Establishment and Characterization of a New Canine Mast Cell Tumor Cell Line

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Abstract. A new cell line (CoMS) was established from a 3-year-old male mongrel dog with mast cell tumor of the oral mucosa. CoMS cells grow in suspension with a doubling time of 27.0 ± 0.7 hr. The cytoplasmic granules were formalin-sensitive, showed diverse appearances in their ultrastructural findings and contained heparin proteoglycan and neutral protease chymase. Calcium ionophore A23187, substance P and concanavalin A caused significant histamine release from CoMS cells, while compound 48/80 failed to release histamine. This cell line will make an available source for studies on canine mast cell tumors.

Key words: canine, cell line, mast cell tumor.

Mast cell tumor (MCT) is one of the common neoplasms in dogs, accounting for 7% to 21% of all skin tumors and 11% to 27% of all malignant skin tumors [19]. There are still some dilemmas caused by its unpredictable biological behaviors with regard to diagnosis and treatment of MCT [9]. Studies on several neoplastic cell lines of human or rodent mast cells such as HMC-1, P-815 and RBL-2H3 have provided many informations about mast cell biology [17, 18, 20]. There will be anatomical, biochemical, immunological and pharmacological differences between human, rodent and canine mast cells. Available cell lines of canine MCT are therefore required for the research on this disease.

In this paper, we describe a successful establishment of a new canine MCT cell line named CoMS in continuous culture and report morphology, histamine release activity and granular components such as proteases and heparin of CoMS cells.

CoMS cell line was obtained from a mucosal mass in the lower lip of a 3-year-old male mongrel dog with MCT. The dog was referred to the Veterinary Teaching Hospital of Hokkaido University with massive swelling of a left lower lip and both cervical lymph nodes. The clinical management were consisted of surgical excision, radiotherapy and chemotherapy. In spite of these treatments, the dog died of deterioration of general conditions 56 days after the first admission. Multiple gastroduodenal ulcers and multiple distant metastases to the internal organs such as the liver, spleen, kidneys and lungs but no metastasis to the skin were observed at necropsy.

The specimen of CoMS collected at surgery was initially maintained in vivo passage in 5-week-old female C.B-17 severe combined immunodeficiency (SCID) mice (Clea Lab., Tokyo, Japan) by subcutaneous implantation, then the tumor tissues excised from the mice were used for establishment of the cell line in vitro.

These tumor tissues were minced finely in RPMI 1640 medium containing 2 mg/ml sodium bicarbonate, 25 mM Hepes buffer, 100 U/ml penicillin and 100 μg/ml streptomycin, filtrated through a stainless mesh and washed two times in phosphate buffered saline. The isolated cells were then suspended and incubated in the above RPMI 1640 medium with 10% heat inactivated fetal calf serum (FCS) at 37°C in a humidified atmosphere of 5% CO₂ and were passage every 4 to 6 days. The cell line named CoMS has been in continuous culture over 60 passages. We used the cells from 40th to 50th passage in all the following examinations.

For calculating the doubling time, cells were plated at 5 × 10⁵ cells/ml in RPMI 1640 containing 10% FCS. Cell growth and viability were measured by trypan blue exclusion test using a hemocytometer every 24 hr, and growth curve was described. As a result, CoMS cells had a log-phase doubling time of 27.0 ± 0.7 hr.

For metachromatic staining, cell smear preparations were fixed in Mota’s lead acetate, Carnoy’s solution or 4% neutral buffered formaldehyde for 5 min, washed in distilled water, and stained for 40 min with 0.5% toluidine blue at pH 0.5 [3]. CoMS cells were stained metachromatically after fixation in Mota’s lead acetate and Carnoy’s solution, however, after fixation in 4% neutral buffered formaldehyde they were not stained metachromatically (Fig. 1).

Ultrastructure of the cells was examined by transmission electron microscopy using methods outlined previously [14]. CoMS cells were generally round or oval and diameters ranged from 10.2 to 15.2 μm (Fig. 2). The nucleus was usually oval or irregular, and the cell surface having cytoplasmic projections was characteristic of the cells. Intracytoplasmic granules appeared variable in size and shape. Many granules had heterogeneous electron-dense particulate material, though others were entirely filled homogenous electron-dense material.

Study of histamine content and release was examined as described previously [4]. Compound 48/80 (ICN Biomedicals Inc., Ohio, U.S.A.), calcium ionophore A23187 (CAL-BIOCHEM, Darmstadt, Germany), substance P (BACHEM AG, Bubendorf, Switzerland), anti-canine IgE (ViroStat, Portland, ME, U.S.A.) and concanavalin A (Wako Pure...
Chemical Ind., Osaka, Japan) were used as stimuli. On the basis of previous reports [5, 8], canine pooled serum from a colony of clinically normal beagle dogs (n=6) was used as a source of IgE. To assess anti-canine IgE or concanavalin A induced histamine release, CoMS cells were passively sensitized by incubation for 24 hr at 37°C in RPMI 1640 medium containing 10% canine serum. Incubations for activating cells were carried out at 37°C for 20 min with concanavalin A and at 37°C for 30 min with other stimuli. Histamine was measured by an o-phthalaldehyde (OPT) spectrofluorimetric method [13]. Histamine release was expressed as a percentage of the histamine in the supernatant to the total histamine in the cells and supernatant after stimulation, and was corrected for spontaneous release. All values collected 3 experiments by duplicate were given as means ± standard deviation. The cytotoxicity of each stimulus at different concentrations was assessed by trypan blue exclusion test. Wright stain was used for the morphological examination of the stimulated cells. As a result, CoMS cells contained 0.040 ± 0.001 µg/cell (n=18). Spontaneous release for all experiments was 7.2 ± 0.9%. Compound 48/80 induced only significant histamine release (7.5 ± 3.4%) at the highest concentration of 100 µg/ml that was cytotoxic (9.8 ± 2.3%) (Fig. 3). Calcium ionophore A23187 induced a concentration-dependent histamine release, and maximum histamine release was 27.5 ± 3.8% at a concentration of 100 µM. Histamine release by substance P was concentration-dependent, and maximum histamine release was 13.7 ± 1.2% at a concentration of 100 µM. Anti-canine IgE did not induce significant histamine release at any concentration, however appeared to induce slight release at the highest concentration of 100 µM (3.7 ± 2.0%). Concanavalin A produced dose dependent release with or without passively sensitized by canine serum, and maximal stimulation was 25.7 ± 6.2% at a concentration of 10 µg/ml. Marked morphological changes were observed after stimulation of calcium ionophore A23187 and concanavalin A (Fig. 4).

Tryptic and chymotryptic activities were measured as described previously [2]. Briefly, the cell pellets containing 1 x 10⁶ cells were extracted in 10 mM bis-Tris (pH 6.1) containing 2 M NaCl and sonicated for 45 sec. The resulting supernatants were assayed for tryp tic activity with 80 µg/ml N benzoyl-L-val-gly-arg-p-nitroanilide (Sigma Chemical Co., St. Louis, MO, U.S.A.) in 60 mM Tris-HCl (pH 7.8), and, for chymotryptic activity, 80 µg/ml N-succinyl-L-phenylalanyl-L-pro-L-phenylalanine (BACHEM AG) in 30 mM Tris-HCl (pH 8.0). Both assays were performed at 37°C by following the change in optical density at 394 nm. Specific activities of 1,740 OD/min·mg·mg⁻¹ (tryp tase) and 110

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Fig. 1. Photomicrographs of CoMS cells stained with toluidine blue. Original magnifications × 400. A: Metachromatic granules are present in the cells fixed in Mota’s lead acetate. B: The cells contain few granules after fixation in 4% neutral buffered formaldehyde.

Fig. 2. Transmission electron micrographs of CoMS cells. A: An oval nucleus and a prominent nucleolus are present, and the cell surface show cytoplasmic projections. Bar=2 µm. B: Intracytoplasmic granules having heterogeneous electron dense particulate materials are seen. Bar=600 nm.
cells contained neutral protease chymase (65.5 ± 23.8 ng/10^6 cells chymotriptic activity) but little trypsin (0.2 ± 0.1 ng/10^6 cells trypsin activity).

To detect heparin localized in cytoplasmic granules, preparations of the cells on a glass slide were incubated with 0.02% berberine sulfate (Hemisulfate Salt, Sigma Chemical Co.) in 1% citric acid water (pH 4.0) for 1 hr at 37°C [11]. The cells were then mounted in glycerol without washing and viewed immediately with a fluorescence photomicroscope. CoMS cells contained yellow fluorescent granules indicating the presence of heparin in the cytoplasm (Fig. 5).

Previous studies of mast cells on formalin sensitivity, morphology, histamine release activity and granular component made it clear the diversity of species-specific mast cell subtypes. In this study, CoMS cells showed the formalin-sensitivity, diverse cytoplasmic granules in their ultrastructural findings and the lack of response to compound 48/80. These characteristics were similar to those of the most of canine mast cells in the gastric mucosa [14, 15] and the airway lumen [16], but not in the skin [4]. This may be related to that the original lesion where isolated CoMS cells is the mucosal site in the lower lip. In addition, CoMS cells predominantly contained chymase as their granular component. Mast cell-specific proteases tryptase (T) and chymase (C) divide human or canine mast cells into three subtypes:T-, TC- and C-mast cells [7]. CoMS cells may therefore originate in C-mast cells. However, it should be considered the above characteristics of CoMS cells may reflect not only the nature of their progenitor mast cell in the original lesion but also change of its nature caused by neoplastic transformation.

IgE receptor mediated stimulation by anti-canine IgE failed to induce significant histamine release from CoMS cells. This result suggests that CoMS cells have few functional IgE receptors like the neoplastic mast cell lines such as human HMC-1, mouse P-815 and canine BR [4, 10, 12]. Concanavalin A elicited cell-to-cell aggregation of CoMS cells and significant histamine release independent on IgE. It is generally accepted that concanavalin A causes histamine release by binding to adjacent IgE molecules to pro-

Fig. 3. Histamine release from CoMS cells induced by each stimulus as described in the text. Before activation by concanavalin A, the cells were incubated in complete medium containing either canine serum 10% (open squares) or no serum (closed squares). #: indicate cytotoxicity.

OD/ min·mL⁻¹·mg⁻¹ (chymase) were used to compute mg of protease activity per sample. The protein content of each supernatant was measured by the bicinchoninic acid assay using commercial based kit (BCA Protein Assay Kit, PIERCE, Rockford, Illinois, U.S.A.). Results were expressed as ng of protease per 10^6 cells. As a result, CoMS

Fig. 4. Photomicrographs of stimulated CoMS cells stained with Wright's. Original magnifications × 400. A: Non-stimulated cells (control). B: Calcium ionophore A23187-stimulated cells show a decrease in cytoplasmic granules and vacuoles in their cytoplasms. C: Concanavalin A-stimulated cells showed dose-dependently high aggregation.
remote aggregation of IgE receptors [15], while it is also reported that cell-to-cell aggregation through adhesion molecules induces degranulation in mast cells [1, 6]. Histamine release from CoMS cells on stimulation with concanavalin A may be therefore caused by cell-to-cell aggregation through certain adhesion molecules rather than caused by a cross-link Fc region from IgE.

Diagnosis and treatment of canine MCT have still some problems caused by its peculiar biological behaviors. Human tumor therapy tends to target biological properties having each tumor cell type. We believe that CoMS cell line is an available source to study new therapeutic strategies against canine MCT.

REFERENCES


Fig. 5. Fluorescence photomicrograph of CoMS cells stained with berberine sulfate. Original magnifications x 400. A: Squash preparation. Fluorescent materials in the nucleus and the cytoplasm are clearly observed. B: Slide preparation. The cytoplasm is filled with homogeneous fluorescent granules and the nucleus is covered with those fluorescent materials.