Proc. Natl. Acad. Sci. USA
Vol. 85, pp. 2284–2287, April 1988
Immunology

Identification of chondroitin sulfate E proteoglycans and heparin proteoglycans in the secretory granules of human lung mast cells

(glycosaminoglycans/IgE/histamine)

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Contributed by K. Frank Austen, December 10, 1987

ABSTRACT **The predominant subclasses of mast cells in both the rat and the mouse can be distinguished from one another by their preferential synthesis of ^{35}S-labeled proteoglycans that contain either heparin or oversulfated chondroitin sulfate glycosaminoglycans. Although [^{35}S]heparin proteoglycans have been isolated from human lung mast cells of 40–70% purity and from a skin biopsy specimen of a patient with urticaria pigmentosa, no highly sulfated chondroitin sulfate proteoglycan has been isolated from any enriched or highly purified population of human mast cells. We here demonstrate that human lung mast cells of 96% purity incorporate [^{35}S] sulfate into separate heparin and chondroitin sulfate proteoglycans in an ≈2:1 ratio. As assessed by HPLC of the chondroitinase ABC digests, the chondroitin [^{35}S]sulfate proteoglycans isolated from these human lung mast cells contain the same unusual chondroitin sulfate E disaccharide that is present in proteoglycans produced by interleukin 3-dependent mucosal-like mouse mast cells. Both the chondroitin [^{35}S]sulfate E proteoglycans and the [^{35}S]heparin proteoglycans were exocytosed from the [^{35}S]sulfate-labeled cells via perturbation of the IgE receptor, indicating that both types of ^{35}S-labeled proteoglycans reside in the secretory granules of these human lung mast cells.**

Two subclasses of mast cells can be distinguished from one another in the mouse and rat by the type of ^{35}S-labeled proteoglycans that they preferentially synthesize and store in their secretory granules. Rat (1) and mouse (2) serosal cavity-derived connective tissue mast cells (CTMC) synthesize heparin proteoglycans. In contrast, rat intestinal mucosa-derived mast cells (MMC) synthesize highly sulfated proteoglycans that contain chondroitin sulfate di-B/E glycosaminoglycans (3). Obtaining enough mouse MMC to characterize their secretory granule proteoglycans has thus far not been possible, but MMC-like bone marrow-derived mast cells (BMMC) have been obtained *in vitro* by culturing progenitors in medium that contains interleukin-3 (IL-3) (4, 5). These *in vitro*-differentiated BMMC preferentially synthesize chondroitin sulfate E proteoglycans (2, 6). Although the rodent MMC and CTMC were generally believed to be separately derived populations of mast cells, we recently demonstrated that when IL-3-dependent mouse MMC-like mast cells were cocultured for 14–28 days with mouse skin-derived 3T3 fibroblasts, they became histochemically and morphologically similar to CTMC, increased their content of histamine and carboxypeptidase A, and augmented their synthesis of [^{35}S]heparin proteoglycans (7–10). In addition, when mast cell-deficient WBB6F$_1$ W/W^v mice were reconstituted with BMMC from WBB6F$_1$ $^{+/+}$ mice, the mast cells recovered from the serosal cavity synthesized heparin proteoglycans in preference to chondroitin sulfate E proteoglycans (11). We concluded from these data that the proteoglycan type present in the secretory granules of a particular mast cell depends on the stage of cellular differentiation and/or the microenvironment in which the cell resides. Indeed, even though isolated rat CTMC incorporate [^{35}S]sulfate only into heparin proteoglycans, these cells contain appreciable amounts of nonradiolabeled chondroitin sulfate di-B/E proteoglycans in their secretory granules (12), which presumably were synthesized at an earlier stage of cellular development.

Human lung-derived mast cells of 40–70% purity (13) and skin biopsy specimens from a patient with urticaria pigmentosa (14) have been reported to synthesize [^{35}S]heparin proteoglycans. In contrast, human MMC have been proposed to preferentially synthesize chondroitin sulfate proteoglycans because [^{35}S]sulfate-labeled human colon biopsies spontaneously release chondroitin [^{35}S]sulfate E proteoglycans into the culture medium along with histamine (15). However, because mast cells do not constitutively exocytose appreciable quantities of their secretory granule components and because human monocytes can also be induced *in vitro* to synthesize chondroitin sulfate E proteoglycans (16), the source of these oversulfated chondroitin sulfate proteoglycans could not be conclusively determined. We now demonstrate that human lung mast cells of 96% purity synthesize and store in their secretory granules two distinct ^{35}S-labeled proteoglycans, one bearing [^{35}S]heparin glycosaminoglycans and the other bearing chondroitin [^{35}S]sulfate E glycosaminoglycans.

MATERIALS AND METHODS

Isolation of ^{35}S-Labeled Proteoglycans from Human Lung Mast Cells. Grossly normal human lung tissues, removed from six patients during surgery for pulmonary carcinoma, were dissected free of blood vessels, major airways, and pleura. The tissues were fragmented, and the individual cells were dispersed by two 30-min incubations with a solution containing 2 mg of Pronase and 0.5 mg of chymopapain per g (wet wt) of tissue and then incubated twice more for 30 min each with a solution containing 1 mg of collagenase and 10 units of elastase per g (wet wt) of tissue (17, 18). The dispersed cells were washed, and the mast cells were enriched by countercurrent centrifugation (18) and discontinuous Percoll density-gradient centrifugation (19) to a purity of 96 ± 3% (mean ± SD, n = 6). Purified mast cells were resuspended at a concentration of ≈1 × 10^6 cells per ml in Eagle's minimal essential medium containing 10% fetal calf serum, penicillin at

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Abbreviations: BMMC, bone marrow-derived mast cell; CTMC, connective tissue mast cell; IL-3, interleukin 3; MMC, mucosal mast cell.

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100 units/ml, streptomycin at 100 μg/ml, gentamycin at 10 μg/ml (GIBCO), and 100 μCi of [^{35}S]sulfate per ml (1 Ci = 37 GBq; New England Nuclear), and incubated at 37°C for 6–14 hr. The ^{35}S-labeled human lung mast cells were pelleted, washed once with [^{35}S]sulfate-free culture medium, and stored frozen at −70°C until used. The cell pellets were subsequently extracted by the sequential addition of 25 μl of 1% Zwittergent 3–12 detergent (Calbiochem) containing protease inhibitors and 225 μl of 4 M guanidine hydrochloride (2). A small fraction of each mast cell extract was chromatographed on 10-ml Sephadex G25/PD10 columns (Pharmacia) to determine the total incorporation of [^{35}S]sulfate into macromolecules (2). Twelve milliliters of 4 M guanidine hydrochloride containing 500 μg of heparin glycosaminoglycan (Sigma) carrier and CsCl (final density = 1.4 g/ml) were added to the remainder of the extracts. After centrifugation for 48 hr at ≈100,000 × *g* (20), the bottom 50% of each gradient was dialyzed against water for ≈6 hr, dialyzed against 0.1 M ammonium bicarbonate for ≈72 hr, lyophilized, resuspended in water, and used for all analytical assessments described below.

Proteoglycan Analysis. The nature of the ^{35}S-labeled glycosaminoglycans that were bound to the human lung mast cell-derived ^{35}S-labeled proteoglycans was determined by quantifying their susceptibility to degradation by nitrous acid (21) and chondroitinase ABC (Miles) (22). Hydrolysates and digests were chromatographed on Sephadex G25/PD-10 columns. [^{35}S]Heparin and chondroitin [^{35}S]sulfate measurements were based on the shift of radioactivity from the excluded volume to the included volume of the column (2) after nitrous acid and chondroitinase ABC treatments, respectively. To determine the type of chondroitin sulfate in these preparations, density-gradient purified samples were incubated with chondroitinase ABC, and the digests were precipitated with ethanol to remove contaminating protein and undigested ^{35}S-labeled macromolecules. The unsaturated ^{35}S-labeled disaccharides in the supernatants were chromatographed on Whatman Partisil 10 PAC amino-cyano HPLC columns (23). Authentic ΔDi-4S (Miles), ΔDi-6S (Miles), and ΔDi-diS_E [disulfated disaccharide possessing *N*-acetylgalactosamine 4,6-disulfate prepared from mouse BMMC ^{35}S-labeled proteoglycans (2)] were used to standardize the HPLC column.

The hydrodynamic sizes of the ^{35}S-labeled proteoglycans present in the detergent/guanidine hydrochloride extracts of ^{35}S-labeled mast cells were determined by Sepharose CL-4B (Pharmacia) chromatography. Density-gradient purified ^{35}S-labeled proteoglycans (50-μl samples) were applied to replicate 0.6 × 100-cm columns of Sepharose CL-4B that had been equilibrated in 4 M guanidine hydrochloride/0.1 M sodium sulfate/0.1 M Tris hydrochloride, pH 7.0; 0.5-ml fractions were collected and analyzed for radioactivity by β-scintillation counting. For some preparations replicate samples of ^{35}S-labeled proteoglycans were incubated with chondroitinase ABC before Sepharose CL-4B chromatography to determine whether there were two distinct proteoglycans or one hybrid proteoglycan possessing two or more types of glycosaminoglycans.

Immunologic Activation of Human Lung-Derived ^{35}S-Labeled Mast Cells. After radiolabeling cells with [^{35}S]sulfate, in one experiment mast cells were suspended in buffer containing 44% ^2H_2O and challenged for 30 min at 37°C with either buffer alone or buffer containing goat anti-human IgE at 1 μg/ml (24). The treated mast cells were centrifuged, the supernatants were removed, and cell pellets were resuspended in buffer and disrupted by sonication. Samples of the supernatants and cell pellets were removed and assayed for their total histamine by the automated fluorometric technique of Siraganian (25). The ^{35}S-labeled proteoglycans that were exocytosed into the supernatants and the ^{35}S-labeled proteoglycans that remained cell-associated were separately counted and characterized. Percent releases of histamine and ^{35}S-labeled proteoglycans were calculated as the amount in supernatant divided by the amount in supernatant and cell pellet times 100. Net percent release was calculated as anti-IgE-induced release minus spontaneous release.

RESULTS

Analysis of the ^{35}S-Labeled Proteoglycans Synthesized by Highly Purified Populations of Human Lung Mast Cells. When incubated with [^{35}S]sulfate, 10^6 human lung mast cells of 96% purity incorporated 2700 ± 1500 cpm (mean ± SD, n = 6) per hr into cell-associated macromolecules. Eighty-five ± two percent (mean ± SD, n = 4) of these ^{35}S-labeled macromolecules were recovered in the bottom fractions of the CsCl density gradients after ultracentrifugation, thus indicating the preferential incorporation of this radioisotope into proteoglycans. As assessed by Sephadex G-25 chromatography of separate nitrous acid-treated and chondroitinase ABC-treated samples, 50 ± 14% (mean ± SD, n = 5) and 32 ± 14% of the ^{35}S-labeled glycosaminoglycans in these preparations were [^{35}S]heparin and chondroitin [^{35}S]sulfate glycosaminoglycans, respectively (see representative experiment, Fig. 1). HPLC revealed that 83 ± 4% (mean ± SD, n = 4) and 16 ± 4% of the total unsaturated chondroitin [^{35}S]sulfate disaccharides that were obtained by chondroitinase ABC treatment eluted at the retention times of authentic ΔDi-4S (monosulfated disaccharide unit of chondroitin 4-sulfate) and ΔDi-diS_E (disulfated disaccharide possessing *N*a-

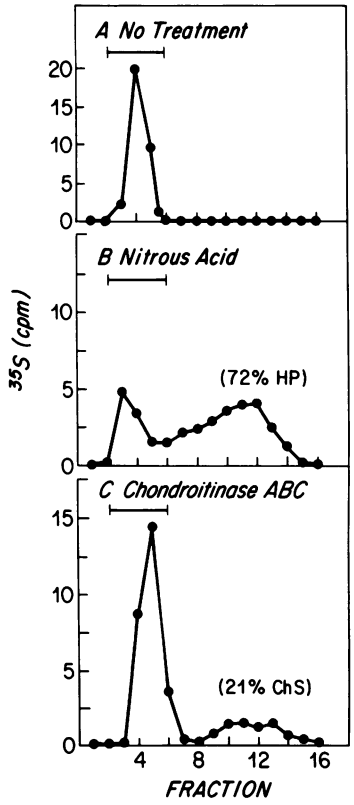

FIG. 1. Sephadex G25/PD-10 chromatography of a representative preparation of human lung mast cell ^{35}S-labeled proteoglycans chromatographed before (*A*) and after incubation with nitrous acid (*B*) or chondroitinase ABC (*C*). Brackets indicate the ^{35}S-labeled macromolecules that filter in the void volumes of the columns. Numbers in parentheses indicate the percent of ^{35}S radioactivity incorporated into heparin (HP) (*B*) and chondroitin sulfate (ChS) (*C*).

cetylgalactosamine 4,6-disulfate), respectively (see representative experiment, Fig. 2).

The human lung mast cell-derived ^{35}S-labeled proteoglycans filtered on Sepharose CL-4B chromatography as a broad peak (Fig. 3*A*), indicative of proteoglycans with molecular weights ranging from ≈150,000 to ≈250,000. When a duplicate sample was chondroitinase ABC-treated and the digest filtered on the same gel filtration column, ≈40% of the ^{35}S-labeled proteoglycans were degraded to ^{35}S-labeled disaccharide-sized molecules (Fig. 3*B*). More importantly, the K_{av} (fraction of stationary gel volume available for solute diffusion) of the remaining undigested ^{35}S-labeled proteoglycans remained unchanged, indicating that the majority of the [^{35}S]heparin proteoglycans did not have chondroitinase ABC-susceptible glycosaminoglycans attached to them. When a separate preparation of ^{35}S-labeled mast cells was similarly analyzed, 35% of the ^{35}S-labeled proteoglycans were degraded to ^{35}S-labeled disaccharides by chondroitinase ABC, but the elution profile of the undigested [^{35}S]heparin proteoglycans did not change (data not shown).

IgE-Mediated Release of ^{35}S-Labeled Proteoglycans and Histamine from Human Lung ^{35}S-Labeled Mast Cells. In an experiment in which ^{35}S-labeled mast cells were challenged with anti-IgE, the net percent release of histamine and ^{35}S-labeled proteoglycans was 45 and 17, respectively. As assessed by their susceptibility to degradation by nitrous acid and chondroitinase ABC, 60% and 33% of the ^{35}S-labeled proteoglycans that were exocytosed into the supernatant contained [^{35}S]heparin and chondroitin [^{35}S]sulfate glycosaminoglycans, respectively.

DISCUSSION

The evidence for heterogeneity of mast cells in rodents is well documented, but the evidence for mast cell heterogeneity in humans has not been as definitive. Although fixation properties of the mast cells in human skin differ from those in the intestine (26, 27), all mast cells in the human are alcian blue$^+$/safranin$^-$. In contrast, rat and mouse MMC are alcian blue$^+$/safranin$^-$, and their CTMC are alcian blue$^+$/safranin$^+$. Dispersed and minimally purified human intestinal MMC are similar to human lung mast cells in ultrastructure, histamine content, anti-human IgE dose–response curves, kinetics of histamine release, nonresponsiveness to fMet-Leu-Phe, and metabolism of arachidonic acid to prostaglandin D_2 and leukotriene C_4 (28). On the other hand, dispersed and partially purified human skin mast cells differ from isolated lung mast cells as follows: their activation–secretion responses to fMet-Leu-Phe, C5a anaphylatoxin, compound 48/80, and substance P (28–31); optimal temperature for release of histamine by anti-IgE (31); carboxypeptidase A content (32); and the amount of leukotriene C_4 generated during activation (31). As assessed by immunohistochemical techniques, both human skin and human lung mast cells contain tryptase, but only the skin-derived cells contain appreciable amounts of chymase (33, 34). Because tryptase$^+$/chymase$^-$ mast cells are deficient in the tissues of humans with T-cell deficiencies, it was proposed that chymase$^-$ human mast cells and chymase$^+$ mast cells differentiate along divergent pathways, with chymase$^-$ mast cells being analogous to rat and mouse MMC in their T-cell dependence (35). However, dispersed and partially purified (putative chymase$^-$) human lung mast cells can be cocultured for extended periods of time with fibroblasts in the absence of T-cell-derived factors (36). Human lung mast cells also have a histamine content similar to that of human skin mast cells (14) and preferentially synthesize heparin proteoglycans rather than chondroitin sulfate proteoglycans. Thus, human lung mast cells do not seem to be equivalent to rat and mouse MMC, even though human lung mast cells are more similar to human intestinal mast cells than to human skin mast cells.

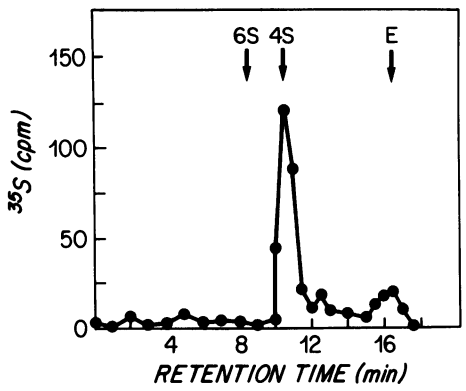

FIG. 2. HPLC of the ^{35}S-labeled disaccharides that were obtained by chondroitinase ABC digestion of a representative preparation of human lung mast cell ^{35}S-labeled proteoglycans. The retention times of authentic ΔDi-4S (monosulfated disaccharide unit of chondroitin 4-sulfate) (4S), ΔDi-6S (monosulfated disaccharide unit of chondroitin 6-sulfate) (6S), and ΔDi-diS_E (disulfated disaccharide possessing *N*-acetylgalactosamine 4,6-disulfate) (E) are indicated.

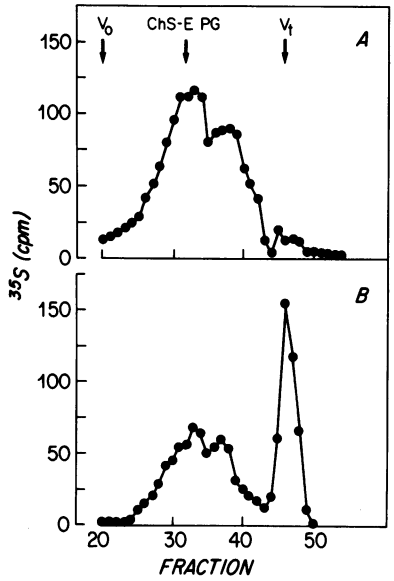

FIG. 3. Sepharose CL-4B chromatography of human lung mast cell-derived ^{35}S-labeled proteoglycans before (*A*) and after chondroitinase ABC treatment (*B*). Elution position of the mouse BMMC-derived chondroitin [^{35}S]sulfate E proteoglycan (ChS-E PG) of M_r 200,000–250,000 is indicated. V_0, void volume; V_t, total volume.

Chondroitin sulfate E and di-B proteoglycans have become useful phenotypic markers for distinguishing rat and mouse T-cell factor-dependent MMC (1–3) from the heparin proteoglycan-rich CTMC and for studying the differentiation of MMC-like mouse BMMC to CTMC-like mast cells (7, 9, 11). We demonstrate in the present study that human lung mast cells of 96% purity incorporate [^{35}S]sulfate into separate [^{35}S]heparin proteoglycans and chondroitin [^{35}S]sulfate E proteoglycans in an approximate 2:1 ratio (Figs. 1–3). Considering the high degree of purity of these mast cell preparations, it is unlikely that the 4% of contaminating cells synthesized the nonheparin proteoglycans. Exocytosis of [^{35}S]heparin proteoglycans and chondroitin [^{35}S]sulfate proteoglycans via perturbation of the IgE receptor in an approximate 2:1 ratio indicates that both types of ^{35}S-labeled proteoglycans reside in the secretory granules of human lung mast cells. When the ^{35}S-labeled proteoglycans were incu-

bated with chondroitinase ABC and chromatographed on Sepharose CL-4B, 35–40% of the macromolecular ^{35}S radioactivity was converted to disaccharide-sized ^{35}S-labeled macromolecules without a change in the hydrodynamic size of the [^{35}S]heparin proteoglycans (Fig. 3). It was concluded that there must be two distinct proteoglycans of similar size in these highly purified human mast cell preparations [as also occurs in mouse BMMC (37) and human basophilic leukocytes from patients with myelogenous leukemia (38)] rather than one hybrid proteoglycan [as occurs in rat basophilic leukemia-1 cells (39)].

The extent of similarity between the two human mast cell proteoglycan peptide cores that contain these different glycosaminoglycans is unknown. RNA blot analysis of total RNA from different populations of normal and transformed rat and mouse CTMC and MMC (40) has revealed that each cell contains substantial amounts of a 1.3-kb mRNA that encodes a serine/glycine-rich proteoglycan peptide core (41). In these species one gene is used to encode a common proteoglycan peptide core of M_r 18,600 despite the fact that the M_r of the secretory granule proteoglycan can vary from 100,000 to 750,000 and the glycosaminoglycans can be heparin, chondroitin sulfate A, chondroitin sulfate di-B, or chondroitin sulfate E. It remains to be determined whether or not there are two distinct types of mast cells in the human lung that each synthesize their own type of proteoglycan. However, based on the *in vitro* and *in vivo* studies of rat and mouse mast cells, it is more likely that a spectrum of mast cells exists in human tissues with the relative predominance of heparin proteoglycans and chondroitin sulfate E proteoglycans being regulated by differentiation factors that originate from the mast cell microenvironment.

This work was supported by Grants AI-00703, AI-07290, AI-22531, AI-23401, AI-23483, and HL-36110 from the National Institutes of Health. R.L.S. is a recipient of an American Heart Established Investigator Award.

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