

## 7 Summary

### **Immunohistochemical study of canine cutaneous histiocytoma with characterization of the regression and proliferation behavior in these tumors**

The canine cutaneous histiocytoma is a commonly encountered benign skin tumor that frequently occurs in young dogs. Histiocytomas appear as rapidly growing, dome-shaped tumors, mostly on the head and extremities, and often show superficial ulceration. One major characteristic of these tumors is the ability to undergo spontaneous regression, which is accompanied by massive infiltration of inflammatory cells. The aim of this study is to investigate the role of the different lymphocyte populations, tumor size, ulceration, necrosis, and proliferation in the regression of histiocytomas.

This examination is based on 191 paraffin-embedded and formalin-fixed histiocytomas from dogs under two years of age taken from archive material of the Institute of Veterinary Pathology of the Freie Universität Berlin. The tumors are assigned to three different size classes ( $< 1 \text{ cm}^2$ ;  $1\text{-}2 \text{ cm}^2$ ;  $>2 \text{ cm}^2$ ) according to their appearance on the paraffin sections. In addition, degree of ulceration and necrosis are scored on an ordinal scale for each tumor from haematoxylin and eosin (HE) stained slices. To date, these parameters have not been the object of intensive research. In this study, the appearance of a large sample size of tumors is statistically evaluated and related to immunohistological results for the first time. The different subpopulations of the infiltrating lymphocytes are identified immunohistochemically by specific antibodies against CD3- and CD79 $\alpha$ -antigens. It is assumed that T-lymphocytes are connected directly to the development and progression of self-healing. For that reason all of the 191 histiocytomas are divided into four stages of regression on the basis of their quantity of CD3+-T-cells. Additionally, the quantity, distribution and relation to necrosis, ulceration and T-lymphocytes of infiltrating CD79+-B-cells were compared in 60 cases: 30 tumors without and 30 with a high degree of ulceration. Furthermore the proliferation behaviour of histiocytomas through the course of regression is documented for the first time. Thereby the actively proliferating cells in 15 slices from each of the regression stages (I to IV) are marked with the antibody MIB1 in order to calculate a proliferation index.

The CD79 $\alpha$ - and MIB1-antigens are immunohistologically verified for this study by modified B-SA staining. Only through this method can the CD79 $\alpha$  antibody be used reliably and suc-

cessfully on paraffin-embedded sections. Preliminary tests show that this technique enables improved staining of target cells as well as better conservation of tissue structure than formerly established B-SA and ABC techniques. Also this modified method improves the illustration of the MIB1 antibody. In the modified B-SA staining procedure slices are initially incubated for 35 minutes in a water bath in Target Retrieval Solution at 95°C temperature for antigen demasking. Antigen pre-treatment in the water bath enables a constant and gentle warming of the slices and is for that reason preferred to microwave heating treatment. The Target Solution shows improved results compared to pre-treatment in citrat or EDTA solution. Afterwards, the slices are incubated with the respective primary antibodies for 40 minutes at room temperature and subsequently at 4°C overnight. The CD79 $\alpha$  antibody is used at a dilution of 1:50 and the MIB1 antibody at a dilution of 1:80.

The examinations show that small tumors (< 1 cm<sup>2</sup> in diameter) originate with significantly higher frequency from the head and neck areas and seldom from the extremities. They also show statistically fewer necroses and less ulceration than larger tumors. Large histiocytomas (>2 cm<sup>2</sup>) have a significantly higher rate of strong ulceration (> 30 % of epithel). Also, number of necroses slightly increases with size of tumors. Immunohistological examinations demonstrate a significant increase of T- and B-lymphocytes and proliferation rate in the course of the regression. As expected, CD3+ T-cells dominate the tumor-infiltrating lymphocytes. Their number within the tumor decreases significantly from periphery to the epithel. Additionally, necrosis increases remarkably through the course of regression. In contrast to previous studies, there are also many CD79+ B-lymphocytes found in the tissue. These correlate with the number of CD3+ T cells, but occur more frequently in the center of the tumor. Since symptoms of an advanced regression are also found in small tumors, regression presumably occurs at the beginning of tumor growth. No correlation is found between occurrence of secondary inflammation and progression of the regression. Despite of the benign nature of the histiocytoma, proliferation rate all over the tumor is surprisingly high, varying within the regression stages between 10-23 % (medians). Tumor cell proliferation at first increases significantly with the regression, but slowly decreases at later regression stages. Tumor size, number of necroses and degree of ulceration are not correlated with proliferation rate.

The results indicate a relation of both tumor growth and necrosis to the regression. Moreover, self healing already starts at the beginning of the tumor growth and is mediated not only by T-cell cytotoxicity but presumably also by B-cell activity. The high proliferation rate could be

responsible for the initiation of immunity both by an increase of tumor antigen activity as well as by the increased mitotic rate itself. The “aggressive” tumor growth might therefore indirectly lead to its own destruction.