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Diagnosis and classification of spontaneously developed and radiation-induced murine haematopoietic neoplasms. The murine models for the research on the human haematopoietic neoplasms

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The Haematopathology Subcommittee of Mouse Models of Human Cancer Consortium (MMHC) proposed a classification that can be readily compared with the human WHO classification 2001 [1] and appropriately delineates the diseases that occur in mice. The mouse lymphoid and nonlymphoid neoplasms develop spontaneously in certain strains and in genetically engineered mice (GEM) or follow induction with ionising radiation or chemical carcinogens or viruses. In the study, the haematopoietic neoplasms that developed in the three investigated mouse strains were identified according to the above classification. They can be useful as mouse models of human lymphoid and nonlymphoid haematopoietic neoplasms.

Key words: T-cell lymphoma, B-cell lymphoma; models, mice

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Introduction

Uniform classification of tumours of murine haematopoietic system has been extensively studied.[1,2] The precise diagnosis will make it possible to compare and contrast murine diseases with human lesions and enable modelling human haematopoietic neoplasms in mice.

Classifications of murine haematopoietic neoplasms has been changing over the last decades. The first classification formulated

by Dunn (1954) was based on the morphology of neoplastic cells and showed similarities to Rappaport's (1966) nomenclature of human lymphomas.[3,4]

In 1981, Pattengale and Taylor proposed a histopathological and immunological scheme of murine lymphomas based on the concept stating that human and murine lymphoid cells of neoplasms represent neoplastic conversion of T or B cell lineage.[5,6] That classification showed a very close relation to Lukes and Collins' (1974) and Kiel's classification (1981).[7,8] Pattengale and Taylor adopted the term of "lymphoid neoplasm" for all haematopoietic neoplasms containing transformed cells that have fully or partially differentiated into T-cells or B-cells or natural killer cells and showing monoclonal proliferation.

The latest classification of murine lymphoid neoplasms was recommended by Haematopathology Subcommittee of MMHCC (Mouse Models of Human Cancers Consortium) and published by Morse *et al.* (2002).[9] It is worth stressing that the classification can be compared with the latest WHO classification of human haematopoietic neoplasms.[1] The classification is also known as "Bethesda proposals for classification of lymphoid neoplasms in mice".[9]

In 2002, the same Subcommittee of MMHCC formulated the latest classification for murine nonlymphoid haematopoietic neoplasms. It was published by Scott C. Kogan *et al.* and it is known as "Bethesda proposals for classification of nonlymphoid neoplasms in mice".[10] The term "nonlymphoid haematopoietic neoplasm" was adopted for haematopoietic neoplasms arising from other lineages than lymphoid ones. The Haematopoietic Subcommittee of MMHCC recommends recognition of the following types of murine lymphoid neoplasms:

B-cell neoplasms
Precursor B-cell neoplasm
Precursor B-cell lymphoblastic lymphoma/leukaemia
Mature B-cell neoplasms
Small B-cell lymphoma
Splenic marginal zone B-cell lymphoma
Follicular B-cell lymphoma
Diffuse large B-cell lymphoma
- Centroblastic
- Immunoblastic
- Histiocyte associated
Classic Burkitt's lymphoma
Burkitt's-like lymphoma
Plasma cell neoplasm
B natural killer cell lymphoma
T-cell - neoplasms
Precursor T-cell neoplasms
Precursor T-cell lymphoblastic lymphoma/leukaemia
Mature T-cell neoplasm
Small T-cell lymphoma
T-natural killer cell lymphoma
T-cell neoplasm, character undetermined
Large cell anaplastic lymphoma

The marked types were not recognized in Pattengale and Taylor classification as separate categories. *E.g.*, the diffuse large B-cell lymphoma was a subtype of follicular centre cell lymphoma from large cells.

It is also worth pointing out that some types of murine lymphomas occur only in mice as special models. E.g. B-natural killer lymphomas can develop only in thymectomized (SL/Kh x AKR/Ms) F1 mice.[11]

The Haematopoietic Subcommittee of MMHCC recommends also four categories of murine nonlymphoid haematopoietic neoplasms with subtypes:

1. Nonlymphoid leukemias
- Myeloid leukemias (granulocytic leukemia)
- Erythroid leukemia
- Megakaryocytic leukemia
- Biphenotypic leukemia
2. Nonlymphoid haematopoietic sarcomas
- Granulocytic sarcoma
- Histiocytic sarcoma
- Mast cell sarcoma
3. Myeloid dysplasias
4. Myeloid proliferations (nonreactive)

The diseases represented in that classification are not very readily compared with human lesions. It is due to the fact that some nonlymphoid haematopoietic neoplasms have not been clearly described in mice. Additional murine models of human nonlymphoid haematopoietic neoplasms are still anticipated.

The purpose of this paper is to show that the murine haematopoietic neoplasms developed in our three investigated mouse strains and identified according to the latest classification can be recognized as murine models of human haematopoietic neoplasms.

Material and methods

Tumours

The murine haematopoietic neoplasms developed:

- spontaneously in recombinant congenic strain OcB/Dem – 20 cases. The OcB/Dem mice were bred from the pairs of mice sent to Cancer Centre in Warsaw from the Netherlands Cancer Institute,[12]
- spontaneously in AKR/W mice carrying endogenous ecotropic provirus which induced potential lymphomas – 46 cases, [13]
- in back-cross (CcS17 x CcS2) x CcS/Dem mice exposed to γ radiation – 72 cases. Mice were exposed to four whole-body γ irradiation with the doses of 1.7 Gy at one week intervals.[14]

All mice were sacrificed when they were visibly sick with poor grooming, hunched posture, weight loss and enlargement of the thymus, lymph nodes or/and spleen detected by palpation.

Pathological procedures

Autopsies were done on each animal. The thymus, mesenteric lymph nodes, spleen, liver as well as other organs with visible neoplastic lesions were fixed in EAFS (ethanol, acetic acid, formol, 0.9% NaCl), and embedded in paraffin; 4 μm thick paraffin sections were stained with H&E or prepared for immunohistochemistry.

Immunophenotyping was performed using two techniques of immunohistochemistry ABComplex and MOM® (Mouse on Mouse) – (immunodetection kit designed to localize murine primary antibodies on mouse tissue), and flow cytometry with the appropriate monoclonal antibodies.

The specific monoclonal antibodies (MAbs) for flow cytometry were conjugated with FITC (CD90.1, CD90.2, CD3ε, CD8, CD5, CD19, CD45R) or PE (CD4, TCRαβ, RAM KAPPA – (rat anti mouse κ), and for immunohistochemistry, they were biotin or pure (anti IgM, anti IgD, anti Igκ, anti Igλ1λ2λ3, Gr-1).[15] All antibodies were produced by PharMingen Germany.

Additionally, two histochemical stainings were performed on the air-dried imprints of tumours, of the spleen, liver or of other organs with neoplastic lesions: naphtol ASBI method - acid phosphatase focal staining is considered to be a specific marker for T lymphoblasts and ASD method - assessment of chloroacetate esterase activity is needed if granulocytic leukemia is to be diagnosed.

Blood smears were stained with Giemsa and the number of neoplastic haematopoietic cells was estimated.

Results

We recognized three types of lymphoid and two types of nonlymphoid haematopoietic neoplasms among 138 classified haematopoietic neoplasms:

LYMPHOID NEOPLASMS:
T-cell derived lymphoma:
- precursor T-cell lymphoblastic lymphoma/leukaemia

B-cell derived lymphoma:
- follicular B-cell lymphoma

- diffuse large B-cell lyphoma subtype centroblastic (CB)

NONLYMPHOID HAEMATOPOIETIC NEOPLASMS:

- granulocytic leukaemia
- granulocytic sarcoma

Precursor T-cell lymphoblastic lymphoma

Human counterpart of mouse precursor T-cell lymphoblastic lymphoma is the lymphoma of the same nomenclature. The microscopic characteristic of examined lymphomas was as follow:

- cells were monomorphic, medium size with scant cytoplasm;
- nuclei were round with fine immature chromatin;
- cells exhibited numerous mitosis;
- 1-2 nucleoli were placed in the centre of the nucleus;
- the spleen was filled up with sheets of neoplastic lymphoid cells;
- in the liver - sheets of neoplastic cells were placed in sinusoids and/or around the vessels.

The detailed immunophenotypes of the examined precursor T-cell lymphoblastic lymphomas are demonstrated in Table 1. The lymphomas exhibited one out of three immunophenotypes (CD4/CD8)$^+$; CD4$^+$/CD8$^-$; CD4$^-$/CD8$^+$. The double positive phenotype was the most frequent conversely phenotype CD4$^-$/CD8$^-$. The expression of CD4 or/and CD8 and CD90 demonstrated by FACS scan analyses is shown in Figures 1A, B, C. Those lymphomas were positive for acid phosphatase staining. Representative images of T-cell lymphoblastic lymphoma are shown in Figures 2A, B, C.

Follicular B-cell lymphoma

Human counterpart is also follicular B-cell lymphoma.

Microscopic changes were as follow:

- diffuse pattern of lymphoma;
- neoplastic cells were small – centrocytic or large – centroblastic;
- cytoplasm was scant;
- nuclei were cleaved, usually with characteristic "heart" shape or noncleaved, round;
- 2-3 nucleoli were prominent and adherent to the nuclear membrane;
- mitoses were visible only among centroblastic cells.

Follicular B-cell lymphomas showed immunophenotype of B-cells – sIg$^+$, B220$^+$, CD19$^+$. Clonality was confirmed by the ex-

Table 1. Immunophenotypes of tested mouse haematopoietic neoplasms

Marker	T-cell derived lymphomas	B-cell derived lymphomas	Granulocytic leukemia / Granulocytic sarcoma
CD90/Thy.1.	+	–	–
CD3ε	+	–	–
CD4	+ or –	–	–
CD8	+ or –	–	–
CD5	+	–	–
TCRαβ	+	–	–
IgM	–	+	–
IgD	–	+/–	–
Igκ / RAM KAPPA	–	+	–
Igλ1λ2λ3	–	–/+	–
CD19	–	+/–	–
CD45R B220	–	+	–
Gr-1	–	–	+

+ - positive
+/– - more often positive than negative
–/+ - more often negative than positive
– - negative

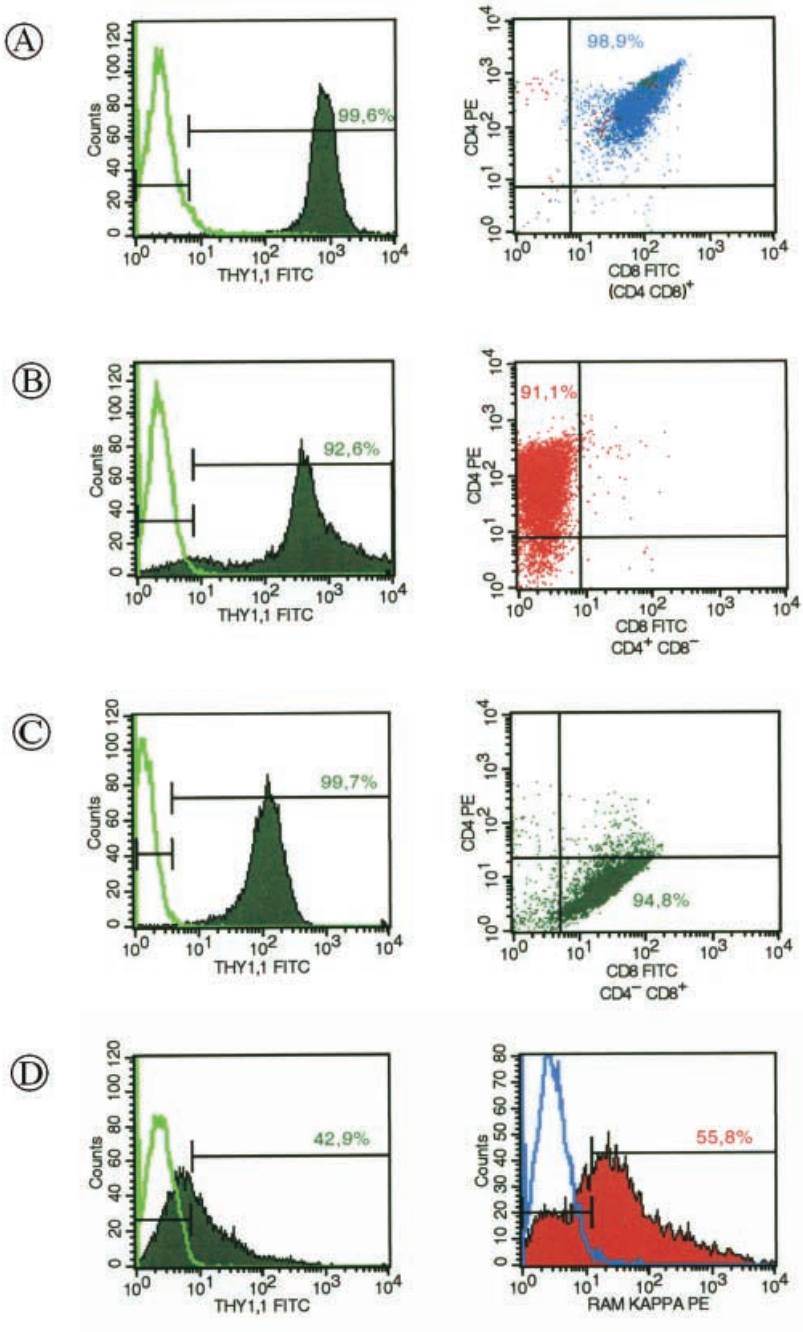

Figure 1. FACS analysis:
Tumor of thymus, T-cell lymphoma (A), (B), (C).
Positive reaction with CD 90.1 (Thy 1.1) FITC (left) co-expression $(CD4/CD8)^+$ or expression CD4+ $CD8^-$ or CD4– $CD8^+$ (right).
Tumor of mesenteric lymph nodes, B-cell lymphoma (D).
Negative with CD90 FITC (left) and positive reaction with RAM KAPPA PE (right).

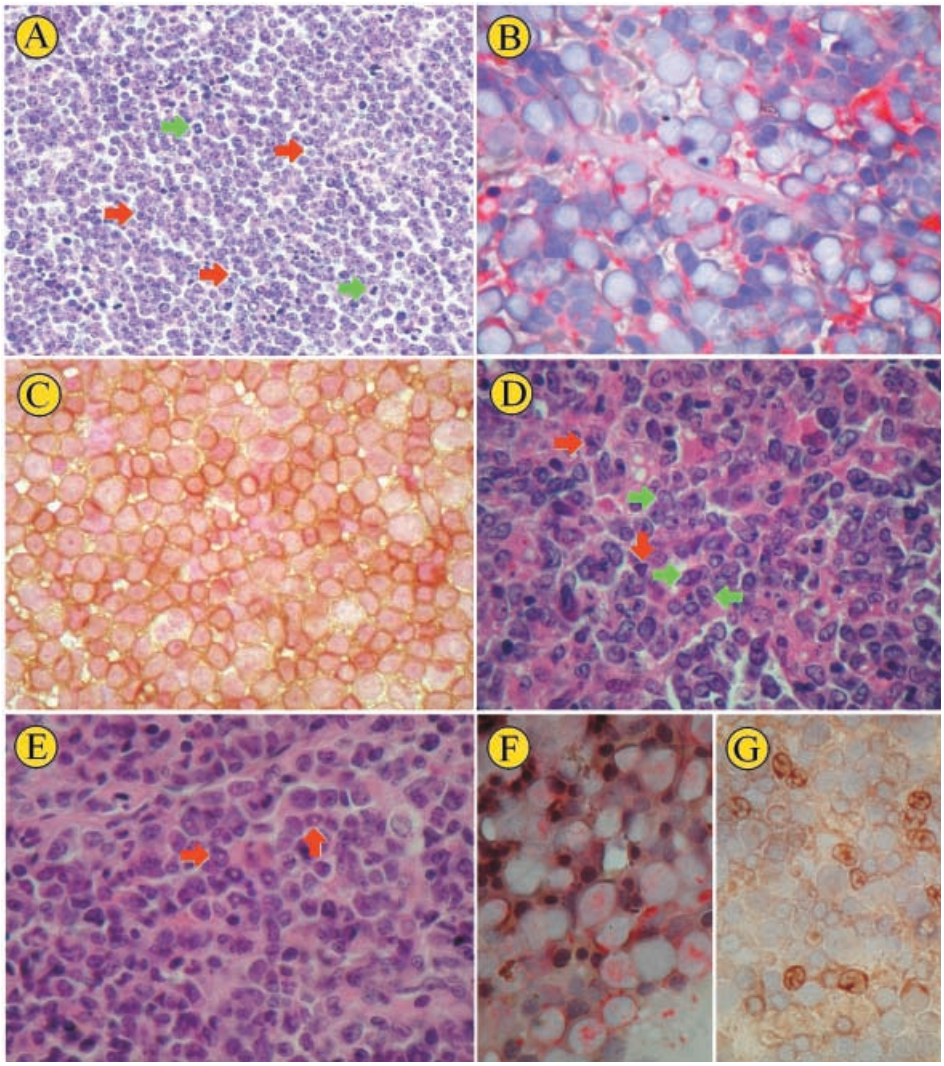

Figure 2. Precursor T-cell lymphoblastic lymphoma/leukaemia (A), (B), (C).
(A) Tumor of thymus - uniform population of medium sized cells with central prominent nucleoli (red arrow) and numerous mitosis (green arrow). H&E x400.
(B) Tumor of mesenteric lymph nodes - focal positive acid phosphatase reaction on air-dried imprint ASBI method x1000.
(C) Tumor of thymus - immunohistochemical staining on air-dried imprints positive for CD90. ABC method x1000.
Follicular B-cell lymphoma (D).
Tumor of mesenteric lymph nodes. Population of large centroblasts (green arrow). and cleaved "heart" shaped centrocytes (red arrow) H&E x1000.
Granulocytic leukaemia (E), (F), (G)
(E) Tumor of mesenteric lymph nodes – infiltration of ring forms of granulocytes (red arrow) H&E x1000.
(F) Tumor of mesenteric lymph nodes – ASD positive staining for chloroacetate esterase activity on air-dried imprint. ASD method x1000.
(G) Spleen – numerous ring shaped forms of granulocytes; positive reaction with Gr-1. ABC method x1000.

pression of one or two heavy chains of immunoglobulin μ, δ (IgM or IgD) and one of light chains κ, λ (Igκ or Igλ).

The detailed immunophenotype of follicular B-cell lymphoma is shown in Table 1. Those lymphomas exhibited the expression of RAM KAPPA and no expression of CD90 (Thy 1.) (Figure 1D). Representative image of follicular lymphoma is shown in Figure 2D.

Diffuse large B-cell lymphoma

The human counterpart is also diffuse large Bcell lymphoma – variant centroblastic. We classified B-cell derived lymphomas with more than 50% of neoplastic lymphoid cells as diffuse large B-cell lymphoma – centroblastic. Those lymphomas showed immunophenotype of mature B-cell lymphoma sIg+, B220+, CD19+, Igκ/RAM KAPPA+.

Granulocytic leukemia

The most severely affected organs were the spleen and mesenteric lymph nodes. The thymus was not involved. The immature forms of granulocytes were found in the peripheral blood and in the periportal, sinusoidal liver and around the liver vessels. Infiltration into the kidney and lung was not observed in our material. Leukaemic cells were bean or, more often ring-shaped and were in one stage of maturation. Representative images of granulocytic leukemia are shown in Figures 2E, F, G.

Granulocytic sarcoma

The lesion was primarily a solid tumour developed in a mesenteric lymph node mass without "spillover" to the peripheral blood. Granulocytic leukaemia and granulocytic sarcoma exhibited positive staining for chloroacetate esterase and showed the expression of Ly-6G Gr-1 in ABC method (Table 1).

Assessment of murine tumours of the investigated mouse strains as a model of human haematopoietic neoplasms

The vast majority of examined haematopoietic neoplasms were derived from lymphoid lineages -127 cases, while nonlymphoid haematopoietic neoplasms developed only in 11 cases. The occurrence of haematopoietic neoplasms in examined strains is shown in Table 2.

In all examined strains, a T-cell derived lymphoma – a precursor T-cell lymphoblastic lymphoma - was the prevalent type of lymphoid neoplasms - 106 cases.

As expected, in the AKR/W mice that type occurred in significantly higher proportion than in the other strains, due to the fact that the AKR/W mice are the most appropriate mouse models of the human precursor T-cell lymphoblastic lymphoma.

Two types of B-cell derived lymphomas, follicular B-cell lymphoma and diffuse large B-cell lymphoma occurred in 21 cases. Those lymphomas developed in the OcB and irradiated Bc(CcS17x CcS2)xCcS2/Dem strains more frequently than in the AKR/W mice. Both mouse strains could be recommended as mouse models for studying human counterpart of those B-cell derived lymphomas.

Granulocytic leukaemia and granulocytic sarcoma were diagnosed mainly in the Bc(CcS17xCcS2)xCcS2/Dem strain. Those

Table 2. The occurrence of neoplasms with given immunophenotype in examined mouse strains

Mouse strains	Nº of tumours	T-cell derived lymphoma	B-cell derived lymphoma	Nonlymphoid haematopoietic neoplasms
OcB/Dem	20	12	7	1
AKR/W	46	45	1	0
Bc (CcS17 x CcS2) x CcS2/Dem	72	49	13	10
TOTAL	138	106	21	11

animals were prepared especially to test the genetic control of susceptibility to radiation induced B-cell derived lymphomas and granulocytic leukaemia. Therefore, the nonlymphoid haematopoietic neoplasms developed in that strain could be the potential mouse model of human neoplasms of the same nomenclature.

Discussion

The mouse precursor T-cell lymphoblastic lymphoma is a very appropriate and well-known counterpart of human lymphoma of the same nomenclature, observed especially in children and young adults.[16] However, it should be stressed that the nuclear convolution, often observed in the human lymphomas, is not seen in the analogous murine lymphomas.

Those lymphomas have been extensively studied in the AKR mice.

Follicular B-cell lymphomas, which developed spontaneously in the OcB/Dem mice and, as a result of irradiation, in the Bc (CcS17 x CcS2) x CcS2/Dem mice, showed a diffuse pattern. While that pattern is common in mice, the follicular structure is usually recognised in human, and the diffuse variant is seen very rarely. Despite those differences, the cytology of these murine and human lymphomas is similar.

The other described type of B-cell derived lymphoma – diffuse large B-cell lymphoma in mice always requires a differential diagnosis with the progression of follicular B-cell lymphoma or progression of splenic marginal zone lymphoma.[17]

It is worth stressing that there is an obstacle in confirmation of clonality of B-cell derived lymphomas in mice, due to the fact that most (approximately 95%) murine light chains are the κ type. Therefore, more informative is the restriction to IgM or IgD than the restriction to light chain κ.

There is also the difficulty in the diagnosis of granulocytic leukemia and granulocytic sarcoma because, in mice, extramedullary haematopoiesis continues in the spleen throughout life. The infiltration in the liver parenchyma by immature forms of granulocytes is more informative than the sheets of immature granulocytes in the spleen. Unfortunately, in our material, in some cases, the liver was not involved and there were no immature granulocytes in the peripheral blood. In those cases, the restriction to one stage of maturation of granulocytes in the spleen was the only criterion for diagnosis.

Due to the fact that the investigated strains developed haematopoietic neoplasms that can be identified according to the classification proposed by the Haematopathology Subcommittee of Mouse Models of Human Cancer Consortium, they can be used as murine models of the human diseases. The observations made on these haematopoietic neoplasms can be translated to their human counterparts.

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