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Lymphomas and leukemias in mice

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Abstract

Lymphomas are among the most common tumors in many strains and stocks of mice, especially those used in safety assessment. CD-1, C57BL/6, B6C3F1 and B6;129 mice develop 10–50% incidences of lymphomas in aging mice. Most of the tumors are B-cell lymphomas of the follicular type, arising in spleen, mesenteric lymph node and/or Peyer's patches. Lymphomas and leukemias may be induced by chemicals, retroviruses and irradiation. Genetics also play a major role in mouse lymphomagenesis and leukemogenesis. The most potent chemical carcinogens require only a single injection in young mice to induce a high incidence of lymphomas, often thymic T-cell lymphoblastic lymphomas. Several genetically engineered mouse lines have high incidences of these tumors. In 2-year carcinogenesis bioassays, increases of incidences of B-cell lymphomas (which occur naturally in controls) are indicative of less potent carcinogens. Classifications of the lymphomas and leukemias have evolved over the years. The practical WHO toxicologic pathology lymphoma and leukemia classification was developed by collaboration between the US STP, RITA, BSTP and JSTP. A more recent mouse lymphoma and leukemia classification was published by a committee of the US NCI Mouse Models of Human Cancers Consortium. This classification follows closely the more detailed human WHO classification and can be used for mouse models of lymphoma and leukemia.

Keywords: Lymphoma; Leukaemia; Mice; Spleen; Carcinogenesis; Lymphocytes; Immunohistochemistry

Introduction

Lymphomas are common naturally occurring tumors of mice and are common endpoints in carcinogenesis bioassays. They are the fifth or sixth most common target site of carcinogens in mice (Gold et al., 2001; NTP, http://ntp-server.niehs.nih.gov/). Interpretation of hyperplastic lymphoid lesions can be difficult and may represent a diagnostic challenge in bioassays when lymphoid hyperplasia is common in a study or when the immune system is a target site of a test chemical. Nomenclature of the lymphomas and leukemias of mice have evolved over the past 30 years. Initially, reticulum cell sarcomas were found in mice until pathologists noted the similarity of mouse lymphomas to B-cell lymphomas of humans and the fact that the mouse tumor cells produced immunoglobulins (Frith et al., 1993, 1996). More recently, the WHO mouse nomenclature, written by a committee of international pathologists, has been used by toxicologic pathologists (Frith et al., 2001). This tumor classification is most useful for safety assessment studies with mice. A humanized nomenclature based on the WHO human hematopoietic tumor classification (Jaffe et al., 2001) was developed by a committee of medical and veterinary pathologists for

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utilization in mouse model studies (Morse et al., 2002; Kogan et al., 2002). It represents a comprehensive list of tumors found in humans and genetically engineered mice, although not practical for use in toxicology studies.

Causes of lymphomas and leukemias

Retroviruses, irradiation and chemicals can cause lymphomas or leukemias in mice (Taddesse-Heath et al., 2000; Gold et al., 2001; Boorman et al., 2000). More recently, genetic engineering has led to many mouse lines with lymphomas and leukemias (Teitell and Pandolfi, 2004; Kogan et al., 2002; Kogan, 2004; Morse et al., 2002).

Lymphomas in control mice

The incidence of lymphomas in control mice varies by strain, stock, sex and age (Fig. 1). Lymphomas in CD-1 (Giknis and Clifford, http://criver.com/flex_content_area/documents/RM_TD_SpontaneousNeoplasticCD1_2005.pdf; Maita et al., 1988; Son and Gopinath, 2004), B6C3F1 (NTP, http://ntp-server.niehs.nih.gov/; Tarone et al., 1981; Haseman et al., 1984, 1997, 1999), B6;129 (Haines et al., 2001) and other lines of mice (Frith and Wiley, 1981; Frith et al., 1996; Haines et al., 2001, Ward et al., 2000) have been reported. The incidence of tumors also follows a binomial or nonbinomial distribution (Haseman et al., 1999; Tarone et al., 1981). Statistical evaluations of various types must be performed as for other tissues. Since lymphomas occur in average incidences of 5–24% for CD-1 and B6C3F1 mice, it is important to use various controls including the specific experiment untreated controls and facility historical controls (Haseman et al., 1997).

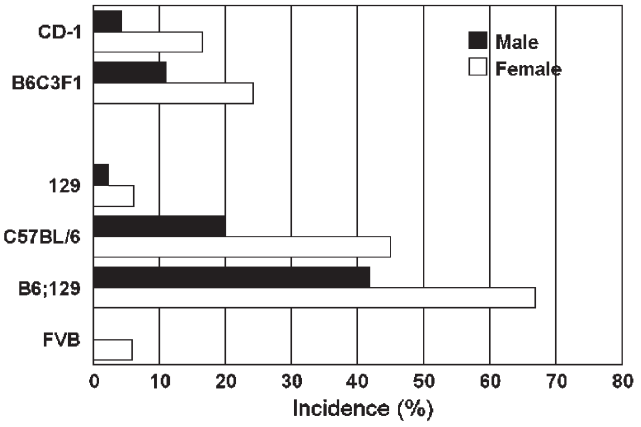

Fig. 1. Average incidences of lymphomas in common strains of mice used in toxicology studies (CD-1, B6C3F1) or genetically engineered mouse studies (B6;129, C57BL/6, FVB). Average tumor incidence values are from papers noted in the reference list. Note that female mice always have higher incidences of lymphomas than do males.

Are tumors in control mice different from those in treated mice?

One must compare the type of lymphoma or leukemia in the control mice to those in treated mice. Often induced tumors have a different cell and tissue of origin and organ distribution than those in control mice. Increased mortality in a study due to a specific lymphoma type also suggests that lymphomas may be different from those in controls. For example, 1,3-butadiene and DMBA induced thymic T-cell lymphoblastic lymphomas in B6C3F1 and B6;129 mice while controls have B-cell lymphomas in spleen and mesenteric nodes (Buters et al., 1999; Melnick and Sills, 2001). B6C3F1, C57BL/6, B6;129 and FVB mice do not develop thymic lymphomas in young mice while 5–10% of aging CD-1 mice develop lymphomas in young mice (Son and Gopinath, 2004). There is some evidence that the lymphoblastic lymphomas (Fig. 2) in young mice are of thymic origin and may sometimes be associated with retroviruses (Taddesse-Heath et al., 2000). Thus, carcinogens causing thymic lymphomas in young CD-1 mice may interact with viral carcinogenesis while carcinogens causing thymic lymphomas in young B6C3F1 mice may not (Melnick and Sills, 2001).

Based on the NTP database (http://ntp-server.niehs.nih.gov/), there are two patterns of induction of lymphomas in mice. These types include (1) early induction associated with increased mortality and decreased survival and (2) increased incidence of lymphomas at the

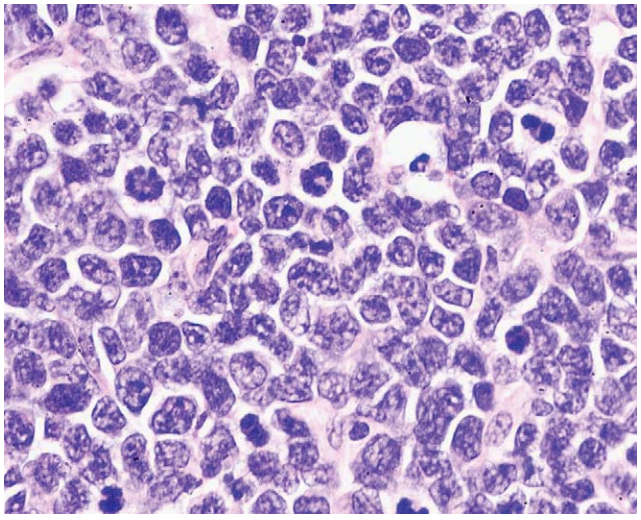

Fig. 2. T-cell lymphoblastic lymphoma in a CD-1 mouse.

end of the 2-year bioassay. The early induced tumors are often thymic T-cell lymphomas while the later are typical naturally occurring B-cell lymphomas from spleen, Peyer's patches or mesenteric lymph node. The early tumors can be associated also with tumors induced in other tissues, as for 1,3-butadiene (Melnick and Sills, 2001).

Diagnosis and classification of lymphomas and leukemias

The classification and nomenclature for diagnosis of leukemias and lymphomas in mice have evolved over the years. Often the gross findings are ignored in bioassays, yet it can provide valuable information for comparison on induced and spontaneous tumors. Lymphomas of mice can be found in the specific gross distributions (Table 1). The WHO classification of mouse lymphomas (Morse et al., 2002; Borowsky et al., 2004) is listed in Table 2. The follicular/pleomorphic lymphoma, the most common lymphoma in most strains of mice, was previously termed reticulum cell sarcoma, centrocytic/centroblastic lymphoma and follicular center cell lymphoma (Jones et al., 1990; Frith et al., 1996). It most commonly arises in spleen, mesenteric lymph node and/or Peyer's patches of small intestine. Follicular lymphoma is often composed of variable populations of centroblasts and centrocytes in various proportions (Fig. 3). These cells are the main cell types in follicles and germinal centers. Besides these blasts and mature lymphocytes, some cells have cleaved (folded) nuclear membranes; hence the term "cleaved" lymphocytes. Tumor cells often express CD45R (B220) and Pax-5 and can produce immunoglobulins, which react with antibodies to human kappa light chains (Morse et al., 2002).

A relatively newly described lymphoma is the splenic marginal zone lymphoma (Ward et al., 1999; Morse et al., 2002). It occurs in low incidence (1–2%) in most mouse strains and can be best differentiated from follicular lymphoma in its early stages as it arises from the marginal zone. It is most common in p53 null mice (Ward et al., 1999).

The NCI mouse models of human cancers consortium classification, which is based on the WHO human classification (Jaffe et al., 2001), uses additional diagnoses for lymphomas resembling those in humans Table 3. Several of these tumors are primarily found in genetically engineered mouse (GEM) models of lymphoma or congenic lines of mice (Kogan, 2004; Morse et al., 2001; Teitell and Pandolfi, 2004; Ward et al., 1999). Two web sites have virtual slides of a variety of mouse lymphomas based on this classification (http://imagearchive.compmed.ucdavis.edu/; http://www.path.uiowa.edu/virtualslidebox/cancer_pathology/content.html). In particular, the diffuse large B-cell lymphoma (DLBCL) is composed of a majority of centroblastic (lymphoblastic), immunoblastic or histiocyte-associated cells, seen mostly in congenic mice and some GEM lines. The true DLBCL in aging CD-1 and

Table 2. Classification of hematopoietic tumors in mice (WHO, Frith et al., 2001)

Leukemia, granulocytic
Leukemia, erythroid
Leukemia, megakaryocytic
Mast cell tumor
Histiocytic sarcoma
Lymphoma
Small lymphocyte (T- or B-cell)
Lymphoblastic (T- or B-cell)
Follicular/pleomorphic (B-cell)
Splenic marginal zone (B-cell)
Plasmacytic (B-cell)
Immunoblastic (B-cell)

Table 1. Gross necropsy classification of mouse hematopoietic tumors

Gross distribution	Origin of T-cell lymphoma	Origin of B-cell lymphoma	Origin of histiocytic sarcoma	Origin of myeloid leukemia
Systemic (generalized)	Common	Rare	Rare	Rare
Thymic	Primarily	Rare	Rare	Rare
Spleen	Rare	Common–follicular or marginal zone	Occasionally – red pulp origin	Common
Peyer's patches	Rare	Common	Occasional	Rare
Mesenteric lymph nodes	Rare	Common	Occasional	Rare
Liver	Rare	Rare	Common	Rare
Uterus	Rare	Rare	Common	Rare
Peritoneum	Rare	Rare	Common	Rare
Skin	Rare	Rare	Occasional	Rare
Bone marrow	Rare	Rare	Rare	Occasional

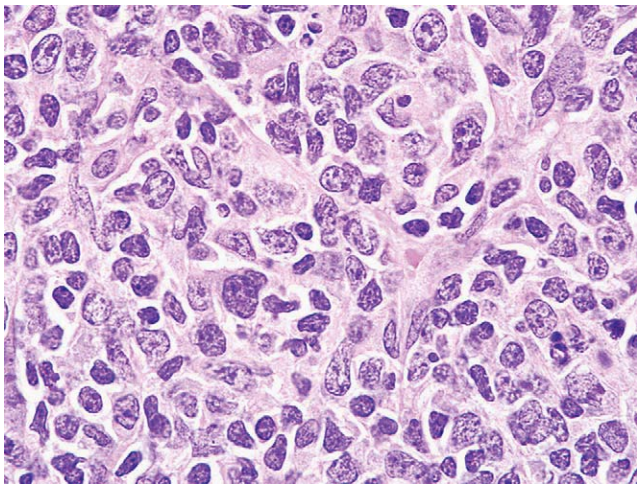

Fig. 3. Follicular (pleomorphic) lymphoma, in a B6C3F1 mouse, is composed of a mixture of cell types (centroblasts and centrocytes), which vary in size and shape.

Table 3. An extended classification of B-cell lymphomas in mice (Morse et al., 2001)

Precursor B-cell neoplasm
Precursor B-cell lymphoblastic lymphoma/leukemia (pre-B LBL)
Mature B-cell neoplasms
Small B-cell lymphoma (SBL)
Splenic marginal zone B-cell lymphoma (SMZL)
Follicular B-cell lymphoma (FBL)
Diffuse large B-cell lymphoma (DLBCL)
Morphologic variants
Centroblastic (CB)
Immunoblastic (IB)
Histiocyte associated (HA)
Subtypes
Primary mediastinal (thymic) diffuse large B-cell lymphoma (PM)
Classic Burkitt (BL)
Burkitt-like lymphoma
Plasma cell neoplasm
Plasmacytoma (PCT)
Extraosseous plasmacytoma (PCT-E)
Anaplastic plasmactyoma (PCT-A0
B-natural killer cell lymphoma (BNKL)

B6C3F1 mice is rarely seen. If they arise from follicles or germinal centers, the mouse WHO classification would term them follicular/pleomorphic. The human follicular lymphomas, however, often form discrete structures resembling normal follicles which the mouse follicular lymphomas do not possess. Hence, MD pathologists often term the mouse follicular tumors as diffuse large B-cell lymphomas, human tumors that do not form follicular structures. Since the origin of mouse lymphomas (in strains and stocks of aging mice that are used in toxicology studies) are often in the white pulp of the spleen or the cortex of the mesenteric lymph nodes, we can define the follicular tumors as to their origin. Less is known of the human counterpart. The mouse DLBCL and follicular lymphomas often express differentiated B-cell antigens such as CD45R (B220) (Fig. 4) and Pax-5 (Fig. 5). Some tumors express focal or diffuse immunoglobulin production often readily shown by antibodies to human kappa light chains. T-cell lymphomas, induced by retroviruses, chemicals, irradiation and found in genetically engineered mice, are often of thymic origin, are lymphoblastic and often have metastases to many tissues. Thymic tumors arise via lymphocyte depletion, atypical hyperplasia and subsequent lymphoma.

Myeloid tumors are rare as spontaneous tumors in most mouse lines except for histiocytic sarcoma, which is common in old C57BL/6 mice (Boorman et al., 2000; Kogan et al., 2002; Kogan, 2004; Frith et al., 1996).

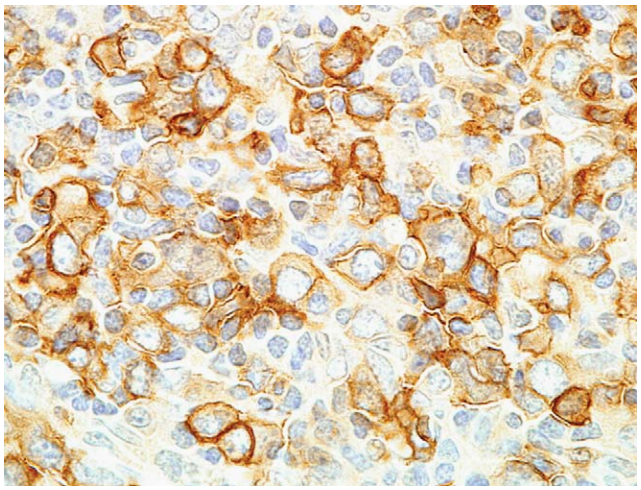

Fig. 4. CD45R cell membrane expression in a typical B-cell follicular lymphoma.

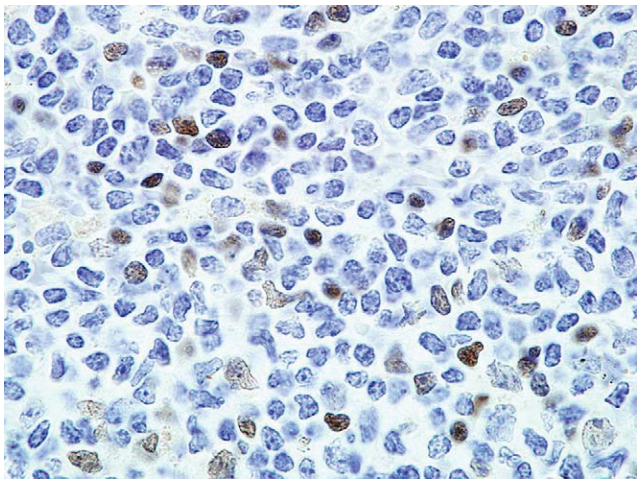

Fig. 5. Pax-5 nuclear expression in a B-cell follicular lymphoma. Note variable expression of the protein in different tumor cells.

Immunohistochemistry for determining tumor antigen expression, Southern blotting for gene rearrangements (Haines et al., 2001) and other techniques can be used to characterize the tumor cells and final tumor classification. These assays can help differentiate spontaneous lymphomas from induced lymphomas. Several excellent web sites offer protocols for mouse antibodies (Columbia University – http://icg.cpmc.columbia.edu/cattoretti/Protocol/Mouse_IHC/Antibodies_for_mouse_IHC.html; NIAID, NIH – http://www.niaid.nih.gov/dir/services/animalcare/VetPathology/mouseha.html; NCI Frederick – http://web.ncifcrf.gov/rtp/lasp/phl/immuno/; NIEHS – http://dir.niehs.nih.gov/dirlep/immuno/)

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