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Histamine and chondroitin sulfate E proteoglycan released by cultured human colonic mucosa: Indication for possible presence of E mast cells

(human intestine)

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ABSTRACT **An association between the release of histamine and chondroitin sulfate E proteoglycan (PG) was demonstrated in human colonic mucosa (HCM). Colonic biopsy samples incorporated [^{35}S]sulfate ($2.7 \times 10^6 \pm 188 \times 10^3$ cpm/mg of wet tissue; mean ± SEM, $n = 5$) into PG, which was partially released into the culture medium during the incubation period. Ascending thin-layer chromatography of the released ^{35}S-labeled PG after its digestion by chondroitin ABC lyase (chondroitinase, EC 4.2.2.4) followed by autoradiography yielded three products that migrated in the position of monosulfated disaccharides of *N*-acetylgalactosamine 4-sulfate and *N*-acetylgalactosamine 6-sulfate and of an oversulfated disaccharide possessing *N*-acetylgalactosamine 4,6-disulfate. Cultured colonic mucosa released 23.6 ± 3.7 ng of histamine per mg of wet tissue (mean ± SEM, $n = 16$) without any specific trigger. Comparison by linear regression analysis of the release of histamine and chondroitin [^{35}S]sulfate E PG revealed a correlation coefficient (r) of 0.7 ($n = 16$; $P < 0.005$). Histological examination of the colonic biopsies revealed the presence of many mast cells in various degrees of degranulation in the mucosa and submucosa, most of which were found in the submucosa. Incubation of the HCM biopsies in the presence of anti-human IgE revealed 58% ± 12% (mean ± SEM, $n = 3$) enhancement in the release of chondroitin [^{35}S]sulfate E PG and 64% ± 10% (mean ± SEM, $n = 4$) of histamine release. The above correlation, the observation that most of the mast cells showed various degrees of degranulation, and the lack of heparin synthesis as opposed to the synthesis and immunological release of chondroitin sulfate E strongly suggest that the E mast cell exists in the human colon.**

There is increasing evidence to suggest that more than one type of mast cell exists within the intestine.

In rats, two subclasses of mast cells, typical and atypical, were identified. Rat serosal and skin mast cells are considered to represent the typical cell type because they possess in their secretory granules both heparin proteoglycan (PG) (1–3) and a neutral protease termed type I (4–6). In contrast, rat atypical mast cells, obtained from collagenase-dissociated small bowel, are distinguished by the presence of less electron-dense granules (7, 8), differential sensitivity to activation by chemical agents (9, 10), and an absence of heparin PG in their secretory granules. These cells contain another, as yet unidentified, PG (11, 12) and neutral protease type II (11, 13).

The growth of a pure population of mast cells in rodent bone marrow cultures stimulated with conditioned media derived from either antigen- or mitogen-activated lymphocytes has been reported (14–19).

Rat mast cells obtained by culture in the presence of T-cell growth factor possess in their secretory granules type II protease and do not contain heparin PG (14). Cultured mouse bone marrow-derived mast cells do not contain heparin PG in their secretory granules but rather chondroitin sulfate E (20). On the other hand, mouse serosal mast cells differentiated *in vivo* contain heparin PG (20). Mast cells containing chondroitin sulfate E PG have been designated as E mast cells (E-MC) in order to distinguish them from heparin-containing mast cells.

Mast cells are present in normal human colonic mucosa (HCM), and their number was shown to increase in patients with inflammatory bowel disease (21–23). However, the exact type of these cells has not yet been determined. In the human gastrointestinal tract, histamine is stored mainly in mast cells (24). In the present study, close association between histamine and chondroitin sulfate E PG release was demonstrated in HCM. In addition, the release of chondroitin sulfate E PG was enhanced after being triggered with anti-human IgE, and histological examination revealed partial mast cell degranulation. Taken together, these findings strongly suggest that E-MC exist in human colonic mucosa.

MATERIALS AND METHODS

Patients. Endoscopic biopsies were obtained from the large bowel of patients undergoing colonoscopy for various reasons and in whom no pathology was found.

Histology. Biopsies were fixed in 10% formalin, and 5-μm sections were sliced from the paraffin blocks. Sections were stained with hematoxylin, eosin, and toluidine blue and were examined for the presence of mast cells by light microscopy.

Radiolabeling of HCM. One to four biopsy samples were placed epithelial-surface-up and cut-surface-down on a stainless steel wire screen, resting over a small well in the center of a 60 × 15 mm plastic organ culture dish (25). The central well was filled with 800 μl of RPMI 1640 medium containing 5% fetal calf serum (enriched medium) and 2500 μCi (1 Ci =

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Abbreviations: E-MC, chondroitin sulfate E-containing mast cell(s); GAG, glycosaminoglycan; PG, proteoglycan; HCM, human colonic mucosa; ΔDi-4S, monosulfated disaccharide unit of chondroitin 4-sulfate [2-acetamido-2-deoxy-3-*O*-(β-D-gluco-4-enepyranosyluronic acid)-4-*O*-sulfo-D-galactose]; ΔDi-6S, monosulfated disaccharide unit of chrondroitin 6-sulfate [2-acetamido-2-deoxy-3-*O*-(β-D-gluco-4-enepyranosyluronic acid)-6-*O*-sulfo-D-galactose]; ΔDi-diS$_E$, oversulfated disaccharide possessing *N*-acetylgalactosamine 4,6-disulfate [2-acetamido-2-deoxy-3-*O*-(β-D-gluco-4-enepyranosyluronic acid)-4-6-di-*O*-sulfo-D-galactose].

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37 GBq) of [^{35}S]sulfate. Organ cultures were incubated for 4 hr at 37°C in humidified 5% CO_2/95% air.

^{35}S-Labeled PG Characterization. The radiolabeled PGs and glycosaminoglycans (GAGs) released into the medium and those remaining associated with the tissue were characterized as follows.

Culture media were subjected to PD-10 chromatography under dissociative conditions to quantitate incorporation of [^{35}S]sulfate into macromolecules.

Tissues were incubated in 5 ml of 0.5 M NaOH at 4°C for 17 hr. The tissue extracts were then neutralized with 0.5 ml of 5 M acetic acid. The ^{35}S-labeled macromolecules from both culture media and tissue extracts were subjected to CsCl density gradient centrifugation (starting density, 1.3 g/ml; containing 25 μg of pig mucosa heparin) for 36 hr at 140,000 × *g* under dissociative conditions. The bottom half of each gradient was dialyzed against water and lyophilized. The lyophilized fractions derived from tissue extracts were subjected to PD-10 chromatography, and the ^{35}S-labeled macromolecules were dialyzed and lyophilized.

The relative hydrodynamic sizes of the ^{35}S-labeled PGs elaborated to the culture medium obtained by density gradient centrifugation were determined by Sepharose CL-4B chromatography following filtration through the column (1.4 × 107 cm) (20). Chondroitin [^{35}S]sulfate E PG from mouse E-MC, [^{35}S]sulfate, ^{125}I-labeled IgG and [^{32}P]DNA (1 kb) were used as reference standards.

^{35}S-labeled GAGs (50 μl) were applied to Sephadex G-200 columns (0.6 × 99 cm) (20). Chondroitin [^{35}S]sulfate E GAG and [^{35}S]sulfate were used as reference standards. The average molecular weight of the ^{35}S-labeled GAG was estimated by the gel filtration method of Wasteson (26).

The ^{35}S-labeled PGs and the ^{35}S-labeled GAGs purified by CsCl density gradient centrifugation were incubated for 4 hr with 0.2 unit of chondroitin ABC lyase (chrondroitinase, EC 4.2.2.4) in 100 μl of Tris·HCl buffer (50 mM Tris·HCl/50 mM NaCl/35 mM sodium acetate, pH 8.0) containing 0.5% bovine serum albumin and 100 μg of both chondroitin sulfate A and chondroitin sulfate C carriers (20, 27, 28). Chondro-6-sulfatase (EC 3.1.6.10; 0.002 unit/μg of carrier), chondro-4-sulfatase (EC 3.1.6.9; 0.002 unit/μg of carrier) were added to several samples during the chondroitin ABC lyase digestion (20). The mobility of the digested products was characterized relative to ^{35}S-labeled disulfated and monosulfated disaccharide derived from chondroitin ABC lyase-digested chondroitin [^{35}S]sulfate E by ascending thin-layer chromatography on precoated cellulose acetate plates; the monosulfated disaccharide unit of chondroitin 4-sulfate is referred to as ΔDi-4S and that of chrondroitin 6-sulfate as ΔDi-6S.

Radiolabeled disaccharides were quantified by scintillation counting after elution with 1 ml of 0.5 M HCl for 2 hr at 55°C.

The susceptibility of the ^{35}S-labeled PG to nitrous acid degradation (29, 30) was used to test for the presence of ^{35}S-labeled heparin and/or heparan sulfate.

Histamine Release to the Cultured Media. One to four biopsies were organ-cultured for 1 hr in medium containing 800 μl of Tyrode's buffer (1 mM Ca^{+2}/0.2 mM Mg^{+2}/0.05% gelatin) at 37°C in humidified 5% CO_2/95% air. Histamine release into the media was quantified by the spectrofluorimetric technique (31).

Measurements of ^{35}S-Labeled PG and Histamine Released to the Medium After Incubation with Anti-Human IgE. A pool of six to eight HCM biopsies were incubated for 1 hr in an enriched medium containing 250 μCi of [^{35}S]sulfate. Each of the biopsies was then transferred into a separate tube containing 200 μl of the enriched medium. Eighty microliters of saline with or without rabbit anti-human IgE, in a dose recommended by the manufacturer (Bio Yeda, Rehovot, Israel) was added, and the samples were incubated for another hour. Supernatants were collected and subjected to PD-10 chromatography, and the amount of ^{35}S-labeled PG released into the medium was quantified. A pool of three HCM biopsies was incubated for 1 hr in 280 μl of saline with or without rabbit anti-human IgE, and histamine release to the media was quantified as described above.

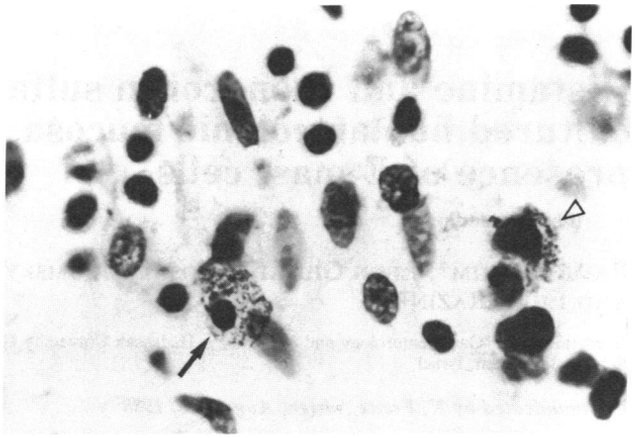

FIG. 1. Biopsy of colonic mucosa showing a mast cell containing prominent secretory granules (black arrow). Note degranulated cells (open arrow). (Toluidine blue; ×1024.)

RESULTS

Histological examination of the tissue revealed the presence of many mast cells, especially in the mucosa and submucosa (Fig. 1). Most of the cells were found to be in various degrees of degranulation.

Within 4 hr of incubation, HCM biopsies incorporated [^{35}S]sulfate into macromolecules, as assessed by PD-10 chromatography, at a rate of 2,712,325 ± 188,188 cpm/mg of wet tissue (mean ± SEM, n = 5). During the incubation, part of the newly synthesized product was secreted to the medium. The ^{35}S-labeled PGs that were released to the cultured medium were isolated by CsCl density gradient centrifugation under dissociative conditions. ^{35}S-labeled PGs in the bottom half of the gradient were filtered on a Sepharose CL-4B column as the predominant polydispersed peak; these PGs had an apparent hydrodynamic size of M_r 200,000–700,000. Sephadex G-200 gel filtration of the ^{35}S-labeled GAGs, liberated by alkali from the PGs released to the culture medium or extracted from tissue, revealed chains with partition coefficient k_{avg} ranging from 0.8 to 0.29. This indicated a M_r of 3,600–19,200. The ^{35}S-labeled PGs released into the medium were resistant to nitrous acid degradation as assessed by exclusion on PD-10 gel filtration of more than 95% of the treated material. In contrast, chondroitin ABC lyase-digested ^{35}S-labeled PGs shifted 58% ± 12% (mean ± SEM, n = 7) of the original macromolecular radioactivity from the excluded volume to an included fraction.

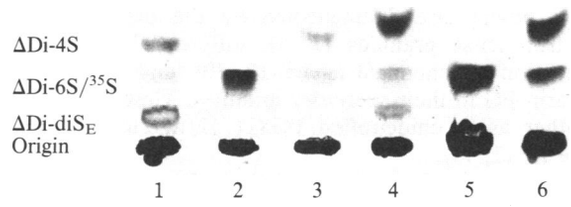

FIG. 2. Ascending thin-layer chromatography of chondroitin ABC lyase-treated ^{35}S-labeled PG in the tissue (lanes 1–3) and that released from HCM (lanes 4–6); lanes 2 and 5 show results of additions of chondro-4-sulfatase, whereas lanes 3 and 6 show results of addition of chondro-6-sulfatase.

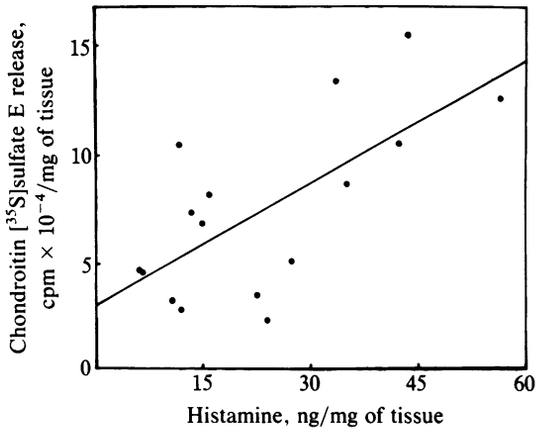

FIG. 3. Correlation of chondroitin [^{35}S]sulfate E PGs and histamine release from cultured colonic mucosa.

Ascending thin-layer chromatography of the chondroitin ABC lyase digest of the released ^{35}S-labeled PGs and the ^{35}S-labeled GAGs extracted from the tissue followed by autoradiography yielded three products. These products migrated in the position of ΔDi-4S, ΔDi-6S, and of an oversulfated disaccharide, possessing *N*-acetylgalactosamine 4,6-disulfate, which we refer to a ΔDi-diS_E (Fig. 2). Quantitation of the radiolabeled digestion products revealed 17.2% ± 3.6 (mean ± SEM, n = 9) of the radioactivity to be associated with the sulfated disaccharide, indicating that 8.6% of the disaccharides present in the GAGs were disulfated.

Colonic tissue released 23.6 ± 3.7 ng of histamine per mg of wet tissue (mean ± SEM, n = 16) without any specific external trigger. Comparison by linear regression analysis (29, 30) of the release of histamine and chondroitin [^{35}S]sulfate E to the culture medium revealed a correlation coefficient (r) of 0.7 (n = 16; $P < 0.005$), with the line intersecting the y axis at a point not statistically different from zero (Fig. 3; $t = 0.76$, $P > 0.05$). The amount of ^{35}S-labeled PG released from the anti-human IgE-treated and -untreated tissues was 13,072 ± 755 cpm and 8241 ± 679 cpm (mean ± SEM, n = 3), respectively, meaning that there was a 58% increase in the labeled PG released after the immunological challenge. Furthermore, the chondroitin sulfate E PG present in HCM did not undergo change in sulfation of its bound GAGs upon exocytosis. The percent enhancement of histamine release from the anti-human IgE-treated tissues was found to be 64% ± 10% (mean ± SEM, n = 4).

DISCUSSION

The intensive investigations carried out in recent years emphasize many similarities between the biochemical characterization of E-MC and the intestinal mucosa mast cells of the rat (20, 15–19). The latter have not been thoroughly investigated in man, mainly because of difficulties in their isolation. In fact, no report on human intestinal mast cell characterization has yet been published. The present investigation proposes that E-MC may be present in the human large intestine.

Histologically human colonic mucosal mast cells exhibited various degrees of degranulation (Fig. 1). Since the existence of two histochemically distinct mast cell populations could not be confirmed in colonic mucosa and submucosa, their initial characterization was performed according to their specific PG synthesis. ^{35}S-labeled GAGs derived from PGs synthesized by the HCM were found to be resistant to nitrous acid degradation. In view of this finding, further studies were directed to determine whether the disaccharides in the ^{35}S-labeled GAGs were identical in their sulfation to those derived from mouse E-MC. Chondroitinase ABC digestion of the ^{35}S-labeled GAGs yielded three cleavage products that comigrated on ascending chromatography with the monosulfated and disulfated disaccharides obtained from mouse E-MC (Fig. 2). The specificity of the two sulfatases for sulfate residues on C-4 and C-6 of the *N*-acetylgalactosamine moiety of chondroitin sulfate (32) confirms the structure of the monosulfated and higher-sulfated disaccharides as the ΔDi-4S, ΔDi-6S, and ΔDi-diS_E (Fig. 2). The wide range in the molecular weight of the chondroitin sulfate E PG (200,000–700,000) can be interpreted in at least two ways: (*i*) enzymatic degradation during or after exocytosis and (*ii*) presence of an E-MC population that is in various degrees of maturation and phases of activation.

Mast cells are the main source for histamine release in the gastrointestinal tract (24). Linear regression analysis of the relationship between the release of histamine and chondroitin [^{35}S]sulfate E PG yielded a correlation of 0.7 (Fig. 3). A 58% increase in the chondroitin [^{35}S]sulfate E PG and 64% in histamine released to the medium was also demonstrated in biopsies treated with anti-human IgE. This IgE-mediated chondroitin sulfate E release does not exclude the possibility that macrophages that express surface IgE Fc fragment receptors (33) are involved in this process or the possibility that the release requires activation of another cell population, probably through enzymes released by the mast cells.

However, the histological finding of partially degranulated mast cells, the correlation between the release of the mast cell granular components, histamine and oversulfated PG, the increase in the release of chondroitin sulfate E after immunological challenge, and the lack of heparin PG synthesis, strongly suggests that E-MC are present in the human large intestine. Modulation of their activity may prove to be therapeutically useful.

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