American Journal of Pathology, Vol. 146, No. 2, February 1995
Copyright © American Society for Investigative Pathology

Technical Advance

Detection of Mouse Mast Cell-Associated Protease mRNA

Heparinase Treatment Greatly Improves RT-PCR of Tissues Containing Mast Cell Heparin

Mindy Tsai, Mary Miyamoto, See-Ying Tam, Zhen-sheng Wang, and Stephen J. Galli

From the Departments of Pathology, Beth Israel Hospital and Harvard Medical School, Boston, Massachusetts

The reverse transcriptase polymerase chain reaction (RT-PCR) procedure is markedly inhibited in specimens of blood that contain commercial heparin as an anticoagulant or in cell preparations containing rat or mouse peritoneal mast cells. However, it was not known whether the levels of endogenous, mast cell-associated heparin that are present in some mammalian tissues are sufficient to interfere with the use of RT-PCR in these settings. We show that RT-PCR detects little or no mRNA transcripts for either mast cell-associated products, such as mouse mast cell-associated protease-2 or -4 (MMCP-2 or MMCP-4) or mast cell carboxypeptidase A, or for mast cell-nonspecific products, such as glyceraldehyde 3-phosphate dehydrogenase, in routinely prepared specimens of cells or tissues that include populations of heparin-containing mast cells. However, signals for mast cell-associated or mast cell-nonspecific transcripts can be readily detected in such specimens if they are treated with heparinase before RT-PCR. RT-PCR after heparinase treatment appears to represent an extremely sensitive method for detecting mast cell-associated transcripts in tissue specimens, permitting the identification of transcripts for mast cell-specific proteases in the skin of genetically mast cell-deficient WBB6F$_1$-W/W^v mice, a tissue that contains few or no mast cells according to histological analysis. ***(Am J Pathol 1995, 146:335–343)***

Several groups, including ours, have used Northern analysis to characterize the expression of transcripts for a variety of mast cell-associated genes in isolated cells or tissues that contain mast cells.[1–6] However, our attempts to use the ordinarily much more sensitive technique of reverse transcriptase polymerase chain reaction (RT-PCR) to analyze the same types of specimens initially proved frustrating, as mast cell-associated transcripts that were readily detectable by RT-PCR in certain populations of immature mouse mast cells were undetectable by RT-PCR in cell populations containing mature mast cells or in tissues, such as mouse skin, that contained high densities of mast cells. Notably, the transcripts of interest often were detectable in these same specimens by Northern analysis, a finding that strongly suggested that the problem in using RT-PCR in these settings was not a lack of adequate levels of mRNA.

In our investigation of this issue, we found four reports indicating that commercial heparin, used as an anticoagulant for blood specimens, can result in attenuation or complete inhibition of target DNA amplification during PCR.[7–10] Indeed, as little as 0.05 U of heparin per reaction tube can suppress the PCR reaction.[9] And, while the studies described herein were

---

Supported in part by United States Public Health Service Grants AI-31982, AI-22674, and AI-23990.

Accepted for publication November 7, 1994.

Address reprint requests to Dr. Stephen J. Galli, Division of Experimental Pathology, Department of Pathology/Research North, Beth Israel Hospital, 330 Brookline Avenue, Boston, MA 02215.

underway, Oberhauser et al[11] reported that the RT-PCR procedure was inhibited in cell preparations that contained mouse or rat peritoneal mast cells and that this inhibition was eliminated by treatment of the specimens with heparinase. However, the implications of these findings for the use of RT-PCR in tissue specimens that contain endogenous heparin of mast cell origin have not, to our knowledge, previously been explored.

Mast cells represent the major (if not the only) source of heparin in mammalian tissues,[12,13] but the content of heparin in individual mast cell populations can vary markedly according to the stage of maturation of the mast cells or their anatomical location (reviewed in references 14–16). For example, immature mast cells derived by placing normal mouse bone marrow cells *in vitro* in IL-3-containing medium (bone marrow-derived cultured mast cells, or BMCMC) express little or no heparin.[17,18] However, when such BMCMC are induced to mature, eg, by injecting the cells into the skin or peritoneal cavity of genetically mast cell-deficient WBB6F$_1$-*W/W^v* mice, they exhibit alterations in a number of their phenotypic characteristics, including the ability to express an increased content of heparin.[19,20] In this respect, such mast cells resemble normal mouse connective tissue-type mast cells (CTMC) of the skin or serosal cavities, which also contain substantial amounts of cytoplasmic granule-associated heparin.[14–20]

Although mast cells clearly represent one source of anticoagulantly active glycosaminoglycans (ie, heparin), several lines of evidence indicate that vascular endothelial cells also can synthesize heparin-like glycosaminoglycans (ie, heparan sulfates) with anticoagulant activity.[12,21–23] Moreover, these vascular endothelial cell-associated glycosaminoglycans can occur in quantities that are sufficient to account for the expression of anticoagulant activity *in vivo*.[23] Indeed, the amount of anticoagulant activity detected in the vasculature of genetically mast cell-deficient *W/W^v* mice is essentially the same as that detected in the congenic normal (+/+) mice.[23] In light of these observations, it is possible that some of the glycosaminoglycans with anticoagulant activity that are present in commercial preparations of heparin are derived from non-mast cell sources or that the presence of anticoagulant glycosaminoglycans of non-mast cell origin might interfere with the performance of RT-PCR in certain tissue specimens.

In the present study, we evaluated the possibility that endogenous, mast cell-associated heparin, like commercial heparin, might interfere with the RT-PCR reaction in tissue specimens that contain significant populations of CTMC.

Materials and Methods

Mice

Female genetically mast cell-deficient WBB6F$_1$-*W/W^v* mice and the congenic normal (+/+) mice (WB/ReJ – *W*/+ × C57BL/6J – *W^v*/+)F$_1$ – *W/W^v* or – +/+ mice) were purchased from The Jackson Laboratory (Bar Harbor, ME) at 4 to 6 weeks of age. Female 4- to 6-week-old or retired breeder BALB/c mice were purchased from The Charles River Laboratories (Wilmington, MA).

Generation of Mouse BMCMC

Primary populations of growth factor-dependent BMCMC were obtained as previously described.[17] Briefly, the femoral bone marrow cells of 4- to 6-week-old female BALB/c or WBB6F$_1$-+/+ mice were maintained in suspension in IL-3-containing conditioned medium, consisting of 10% heat-inactivated fetal calf serum (GIBCO BRL, Grand Island, NY), 5×10^{-5} mol/L 2-mercaptoethanol (Sigma Chemical Co., St. Louis, MO), and 2 mmol/L L-glutamine (GIBCO BRL) in Dulbecco's modified Eagle's medium (GIBCO BRL; complete medium) supplemented with 20% (v/v) supernatants of, for BALB/c BMCMC, concanavalin A-activated spleen cells[17] or, for +/+ BMCMC, WEHI-3 cell-conditioned medium.[17,24] The cells were resuspended in fresh conditioned medium once or twice per week. After 4 to 5 weeks of cultures, at least 95% of cells that remained in the cultures were identifiable as mast cells, as determined by staining with neutral red (Fisher Scientific, Orangeburg, NY).

RNA Extraction

RNA was extracted from BMCMC, peritoneal cells, or ear skin by RNAzol^B methods according to the manufacturer's specifications (Biotecx Laboratories, Houston, TX). Peritoneal cells (containing ~4% mast cells by neutral red staining) were obtained from peritoneal lavage of female BALB/c retired breeder mice. Ear skin was obtained from mice immediately after death by CO_2 inhalation. Skin was harvested with a scalpel and snap-frozen in liquid N_2. Samples were stored at −80 C until processed for RNA extraction. RNA was also extracted, as above, from NIH 3T3 cells, which were used as a representative cell population that lacked endogenous heparin. NIH 3T3 cells were obtained from the American Type Culture Collection (Rockville, MD) and were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum.

Treatment with Heparinase or Heparin

Aliquots of RNA were incubated with 1 U of heparinase I (Sigma) per µg of RNA (in 5 mmol/L Tris pH 7.5, 1 mmol/L CaCl$_2$, 40 U of RNAsin) for 2 hours at 25 C as previously described.[8] For heparin treatment, 0.1 U of heparin (Elkins-Sinn, Cherry Hill, NJ) per µg of RNA was added immediately before performing the RT-PCR reaction.

RT-PCR

cDNA was synthesized from total RNA with the GeneAmp RNA PCR kit (Perkin-Elmer, Norwalk, CT) according to the manufacturer's specifications (except that we used 5 µg of total RNA/specimen, as we expected that the tissues would contain relatively small amounts of mast cell mRNA). Total RNA was reverse transcribed in a 20-µl mixture containing 2.5 µmol/L random hexamer primers, 1× PCR buffer, 5 mmol/L MgCl$_2$, 1 mmol/L dNTP, 20 U of RNAse inhibitor, and 50 U of Moloney murine leukemia virus reverse transcriptase at room temperature for 10 minutes and 42 C for 15 minutes followed by a 5-minute incubation at 99 C. The cDNA was then amplified for 1 minute at 94 C, 2 minutes at 55 C, and 3 minutes at 72 C for 30 thermocycles in a 100-µl volume reaction containing 2 mmol/L MgCl$_2$, 1× PCR buffer, and 2.5 U of *Ampli*Taq DNA polymerase (Perkin-Elmer). Four pairs of oligonucleotide primers were used: 1), MMCP-2 (594 bp),[25] 5′ primer, 5′-GTGATGACTGCTGCACACTG-3′ and 3′ primer 5′-CTTGAAGAGTCTGACTCAGG-3′; 2), MMCP-4 (421 bp),[26] 5′ primer, 5′-GTAATTCCTCTGCCTCGTCCT-3′ and 3′ primer 5′-CCCAAGGGTTATTAGAAGAGCTC-3′; 3), MC-CPA(689 bp),[25] 5′ primer, 5′-ACACAGGATCGAATGTGGAG-3′ and 3′ primer, 5′-TAATGCAGGACTTCATGAGC-3′; and 4), G3PDH (452 bp),[27] 5′ primer, 5′-ACCACAGTCCATGCCATCAC-3′ and 3′ primer, 5′-TCCACCACCCTGTTGCTGTA-3′. PCR products were analyzed on 2% agarose gels. The authenticity of the PCR products was verified by subcloning and DNA sequencing. In some experiments, the identity of the PCR products was confirmed by Southern hybridization with gene-specific cDNA probes that had been verified by DNA sequencing.

Mast Cell Reconstitution of Genetically Mast Cell-Deficient WBB6F$_1$-*W/W^v* Mice

WBB6F$_1$-*W/W^v* mice were selectively and locally repaired of their mast cell deficiency by the injection of *in vitro*-derived, growth factor-dependent immature mast cells (BMCMC) of congenic +/+ mouse origin into one ear.[28,29] Mast cells (0.5×10^6) were injected into the left ears in 20 µl of Dulbecco's medium and 20 µl of medium alone was injected into the right ears. The mice were killed by CO$_2$ inhalation at 7, 19, 35, or 70 days after mast cell reconstitution, a central biopsy of each ear was taken for histological analysis (see below), and the remaining tissue from each ear was processed for total RNA for RT-PCR analysis.

Histological Analysis

The samples of mast cell-reconstituted ears and contralateral control (mast cell-deficient) ears were processed for 1-µ Epon-embedded, Giemsa-stained sections.[28,29] The sections were coded and then were examined under a light microscope at ×400 magnification by an observer who was not aware of the identity of the individual sections, and the number of mast cells per square millimeter of dermis was determined as previously described.[29]

Results

Heparinase Treatment Permits the Use of RT-PCR to Detect Mast Cell-Associated or Mast Cell-Nonspecific Transcripts in Specimens Containing Cells or Tissues with a High Content of Heparin

It has been reported that commercial heparin, added as an anticoagulant during blood sample preparation, inhibits the PCR reaction in these specimens.[7–10] However, the possible effects of endogenous heparin of mast cell origin on gene amplification by RT-PCR in tissue specimens that contain mast cells have not been investigated. We therefore assessed the effects of treatment with heparinase or heparin on the ability to perform RT-PCR using total RNA extracted from 1), mouse BMCMC, a mast cell population that contains little or no heparin;[17,18] 2), total peritoneal cells, which contain ~4% serosal mast cells, a type of CTMC with a high content of heparin;[17–20] and 3), skin, a representative tissue with a high density of heparin-containing CTMC.[19,30]

Figure 1 shows the results of RT-PCR reactions in these specimens with gene-specific primers for mast cell carboxypeptidase A (MC-CPA), a mast cell-specific protease that is expressed in CTMC and BMCMC.[31] In BMCMC, MC-CPA transcripts were readily

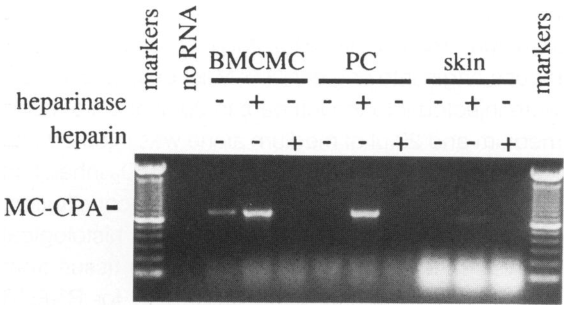

Figure 1. *Inhibition of RT-PCR by heparin. Aliquots of total RNA isolated from BALB/c mouse BMCMC, BALB/c mouse peritoneal cells (PC), or BALB/c mouse ear skin were preincubated with heparinase I (1 U per μg of RNA) or heparin (0.1 U per μg of RNA), and then 5-μg aliquots of the treated (+) or untreated (−) RNA were subjected to the RT-PCR reaction with MC-CPA 5′ and 3′ primer pairs, which generate a 689-bp cDNA. The PCR products were electrophoresed on an agarose gel containing ethidium bromide.*

amplified in either untreated or heparinase-treated RNA, whereas no MC-CPA band was detected in a BMCMC RNA sample that had been preincubated with heparin. By contrast, in accord with the findings of Oberhauser et al[11] with regard to other murine peritoneal mast cell-associated transcripts, MC-CPA transcripts were not detectable in specimens of mouse peritoneal cells unless the RNA samples had been treated with heparinase before RT-PCR. The same was true for specimens of ear skin. Similar findings were obtained when we performed PCR amplification using primers for the housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (G3PDH; Figure 2). Transcripts for G3PDH were detectable in specimens of NIH 3T3 cells or from the skin of genetically mast cell-deficient WBB6F$_1$-*W/W^v* mice whether or not the specimens were pretreated with heparinase, but heparinase pretreatment was required for the RT-PCR detection of G3PDH transcripts in the skin of the congenic normal (WBB6F$_1$-+/+) mice, a tissue that contains a high density of mast cells. These results indicate that mast cell-associated heparin can inhibit the PCR amplification of relatively abundant, mast cell-nonspecific transcripts as well as mast cell-specific transcripts. By contrast, the results obtained with mast cell-deficient *W/W^v* mice indicate that any vascular endothelial cell-derived heparin-like glycosaminoglycans that are present in the skin of these animals[23] do not detectably influence the ability to perform RT-PCR in these specimens.

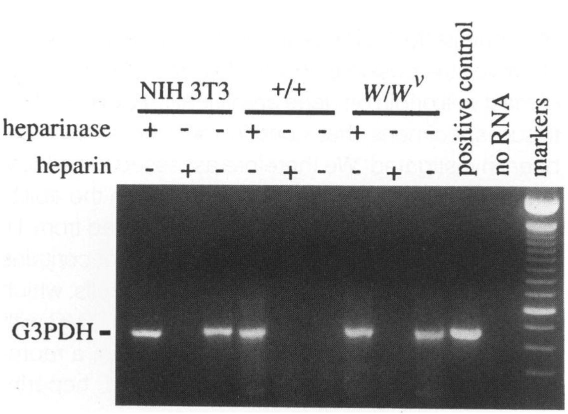

Figure 2. *Inhibition of RT-PCR by heparin. Aliquots of total RNA isolated from NIH 3T3 cells or from ear skin of WBB6F$_1$-+/+ (normal) or genetically mast cell-deficient WBB6F$_1$-W/W^v mice were preincubated with heparinase I (1 U per μg of RNA) or heparin (0.1 U per μg of RNA), and then 5-μg aliquots of the treated (+) or untreated (−) RNA were subjected to the RT-PCR reaction with G3PDH 5′ and 3′ primer pairs, which generate a 452-bp cDNA. The PCR products were electrophoresed on an agarose gel containing ethidium bromide.*

Heparinase Treatment Permits RT-PCR Detection of MMCP-2 and MC-CPA mRNA Expression in the Skin of WBB6F$_1$ Mice

We next assessed the effect of heparinase pretreatment of RNA on our ability to use RT-PCR to detect expression of mRNA for the mast cell-associated proteases, mouse mast cell protease 2 (MMCP-2),[32] or MC-CPA in the skin of WBB6F$_1$-+/+ (normal) or congenic mast cell-deficient (WBB6F$_1$-*W/W^v*) mice. As shown in Figure 3, transcripts of the mast cell-associated proteases MMCP-2 and MC-CPA were detectable by RT-PCR in BALB/c mouse BMCMC with or without heparinase pretreatment, whereas these transcripts were not detected in the skin RNA of +/+ mice unless the specimens had been pretreated with heparinase. In this experiment, we detected little or no signal for MMCP-2 or MC-CPA transcripts in the skin of *W/W^v* mice, a tissue that is profoundly deficient in

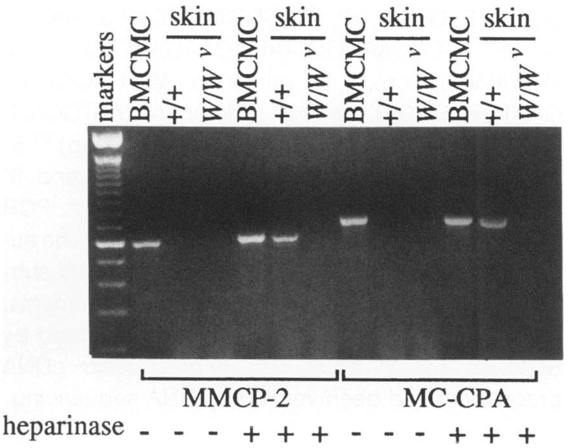

Figure 3. *Detection of MMCP-2 and MC-CPA mRNA in specimens of BALB/c BMCMC or ear skin of WBB6F$_1$-+/+ (normal) or genetically mast cell-deficient WBB6F$_1$-W/W^v mice. Aliquots (5 μg) of heparinase-treated or untreated RNA isolated from BMCMC or ear skin specimens were subjected to RT-PCR reactions with MMCP-2 5′ and 3′ primer pairs, which generated a 594-bp cDNA, or MC-CPA 5′ and 3′ primer pairs, which generated a 689-bp cDNA. The PCR products were electrophoresed on an agarose gel containing ethidium bromide.*

mast cells,[33] even when the RNA had been pretreated with heparinase before RT-PCR (Figure 3).

To determine whether RT-PCR might be used to assess the relative amounts of mRNAs present in different specimens of RNA, we performed RT-PCR using consistent conditions of heparinase pretreatment, reverse transcriptase concentration, cycles of PCR, and temperature, while varying only the amount of total RNA analyzed. As shown in Figure 4, this analysis indicated that similar amounts of G3PDH mRNA were present in RNA specimens from WBB6F$_1$-+/+ or *W/W^v* mouse skin or from IL-3 derived BMCMC. By contrast, we observed distinct effects of RNA dilution on the strength of signals for mast cell-specific MC-CPA in the three different specimens. The MC-CPA signals derived from BMCMC RNA showed little variation over a 500-fold concentration range of starting RNA, the MC-CPA signal in +/+ mouse skin was clearly detectable in as little as 0.01 μg of total RNA, albeit at diminished intensity compared with that observed with larger amounts of RNA, and the MC-CPA signal in the skin of mast cell-deficient *W/W^v* mice was barely detectable in 1 μg of RNA. These results indicate that, at least in some experiments, RT-PCR after heparinase pretreatment is sufficiently sensitive to detect small amounts of MC-CPA mRNA in *W/W^v* mouse skin, presumably reflecting that fact that the skin of adult *W/W^v* mice ordinarily contains ~0.5% of the number of mast cells present in the skin of the congenic normal (+/+) mice.

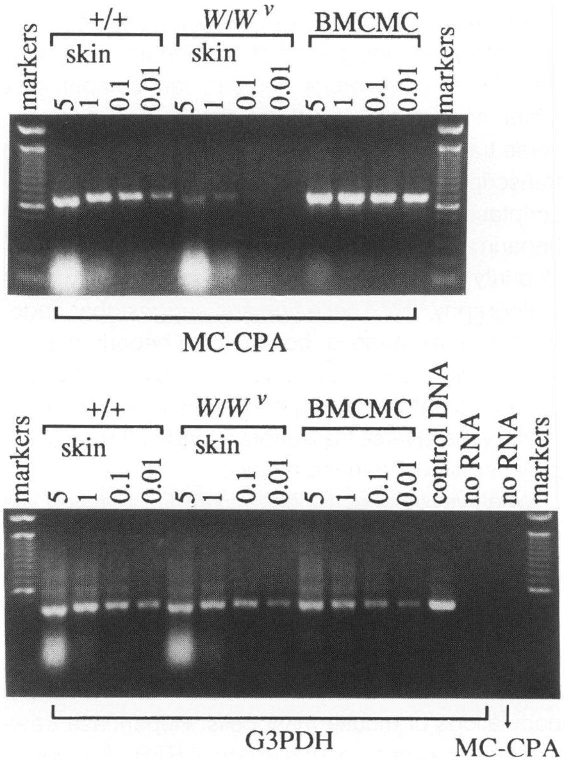

Figure 4. *Effect of RNA dilution on detection of MC-CPA or G3PDH mRNA in heparinase-treated total RNA isolated from BALB/c BMCMC or ear skin of WBB6F$_1$-+/+ (normal) or WBB6F$_1$-W/W^v (mast cell-deficient) mice. RT-PCR was performed with 5.0, 1.0, 0.1, or 0.01 μg of total RNA. The PCR products were electrophoresed on an agarose gel containing ethidium bromide.*

Detection of MMCP-4 mRNA by RT-PCR in the Ears of W/W^v Mice after Their Selective Reconstitution by the Injection of BMCMC of Congenic +/+ Origin

Using RT-PCR with heparinase pretreatment of specimens and Southern hybridization for detection of the PCR products, we examined expression of mRNA for the mast cell-associated protease MMCP-4[26] in the ears of *W/W^v* mice at various intervals after the local injection of BMCMC of congenic +/+ origin. Confirming a recent report,[34] we found that mRNA for MMCP-4 was detectable in BMCMC derived from WBB6F$_1$-+/+ mice (Figure 5). MMCP-4 mRNA signals also could be detected as early as 7 days after reconstitution in the mast cell-injected ears (left ears) of WBB6F$_1$-*W/W^v* mice (Figure 5). At this interval, 10.1 ± 6.2 mast cells/mm^2 were identifiable by light microscopy in the dermis of the ears that had been injected with +/+ BMCMC *versus* 0 in the contralateral control (medium-injected) ears ($P < 0.001$; Table 1). Mast cell numbers gradually increased to 44.6 ± 11.4/mm^2 by 70 days after local mast cell reconstitution. By contrast, the contralateral control (medium-injected) ears remained profoundly mast cell deficient (Table 1). However, using RT-PCR after heparinase treatment, we detected a weak signal for MMCP-4 transcripts in the control (medium-injected)

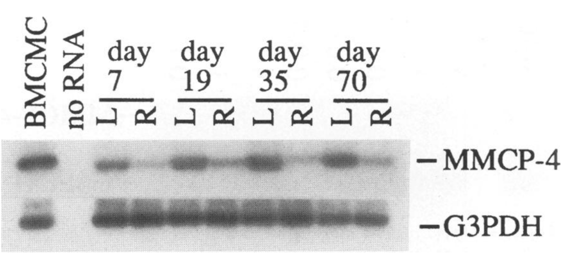

Figure 5. *Detection of MMCP-4 mRNA in the ears of genetically mast cell-deficient WBB6F$_1$-W/W^v mice that had been selectively and locally reconstituted with BMCMC derived from the congenic normal (+/+) mice. Total RNA was isolated from the starting population of +/+ BMCMC or from the +/+ BMCMC-injected (mast cell-reconstituted) left ears (L) or the medium-injected (mast cell-deficient) right ears (R) of WBB6F$_1$-W/W^v mice. The ear specimens were obtained 7, 19, 35, or 70 days after injection of +/+ BMCMC or medium. Heparinase-treated RNA (5 μg) was reverse transcribed for each specimen. RT-PCR products were transferred to a nylon membrane and hybridized with a ^{32}P-labeled MMCP-4 or G3PDH cDNA probe.*

Table 1. *Number of Mast Cells/mm^2 of Dermis in the Mast Cell-Reconstituted (Left) of Medium-Injected Control (Right) Ears of W/W^v Mice at Various Intervals after the Injection of +/+ BMCMC or Medium*

Days after injection	No. of mast cells/mm^2 dermis: Left ears (*mast cell injected*)	No. of mast cells/mm^2 dermis: Right ears (*medium injected*)	*P* value, left *versus* right
7	10.1 ± 6.2	0	<0.001
19	25.9 ± 8.3	0.6 ± 0.4	<0.001
35	40.1 ± 10.6†	0.5 ± 0.5	<0.001
70	44.6 ± 11.4†	1.9 ± 1.2	<0.001

*The data, expressed as the mean ± SEM, are from the same mice (*n = 5 for each interval*) that were analyzed in Figures 5 and 6.
†*P* < 0.05 *versus* day 7 value in same treatment group.

ears of the W/W^v mice, presumably reflecting the contribution of the rare mast cells present in these tissues. Figure 5 and Table 1 show the results of one of two different experiments that gave similar findings.

We then assessed the expression of MMCP-4 mRNA in the mast cell-reconstituted or contralateral control ears semiquantitatively, by performing serial dilutions of the total RNA (5, 1, 0.1, 0.01, and 0.001 µg) and then amplifying this RNA under standard conditions of RT-PCR (Figure 6). We found that the MMCP-4 mRNA signal was stronger in the mast cell-reconstituted ears than in the control ears, as a signal was detected in dilutions down to 0.1 µg of total RNA from the mast cell-reconstituted ears but down to only 1 µg of total RNA from the contralateral control ears. By contrast, dilution of RNA from left (mast cell-reconstituted) *versus* right (control, mast cell-deficient) ears resulted in a very similar dilution of the strength of the RT-PCR signal for G3DPH. These results indicate that, as expected on the basis of histological analysis, levels of MMCP-4 mRNA were higher in the mast cell-reconstituted than in the medium-injected ears of these W/W^v mice.

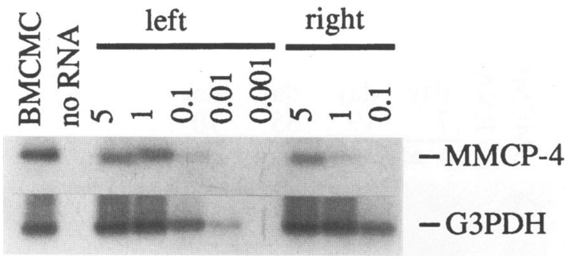

Figure 6. *Effect of RNA dilution on detection of MMCP-4 or G3PDH mRNA in heparinase-treated total RNA isolated from ear skin of genetically mast cell-deficient WBB6F$_1$-W/W^v mice that had been selectively and locally reconstituted with BMCMC derived from the congenic normal (+/+) mice. RT-PCR was performed with 5.0, 1.0, 0.1, 0.01, or 0.001 µg of total RNA isolated from the +/+ BMCMC-injected (mast cell-reconstituted) left ears or 5, 1, or 0.1 µg of total RNA isolated from medium-injected (mast cell-deficient) right ears of WBB6F$_1$-W/W^v mice. The ear specimens were obtained 70 days after injection of +/+ BMCMC or medium. Heparinase-treated RNA (5 µg) was reverse transcribed for each specimen. RT-PCR products were transferred to a nylon membrane and hybridized with a ^{32}P-labeled MMCP-4 or G3PDH cDNA probe.*

Discussion

Our findings confirm those of Oberhauser et al[11] in showing that endogenous, mouse peritoneal mast cell-associated heparin, like commercial heparin,[7–10] can significantly interfere with the RT-PCR method. However, we also found that native populations of mast cells in normal vascularized tissues can contain sufficient heparin to interfere with the performance of RT-PCR in these specimens. Although we did not investigate how endogenous heparin interfered with the RT-PCR procedure, the mechanism probably reflects an interaction between critical components of the reaction mixture with the highly anionic sulfated glycosaminoglycan side chains of the heparin proteoglycan. Thus, commercial heparin can competitively inhibit several cellular DNA polymerases,[35] HIV reverse transcriptase,[36] simian sarcoma virus reverse transcriptase,[37] murine leukemia virus reverse transcriptase,[8] and *Taq* DNA polymerase.[8] Commercial heparin also can be used, when bound to Sepharose, to purify DNA or RNA polymerases by affinity chromatography.[38,39] These findings suggest that endogenous or exogenous (commercial) heparin may interfere with the RT-PCR reaction by binding to (and interfering with the activity of) the moloney murine leukemia virus reverse transcriptase and/or the *Taq* DNA polymerase used in the method.

Whatever its effect(s) on the RT-PCR reaction, we confirmed[11] that the inhibitory action of mouse peritoneal mast cell-associated heparin can be significantly diminished or abolished by treatment of the specimens with heparinase before performing the RT-PCR procedure and showed that the same was true for specimens of normal skin that contained native populations of mouse mast cells. Heparinase treatment greatly improved the ability of RT-PCR to detect presumably mast cell-specific transcripts (such as those for the proteases MMCP-2, MMCP-4, or MC-CPA) as well as mast cell-nonspecific transcripts (such as that for G3PDH) in specimens that contain serosal or skin CTMCs. The latter point may be of some practical importance, as it indicates that the inhibitory effect of endogenous mast cell-associated heparin on the RT-PCR reaction can significantly impair the ability of this technique to detect even relatively abundant transcripts that are associated with cell types other than the mast cell. Accordingly, we recommend that investigators planning to use RT-PCR to analyze any cells or tissues that have a resident population of heparin-containing mast cells (this includes most human tissues and many tissues of murine rodents[12–16]) consider pretreating the specimens with heparinase before performing RT-PCR.

Our findings also have implications for the analysis of mast cell development or other processes that influence mast cell phenotype. In the mouse, serosal or skin CTMCs acquire the ability to store increasing amounts of heparin during the maturation process (reviewed in references 14–16), and many populations of rat or human mast cells also contain heparin.[13,15] The ability to use RT-PCR to detect mast cell-associated mRNAs will permit a more direct evaluation of whether a lack of expression of a particular transcript in a Northern blot of mast cell-derived RNA reflects the absence of the mRNA or merely the presence of low levels of the transcript.

This point is illustrated by our RT-PCR analysis of genetically mast cell-deficient *W/W^v^* mice. The skin of adult WBB6F$_1$-*W/W^v* mice ordinarily contains <0.5% of the density of dermal mast cells present in the skin of congenic normal (WBB6F$_1$-+/+) mice,[33] and the skin of *W/W^v* mice contains no detectable heparin.[30] By Northern analysis, mRNA for the mast cell-associated proteases MMCP-4 or MC-CPA was not detectable in the skin of WBB6F$_1$-*W/W^v* mice.[6] However, by RT-PCR, signals for both of these proteases were detected in the skin of *W/W^v* mice, albeit at levels substantially less than those present either in the skin of congenic +/+ mice or in *W/W^v* mouse skin sites that had been reconstituted by the injection of BMCMC of congenic +/+ mouse origin. This result indicates that RT-PCR of heparinase-pretreated specimens probably represents a more sensitive method than histological analysis for detecting extremely small populations of mast cells in the tissues of mutant mast cell-deficient mice. This finding also indicates that our RT-PCR approach can be used to characterize the pattern of expression of mast cell-specific mRNAs in tissues, like those of genetically mast cell-deficient mice, that contain very small numbers of mast cells.

Acknowledgments

We thank Susan Fish for excellent technical assistance.

References

1. Reynolds DS, Serafin WE, Faller DV, Wall DA, Abbas AA, Dvorak AM, Austen KF, Stevens RL: Immortalization of murine connective tissue-type mast cells at multiple stages of their differentiation by coculture of splenocytes with fibroblasts that produce Kirsten sarcoma virus. J Biol Chem 1988, 263:12783–12791
2. Plaut M, Pierce JH, Watson CJ, Hanley-Hyde J, Nordan RP, Paul WE: Mast cell lines produce lymphokines in response to cross-linkage of Fc$_\epsilon$RI or to calcium ionophores. Nature 1989, 339:64–67
3. Burd PR, Rogers HW, Gordon JR, Martin CA, Jayaraman S, Wilson SD, Dvorak AM, Galli SJ, Dorf ME: Interleukin 3-dependent and -independent mast cells stimulated with IgE and antigen express multiple cytokines. J Exp Med 1989, 170:245–257
4. Gordon JR, Galli SJ: Mast cells as a source of both preformed and immunologically inducible TNF-α/cachectin. Nature 1990, 346:274–276
5. Gordon JR, Galli SJ: Release of both preformed and newly synthesized tumor necrosis factor-α (TNF-α)/cachectin by mouse mast cells stimulated via the Fc$_\epsilon$RI: a mechanism for the sustained action of mast cell-derived TNF-α during IgE-dependent biological responses. J Exp Med 1991, 174:103–107
6. Stevens RL, Friend DS, McNeil HP, Schiller V, Ghildyal N, Austen KF: Strain-specific and tissue-specific expression of mouse mast cell secretory granule proteases. Proc Natl Acad Sci USA 1994, 91:128–132
7. Beutler E, Gelbart T, Kuhl W: Interference of heparin with the polymerase chain reaction. BioTechniques 1990, 9:166
8. Izraeli S, Pfleiderer C, Lion T: Detection of gene expression by PCR amplification of RNA derived from frozen heparinized whole blood. Nucleic Acids Res 1991, 19:6051
9. Holodniy M, Kim S, Katzenstein D, Konrad M, Groves E, Merigan TC: Inhibition of human immunodeficiency virus gene amplification by heparin. J Clin Microbiol 1991, 29:676–679
10. Wang J-T, Wang T-H, Sheu J-C, Lin S-M, Lin J-T, Chen D-S: Effects of anticoagulants and storage of blood samples on efficacy of the polymerase chain reaction assay for hepatitis C virus. J Clin Microbiol 1992, 30: 750–753
11. Oberhauser AF, Balan V, Fernandez-Badilla CL, Fernandez JM: RT-PCR cloning of Rab3 isoforms expressed in peritoneal mast cells. FEBS Lett 1994, 339: 171–174
12. Kjellén L, Lindahl U: Proteoglycans: structures and interactions. Annu Rev Biochem 1991, 60:443–475

13. Schwartz L, Huff T: Biology of mast cells and basophils. Allergy: Principles and Practice. Edited by E Middleton Jr, CE Reed, EF Ellis, NF Adkinson Jr, JW Yunginger, WW Busse. St. Louis, Mosby, 1993, pp 135–168
14. Kitamura Y: Heterogeneity of mast cells and phenotypic changes between subpopulations. Annu Rev Immunol 1989, 7:59–76
15. Galli SJ: New insights into "the riddle of the mast cells" microenvironmental regulation of mast cell development and phenotypic heterogeneity. Lab Invest 1990, 62:5–33
16. Stevens RL, Austen KF: Recent advances in the cellular and molecular biology of mast cells. Immunol Today 1989, 10:381–386
17. Galli SJ, Dvorak AM, Marcum JA, Ishizaka T, Nabel G, der Simonian H, Pyne K, Goldin JM, Rosenberg RD, Cantor H, Dvorak HF: Mast cell clones: a model for the analysis of cellular maturation. J Cell Biol 1982, 95:435–444
18. Razin E, Stevens RL, Akiyama F, Schmidt K, Austen KF: Culture from mouse bone marrow of a subclass of mast cells possessing a distinct chondroitin sulfate proteoglycan with glycosaminoglycans rich in *N*-acetylgalactosamine-4,6-disulfate. J Biol Chem 1982, 257:7229–7236
19. Nakano T, Sonoda T, Hayashi C, Yamatodani A, Kanayama Y, Yamamura T, Asai H, Yonezawa T, Kitamura Y, Galli SJ: Fate of bone marrow-derived cultured mast cells after intracutaneous, intraperitoneal, and intravenous transfer into genetically mast cell-deficient W/W^v mice: evidence that cultured mast cells can give rise to both connective tissue type and mucosal mast cells. J Exp Med 1985, 162:1025–1043
20. Otsu K, Nakano T, Kanakura Y, Asai H, Katz HR, Austen KF, Stevens RL, Galli SJ, Kitamura Y: Phenotypic changes of bone marrow-derived mast cells after intraperitoneal transfer into W/W^v mice that are genetically deficient in mast cells. J Exp Med 1987, 165: 615–627
21. Marcum JA, Fritze L, Galli SJ, Karp G, Rosenberg RD: Microvascular heparin-like species with anticoagulant activity. Am J Physiol 1983, 245:H725–H733
22. Marcum JA, Rosenberg RD: Heparin-like molecules with anticoagulant activity are synthesized by cultured endothelial cells. Biochem Biophys Res Commun 1985, 126:365–372
23. Marcum JA, McKenney JB, Galli SJ, Jackman RW, Rosenberg RD: Anticoagulantly active heparin-like molecules from mast cell-deficient mice. Am J Physiol 1986, 19:H879–H888
24. Nagao K, Yokoro K, Aaronson SA: Continuous lines of basophil/mast cells derived from normal mouse bone marrow. Science 1981, 212:333–335
25. Ebi Y, Kanakura Y, Jippo-Kanemoto T, Tsujimura T, Furitsu T, Ikeda H, Adachi S, Kasugai T, Nomura S, Kanayama Y, Yamatodani A, Nishikawa S-I, Matsuzawa Y, Kitamura Y: Low *c-kit* expression of cultured mast cells of *mi/mi* genotype may be involved in their defective responses to fibroblasts that express the ligand for *c-kit*. Blood 1992, 80:1454–1462
26. Serafin WE, Sullivan TP, Conder GA, Ebrahimi A, Marcham P, Johnson SS, Austen KF, Reynolds DS: Cloning of the cDNA and gene for mouse mast cell protease 4: demonstration of its late transcription in mast cell subclasses and analysis of its homology to subclass-specific neutral proteases of the mouse and rat. J Biol Chem 1991, 266:1934–1941
27. Martinelli R, Salvatore F: The complete sequence of a full length cDNA for human liver glyceraldehyde-3-phosphate dehydrogenase: evidence for multiple mRNA species. Nucleic Acids Res 1984, 12:9179–9189
28. Wershil BK, Mekori YA, Murakami T, Galli SJ: ^{125}I-Fibrin deposition in IgE-dependent immediate hypersensitivity reactions in mouse skin: demonstration of the role of mast cells using genetically mast cell-deficient mice locally reconstituted with cultured mast cells. J Immunol 1987, 139:2605–2614
29. Yano H, Wershil BK, Arizono N, Galli SJ: Substance P-induced augmentation of cutaneous vascular permeability and granulocyte infiltration in mice is mast cell dependent. J Clin Invest 1989, 84:1276–1286
30. Nakamura N, Kojima J, Okamoto S, Kitamura Y: Absence of heparin in glycosaminoglycan fractions isolated from the skin of genetically mast cell-depleted W/W^v mice. Biochem Int 1981, 3:449–456
31. Reynolds DS, Stevens LS, Gurley DS, Lane WS, Austen KF, Serafin WE: Isolation and molecular cloning of mast cell carboxypeptidase A: a novel member of the carboxypeptidase gene family. J Biol Chem 1989, 264:20094–20099
32. Serafin WE, Reynolds DS, Rogelj S, Lane WS, Conder GA, Johnson SS, Austen KF, Stevens RL: Identification and molecular cloning of a novel mouse mucosal mast cell serine protease. J Biol Chem 1990, 265:423–429
33. Kitamura Y, Go S, Hatanaka K: Decrease of mast cells in W/W^v mice and their increase by bone marrow transplantation. Blood 1978, 52:447–452
34. Eklund KK, Ghildyal N, Austen KF, Friend DS, Schiller V, Stevens RL: Mouse bone marrow-derived mast cells (mBMMC) obtained *in vitro* from mice that are mast cell-deficient *in vivo* express the same panel of granule proteases as mBMMC and serosal mast cells from their normal littermates. J Exp Med 1994, 180:67–73
35. Furukawa K, Bhavanandan VP: Influences of anionic polysaccharides on DNA synthesis in isolated nuclei and by DNA polymerases α: correlation of observed effects with properties of the polysaccharides. Biochim Biophys Acta 1983, 740:466–475
36. Ito M, Baba M, Sato A, Pauwels R, De Clercq E, Shigata S: Inhibitory effect of dextran sulfate and heparin on the replication of human immunodeficiency virus

(HIV) *in vitro*. Antiviral Res 1987, 7:361–367

37. De Cioccio D, Srivastava BIS: Inhibition of deoxynucleotide-polymerizing enzyme activities of human cells and of simian sarcoma virus by heparin. Cancer Res 1978, 38:2401–2407
38. Brennessel BA, Buhrer DP, Gottlieb AA: Use of insoluble heparin for isolation of DNA polymerase enzymes from murine myeloma. Anal Biochem 1978, 87: 411–417
39. Nasheuer H-P, Grosse F: Immunoaffinity-purified DNA polymerase α displays novel properties. Biochemistry 1987, 26:8458–8466