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IMMUNOPHENOTYPE OF SPONTANEOUS HEMATOLYMPHOID TUMORS OCCURRING IN YOUNG AND AGING FEMALE CD-1 MICE

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Abstract

A few reports indicated the incidence of hematology lymphoid neoplasms in old CD-1 mice, but the cellular lineage of CD-1 mouse neoplasms has not been published. In this study, immunohistochemistry (IHC) was used to characterize the cellular lineage of spontaneous hematology lymphoid neoplasms arising in young female CD-1 mice used as health monitoring sentinels and aging female CD-1 mice used as controls in 80 wk carcinogenesis studies. Lymphoblastic lymphomas of T-cell and B-cell lineage were common in mice 12 mo or less of age, whereas a wide range of non-lymphoblastic B-cell lymphomas and lymphoblastic T-cell lymphomas were common in mice > 12 mo old. Renal hyaline droplets positive for lysozyme were observed in aged mice with a histiocytic-associated large B-cell lymphoma (HA-BCL) and a myeloid leukemia. Endogenous ecotropic MuLV genes have been recovered from CD-1 mice, but MuLV protein expression has not been previously demonstrated. We reported for the first time the expression of MuLV protein by IHC in lymphomas and some normal tissues of both young and aging CD-1 mice. This report should help to differentiate spontaneous lymphomas and leukemias in CD-1 mice from those induced by chemicals and other methods.

Keywords

CD-1 mice; female; hematology lymphoid neoplasms; immunohistochemistry

Introduction

The CD-1(ICR) mouse is a multipurpose outbred research mouse. It is the most commonly outbred mouse stock that is used in aging, oncology and toxicologic safety and efficacy testing (Maita et al., 1988; Son, 2003; Baldrick and Reeve, 2007; Bradley et al., 2012). There are a few reports that indicate the incidence of hematology lymphoid tumors in CD-1 mice (Maita et al., 1988; Son and Gopinath, 2004; Ward, 2006; Baldrick and Reeve, 2007; Giknis and Clifford, 2010; and Bradley et al., 2012). Only one of these reports (Son and Gopinath,

2004) addresses the incidence of these tumors in mice less than 12 mo of age, where the other reports note the incidence in mice on 2 year- long studies. Furthermore, only one publication has attempted to provide a morphologic classification other than the collective term lymphoma for these tumors in CD-1 mice (Bradley et al., 2012). The purpose of this study was to further characterize and classify hematolymphoid tumors with immunohistochemistry based on cellular lineage that occur in young and aged CD-1 mice and to determine whether a retrovirus was expressed in these tumor burdened CD-1 mice.

Materials and Methods

Animals

Tissue samples were collected from a total of 59 female mice. Twenty-four mice were maintained in the St. Jude Children's Research Hospital (SJCRH) sentinel program and were sacrificed or died between 2008 and 2014. Sentinel mice were generally maintained until 12–14 months of age then often disposed of and not necropsied unless ill. Of 218 female sentinel CD-1 mice necropsied over 6 years at SJCRH, 24 had lymphoma. Thirty-two mice were obtained from the control group of an 80 wk dietary carcinogenesis study evaluated between October 2009 and April 2011 and conducted by Charles River Edinburgh (CRE). It was previously reported that 17% of males and females in the CRE 80 and 104 week studies with CD-1 mice developed hematopoietic tumors (Bradley et al, 2012). The animals were purpose-bred for laboratory use, the SJCRH mice came from Charles River USA breeding facilities at Portage, MI and Kingston, NY and the CRE mice came from Charles River European suppliers (Charles River UK Ltd, Margate, Kent). All control mice incorporated into the CRE studies were obtained from groups of mice that had been sham dosed by giving untreated diet *ad libitum*. For compatibility reasons only CD-1 female mice are used in the SJCRH sentinel program. Consequently all the mice in this study from both sources were females. Male mice were not studied from the 80 or 104 week studies, in part, because they were reported to have a lower incidence of similar morphologic types of lymphomas as did females than reported by one of our co-authors (Bradley et al, 2012). The mice were housed in groups of 3 or 5 animals per cage. The temperature and humidity of the SJCRH animal rooms were automatically controlled at 20.6–22.8 °C and 30–70% respectively with a minimum of 15 air changes per hr. An automatic 12 hr light/dark cycle of 0600 -1800 hr was maintained. Animals were provided with reverse osmosis purified water chlorinated at 8 ppm *ad libitum* and fed an *ad libitum* commercial rodent diet (Autoclavable Rodent Breeder Diet 5013, LabDiets, Richmond, IN). The temperature and humidity of the CRE animal rooms were automatically controlled at 19–23°C and 40–70% respectively, with a minimum of 15 air changes per hr. An automatic light cycle of 0700 -1900 (12-hour cycle) was maintained. The CRE animals were provided water *ad libitum* and fed *ad libitum* medicated commercial rodent diet (Rat and Mouse Modified No. 1 Diet SQC Expanded, Special Diets Services Ltd, 1 Stepfield, Witham, Essex UK).

The SJCRH study was conducted with approval of the IACUC in accordance with NIH guidelines. The CRE studies were conducted in accordance with the UK Animals (Scientific Procedure) Act 1986, which conforms to the European Convention for the Protection of

Vertebrate Animals Used for Experimental and other Scientific Purposes (Strasbourg, Council of Europe).

Histopathology

Decedent animals and live animals humanely euthanized were necropsied and tissues from all organ systems were collected and fixed in 10% neutral buffered formalin. All tissues were embedded in paraffin, sectioned at 4–5 μ m and stained with hematoxylin and eosin. The histopathology and immunohistochemistry of all the lymphoid tissues and bone marrow, when available, were examined blindly by two veterinary pathologists (JER and JMW) and all hematolymphoid proliferations were classified morphologically according to the recommendation of the Mouse Models of Human Cancer Consortium (Morse III et al., 2002; Kogan et al., 2002). The diagnoses reported represent the consensus opinions of both pathologists along with the concurrence of the CRE veterinary pathologist (AB).

Additional unstained sections were processed for immunohistochemistry (IHC). Tissues from all SJCRH and CRE cases were immunostained for T- and B-cell antigens and select cases for macrophage and myeloid antigens. The IHC procedures and antibodies used for the hematolymphoid antigens were as previously described (Rehg et al., 2012) (Table 1). For the presence of Rauscher MuLV p30, tissues from 16 SJCRH and 9 CRE mice were stained. The goat anti-p30 antibody was provided by Dr. Sandra Ruscetti, of the National Cancer Institute, Frederick, Maryland (O'Brien et al., 1978) and IHC p30 procedures were as previously described (Hartley et al., 2008).

Results

Table 2 presents the spontaneous histopathological findings recorded for mice from the SJCRH sentinel program and an 80 wk study at CRE. The hematolymphoid neoplasms in the SJCRH mice, except for two, occurred in mice 12 mo old or less and were classified into four groups whereas 30 of the 32 neoplasms in the CRE mice occurred in mice older than 12 mo. Some hematolymphoid neoplasms (lymphoblastic lymphoma, follicular lymphoma and myeloid leukemia) were common to both groups whereas other neoplasms were only observed in the CRE mice, and these mice were generally 20 mo of age or less.

The 32 cases of lymphoblastic lymphoma (LBL) in mice less than and greater than 12 mo of age were characterized by medium sized blastic cells having scant pale cytoplasm, nuclei with fine stippled chromatin and inconspicuous or small-medium sized nucleoli (Figures 1–2). The nuclei were round or irregular and the cells formed non-cohesive sheets. Tingible body macrophages were scattered among the tumor cells giving a starry sky appearance. Morphologically, the 17 cases of T-cell LBL (T-LBL) and 15 B-cell LBL (B-LBL) cases were indistinguishable except perhaps for the involvement of thymus in T-LBL. Often it was not possible to determine if the thymus was involved or mediastinal lymph nodes or both. However, immunohistochemistry with the use of antibodies to T-cell and B-cell antigens, the lymphoblastic lymphomas were further classified by their cellular lineage into T-LBL or B-LBL. The lymphoblastic lymphomas tend to involve the spleen, multiple lymph nodes, liver, lungs, kidneys, ovaries and the CNS, but T-LBL (53%, 9/17) was more likely than B-LBL (7%, 1/15) to involve the small intestine (Table 3). Lymphoblastic lymphoma was

more common (96%; 23/24) in mice 12 mo old or less than in mice over 12 mo old (33%; 10/30) and T-LBL was more common (67%, 16/24) than B-LBL (29%; 7/24) in the young age group. Antigen expression among the different hematolymphoid tumors was characteristic of specific lineages and the maturation stages of the different class of tumors (Table 4). The T-LBLs labeled with CD3 (100%, 17/17) (Figure 3), CD5 (76%, 13/17), CD43 (100%, 17/17) and TDT (82%, 14/17) (Figure 4). The B-LBLs labeled with PAX5 (100%, 15/15), CD45R/B220 (40%, 6/15) (Figure 5), IgM (53%, 8/15), CD43 (53%, 8/15), TDT (13%, 2/15), but they did not label with kappa light chain (IgK, 0%, 0/15). Based on the expression of IgM, the B-LBLs consisted of two maturation subtypes, pro-B (47%, 7/15) which lack IgM immunoreactivity and pre-B (53%, 8/15). The pro-B LBL labeled with CD45R/B220 (53%, 4/7), PAX5 (100%, 7/7), CD43 (70%, 5/7), but they did not label with IgM (0%, 0/7) or IgK (0%, 0/7). The pre-B LBL labeled with CD45R/B220 (25%, 2/8), PAX5 (100%, 8/8), CD43 (38%, 3/8), IgM (100%, 8/8), IgK (0%, 0/8). One lymphoma of both B-LBL subtypes labeled with TDT. Unlike T-LBL where the incidence decreased with age (young 63%, 15/24; aging 6%, 2/32), the incidence of B-LBL was generally the same in young (25%; 6/24) and aging (28%; 9/32) mice.

Follicular lymphoma was found in 7 mice greater than 12 mo of age. The tumors involved the spleen, lymph nodes, and other tissues (Table 3). In the spleen, the neoplastic cells caused enlarged white pulp areas. The tumors were comprised of small, medium and large cells of variable proportions with moderate cytoplasm (Figure 6). The small cells had round, irregular or cleaved dense nuclei in centrocytes. The medium and large tumor cells had vesicular nuclei with nucleoli juxtaposed to the nuclear membrane (centroblasts) or central / elongated prominent nucleoli (immunoblasts). Cleaved nuclei were often seen. Macrophages, plasma cells and T-lymphocytes in variable proportions were also commonly admixed among the neoplastic lymphocytes. The lymphoma cells were usually positive for PAX5 (100%, 7/7), CD45R/B220 (86%, 6/7), PNA (86%, 6/7), BCL6 (86%, 6/7) and less so for IgM (57%, 4/7) and IgK (71%, 5/7).

Histiocyte-associated B-cell lymphoma (HA-BCL) was diagnosed in 10 mice. The tumors were often found in several lymph nodes and spleen. Histologically, the lymphomas were composed of a mixture of immature lymphocytes with large to small nucleoli. A few cases contained mostly lymphoblasts. Interspersed along the immature lymphoid cells were histiocytic cells with abundant pale eosinophilic cytoplasm (Figure 7). The tumor lymphocytes were positive for PAX5 (100%, 9/9) (Figure 8), CD45R/B220 (67%, 6/9), IgM (67%, 6/9) and IgK (33%, 3/9) while histiocytes were ionized calcium-binding adapter molecule 1 (IBA1) positive (9/9, 100%) (Figure 9). Two of the nine (22%) HA-BCLs had abundant infiltrating CD3+ T cells (Figure 10).

The marginal zone lymphoma was morphologically similar to the two small lymphocyte lymphomas and the tumor was seen in the spleen, lymph nodes and many other tissues (Table 3). However, the tumor cells were associated with the marginal zone of all of the follicles and they infiltrated into the red pulp bridging follicle to follicle. The tumor cells expressed PAX5 and IgM but not CD45R/B220.

Small lymphocyte lymphoma, was only observed in two mice older than 18 mo of age. The tumors were characterized by small to medium lymphocytes with round condensed nuclei and a scant pale rim of cytoplasm. The tumor pattern was that of non-cohesive cells with rare to a few mitotic figures. These tumors involved multiple lymphoid organs. With use of immunohistochemistry and antibodies to T- and B-lymphocyte markers the two tumors were further classified as a small cell lymphoma of T-cell lineage and a small cell lymphoma of B-cell lineage. The cells of T-small cell lymphoma expressed CD3, CD43 and CD5 whereas the cells of the B-small cell lymphoma expressed PAX5, CD45R/B220, IgM and IgK but not CD3.

Plasma cell lymphoma was observed in only one 21 mo old CRE mouse. The tumor involved multiple lymphoid organs and consisted of cells with abundant cytoplasm. The tumor cells had round nuclei and were arranged in non-cohesive sheets. Morphologically, the cells could have been considered to be mature small lymphocytes but with immunohistochemistry the tumor cells were confirmed to be of plasma cell lineage. The tumor cells expressed the plasma cell markers CD43, CD138 and IFR4.

Myeloid leukemia developed in four mice 12 to 18 mo old with 75% occurring in CRE mice greater than 12 mo of age. These neoplasms were composed of large immature myeloid cells often with ring forms (Figure 11). Tumor cells were usually positive for CD43 (100%, 4/4), myeloperoxidase (75%, 3/4), YM 1 (75%, 3/4) and RUNX1 (75%, 3/4) (Figure 12), but only one was immunoreactive for lysozyme (25%, 1/4) (Table 4). The tumors were often in many tissues: spleen, liver, lungs, lymph nodes, bone marrow (50%, 2/4), and other tissue (Table 3).

Histiocytic sarcoma was observed in two aged CRE mice 20 mo of age. The tumors consisted of infiltrates of monomorphic or pleomorphic histiocytes with abundant pink cytoplasm. The tumor cells had round, ovoid and irregular vesicular nuclei with small nucleoli. Multinucleated giant cells were scattered among the neoplastic tumor cells. The tumors had a high mitotic index and the tumors involved multiple organs. Although the tumor cells expressed IBA1, renal tubule hyaline droplets commonly associated with murine histiocytic sarcoma were not observed in these two mice.

In SJCRH mice with lymphoma or myeloid leukemia, MuLV p30 antigen was found by IHC in tumor cells (5/10 cases studied) (Figure 13) and in normal megakaryocytes in spleen or bone marrow (5/10) (Figure 14). B cell follicles in lymph node or spleen of a few mice showed p30 (Figure 15). MuLVp30 antigen was also found in salivary gland epithelium of 5/5 mice with lymphoma or in 1 mouse with myeloid leukemia (Figure 16), pancreatic acinar cells of 7/7 mice, but not in megakaryocytes of the 13 month old normal CD1 mice, in tissues of five 4 week old CD-1 mice nor in tissues of FVB mice, which have been reported to be free of MuLV (Teddessee-Heath et al, 2000; Morse III, 2007). Antigen was found in tumor cells of 3/9 CRE lymphoma cases and in megakaryocytes of 1/9 CRE mice with lymphoma.

Renal tubular hyaline droplets (Figure 17) were found in 6 of 9 CRE mice with HA –BCL, but not in any SJCRH mice with any lymphoma or leukemia. One positive renal droplet case

had both HA-BCL and focal histiocytic sarcoma. The renal droplets were positive for lysozyme in 5/5 mice (Figure 18). One CRE myeloid leukemia had hyaline droplets, which had strong immunoreactivity for lysozyme and a few granules with weak immunoreactivity for YM1.

Discussion

In 2004 it was reported that 17% of 101 CD-1 male and female mice less than 12 mo of age developed lymphoma (Son and Gopinath, 2004). The morphologic features and cellular lineage of these lymphomas were not determined. In 2012, a report describes a range of hematolymphoid tumors of undetermined cellular lineage but with morphologic features similar to those in our report that were present in 355 control mice collected at the end of several 24 mo carcinogenicity studies (Bradley et al., 2012). Our data clearly demonstrate that with the use of immunohistochemistry the lymphoblastic lymphomas and small lymphocyte lymphomas that arise in young and old CD-1 mice consist of both T-lymphocyte and B-lymphocyte lineages. The data further demonstrate that the lymphoblastic lymphomas that occur in CD-1 mice less than 12 mo of age are predominantly of T-lymphocyte lineage and that B-lymphoblastic lymphomas are equally prevalent in CD-1 mice less than and older than 12 mo. of age. This age and cellular distribution of lymphoblastic lymphoma in CD-1 mice is similar to that in human patients. T-cell lymphoblastic lymphoma is more prevalent than B-cell lymphoblastic lymphoma in pediatric patients (Cortelazzo et al., 2011) and has a higher rate of mediastinal tumor (Cortelazzo et al., 2011).

HA-BCL is found in variable proportions in congenic mice and recombinant inbred mice (Morse III et al., 2001, Hao et al., 2010). It is uncommon in the most popular strains and stocks of mice, but our data suggest it is a common subtype of lymphoma in CD-1 mice. Other reports on lymphomas in CD-1 mice have not reported HA-BCL, but HA-BCL in these reports may be diagnosed under the collective term lymphoma or pleomorphic / follicular lymphoma (Decker et al., 2012; Bradley et al., 2012). Mechanisms for the involvement of apparent non-neoplastic histiocytes in these tumors are not known.

Infiltrating non-neoplastic T- cells have been reported in mouse lymphomas and appear to be common in aging mice (Haines et al., 2001). Human T cell and histiocyte- rich, large B cell lymphomas are considered an entity in the WHO classification of human hematolymphoid tumors (De Wolf-Peeters et al., 2008). Although the HA-BCLs in this study have some similarity to the human T cell/histiocyte-rich large B-cell lymphoma, the histiocyte and lymphocyte populations in the human T cell/histiocyte-rich large B-cell lymphoma are far more extensive than either population in the mouse HA-BCLs.

T-cell lymphoblastic lymphomas in either the SJ or the CRE mice probably arose from thymic lymphocytes as they often do in chemically induced thymic lymphomas or in genetically engineered mice (Ward, 2006). B-cell lymphoblastic lymphomas in the study mice may have arisen from a B-cell progenitor and the small B-cell lymphoma from either a naïve or antigen experienced B-cell in spleen or other lymphoid tissues. Follicular lymphoma and HA-BCL arise from germinal center B-cells and diffuse large B-cell

lymphomas (DLBCL) from peripheral B-cells of either germinal center or activated post-germinal center cells (Stein et al., 2008). Splenic marginal zone lymphomas in the mouse are of a B-cell of an unknown stage of B-cell differentiation. The small T-cell lymphoma may have arisen from circulating T-lymphocytes.

Renal hyaline droplets were first reported in rats and mice with histiocytic sarcomas (Hard and Snowden 1991) but recently were reported in CD-1 mice with lymphoma (Decker et al 2012). The figure in the Decker report appears to be a lymphocytic lymphoma and not a HA-BCL. The CD-1 mice with lymphoma and renal hyaline droplets in the 2012 report had some evidence of immunoglobulins in tumor cells but no lysozyme in the droplets with their IHC studies being inconclusive (Decker et al., 2012). The cause of the renal droplets in mice with lymphoma that are not a HA-BCL or histiocytic sarcoma is not definitively known since they seem to be of tumor histiocyte origin. HA-BCLs show many histiocytes which are probably a source of lysozyme for renal accumulation as hyaline droplets, which others (Hard and Snowden 1991) showed contain lysozyme. Assuming the renal droplets in HA-BCL contain abundant lysozyme, we postulate as has been postulated for histiocytic sarcoma, that the lysozyme accumulates in the renal tubules as a result of absorption of glomerular filtration fluid containing the enzyme released into the blood by the abundant histiocytes in the HA-BCL.

Renal hyaline droplets positive for lysozyme were also present in the renal tubules of one of the four myeloid leukemias. Cytoplasmic lysozyme droplets have been reported to accumulate in the renal tubules of Wistar-Frith rats injected intraperitoneally with rat myeloid leukemia (choroleukemic) cells (Klockars et al., 1974). YM1 and YM2 chitinase-like lectins have been reported to accumulate as eosinophilic droplets in renal tubules of a mouse with experimental myeloid leukemia (Marchesi et al., 2003; Marchesi et al., 2006). In the latter report the renal tubular eosinophilic droplets co-expressed YM1 protein and lysozyme, but the myeloid tumor cells in this single case did not express lysozyme suggesting the myeloid tumor cells were not the source of the lysozyme. In three of the four myeloid leukemias in our study, the cells expressed YM1 (now CHIL3, Regh et al., 2012; Table 1), but only the myeloid leukemia immunoreactive for lysozyme had renal tubular hyaline droplets that co-localized lysozyme and YM1, the former with strong intensity and the latter with slight positivity. To the authors' knowledge the present report is the first illustration of renal lysozyme positive eosinophilic droplets associated with spontaneous mouse myeloid leukemia that reveal localization of lysozyme.

Murine leukemia virus (MuLV) was first discovered in the 1950's (Gross, 1951). Proviruses that encode infectious ecotropic and xenotropic MuLV are found in the genomes of some strains or stocks of mice but not others (Morse III, 2007). The MuLV genome is reported to be present in retired CD-1 breeder mice (Taddesse-Heath et al., 2000), but MuLV expression of major core proteins (p30 or p70) in the CD-1 mouse has not been previously reported. Ecotropic MuLV infection of adult mice has been shown to increase B-cell proliferation, serum immunoglobulin M levels and expression of transcripts associated with B-cell activation (Lee et al., 1978).

Ecotropic MuLV has also been shown to induce a variety of lymphoma subtypes in outbred and inbred mice (Hartley et al., 2008). Our data suggest that the expression of MuLV protein in CD-1 mice should be considered in interpretation of the use of CD-1 mice or their tissues in various toxicologic studies related to carcinogenesis, propagation of other viruses, biological responses to cell signaling and RNA interference.

This study has characterized the subtypes and cell lineages of lymphomas that commonly develop in young and aging female CD-1 mice. We also demonstrated that renal hyaline droplets containing lysozyme may be seen in association with histiocytic-associated B-cell lymphomas and that the MuLV major core protein p30 is present in CD-1 lymphomas and normal tissues. The data also suggest that additional studies are warranted to determine the significance of the retroviral infection in CD-1 mice.

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Abbreviations

B-LBL	B-cell lymphoblastic lymphoma
CD	cluster of differentiation
CRE	Charles River Edinburgh
DLBCL	diffuse large B-cell lymphoma
IgK	kappa light chain immunoglobulin
IHC	immunohistochemistry
HA-BCL	histiocyte- associated B-cell lymphoma
LBL	lymphoblastic lymphoma
MuLV	mouse leukemia virus
PALS	periarteriolar lymphoid sheath
PAX5	paired box protein
PNA	peanut agglutinin
SJCRH	St. Jude Children's Research Hospital
T-LBL	T-cell lymphoblastic lymphoma
WHO	World Health Organization

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FIGURE 1. Lymphoblastic T-cell lymphoma in an 8 month old sentinel mouse with a thymic mass. Note the uniform population of round cells with medium-sized nucleoli and starry sky macrophages. H&E.

FIGURE 2. Lymphoblastic B-cell lymphoma in the thymus of a 6 month old sentinel mouse. Note similarity of cell types to those in Figure 1. H&E.

FIGURE 3.
CD3+ cells in a lymphoblastic T-cell lymphoma in an 8 month old female sentinel mouse.
Metastatic tumor cells are found in the PALS and red pulp of the spleen.

FIGURE 4.
TDT+ cell nuclei in a lymphoblastic T-cell lymphoma in an 8 month old sentinel mouse.

FIGURE 5. CD45R/B220+ cell membranes of tumor cells in a lymphoblastic B-cell lymphoma in a 6 month old mouse. Note normal CD45R/B220+ small lymphocytes in lower left corner.

FIGURE 6. Follicular lymphoma in a Peyer's patch of a 21 month old mouse. Note pleomorphism of cell types.

FIGURE 7.

Histiocyte-associated B-cell lymphoma in a lymph node of a 21 month old mouse. Note the pale large histiocytes with abundant eosinophilic cytoplasm among the round blastic lymphocytic tumor cells. H&E.

FIGURE 8.
PAX5 in nuclei of tumor cells of a histiocyte-associated B-cell lymphoma in a 16 month old mouse.

FIGURE 9.
IBA1 in histiocytes in the histiocyte-associated B-cell lymphoma in Figure 8.

FIGURE 10.
CD3 in normal lymphocytes infiltrating in the histiocyte-associated B-cell lymphoma in a 21 month old mouse.

FIGURE 11.
Myeloid leukemia in a 16 month old mouse. Note undifferentiated cells, with some blasts and ring forms.

FIGURE 12.
RUNX1 in the nuclei of tumor cells of myeloid leukemia in Figure 11.

FIGURE 13.
Rauscher leukemia virus (MuLV) p30 in myeloid leukemia of a 12 month old sentinel mouse.

FIGURE 14.
Rauscher leukemia virus (MuLV) p30 in splenic megakaryocytes in a 20 month old mouse.

FIGURE 15.
Rauscher leukemia virus (MuLV) p30 in the follicles of a normal lymph node in a 5 month old sentinel mouse with B-cell lymphoblastic lymphoma in other tissues.

FIGURE 16.
Rauscher leukemia virus (MuLV) p30 in the parotid salivary gland of a 12month old
sentinel with myeloid leukemia.

FIGURE 17. Renal tubular hyaline droplets in a 17 month old mouse with histiocyte-associated B cell lymphoma.

FIGURE 18.
Lysozyme in hyaline droplets of the mouse with histiocyte-associated B-cell lymphoma in
Figure 17.

Table 1
Commonly Used Antibodies and Sources for Mouse Hematolymphoid Antigens Detectable in Paraffin Sections

Antigen	Major cells expressing antigen	Antibody	Clone	Dilution	Source	Epitope retrieval
BCL6	Germinal center (GC) B-cells (centroblasts and centrocytes), GC or post GC B-cell neoplasms	Rabbit anti-human	n/a	100	Santa Cruz	HIER, Target Retrieval (DAKO pH 6)
CD3	T-cells and many T-cell neoplasms	Goat anti-mouse	n/a	350	Santa Cruz	HIER, Target Retrieval (DAKO pH 6)
CD5	T-cells and many T-cell lymphomas	Rat anti-mouse	53-7.3	200	BD Biosciences	HIER, Target Retrieval (DAKO pH 6)
CD43	Early B-progenitors, T-cells, myeloid cells, plasma cells, pro B-lymphoblastic lymphoma, T-cell lymphomas, myeloid leukemia, plasmacytomas All hematolymphoid cells; low to no expression on plasma cell neoplasms	Rat anti-mouse	S7	20	BD Biosciences	HIER, Target Retrieval (DAKO pH 6)
CD45R/B220	B-cells, subsets of T-cells, dendritic cells and NK cells	Rat anti-mouse	RA3-6B2	4000	BD Biosciences	HIER, Citrate pH 6
CD138	Plasma cells	Rat anti-mouse	281-2	1000	BD Biosciences	HIER, ER1 (Leica, pH 6)
IBA-1	Histiocytes/macrophages	Rabbit anti-human	n/a	500	Biocare Medical	HIER, Citrate pH 6
IFR4/MUM1	Normal and neoplastic plasma cells; post GC B-cell lymphomas.	Goat anti-mouse	n/a	6000	Santa Cruz	HIER, Target Retrieval (DAKO pH 6)
IgM	Immunoglobulin heavy chains, some immature B-cells; mature B-cells; plasma cells	Rat anti-mouse	II/41	60	BD Biosciences	HIER, Target Retrieval (DAKO pH 6)
IgK	Immunoglobulin kappa light chains, some immature B-cells; mature B-cells; plasma cells	Goat anti-mouse	n/a	2000	Southern Biotechnology	HIER, Target Retrieval (DAKO pH 6)
Lysozyme (LYZ)	Histiocytes/monocytes and myeloid cells (benign and neoplastic)	Rabbit anti-human	n/a	4000	DAKO	HIER, Citrate pH 6
Myeloperoxidase (MPO)	Myeloid cells benign and neoplastic	Rabbit anti-human	n/a	1500	DAKO	HIER, Citrate pH 6
PAX5	B-cells; B lymphomas; not expressed in plasma cells	Goat anti-human	n/a	300	Santa Cruz	HIER, Citrate pH 6
PNA Lectin	GC cells & FDC	n/a	n/a	200	Vector	HIER, Citrate pH 6
RUNX1/AML	Myeloid cells, megakaryocytes and lymphoid cells	Rabbit anti-human	n/a	600	Active Motif	HIER, ER1 (Leica, pH 6)
TDT	Immature thymic lymphoid cells, lymphoblastic neoplasms	Rabbit anti-human	n/a	50	Supertechs	HIER, Citrate pH 6
YM1 (CHIL3)	Neutrophils; Th2 activated macrophages	Goat anti-mouse	n/a	3000	R&D	HIER, Target Retrieval (DAKO pH 6)

Table 2

Hematolymphoid Neoplasms in Young and Aging Female CD-1 Mice

ST. JUDE SENTINEL PROGRAM N = 24				UK CRL BIOASSAY PROGRAM N = 32*			
Age (Mo)	N	Classification	Age (Mo)	N	Classification	Age (Mo)	N
5-8	15	T-lymphoblastic lymphoma	11, 16	2	T-lymphoblastic lymphoma		
5-10	6	B-lymphoblastic lymphoma	11,13-19	9	B-lymphoblastic lymphoma		
14	2	Follicular lymphoma	14-21	5	Follicular lymphoma		
12	1	Myeloid leukemia	13-21	9	Histiocyte associated B-lymphoma		
			21	1	Marginal zone lymphoma		
			20	1	Small B-cell lymphoma		
			20	1	Small T-cell lymphoma		
			21	1	Plasma cell lymphoma		
			15, 16, 18	3	Myeloid leukemia		
			20,21	2	Histiocytic sarcoma		

* The number of tumors exceeds the number of mice because two mice each had two hematolymphoid tumors.

TABLE 3

Hematolymphoid Neoplasm Tissue Distribution in Female CD-1 Mice

Tumor Type	No ¹	Adrenal ²	BM ³	CNS	Colon	Eyes	Heart	Small Intestine	Kidney	Liver	Lungs	Lymph nodes	Ovary	Pancreas	Salivary Glands	Spleen	Stomach	Thymus
T-cell LBL	17	8	15	13	5	8	10	9	15	17	16	17	13	7	4	16	8	17
B-cell LBL	15	5	14	11	5	5	5	1	9	11	14	3	11	6	6	3	12	9
Follicular lymphoma	7	2	3	0	0	0	0	2	4	3	4	6	2	2	1	6	4	3
Histiocytic-associated B-lymphoma	9	4	9	2	2	1	2	2	9	1	1	1	2	3	5	9	7	9
Marginal Zone lymphoma	1	0	1	0	0	1	0	0	1	1	1	1	1	0	1	1	1	0
Small B-cell Lymphoma	1	1	1	1	1	0	0	1	1	1	1	1	1	0	1	1	1	0
Small T-cell lymphoma	1	1	1	1	0	1	0	0	1	1	1	1	1	0	0	1	1	1
Plasma cell lymphoma	1	0	0	0	0	0	0	0	1	0	1	1	0	0	1	1	0	1
Myeloid Leukemia	4	2	4	2	2	2	3	3	3	4	3	4	2	2	1	4	3	2

¹Number of tumors evaluated.²The number of the organ having tumor.³BM = bone marrow.

TABLE 4

Immunophenotype of Hematolymphoid Neoplasms in Female CD-1 Mice

Tumor Type	No ¹	PAX5 ²	CD45R	CD3	CD5	CD43	CD138	TDT	IgM	IgK	BCL6	PNA	MPO	RUNX1	LYZ	IBA1	IFR4
T-cell LBL	17	0	0	17	13	17	ND ³	14	ND	ND	ND	ND	ND	ND	ND	ND	ND
B-cell LBL	15	15	6	0	ND	8	12	2	8	0	ND	ND	ND	ND	ND	ND	ND
Follicular lymphoma	7	7	6	0	ND	ND	ND	ND	4	5	6	6	ND	ND	ND	ND	ND
Histiocytic-associated B-lymphoma	9	9	6	0	ND	ND	ND	ND	4	2	0	0	ND	ND	ND	9	ND
Marginal zone lymphoma	1	1	0	0	0	0	ND	ND	1	0	ND	ND	ND	ND	ND	ND	ND
Small T-cell lymphoma	1	0	0	1	0	1	ND	0	ND	ND	ND	ND	ND	ND	ND	ND	ND
Small B-cell lymphoma	1	1	1	0	0	0	ND	ND	1	1	ND	ND	ND	ND	ND	ND	ND
Plasma cell lymphoma	1	0	0	0	ND	1	1	ND	0	1	ND	ND	ND	ND	ND	ND	1
Myeloid leukemia	4	0	0	0	ND	4	ND	0	ND	ND	ND	ND	4	4	1	0	ND

¹. Number of tumors evaluated.². Number of tumors expressing antigen.³. Not Done.