Hemopoietic and Lymphoid Organs in AKR/JY Mice with Thymic Lymphoma

G. V. Karpova, T. I. Fomina, E. V. Abramova, N. V. Bel'skaya, E. S. Trofimova, and V. M. Perel'muter*

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 134, No. 7, pp. 79-82, July, 2002
Original article submitted February 19, 2002

Cytological and histological studies of the thymus, bone marrow, peripheral blood, spleen, and lymph nodes in 6-9-month-old AKR/JY mice showed that the risk of thymic lymphoma in these animals increased with age. Generalization of the tumor process was observed in mice aged over 8 months and first involved the spleen, then the lymph nodes, bone marrow, and liver. A case of primary lymphoma in the spleen was detected.

Key Words: *AKR/JY mice; hemopoietic and lymphoid organs*

AKR/JY mice aged over 6 months (old animals) spontaneously develop thymic tumors (lymphomas) induced by Gross retrovirus. This pathology called lymphoid leukemia, T-cellular thymus-dependent leukemia, lymphoid thymoma, or T-cellular thymic lymphoma [4,6, 10-15] is responsible for 90% deaths in this strain [1,14]. Progression of malignant lymphoma is characterized by generalization, i.e. involvement of the lymph nodes, spleen, bone marrow (leukemization), and other organs [6]. The data on the state of peripheral blood cells, bone marrow, and lymphoid organs in aged AKR/JY mice are scanty and contradictory [2,3,8].

This paper presents the results of morphological analysis of the thymus, bone marrow, spleen, and lymph nodes in old AKR/JY mice during the development of thymic lymphoma.

MATERIALS AND METHODS

Experiments were carried out on 105 AKR/JY mice from the collection of the Laboratory of Experimental Biomodeling, Institute of Pharmacology. The strain was obtained at the Laboratory of Experimental Biomodeling of Russian Academy of Medical Sciences (Moscow region) in 1985 and was maintained by inbreeding. The animals were kept in accordance with the regulations of the European Convention for Protection of Vertebrates used for Experimental and Other Research Purposes (Strasbourg, 1986).

The morphology of hemopoietic and lymphoid organs was studied in 55 AKR/JY mice (39 females and 16 males) aged 6-9 months (generations 186-192); the life span and mortality rate were evaluated on 50 animals (generations 164-196). Peripheral blood parameters (counts of erythrocytes, reticulocytes, leukocytes, and differential leukocyte count) were studied in mice aged 6 (7 females and 6 males), 7 (8 females and 5 males), 8 (12 females), and 9 months (12 females and 5 males). The total count of bone marrow karyocytes, thymus and spleen weights, and cytograms were evaluated. The animals were sacrificed by cervical dislocation.

Preparations for cytological analysis of the thymus, spleen, and bone marrow (smears) were prepared from fragments homogenized with autologous serum (1:1), fixed in methyl alcohol, and stained with azur-2-eosin after Nocht—Maximov [9]. The morphology of lymphoid organs and liver was evaluated on histological preparations (fixation in Carnoy fluid, hematoxylin and eosin staining, and Brachet staining) from animals aged 7, 8, and 9 months.

The results were statistically processed by methods of variational statistics [5].

Institute of Pharmacology, Tomsk Research Center, Siberian Division of Russian Academy of Medical Sciences; *Department of Pathological Anatomy, Siberian State Medical University, Tomsk

0007-4888/02/1341-069$27.00 © 2002 Plenum Publishing Corporation

RESULTS

The mean life span of AKR/JY mice is 276±54 days. The animals start dying at the age of 6 months, and by the age of 13 months 90% animals die, the majority of animals (72%) die at the age of 6-9 months. The main cause of death is malignant thymic lymphoma.

Body weight gain decreases in AKR/JY mice with age (from 6th to 9th months of life). The counts of erythrocytes, reticulocytes, lymphocytes decreased and the percentage of mature neutrophils increased. The total count of bone marrow cells and the percent ratio of individual cells in the myelogram did not appreciably change in mice without leukemic transformation of thymic lymphoma; the mean weights of the thymus and spleen increased (Table 1).

Histological and cytological studies showed development of thymic lymphoma in 16 animals (15.4% at the age of 7 months, 25% at 8 months, and 64.7% at 9 months, Table 2). On histological preparations the tumor was represented by lymphoblasts (large or small). Their nuclei had irregular contours and were surrounded by a narrow rim of pyroninophilic cytoplasm. Macrophages with a wide cytoplasm containing phagocytosed cells were also seen among tumor cells. Lymphoma cells in the thymus had lymphoblastic morphology, macroforms (in comparison with large and medium-sized thymocytes in the intact thymus) constituted 12.5-86.5% cells of the organ.

Generalization of the tumor process was evaluated by the size and morphology of lymph nodes, spleen, bone marrow, and liver. Metastatic cells of lymphoblastic morphology were detected in the bone marrow of 6 animals with thymic lymphomas aged 8 (n=1) and 9 (n=5) months. The thymus and spleen were almost totally (80-90%) destroyed in these animals, the percentage of pathological cells in the bone marrow varied from 9.0 to 90.4; the total leukocyte count was 6.0-36.2 10^9/liter, the content of neutrophils was 35-85% and that of blasts 0.30% (Table 2).

The thymus was involved in the tumor process in all animals with lymphomas; in only 1 case (7 months) only one lobe was involved. The weight of the thymus was <100 mg in 4 animals with lymphomas, 100-200 mg in 5 animals, and >200 mg in others.

Hyperplasia of the organ was observed in 14 animals aged 7 and 8 months (7 animals per age group): the weight, number, and size of the thymus lobes increased, but the relative cellularity of zones, clear-cut interface between the cortical and medullary matter, and moderate proliferative activity of cortical lymphocytes were preserved. In other mice signs of age-associated involution of the thymus were observed (despite its great weight): low number of lymphocytes in the cortical and medullary matter, blurred interface between the zones, and development of fatty tissue.

Morphological analysis of the spleen showed infiltration with tumor cells and almost complete replacement of the tissue with lymphoblastic elements

TABLE 1. Parameters of Hemopoietic and Lymphoid Organs in Aged AKR/JY Mice (X±m).

Parameter	Age, months			
	6 (n=13)	7 (n=13)	8 (n=12)	9 (n=17)
Mouse weight, g	29±3	34±2	25±1	28±1
Weight, mg				
thymus	79±8	83±10	105±34	201±49
spleen	64±5	58±4	98±20	166±40
Peripheral blood				
erythrocytes, 10^{12}/liter	6.8±0.4	5.9±0.3	5.9±0.1	4.8±0.3
reticulocytes, $^0/_{00}$	43±2	32±2	37±2	26±2
leukocytes, 10^9/liter	13±1	9±1	12±2	6.0±0.3
neutrophils, %	38±5	40±3	49±4	49±7
lymphocytes, %	49±5	42±2	36±3	33±7
Bone marrow				
total cell count, 10^6/femur	22.0±0.5	19.0±1.5	17±1	23±1
immature neutrophils, %	9±1	8.0±0.4	7±1	8±1
mature neutrophils, %	36±2	35±2	33±5	33±2
lymphocytes, %	17±1	16±1	12±1	14±2
erythronormoblasts, %	26±2	27±2	26±4	24±3

TABLE 2. Individual Characteristics of AKR/JY Mice with Thymic Lymphoma

Parameter	Age, months (sex)															
	7		8					9								
	1 (f)	2 (f)	3 (f)	4 (f)	5 (f)	6 (f)	7 (f)	8 (f)	9 (f)	10 (f)	11 (f)	12 (f)	13 (f)	14 (f)	15 (f)	16 (f)
Mouse weight, g	23.9	28.2	26.8	26.1	23.3	28.2	28.6	33.0	27.7	30.6	39.1	24.9	32.0	26.0	21.4	24.1
Weight, mg																
thymus	90	153	92	120	460	75	81	143	182	185	204	226	275	382	610	730
spleen	90	64	89	80	295	60	119	425	113	87	154	139	85	60	290	670
Blasts, %																
thymus	11.5	52.0	16.5	52.5	78.5	12.8	17.6	84.8	84.8	70.8	75.0	63.6	86.5	77.0	81.2	86.4
spleen	16.5	9.6	12.0	9.5	82.5	12.8	38.0	81.6	28.0	12.0	64.4	48.0	34.4	1.2	94.0	79.6
peripheral blood	—	S	S	S	0	1	S	7	1	2	10	4	2	7	7	30
bone marrow	2.4	2.3	1.6	1.5	69.6	2.0	2.0	31.5	2.0	1.6	2.0	9.0	12.5	1.5	77.2	90.4
Peripheral blood																
leukocytes, 10^9/liter	N.d.	5.0	7.7	11.2	23.5	11.6	5.0	6.2	8.4	11.2	4.7	5.2	6.0	6.7	36.2	15.7
neutrophils, %	N.d.	50	55	34	61	39	60	85	48	51	56	34	55	33	65	35
lymphocytes, %	N.d.	31	25	47	25	52	28	2	45	34	16	48	31	45	12	23
Infiltration																
lymph nodes	—	—	—	—	+	—	—	+	—	—	+	+	+	—	+	+
liver	—	—	—	—	+	—	—	+	—	—	+	+	—	—	+	+
spleen	—	—	—	—	+	—	+	+	+	—	+	+	+	—	+	+

Note. Nd: not detected; s: solitary; —: absent; +: present.

in 9 animals with thymic lymphoma (Table 2). In 3 animals (aged 7, 8, 9 months) splenic hyperplasia manifested in increased number and size of lymphoid follicles and cell proliferation in T-dependent zones. In 1 case (9-month-old mouse) lymphoblastic lymphoma developed in the spleen (spleen weight 255 mg, content of blasts in the splenogram 75.6%) in the absence of changes in the thymogram and histological signs of age-associated thymus involution (weight 64 mg, content of blasts 18.8%).

The lymph nodes (axillary, inguinal, and mesenteric) were enlarged in 7 animals with thymic lymphomas (1 mouse aged 8 months and 6 mice aged 9 months). Histological study of enlarged lymph nodes showed obliteration and replacement of the node tissue with tumor cells.

Morphological study of other organs showed lymphoblastic infiltration of the liver in 5 mice aged 9 months and in 1 mouse aged 8 months with thymic lymphomas.

Hence, the risk of thymic lymphoma in AKR/JY mice increases with age. Generalization of the tumor process usually occurs in animals aged over 8 months and first involves the spleen, and then the lymph nodes, bone marrow, and liver. The risk of primary lymphoma in the spleen should also be taken into account.

REFERENCES

1. Z. K. Blandova, V. A. Dushkin, A. M. Malashenko, and E. F. Schmidt, *Laboratory Animal Strains for Biomedical Studies* [in Russian], Moscow (1983).
2. S. A. Kozlov, V. N. Pan'kov, S. A. Konyshev, *et al.*, *Gematol. Transfuziol.*, **42**, No. 2, 19-22 (1997).
3. L. A. Kosnyreva, *Regularities of Hemopoiesis Regulation in AKR/JY Mice during the Preuleukemic Period*, Abstract of Cand. Med. Sci. Dissertation, Tomsk (1999).
4. N. I. Kochergina and A. A. Yarilin, *Eksp. Onkol.*, **8**, No. 2, 57-60 (1986).
5. G. F. Lakin, *Biometry* [in Russian], Moscow (1980).
6. I. A. Lisukov, I. G. Tsyrlova, and V. A. Kozlov, *Eksp. Onkol.*, No. 1, 71-73 (1986).
7. N. A. Probatova, N. N. Tupitsyn, and E. V. Fleishman, *Arkh. Patol.*, No. 4, 61-71 (1998).
8. A. I. Svirnovskii, T. V. Shimanskaya, A. V. Bakun, *et al.*, *Pressing Problems in Standardization of Laboratory Animals for Biomedical Studies* [in Russian], Moscow (1988), pp. 152-154.
9. *Handbook of Laboratory Methods of Investigation*, Ed. V. V. Men'shikov [in Russian], Moscow 91987).
10. I. G. Tsyrlova, *Vestn. Akad. Med. Nauk SSSR*, No. 12, 34-39 (1991).
11. J. M. Angel, D. C. Morisot, and E. R. Richie, *J. Natl. Cancer Inst.*, **8**, No. 21, 1652-1655 (1989).
12. A. Barker and S. Waksal, *Cell Immunol.*, **12**, No. 1, 140-149 (1974).
13. M. Bortin and R. Truitt, *Biomedicine*, **26**, No. 3, 309-312 (1977).
14. L. Gross, *Proc. Soc. Exp. Biol. Med.*, **76**, No. 1, 27-30 (1951).
15. E. F. Hays, G. C. Bristol, J. P. Lugo, and X. F. Wang, *Exp. Haematol.*, No. 7, 1116-1121 (1989).