INVOLVEMENT OF EPITHELIAL DENDRITIC CELLS (LANGERHANS CELLS) AND MACROPHAGES IN IMMUNE MECHANISMS IN SELECTED TUMOURS OF DOG SKIN

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Abstract

The studies attempted to demonstrate the involvement of epithelial dendritic cells and macrophages in mechanisms of anti-neoplastic immune surveillance in dogs and to define whether local antigen presentation and activation of lymphocytes T takes place in the skin, similarly as it develops in the draining of lymph nodes. Eight skin specimens of basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) for the presence of the skin immune system cells above the tumour were examined. Sections of the sampled tissue were used to produce paraffin histopathological sections and ultrastructural preparations. The material was then examined under transmission electron microscope. Langerhans cells, macrophages, mastocytes, and lymphocytes in perilesional skin close to BCC and SCC were found. The presence of numerous dendritic cells, macrophages, as well as mast cells and lymphocytes found in the direct vicinity of the tumour, indicate that the skin immune system is involved in the local immune response.

Key words: dogs, Langerhans cells, macrophages, skin, epithelial tumours.

The skin’s immune system (SIS), termed as sometimes the skin associated lymphoid tissue (SALT), consists of dendritic cells, keratinocytes, lymphocytes T, vascular endothelium cells, macrophages, granulocytes, mast cells, and melanocytes. The principal aim of the SIS function is the development of appropriate mechanisms of detection and elimination of virus-infected cells and transformed cells, including neoplastically transformed cells (9, 10).

The most effective group of cells that recognise and present antigens (antigen presenting cells, APC) to lymphocytes T involves dendritic cells (DC). There are two subpopulations of DC originating from the same bone marrow precursor CD34+ cell, which differentiating into CD33+ cells provides the origin of myeloid cell line, while transforming into CD10+ cells provides the origin of the lymphoid cell line. The CD33+ cells transform further into CD14+ cells from which macrophages and tissue DC originate, or to CD1a+ cells, which provides the origin for Langerhans cells (LCs) or epidermal dendritic cells (14). Morphological markers of LCs in humans and in most of animal species involve typical organelles, termed Birbeck granules. The exceptions include dogs in which the structures appear sporadically only (8, 13). LCs may present antigens using molecules of main histocompatibility complex (MHC) of either class II or class I (14), in contrast to macrophages, which carry no MHC class II molecules, but in specific circumstances they may synthesise them under the effect of interferon γ (IF-γ) (9). Nevertheless, the principal function of macrophages in the course of inflammatory process involves not antigen presentation but phagocytosis (9), which can be executed due to numerous hydrolyses present in late endosomes and in lysosomes, serving for intracellular digestion. Macrophages release also cytokines, i.e. interleukins (ILs – 1, 4, 6, 8, 10, and 12), participating in immune reactions, and growth and differentiation factors (platelet derived growth factor - PDGF, TGFβ) (9).

Keratinocytes, forming 95% of epidermal cells, also represent a significant part of the immune system. Their function is linked to a release by the cells of several cytokines, both immunostimulatory ones: IL-1, IL-3, IL-8 belonging to chemokines, IL-12, granulocyte-macrophage colony stimulating factor (GM-CSF), and immunosuppressive ones (tumour growth factor β–TGFβ, IL-10 and neuropeptide α MSH). Tumour necrosis factor (TNF) manifests both
immunostimulatory and immunosuppressive activity, depending on the stage of cellular reaction (9, 10).

This study attempted to demonstrate the involvement of epithelial dendritic cells and macrophages in mechanisms of tumour immunosurveillance in dogs and to define whether the local presentation of antigen and activation of lymphocytes T takes place in the skin and draining lymph nodes.

**Material and Methods**

The study was performed on 8 surgically excised malignant epithelial skin tumours in dogs, i.e. basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) excised together with adherent tissues. The tissue samples were fixed in 7% formalin and paraffin sections stained with haematoxylin and eosin (HE), and then were prepared for histopathological examinations. Skin samples for ultrastructural studies were fixed first in 3.5% glutaraldehyde, in 0.1 M phosphate buffer, pH 7.2–7.4, and then in 1% osmium tetroxide. Afterwards, the samples were dehydrated in alcohol-acetone and embedded in Epon 812. Semithin sections were stained with 1% toluidine blue, while ultrathin sections were contrasted with uranyl acetate and lead citrate. The material was analysed under a transmission electron microscope (TEM), Tesla BS – 500.

**Results**

Under a light microscope, basal cell carcinoma was composed of nests of basaloid cells (Fig. 1) with peripheral palisading cells (Fig. 2). The cells have hyperchromatic nuclei and scant cytoplasm. The nests of tumour cells were surrounded by stroma (Fig. 2). Mitotic figures were common. Squamous cell carcinoma was characterised by invasion of malignant keratinocytes through the basement membrane and into the dermis. Cellular atypia, including pleomorphism, hyperchromatic nuclei, and mitoses, was prominent. The neoplastic cells was poorly differentiated (Fig. 3).

![Fig. 1. Nest of basaloid cells. HE. Scale bar = 10 µm](image1)

![Fig. 2. Basal cell carcinoma. Peripheral palisading cells (arrows). HE. Scale bar = 10 µm](image2)

![Fig. 3. Squamous cell carcinoma. Poorly differentiated neoplastic cells. HE. Scale bar = 10 µm](image3)

![Fig. 4. Stimulated dendritic cell with clearly marked lysosomal fraction (white arrows). Surface zone of the cell manifests multiple long cytoplasmic projections (black arrow). TEM. Scale bar = 1 µm](image4)

In ultrastructural preparation of tumour-adhering tissue, in all cases numerous DC were observed, characterised by a lucid cell nucleus with euchromatin (Figs 4, 5) domination and evidently developed, active nucleolus along with exposed granular and fibrillar zones (Fig. 5).
Fig. 5. Partial destruction of dendritic cells manifested by gradual smoothening of cytoplasmic projections at the surface. Partial separation of one of the projections (black arrow). In the subsuperficial zone note the decomposing vacuoles (white arrow). TEM. Scale bar = 1 µm

Fig. 6. Mast cell with typical cytoplasmic granules. TEM. Scale bar = 1 µm

Fig. 7. A decomposing macrophage. Note active lysosomes (short arrows) and damaged mitochondria with clarified matrix and disappearance of mitochondrial cristae (long arrows). The cell is so damaged that the pattern is poorly legible. TEM. Scale bar = 1 µm

Fig. 8. Lymphocyte (long arrow) and a fragment of decomposing dendritic cell (short arrow). TEM. Scale bar = 0.5 µm

Stratified arrangement of heterochromatin was noted mainly by the nuclear envelope (Fig. 5). Cytoplasm of dendritic cells in the perinuclear zone contained high concentrations of active lysosomes and phagosomes along with vast rough endoplasmic reticulum, arranged into typical stellate structures (Fig. 4), less frequently individually dispersed or in parallel next to each other. The cytoplasm of such cells contained also numerous large vacuoles filled with a granular material and residual bodies representing lysosomes in the course of digestion (Fig. 5). Moreover, active DC demonstrated swollen mitochondria with a clarified matrix and partial disappearance of mitochondrial cristae (Fig. 5). The cell membrane of LCs manifested a variable extent of folding with numerous long cytoplasmic projections (Fig. 4), which fused with each other forming subsuperficial vacuoles. Around some DC cells there could be observed cells, which were undergoing decomposition, devoid of the surrounding cell membrane, and organelles of earlier decomposed cells, loosely positioned between fibres of the dermis.

In close neighbourhood of DC, mast cells with insular arrangement of heterochromatin, filled with numerous granules and manifesting variable extent of secretion concentration (Fig. 6), and stimulated macrophages with active lysosomes, mitochondria with a clarified matrix and disappearance of mitochondrial cristae (Fig. 7) were noted. A high degree of cellular damage made the patterns poorly legible. The mast cells in “contact” with macrophages presented a condition of degranulation. Apart from DC, macrophages, and mast cells, individual lymphocytes were seen in TEM images (Fig. 8).

Discussion

Migration of LCs following contact with antigen represents a dynamic process and follows a strictly defined scheme, both in the course of neoplastic processes and in allergic diseases. However, in the case
of neoplastic cells, the contact with antigen may, for several reasons, be markedly inhibited. The specific character of neoplastic disease has to be taken into account, in which tumour cells attempting to spread to the possibly highest extent, try to bypass or to neutralise the host immune system. In this aim, some tumours secrete substances of immunosuppressive activity (TGFβ), preventing the activation of DC. The substance that is produced by tumour cells, which inhibits cell migration to the tumour prevents the access of LCs to the neoplastic antigens. A situation is also possible in which the LCs penetrate the tumour and sample the antigens, but are unable to emigrate from the tumour to lymph nodes. In this way, the antigen is not presented to lymphocytes Th (11). It should also be remembered that the process of skin carcinogenesis results, i.e., from mutagenic effects of UV light in epidermal cells (18) and from the development of a specific type immunosuppression, which make tumour progression possible (5, 16). Even low doses of radiation, which do not induce noticeable lesions in LCs may induce a condition of locally impoverished immunity since they alter the proliferation of regulatory (suppressive) lymphocytes T, release of TNFα, IL-1, IL-10, and prostaglandins, change local microenvironment inhibiting immune surveillance in the skin as well as promoting a tolerance to the development of neoplastic lesions and metastases (19). Nevertheless, in specific circumstances, strong cell-mediated immune response in the skin with involvement of LCs, lymphocytes T CD 3+ and CD 4+, and cytokines, e.g. interleukin 2, results in a spontaneous regression of skin tumours and their treatment (6, 7, 20).

Many authors have attempted to describe the significance of LCs in the course of skin neoplasia, taking into account their number and morphology both within the very tumour, and in epithelial and mesenchymal tissues, which surround the neoplastic growth (1-4). In most cases, the number of DC in the tumour's vicinity markedly decreased while data related to their number in the vicinity of the neoplastic growth have proven divergent. Additionally, LCs, particularly those within the tumour, underwent significant alterations. They became more spherical, manifested shorter and broken projections and significantly reduced Birbeck granules (2, 3). The alterations observed in LCs represented, according to the authors, a response to the release by the tumour vascular endothelium growth factor, which inhibited maturation of LCs. The altered shape of LCs could also result from effects of UV irradiation, IL-10, TNFβ (3, 4) or prostaglandins (PGE), neuropeptides (αMSH), and cytokines (IL-10) synthesised by the altered keratinocytes of a variable extent immunosuppressive effects (9, 19).

In our studies we have paid attention to another aspect of the problem. Using ultrastructural analysis we have attempted to demonstrate that in skin tumours, similarly as it was documented for skin diseases with significant involvement of cell-mediated immunity (12), the presentation of antigen may already take place in the dermis with no need for LCs migration to the draining lymph nodes. The problem has earlier been dealt with by Murphy et al. (15). On the basis of their observations, the suggestion can be advanced that local immune response develops only in cases of specific type basal cell carcinoma.

In our studies however, an evident activation of LCs and macrophages and of “co-operating” with them, mast cells have been noted close to tumour cells in any of the studied skin tumours. The presence of high numbers of DC, macrophages, as well as mast cells and lymphocytes in the vicinity of the tumour, points to involvement of the skin’s immune system in the local immunosurveillance, but does not provide unequivocal response to the original problem. It is possible that the devoid of Birbeck granules DC noted close to the tumour represent in fact migrating indeterminate cells, in which the organelles are not present (9). However, considering the fact that we deal with the dog skin, it may be possible that they represent interdigitating cells, prepared for antigen presentation beyond a lymph node. This has seemed to be indicated by their augmented activity noted in ultrastructural patterns. In order to confirm the assumption it would be necessary, similarly to what was done by other authors, to demonstrate the presence of surface antigens typical for individual types of DC (13, 17).

Describing ultrastructural pattern of follicular stem cell carcinoma in dogs, Mikaelian and Wong (13) detected in neoplastic foci the presence of DC with a narrow margin of lucid cytoplasm, oval cell nucleus with granules of peripherally located heterochromatin, individual organelles, including rough endoplasmic reticulum, mitochondria with tubular cristae, ribosomes, and glycogen granules. The cells contained no desmosomes, Birbeck granules or melanosomes, but manifested positive reaction for CD18 antigen and lysozyme. Such traits and location of the cells within the tumours have permitted the authors to conclude that they have dealt with LCs.

Shinzato et al. (17) attempted to demonstrate the difference between LCs and interdigitating cells and observed evident expression of CD1a antigen and protein S 100 on the surface of LCs, while interdigitating cells were, according to the authors, CD1a negative, presenting only a pronounced expression of protein S 100.

Our studies demonstrated the augmented activity of cells, which infiltrated the tumour and thus confirmed the active role of the immune system in the process of combating the tumour. However, the ultrastructural patterns have been unable to visualise the dynamic nature of LCs migration. They also have not been able to define surface antigens of the cells, which might permit a conclusion whether we have dealt with an undefined cell, which migrated through the skin following contact with an antigen, or with the interdigitating cell capable of presenting antigen in a site untypical for the cell. As is already mentioned above, resolution of the problem would require immunocytochemical studies and several analyses, e.g. using real-time PCR.
References

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