Bull. Tokyo Med. Dent. Univ., 30: 95–107, 1983

RELATION BETWEEN FRIEND LEUKEMIA VIRUS-INDUCED LEUKEMIA AND GENETIC CONTROL OF THE HOST

BY

Masanobu KITAGAWA, Osamu MATSUBARA, and Tsutomu KASUGA[*1)]

ABSTRACT

Hematological assays of inbred specific pathogen-free (SPF) mice of ten different strains inoculated with Friend leukemia virus (FLV) were performed chronologically to assess whether the genetic control of the host may play an important role in viral oncogenicity. Mice strains C57BL/6J, B10 (H-2^b) and B10D2 (H-2^d) were FLV-resistant, BALB/c, DBA/2N (H-2^d), RFM (H-2^f), AKR and 80% of CBA/JN (H-2^k) were FLV-sensitive (polycythemia) and C3H/He, B10Br and 20% of CBA/JN (H-2^k) were FLV-sensitive (anemia). Only the AKR strain mice showed a spontaneous regression of splenomegaly. These results indicate that there is not a strong but a weak correlation between the H-2 haplotype and the reaction to FLV. The main phenomenon in the anemic mice was the monotonous proliferation of the naked blastic cell, whereas that in the polycythemic mice was the enormous increase of the mature erythroblast and the decrease of the naked blastic cell in the later phase. These facts suggest that the naked blastic cell in the mice with polycythemia are reactive and that in the mice with anemia truly neoplastic.

Key words: Friend virus, leukemogenic viruses, retrovirus infection, host-virus relation, histocompatibility loci, experimental leukemia, anemia, polycythemia

INTRODUCTION

The mechanism of viral carcinogenesis, especially of the viral infection and integration with the host, has been well studied, but still clarified fragmentarily. Wondering whether the genetic control of the host might play an important role in viral oncogenesis, our purpose was to examine the experimental leukemia induced by the Friend leukemia virus (FLV) (Friend [1]; Kasuga and Oota [2]) by using various strains of mice. FLV is a type-C retrovirus that induces a rapid multistage leukemia in the susceptible mice. FLV is a complex of two viruses consisting of a replication-defective spleen focus-forming virus (SFFV) (Axelrad and Steeves [3]) and replication-competent helper virus (F-MuLV) (Tambourin and Wendling [4]). As leukemia progresses, the disease shows a biphasic pattern. Whereas both splenomegaly and the rapid changes in erythropoiesis are observed in the early phase (Mager *et al.* [5]; Mirand [6]), the later phase is characterized by the development of the clones of the tumorigenic cells which can be established into the Friend leukemia cell lines (Oboshi [7]; Ikawa and Sugano [8]). Afterwards, new two strains of FLV have been separated. They were FV-P

*1) 北川昌伸・松原　修・春日　孟: 2nd Department of Pathology (Chief: Prof. T. KASUGA), Faculty of Medicine, Tokyo Medical and Dental University (Tokyo Ika Shika Daigaku)
Received for publication, June 1, 1983.

(Mirand [9]) and FV-A (Mager *et al.* [5]), both of which consist of SFFV and F-MuLV, and induce different types of Friend disease (Marcy *et al.* [10]). Recently, Shibuya *et al.* [11] reported that the *Fv-5* gene of the host mouse determines the induction of early anemia or polycythemia even when FV-P alone was used. Still controversial is the correlation between the genetic control and the induction of leukemia and also the cytological characterization of the tumorigenic cell in the FLV-induced leukemia. Moreover, there has been no report about the long-term observation of the susceptible mice of various strains in the same experimental procedure. The hematopathological assay was performed chronologically until the time of death for the purpose of assessing the relation between the FLV-induced leukemia and the H-2 haplotype of the mouse and also to examine the character of the tumorigenic cell in the various strains of mice.

Materials and Methods

I, *Mice* Ten strains of mice were obtained from the following farms: C3H/He, RFM, B10, B10Br, B10D2 and C57BL/6J mice from the National Institute of Radiological Sciences in Chiba and DBA/2N, BALB/c, CBA/JN and AKR from Charles River Japan, Inc. Ten female SPF mice of each strain were employed for the experiment. They were housed five per cage and fed with radiologically sterilized laboratory pellet and tap water *ad libitum*. All were bred under the condition of SPF to rule out the influence of intestinal bacterial flora.

II, *Virus and virus infection* A complex of FLV was obtained from the supernatant fluid of the homogenized spleen taken from six- to 12-week-old female C3H/He strain mice that had been serially infected. FLV-infected mice were initially obtained from Dr. K. Hirashima of the National Institute of Radiological Sciences in Chiba. The FLV had more than 300 cell-free passages in the C3H/He strain mice for seven years in the Institute after they had been obtained from Dr. C. Friend in 1960 (Kasuga and Oota [2]; Hirashima [12]). The leukemogenicity of the virus had been well sustained and was confirmed both by splenomegaly and hematological assays. The spleen of the FLV-infected C3H/He strain mice, weighing 2 to 3 g, was placed in the physiological saline aseptically, diluted to 10% weight per volume and homogenized for two minutes under moderate power by Polytron (Kinematica, Swiss). After centrifugation at 3,500 rpm for 20 minutes, the supernatant fluid was gradedly filtrated through the Millipore filter of 0.45 nm and 0.22 nm mesh. The clear filtrate (0.1 m*l*) was injected into the abdominal cavity of the experimental 9-week-old mouse.

III, *Hematological study* The leukemogenicity of FLV in ten various inbred strains of mice was studied by hematological assays of the blood from the tail vein. The parameters such as hematocrit (Ht) (%), the red blood cell count (RBC) (/mm^3), the white blood cell count (WBC) (/mm^3) and the peripheral blood smear, were examined twice a week from the time of injection (0 day) till the time of death in the case of the FLV-sensitive strains or till the time of more than 100 days after in the case of the FLV-resistant strains. The mean corpuscle volume of the red blood cell (MCV) ($10^{-15}l$) was calculated each time. The peripheral blood smear was stained with hematoxylin-eosin and May-Grünwald Giemsa stain.

Results

The result of the hematological assays

Table 1. Chronological Changes of Hematological Findings in Various Mice Strains

Mice strain	Parameter	Days after injection			
		Control[1]	20		24
BALB/c	RBC 10^4	1,300±0	1,210±14		1,327±183
	WBC 10^2	166±34	3,050±127		1,060±191
	Ht %	62.5±2.1	64.5±0.7		69.7±7.0
	MCV 10^{-15}	48.1±1.6	53.4±1.2		52.6±2.1
		Control	20		24
DBA/2N	RBC	1,033±74	1,375±64		1,348±315
	WBC	160±48	2,970±1,230		1,670±806
	Ht	49.5±0.7	71.0±2.8		69.5±7.8
	MCV	48.1±2.8	51.7±0.4		52.8±5.8
		Control	31		96
RFM	RBC	1,193±60	1,680		1,388±40
	WBC	123±5	4,000		3,750±141
	Ht	56.5±2.1	85.0		86.5±0.7
	MCV	47.4±0.6	50.6		62.3±1.3
		Control	23	33	58
AKR	RBC	1,010±10	1,053±87	1,130±42	1,004±153
	WBC	149±15	592±328	213±23	504±432
	Ht	53.8±0.5	67.3±5.5	61.0±5.3	67.3±13.6
	MCV	53.1±0.5	63.9±0.6	56.7±0.9	66.9±7.5
		Control	7		21
CBA/JN	RBC	1,100±4	1,090±131		p[2] 1,200
					a[3] 995
	WBC	135±49	285±42		p 1,300
					a 2,300
	Ht	53.0±0	50.0±0		p 64.0
					a 46.0
	MCV	48.3±0.1	46.1±5.5		p 52.5
					a 50.3
		Control	21		35
C3H/He	RBC	1,000±49	416±28		291±25
	WBC	100±1	1,193±599		2,664±281
	Ht	50.7±2.1	21.7±0.6		20.0±3.0
	MCV	50.7±2.2	52.2±4.7		68.4±6.0
		Control	72		107
B10Br	RBC	1,148±39	640[4]		288
	WBC	190±80	134		2,380
	Ht	53.0±1.4	36.0		20.0
	MCV	46.2±0.3	56.3		69.4

After the FLV was injected into the various mice strains, the RBC and WBC count, hematocrit value and the MCV were determined chronologically. All mice were females and 9 weeks old at the time of injection. One to four mice were assayed in each strain. The values in this table show the mean ± standard deviation or the representative one when only one mouse can be determined.

1) Data in the preinjection time are shown as controls.
2) Polycythemic mice
3) Anemic mice
4) Two out of ten B10Br strain mice are affected.

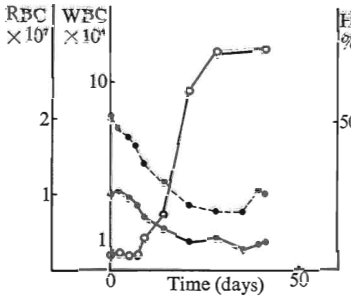

Fig. 1a. C3H/He

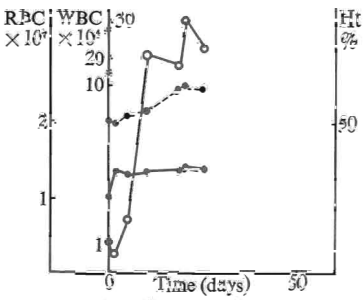

Fig. 1b. DBA/2N

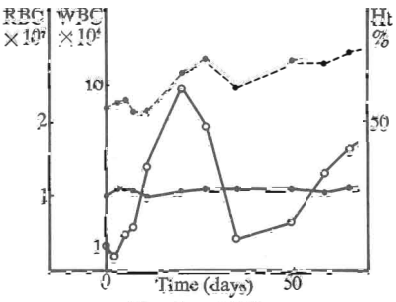

Fig. 1c. AKR

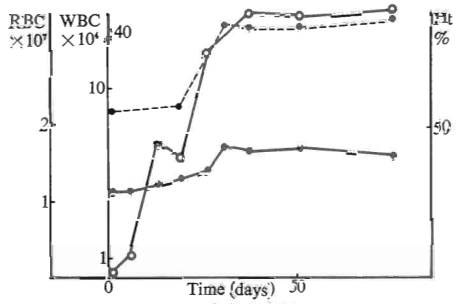

Fig. 1d. RFM

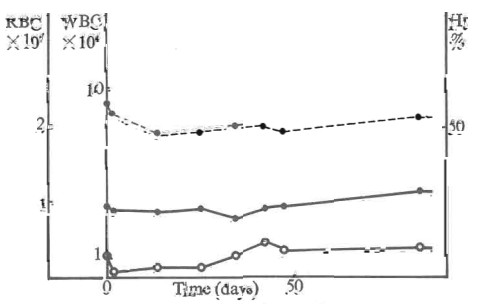

Fig. 1e.C57BL/6J

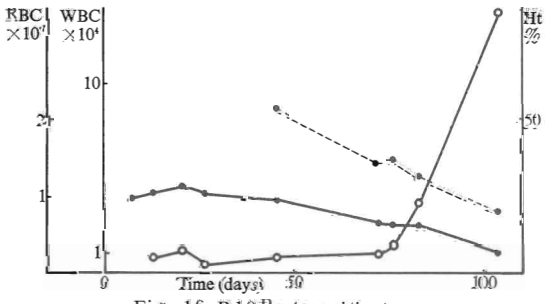

Fig. 1f. B10Br (sensitive)

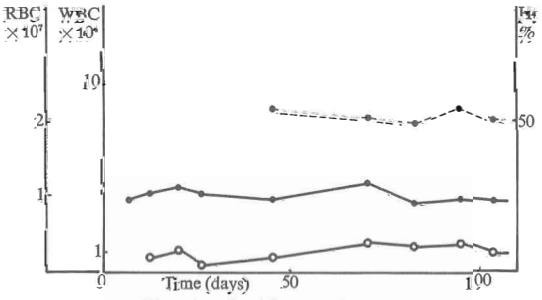

Fig. 1g. B10Br (resistant)

Fig. 1. Chronological changes of the hematocrit value (represented by the dotted line -------), the RBC count (represented by the solid line ———) and the WBC count (represented by the open circles connected by the solid line O—O) in the various strains of mice. Fig. 1a shows the change in C3H/He strain, 1b in the DBA/2N, 1c in the AKR, 1d in the RFM, 1e in the C57BL/6J, 1f in the FLV-sensitive mice of the B10Br strain and 1g in the FLV-resistant mice of the B10Br strain.

in each strain is shown in Table 1. The BALB/c, DBA/2N, RFM, AKR, CBA/JN, C3H/He and two out of ten B10Br strain mice were FLV-sensitive and the C57BL/6J, B10, B10D2 and eight out of ten B10Br strain mice were FLV-resistant.

The chronological change of the hematocrit value, the RBC count, and the WBC count in the C3H/He, DBA/2N, AKR, RFM, C57BL/6J and B10Br strain mice is shown in Fig. 1 a-g. In most of the FLV-sensitive strains, leukemia began to appear one to three weeks after the injection, however, one of them, the B10Br strain, became sick about ten weeks after. Although the normal level of the WBC count was less than 20,000/mm³, the WBC count became over 100,000/mm³ and the blastic cell appeared in the peripheral blood when

leukemia occurred. We interpreted the rapid increase of the WBC count accompanied by the appearance of the blastic cell as the onset of this disease. Physical examination revealed splenomegaly in every mouse with leukemia. Almost all mice which suffered from leukemia died about one to two months later. Only the AKR strain showed a spontaneous regression of splenomegaly. Six out of ten AKR strain mice died with hepatomegaly about two months later. The life span of each strain was about 20 to 40 days in the DBA/2N, BALB/c, CBA/JN and C3H/He strain mice, about one to two months in the AKR strain mice, about one to six months in the RFM strain mice and about three to six months in the FLV-sensitive B10Br strain mice.

All of the DBA/2N, RFM, CBA/JN, AKR and C3H/He strain mice suffered from leukemia and nine out of ten BALB/c strain mice and two out of ten B10Br strain mice died of this disease. On the contrary, all of the B10, B10D2 and C57BL/6J strain mice were resistant to FLV and had no remarkable findings for more than 100 days.

Table 2. Chronological Changes of Hematocrit Value (%)

Mice strain	Control	Onset	Before death
BALB/c	62.5±2.1	64.5±0.7	72.4± 5.4
DBA/2N	49.5±0.7	71.0±2.8	71.2± 3.3
RFM	56.5±2.1	85.0	86.5± 0.7
AKR	53.8±0.5	67.3±5.5 (61.0±5.3)[1]	67.3±13.6[2]
CBA/JN	53.0±0	48.5±3.5	64.0± 1.4[3] 46.0[4]
C3H/He	50.7±2.1	21.7±0.6	20.0± 3.0
B10Br	53.0±1.4	36.0	20.0[5]

The hematocrit values increase in the BALB/c, DBA/2N, RFM, AKR and a part of the CBA/JN mice strains. They decrease in the C3H/He, B10Br and two out of ten CBA/JN strain mice. The AKR strain mice show a spontaneous regression of splenomegaly. Hematocrit value also decreases at that time[1] and then increases again before death[2]. The CBA/JN strain mice show both polycythemic[3] and anemic[4] patterns. Two out of ten B10Br strain mice are FLV-sensitive[5] and affected with the disease much later than the other FLV-sensitive strains.

The chronological changes of the hematocrit value of the mice are summarized in Table 2. The early phase was characterized by the change of the hematocrit value and the RBC count, namely, anemic or polycythemic condition. The BALB/c, DBA/2N, AKR and eight out of ten CBA/JN strain mice clearly showed polycythemia. The C3H/He, B10Br and 20% of CBA/JN strain mice showed anemia. Only the CBA/JN strain mice showed both the polycythemic (8/10) and anemic (2/10) patterns. In the B10Br strain

Table 3. Chronological Changes of Value of MCV ($10^{-15}l$)

Mice strain	Control	Onset	Before death
BALB/c	48.1±1.6	53.4±1.2	52.4±2.1
DBA/2N	48.1±2.8	51.7±0.4	52.8±5.8
RFM	47.4±0.6	50.6	62.3±1.3
AKR	53.1±0.5	63.9±0.6 (56.7±0.9)[1]	66.9±7.5[2]
CBA/JN	48.3±0.1	46.1±5.5	52.3±0.3[3] 50.3[4]
C3H/He	50.7±2.2	52.2±4.7	68.4±6.0
B10Br	46.2±0.3	56.3	69.4[5]

The values of the MCV of both the polycythemic and anemic mice strains increase significantly after the onset. Significant differences from the control at $P<0.01$ and $P<0.05$ by Student's t test. (BALB/c; $t=6.39>t_6(0.01)=3.707$, DBA/2N; $t=2.774>t_5(0.05)=2.571$, RFM; $t=13.4>t_3(0.01)=5.841$, AKR; $t=5.87>t_5(0.01)=4.032$, CBA/JN (polycythemia); $t=17.9>t_2(0.01)=9.925$, CBA/JN (anemia); Only one mouse was counted. C3H/He; $t=12.7>t_4(0.01)=4.604$, B10Br; Only one mouse was counted.) In the AKR strain mice, the value of the MCV increases once, recovers to the control level during a spontaneous regression of splenomegaly[1], and then increases again before death[2]. That of the CBA/JN strain mice increases both in the polycythemic[3] and anemic[4] mice. Two out of the B10Br strain mice are FLV-sensitive[5].

Table 4. Chronological Changes of Cellular Population in the Peripheral Blood

Mice strain	Days after injection	Total WBC count (10^2/mm^3)	Myelogenic cells	Atypical mono-lymphoid cells	Lymphoid cells	Erythroid cells	Naked blastic cells
BALB/c	0	166	33(20)	—	133(80)	—	—
	10	800	72(9)	8(1)	344(43)	80(10)	296(37)
	20	3,050	199(7)	92(3)	45(1)	379(12)	2,334(77)
	31	1,500	345(23)	105(7)	660(44)	390(26)	180(12)
DBA/2N	0	160	26(16)	—	134(84)	—	—
	10	2,066	11(1)	158(8)	30(1)	145(7)	1,723(83)
	20	2,970	19(1)	365(12)	108(4)	1,546(52)	391(31)
	24	1,670	50(3)	512(31)	28(2)	863(52)	217(13)
RFM	2	37	10(26)	—	27(74)	—	—
	19	780	16(2)	86(11)	78(10)	86(11)	515(66)
	31	4,000	40(1)	280(7)	80(2)	920(23)	2,680(67)
	76	8,860	—	1,329(15)	266(3)	3,544(40)	3,721(42)
AKR	0	145	23(16)	—	122(83)	—	—
	19	958	98(10)	17(2)	257(27)	322(34)	255(27)
	33	213	30(14)	2(1)	133(62)	36(17)	12(6)
	65	650	46(7)	30(5)	222(34)	236(36)	116(18)
CBA/JN	0	100	9(9)	—	91(91)	—	—
p[1]	10	1,380	83(6)	—	442(32)	331(24)	524(38)
	17	1,050	22(2)	15(1)	323(31)	373(36)	316(30)
	21	1,300	39(3)	13(1)	52(4)	611(47)	585(45)
a[2]	10	1,290	26(2)	13(1)	245(19)	39(3)	968(75)
	21	3,040	61(2)	30(1)	274(9)	426(14)	2,250(74)
C3H/He	0	100	13(13)	—	87(87)	—	—
	14	374	12(3)	—	72(19)	—	289(77)
	21	1,042	21(2)	21(2)	83(8)	208(20)	708(68)
	28	1,456	15(1)	44(3)	102(7)	175(12)	1,121(77)
B10Br	0	190	49(26)	—	141(74)	—	—
	70	271	18(8)	—	203(92)	—	—
	83	438	25(6)	—	355(81)	—	53(12)
	107	2,380	24(1)	—	119(5)	262(11)	1,975(83)

The chronological changes of the cellular population are examined by the peripheral blood smear stained with May-Grünwald Giemsa stain. The anemic strains show an increase mainly of the naked blastic cell, while the polycythemic strains are characterized by the increase of the mature erythroblast and the atypical mono-lymphoid cell in the later phase of the disease. Not only the absolute value of each component but also the relative counts (shown in ( )) are characteristic in each strain. In the CBA/JN strain mice, the polycythemic pattern[1] and anemic pattern[2] are observed.

mice, two out of ten (20%) were FLV-sensitive and the twice-repeated experiments showed the same results. The onset time in the B10Br strain mice was about ten weeks after the viral injection and for the other strain mice it was later than that.

The value of the MCV in most of the mice of both the polycythemic and anemic strains was larger than that of the mice of virus-free condition (Significant differences from the control at $P<0.01$ and $P<0.05$ by Student's t test.), as shown in Table 3. The value of the MCV did not change in the FLV-resistant strains and returned to that of the control value in the mice with a spontaneous regression.

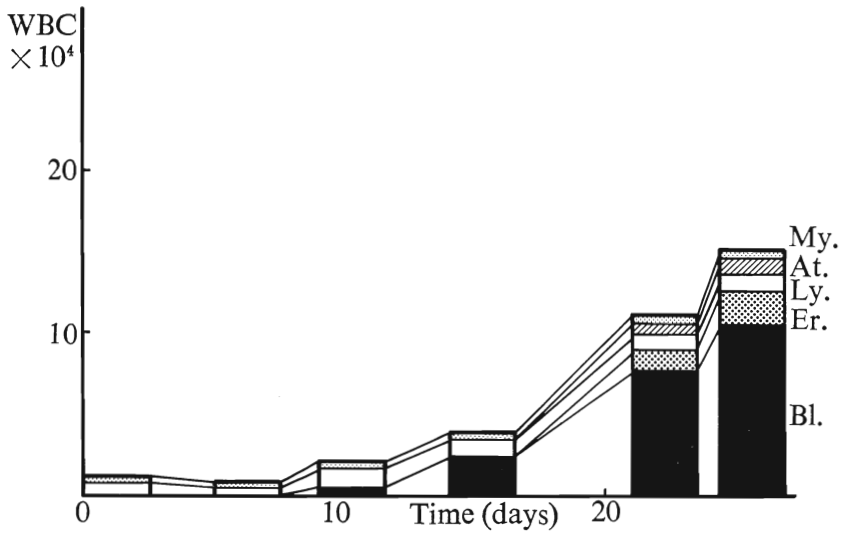

Fig. 2a. Changes of Cellular Population in the C3H/He Strain Mice.

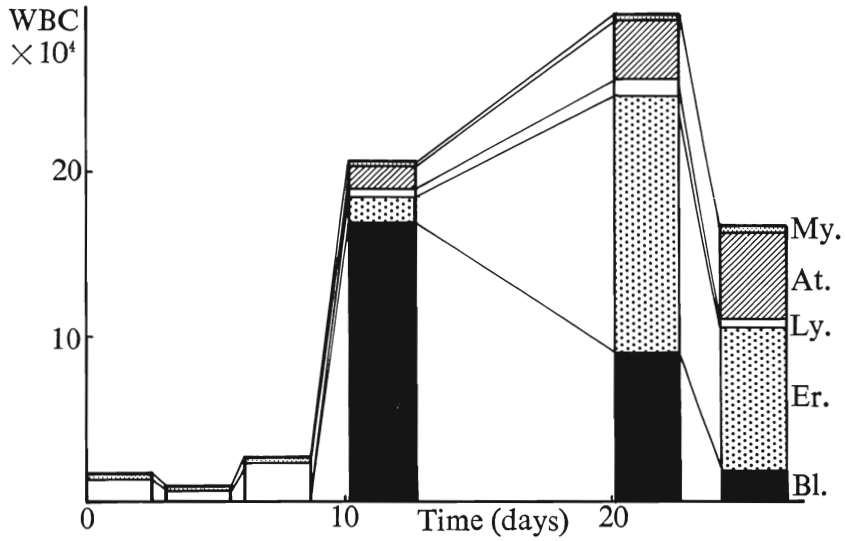

Fig. 2b. Changes of Cellular Population in the DBA/2N Strain Mice.

Fig. 2. Changes of cellular population in the peripheral blood are shown. Fig. 2a shows the change in the anemic mouse strain, C3H/He, and 2b in the polycythemic strain, DBA/2N.
My.: Myelogenic cell At.: Atypical mono-lymphoid cell Ly.: Lymphoid cell Er.: Erythroid cell Bl.:Naked blastic cell

The relation between the number of blastic cell and the other components in the peripheral blood is shown in Table 4. The myelogenic and lymphoid cells increased a little in number. This seemed to be a reactive phenomenon, for these cells showed neither atypism nor monotonous proliferation. Fig. 2 a and b show the number of different cell types in the anemic strain, C3H/He, and in the polycythemic strain, DBA/2N.

The microscopical examination of the

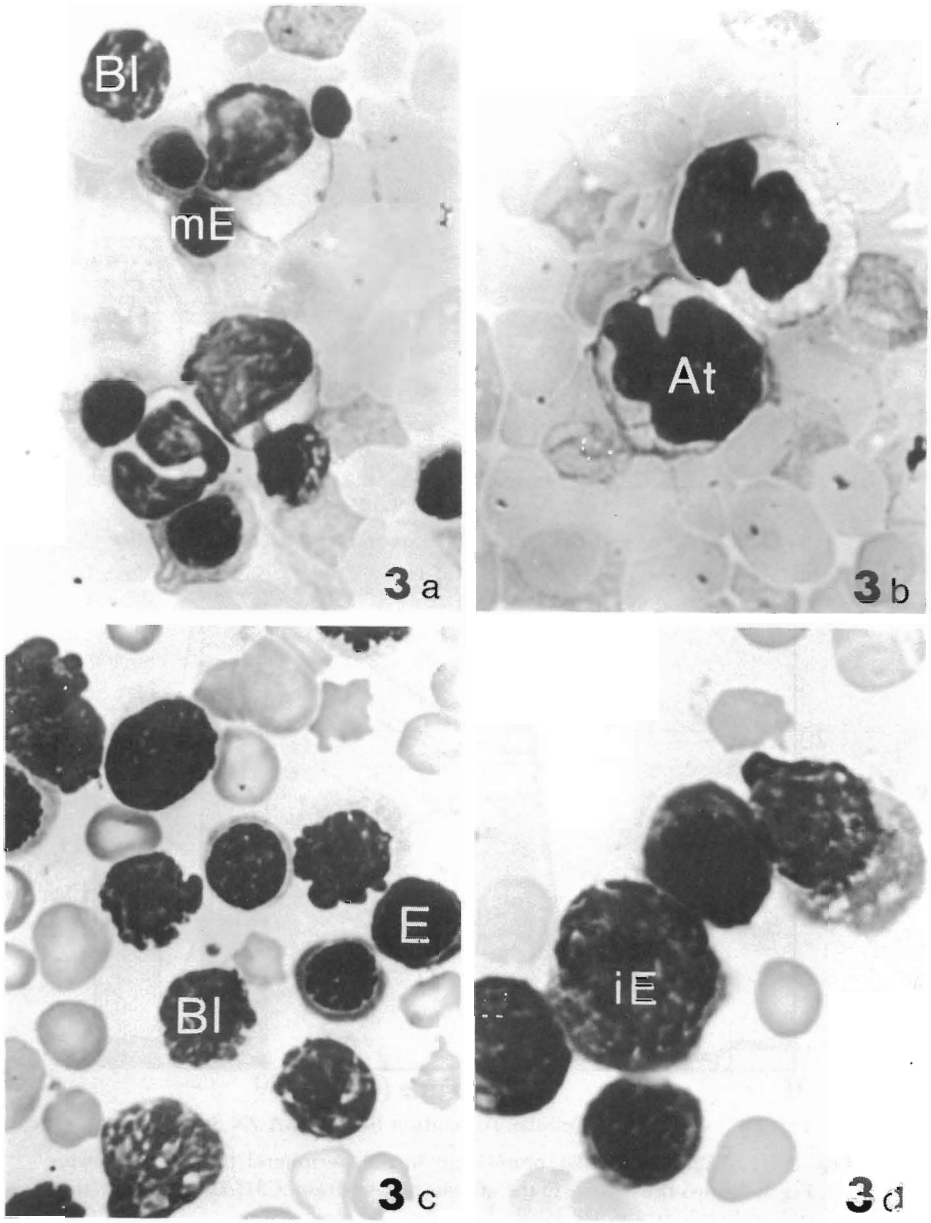

Fig. 3a. Peripheral blood smear in the DBA/2N strain (24 days after injection) (×1400). b. Atypical mono-lymphoid cells in the DBA/2N strain (×1780). c. Peripheral blood smear in the C3H/He strain (35 days after injection) (×1400). d. Immature erythroblasts in the C3H/He strain (×1780).
Bl: Naked blastic cell E: Erythroblast mE: Mature erythroblast iE: Immature erythroblast At: Atypical mono-lymphoid cell

peripheral blood smear revealed three important findings.

1) Character of the blastic cell: Most of the blastic cells had only a naked nucleus. This tendency was more apparent in the anemic mice strains, C3H/He and B10Br. The blastic cells in these strains were larger than those in the polycythemic strains (Fig. 3 a-d).

2) Erythroblast: As the disease progressed, the erythroblast appeared in the peripheral blood. In the anemic strains, C3H/He, B10Br and 20% of CBA/JN, a small number of the relatively immature erythroblast and the mature erythroblast was observed. On the contrary, in the polycythemic strains, BALB/c, DBA/2N, RFM, AKR and 80% of CBA/JN, the erythroblast of various stages in maturation coexisted with the naked blastic cell from the early phase of the disease (Fig. 3 a and c). The mature erythroblast increased in number as the disease progressed and finally outnumbered the naked blastic cell in the polycythemic strains.

3) Atypical mono-lymphoid cell in the polycythemic strains: In some strains of the polycythemic strain mice, BALB/c, DBA/2N and RFM, not only the naked blastic cell but also the atypical mono-lymphoid cell proliferated in the later phase, namely, three weeks after the injection. This atypical mono-lymphoid cell had a large cleaved nucleus and a relatively large cytoplasm without any granules (Fig. 3 b and d). The cytoplasm was negative for periodic acid Schiff or peroxidase stain. In the AKR strain mice which showed a spontaneous regression of splenomegaly about 30 days after the onset, the atypical mono-lymphoid cell did not increase remarkably in number.

The AKR strain mice were FLV-sensitive and showed polycythemia about ten days after the injection of the virus. These mice showed a spontaneous regression of splenomegaly about 20 to 30 days after the onset. The WBC count increased up to about 100,000/mm^3, and then returned to the control value. The polycythemia and the increased MCV also regressed. About 40 to 60 days after the injection, the WBC count increased again in all the AKR strain mice. These mice showed marked hepatomegaly of 4 to 6 g, while the normal liver weighed less than 1.5 g. Splenomegaly regressed and was not observed at the time of death. The cellular components of the WBC, when the count increased, were similar to those of the other polycythemic mice strains.

Table 5. H-2 Haplotypes and Sensitivity to FLV

Mice strain	Sensitivity	Life span (days)
H-2^k		
C3H/He	S (10/10) A	22 to 40
B10Br	pS (2/10) A	79 to 178[1)]
CBA/JN	S (10/10) A or P	16 to 21
AKR	S (10/10) P	40 to 70[2)]
H-2^d		
BALB/c	S (0/10) P	20 to 31
DBA/2N	S (10/10) P	20 to 37
B10D2	R (0/10) —	—
H-2^b		
C57BL/6J	R (0/10) —	—
B10	R (0/10) —	—
H-2^f		
RFM	S (10/10) P	31 to 175

The mice strains of the H-2^b type are FLV-resistant, those of the H-2^d type are FLV-sensitive (polycythemia) or FLV-resistant. Anemic patterns are observed only in those of the H-2^k type. Two out of ten B10Br strain mice are FLV-sensitive and affected much later than the other strains[1)]. Spontaneous regression of splenomegaly is seen in the AKR strain mice of the H-2^k type which show a polycythemic pattern at the time of the onset. Four out of ten AKR strain mice are still alive 70 days after the injection[2)].

S: Sensitive to FLV
pS: Partially sensitive to FLV
R: Resistant to FLV
A: Anemic mice
P: Polycythemic mice

The relation between the H-2 haplotype and the reaction to FLV is summarized in Table 5. The H-2 haplotypes of each strain were as follows: H-2^k for C3H/He, B10Br, CBA/JN and AKR; H-2^d for BALB/c, DBA/2N and B10D2; H-2^b for C57BL/6J and B10 and H-2^f for RFM. The present study revealed that all the strains of the H-2^b type were FLV-resistant, that those of the H-2^d type were FLV-sensitive (polycythemia) or FLV-resistant, that an anemic pattern was observed only in those of the H-2^k type and that the spontaneous regression of splenomegaly with a polycythemic pattern occurred in one AKR mice strain of the H-2^k type.

Discussion

I, *Two patterns of hematological kinetics* The present study revealed that in the polycythemic mice strains, the mature erythroblast increased in number in association with the increasing number of the atypical mono-lymphoid cell in the later phase and that the change in the anemic strains was characterized by the monotonous proliferation of the naked blastic cell. The character of the tumorigenic cell in the later phase of this disease has not been fully clarified in the previous studies. Since the erythroblast in the polycythemic strains showed various stages of maturity, it might not be disturbed to differentiate nor to synthesize hemoglobin. The studies *in vitro* (Shibuya *et al.* [13]) brought about the same result as our observation. Calculation of the value of the MCV in this study could lead to new findings that the macrocytic change of the RBC occurred in the mice with FLV-induced leukemia. Macrocytic anemia which occurred in the anemic strains seemed to be the result of disturbed differentiation in the relatively immature erythroblast (Peschle *et al.* [14]; Steinheider *et al.* [15]) or caused by the bone marrow involvement of the leukemic cells. In the C3H/He strain mice, the naked blastic cell seemed to be of an erythroblastic origin because it resembled the immature erythroblast in the nuclear chromatin pattern and size and because the nucleus of the immature erythroblast sometimes separated from the cytoplasm and became naked. These facts suggest that the anemia in the C3H/He strain mice was the result of the disturbed differentiation of the erythroblast. On the contrary, because the nucleus of the naked blastic cell in the B10Br strain mice resembled that of the lymphoid cell, the anemia seemed to be related to the leukemic infiltration of the bone marrow.

II, *H-2 haplotype of the mouse and Friend disease* According to the previous report, several host genes, Fv-1 (Rowe *et al.* [16]), Fv-2 (Mac *et al.* [17]), W (Steeves *et al.* [18]), Steel (Bennet *et al.* [19]) and H-2 (Lilly [20]), are known to control the susceptibility to FLV-induced leukemia. About the H-2 haplotype, Lilly and Pincus [21] reported that the H-2 haplotype and the gene for splenomagaly segregated independently at a high virus dosage, however, relative susceptibility to a moderate to low dose of FLV was influenced by the H-2 haplotype. The result regarding the H-2 haplotype and sensitivity to FLV, as shown in Table 5, leads to the conclusion that the H-2 haplotype has not a strong correlation but a weak correlation to the reaction to FLV. The new findings, which were different from the previous reports in our study, were as follows; 1) All AKR strain mice were FLV-sensitive, although the previous report showed that they were partially susceptible (Klein [22]). 2) The B10Br strain mice were partially sensitive and were affected much later than the other

strains, although the previous report showed that they were FLV-resistant (Klein [22]). 3) The RFM strain mice had a new haplotype (H-2^f) and were FLV-sensitive (polycythemia). Klein [22] reported that four (AKR, CBA, C3H/Bi and RF/J) out of the seven mice strains of the H-2^k type, three (BALB/c, DBA/2, and NZB) out of the five mice strains of the H-2^d type and two (A.BY and C3H.SW) out of the four mice strains of the H-2^b type were FLV-sensitive or relatively sensitive.

These differences may be explained by the different experimental procedures as follows: 1) Condition of SPF or conventional condition, 2) strains of FLV, 3) viral dosage, 4) analysis method of susceptibility, namely whether the hematological assay is performed or not, 5) duration of observation and 6) age and sex of mice. The two factors among them, namely, SPF or conventional conditions, and long- or short-term observation, must be the most important for this difference in the result regarding the susceptibility of the mice strains to FLV.

III, *Friend disease in mice strains of H-2 ^{k} type* The mice strains of the H-2^k type were FLV-sensitive or partially FLV-sensitive and showed interesting patterns of the disease.

The C3H/He and two out of the ten B10Br strain mice showed the anemic pattern of this disease. The previous report showed that the B10Br strain mice were resistant of FLV (Klein [22]). The naked blastic cell in the B10Br strain mice resembled the nucleus of the lymphoid cell, whereas that in the other anemic strain resembled the nucleus of the erythroblast. So, it is difficult to decide whether or not the tumor cell in the B10Br strain mice might be the same as the tumor cell in the other anemic strains. Further study may be necessary.

The CBA/JN strain mice showed both the polycythemic and anemic patterns. It is difficult how to interpret these findings. According to the previous report, the CBA strain mice showed anemia in the early phase even when FV-P was injected (Shibuya *et al.* [11]). A larger number of mice and genetical analysis may be necessary to determine the character of the reaction to FLV in this strain.

The AKR strain mice showed a polycythemic pattern and spontaneous regression of splenomegaly. It is interesting that these mice showed marked hepatomegaly at the time of death. An increasing number of blastic cells both at the time of splenomegaly and at the time of hepatomegaly seemed to be the cells of an erythroblastic origin. Whether or not these cells originated from the same cell is a difficult problem and should be clarified later.

IV, *Typing of the tumor cell* In the polycythemic strains, the naked blastic cell decreased in number as the disease progressed and the atypical mono-lymphoid cell increased in number instead of the former cell. In the AKR strain mice, the polythemic pattern of the disease was observed in the early phase and a spontaneous regression of splenomegaly occurred about 30 days after the onset. These facts suggest that the naked blastic cell or the erythroid cell in the polycythemic strains had a reactive character rather than of a neoplastic origin. We think that the atypical mono-lymphoid cell may be the real tumor cell in the polycythemic strains. The previous report suggested that the large monocytoid cell might be the lymphoreticular-origin stem cell (Buffett and Furth [23]; Oboshi [7]; Kasuga and Oota [2]), whereas the studies *in vitro* suggested that the atypical mono-lymphoid cell might be the immature myeloid cell appearing only re-

actively (Heard *et al.* [24]). The problems, which component may be the real tumor and which may be only the reactive proliferation, could not be clarified by microscopical examination only and other methods are necessary.

In the anemic strains, the naked blastic cell increased in number till the time of death and the other components varied only a little at any time. So, the naked blastic cell was thought to be the tumor cell.

It is difficult to decide whether or not the naked blastic cell in both the polycythemic and anemic strains may be all neoplastic, but it is assumed that the cell with viral infection may become fragile and that this cell may have a tendency to rupture easily in the peripheral blood smear. The problem whether or not the naked blastic cell in each strain may be different in origin could not be clarified by this study from only the peripheral blood smear. It will be explored by electron microscopical and histochemical observation.

V, *Increase of MCV* The value of MCV was calculated to know whether or not the viral infection had an influence on the mature erythrocyte. The value of the MCV increased significantly in most mice of both the polycythemic and anemic strains. The FLV-resistant mice showed neither erythroblastosis nor an increase of MCV. In the AKR strain mice, the value of the MCV increased first, decreased later and returned to that of the control value after regression. Thus, the increase of the value of the MCV seems to be related not to the aging effect but to the viral infection. It is expected that the FLV disturbs the erythroid cell to differentiate and that the large RBC was produced or some nutritional disturbance of the erythrocyte may have occurred.

The mature erythroblast with the ability of hemoglobin synthesis may not have been counted as the RBC but as the WBC, and thus, the increase of the value of hematocrit will be larger than the value calculated only from the increase of the RBC count. But this factor had only little effect on the increase of the value of the MCV.

ACKNOWLEDGEMENT

We are grateful to Dr. K. Hirashima of the Department of Radiation Health of the National Institute of Radiological Sciences in Chiba for providing us the virus in the C3H/He strain mice, to Dr. K. Furuse of the Division of Physiology and Pathology of the above Institute and the staff of the Animal and Plant Supply Section in Technical Service of the above Institute for supplying the mice for this experiment, to Mrs. S. Seki of our 2nd Department of Pathology for her skillful assistance and to Dr. T. Sato of our 1st Department of Internal Medicine for the review of this manuscript.

REFERENCES

1) Friend, C.: Cell-free transmission in adult Swiss mice of a disease having the character of a leukemia. J. Exp. Med., 105: 307–318, 1957.
2) Kasuga, T., and Oota, K.: Pathological study of Friend disease. Gan-no-Rinsho, 8: 261–265, 1962 (in Japanese).
3) Axelrad, A. A., and Steeves, R. A.: Assay for Friend leukemia virus. Rapid quantitative method based on enumeration of macroscopic foci. Virology, 24: 513–516, 1964.
4) Tambourin, P., and Wendling, F.: Malignant transformation and erythroid differentiation by polycythemia-inducing Friend virus. Nature, 234: 230–233, 1971.
5) Mager, D. L., *et al.*: Quantitative colony method for tumorigenic cells transformed by two distinct FLV. Proc. Natl. Acad. Sci. USA, 78: 1703–1707, 1981.
6) Mirand, E. A.: Virus-induced erythropoiesis in hypertransfused-polycythemic mice. Science, 156: 832–833, 1967.
7) Oboshi, S., and Murayama, K.: *In vitro* cul-

ture and cloning of ascites sarcoma cells of Friend's disease origin. Gann, 58: 235–247, 1967.
8) Ikawa, Y., *et al.*: Phenotypic variants of differentation-inducible Friend leukemia lines. Bibl. Haematol., 43: 37–47, 1976.
9) Mirand, E. A., *et al.*: Effect of Friend virus in Swiss and DBA/1 mice on Fe^{59} uptake. Proc. Soc. Exp. Biol. Med., 106: 423–426, 1961.
10) Marcy, E., *et al.*: Anemia and polycythemia-inducing isolates of Friend spleen focus-forming virus. J. Exp. Med., 151: 1477–1492, 1980.
11) Shibuya, T., and Mak, T. W.: A host gene controlling early anemia or polycythemia induced by Friend erythroleukemia virus. Nature, 296: 517–519, 1982.
12) Hirashima, K., and Kumatori, T.: The changes of the susceptibility of the hematopoietic stem cells to Friend leukemia virus. *In* Proceedings of the Fourth International Conference on Erythropoiesis, edited by Nakao, K., Fisher, J. W., and Takaku, F., University of Tokyo Press, Tokyo, 1975, pp. 121–130.
13) Shibuya, T., and Mak, T. W.: Induction of erythroid tumorigenic colonies by Friend helper virus F-MuLV alone and isolation of a new class of Friend erythroleukemic cells. J. Cell. Physiol., Supple., 1: 185–194, 1982.
14) Peschle, C., *et al.*: Kinetics of erythroid precursors in mice infected with the anemic or the polycythemic strains of Friend leukemia virus. Proc. Natl. Acad. Sci. USA, 77: 2054–2058, 1980.
15) Steinheider, G., *et al.*: Comparison of the biological effect of anemia-inducing and polycythemia-inducing Friend virus complex. Experientia, 35: 1173–1175, 1979.
16) Rowe, W. P., and Sato, H.: Genetic mapping of the Fv-1 locus of the mouse. Science, 180: 640–641, 1973.
17) Mak, T. W., *et al.*: Fv-2 locus control expression of Friend spleen focus-forming virus-specific expression of normal and infected mice. Proc. Natl. Acad. Sic. USA, 76: 5809–5812, 1968.
18) Steeves, R. A., *et al.*: Genetic controls by the W locus of susceptibility to SFFV. Nature, 218: 372–374, 1968.
19) Bennet, M., *et al.*: Mutant Sl alleles of mice affect susceptibility to Friend spleen focus-forming virus. Science, 162: 564–565, 1968.
20) Lilly, F.: The effect of histocompatibility-2 type on response to the Friend leukemia virus in mice. J. Exp. Med., 127: 465–473, 1968.
21) Lilly, F., and Pincus, T.: Genetic controls of murine viral leukemogenesis. *In* Adv. Cancer Res. 17, edited by Klein, G., and Weinhouse, S., Academic Press, New York and London, 1973, pp. 231–277.
22) Klein, J.: Biology of the mouse histocompatibility-2 complex. Springer Verlag, New York, 1975, pp. 398.
23) Buffett, R. F., and Furth, J.: A transplantable reticulum-cell sarcoma variant of Friend's viral leukemia. Cancer Res., 19: 1063–1069, 1959.
24) Heard, J. M., *et al.*: Comparative effects of polythemia-inducing Friend leukemia virus and Moloney leukemia virus on hematopoietic progenitors in murine long-term bone marrow cultures. Cancer Res., 41: 3260–3265, 1981.