IN Volvement OF CD8+ Cells IN protective mechanisms in canine mammary adenocarcinomas (short communication)

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Abstract

The study aimed at demonstration of involvement of CD8+ cells in protective mechanisms in canine mammary gland carcinomas. Material for the studies involved metastases to lungs sampled at autopsy from crossbreed bitches aged 11 to 13 years. The tumours were verified histopathologically as adenocarcinomas. The presence of CD8+ cells infiltrating the examined tumour tissues was estimated in cryo-sections by immunofluorescence methods. Parental tumours free of metastases were accompanied by slight infiltration of CD8+ cells. In the case of metastasing tumours, and especially in their pulmonary metastases, the increased number of CD8+ cells was observed. Taken together, metastasing potential of canine mammary adenocarcinomas was positively correlated with the number of CD8+ cells in the tumour stroma.

Key words: bitch, CD8+, adenocarcinoma, mammary gland.

Tumours consist of parenchyma and, usually, stroma (extracellular matrix, ECM). The classification of tumours is determined by the type of parenchyma cells. Nevertheless, especially blood vessels and lymphocytes situated in the stroma influence the future fate of the parenchyma cells (5). Moreover, the presence of infiltrating cells within the stroma, particularly in cancers, manifests defence abilities of the host.

The considerable diversity of tumour infiltrating lymphocytes (TIL) was noticed (6). It was reported that CD3+ CD8+ cells and CD3+ CD4+ cells are mainly included in TIL of adenocarcinoma (3). Moreover, presence of CD19+ B cells has been noted, while CD3– CD16+ NK cells were rare. TIL are being used in anti-neoplastic therapy, which following stimulation with interleukin 2 (IL-2) results in augmented cytotoxic activity and elevated number of tumour-enclosed cytotoxic T cells (CTL). Experiments on animals indicate that TIL may be even 100-fold more effective in tumour treatment than LAK (lymphokine activated killer) cells (4).

Among lymphocytes infiltrating tumour tissues, cytotoxic T cells CD8+ play an important role. They manifest the ability of killing virus-infected or tumour cells by recognising antigens in association with autologous class I MHC (major histocompatibility complex) molecules, e.g., tumour cells induced by HPV or HBV viruses (1, 8, 9). Activated CD8+ cells secrete perforins and granzymes (serine esterases). Perforin inserting itself into cell membrane of a target cell, in presence of Ca2+ ions forms a transmembrane channel which disrupt ionic balance of the cells and facilitates the penetration of the target cell by CTL-released enzymes. Moreover, the FasL (CD95L) molecule, present on the surface of CD8+ cells, interacts with Fas (CD95) molecule, appearing on the surface of the targeted cell, and induces apoptosis of the latter (4, 7).

The involvement of CD8+ cells in protective mechanisms in canine mammary adenocarcinomas and their metastases has not been fully examined and its recognition was the aim of the present studies.

Material and Methods

Sample collection. Material for the studies included primary tumours of mammary gland and their metastases to lungs, sampled at autopsy from crossbreed bitches aged 11 to 13 years. The tumours were verified as adenocarcinomas using histopathological examination. After collection, the fragments of tissues were frozen in liquid nitrogen and stored at -20°C.

Reagents and basic equipment. Rat anti-canine CD8 antibody was purchased from AbD Serotec (Oxford, UK). Alexa Fluor® 488 goat anti rat IgG (H+L) antibody was bought from Molecular Probes (Eugene, Oregon, USA).
Fig. 1. Mammary adenocarcinoma with no metastases and slight CD8+ cell infiltration (arrow). 400x.

Fig. 2. Mammary adenocarcinoma (parental tumour), which formed metastases with moderate infiltration of CD8+ cells (arrows). 400x.

Fig. 3. Metastasis of mammary adenocarcinoma to lung with strong infiltration of CD8+ cells (arrows). 400x.

Fig. 4. Negative control: normal inguinal lymph node. 100x.
Image-iT FX signal enhancer, ProLong Gold anti-fade reagent were bought from Invitrogen (Carlsbad, California).

**Indirect immunostaining of frozen tissue sections.** Tumour cryo-sections, 5-10 µm thick, were fixed in cold-dry acetone for 15 min. The slides were then washed 3 times in PBS, incubated for 20 min with Image-iT FX signal enhancer and washed again in PBS. After that, cryo-sections were incubated overnight at 4°C with primary antibody (rat anti canine CD8+) at 1:30 dilution. Next day, the sections were washed 3 times in PBS and incubated with the second antibody (Alexa Fluor® 488 goat anti rat IgG) at 1:400 dilution at 37°C. After 1.5 h incubation, the, the slides were washed 3 times in PBS and counterstained for 5 min with propidium iodide (2 µg/mL). After that, the sections were washed in PBS and mounted in ProLong Gold anti-fade reagent. All fluorophore labelled tissue sections were analysed using an AxioImager.M1 Zeiss fluorescence microscope equipped with AxioVision Rel.4.5 software.

**Results and Discussion**

It was demonstrated that parental tumours free of metastases were accompanied by slight infiltration of CD8+ cells (Fig. 1). In the case of parental metastasing tumours, the increased number of CD8+ cells was observed (Fig. 2), while in the pulmonary metastases even clusters of the numerous CD8+ cells were visible (Fig. 3).

Samples of normal inguinal lymph nodes from negative control (without primary antibody) (Fig. 4) and from positive control (Fig. 5).

As mentioned above, infiltration of immune cells into tumour stroma represents a defence reaction of the host against the tumour. The reactions are rare in advanced cancer diseases, *e.g.* in highly differentiated human mammary cancer the stroma is more dense and parvocellular than in cancers of low differentiation, in which slender stroma is infiltrated by numerous immune cells (5).

On one hand, the presence of immune cells in tumour ECM is favourable for the host, as it leads to partial elimination of tumour cells. This reflects the fact that tumours carry specific antigens, capable of inducing both humoral and cell-mediated immune reactions. On the other hand, the presence of infiltrating cells in tumour tissue may promote tumour cells migration and metastases formation. It was proved that the developing inflammatory reaction with oedematous lesions and tissue necrosis facilitates the penetration of tumour cells to tissue spaces and their further intravascular import by reverse diapedesis (5). Bilik et al. (2) demonstrated positive correlation between number of CD8+ lymphocytes which infiltrated stroma of human breast cancer and number of metastases to axillary lymph nodes. Similarly, Chin *et al.* (3) found that augmented ratio of CD4/CD8 cells in lymphocyte infiltrate of human ductal mammary carcinoma may point to facilitated metastase development in lymph nodes. Thus, the high expression of CD4+ may lead to the progression of the parental tumour.

Our studies have shown that metastasing potential of canine mammary adenocarcinoma reveals positive correlation with the number of CD8+ cells, located in its stroma. The observations should be extended to other cell populations in ECM (CD4+, NK).

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References


