Journal of Dairy Research

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Phenotypic evaluation of mast cells in bovine mammary tissue and mastitis in the context of fibrosis

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Research Article

Cite this article: Özgüden-Akkoc CG, Mutlu AM, Keskin A, Yumuşak E and Akkoc A (2023). Phenotypic evaluation of mast cells in bovine mammary tissue and mastitis in the context of fibrosis. *Journal of Dairy Research* **90**, 387–392. https://doi.org/10.1017/S0022029923000651

Received: 2 January 2023
Revised: 2 October 2023
Accepted: 7 October 2023
First published online: 8 January 2024

Keywords:
Bovine; fibrosis; immunohistochemistry; mast cells; mastitis

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Abstract

This research paper addresses the hypothesis that mast cells (MCs) contribute to the formation of mammary fibrosis. MCs are important immune regulatory and immune modulatory cells that play major roles in the inflammatory process. Since there is no detailed knowledge, this research study aimed to comparatively investigate the presence, localization, and immunophenotypes of MCs in healthy and mastitic mammary tissues. A total of 264 mammary samples were evaluated for the examination of mast cells and fibrosis. The mean mast cell number in both acute and chronic mastitis samples were very significantly higher than the control group $P < 0.001$). A 7.9-fold increase in the number of mast cells was found when the chronic mastitis group was compared with the control (healthy) group. Immunohistochemistry revealed presence of all three immune phenotypes in control and mastitic mammary samples (tryptase + (MC_T), chymase + (MC_C) and both chymase and tryptase + (MC_{TC}). The mean MC_T, MC_C, and MC_{TC} numbers in the chronic mastitis group were found to be significantly higher than the control ($P < 0.001$ for all three phenotypes) but did not differ significantly between control and acute mastitis samples. When the mean numbers of MC_T, MC_C, and MC_{TC} in the control group and chronic mastitis group were compared, a 10.5, 7.8, and a 4.1-fold increase was observed, respectively. The amount of connective tissue was strongly increased in tissues with chronic mastitis and a 3.01-fold increase was detected compared to the control group. A statistically significant relation was also found between the amount of fibrosis and the increased number of total MCs ($P < 0.001$).

Mastitis is pathological inflammation of the mammary tissue mostly caused by infectious agents. It is considered the most common disease leading to severe financial losses due to disposal of infected milk, veterinarian costs, treatment and hygiene expenses, as well as early culling of affected animals from the herd. The total financial loss due to a dairy cattle suffering from mastitis is between €65 and €182 per cow/per year (Huijps *et al.*, 2008). Clinical and subclinical mastitis are fundamental entitles in dairy cattle and clinical mastitis is characterized by sudden onset, alterations of milk quality and appearance, decreased milk production, and the presence of the basic signs of inflammation. The diagnosis of acute mastitis is fairly easy and rapid, and treatment interventions are largely successful. On the contrary, subclinical mastitis is insidious and there are no visible signs of inflammation except a decrease in milk production (Sharun *et al.*, 2021). Inefficient treatment protocols as well as the prolonged inflammatory process in subclinical mastitis causes loss of functional units and progressive fibrosis in the mammary tissue. Fibrosis can be briefly defined as an increase in extracellular matrix (ECM) components and the proliferation of connective tissue cells. It occurs irreversibly following chronic inflammation in various tissues (Dees *et al.*, 2020). The replacement of the parenchyma with fibrotic tissue leads to dysfunction, which in the mammary tissue is characterized by a severe decrease or cessation of milk production. It is not possible to completely prevent or reverse fibrosis within the current medical capabilities. New and effective therapeutic approaches can only be possible with the understanding of the pathogenesis of fibrosis. One of the novel candidates in the combat against fibrosis may be MCs and their unique cytoplasmic proteases such as tryptase and chymase (Asano-Kato *et al.*, 2005; Kosanovic *et al.*, 2015). MCs originate from pluripotent hematopoietic progenitor cells of the bone marrow (Elieh Ali Komi *et al.*, 2020). Immature precursor MCs remain in circulation for a short period and then travel to tissues where they acquire the morphological and biochemical characteristics of mature MCs under the influence of growth factors such as stem cell factor, interleukin-3 (IL-3), IL-4, IL-10, IL-33, stromal cell-derived factor-1, nerve growth factor and transforming growth factor-β (TGF-β) (Elieh Ali Komi *et al.*, 2020). MCs are

divided into 3 different immunophenotypes as tryptase + mast cells (MC_T), chymase (+) mast cells (MC_C), and tryptase-chymase + mast cells (MC_{TC}) according to their cytoplasmic enzyme contents (Moon *et al*., 2014). Although the presence of MCs in developing and adult mammary tissues of heifers and cows has been examined histochemically and by electron microscopy, no data on the immunophenotypes of MCs were found (Nielsen, 1975; Beaudry *et al*., 2016). MCs were only nominally mentioned among other inflammatory cells in studies on the histopathological features of bovine mastitis (Nickerson and Heald, 1982), but no detailed information was provided. MCs have the function of killing non-phagocytosed bacterial clusters in the extracellular space through the production of extracellular traps containing DNA fragments, histone proteins, tryptase enzymes and antibacterial peptides (Möllerherm *et al*., 2016). Therefore, MCs may be important in mastitis, which is mostly caused by bacteria. Though MCs have cardinal function in innate and acquired immune responses, their effect on fibrosis has been questioned in recent years (Summers *et al*., 2012; Lombardo *et al*., 2019). There are studies examining the possible role of MCs and their mediators in experimentally induced fibrosis in various organs such as the lung, kidney, liver and heart (Matsumoto *et al*., 2003; Komeda *et al*., 2010; Summers *et al*., 2012; Overed-Sayer *et al*., 2020) and it has been reported that the severity of fibrosis is significantly reduced in tissues where MCs are inhibited. Furthermore, results of studies in mast cell-deficient mouse models revealed that these animals are protected from renal and pulmonary fibrosis, whilst reintroduction of wild-type bone marrow-derived mast cells into mast cell-deficient mice restored fibrosis (Summers *et al*., 2012; Veerappan *et al*., 2013).

The effects of MC tryptase and chymase enzymes on the fibrotic process have been examined. Tryptase enzyme has been shown to increase fibroblast proliferation, migration and collagen synthesis. Increased MC_T with elevated pulmonary tryptase levels has been reported in pulmonary fibrosis (Wygrecka *et al*., 2013). Similarly, a significantly increased number of MC_C phenotype MCs were reported in hypertrophic cutaneous lesions by Chen *et al*. (2017). They also demonstrated that chymase augments dermal fibroblast proliferation and collagen synthesis in vitro. Similarly, chymase treatment also induces proliferation and collagen synthesis in cardiac fibroblasts (Zhao *et al*., 2008). Matsumoto *et al*. (2003) reported that the number of MCs in cardiac tissue was increased in dogs with fibrosis and emphasized that chymase inhibition both prevents fibrosis and improves diastolic dysfunction. Kanemitsu *et al*. (2008) demonstrated that chronic inhibition of chymase prevented fibrosis after the myocardial infarction model. Although the role of MCs in naturally occurring or experimentally induced fibrosis in various animals has been studied, there are no data on bovine mammary tissue. Therefore, we aimed to investigate the presence of MCs and identify their immune phenotypes in healthy and mastitic mammary tissues with fibrosis. We believe that it is essential to examine whether mammary fibrosis is associated with an increased number of MCs for further clinical studies aimed at treatment or alleviation of fibrotic changes.

Material and methods

A total of 264 bovine mammary samples were collected from a local slaughterhouse (Bursa, Turkey). The presence of mastitis in the samples, the number of healthy, acute-chronic mastitic tissues and the parameters used in this classification are presented in the first part of the results section. The study was approved by the University of Bursa Uludağ Institutional Animal Care and Use Committee (Protocol Number: 2019-07/04). Sampling, tissue processing and staining for microscopical evaluation are detailed in the Supplementary file.

Masson's trichrome staining procedure was used to demonstrate connective tissue and fibrosis in mammary tissues. The amount of connective tissue was analysed in digital images taken from 10 randomly selected fields at ×200 magnification in a microscope using Olympus Stream Motion software (Shinjuku, Tokyo, Japan). The image analysis protocol is detailed in Supplementary Figs S1–S3.

MCs in mammary samples were demonstrated by toluidine blue staining. The number, localization and activity (granulated/degranulated) of MCs in 10 randomly selected fields were evaluated in a blinded manner. MCs were considered as degranulated if there was an extensive dispersion of more than 15 extruded vesicles localized near the cell, or an extensive loss of granule staining, giving the cell a 'ghostly' look. The number of granulated, degranulated and total MCs were expressed as mean numbers and fold increases for each group.

The immunohistochemical staining was performed using UltraVision Detection System anti-polyvalent HRP/DAB (TP-015-HD, Thermo Fisher Scientific, Waltham, MA, USA) to detect the localization of MC_T, MC_C and MC_{TC}. Isotope IgG antibody was applied to achieve negative control staining and skin samples were used as the positive control. Detailed immunohistochemistry protocol is given in the Supplementary File.

Statistical data analysis was performed using IBM SPSS Statistics version 20. Data was tested with the Shapiro–Wilk test for normal distribution. Group comparisons were performed using the one-way analysis of variance (ANOVA) test when data were normally distributed, otherwise, the Kruskal–Wallis test was used. Results were considered significant at $P < 0.05$. In addition, the Pearson correlation test was used to determine the correlation between mast cells and fibrotic lesions in chronic mastitis samples.

Results

Healthy and functionally active mammary glands had a soft consistency; cross-sectional surfaces of healthy samples displayed pinkish-cream colour and a well-developed lobuloalveolar structure. Milk oozing was evident from the cut surfaces of these samples. Supramammary lymph nodes of normal size and consistency were observed. In cases of acute mastitis, redness, presence of oedema, purulent exudation and clotted milk were noted in the cut surfaces of mammary samples. In addition to these common findings, necrotic changes with discolorations were detected in some cases. Firm mammary lobules with thick, white interlobular fibrous tissues were primary findings in chronic mastitis cases (online Supplementary File Fig. S4). No milk production was observed in those samples.

Histopathologically, no prominent lesions were observed in 119 out of 264 mammary samples (45.7%: online Supplementary Figs S5A, B). Hyperaemic blood vessels, oedema in interlobular spaces, neutrophil leucocyte infiltrations of varying severity in alveolar and duct lumens, degenerated and necrotic alveolar and tubular epithelial cells were noticed in acutely inflamed samples (101/264, 38%: online Supplementary Figs S6A, B). Chronic mastitis was diagnosed in 44 out of 264 (16.6%) of mammary samples. Microscopic examination of

https://doi.org/10.1017/S0022029923000651 Published online by Cambridge University Press

mammary samples with chronic mastitis revealed fibrous tissue proliferation together with mononuclear cell infiltrations in the interstitial spaces and severe atrophy in alveolar structures (online Supplementary Figs S7A, B).

MCs with metachromatic granules were visualized by toluidine blue staining in tissue sections and they were localized around blood and lymph vessels, interstitial spaces, alveoli and ducts in healthy mammary tissues (online Supplementary Figs S8A, B). In normal bovine mammary tissue, a small number of MCs were found, in particular around vessels of connective tissue. MC numbers were significantly increased in tissue samples with acute and chronic mastitis when compared with healthy controls. In contrast to healthy samples, the localization of mast cells was shifted in mastitic tissues, in that a large number of mast cells were observed not only in the interstitial area but also in the parenchymal tissue. The mean number of MCs in healthy tissues was found to be 4.14. The mean number of MCs in acute mastitis cases was significantly increased (10.05) and there was a 2.42-fold increase in the number of MCs compared to healthy mammary tissues ($P < 0.001$). The mean number of MCs in tissue samples with chronic mastitis was found to be 32.7 and a 7.9-fold increase was detected when compared with healthy controls ($P < 0.001$). Furthermore, the activity of MCs was evaluated according to whether they were degranulated or not. The mean number of degranulated MCs in healthy controls, acute mastitis and chronic mastitis cases were 1, 2.5, and 6.8, respectively. Therefore, the mean number of degranulated MCs was 2.5-fold higher in acute mastitis cases and 6.8-fold higher in chronic mastitis cases

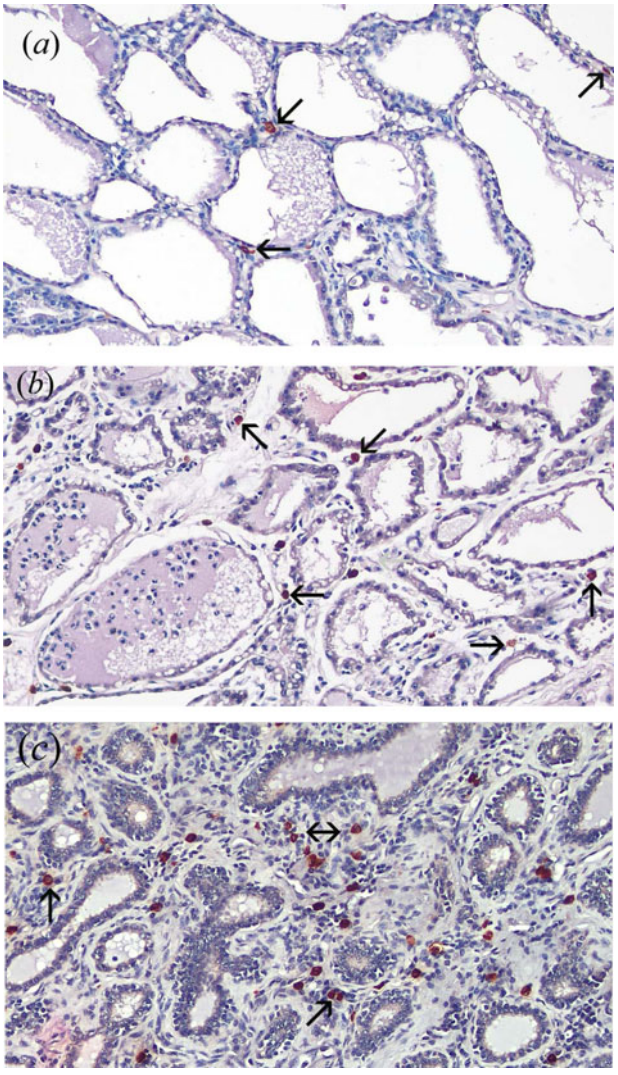

Figure 1. Tryptase positive mast cells (arrows and double-headed arrows) around the alveoli in (a) healthy mammary tissue, (b) mammary tissue with acute mastitis showing increased number as well as presence in neutrophil infiltrations within the lumen of alveoli and (c) in the interstitial areas of mammary tissue with chronic fibrotic mastitis, showing greatly increased number. Streptavidin–biotin–peroxidase method, DAB chromogen, ×200 magnification.

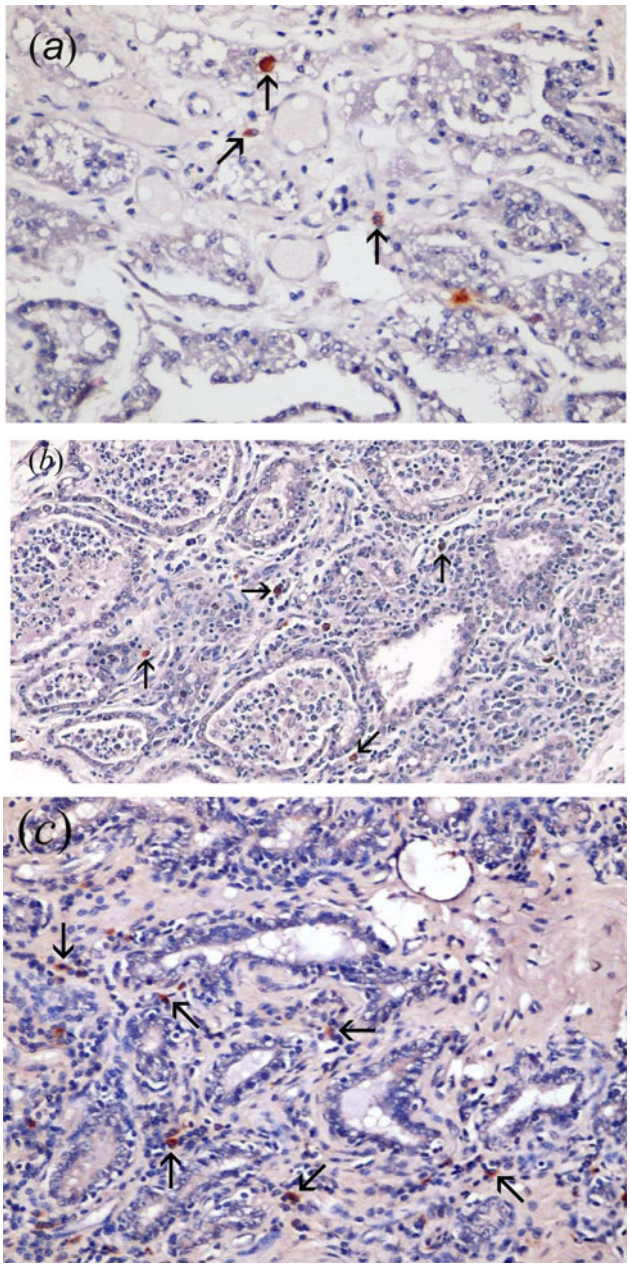

Figure 2. Chymase-positive mast cells (arrows) around the alveoli in (a) healthy mammary tissue, (b) mammary tissue with acute mastitis showing increased number as well as presence in neutrophil infiltrations within the lumen of alveoli and (c) in the interstitial areas of mammary tissue with chronic fibrotic mastitis, showing greatly increased number. Streptavidin–biotin–peroxidase method, DAB chromogen, ×200 magnification.

https://doi.org/10.1017/S0022029923000651 Published online by Cambridge University Press

compared to healthy controls. The mean number of intact and degranulated MCs and fold increases in healthy, acute and chronic mastitis mammary tissues are summarized in online Supplementary Table S1 and the *P* values are summarized in online Supplementary Table S2.

Immunohistochemical staining results revealed that all three phenotypes of MCs (MC_T, MC_C, and MC_{TC}) were detected in healthy controls and mastitic samples (Figs. 1–3). The mean number of MC_T in healthy controls, acute and chronic mastitis cases were 1.8, 4.4, and 19, respectively. No statistically significant difference was found when MC_T in healthy controls was compared with acute mastitis cases ($P > 0.05$). A statistically significant difference was found when MC_T in healthy controls was compared with samples with chronic mastitis ($P < 0.001$). Similarly, when MC_T cells in acute and chronic mastitis were compared with each other, the difference was found to be statistically significant ($P < 0.001$).

The mean number of MC_C in healthy controls, acute and chronic mastitis cases were 1, 2.6, and 7.8, respectively. When the mean number of MC_C observed in healthy controls was compared with the mean number of MC_C in samples with acute and chronic mastitis, the increases observed in both acute and chronic mastitis were statistically significant (both $P < 0.001$). Finally, the mean numbers of MC_{TC} phenotypes were compared in healthy, acute, and chronic mastitis groups. No statistically significant difference was found between healthy control and acute mastitis samples ($P > 0.05$), but a statistically significant difference was found between healthy and chronic mastitis groups ($P < 0.01$). When the mean MC_{TC} number of acute mastitis and chronic mastitis samples were compared with each other, the difference was statistically significant ($P > 0.01$). The mean number of MC phenotypes and fold increases in healthy, acute and chronic mastitic samples are summarized in the Supplementary file (Online Supplementary Table S3 and *P* values in Table S4).

Fibrotic changes in mammary samples were evaluated by Masson's trichrome staining and measured by ImageJ analysis software. The average amounts of connective tissue measured in controls and samples with acute mastitis were found to be 9.9 and 12.35%, respectively, and no statistical significance was found. The average amount of connective tissue in chronic mastitic samples reached 29.83%. Compared to the control group, the average amount of connective tissue increased 3.01-fold in chronic mastitic samples. The possible relationship between MCs and fibrosis in samples with chronic mastitis was also investigated by correlation analyses and it was found that there was a positive correlation between the increasing number of total MCs and the severity of fibrosis (Pearson's correlation coefficient (*r*) for total MCs: 0.76; $P < 0.05$). A similar positive correlation was also obtained from the total number of granulated and degranulated mast cells with tissue fibrosis (Pearson's correlation coefficient (*r*) for granulated MCs 0.69 and for degranulated MCs 0.55, both $P < 0.05$).

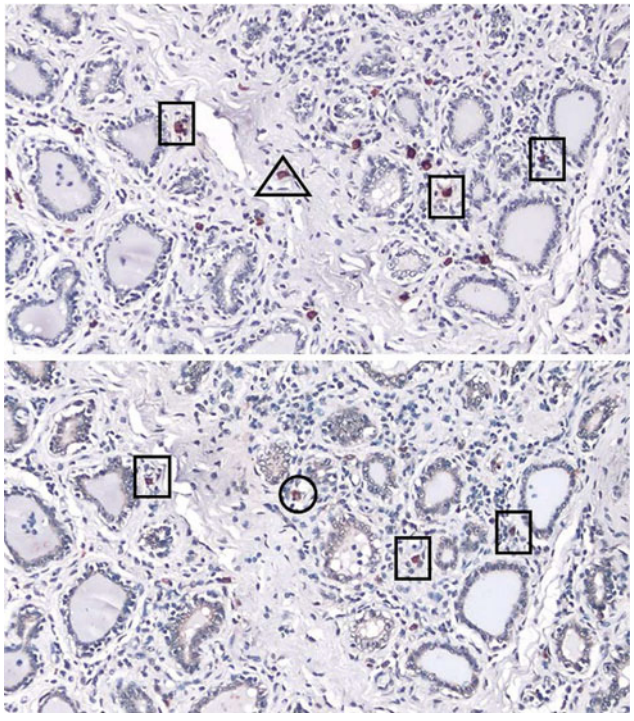

Figure 3. Determination of tryptase–chymase positive mast cell phenotype in serial sections of mammary tissues. Determination of tryptase–chymase + mast cell phenotype according to tryptase (top picture) and chymase (bottom picture) staining results. Mast cells in rectangles are those stained positively with both tryptase and chymase antibodies and have tryptase-chymase + mast cell phenotype. Cells marked with triangles and circles are mast cells with tryptase + and chymase + phenotypes, respectively. Streptavidin-biotin peroxidase, DAB, ×200 magnification.

Discussion

We have investigated the presence and immune phenotypes of MCs in normal bovine mammary tissue, and results were compared with acute and chronic mastitic samples displaying non-fibrotic and fibrotic changes, respectively. To the best of our knowledge, this is the first detailed documentation of the existence of MCs in bovine mammary tissue and mastitis. In the following sections, since there is no data on the presence and immune phenotypes of MCs in bovine mammary tissues, the results are mainly discussed in relation to results of previous human and laboratory animal studies.

The majority of mastitis cases in dairy cattle are subclinical and the inflammation in the mammary tissue progresses insidiously, fibrosis is inevitable in cases that are not diagnosed in the early stages of inflammation or not completely treated. MCs are innate immune cells and have pivotal roles in the modulation of acute and chronic inflammation. These heterogeneous cells are classified according to their histochemical staining features and in their content of preformed biological granules. This heterogeneity directly affects their functions and responses to inflammatory stimuli. Moreover, this heterogeneity is remarkable even in different micro-anatomical localization of organs (Bradding, 2009). For example, different lung compartments in humans contain mast cells with distinct granules, which can be classified as MCs containing either tryptase only (MC_T), chymase only (MC_C), or both tryptase and chymase (MC_{TC}). Weidner and Austen (1993), reported that MC_{TC} and MC_T phenotypes were present in human breast tissue, but the MC_C phenotype was not found. The results of our study showed that MCs with three distinct phenotypes are present in bovine mammary tissue, which is different from human. In bovine mammary tissue, MCs are localized around stromal blood and lymph vessels, in the interalveolar interstitial tissue and around the duct system. Microanatomically, it was noted that MC immune phenotypes did not exhibit a specific distribution pattern and all phenotypes were present in the indicated histologic compartments.

The presence of a large number of MCs in tissues in close contact with the external environment increases the importance of

https://doi.org/10.1017/S0022029923000651 Published online by Cambridge University Press

the evaluation of these cells in mammary tissue. In this study, the parenchyma of the mammary tissue was the primarily examined compartment. Although we have histochemically examined MC distribution in mammary papillae (results were not included), immunohistochemical examinations have not been performed. Studies on the localization of MCs even in healthy mammary tissue are extremely limited and there is no data on their presence, immune phenotypes and possible roles in mastitis. In the study by Beaudry *et al.* (2016), the localization and numbers of MCs in bovine prepubertal mammary tissue were investigated. Although their study provides valuable data on the roles of MCs in the development of mammary tissue, no data on the phenotype of these cells were provided. Additionally, one of the most important differences between the studies is our examination of histochemical and immune phenotypes of MCs in both healthy and mastitic tissues. The presence of MCs in pregnant, lactating and involuting mouse mammary glands has also been documented (Szewczyk *et al.*, 2000; Lilla *et al.*, 2009). In the study by Lilla *et al.* (2009) only the chymase positive phenotype of MCs was targeted and the presence of this phenotype in healthy mammary tissue of mice was demonstrated, but no data on other phenotypes were included. Szewczyk *et al.* (2000) examined the presence and number of mast cells histochemically in the development and involution of mammary tissue in mice, but they did not provide any data on mast cell immune phenotypes. Our research differs from the study of Szewczyk *et al.* (2000) since it provides data on mast cells and their immune phenotypes in mammary samples, as well as including healthy and mastitic tissues.

Fibrosis can be defined as the gradual loss of parenchymal units and an increase in connective tissue cells and non-functional extracellular matrix proteins. This process is irreversible and there is no effective treatment. MCs can synthesize a wide range of mediators with profibrotic properties such as TGF-β, platelet-derived growth factor and fibroblast growth factor (Dees *et al.*, 2020). These mediators stimulate fibroblasts to produce ECM components. In addition, MC tryptase promotes collagen synthesis by fibroblasts. The upregulated proliferation of conjunctival fibroblasts by tryptase treatment in a dose-dependent manner has been demonstrated (Asano-Kato *et al.*, 2005) and the authors also reported that inhibition of tryptase activity *via* specific receptor blockers prevented tyrptase-induced fibroblast proliferation. Frungieri *et al.* (2005) have demonstrated that MCs actively contribute to liver fibrosis *via* the release of tryptas, and Lombardo *et al.* (2019) reported a direct correlation between the number of mast cells and severity of hepatic fibrosis. The results of previous experimental studies have revealed that tryptase phenotype MCs are more prominent than the other two MC phenotypes in the formation of fibrosis in different organs. In our study, an increased number of MCs with tryptase phenotype were found in fibrotic mammary tissue, which is consistent with previous studies. Chymase is another important component of MCs involved in fibrosis in various organs (Matsumoto *et al.*, 2003; Komeda *et al.*, 2010; Pons *et al.*, 2017). Pons *et al.* (2017) showed that MC chymase contributes to renal fibrosis through the epithelial-mesenchymal transition in the ureteral obstruction model. Matsumoto *et al.* (2003) demonstrated that chymase inhibition is important in the prevention of cardiac fibrosis in dogs. Similarly, Komeda *et al.* (2010) reported that chymase inhibition resulted in the attenuation of liver fibrosis in hamsters. Our results provide scientific background for further clinical approaches targeting fibrosis in bovine mammary gland.

Since the degranulation of MCs is a useful indicator of their activity, we also evaluated the number of degranulated MCs in the tissue sections and showed that it was greatly increased in acute and chronic mastitis. This indicates that the increased number and activity of MCs in tissues with mastitis (acute and/or chronic) may play important roles in the pathogenesis of bovine mastitis.

In conclusion, we found that the total number of MCs was significantly increased in mammary samples with mastitis and the mean number of MCs was highest in chronic fibrotic mastitis cases. We demonstrated statistical correlations between increased densities of MCs, degree of fibrosis and parenchymal collagen density in chronic mastitic samples. These observations shed light on the possible role of MCs in the pathogenesis of mammary fibrosis. Further studies are needed to understand the clinical significance of MC inhibition in the prevention or alleviation of fibrotic changes in bovine mastitis.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0022029923000651

Acknowledgment. This work was supported by The Scientific and Technological Research Council of Türkiye (TUBITAK-TOVAG-Research Project ID: 118O893).

References

Asano-Kato N, Fukagawa K, Okada N, Dogru M, Tsubota K and Fujishima H (2005) Tryptase increases proliferative activity of human conjunctival fibroblasts through protease-activated receptor-2. *Investigative Ophthalmology Vision Science* **46**, 4622–4626.

Beaudry KL, Parsons CL, Ellis SE and Akers RM (2016) Localization and quantitation of macrophages, mast cells, and eosinophils in the developing bovine mammary gland. *Journal of Dairy Science* **99**, 796–804.

Bradding P (2009) Human lung mast cell heterogeneity. *Thorax* **64**, 278–280.

Chen H, Xu Y, Yang G, Zhang Q, Huang X, Yu L and Dong X (2017) Mast cell chymase promotes hypertrophic scar fibroblast proliferation and collagen synthesis by activating TGF-β1/Smads signaling pathway. *Experimental and Therapeutic Medicine* **14**, 4438–4442.

Dees C, Chakraborty D and Distler J (2020) Cellular and molecular mechanisms in fibrosis. *Experimental Dermatology* **30**, 121–131.

Elieh Ali Komi D, Wöhrl S and Bielory L (2020) Mast cell biology at molecular level: a comprehensive review. *Clinical Reviews in Allergy & Immunology* **58**, 342–365.

Frungieri MB, Albrecht M, Raemsch R and Mayerhofer A (2005) The action of the mast cell product tryptase on cyclooxygenase-2 (COX2) and subsequent fibroblast proliferation involves activation of the extracellular signal-regulated kinase isoforms 1 and 2 (erk1/2). *Cell Signalling* **17**, 525–533.

Huijps K, Lam JGM T and Hogeveen H (2008) Costs of mastitis: facts and perception. *Journal of Dairy Research* **75**, 113–120.

Kanemitsu H, Takai S, Tsuneyoshi H, Yoshikawa E, Nishina T, Miyazaki M, Ikeda T and Komeda M (2008) Chronic chymase inhibition preserves cardiac function after left ventricular repair in rats. *European Journal of Cardio-thoracic Surgery* **33**, 25–31.

Komeda K, Takai S, Jin D, Tashiro K, Hayashi M, Tanigawa N and Miyazaki M (2010) Chymase inhibition attenuates tetrachloride-induced liver fibrosis in hamsters. *Hepatology Research* **40**, 832–840.

Kosanovic D, Luitel H, Dahal BK, Cornitescu T, Janssen W, Danser AH, Garrelds IM, De Mey JG, Fazzi G, Schiffers P, Iglarz M, Fischli W, Ghofrani HA, Weissmann N, Grimminger F, Seeger W, Reiss I and Schermuly RT (2015) Chymase: a multifunctional player in pulmonary hypertension associated with lung fibrosis. *European Respiratory Journal* **46**, 1084–1094.

Lilla JN, Joshi RV, Craik CS and Werb Z (2009) Active plasma kallikrein localizes to mast cells and regulates epithelial cell apoptosis, adipocyte differentiation, and stromal remodeling during mammary gland involution. *Journal of Biological Chemistry* **284**, 13792–13803.

Lombardo J, Broadwater D, Collins R, Cebe K, Brady R and Harrison S (2019) Hepatic mast cell concentration directly correlates to stage of fibrosis in NASH. *Human Pathology* **86**, 129–135.

Matsumoto T, Wada A, Tsutamoto T, Ohnishi M, Isono T and Kinoshita M (2003) Chymase inhibition prevents cardiac fibrosis and improves diastolic dysfunction in the progression of heart failure. *Circulation* **107**, 2555–2558.

Möllerherm H, von Köckritz-Blickwede M and Branitzki-Heinemann K (2016) Antimicrobial activity of mast cells: role and relevance of extracellular DNA traps. *Frontiers in Immunology* **7**, 265.

Moon TC, Befus AD and Kulka M (2014) Mast cell mediators: their differential release and the secretory pathways involved. *Frontiers in Immunology* **5**, 569.

Nickerson SC and Heald CW (1982) Cells in local reaction to experimental *Staphylococcus aureus* infection in bovine mammary gland. *Journal of Dairy Science* **65**, 105–116.

Nielsen EH (1975) The fine structure of mast cells in the normal bovine mammary gland. *Anatomia Histologia Embryologia* **4**, 360–367.

Overed-Sayer C, Miranda E, Dunmore R, Liarte Marin E, Beloki L, Rassl D and Clarke D (2020) Inhibition of mast cells: a novel mechanism by which nintedanib may elicit anti-fibrotic effects. *Thorax* **75**, 754–763.

Pons M, Ali L, Beghdadi W, Danelli L, Alison M, Madjène LC, Calvo J, Claver J, Vibhushan S, Åbrink M, Pejler G, Poli-Mérol ML, Peuchmaur M, El Ghoneimi A and Blank U (2017) Mast cells and MCPT4 chymase promote renal impairment after partial ureteral obstruction. *Frontiers in Immunology* **4**, 450.

Sharun K, Dhama K, Tiwari R, Gugjoo MB, Yatoo MI, Patel SK, Pathak M, Karthik K, Khurana SK, Singh R, Puvvala B, Amarpal, Singh R, Singh KP and Chaicumpai W (2021) Advances in therapeutic and managemental approaches of bovine mastitis: a comprehensive review. *Veterinary Quarterly* **41**, 107–136.

Summers SA, Gan PY, Dewage L, Ma FT, Ooi JD, O'Sullivan KM, Nikolic-Paterson DJ, Kitching AR and Holdsworth SR (2012) Mast cell activation and degranulation promotes renal fibrosis in experimental unilateral ureteric obstruction. *Kidney International* **82**, 676–685.

Szewczyk G, Szukiewicz D, Zaczek R and Maslinska D (2000) Mast cells in the mouse mammary gland correlation with the development of lactiferous structures. *Folia Biologica (Krakow)* **48**, 13–17.

Veerappan A, O'Connor NJ, Brazin J, Reid AC, Jung A, McGee D, Summers B, Branch-Elliman D, Stiles B, Worgall S, Kaner RJ and Silver RB (2013) Mast cells: a pivotal role in pulmonary fibrosis. *DNA and Cell Biology* **32**, 206–218.

Weidner N and Austen KF (1993) Heterogeneity of mast cells at multiple body sites. Fluorescent determination of avidin binding and immunofluorescent determination of chymase, tryptase, and carboxypeptidase content. *Pathology-Research and Practice* **189**, 156–162.

Wygrecka M, Dahal BK, Kosanovic D, Petersen F, Taborski B, von Gerlach S, Didiasova M, Zakrzewicz D, Preissner KT, Schermuly RT and Markart P (2013) Mast cells and fibroblasts work in concert to aggravate pulmonary fibrosis: role of transmembrane SCF and the PAR-2/PKC-α/Raf-1/p44/42 signaling pathway. *The American Journal of Pathology* **182**, 2094–2108.

Zhao XY, Zhao LY, Zheng QS, Su JL, Guan H, Shang FJ, Niu XL, He YP and Lu XL (2008) Chymase induces profibrotic response *via* transforming growth factor-beta 1/Smad activation in rat cardiac fibroblasts. *Molecular and Cellular Biochemistry* **310**, 159–166.

https://doi.org/10.1017/S0022029923000651 Published online by Cambridge University Press