

# Immunohistochemical evaluation of lymphocyte types infiltrate into the canine seminomas

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## Key words:

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## Abstract:

**BACKGROUND:** Seminoma is frequently observed in human and canine testes especially in cryptorchids. Canine and human seminomas are typically associated with leukocytic infiltration. **OBJECTIVES:** We aimed to identify the type of lymphocytes that infiltrate to seminomas. **METHODS:** Tumor infiltrating lymphocytes were evaluated by immunohistological techniques in 5 dogs with diffuse seminoma. Routine pathological examination of these specimens showed diffuse seminoma with tumor infiltrating lymphocytes in all cases. **RESULTS:** Lymphocytes in seminomas were predominantly T cytotoxic (CD3+, CD8+) cells. These cells were distributed diffusely, but patchy around the vessels. B-cells were also identified as some rare single cells diffusely scattered in the tumoral parenchyma. **CONCLUSIONS:** The results of this study showed that the lymphocyte infiltrating cells are mainly T lymphocytes particularly CD8 cytotoxic type.

## Introduction

Seminoma is frequently observed in human and canine testes (MacLachlan and Kennedy, 2002; Grieco et al., 2004). In the WHO classification of animal canine seminomas, these types of tumors are reported as having an intratubular or diffuse pattern (Kennedy et al., 1998, Grieco et al., 2007). Canine Seminomas, often developing in old dogs, are less aggressive than their human counterparts (Scully and Coffin, 1952; Benazzi et al., 1995; Grieco et al., 2004). Nevertheless, the low malignancy and low metastatic potential of most canine seminomas, together with their tendency to develop in older animals, has suggested to several authors that they usually represent the counterpart of human spermatocytic seminomas (DeVico et al., 1994; Maiolino et al., 2004; Grieco et al., 2007). Both canine and human seminomas are typically associated with leukocytic infiltration. Testicular seminoma is characterized by prominent lymphoid infiltrates that were predominantly T cells, but B cells were also identified as some follicular aggregates in tumoral parenchyma. T cells

were distributed diffusely with the predominance of the CD 8<sup>+</sup> phenotype (Grieco et al., 2004). The presence of a large number of activated T cells, despite the complete absence of MHC I and MHC II antigens, is an Immunological paradox (Nouri et al., 1993). Some investigators believe that tumor infiltrating lymphocytes contain a high percentage of  $\gamma/\delta$  T cells, ranging from 17.3 to 35.1%.  $\gamma/\delta$  T cells often accumulate within the granulomatous inflammation of tumor tissues (Xia Zhao). In contrast to the distribution of T cells, B cells tended to accumulate and occasionally formed lymphoid follicles showing a phenotypic pattern of B cell antigens. This was comparable with secondary lymphoid follicles in lymphoid organs (Takashi et al., 1992).

## Materials and Methods

The materials consisted of formalin-fixed paraffin embedded tissue blocks of five formalin fixed, testicular canine tumors, all of them diagnosed as diffuse seminomas. The tumors were derived from

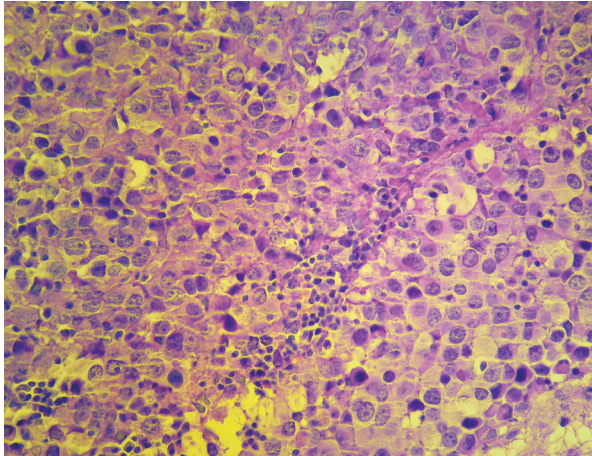


Figure 1. Diffuse seminoma. Leukocytic aggregate, mainly composed of lymphocytes, partly surrounding a fibrous tissue. (HE  $\times$  400).

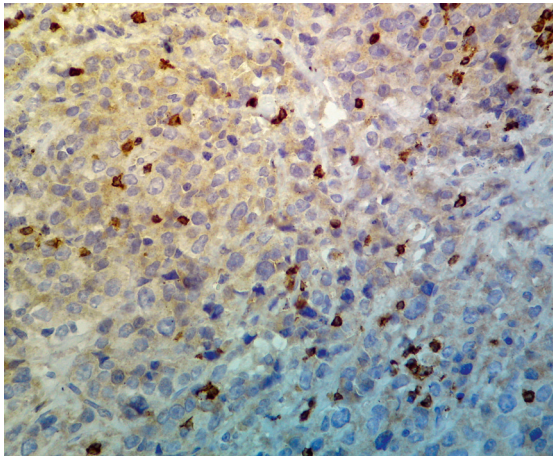


Figure 3. Diffuse seminoma. Immunolabelling for CD79a. B lymphocytes were detectable in the diffuse singly among neoplastic cells (IHC  $\times$  450).

dogs ranging in age from 8 to 11 years old. Five micrometer sections from each block were prepared for Hematoxylin and Eosin (H&E) and also for immunohistochemical studies. Besides H & E, immunohistochemical staining was performed manually, using the avidin-biotin-complex procedure with the following antibodies: Immunoglobulin associated alpha molecule or CD79a (clone HM57; dilution 1:50, high temperature citrate buffer pretreatment; (DAKO); CD3 (polyclonal rabbit anti-human antibody; dilution 1:200); CD8 (polyclonal rabbit anti-human antibody; dilution 1:200). Staining intensity and distribution were reported as strongly or weakly positive; and as diffuse or focal.

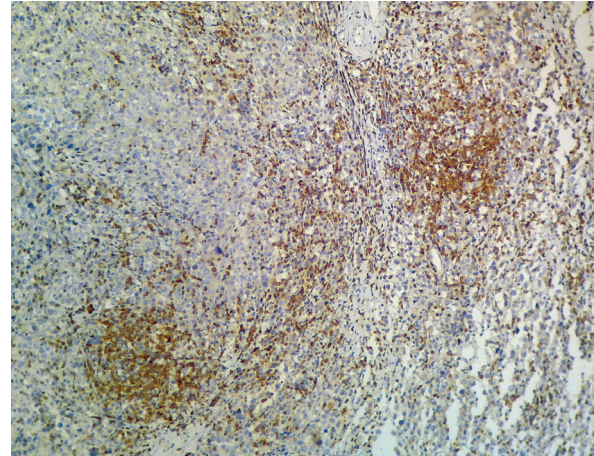


Figure 2. Diffuse seminoma. Immunolabelling for CD8. The majority of the infiltrating cells aggregated in the lymphoid patches and also scattered in the tumoral parenchyma (IHC  $\times$  100).

## Results

This study characterized lymphocytes infiltrating in 5 canine with diffuse seminomas. Antibodies were directed against CD79a, CD3 and CD8. Infiltrating lymphocytes were aggregated around vessels and also scattered singly among the neoplastic cells (Figure 1). The more profuse infiltrates often had the patchy aggregates of lymphocytes, without any obviously granulomatous inflammation. Immunohistochemically, all the samples showed generally similar results. Most of the infiltrating cells were strongly positive for CD3 and CD8 with patchy or diffuse pattern for both of them. Infiltrating cells were mainly T lymphocytes (CD3<sup>+</sup>), particularly of the CD8<sup>+</sup> subset. These cells were aggregated in the lymphoid patches and also scattered in the tumoral parenchyma (Figure 2). B lymphocytes (CD79a<sup>+</sup>) were detectable as diffuse and singly among neoplastic cells. The rare scattered B cells were strongly positive for CD79a. These cells were not aggregated in the lymphoid patches, but singly scattered in the tumoral parenchyma (Figure 3).

## Discussion

Focal aggregates of lymphocytes are a common feature in both canine and human seminomas. In the diffuse seminomas, infiltrating cells were localized at the periphery of the tumor, or around vessels, or

randomly and singly scattered amongst the neoplastic cells. Similar findings were observed around the tubules in the intratubular cases. On the other hand, even in the purely intratubular cases, lymphocytes might partly aggregate around the neoplastic tubular structures (Bols et al., 2000). In this study, leukocytic aggregates were detected around the connective tissue and vascular structures. Then, in all cases, there were patchy patterns of infiltrated lymphocytes, besides their diffuse appearance. According to an immunohistological study in seminoma samples in dogs, almost all of the infiltrating leukocytes and tumor cells were positive for MHC I and only leukocytes were positive for MHC II antigens (Grieco et al., 2004). In the testis, which is an immunologically privileged area, immunological reactions against seminal cells may be triggered only when they spread out of the tubules (Bols et al., 2000). In fact, the seminiferous tubules are protected from immune attack by Fas ligand (FasL) molecules expressed by Sertoli cells (Bellgrau et al., 1995; Braendstrup et al., 2009). The presence of CD8<sup>+</sup> lymphocytes, together with the large number of both infiltrating and neoplastic cells expressing MHC I, suggests that the lymphocytes mediate a cytotoxic reaction against the neoplastic cells. This hypothesis may underlie the favorable prognosis frequently associated with canine seminomas (Grieco et al., 2004). The total loss of basement membrane in diffuse seminomas may explain the random distribution of leukocytic aggregates in these cases (Benazzi et al, 1995). In human seminomas, leukocytic infiltrates were mainly composed of T lymphocytes (Bell et al., 1987; Nakanoma et al., 1992; Cope et al., 1999). B lymphocytes were present in each infiltrate and are not a constant feature. Moreover, in the follicular infiltrates of B lymphocytes were centrally located, while T lymphocytes occupied the periphery (Nakanoma et al., 1992; Torres et al., 1997), in contrast with the findings of this study that the B lymphocytes were not centrally located and not in follicular formation, while T lymphocytes were similarly distributed as patchy or diffuse in the central and periphery regions. This study demonstrated that in canine seminomas, T lymphocytes with the CD8<sup>+</sup> subset were the most frequently represented infiltrating cells. Similar findings were observed in

human seminomas (Bell et al., 1987; Nakanoma et al., 1992; Cope et al., 1999). Thus, the host reaction against neoplastic cells is mainly cytotoxic in canine seminomas. Reactions between malignant cells and the host immune system are believed to play an important role in regulating tumor cell growth, and lymphocytes found in neoplastic tissues are believed to exert this function (Nakanoma et al., 1992). Cell-to-cell contact is essential for some phases of the immune response, including lysis of neoplastic cells by cytotoxic T lymphocytes (Tomita et al., 1993; Grieco, 2004). These considerations might explain the biological behavior seen in canine seminomas, which are not highly malignant and rarely metastasize. We consistently observed a significant number of CD3 and CD8 positive cells in leukocytic aggregate regions which also scattered diffusely. Moreover, neoplastic cells expressing CD79a, were recognized in rare scattered lymphocytes and not in leukocytic aggregate regions. These findings, associated with the prevalence of cytotoxic CD8<sup>+</sup> lymphocytes, suggest that in canine seminomas inflammatory cells play an active anti tumoral role.

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## ارزیابی ایمونوهیستوشیمیایی انواع لنفوسیت‌هایی که به سمینوماهای سگ ارتشاح می‌یابند

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### چکیده

**زمینه مطالعه:** سمینوما بطور شایعی در بیضه انسان و سگ‌ها بخصوص افراد نهران بیضه دیده می‌شود. در تومورهای سمینوما، انسان و سگ‌ها ارتشاح لکوسیت‌ها به طور بارزی صورت می‌گیرد. **هدف:** در این تحقیق مشخص شدن نوع سلول‌های لنفوسیت ارتشاحی به بستر تومور سمینوما اهمیت دارد. **روش کار:** ارزیابی ایمونوهیستوشیمیایی این لنفوسیت‌ها که در مورد پنج بلوک سمینوما، منتشر دارای ارتشاح لنفوسیتی انجام گردید، آنتی‌بادی علیه مولکول‌های CD79a، CD8، CD3، بروش ایمونوهیستوشیمیایی بکار برده شد. **نتایج:** لنفوسیت‌های T با مشخصه مثبت بودن CD8 و CD3 در جمعیت لکوسیت‌های ارتشاحی بیشترین فراوانی را نشان دادند. این سلول‌ها ضمن اینکه الگوی منتشر داشتند ولی بیشتر در اطراف عروق مجتمع بودند. هر چند، لنفوسیت‌های B نیز بصورت سلول‌های تکی، اندک و منتشر در پارانشیم تومور دیده شد. **نتیجه‌گیری نهایی:** نتایج حاصل از این تحقیق نشان می‌دهد که سلول‌های لنفوسیت ارتشاحی به پارانشیم تومور سمینوما، سگ اغلب از نوع T بخصوص کشنده سلولی و CD8 مثبت می‌باشد.

واژه‌های کلیدی: لنفوسیت، سمینوما، ایمونوهیستوشیمی.

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