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LYMPHOSARCOMA (MALIGNANT LYMPHOMA) – FROM CATS TO KOALAS

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Introduction
Lymphoid tumours are readily diagnosed in most domestic and wildlife vertebrate species possessing an adaptive immune system. Moreover, in many species, including the dog,¹ the cat² and the koala,³ lymphoid tumours are the most commonly detected neoplasms. Yet, despite all the information available on lymphoid tumours in both people and animals, there remains much ignorance and confusion about causation, diagnosis and classification, prognosis and treatment. This presentation aims to clarify the present state of understanding of lymphoid tumours in animals, particularly in the dog and cat. My experiences with lymphoid neoplasia in the koala (Phascolarctos cinereus), a marsupial, will also be drawn upon in order to show how a common approach can be developed to understand causation, disease expression and diagnosis in all species.

Nomenclature – origin and definition
Any current confusion over nomenclature of lymphoid neoplasms rests with the early scientists. It wasn’t until the mid 1800’s that Virchow coined the terms leukemia and lymphosarcoma for lymphoid neoplasia in people.⁴,⁵ This was quickly followed by the term malignant lymphoma for lymphosarcoma in 1871. However, until about the 1930’s, the term lymphosarcoma was used to distinguish those lymphoid tumours in people that were not Hodgkin’s Disease (Hodgkin’s lymphoma), whilst malignant lymphoma was used to cover both. This caused much confusion until medical pathologists finally agreed in the mid 1900’s to use the terms Non-Hodgkin’s and Hodgkin’s Lymphoma (lymphoma was used instead of malignant lymphoma because all tumours identified are malignant ie the word ‘malignant’ is redundant). Naturally, when the nomenclature was extrapolated to the naming of animal lymphoid neoplasms there was no need to be concerned about the distinction between Non-Hodgkin’s and Hodgkin’s Lymphoma, as the latter condition is very rare (or possibly does not exist). Today lymphosarcoma, malignant lymphoma and simply lymphoma are all used interchangeably to describe solid malignant lymphoid neoplasms in animals.⁵ Some of these may also express a leukaemic manifestation, although lymphoid leukaemia may occur as a distinct entity. Table 1 presents a list of terms and their definitions in relation to lymphoid neoplasia

Diagnosis, classification systems, and prognosis
The diagnosis of lymphosarcoma can be difficult due to great variation in the clinical presentation related to which organs or tissues are infiltrated by the neoplastic cells and the possible presence of paraneoplastic effects. A good clinical approach requires identifying the organs or tissues affected, determining the presence of diffuse infiltrates or solid masses, and laboratory confirmation through clinical pathological data, and cytopathological and/or histopathological
examination. Lymphosarcoma, because it is composed of round cells with little connective stroma, lends itself well to cytopathological examination and diagnosis.

Classification systems for lymphosarcoma are as numerous as the terms used for lymphoid neoplasia, but they can shed some light on cytogenesis, pathogenesis, sometimes aetiology and sometimes prognosis. Parodi provides an excellent summary of classification systems used by veterinary pathologists; however, clinicians are often more interested in those that focus on clinical staging utilizing organ/tissue involvement and the impact on the health of the animal. Perhaps an exception to this attitude is the classification systems that utilize immunophenotyping and cytogenetic markers. This is because the determination of cell lineage (particularly B, T and Null Killer [NK] lymphocyte lineage) appears to have distinct implications for prognosis related to chemotherapeutic and radiotherapeutic treatment in people.

Immunophenotyping is now performed extensively for animals with lymphosarcoma, but unfortunately its promise as a prognostic indicator appears not to be as clear-cut as in people. In dogs, some studies have suggested that differentiation of lymphosarcoma into T or B lymphocytes does have implications for prognosis, especially in response to chemotherapy and overall survival times, with T lymphocyte tumours having a poorer prognosis (Ponce et al, 2004; Vail 2000). However, in the cat immunophenotyping on its own appears not to be a strong prognostic indicator, although Malik et al, in their extensive study of a 100 affected cats, suggested that tumours of T lymphocytes provide a more favourable prognosis in relation to long term survival in response to therapy. Therefore, it might be wise at this stage, for dogs and cats, to approach the issue of prognosis related to therapy as being related to a combination of a myriad of factors (such as site, age, breed and clinical stage), with immunophenotyping being only one. Immunophenotyping has been done for lymphosarcoma in the koala, but more to understand pathogenesis rather than prognosis for therapy. However, T cell tumours appear to be more common, and were more commonly related to a leukaemic manifestation, which is regarded as an indication of advanced disease and, therefore, an unfavourable.

**Mechanisms of carcinogenesis and causes of lymphosarcoma (Figure 1)**

Causes of neoplasia in general are poorly understood, but there is a growing reality for most that causation is complex and probably involves multiple interacting factors before neoplasia is established and progressive. This seems logical when one appreciates the complexity of mechanisms involved in carcinogenesis that lead to genetic alteration/damage (mutation), growth and invasion. Causes can include hereditary/familial disease (eg canine breed predisposition to certain types of neoplasms, such as Boxer dogs) physical agents (eg ultraviolet [UV] irradiation and skin tumours, electromagnetic fields), chemical agents (eg hormonally-related canine mammary tumours),
infectious agents (especially retroviruses), or a combination (eg UV irradiation and papillomaviruses;\textsuperscript{12} UV irradiation and chemical promoters\textsuperscript{13}).

For the production of lymphoid neoplasia in people, the emphasis has been on the role of viruses, despite that other causes probably exist.\textsuperscript{14} This situation also exists for lymphoid neoplasia in animals, where a myriad of oncoviruses have been identified. In the cat, the emphasis has been on the investigations of the roles of Feline Leukaemia Virus and Feline Immunodeficiency Virus,\textsuperscript{15} with the latter possibly operating through suppression of immune surveillance rather than through initiation or promotion. The situation for lymphomagenesis in the cat, however, is likely to be more complex with the viruses probably interacting with both environmental and host factors. This certainly seems to be the case for lymphoid neoplasia in the koala, where a koala retrovirus (KoRV) has been implicated, but is widespread in the koala population.\textsuperscript{16,17} In the dog, viruses are yet to be implicated in lymphoid neoplasia, and the only triggers suspected to date are possible familial predisposition (eg Boxer), pesticides and magnetic fields.\textsuperscript{7,18}

**Paraneoplastic syndromes associated with lymphosarcoma**

Paraneoplastic syndromes are referred to as systemic effects of neoplasia that cannot be explained simply by the physical growth of primary or metastatic masses. Although not completely understood, paraneoplastic syndromes are mainly thought to occur due to genetic alteration that allows the production of a chemical that has biological activity (eg cytokines or ‘hormones’) or due to the expression of neoplastic antigens that are regarded as ‘foreign’ by the immune system. A number of paraneoplastic syndromes have been recorded for lymphosarcoma in both people and animals, and many appear to relate to ‘autoimmunity’ affecting skin, nerves, haematopoietic cells, joints and kidney.\textsuperscript{18,19} Reasons for the autoimmunity are not completely understood, but may involve loss of immune tolerance, autoantibody production by autoreactive B lymphocytes or chronic antigenic stimulation.\textsuperscript{19} There is also the distinct possibility that antibodies directed at tumour specific antigens on neoplastic lymphocytes can be cross-reactive with antigenic determinants (epitopes) on normal body cells such as erythrocytes (immune-mediated haemolytic anaemia) and synovial membrane cells (immune-mediated arthritis).\textsuperscript{20}

In addition, lymphosarcoma is also known to be associated with the production of chemicals. Some of these are related to partial or full immunoglobulin production (dysproteinemia), but others appear to be cytokines (eg possible interleukin-5 production leading to hypereosinophilia\textsuperscript{21}) or hormonally-active substances, such as those that can cause persistent hypercalcaemia.

**Where will the future focus be for improved understanding of lymphosarcoma?**

In those species where individual animals are highly prized, such as small companion animals, the development of improved treatment protocols will remain
a key focus. In tandem with this will be the pursuit of better prognostic indicators, which may yet lead to additional classification/grading and staging systems. For lymphosarcoma, immunophenotypic analysis will continue to be developed, but clonal analysis is also likely to be pursued as the clonal theory for carcinogenesis that is a neoplasm results from the progeny of a single cell, appears to hold true for most lymphoid neoplasia. This is despite the fact that bi- and multi-clonality have been detected, mainly through the determination of more than one immunoglobulin product. Of course, this may well be due to acknowledged heterogeneity of malignant neoplasia that develops with the formation of subclones due to progressive mutations.

However, what will be the focus for those species, such as the koala, where most animals are unlikely to be treated for lymphoid neoplasia when it develops? Logic dictates that research will be aimed at preventing development, and this will require improved understanding of the mechanisms of carcinogenesis. The outcome of such research will have implications for the prevention and control in all species. For lymphoid neoplasia, this will require a better awareness of how cell mutations occur through heritable components, because of viruses and because of exposure to irradiation and chemicals such as pesticides. A component of this will be the further delineation of cellular proto-oncogenes and how viruses (who carry their own oncogenes that were originally derived from mammalian cell DNA), chemical carcinogens and irradiation can cause their conversion to oncogenes, which encode for oncoproteins that cause cell transformation. For example, the protooncogene myc was originally isolated from avian myelocytomatosis virus – called v-myc. Its cellular counterpart is called c-myc that has been found to be overexpressed in B cell lymphosarcomas in both people and mice.

Additional areas for study will be genes that code for DNA repair enzymes and tumour suppressor genes, such as p53, that are important for delaying entrance into cell multiplication to allow for DNA repair. To date alterations/mutations to p53 have been detected in lymphoid neoplasms in people, particularly the overexpression of non-functional p53. The situation in canine lymphosarcoma is not as clear, but p53 overexpression may occur for T-cell phenotypes. Mutations affecting the p53 gene have also been identified in feline lymphosarcoma cell lines. Genes that control programmed cell death (apoptosis) may also be altered in neoplasia and allow continued cell survival and tumour mass growth. Some lymphoid neoplasms, for example, are characterized by increased expression of the proto-oncogene, bcl-2, that blocks apoptosis, which may in part be due to altered p53 function. An additional area of study may be the determination of factors, especially matrix metalloproteinases, that allow angiogenesis and invasion, both important features of malignancy. Defects in immunesurveillance (tumour immunity) leading to reduced tumour cytotoxicity (either through defects in the minor mechanism of antibody-dependent cell-mediated cytotoxicity or defects in the major mechanism of producing cytotoxic T lymphocytes) will also continue to
figure prominently in future research. This is particularly so, because additional understanding will lead to improved immunotherapies.

References
Table 1. Terms used to describe lymphoid neoplasia

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
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<tbody>
<tr>
<td>Lymphosarcoma (synonyms: malignant lymphoma; lymphoma; in man non-Hodgkins lymphoma)</td>
<td>Solid mass or masses composed of neoplastic lymphoid cells</td>
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<tr>
<td>Hodgkin’s Disease (Hodgkin’s Lymphoma)</td>
<td>A type of Lymphoma in people characterized by neoplastic lymphoid cells, various inflammatory cells and so called Reed-Sternberg cells. It is very uncommon in animals</td>
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<tr>
<td>Multiple Myeloma</td>
<td>Solid masses of malignant plasma cells (in bones and sometimes visceral tissues)</td>
</tr>
<tr>
<td>Plasmacytoma</td>
<td>Solid mass of benign plasma cells (commonly in skin)</td>
</tr>
<tr>
<td>Lymphoid Leukaemia</td>
<td>Leukaemia refers to the presence of neoplastic cells in the circulating blood. This implies that bone marrow is producing the neoplastic cells. Lymphoid leukaemia may occur as a primary condition or develop in the later stages of lymphosarcoma.</td>
</tr>
<tr>
<td>Acute Lymphoblastic Leukaemia (ALL)</td>
<td>As a primary condition, characterized by acute onset and at least 30% of the circulating neoplastic cells being lymphoblasts.</td>
</tr>
<tr>
<td>Chronic Lymphocytic Leukaemia (CLL)</td>
<td>As a primary condition, characterized by a chronic time course and a predominance of small lymphocytes as the circulating neoplastic cells</td>
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Figure 1 Mechanisms and causes of carcinogenesis

<table>
<thead>
<tr>
<th>Causes</th>
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<tr>
<td>Theory of multistep carcinogenesis, agents for:</td>
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<tr>
<td>1. Initiation (permanent mutation)</td>
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<tr>
<td>2. Promotion (promote growth of tumour cells and allow for further DNA alteration)</td>
</tr>
<tr>
<td>3. Progression (further mutations that allow for invasiveness and tumour heterogeneity)</td>
</tr>
<tr>
<td>Causes of carcinogenesis often operate in tandem as initiating, promoting and progressing agents. They include multicellular and microbial organisms, chemicals, physical agents, heritable factors, and immune-modifiers</td>
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<table>
<thead>
<tr>
<th>Mechanisms</th>
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<tr>
<td>Initial mutation (chromosomal and gene-based alterations) nb some may be naturally occurring ie passive process:</td>
</tr>
<tr>
<td>1. Activation of proto-oncogenes to oncogenes. Proto-oncogenes regulate cellular responses to external signals that stimulate growth and differentiation (including control of apoptosis) Expression of aberrant proteins (oncoproteins) leading to clonal expansion and additional mutations</td>
</tr>
<tr>
<td>2. Alterations in DNA repair genes and resultant defective enzymes. These operate during passive mutations and in normal processes such as Ig production and T Cell receptor gene arrangement in immune responses</td>
</tr>
<tr>
<td>3. Loss of tumour suppressor gene function. These genes encode proteins that restrict or inhibit cell proliferation (including apoptosis eg P53 and and interaction with proto-oncogene bcl-2). They may play a role in maintaining cell to cell contact</td>
</tr>
<tr>
<td>Continued growth and spread (malignancy) occur with additional mutations (heterogeneity of malignancy) angiogenesis and invasion. Diminished or altered immune surveillance can allow enhanced growth and spread</td>
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