺-ionophore (A23187) and IgE-mediated degradation of BMCC.** Cultured mast cells were centrifuged for 5 minutes at 500 x g, suspended at a concentration of 1 x 10⁵ cells/0.5 mL Tyrode's buffer (137 mmol/L NaCl, 2.7 mmol/L KCl, 0.4 mmol/L NaH₂PO₄, 10 mmol/L HEPES), containing 0.5 mmol/L Ca²⁺, 0.2 mmol/L Mg²⁺, 5 mmol/L glucose, and 0.05% gelatin (modified Tyrode's buffer), and incubated for 45 minutes at 37°C with the calcium-ionophore A23187 in doses ranging from 0 to 0.5 µmol/L. Alternatively, mast cells (1 x 10⁴) in 0.2 mL Tyrode's buffer were sensitized by incubation for 1 hour at 37°C with 0.5 µg of mouse monoclonal IgE directed against dinitrophenyl-conjugated bovine serum albumin (DNP-BSA), kindly provided by Dr F.T. Liu, the Medical Biology Institute, La Jolla, CA. Sensitized cells were washed twice with 1.5 mL of modified Tyrode's buffer, sedimented at 400 x g at room temperature, and incubated for 15 minutes at 37°C in 0.5 mL of prewarmed (37°C) modified Tyrode's buffer containing 75 to 100 ng of DNP-BSA (18 mol DNP/mol BSA). Reactions were terminated by centrifugation at 400 x g for 5 minutes at room temperature and removal of the supernatants, which were collected. Cell pellets were resuspended in modified Tyrode's buffer and the cells lysed by three cycles of freezing in liquid nitrogen and thawing. All experiments were performed in duplicate and under sterile conditions. Exocytosis of secretory granule constituents was assessed by measurements of β-hexosaminidase activity in the supernatants relative to the total cellular activity. Only cultures with greater than 90% cell viability, as determined by the trypan blue exclusion test, were used for the release experiments.

**Preparation of sulfate-labeled ECM coated dishes.** Corneal endothelial cells were plated at an initial density of 5 x 10⁴ cells per 35-mm dish and were maintained as described above, except that 5% dextran T-40 was included in the growth medium. Na₃[³⁵S]O₃ (540 to 590 mCi/mmol) was added 3 and 7 days after seeding (40 µCi/mL) and the cultures were incubated with the label with no medium change. Five to seven days after reaching confluence (10 to 12 days after seeding), the cell layer was dissolved by exposure (10 minutes, 22°C) to 0.5% Triton X-100 in phosphate-buffered saline (PBS) containing 0.025 M NaH₂O₇, followed by four washes in PBS. The underlying ECM remained intact, free of cytoskeletal elements, nuclei, and cellular debris and firmly attached to the entire area of the tissue culture dish. Previous studies have shown that 70% to 75% of the total ECM-bound radioactivity was incorporated into HS side chains. Similar results were obtained with ECM incubated with bone endothelial cells, except that this matrix was loosely attached to the tissue culture dish and often contained some cellular debris.

**Degradation of sulfated proteoglycans.** [³⁵S]SO₄²⁻-labeled ECM was incubated (37°C, 10% CO₂ incubator, 48 hours) with cell lysates (10% cells/mL modified Tyrode's buffer) prepared by four cycles of freezing in liquid nitrogen and thawing at 37°C, followed by centrifugation at 10,000 x g for 5 minutes. The labeled ECM was also incubated with supernatants of BMMC stimulated immunologically, or by Ca²⁺-ionophore (A23187), as described. For optimal results, phosphate citrate buffer (50 mmol/L, pH 6.0) was added to BMMC lysates and supernatants incubated with the labeled ECM. Phosphate buffer (20 mmol/L, pH 6.2) was added to intact BMCC suspended in Tyrode's buffer or growth medium. The final pH during incubations with ECM was 6.6 and 6.8, respectively. To evaluate the occurrence of proteoglycan degradation, the incubation medium was collected and applied for gel filtration on Sepharose 6B columns (0.9 x 30 cm). Fractions (0.2 mL) were eluted with PBS at a flow rate of 5 mL/h and counted for radioactivity using Bio-fluor scintillation fluid. The excluded volume (Vo) was marked by blue dextran, and the total included volume (Vi) by phenol red. The latter was shown to comigrate with free [³⁵S]SO₄²⁻. Similar elution profiles (Kav values) and recoveries were obtained whether the centrifuged media were subjected to gel filtration under dissociation conditions (4 mol/L guanidine-HCl in 0.1 mol/L sodium acetate, pH 5.5) or eluted with PBS. Intact, ECM-derived HS proteoglycans were eluted from Sepharose 6B with or next to Vo (peak I, Kav < 0.2), while heparanase-mediated degradation fragments of HS migrated as peak II (0.5 < Kav < 0.75). No such low molecular weight (mol wt) peak II material was found in ECM that was not exposed to heparanase. Heparanase activity is presented by the actual elution profile (Sepharose 6B) of labeled material released from ECM. In some experiments, heparanase activity is expressed by the total amount of counts per minute eluted in peak II, multiplied by the Kav of this peak. Although this definition of enzyme activity compensates for variations in the size of peak II fragments, it is arbitrary due to the nonlinear relationship between Kav and the mol wts of the eluted fragments. Recoveries of labeled...
material applied on the columns ranged from 85% to 95% in different experiments. Each experiment was performed at least three times, and the variation of elution positions (Kav values) did not exceed 15%.

Enzyme activity. β-Hexosaminidase was assayed by hydrolysis of p-nitrophenyl-β-D-2-acetamido-2-deoxyglucopyranoside. One unit of enzyme cleaves 1 μmol of substrate per hour at 37°C. Chondroitinase activity was measured as described. Lactate dehydrogenase (LDH) activity was determined as previously described.

RESULTS

Degradation of sulfated proteoglycans in the ECM by bone marrow-derived, mast cell heparanase. Lysates prepared by freezing and thawing of cultured BMMC were incubated (48 hours, 37°C) with sulfate-labeled ECM in the absence or presence of 20 μg/mL heparin, or chondroitin sulfate A. Sulfate-labeled degradation products released into the incubation medium were analyzed by gel filtration on Sepharose 6B columns. In the presence of the incubation buffer alone there was a constant release of labeled material, which consisted almost entirely (greater than 90%) of large mol wt fragments (peak 1) eluted with, or next to Vt. In contrast, incubation of the labeled ECM with mast cell lysates resulted in release of low mol wt sulfate-labeled fragments (peak II, Kav 0.75) (Fig 1). These low mol wt fragments were shown to be degradation products of HS, as evidenced by being: (1) five- to sixfold smaller than intact HS side chains (Kav ~ 0.33) released from ECM by treatment with alkaline borohydride or papain (Fig 1); (2) resistant to further digestion by chondroitinase ABC; and (3) susceptible to deamination with nitrous acid. Similar results were obtained with metabolically labeled ECM produced by corneal and vascular endothelial cells (data not shown).

Release of peak II labeled degradation fragments from ECM increased as a function of the amount of BMMC lysates (up to 2 x 10^6 lysed cells/mL) (Fig 2A) and time of incubation (up to 48 hours) with the ECM (Fig 2B). Fifty-five to sixty percent of the total ECM-incorporated sulfate-labeled material was released by 2 x 10^6 lysed BMMC during a 48-hour incubation period.

Addition of heparin (20 μg/mL) to mast cell lysates before incubation with the ECM resulted in complete inhibition of peak II production (Fig 1). This was associated with a corresponding increase in peak I material (Kav < 0.2), representing nearly intact, ECM-derived proteoglycans (mol wt ~ 5 x 10^5 daltons). Complete inhibition of peak II production was also obtained by incubating the labeled ECM with BMMC lysates at pH 8, as compared with pH 6.6 to 6.8 in all the other experiments (Fig 1). Degradation of the ECM-HS also occurred at pH 7.2, but to an extent that was 60% to 70% of that obtained at pH 6.6. Similar results were obtained when soluble ECM-derived HS proteoglycans (HSPG), rather than intact ECM, were used as substrate in the heparanase assay. Addition of chondroitin sulfate A (20 μg/mL) to mast cell lysates incubated with sulfate-labeled ECM had no effect on peak II production (Fig 1). Size of sulfate-labeled fragments released from ECM by various GAG degrading enzymes and cells was compared. Lysates of mast cells and of highly metastatic mouse lymphoma cells (ESb) released from ECM that labeled degradation products were five- to sixfold smaller than intact HS side chains. Fragments released by bacterial heparitinase (β-eliminase), were smaller (Kav ~ 0.8 on Sepharose 6B). Degradation of the ECM HS by BMMC lysates was two- to threefold higher than by lysates of ESb lymphoma cells. This may be due to a higher proteolytic activity in BMMC, which facilitates degradation of the ECM HS by heparanase.

Degradation of sulfate-labeled ECM also was obtained during incubation with viable BMMC, although to an extent that was seven- to eightfold lower than that observed with lysed cells (Fig 3). Approximately 50% of the cells suspended in RPMI/WEHI medium attached to the ECM, adopting a flattened morphology. The other cells remained floating, and 94% of the entire cell population retained viability 24 hours after seeding. After this incubation period, degradation products released into the medium were analyzed by gel filtration through Sepharose 6B. An elution pattern, similar to that obtained with cell lysates, was observed. The amount of radioactivity in peak II increased as a function of the cell number (Fig 3). The average size of HS cleavage products increased (Kav ranging from 0.65 to 0.45) as the amount of cells decreased, reflecting less favorable degradation conditions. Little (less than 5%) or no heparanase activity was detected in medium conditioned for 3 days by cultured BMMC, as well as in the supernate fraction of BMMC incubated (24 hours, 37°C) in regular tissue culture dishes under the heparanase assay conditions (2.5 x 10^6 cells/mL, pH 6.8). No chondroitinase activity was detected in these lysates (2 x 10^3 cells/mL) using unlabeled chondroitin sulfate C as substrate.
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Release of heparanase activity by degranulating bone marrow-derived mast cells. Mast cell granules contain a large variety of enzymes, packed densely by proteoglycans contained in them. We investigated whether heparanase is contained in these highly organized structures, and hence available for controlled secretion. As demonstrated in Figs 4 and 5, degranulation of BMMC induced either with the Ca\(^{2+}\) ionophore A23187 (Fig 4), or immunologically (Fig 5), resulted in release of heparanase activity, as determined by incubation of the supernate fractions of triggered cells with sulfate-labeled ECM and analyzing the size of degradation products by gel filtration. HS degradation was manifested by appearance of the characteristic peak II material (0.65 < Kav < 0.75). A three- to fourfold higher activity was detected in the supernate fractions of cells that were challenged with either IgE in the presence of DNP-BSA or exposed to Ca\(^{2+}\) ionophore, as compared with cells that were not exposed to degranulation-inducing agents. Heparanase activity released by cells challenged with IgE in the absence of DNP-BSA was similar to the basal level of enzyme detected in the absence of IgE (Fig 5). Low levels of heparanase activity in unstimulated cells might be attributed to a certain degree of spontaneous degranulation resulting from the experimental procedure involving centrifugation and dispersion of cells. Heparanase secretion by degranulating cells was accompanied by a concomitant release of \(\beta\)-hexosaminidase, a granular enzyme, indicating the granular origin of heparanase (4.0% + 0.7% spontaneous release, as compared with about 40% release of both \(\beta\)-hexosaminidase and heparanase induced by Ca\(^{2+}\)-ionophore). HS degradation activity in supernates of activated mast cells was abolished completely in the presence of heparin (10 \(\mu\)g/mL), but there was no effect to chondroitin sulfate A at the same concentration. These results indicate that the enzyme secreted during degranulation of BMMC exhibits characteristics similar to those of the enzyme found in the cell lysates (Fig 1).

The presence of heparanase activity was also tested in two cell lines related to mast cells. Surprisingly, no enzyme activity was detected in both Abelson transformed BMMC and in a rat basophilic leukemia cell line (RBL), as evidenced by lack of peak II material after incubation of cell lysates with sulfate-labeled ECM (not shown).

Inhibitory effect of sulfated proteoglycans and modified heparins on mast cell heparanase. Several sulfated proteoglycans were tested for their ability to inhibit mast cell heparanase. Native heparin, HS, chondroitin sulfate A,
chondroitin sulfate C, or chondroitin sulfate E were added at increasing concentrations (0.5, 2, 10, 20 \mu g/mL) to mast cell lysates, before incubation with sulfate-labeled ECM. HS degradation products released from ECM into the incubation medium were analyzed by gel filtration on Sepharose 6B. Native heparin exhibited the highest inhibitory effect on mast cell heparanase activity, measured as radioactivity released from ECM and eluted in peak II (Fig 6A). At a concentration as low as 0.5 \mu g heparin/mL, radioactivity eluted in peak II was only 39% of that observed in the absence of heparin, but there was little (5% to 10% inhibition) or no effect to HS, chondroitin sulfate E, chondroitin sulfate A, and chondroitin sulfate C at this concentration. At 2 \mu g heparin/mL the amount of low mol wt (peak II) radioactive fragments decreased to 15% (similar to that observed in corresponding fractions in the absence of heparanase activity), as compared with 56%, 82%, 89%, and 100% peak II radioactivity at the same concentration of HS, chondroitin sulfate E, chondroitin sulfate A, and chondroitin sulfate C, respectively. At concentrations higher than 10
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...expression of heparanase occurred also on incubation of viable BMMC cells with ECM, although to a much lower extent as compared with that found with lysed or degranulated BMMC. No significant heparanase activity was detected in the cell conditioned medium.

The BMMC heparanase described in this study degraded the ECM-HS at four to five intrachain sites, yielding fragments of approximately 5,000 to 7,000 daltons. The heparan sulfate nature of the degradation products was demonstrated by their susceptibility to deamination with nitrous acid and precipitation with 0.05% cetylpyridinium chloride in 0.6 mol/L NaCl. The released fragments were resistant to further degradation with papain, or chondroitinase ABC. Degradation of HS side chains in ECM was inhibited by heparin, as previously observed with heparanases of other cell types.

DISCUSSION

In this study we demonstrate expression of an ECM-HS degrading endoglycosidase (heparanase) in BMMC. The enzyme is stored within the mast cell granules, as it was readily released on IgE-Ag or calcium ionophore (A23187)-induced degranulation, concomitantly with another mast cell granule marker, the enzyme β-hexosaminidase. The activated cells secreted a preformed enzyme, as evidenced by its presence in lysates of both unstimulated and stimulated mast cells. Expression of heparanase occurred also on incubation of viable BMMC cells with ECM, although to a much lower extent as compared with that found with lysed or degranulated BMMC. No significant heparanase activity was detected in the cell conditioned medium.

Fig 6. Effect of various GAGs and modified heparin species on degradation of sulfate-labeled ECM by BMMC heparanase. (A) Effect of GAGs. (25S-ECM was incubated (24 hours, 37°C) with lysates of 4 × 10⁶ BMMC in the absence or presence of the indicated concentrations of chondroitin sulfate A (△), chondroitin sulfate C (○), chondroitin sulfate E (●), HS (□), or heparin (◇). (B) Effect of modified heparins. 35S-labeled ECM was incubated (24 hours, 37°C) with lysates of 4 × 10⁶ BMMC in the absence or presence of the indicated amounts of N-acetylated, O-desulfated heparin (○); N/O-desulfated heparin (●); N-sulfated, N/O desulfated heparin (△); N-acetylated, O-sulfated heparin (△); HS (□); or native heparin (◇). The different GAGs were added at the appropriate concentration to 1 mL BMMC lysates before incubation with the labeled ECM. Incubation media were collected and assayed for sulfated degradation products, as described in Materials and Methods. Heparanase activity is expressed as the total amount of counts per minute eluted in peak II multiplied by the Kav of this peak.

μg/mL, HS yielded up to 80% inhibition, while chondroitin sulfate E exerted approximately 35% inhibition of the mast cell heparanase. Chondroitin sulfates A and C exerted no significant inhibition, even at concentrations as high as 20 μg/mL (Fig 6A).

Structural requirements for inhibition of the mast cell heparanase were analyzed by using various chemically modified species of heparin. For this purpose heparin was first either totally desulfated or N-desulfated. These heparins were then left with their N-position exposed, or were further N-acetylated or N-desulfated, as described. As demonstrated in Fig 6B, native heparin exhibited the highest inhibitory effect on the mast cell heparanase, yielding almost complete inhibition of HS degradation at a concentration of 2 μg/mL. In comparison, N-acetylated, O-sulfated heparin exhibited 50% inhibition, whereas O-desulfated, N-sulfated heparin or N-acetylated, O-desulfated heparin yielded about 15% inhibition at 2 μg/mL. Totally desulfated heparin exhibited almost no inhibitory effect on the enzyme activity, even at a concentration of 10 μg/mL.
intact or lysed BMMC did not exceed 60% of the total ECM incorporated radioactivity. In some experiments, purified HS-PG, rather than intact ECM, was used as a substrate for the mast cell heparanase. Similar results were obtained, ruling out the possibility of a latent heparanase residing in the ECM itself and being activated by the mast cells.

Using chemically modified heparin species, we characterized some of the structural requirements for inhibition of the BMMC heparanase. Our results indicate that this inhibition depends on the degree of sulfation of the heparin molecule and the position of sulfate groups. The presence of a sulfate group in either the N-, or O-position, was essential for heparanase inhibition. N-acetylated, O-sulfated species of heparin apparently were more potent inhibitors as compared with O-desulfated, N-desulfated heparin. No inhibition was displayed by totally desulfated heparins, such as N/O desulfated heparin, or N-acetylated, O-desulfated heparin. Most potent inhibition was exerted by native heparin, at concentrations as low as 2 μg/mL. HS was less effective, yielding almost complete inhibition only at a concentration of 10 μg/mL, and resembling in its potency that of N-acetylated, O-sulfated heparin. Except for elevated susceptibility to inhibition by HS and chondroitin sulfate E, this inhibitory pattern of the mast cell heparanase is similar to that described for heparanase of platelets, neutrophils, ESb inhibitory pattern of the mast cell heparanase is similar to heparanase of platelets, neutrophils, ESb inhibitors.

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Heparanase activity. This latter result may suggest suppression of the mast cell heparanase described in this study exhibited degradation activity toward HS, with little activity against degradation activity toward HS, with little activity against heparanase activity. This latter result may suggest suppression of heparanase expression by Abelson gene products.

 Mast cells have long been described as residing in the neighborhood of capillaries and venules. These cells accumulate at the site of tumor implantation before the ingrowth of new capillaries, and it has been proposed that mast cells may influence the growth of vascular endothelial cells. Mast cells, or heparin, a mast cell product, have been shown to increase endothelial cell locomotion and to potentiate angiogenesis. This effect of heparin has been attributed to its ability to enhance both the activity and production of collagenase, potentiate the proliferative effect of acidic FGF on cultured endothelial cells, and protect basic FGF from inactivation. Because capillary growth involves enzymatic degradation of basement membrane and ECM, as well as endothelial cell locomotion and proliferation, we propose that heparanase, released on degranulation of mast cells, may play a role in tumor angiogenesis and growth through dissolution of basement membranes and the associated release of endothelial cell growth factors that are firmly bound to HS. The cultured mast cells used in this study differ from connective tissue mast cells in that they synthesize chondroitin sulfate E, rather than heparin. However, recently an inter-relationship between BMMC and connective tissue mast cells has been demonstrated, indicating that the phenotypic changes in mast cells are dependent on factors derived from their microenvironment. Therefore, BMMC may function as a bipotent precursor for connective tissue mast cells and vice versa. Because connective tissue mast cells synthesize heparin, it is likely that heparanase will be neutralized by heparin, when released on degranulation. However, it is possible that the mast cell heparin is degraded and/or dissociated from heparanase by other cells or conditions existing in the microenvironment of these cells.

 Degranulating mast cells have been observed in areas of neovascularization, such as the synovial fluid of arthritic joints, around newly formed blood vessels in the neovascularization area of healed lesions of portwine stains, or at the later stages of neoplasm growth in areas of connective tissue lysis at the tumor-host junction. Mast cell degranulation may initiate a cascade of events by secretion of granule constituents, such as heparanase, tryptase (participating in collagenase activation), and other proteolytic enzymes, heparin, histamine, and serotonin. These enzymes and substances have been reported to induce matrix dissolution and display mitogenic and angiogenic activities. HS proteoglycans interact with different attachment sites on plasma membranes and with macromolecules, such as collagen, laminin, and fibronectin in the ECM. The involvement of this proteoglycan in the self-assembly and insolubility of the ECM, as well as in cell adhesion and locomotion, suggests a role for the mast cell heparanase in chronic inflammation and tumor progression.

REFERENCES


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Mast Cell Heparanase


Degranulating mast cells secrete an endoglycosidase that degrades heparan sulfate in subendothelial extracellular matrix

P Bashkin, E Razin, A Eldor and I Vlodavsky