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Bovine mast cells: distribution, density, heterogeneity, and influence of fixation techniques

Received: 9 June 1997 / Accepted: 3 February 1998

Abstract Mast cells can be distinguished according to various characteristics: rodent mast cells have been sub-typed by histochemical criteria, whereas canine and human mast cells have been classified according to their proteases. Comparisons of mast cells from different species have therefore resulted in contradictory and confusing opinions on mast cell heterogeneity. Thus, it is essential to obtain species-specific data on mast cell density and heterogeneity. The present study was carried out to determine the physiological distribution of mast cell numbers and types in bovines according to tissue location, staining, and fixation methods. Samples were fixed in formalin or Carnoy’s fluid. The average number of mast cells was determined by using a metachromatic staining method. Protease content of mast cells was examined with a double-enzyme-immunohistochemical staining technique. Three mast cell subtypes were distinguished: T-, TC-, and C-mast cells. The T-mast cell was the predominant subtype in nearly all investigated organs and tissue locations. Only tryptase-positive mast cells could be demonstrated in bovine skin and uterus. No chymase activity was found in these organs, regardless of the fixation type. A larger number of mast cells was observed after fixation in Carnoy’s fluid. The three different mast cell subtypes were only demonstrated in formalin-fixed tissue; chymase-positive mast cells were not found after fixation in Carnoy’s fluid. Increasing experimental data suggest that mast cell subtypes have different functions in promoting and modulating inflammation and in remodeling the extracellular matrix. Since mast cell tryptase and chymase have different functional properties, these results may clarify the different reaction patterns observed in various organs and species.

Key words Mast cells · Heterogeneity · Tryptase · Chymase · Enzyme histochemistry · Immunohistochemistry · Bovine

Introduction

Although the immunopathological role of mast cells has been acknowledged, these cells have aroused much controversy and confusion. One explanation for the sometimes contradictory opinions on mast cell function arises from their heterogeneity, which can express itself as differences in histochemical, biochemical, and functional characteristics.

The main criterion for mast cell differentiation in rodents is formalin resistance. In humans, mast cells are distinguished by their content of the mast-cell-specific proteases, chymase and tryptase. Three mast cell subtypes in humans are differentiated: mast cells that contain tryptase (T-MC) only, mast cells that contain tryptase, chymase, carboxypeptidase, and cathepsin G (TC-MC), and mast cells that contain only chymase (C-MC); Irani et al. 1986, 1989, 1991; Schechter et al. 1990; Weidner and Austen 1993).

In contrast to humans and rodents, few data exist regarding mast cell density and formalin sensitivity in ruminants. Hunt et al. (1991) have examined bovine skin and lung mast cells by using two different fixatives and the toluidine blue staining method and have demonstrated formalin sensitivity in the majority of lung and skin mast cells. Similar results have been obtained by investigating mast cells in the lower respiratory tract of sheep (Chen et al. 1990), a study that has also shown that the density of formalin-sensitive mast cells in ovine airways is greater than that in the airways of dogs (Gold et al. 1977) and
the APAAP complex was performed twice. Between each step, Dianova) for 30 min. Incubation with the mouse anti-rabbit IgG was followed by incubation with the APAAP complex (1:100, anti-rabbit IgG (1:200, Dianova) was applied for 30 min. This step with bovine mast cell tryptase was demonstrated in a previous study (Welle et al. 1995). No counterstaining specificity of the kit for the detection of mast cell chymase was demonstrated in a previous study (Mair et al. 1988, Kube et al. 1998).

In ruminants, no systemic studies have been performed regarding mast cell heterogeneity based on their protease content. Chymase is present in mast cells of the ovine gastrointestinal tract (Huntley et al. 1986), and the number of chymase-positive mast cells increases after infection with nematodes (Huntley et al. 1987). The involvement of mast cells in the pathogenesis of bovine disease underlines the necessity for more detailed studies of mast cell heterogeneity in this species. Our intention here has been to gather species-specific data on (1) the mast cell density in various organs and tissue locations, (2) the heterogeneity based on their protease content, and (3) the relative distribution of mast cell subtypes under different fixation conditions. The surveyed data reflect the physiological distribution pattern of mast cells in the investigated organs of cattle and provide reference values for evaluating the involvement of mast cell subtypes in pathological conditions.

Materials and methods

Fresh bovine tissue specimens from 10 Holstein-Frisian cows of approximately 5 years of age were collected at a slaughterhouse. The cows were slaughtered for various reasons (e.g., broken leg, insufficient milk production). Samples were obtained from the forestomach, small bowel, uterus, lung, skin, and mesenterial lymph node. Light-microscopical analysis indicated that all samples were free of any pathological lesions. Tissue samples were fixed in 4% formalin overnight and neighboring samples were fixed in Carnoy’s fluid for 4 h. After fixation, the tissues were dehydrated in an automatic processor and embedded in paraffin. Serial sections were cut at a thickness of 4 μm.

Serial sections from each tissue sample were stained with hematoxylin and eosin, methylene blue to demonstrate the sulfated acid glycosaminoglycans in mast cell granules, and a double labelling technique for the demonstration of mast cell proteases. An enzyme-histochemical reaction was used for the detection of chymase activity and an immunohistochemical staining method with a polyclonal antibody for the detection of tryptase. The double labelling procedure was performed as follows. After being deparaffinized in xylol and acetone for 15 min each, the sections were immersed in TRIS-buffered saline (TBS), pH 7.4, and subsequently in deionized water. The enzyme-histochemical reaction for the detection of chymase was performed with a commercially available detection kit (Sigma) and naphthol-AS-D-chloroacetate as the substrate. The only modification to the protocol provided by the company was the use of Fast Blue BB (Base) instead of Fast Red Violet LB (Base). The specificity of the kit for the detection of mast cell chymase was demonstrated in a previous study (Welle et al. 1995). No counterstaining was performed. The immunohistochemical staining for mast cell tryptase was performed at room temperature immediately after the enzyme-histochemical staining and predigestion with 0.1% protease XIV (Sigma) for 4 min at 37°C. Tryptase was detected with a polyclonal rabbit anti-human skin tryptase antibody (Harvima et al. 1988) at a dilution of 1:2000. Cross-reactivity of the antibody with bovine mast cell tryptase was demonstrated in a previous study (Welle et al. 1995). After an incubation period of 30 min, a mouse anti-rabbit IgG (1:200, Dianova) was applied for 30 min. This step was followed by incubation with the APAAP complex (1:100, Dianova) for 30 min. Incubation with the mouse anti-rabbit IgG and the APAAP complex was performed twice. Between each step, the sections were washed thoroughly in TBS (pH 7.4). The antibody and the APAAP complex were diluted in RPMI buffer (pH 7.4). The alkaline phosphatase substrate was prepared by dissolving 20 mg naphthol-AS-MX-phosphate (Dianova) in 2 ml dimethylformamide and then adding 98 ml TBS (pH 8.2). Immediately before this preparation was used, Fast Red TR (100 mg) and Levamisole (1 M, 2408 mg) were added. Incubation with the substrate was performed for 30 min with continual agitation. Sections were then rinsed in tap water and mounted in Kaiser’s glycerin-gelatin. Negative controls for each tissue specimen were performed by omitting the primary antibody. Only cells with all or part of a nucleus visible were counted in order to distinguish mast cells from neutrophilic granulocytes. The quantitation of the cells was performed by using a square eyepiece graticule (objective x40, eyepiece x12.5, 10×10 squares with a total side length of 0.25 mm). Each tissue was subdivided into various locations and, if possible, at least 1000–1800 fields (~1 mm²) at each tissue location were counted.

Mast cell density was evaluated separately for each staining method and the median, the 25th, and the 75th quartile of data obtained from each tissue location was calculated (mast cell density was expressed as the number of cells per mm²). Furthermore, the median percentages of T-, TC-, and C-mast cells was evaluated. All data were evaluated in specimens fixed in formalin or in Carnoy’s fluid.

The non-parametric Wilcoxon signrank sum test was carried out between the mast cell numbers obtained by double labelling and by methylene blue stain after formalin fixation (F) or fixation in Carnoy’s fluid (C). In addition, the test was carried out between the mast cell numbers obtained with methylene blue staining and fixation in Carnoy’s fluid or formalin fixation (MB). Furthermore, the test was carried out after double labelling and fixation in Carnoy’s fluid and formalin fixation (DL). P values were considered significant when less than 0.05.

Results

The highest mast cell density was found in all organs perivascularly after methylene blue staining (Fig. 1) and double labelling (Fig. 2). After formalin fixation, three mast cell subtypes were identified in bovines: T-mast cells, which contained only tryptase and stained red with the polyclonal rabbit anti-human skin tryptase antibody (Figs. 2, 3), C-mast cells, which contained only chymase and stained blue by the enzyme-histochemical detection method with naphthol-AS-D chloroacetate as substrate for chymase (Fig. 3), and finally the TC-mast cell subtype, which contained both tryptase and chymase and stained red and blue (Fig. 3). Bovine mast cells did not display any chymase activity in tissue specimens fixed in Carnoy’s fluid. The results for each organ are below.

Forestomach

In the forestomach, the highest mast cell density was detected in the lamina propria mucosae, whereas the lowest mast cell numbers were observed in the lamina muscularis. After fixation in Carnoy’s fluid, a larger number of metachromatically staining mast cells was detected, and the values were significant in the medial and basal lamina propria. In contrast, the mast cell numbers, detectable by the double labelling, were not influenced by the fixation method used. The median values and the error bars representing the 25th and 75th percentile obtained by each staining and fixation method and the results of the statistical analysis are shown in Fig. 4a.