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

By Pnina Bashkin, Ehud Razin, Amiram Eldor, and Israel Vlodavsky

Mast cells are widely distributed in perivascular connective tissues, especially in areas of active tumor growth and vascular reactivity. Incubation of metabolically [35S]O
2
-labeled subendothelial extracellular matrix (ECM) with lysates of bone marrow-derived mouse mast cells (BMMC) resulted in extensive degradation of heparan sulfate (HS) into fragments 5 to 6 times smaller than intact HS side chains. A much lower activity (seven- to eightfold) was expressed by intact BMMC incubated in contact with the ECM. These fragments were not produced in the presence of heparin, were sensitive to deamination with nitrous acid, and resistant to further degradation with papain or chondroitin ABC. These results indicate that an endoglycosidase (heparanase) is involved in BMMC-mediated degradation of HS in the subendothelial ECM. Heparanase activity was not detected in medium conditioned by cultured BMMC, or in lysates of Abelson transformed BMMC and rat basophilic leukemia (RBL) cells. Both heparanase and β-hexosaminidase, a mast cell granule enzyme, were released on degradation of BMMC induced by the calcium ionophore A23187, or by exposure to IgE-Ag, suggesting that heparanase is localized in the cell granules. Under these conditions, less than 5% of the cellular content of lactate dehydrogenase were released. Degradation of the ECM-HS by the mast cell heparanase and the associated release of HS-bound endothelial cell growth factors that are stored in ECM (Vlodavsky et al, Proc Natl Acad Sci USA 84:2292, 1987; Bashkin et al, Biochemistry 28:1737, 1989) may play a role in the proposed mast cell-mediated stimulation of neovascularization.

© 1990 by The American Society of Hematology.

From the Departments of Oncology and Hematology, Hadassah University Hospital; and Institute of Biochemistry, Hebrew University Hadassah Medical School, Jerusalem, Israel.

Submitted July 7, 1989; accepted February 9, 1990.

Supported by PHS Grant CA30289 awarded to I.V. by the National Cancer Institute DHHS; by grants from G.I.F., The German-Israeli Foundation for Scientific Research and Development, awarded to I.V. and E.R.; and by a grant from the Israel Cancer Research Fund. I.V. is a Leukemia Society of America Scholar.

Address reprint requests to Israel Vlodavsky, PhD, Department of Oncology, Hadassah Medical Center, P.O.B. 12000, Jerusalem, 91120 Israel.

The publication costs of this article were defrayed in part by charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. section 1734 solely to indicate this fact.

© 1990 by The American Society of Hematology.

0006-4971/90/7511-0009$3.00/0

Blood, Vol 75, No 11 (June 1), 1990: pp 2204-2212
Becton Dickinson (Oxnard, CA). Trypsin type III, chondroitinase ABC, chondroitin sulfate A and C, Ca\textsuperscript{2+}-ionophore A23187, Triton X-100, and dextran T-40 were from Sigma Chemicals (St Louis, MO). Sodium heparin was from Diosynth (Oss, Holland). Heparin sulfate was from Seikagaku Kogyo Co (Tokyo, Japan). N/O-desulfated heparin, O-desulfated, N-resulfated heparin, N-acetylated heparin, and N-acetylated, O-desulfated heparin were prepared\textsuperscript{22} and kindly provided by Dr Lina Wasserman (Rogoff-Wellcome Medical Research Institute, Beilinson Medical Center, 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, Milwaukee, WI) as described.\textsuperscript{22} Chondroitin sulfate E, derived from shark cartilage, was a kind gift from Dr N Nesvig, Ochanomizu University, Tokyo, Japan. Sepharose 6B was from Pharmacia Fine Chemicals (Uppsala, Sweden). Na\textsubscript{2}O\textsubscript{3}SO\textsubscript{4} 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 mast cell cultures were obtained from femurs of 2-month-old male Balb/c mice, as described.\textsuperscript{22} Cells were cultured for 14 days at 37°C, at a starting density of 1 x 10\textsuperscript{5} 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-mercaptoethanol, pH 7.2. Cultures were maintained at 37°C in a humidified incubator with 5% CO\textsubscript{2}. 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\textsuperscript{23} were cultured in RPMI 1640 medium supplemented with 10% FCS and 50 μmol/L 2-mercaptoethanol. Cultures of bovine corneal endothelial cells were established from steer eyes as described,\textsuperscript{24} 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% CO\textsubscript{2} humidified incubator. Partially purified bovine brain fibroblast growth factor (FGF, 100 ng/mL)\textsuperscript{25} 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.\textsuperscript{21}

**Ca\textsuperscript{2+}-ionophore (A23187) and IgE-mediated degradation of BMMC.** Cultured mast cells were centrifuged for 5 minutes at 500 x g, suspended at a concentration of 1 x 10\textsuperscript{6} cells/0.5 mL Tyrode's buffer (137 mmol/L NaCl, 2.7 mmol/L KCl, 0.4 mmol/L Na\textsubscript{2}HPO\textsubscript{4}, 10 mmol/L HEPES), containing 0.5 mmol/L Ca\textsuperscript{2+}, 0.2 mmol/L Mg\textsuperscript{2+}, 5 mmol/L 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.\textsuperscript{13} Alternatively, mast cells (1 x 10\textsuperscript{6}) 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),\textsuperscript{13} 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 b-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 10\textsuperscript{4} cells per 35-mm dish and were maintained as described above, except that 5% dextran T-40 was included in the growth medium. Na\textsubscript{2}S\textsuperscript{35}SO\textsubscript{4} (540 to 590 mCi/mmol) was added 3 and 7 days after seeding (40 μCi/mL) and the cultures were incubated with the label for 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) (vol/vol) containing 0.025 N NH\textsubscript{2}OH, followed by four washes in PBS.\textsuperscript{23-26} 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.\textsuperscript{22} Previous studies have shown that 70% to 75% of the total ECM-bound radioactivity was incorporated into HS side chains.\textsuperscript{19,23} Similar results were obtained with the ECM produced by vascular 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]Sulfate-labeled ECM was incubated (37°C, 10% CO\textsubscript{2} 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 Ca\textsuperscript{2+}-ionophore (A23187), as described.\textsuperscript{21} For optimal results, phosphate citrate buffer (20 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 BMMC 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]Sulfate. 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 I) eluted with, or next to V0. 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 × 10⁶ 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 × 10⁶ 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 × 10⁵ 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 × 10⁶ cells/mL, pH 6.8). No chondroitinase activity was detected in these lysates (2 × 10⁷ cells/mL) using unlabeled chondroitin sulfate C as substrate.
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% spontaneous release, 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 μg/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 μg 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 μg 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
MAST CELL HEPARANASE

Fig 6. Effect of various GAGs and modified heparin species on degradation of sulfate-labeled ECM by BMMC heparanase. (A) Effect of GAGs. 35S-ECM was incubated (24 hours, 37°C) with lysates of 4 x 10^6 BMMC in the absence or presence of the indicated concentrations of chondroitin sulfate A (A), chondroitin sulfate C (C), chondroitin sulfate E (E), HS (W), or heparin (W). 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.

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 8-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.

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.35,36 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.39 We have previously demonstrated that requirements for heparanase-mediated degradation of HS in a multimolecular structure such as the ECM differ from those involved in degradation of the same molecule in a soluble form. Proteolytic degradation of the protein core and of adjacent proteins is often necessary as a first step to enable subsequent degradation of the glycosaminoglycan chains by the endoglycosidase.37 To take into account the involvement of such a proteolytic activity, we have used a naturally produced substrate whose degradation may better resemble the situation in vivo, as compared with a purified soluble HSPG. Higher proteolytic activity in BMMC lysates as compared with ESb lymphoma cell lysates may therefore be held responsible for a more efficient degradation of the ECM' HS by lysed BMMC as compared with ESb lymphoma cell lysates. While the extent of ECM labeling varied (up to twofold) in different experiments, the variation between ECM-coated dishes of the same batch was negligible. The amount of sulfate-labeled material released from ECM by

100
80
60
40
20
0
0 10 20
Heparanase activity (% control)
Concentration (ug/ml)

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-resulfated, as described.25 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-resulfated 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.

**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 8-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
intact or lysed BMMC did not exceed 60% of the total ECM incorporated radioactivity. In some experiments, purified HSPG, 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 lymphoma cells, and B-16 melanoma. Substrate specificity studies suggest that there are at least three different types of mammalian cell endo-P-D-glucuronidases. The human platelet enzyme depolymerizes both HS, and heparin, and cleaves the P-glucuronidic linkage in the antithrombin-binding octasaccharide of heparin molecules. Another endoglucuronidase from mouse mastocytoma displays strict specificity toward macromolecular heparin proteoglycans, degrading it into fragments similar in size to commercial heparin. The mastocytoma enzyme has little or no activity against HS and does not cleave the antithrombin-binding regions of heparin. In contrast, the mast cell heparanase described in this study exhibited degradation activity toward HS, with little activity against commercial heparin. Our studies with both RBL cells and Abelson transformed BMMC failed to detect any 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 degradation 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

1. Riley JF: The relationship of the tissue mast cells to the blood vessels in the rat. J Path Bact 65:461, 1953

From www.bloodjournal.org by guest on October 23, 2017. For personal use only.


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

P Bashkin, E Razin, A Eldor and I Vlodavsky