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Ancient origin of mast cells

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ABSTRACT

The sentinel roles of mammalian mast cells (MCs) in varied infections raised the question of their evolutionary origin. We discovered that the test cells in the sea squirt *Ciona intestinalis* morphologically and histochemically resembled cutaneous human MCs. Like the latter, *C. intestinalis* test cells stored histamine and varied heparin·serine protease complexes in their granules. Moreover, they exocytosed these preformed mediators when exposed to compound 48/80. In support of the histamine data, a *C. intestinalis*-derived cDNA was isolated that resembled that which encodes histidine decarboxylase in human MCs. Like heparin-expressing mammalian MCs, activated test cells produced prostaglandin D_2 and contained cDNAs that encode a protein that resembles the synthase needed for its biosynthesis in human MCs. The accumulated morphological, histochemical, biochemical, and molecular biology data suggest that the test cells in *C. intestinalis* are the counterparts of mammalian MCs that reside in varied connective tissues. The accumulated data point to an ancient origin of MCs that predates the emergence of the chordates >500 million years ago, well before the development of adaptive immunity. The remarkable conservation of MCs throughout evolution is consistent with their importance in innate immunity.

1. Introduction

Mast cells (MCs) are important effector cells that participate in innate and adaptive immunity [1]. No human has been found who lacks MCs. Located at strategic sites within epithelial and mucosal surfaces, MCs perform sentinel roles in combating numerous pathogens, in part, via their exocytosed heparin·protease complexes [2–5]. Human MCs and their progenitors are highly susceptible to M-tropic strains of HIV-1 [6,7], and the loss of HIV-1-infected MCs in the gastrointestinal tract and other tissue sites [8] contributes to the development of AIDS.

When activated by complement anaphylatoxins or by varied pathogen-derived products, mammalian MCs quickly release their granule (e.g., histamine [9] and heparin·serine protease complexes [10–14]) and lipid (e.g., prostaglandin D_2 [PGD_2] [15] and leukotriene C_4 [LTC_4] [16]) mediators to initiate the acute phase of the inflammatory response against the infectious organism. Given their central roles in innate and acquired immunity, surprisingly little is known concerning the origin and evolution of different polarized subsets of MCs and their granule and lipid mediators. MCs have been identified in zebrafish [17]. Nevertheless, no protostome has been shown that possesses cells that resemble any subset of MCs in mice and humans. We therefore looked for MC-like cells in *Ciona intestinalis* which is a member of the chordate lineage that gave rise to vertebrates [18].

2. Materials and methods

2.1. Histochemistry and ultrastructure of the test cells in C. intestinalis, and biochemical characterization of the heparin glycosaminoglycans present in their secretory granules

C. intestinalis were collected at the Marine Biological Laboratory in Woods Hole, Massachusetts. The test cells that surround the oocytes of this sea squirt were isolated for *in vitro* study by mild physical trauma of the liberated eggs, following by sedimentation of their dense granulated test cells. The heparin proteoglycans and glycosaminoglycans were isolated from lysed test cells using the zwittergent 3–12 detergent/$CsCl_2$ density-gradient method we previously developed for the isolation of proteoglycans and glycosaminoglycans from rodent and human MCs [19]. Employing the experimental procedures developed for analyzing mammalian heparin glycosaminoglycans [20], purified test cell-derived heparin

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was digested with heparitinases (Seikagaku Corp.) and the resulting disaccharides were subjected to high performance liquid chromatography. After chromatographic separation of the generated disaccharides, they were reacted with 2-cyanoacetoamide as a post-column reagent. They were identified and quantitated based on comparisons of their elution positions and peak heights with those of known amounts of standard disaccharides.

2.2. Release of enzymatically active serine proteases from activated C. intestinalis test cells

C. intestinalis test cells ($\sim 1 \times 10^6$ cells/ml) were activated by exposure to 0.2 mM compound 48/80 (Sigma–Aldrich), as previously described for heparin-expressing mouse MCs [21] and *Styela plicata* test cells [22]. The treated cells were subjected to electron microscopy, using standard methodology [23,24]. In other experiments, supernatants were collected from the compound 48/80-activated test cells. The presence of enzymatically active serine proteases in the supernatants was detected using 5 nM of the biotinylated probe Phe-Pro-Arg-chloromethylketone (PPACK, Santa Cruz Biotech.), employing the experimental protocol recommended by the manufacturer. PPACK binds irreversibly to the active sites of serine proteases [25,26]. Thus, the PPACK-labeled serine proteases in the test cells of *C. intestinalis* were separated by SDS–PAGE, transferred to Protran BA83 nitrocellulose membranes (Whatman), blocked in 5% nonfat milk for 1 h, and incubated for 3 h with horseradish peroxidase (HRP)-conjugated streptavidin which binds with high affinity to the biotin moiety of the biontinylated-PPACK probe. The treated blots were washed three times (10 min each) in phosphate-buffered saline containing 0.1% Tween 20. They were then developed in enhanced chemiluminescence (ECL) reagent (Millipore) for 2–5 min. Labeled serine proteases were detected by exposing the blots to Blue XB-1 film (Kodak).

2.3. Release of histamine from activated C. intestinalis test cells, and cloning of the putative C. intestinalis ortholog of human histidine decarboxylase (HDC)

The histamine enzyme-linked immunosorbent assay (ELISA) created by Bertin Pharma/SPI Bio and distributed by Cayman Chemical was used to measure histamine levels in lysates of enriched test cells, again using the manufacturer's protocol. This ELISA is based on the competition between unlabeled derivatized histamine and aceylcholinesterase that has been linked to histamine (defined as the tracer) for a mouse monoclonal antihistamine antibody that is bound to a 96-well plate.

HDC [27] participates in the biosynthesis of histamine in mammalian MCs. A previously created cDNA library [28] was used to isolate its putative *C. intestinalis* ortholog (CiHDC). A search of the Expressed Sequence Tags (ESTs) in the library revealed a clone (designated *cidg826g09*) whose partial nucleotide sequence resembled that of human and mouse HDC. Using a standard molecular biology approach, the nucleotide sequence of the entire coding domain of CiHDC was determined, as well as the amino acid sequence of its translated protein. Finally, a guinea pig polyclonal anti-HDC antibody (Thermo Scientific) that recognizes human, mouse, chicken, and amphibian HDC was used to detect the presence of the putative CiHDC protein in test cells. Test cells were lysed in SDS–PAGE loading buffer. The liberated proteins were denatured at 95 °C for 5 min, fractionated on a 10% NuPAGE gel (Invitrogen), and immunoblotted with the anti-HDC antibody.

2.4. Release of prostaglandin D_2 (PGD_2) from activated C. intestinalis test cells, and cloning of the putative C. intestinalis ortholog of human hematopoietic-type PGD_2 synthase (HPGDS)

To determine if *C. intestinalis* test cells have the ability to generate PGD_2 and/or LTC_4 upon cellular activation, $\sim 10^6$ cells were placed in 200 µl of Hank's buffer containing 2 mg/ml of bovine serum albumin, 5 µM calcium ionophore A23187 (Calbiochem), and 10 µM arachidonic acid. One hour latter, the reactions were terminated by the addition of 2 volumes of methanol. After centrifugation at 12,000*g* for 10 min, half of the supernatants were analyzed for LTC_4 by reverse phase-high performance liquid chromatography [29]. The other half were dried under reduced pressure using a rotoevaporator, resuspended in ELISA buffer, and evaluated for their PGD_2 content using an ELISA kit (Cayman Chemical).

Rat, mouse, and human HPGDS catalyze the conversion of prostaglandin H_2 to PGD_2 [30] in heparin-expressing mammalian MCs [15]. We queried the EST database to isolate its putative *C. intestinalis* ortholog (CiHPGDS). The nucleotide sequences of the *C. intestinalis* ESTs BW487468 and BW062636 revealed significant homology to the respective 5′ and 3′ ends of the human HPGDS transcript noted at GenBank accession NP_055300. The nucleotide sequences that encode the entire coding regions of allelic isoforms of CiHPGDS were next determined using a test cell-enriched mRNA preparation and primers that were based on the above *C. intestinalis* ESTs. To that end, total RNA (1 µg) from test cells was reverse transcribed into cDNAs using SuperScript II reverse transcriptase (Life Technologies) and random hexamers. The CiHPGDS cDNAs were amplified using high fidelity Pfu polymerase (Stratagene). The forward and reverse primers used were 5′-ATGCCAGTTTAC AAGTTATACTACTTC-3′ and 5′-TTACATATTTGTCTTTGGTCTTGTG G-3′, respectively. The polymerase-chain reaction (PCR) cycling condition consisted of 35 cycles of denaturation (95 °C, 15 s), annealing (55 °C, 15 s), and extension (70 °C, 1 min). The resulting PCR products (~600 bp) were separated on 1% agarose gels, purified, and inserted in the pCR2.1 TOPO cloning vector (Life Technologies). The entire inserts from multiple independent clones were subjected to DNA sequencing.

3. Results and discussion

3.1. Histochemistry and ultrastructure of the test cells C. intestinalis test cells, and biochemical characterization of their granular heparin·protease complexes

All mammalian MCs possess non-segmented nuclei [23] and become metachromatic when stained with toluidine blue [31]. We discovered that the test cells in *C. intestinalis* histochemically and morphologically resembled the MCs that reside in the skin and other connective tissues of mammals in that they contained non-segmented nuclei and electron-dense granules that became metachromatic when stained with toluidine blue (Fig. 1A).

The T-cell-independent polarized subset of MCs that constitutively reside in the peritoneal cavity, skin, and other connective tissues of mice preferentially store heparin in their granules [32,33]. *S. plicata* contains a heparin glycosaminoglycan that structurally resembles that in the latter population of mammalian MCs [22]. In this urochordate, heparin is released upon cellular activation. We discovered that the test cells in *C. intestinalis* also contain classical heparin (Fig. 1B).

3.2. Release of enzymatically active serine proteases from activated C. intestinalis test cells

Heparin is essential for the packaging of tetramer-forming tryptases and other serine proteases inside the secretory granules of