Immunophenotypes of spontaneous lymphomas in inbred mice – a contribution to mouse models of human lymphoid neoplasms

HANNA SZYMAŃSKA[1], JOANNA PISKOROWSKA[1], ELŻBIETA KRYSIAK[1], HENRYK MARIAN SKURZAK[2], EWA KRASZEWSKA[3], ALINA CZARNOMSKA[1]

Maria Skłodowska-Curie Memorial Cancer Center and Institute of Oncology, Warsaw, Poland:
[1]Department of Genetics and Laboratory Animal Breeding; [2]Department of Immunology; [3]Department of Biostatistics

Abstract

The Haematopathology Subcommittee of the Mouse Models of Human Cancer Consortium has proposed that mouse lymphoid neoplasms be used as models of human lymphomas. As a contribution to the proposed classification of mouse lymphomas according to the WHO classification of human lymphoid neoplasms, we evaluated the immunophenotypes of spontaneously developed lymphomas in group of 99 AKR/W mice, 108 C57BL/6W mice, 51 C57BL/10W mice, 46 BALB/cW and 57 129/SvW mice. Two immunohistochemical methods, ABComplex and MOM®, and flow cytometry with 13 monoclonal antibodies were used. In AKR/W mice, 99% of spontaneous lymphomas were Tcell derived, while this immunophenotype accounted for only 40% of lymphoid neoplasms in BALB/cW mice, 35% in C57BL/6W, 6% in C57BL/10W and 13% in 129/SvW mice. The T-cell derived lymphomas appeared earlier in AKR/W mice (median age 271 days) than in the other strains (median age 628-866 days) ($p<0.0001$). The incidence of B-cell lymphomas was highest in C57BL/10W mice (94%), 88% in 129/SvW mice, 65% in C57BL/6W mice, 60% in BALB/cW mice and only 0.01% in AKR/W mice. B-cell lymphomas developed only in aging mice (median age 787 days). The T-cell lymphomas had five different immunophenotypes, while the B-cell lymphomas had only one immunophenotype characteristic for mature B-cells. The lymphomas developed in these common mouse strains could be useful as models for human lymphomas.

Key words: *lymphoma immunophenotypes, aging inbred mice*

(Centr Eur J Immunol 2004; 29 (3-4): 78-84)

Introduction

The Haematopathology Subcommittee of the Mouse Models of Human Cancer Consortium [1] has proposed a new classification of mouse lymphoid neoplasms (Morse et al., 2002) [2, 3] which follows the WHO classification of human neoplasms [4, 5] The essential elements of the classification are morphology, immunophenotype and genetic abnormalities of lymphomas. The Haematopathology Subcommittee of MMHCC recommends introduction of the modalities used to classify human lymphomas in studies of mouse haematopoietic tumours. Immunohistochemical stains and flow cytometry provide, apart from morphology, valuable information to characterise the mouse lymphoid neoplasms. The Haematopathology Subcommittee of MMHCC evaluated lymphomas developing in mice as presumptive mouse models of human lymphoid neoplasms. The dependences of immunophenotypes on the inbred strains and age of mice were described by Pattengale [6].

The aim of the study was to compare the incidence of spontaneously developed lymphomas and distinguish the

Correspondence: Hanna Szymańska, PhD, The Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Department of Genetics and Laboratory Animal Breeding Roentgen Str 5, 02-781 Warsaw, Poland, e-mail: hanszym@yahoo.com

immunophenotypes of the lymphomas in mice of five selected strains, from the point of view of their usefulness as mouse models for human lymphoid neoplasms.

Material and methods

361 mice of five inbred strains AKR/W, BALB/cW, C57BL/10W, C57BL/6W and 129/SvW were used in the experiment. The aging animals showed a high number of spontaneously developed lymphoid neoplasms. The mice maintained at the Institute of Oncology in Warsaw were housed in the barrier system in SPF condition. They were bacteriologically, virologically and parasitologically controlled. The pelleted food (LABOFEED H), cages with bedding material and bottles with water were sterilised.

The mice received human care and ethical treatment. They were sacrificed when visibly sick with enlargement of the thymus, lymph nodes or/and spleen detected by palpation and other clinical symptoms as poor grooming and hunched posture.

All visibly sick mice were autopsied. 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). The fixed tissues were embedded in paraffin, sectioned at 4?m and then stained with H&E.

Immunophenotyping was performed using two techniques of immunohistochemistry: ABComplex and MOM® (Mouse on Mouse) - (immunodetection kit designed for detecting mouse primary antibodies on mouse tissue) according to the scheme recommended by the producer; and by flow cytometry with the appropriate 13 monoclonal antibodies [7]. Immunohistochemical staining was performed on paraffin sections using antigen retrieval unmasking method - microwave oven heating and /or air-dried imprints. The neoplastic single-cell suspension ($1x10^6$/1 ml) was analyzed by FACSCalibur Becton-Dickinson. The procedure for FACS analysis was reported previously in details [8, 9] Appropriate gates were set to calculate the percentage of positive cells. The expression of cytoplasmic cCD3ε was analysed by flow cytometry after using fixation and permeabilisation system Leucoperm™ (Serotec) Cat N°BUF09 according to the Serotec procedure.

The specific monoclonal antibodies (MAbs) for flow cytometry were labelled directly with FITC: CD90.2/CD90.1, CD3ε, CD8, CD5, CD19, CD45R-(B220), GAM (Goat anti Mouse Immunoglobulin) or with PE: CD4, TCRβ, RAM KAPPA – (rat anti mouse κ); for immunohistochemistry MAbs were biotin conjugated: anti-IgM, anti-Igκ, anti- Igλ1λ2λ3, or pure anti-IgD. CD90.1 was used only for lymphomas from AKR/W strain, while antibody CD90.2 in case of lymphomas from the other strains [10] All antibodies were derived from BD PharMingen Germany.

Blood and bone marrow smears were stained with modified Giemsa stain (Sigma) and the number of neoplastic haematopoietic cells was estimated.

Statistical analysis

Incidence of lymphomas, B-cell, T-cell and both, between strains were compared using overall two-sided chi2 or Fisher's exact test at 0.05 level of significance. In case of significant results paired comparisons were done between every two different strains. According to Bonferroni correction, 0.005 level of significance was chosen for pairwise tests.

Age of mice with T-cell and B-cell lymphoma was compared using two-sided Mann-Whitney test with 0.05 level of significance. Comparison was done for both genders and separately for females and males.

Results

140 spontaneously developed lymphoid neoplasms were identified in mice of the five examined strains.

98 cases were recognised as T-cell lymphomas while 42 cases were B-cell derived lymphomas.

The tested strains differed in the incidence of lymphomas (overall χ^2 test $p<0.001$). The pairwise comparisons indicated significantly higher occurrence of lymphomas in AKR/W strain compared with the other tested strains (χ^2 test $p<0.001$). Moreover, there was a statistically significant lower percentage of incidence of lymphomas in C57BL/6W strain than in C57BL/10W strain (χ^2 test $p=0.005$).

The examined strains differed in the incidence of T-cell and B-cell lymphomas (overall Fisher's exact test: $p<0.001$). The number of T-cell lymphomas in AKR/W strain was considerably higher than in the other strains: BALB/cW, C57BL/6W, C57BL/10W, 129/SvW (Fisher's exact test $p<0.001$).

Adequately, the occurrence of B-cell lymphomas in AKR/W strain was significantly lower than in C57BL/6W and C57BL/10W strains ($p<0.001$ AKR/W with C57BL/6W to $p=0.006$ AKR/W with C57BL/10W).

The differences in the occurrence of B-cell lymphomas among the mice of strains: BALB/cW, C57BL/6W, C57BL/10W and 129/SvW were not statistically significant at 0.005 level except C57BL/6W inbred mice in comparison with C57BL/10W mice. The percentage of incidence of B-cell lymphomas in C57BL/6W was significantly lower than in C57BL/10W ($p=0.001$).

The age of mice with developed T-cell lymphomas vs. B-cell lymphomas was also compared. Due to the low age and high number of AKR/W mice with developed T-cell lymphoma, the median age, not mean age was considered.

The age of mice from all examined strains with spontaneously developed T-cell lymphoma was significantly lower (median 274 days) than mice with B-cell lymphomas

Table 1. Occurrence of the T or B-cell derived lymphoid neoplasms in examined mouse strains

Strain	Number of examined mice	Mice with lymphoid neoplasms		Type of lymphoid neoplasms			
				T		B	
		number	%	number	%(*)	number	%(**)
AKR	99	87	88	86	99	1	0.01
BALB/c	46	10	22	4	40	6	60
C57BL/6	108	17	17	6	35	11	65
C57BL/10	51	18	35	1	6	17	94
129/Sv	57	8	14	1	13	7	88
all strains	361	140	39	98	70	42	30

** percentage of T-cell lymphoma from number of mice with lymphoma neoplasms*
*** percentage of B-cell lymphoma from number of mice with lymphoma neoplasms*

Table 2. The median age of mice with developed T- or B-cell lymphoma

Strain	Median age of mice with T-cell lymphoma			Median of age of mice with B-cell lymphoma		
	female and male	female	male	female and male	female	male
AKR	271	258	310	460	–	460
BALB/c	743	743	–	829	829	–
C57BL/6	803	730	899	786	753	803
C57BL/10	866	866	–	774	723	813
129/Sv	628	–	628	749	749	896
all strains	274	267	341	787	769	808

(median 787 days) ($p<0.0001$). When we analysed only the ages of BALB/cW, C57BL/6W, C57BL/10W and 129/SvW mice with developed T-cell lymphomas vs. B-cell lymphomas, they were comparable (median 628 days with T-cell lymphomas to 829 days with B-cell lymphomas). The age of females (median 267 days) compared with the age of males (median 341 days) with developed T-cell lymphoma was slightly lower $p=0.05$. The differences in the age of females (median 769) and males (median 808 days) with developed B-cell lymphomas were insignificant $p=0.46$.

The occurrence of the T or B-cell derived lymphoid neoplasm in the examined strains is given in tab. 1.

The median of age of mice with T-or B-cell lymphomas is shown in tab. 2.

T-cell neoplasms demonstrated lymphoblastic morphology and were diagnosed as Precursor T-cell lymphoblastic lymphoma. B-cell neoplasms were recognised as FBL (Follicular B-cell lymphoma) or DLBL (Diffuse large B-cell lymphoma).

Although the spontaneously developed T-cell lymphomas were histologically homogenous, they could be immunologically subdivided in five different immunophenotypes.

The neoplastic lymphoid T-cells were always positive for CD 90.1 or CD 90.2, CD5 and cytoplasmic cCD3ε, while the differences concerned on expression: CD4, CD8 and surface sCD3ε linked with TCRβ. The level of the expression of sCD3ε were connected with TCRβ. The different expression of CD4 and CD8 indicated their derivation from one of the subsets of T-cells: double positive $CD4^+CD8^+$ or double negative $CD4^-CD8^-$ or single positive $CD4^+CD8^-$, $CD4^-CD8^+$ and confirmed their monoclonality.

In AKR/W mice the immunophenotype with expression both $(CD4/CD8)^+$ was the most frequent while the occurrence T-cell lymphomas with immunophenotypes $CD4^-CD8^+$ and $CD4^-CD8^-$ developed in the same number. It is worth stressing that the immunophenotype $CD4^+CD8^-$ was more frequent than the inverse $CD4^-CD8^+$.

Two cases of lymphoid neoplasms developed in

Table 3. Occurrence of the immunophenotypes of T-cell derived lymphomas in the examined strains

Expression of antigens									Incidence of immunophenotypes	
Immuno-phenotype	**CD90.1/ CD90.2**	**sCD3ε/ cCD3ε**	**TCR β**	**(CD4/ CD8)$^+$**	**CD4$^+$ CD8$^-$**	**CD4$^-$ CD8$^+$**	**CD4$^-$ CD8$^-$**	**CD5**	**AKR**	**BALB/c, C57BL/10, C57BL/6, 129/Sv**
1	+	+/+	+	**+**	–	–	–	+	48	3
2	+	+/+	+	–	**+**	–	–	+	24	4
3	+	+/+	+	–	–	**+**	–	+	6	1
4	+	+/+	+	–	–	–	**+**	+	6	2
5	+	**–/+**	**–**	–	–	–	**+**	+	2	0

Table 4. Immunophenotype of B-cell derived lymphomas observed in the examined strains

Expression of antigens										
CD45R (B220)	**RAM KAPPA anti-Igκ**	**anti-Igλ1λ2λ3**	**GAM**	**CD19**	**sIgM**	**sIgD**	**CD5**	**CD90.1/ CD90.2**	**sCD3ε**	**TCRβ**
+	+	–	+	+	+	+	– or low	–	–	–

AKR/W mice with immunophenotype CD4$^-$CD8$^-$ were found to be sCD3ε and TCRβ negative though the lymphomas showed expression cCD3ε. In BALB/cW, C57BL/10W, C57BL/6W and 129/SvW mice analysed together T-cell lymphomas of the particular immunophenotypes developed with the following frequency: CD4$^+$CD8$^-$>CD4$^+$CD8$^+$>CD4$^-$CD8$^+$>CD4$^-$CD8$^-$ However the data can not be compared due to the small numbers of T-cell lymphomas developed in those strains. The occurrence of the distinguished immunophenotypes of T-cell lymphomas in the examined strains is shown in tab. 3. The representative analyses of T-cell lymphomas by flow cytometry are shown in fig. 1 A-K and immunostaing image in fig. 1 L.

B-cell lymphomas demonstrated mature B-cell immunophenotype: CD45R(B220)$^+$, CD19$^+$, sIgM or/and sIgD$^+$, with κ light chain expression, λ light chain expression on B neoplastic cells was not found. The expression of examined antigens of B-cell lymphomas in the tested strains is given in tab. 4. The representative analyses of B-cell lymphomas by flow cytometry are shown in fig. 2 A-G and immunohistochemical staining images in fig. 2 H-I.

In flow cytometry analysis, reactions with CD90.2 and surface CD3ε are low positive due to the small number of non neoplastic T cells in B-cell lymphomas.

Discussion

Mice from the examined strains significantly differed in the incidence of the spontaneous lymphomas, average age of occurrence and their immunophenotype.

Several points of the comparison deserve particular discussion.

First, the survey of the literature indicates that the etiology of lymphomas in the AKR mice is associated with endogenous ecotropic viruses: inherited at two non-linked chromosomal loci Akv-1, Akv-2, the xenotropic virus and recombinant viruses, that results in high incidence of lymphomas in AKR strain [11]. Moreover, the incidence of thymic T-cell lymphomas is influenced by age factor and never occurs in old thymic environment [12, 13].

Actually, the examined AKR/W inbred mice significantly differed in the increase of number of T-cell lymphomas from C57BL/6 and C57BL/10 strains as well as the lower average age of mice with developed T-cell lymphomas. Higher incidence of T-cell versus B-cell lymphomas was observed in AKR/W mice and the result is compared with the data from the literature [14].

Noteworthy, CD4$^+$CD8$^-$ immunophenotype predominated over CD4$^-$CD8$^+$ in the examined mice of all strains. It is interesting, because the literature data indicates that CD4$^+$CD8$^-$ immunophenotype is higher malignant than the opposite immunophenotype [15].

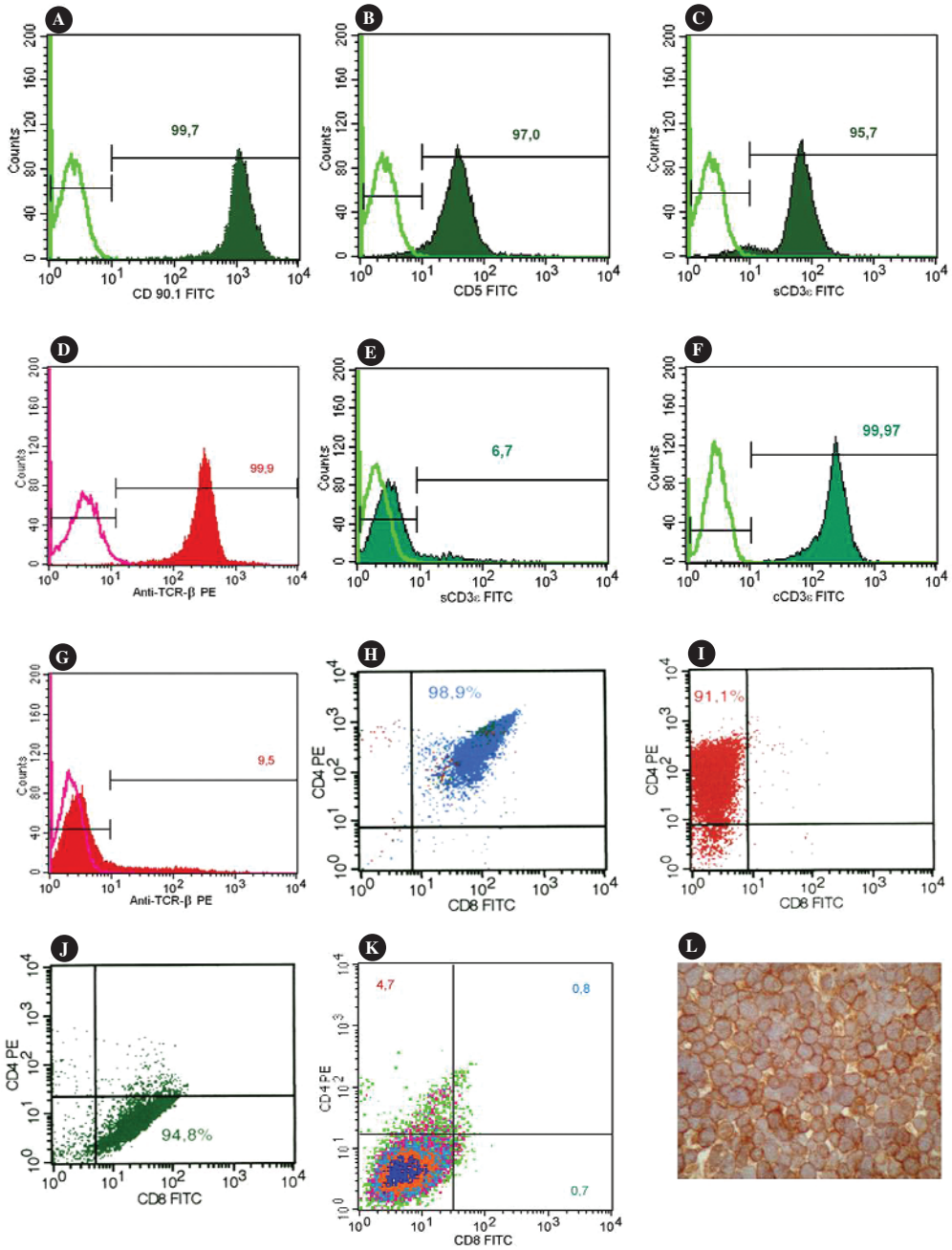

Fig. 1. Representative FACS profiles and immunostaining image. Tumour of thymus, T-cell lymphoma. (**A**) Positive reaction with CD90.1; (**B**) Positive reaction with CD5; (**C**) Surface positive reaction with CD3ε; (**D**) Positive reaction with anti-TCRβ; (**E**) Negative surface reaction with CD3ε; (**F**) Positive cytoplasmic reaction with CD3ε; (**G**) Negative reaction with anti-TCRβ; (**H**) Dot plot – expression (CD4 CD8)$^{+}$; (**I**) Dot plot expression CD4^{+}CD8^{-}; (**J**) Dot plot expression CD4^{-}CD8^{+}; (**K**) Dot plot – CD4^{-}CD8^{-}; (**L**) Immunostaining on air-dried imprints positive for CD90.1 ABC method x 1000

Second, as literature indicates, the aging C57BL/6 and 129/Sv mice develop lesions similar to many different diseases of inbred mice and additionally B-cell lymphomas [16]. Therefore the mice of these strains were selected for our investigation.

Actually, C57BL/6W and 129/SvW mice developed high number of B-cell lymphomas.

Moreover, AKR/W mice developed only haematopoietic neoplasms, predominantly the lymphoid neoplasms and two cases of non-lymphoid neoplasms (granulocytic sarcomas).

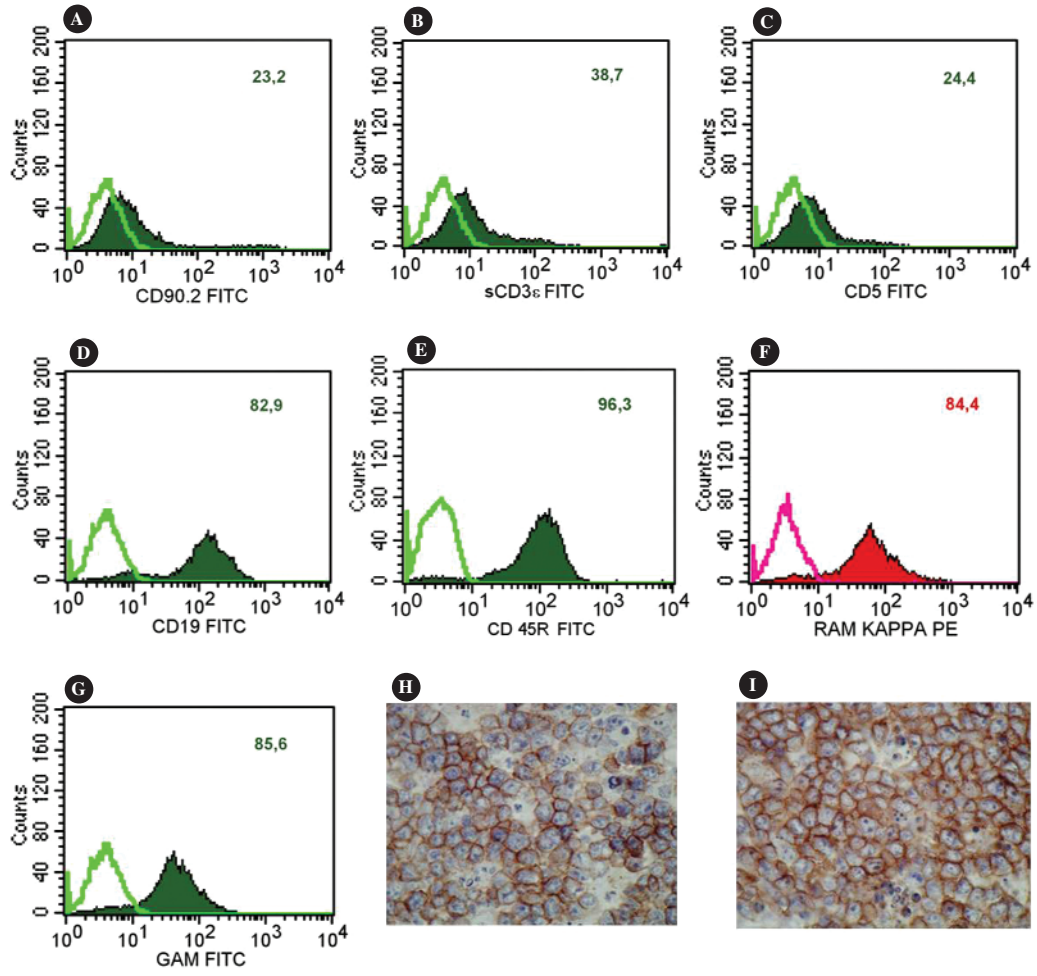

Fig. 2. Representative FACS profiles and immunostaining images. Tumour of mesenteric lymph nodes. (**A**) Low positive reaction with CD90.2; (**B**) Low positive surface reaction with CD3ε; (**C**) Low positive reaction with CD5; (**D**) Positive reaction with CD19; (**E**) Positive reaction with CD45R (B220); (**F**) Positive reaction with RAM KAPPA; (**G**) Positive reaction with GAM; (**H**) Immunostaining on paraffin section, positive reaction with anti-IgM ABC method x 1000; (**I**) Immunostaining on paraffin section, positive reaction with anti-IgD ABC method x 1000

On the contrary BALB/cW, C57BL/10W, C57BL/6W and 129/SvW mice developed besides haematopoietic neoplasms (mainly lymphomas and small number of granulocytic sarcomas) other neoplasms, e.g. tumors of the lungs in BALB/cW mice, tumors of the liver in C57BL/6W and C57BL/10W mice and neoplasms of the uterus in 129/SvW mice.

Third, in human CD5 antigen express on T-cells and a subset of B-cells. Expression of CD5 antigen on neoplastic B-cells is helpful marker for the diagnosis of mantle cell lymphoma (B-cell lymphoma) [17]. However the survey of the literature reveals that in mouse 85% of spontaneous B-cell lymphomas except plasmocytomas are $CD5^+$. Therefore CD5 antibody is not as useful in mice [18].

In our experiment the examined B-cell lymphomas were CD5- or low, while T-cell lymphomas showed expression of CD5 antigen. We found the expression of CD5 antigen as helpful marker to distinguish T and B-cell lymphomas.

Fourth, in mouse the clonality of B-cell lymphomas cannot depend on the restriction to the expression of immunoglobulin κ light chain, because about 95% mouse light chains are the κ type. Expression of the only λ light chain is more informative [19].

However, the examined B-cell lymphomas expressed Ig κ light chain (Ig κ^+/RAM KAPPA$^+$), while the expression of λ light chain were not found. Therefore we did not consider that criterion as a confirmation of the clonality. Only the expression of one or two heavy chains μ, δ (IgM

or IgD) on the surface of lymphoid cells was important to confirm the clonality of B-cell lymphomas.

Fifth, the data from the literature indicates that aged C57BL/6 mice develop clinically malignant lymphoproliterative state which is difficult to differentiate from frank B-cell lymphomas. However, lymphoid hyperplasia is polyclonal with respect to the immunoglobulin heavy chains in the opposite to B-cell lymphoma which is monoclonal [19]. We found that the immunological feature is valuable to differentiate B-cell lymphoid hyperplasia in C57BL/6 mice from frank B-cell lymphomas.

Our investigations proved that it is possible to estimate the number of spontaneously developed T- and B-cell lymphomas in the examined inbred mice.

We also demonstrated that the age of mice when T- or B-cell lymphomas develop can be predicted.

The study of the mouse lymphomas is valuable because understanding their pathogenesis provides opportunities for treating similar diseases in humans. Therefore it is essential to determine whether lymphoid neoplasms developed in the mouse and human are true homologs and deserve identical names. T-cell lymphomas in AKR mice have some clinical and pathologic similarities to the Pre-T LBL (Precursor T-cell lymphoblastic lymphoma/leukemia) observed in children. However, it is worth stressing that convolution of neoplastic cells marked in human is not observed in the mouse homolog [19]. Two sub-types of B-cell lymphomas observed in the examined BALB/cW, C57BL/10W, C57BL/6W and 129/SvW strains also have human counterparts with identical names. However, the comparison deserves a commentary. The histopatological diagnosis of human FBL (Follicular B-cell lymphoma) is based on the recognition of follicular structures generated by transformed B-cells while mouse FBL develops as diffuse variant that is uncommon in humans. In the mouse, DLBL (Diffuse large B-cell lymphoma) is associated with progression of the follicular B-cell lymphoma while in human it develops as separate disease [2].

To conclude, the examined inbred mice with high incidence of spontaneously developed sub-types of T- or B-cell lymphomas could be recommended as the mouse models for studying the human counterparts of those lymphoid neoplasms in experimental and pre-clinical studies.

References

1. Galvez JJ, Cardiff RD, Munn RJ, Borowsky AD (2004): Mouse models of human cancers (Part 2). Comp Med 54 (1): 13-15.
2. Morse HC 3rd, Anver MR, Fredrickson TN, et al.; Hematopathology subcommittee of the Mouse Models of Human Cancers Consortium (2002): Bethesda proposals for classification of lymphoid neoplasms in mice. Blood 100 (1): 246-258.
3. Frith CH, Ward JM, Harleman JH, et al.: Hematopoietic system. In: International Classification of Rodent Tumors, The Mouse. Ed. Mohr U. Springer-Verlag, New York, NY, 2001, 417-451.
4. Jaffe ES, Harris NL, Stein H, Vardiman J, eds: Pathology and genetics of tumours of haematopoietic and lymphoid tissues: WHO Classification of tumours. IARC Press. Lyon, France, 2001.
5. Jaffe ES, Banks PM, Nathwani B, et al. (2004): Recommendations for the reporting of lymphoid neoplasms: A report from the Association of Directors of Anatomic and Surgical Pathology. Mod Pathol 17 (1): 131-135.
6. Pattengale PK: Tumours of the lymphohaematopoietic system. In: Pathology of tumours in laboratory animals. Vol. 2. Tumours of the mouse. Ed. Turusov VS, Mohr U. Lyon 1994, 651-670.
7. Lai L, Alaverdi N, Maltais L, Morse HC 3rd. (1998): Mouse cell surface antigens: nomenclature and immunophenotyping. J Immunol 160 (8): 3861-3868.
8. Szymanska H, Sitarz M, Krysiak E, et al. (1999): Genetics of susceptibility to radiation-induced lymphomas, leukemias and lung tumors studied in recombinant congenic strains. Int J Cancer 83 (5): 674-678.
9. Szymanska H, Piskorowska J, Krysiak E, et al. (2004): Diagnosis and classification of spontaneously developed and radiation induced murine haematopoietic neoplasms. The murine models for the research on the human haematopoietic neoplasms. Radiol Oncol 38 (3): 217-225.
10. International Committee on Standardized Genetic Nomenclature for Mice (1996): Standard normal chromosomes. In: Genetic Variants and Strains of Laboratory Mouse. Ed. Green M.C. 1st Edn. Stutgart, New York.
11. Haran-Ghera N, Peled A, Wu L, at al. (1995): The effects of passive antiviral immunotherapy in AKR mice: I. The susceptibility of AKR mice to spontaneous and induced T cell lymphomagenesis. Leukemia 9 (7): 1199-1206.
12. Utsuyama M, Hirokawa K (2003): Radiation-induced-thymic lymphoma occurs in young, but not in old mice. Exp Mol Pathol 74 (3): 319-325.
13. Kaptzan T, Skutelsky E, Itzhaki O, et al. (2004): Age-dependent differences in the efficacy of cancer immunotherapy in C57BL and AKR mouse strains. Exp Gerontol 39 (7): 1035-1048.
14. Haran-Ghera N, Peled A, Canaani E, et al. (1995): The effects of passive anti-viral immunotherapy in AKR mice: II. Susceptibility to B cell lymphomagenesis. Leukemia 9 (11): 1940-1947.
15. Klein O, Staroselsky A, Huszar M, et al. (1998): Biological behavior and cell properties of new AKR lymphoma malignancy variants. Tissue Cell 30 (1): 95-103.
16. Haines DC, Chattopadhyay S, Ward JM (2001): Pathology of aging B6;129 mice. Toxicol Pathol 29 (6): 653-661.
17. Mioduszewska O (1995): Patologia chłoniaków i ziarnicy złośliwej [Pathology of the lymphatic system. Selected problems] Pol J Pathol 46 (1 Suppl): 1-60.
18. Morse HC, et al. Bethesda proposals for classification of lymphoid neoplasms in mice supplementary information http://emice.nci.nih.gov/emice/contentfiles/emicePublicLFS/MorseSupplement.pdf 2003.10.21
19. Pattengale PK, Taylor CR (1983): Experimental models of lymphoproliferative disease. The mouse as a model for human non-Hodgkin's lymphomas and related leukemias. Am J Pathol 113 (2): 237-265.