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A single gene in mast cells encodes the core peptides of heparin and chondroitin sulfate proteoglycans

(gene family/serine-glycine repeat)

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ABSTRACT **The diversity of the genes encoding mammalian proteoglycan peptide cores was explored using a cDNA clone that encodes the partial sequence of a cell surface/pericellular matrix-localized chondroitin sulfate proteoglycan. Thus we were able to detect the expression of the gene(s) encoding the intracellular chondroitin sulfate proteoglycan produced by a variety of rat and mouse mucosal-like mast cells and the intracellular heparin proteoglycan synthesized by rat serosal mast cells. The cDNA from the proteoglycan cDNA clone pPG-1 was fractionated into two discrete fragments, one of which contained the nucleotides encoding the serine-glycine repeat sequence (pPG-B) and the other of which contained sequences on the 3′ side of the repeat (pPG-M). As assessed by Southern blot analysis, pPG-B identified a large gene family, whereas pPG-M identified a single DNA fragment in the rat genome. When the pPG-1 insert and the two subcloned probes pPG-B and pPG-M were used to analyze RNA extracted from the rat and mouse mucosal-like mast cells and the rat serosal mast cells, the same major RNA species was detected at 1.3 kilobases with both probes. These data suggest that the gene responsible for the peptide core of the extracellular chondroitin sulfate proteoglycan synthesized by the rat yolk sac cell line is also the gene that encodes the core peptides of the secretory granule-localized chondroitin sulfate and heparin proteoglycans.**

Proteoglycans are highly acidic macromolecules that possess at least one sulfated glycosaminoglycan chain covalently bound to a peptide core. Because of their differential reactivity to panels of monoclonal antibodies, their differential peptide mapping after proteolytic treatments, their different sizes after translation, and in some cases their different amino acid sequences, the existence of several families of proteoglycans has been proposed. Proteoglycans can be differentiated further by their location in the cell or in the extracellular and/or pericellular matrix and by the nature of the carbohydrate residues attached to their peptide cores. Proteoglycans localized to extracellular matrices appear to belong to a family that is distinct from the subfamily of more hydrophobic proteoglycans intercalated into the plasma membrane. A third family of proteoglycans includes those protease-resistant proteoglycans found in the secretory granules of cells. The variability of the glycosaminoglycans attached to the peptide cores of the proteoglycans of these three families has led to the proposal that, for each discrete proteoglycan, a different core peptide is synthesized and that the post-translational modifications of the peptide cores are dependent upon the primary amino acid sequence of the core protein.

All populations of *in vivo*- and *in vitro*-differentiated mast cells examined have been found to contain large amounts of a mast cell-specific family of proteoglycans stored in their secretory granules (1). Although the functions of these mast cell proteoglycans are obscure, the fact that they remain bound to the basically charged serine proteases when exocytosed from immunologically activated cells suggests that proteoglycans may participate in the packaging and regulation of the enzymatic activities of these proteases (2–4).

Two types of tissue mast cells can be distinguished in the rat and the mouse by the nature of their predominant secretory granule proteoglycan. Rat (5, 6) and mouse (7) serosal mast cells (SMC), which are prototypic of connective tissue mast cells, contain a M_r 750,000–1,000,000 proteoglycan that consists of heparin glycosaminoglycans attached to a peptide core. This peptide core in rat SMC is composed primarily of glycine and serine residues (6, 8), presumably in tandem serine-glycine repeats.

The second type of tissue mast cell is the T-cell factor-dependent mast cell that is present in increased numbers in the gastrointestinal mucosa of helminth-infected rats and mice (9). As characterized in rats, the mucosal mast cell contains a smaller (M_r 150,000) chondroitin sulfate di-B proteoglycan that is distinct from all other cell surface and matrix-localized chondroitin sulfate proteoglycans both in its high degree of sulfation and its resistance to proteolysis (10). Mouse T-cell factor-dependent bone marrow-derived mast cells (BMMC) have been obtained *in vitro* by culture of progenitor cells in the presence of conditioned medium that contains interleukin 3 (IL-3) (11, 12). The M_r 200,000 chondroitin sulfate E proteoglycan found in the *in vitro* differentiated mouse BMMC also has a glycine- and serine-rich core peptide (13). These *in vitro*-differentiated mouse mast cells are believed to be analogous to the T-cell factor-dependent mast cells that are present in the gastrointestinal mucosa. These cells are also similar to the IL-3-independent mast cell lines derived from mouse bone marrow or fetal liver tissue that have been immortalized by Abelson mouse leukemia virus infection (14). Another transformed cell line that is analogous to the mucosal mast cell is the rat basophilic leukemia (RBL) line (15). RBL cells contain rat mast cell protease II in their secretory granules, as is characteristic of mucosal mast cells *in vivo*, and lack the rat mast cell protease I that is found only in the SMC (16). Although the proteoglycans synthesized by RBL cells are similar to those found in rat mucosal mast cells and mouse BMMC in that they contain peptide cores enriched for serine and glycine, they are different in that at least some are hybrids bearing both chondroitin sulfate di-B and heparin glycosaminoglycan side chains (17).

The chondroitin sulfate proteoglycan isolated from the cell surface/pericellular matrix of a rat yolk sac tumor cell has

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Abbreviations: SMC, serosal mast cell; BMMC, bone marrow-derived mast cell; IL-3, interleukin 3; RBL, rat basophilic leukemia cell; ABFTL, Abelson murine leukemia virus-transformed mast cells.

been purified, and its N-terminal amino acid sequence has been obtained (18). Using oligonucleotide probes specific for the N-terminal sequence, a cDNA specific for the gene that encodes the peptide core of this proteoglycan was isolated (19). This cDNA, designated pPG-1, contained 874 base pairs (bp), 147 bp of which encoded a serine-glycine repeat sequence of 49 amino acids. The pPG-1 cDNA detected a 1.3-kilobase (kb) mRNA species in the rat yolk cell line but failed to detect any mRNA species in RNA obtained from the rat hepatoma 7777 cell line that produces a plasma membrane-localized heparan sulfate proteoglycan. In this study we have used the pPG-1 cDNA sequence, both in total and in gene-specific fragments, to probe RNA samples from a variety of mouse and rat mast cell types. From this analysis and the genomic DNA screening, we determined that the gene encoding the rat yolk sac chondroitin sulfate proteoglycan core peptide also encodes both the intracellular chondroitin sulfate proteoglycan core peptide of mucosal mast cells and the heparin proteoglycan core peptide of serosal mast cells. Thus, these protease-resistant, secretory granule proteoglycans comprise a subfamily of similar, if not identical, core peptides that are part of the larger proteoglycan gene family.

MATERIALS AND METHODS

Cell Culture. The RBL cell line (15), the Abelson murine leukemia virus-transformed mouse mast cell line (ABFTL) (14), and the mouse Ltk$^-$ fibroblast cell line (20) were maintained in Dulbecco's minimum essential medium supplemented with 10% (vol/vol) fetal calf serum (GIBCO). The myelomonocytic WEHI-3 cell line (21) was maintained in RPMI 1640 media supplemented with 10% (vol/vol) fetal calf serum. These cell lines were seeded into 250-ml culture flasks and cultured at 37°C until confluent density was reached. Rat SMC were isolated from Sprague–Dawley rats (The Jackson Laboratory) by peritoneal lavage of each animal with 50 ml of Tyrode's buffer containing 0.1% gelatin (Sigma). The SMC were concentrated by isopyknic and isokinetic sedimentations (2, 22) to >97% purity as assessed by metachromasia after toluidine staining. Approximately 10^6 SMC were obtained per rat.

Plasmid Constructions. The DNA sequence obtained from the pPG-1 cDNA, which was inserted into pBR322 at the *Pst* I site, indicated that the serine-glycine repeat sequences were separated from the 3′ sequences of the cDNA by a *Ssp* I restriction enzyme site. Thus the pPG-1 plasmid was digested to completion with *Pst* I and *Ssp* I (New England Biolabs), and the three resulting DNA fragments were resolved by agarose electrophoresis. The appropriate DNA fragments were purified from the agarose gel and ligated into *Pst* I- and *Ssp* I-digested pBR322, creating the pPG-B (containing the 5′ and the serine-glycine repeat sequences of pPG-1) and pPG-M (containing the 3′ sequences of pPG-1) plasmids (for nucleic acids method, see ref. 23). Positive inserts were identified by hybridization of the bacterial colonies with the specific inserts.

RNA and DNA Isolation and Blot Hybridization. Total RNA was isolated from the cell populations using the method of Chirgwin (24) and was analyzed by formaldehyde/agarose electrophoresis followed by RNA gel blot analysis (25). Genomic DNA was isolated from rat liver tissue and analyzed by Southern blot hybridization (26) using the purified DNA fragments from the pPG-1, pPG-B, and pPG-M plasmids as probes.

RESULTS

Identification of a Multigene Family with the Rat Yolk Sac Cell-Derived Chondroitin Sulfate Proteoglycan cDNA. The proteoglycan genes expressed in mouse and rat mast cells and other cell types were analyzed to determine whether the various proteoglycan core peptides are encoded by different members of a multigene family or if a single gene is responsible for all the production of the core peptides of intracellular, protease-resistant chondroitin sulfate, and heparin mast cell proteoglycans. When the pPG-1 sequence was used to analyze a Southern blot of rat genomic DNA at high stringency (Fig. 1), 15–30 specific DNA fragments were recognized. Thus, any mRNA species detected with the pPG-1 probe could have been synthesized from any gene of this multigene family and not necessarily from the proteoglycan core protein gene identified by the pPG-1 cDNA sequence. It was necessary, therefore, to produce a probe specific for the pPG-1 chondroitin sulfate proteoglycan core peptide gene. The DNA sequence analysis of the pPG-1 cDNA (19) and the amino acid composition data for the mast cell proteoglycans suggested that the most likely portion of the core peptide gene to be conserved within a multigene family would be the stretch of sequences encoding the serine-glycine repeat of 49 amino acids. Thus, a probe specific for the proteoglycan gene would be best derived from either the 5′- or the 3′-coding sequences and not the serine-glycine repeat codons.

We determined from the DNA sequence information that the restriction enzyme *Ssp* I would cleave the cDNA sequences just on the 3′ side of the serine-glycine repeat, providing a 3′-specific probe that contained 81 bases of coding sequence and 404 bases of 3′-untranslated sequences. The other fragment contains unique sequences from the 5′ portion of the gene as well as the nucleotides encoding the serine-glycine repeat. The two *Pst* I/*Ssp* I fragments derived from the pPG-1 plasmids were subcloned into pBR322 and designated pPG-B (containing the serine-glycine repeat) and pPG-M (containing the 3′-coding and -untranslated sequences (Fig. 2).

Demonstration of a Specific Probe for the pPG-1 Chondroitin Sulfate Proteoglycan Gene. The specificity of these probes was tested by Southern blot analysis; rat genomic DNA was hybridized to the DNA inserts from the pPG-B and the pPG-M plasmids. As shown in Fig. 3*A*, the pPG-B insert hybridized to a complex pattern of genomic fragments, ranging from a minimum of 15 discrete bands to

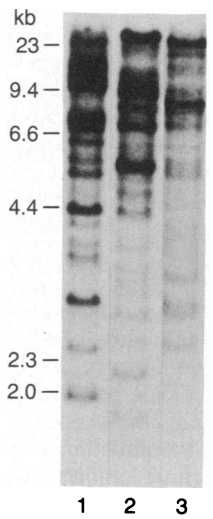

FIG. 1. Southern blot hybridization of rat DNA with the pPG-1 cDNA probe. Rat liver genomic liver DNA (10 μg) was digested with the following restriction enzymes: lane 1, *Bam*HI; lane 2, *Eco*RI; lane 3, *Hin*dIII. The digested DNA was examined by Southern blot using the isolated pPG-1 insert. Molecular size markers, *Hin*dIII-digested λ DNA, are as shown.

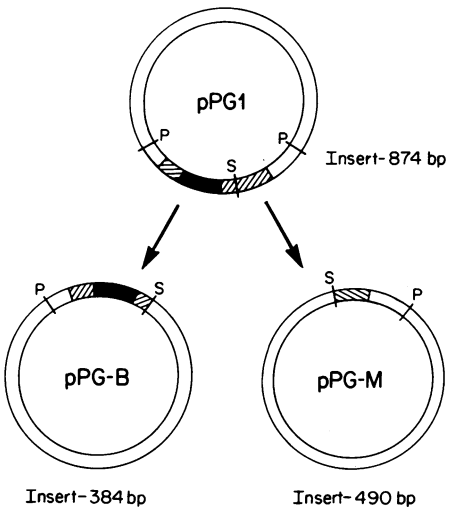

FIG. 2. Construction of pPG-B and pPG-M; subclones of pPG-1 that contain the serine-glycine repeat and the gene-specific sequences. The subclones of pPG-1 containing the serine-glycine repeat and the 3'-specific coding and -untranslated sequences were generated when the pPG-1 plasmid was digested with *Pst* I (P) and *Ssp* I (S), and the resulting DNA fragments were subcloned into *Pst* I- and *Ssp* I-digested pBR322. The serine-glycine repeat is shown as the solid black insert (in pPG-B), the 5'- and 3'-coding sequences as the diagonal lined inserts, and the 3'- and 5'-untranslated sequences as the open inserts within the P and S sites.

more than 30 (depending upon the restriction enzyme used). The number and size of the genomic fragments identified with the pPG-B probe were virtually identical to those seen with the whole pPG-1 probe (Fig. 1). The pPG-M fragment (Fig. 3*B*), in contrast, hybridized to a single genomic fragment. This indicated that the pPG-M insert could be used as a gene-specific probe to examine the expression of the rat yolk sac proteoglycan peptide core in the various types of rat and mouse mast cells.

Analysis of pPG-1 Transcripts in Mouse and Rat Mast Cells. Whether the same proteoglycan gene is responsible for the core peptide incorporated in the variety of proteoglycans that are expressed in mast cells and in the yolk sac tumor cell was determined by hybridizing RNA gel blots of total RNA from different rat and mouse mast cells to the three cDNA probes. The mast cells examined included the RBL and AFBTL cell lines, which produce predominantly intracellular chondroitin sulfate proteoglycans, and freshly isolated rat SMC, which produce heparin proteoglycans. Total RNA from the myelomonocytic WEHI-3 cell line and the mouse Ltk$^-$ fibroblast cell line was also analyzed. RNA gel blots containing RNA from each of these cells showed the same pattern with the pPG-1, the pPG-B, or the pPG-M probe (Fig. 4). A predominant 1.3-kb mRNA species (with the larger RNA species being most likely partially spliced forms of the pPG-1 mRNA) was identified in each of the cell types, except for mouse L cells, in the same molar ratios relative to the actin controls. The same mRNA species was also identified in IL-3-dependent, nontransformed mouse BMMC (data not shown), indicating that the Abelson transformation event did not alter the specific expression of this proteoglycan gene. Thus, the same proteoglycan core peptide gene is transcriptionally active in all of these cell types, including the WEHI-3 cell line, but only minimally in the mouse fibroblast L cell line. The structure, function, and cellular location of the proteoglycan presumably produced by the WEHI-3 cells is not known, and from this data it cannot be determined if it is an extracellular or an intracellular proteoglycan possessing chondroitin sulfate or heparin glycosaminoglycan side chains.

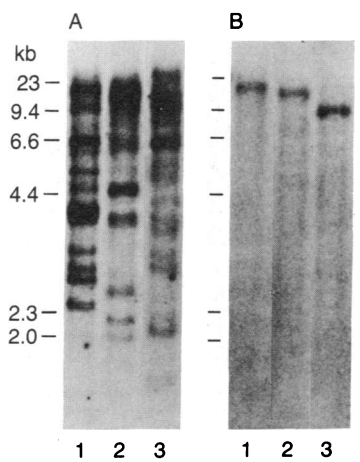

FIG. 3. Southern blot hybridization of rat DNA with the pPG-B (*A*) and pPG-M (*B*) probes. Rat liver genomic liver DNA (10 μg) was digested with the following restriction enzyme: lanes 1, *Bam*HI; lanes 2, *Eco*RI; lanes 3, *Hin*dIII. The digested DNA was examined by Southern blot using as the probes the isolated pPG-1 specific fragments described in Fig. 1. The *Pst* I–*Ssp* I insert from pPG-B was used in *A*, and the *Pst* I–*Ssp* I insert from pPG-M was used in *B*. Molecular size markers, *Hin*dIII-digested λ DNA, are as shown.

DISCUSSION

The proteoglycans produced *in vivo* fall into three major families depending upon their eventual destination. One of these families, the intracellular proteoglycans of mast cells, is made up of at least two subfamilies depending upon the type of glycosaminoglycan attached to the core peptide. Because of subtle differences in the amino acid composition of the core peptides (10), it is assumed that different peptide cores existed for the different intracellular mast cell proteoglycans as well as for the extracellular and pericellular proteoglycans. The fact that rat SMC synthesize only heparin proteoglycans when radiolabeled with [^{35}S]sulfate *ex vivo* but can be induced to synthesize chondroitin sulfate E glycosaminoglycans onto the exogenous glycosaminoglycan accep-

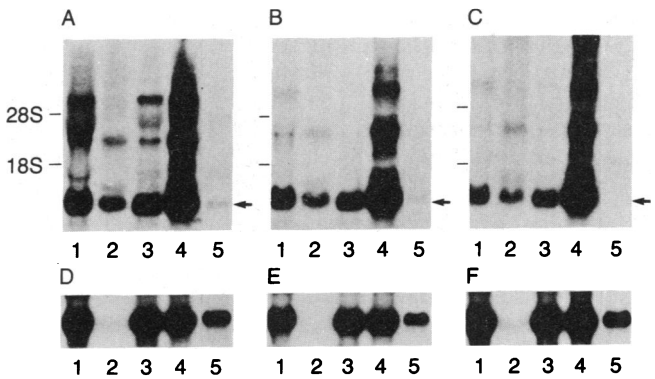

FIG. 4. RNA gel blot analysis of mouse and rat mast cells with the pPG-1, pPG-B, and pPG-M probes. Total RNA was isolated from mouse WEHI-3 cells (lanes 1), freshly isolated rat SMC (lanes 2), the IL-3-independent mouse mast cell line ABFTL (lanes 3), rat RBL cells (lanes 4), and mouse L cell fibroblasts (lanes 5). After electrophoresis and RNA gel blotting, the filters were probed with the pPG1 insert (*A*), the pPG-B insert (*B*), or the pPG-M insert (*C*). The same filters (*A*, *B*, and *C*) were washed and probed with an actin cDNA (*D*, *E*, and *F*, respectively). The arrow indicates the 1.3-kb proteoglycan mRNA species. The position of the migration of the 18S and 28S rRNA is shown.

tor, *p*-nitrophenol-β-D-xyloside (27), lent support to this hypothesis.

In this study we have used a cDNA clone that is specific for the gene encoding the peptide core of the chondroitin sulfate cell surface proteoglycan of rat yolk sac tumor cells to determine if this gene also encodes the intracellular chondroitin sulfate and/or heparin proteoglycan peptide cores in mast cells. The analysis of the gene(s) encoding the intracellular proteoglycans using the pPG-1 cDNA was complicated by the initial finding that the pPG-1 cDNA is related by nucleotide sequence to a large gene family in the rat (Fig. 1). Presumably some of the sequences identified with the pPG-1 cDNA include those encoding the *per* genes that have been shown in *Drosophila* and in mice to contain tracts of serine-glycine repeat sequences (28, 29).

To obtain a nucleic acid probe specific for the pPG-1 gene, two subclones were prepared from the original cDNA; one contained the serine-glycine repeat sequences (pPG-B) and the other contained the presumably specific 3′ coding and untranslated sequences (pPG-M) (Fig. 2). The specificity of these subclones was determined by Southern blot analysis of rat genomic DNA (Fig. 3), which confirmed that the pPG-B sequence was responsible for detecting the multigene family and that the pPG-M probe was specific for a single sequence in the rat genome.

Using the gene-specific probe, pPG-M, and the serine-glycine repeat-containing probe, pPG-B, we analyzed RNA isolated from a variety of mouse and rat mast cells by RNA gel blot hybridization (Fig. 4). We determined that the mouse IL-3-independent BMMC cell line ABFTL, which produces an intracellular chondroitin sulfate E proteoglycan, and the rat SMC, which produces an intracellular heparin proteoglycan, use the same gene to encode a core peptide that the rat yolk sac cell uses in the synthesis of the extracellular chondroitin sulfate proteoglycan. Although SMC might contain a second mRNA encoding a proteoglycan core peptide for heparin side chains, this second message would not contain a serine-glycine repeat that would hybridize to the repeat sequence in the pPG-B probe. The data in this study tend to support a model for which a single gene is used to produce the core peptide of the intracellular mast cell proteoglycans and the extracellular rat yolk sac proteoglycan. These proteoglycans comprise a specific subset of proteoglycans whose definition is independent of both the eventual cellular localization of the mature protein and the type of glycosaminoglycan attached to the core peptide.

The analysis of the mRNA produced by the cells with the pPG-1 specific probes or the actin probe also demonstrated that the SMC constitute a committed population that has maintained relatively high levels of the pPG-1 transcript but has greatly decreased levels of actin mRNA (Fig. 4) and total ribosomal RNA (data not shown). This contrast is most dramatic when the relative levels of pPG-1 and actin mRNA in both SMC and mouse fibroblast L cells are compared. These data suggest that in the maturation stages of the SMC, the transcription of nonmast cell-specific genes is diminished compared to the transcription of those genes encoding proteins necessary for the function of the mature mast cell. Further examination of the differential gene activation of mast cell specific genes via transcriptional regulation should allow us to understand better the maturation of cells in the mast cell lineage.

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