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Effects of Friend leukemia virus (FLV) inoculation in F_1 mice and differentiation of FLV-induced leukemia

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Summary. Effects of Friend leukemia virus (FLV) inoculation in F, specific pathogen free (SPF) mice were examined. Resistance to FLV was dominantly inherited both in F, hybrid mice (BDF,) (FLV-resistant & FLV-sensitive with polycythemia) and F, hybrid mice ($B6C3F_1$) (FLV-resitant & FLV-sensitive with anemia). But the population dynamics of the nucleated cell components of F, mice after FLV inoculation differed from those of FLV-resistant inbred mice. A small number of mature erythroblasts appeared in the peripheral blood of BDF, mice. In $B6C3F_1$ mice, erythroblastosis with splenomegaly and polycythemia occurred. However, all of these findings in BDF, and $B6C3F_1$ mice regressed spontaneously. In F, mice, FLV induced an intermediate reactive pattern of the two patterns that had been induced in the parental strains. The results indicate that FLV may induce leukemia with various degrees of differentiation, according to the genetic difference of the host.

Key words: Friend virus - Retrovirus infection - Host - virus relation - Experimental leukemia

Introduction

Friend leukemia virus (FLV) is a type-C retrovirus that induces leukemia in susceptible mice (Friend, 1957; Kasuga and Oota, 1962) and provides a convenient test system for the investigation of host-virus relation. We have demonstrated the role of T-cells in controlling susceptibility to FLV-induced leukemia (Kitagawa et al., 1986). Genetically, the contribution of host hereditary factors in determining the susceptibility to FLV injection is well established. Several host genes, such as Fv-l (Steeves and Lilly, 1977), Fv-2 (Lilly, 1970; Odaka, 1970; Mak et al., 1980), Fv-3 (Kumar et al., 1978), W (Steeves et al., 1968; MacDonald et al., 1980), Steel (Bennett et al., 1968; McCool et al., 1979; MacDonald et al., 1980; Mak et al., 1980), and H-2 (Lilly, 1968; Lilly and Pincus, 1973; Klein, 1975; MacDonald et al., 1980; Mak et al., 1980) are known to control the susceptibility of mice to FLV-induced leukemia. But, in most of the previous reports, susceptibility was evaluated by splenomegaly with the proliferation of leukemia cells; detailed chronological changes in the composition of the cellular populations of peripheral blood were not fully examined. We have previously reported that FLV induces different types of leukemia, according to the genetic difference of the host (Kitagawa et al., 1983). In order to clarify why the same virus induces different types of leukemia, we decided to examine chronological changes in cellular populations of peripheral blood in various F, mice between FLV-sensitive and -resistant.

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Materials and methods

Mice

Three strains of inbred mice and three F, hybrid mice were obtained from the following farms: C57BL/6 and C3H/He from the National Institute of Radiological Sciences in Chiba, and DBA/2, BALB/c & DBA/2 F, (hereafter called CDF,), C57BL/6 & DBA/2 F, (hereafter called BDF,) and C57BL/6 & C3H/He F, (hereafter called $B6C3F_1$) from Charles River Japan Inc. Ten female specific pathogen free (SPF) mice of each strain were employed for the experiment. They were housed 5 per cage

and fed with radiologically sterilized laboratory pellet and tap water *ad libitum.* All were bred under SPF condition to rule out the influence of intestinal bacterial flora.

Virus and Virus Infection

FLV, originally derived from Dr. C. Friend, was obtained from Dr. Hirashima, National Institute of Radiological Sciences, Chiba, Japan. An FLV complex was obtained from the supernatant fluid of homogenized spleen taken from 6 to 12 week-old female C3H/He strain mice. The virus has been maintained by serial passage in C3H/He mice. The leukemogenicity of the virus has been well maintained and was confirmed both by observation of splenomegaly and by hematological assays. The spleen of FLV-infected C3H/He mice, weighing 2 to 3 g, were placed in the physiological saline aseptically, and 10% weight per volume homogenate was made in a Polytron (Kinematica, Luzern, Swiss) for 2 minutes under moderate speed. The homogenate was centrifuged at 3,500 rpm for 20 minutes and the supernatant filtrated successively through Millipore filters of 0.45 nm and 0.22 nm mesh. The resulting clear filtrate (0.1 ml) was injected into the abdominal cavity of 9 week-old mice.

Hematological Assays

The leukemogenicity of FLV in inbred and F_1 mice was studied by hematological assays of peripheral blood from the tail vein. Parameters such as hematocrit (Ht) values (%), nucleated cell counts (/mm^3), and peripheral blood smears stained with hematoxylin and eosin, and May-Grünwald Giemsa's stain were examined twice a week from the time of injection (0 day) till death, in FLV-sensitive mice, or more than 100 days after injection, in FLV-resistant mice. Results were obtained from pools of 2 to 3 mice per experimental group and, unless otherwise noted, are expressed as the mean value of each parameter. For simplicity, the S.D.'s of mean values were not always presented, but were below 20% in all cases.

Results

Effects of FLV inoculation in Inbred Mice

Inbred mice were classified according to reactive patterns to FLV, into three groups: FLV-resistant (C57BL/6), FLV-sensitive with polycythemia (DBA/2) and FLV-sensitive with anemia (C3H/He).

Fig. 1 shows population dynamics of the nucleated cell components after FLV injection in the three strains of inbred mice. In C57BL/6 mice, the composition and total number of the nucleated cells showed little change (Fig. 1a). In DBA/2 mice, the blastic cell of peripheral blood increased in number by week 2 after injection, but decreased in the later phase of the disease after week 3 till death. However, the mature erythroblasts and the atypical mono-lymphoid cells increased in number in the later phase (Fig. 1b). In contrast, the blastic cells of C3H/He mice showed monotonous and continuous proliferation till the time of death (Fig. 1c).

Effects of FLV inoculation in F_1 Mice

Effects of FLV inoculation on the hematological parameters of F_1 mice is shown in Tables 1 and 2. Table 1 indicates the comparison of Ht values of inbred mice to those of F_1 hybrid mice, and Table 2 the chronological changes in the populations of the nucleated cell components in F_1 mice. Ten mice of each strain were examined, all mice of the same strain showing a similar reactive pattern to FLV. Susceptibility of the same strain was not separated into FLV-sensitive and resistant.

a) (BALB/c & DBA/2) CDF_1; CDF_1 mice, F_1 hybrid mice of polycythemic FLV-sensitive BALB/c and DBA/2 mice were FLV-sensitive with polycythemia (Fig. 2) and showed the same pattern of hematological changes as BALB/c and DBA/2 mice.

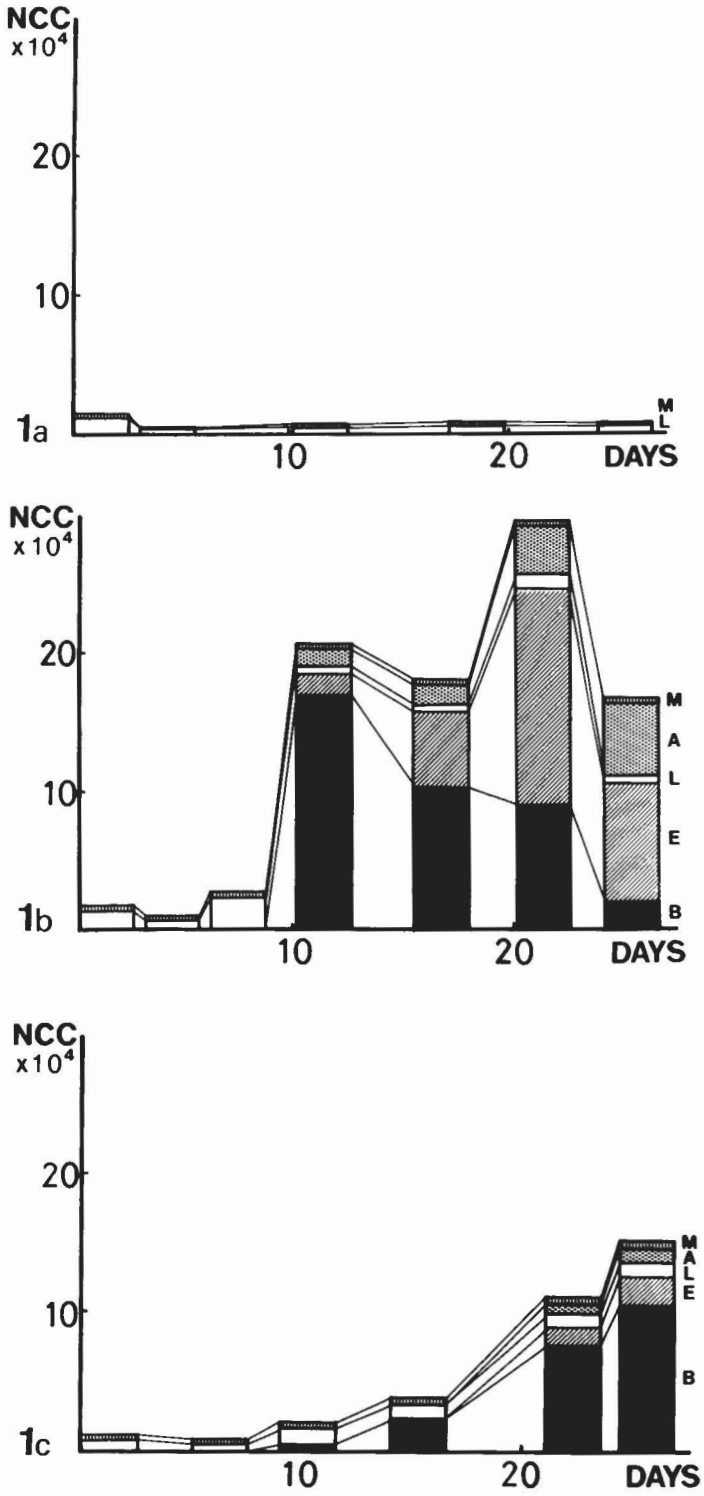
b) (C57BL/6 & DBA/2) BDF_1; BDF_1 mice, F_1 hybrid mice of FLV-resistant C57BL/6 mice and polycythemic FLV-sensitive DBA/2 mice were FLV-resistant. They showed no significant change in Ht values, nor of nucleated cell counts after FLV injection. But a small number of mature erythroblasts, not seen in C57BL/6 mice, appeared by week 2 to 3.

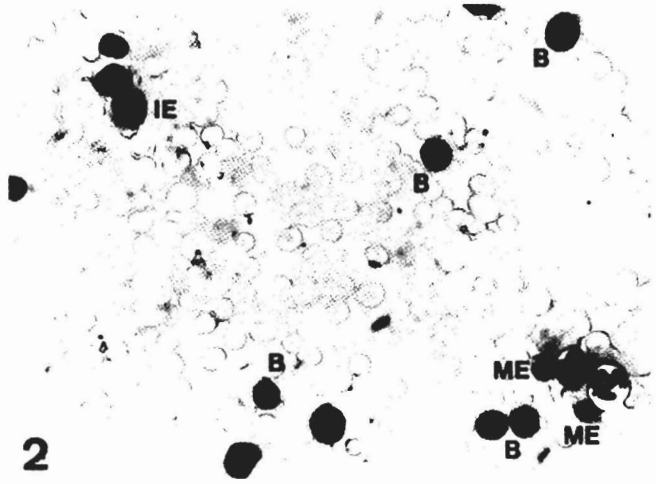
c) (C57BL/6 & C3H/He) $B6C3F_1$; $B6C3F_1$ mice, F_1 hybrid mice of FLV-resistant C57BL/6 mice and anemic FLV-sensitive C3H/He mice showed polycythemia and splenomegaly in week 3 after injection. But $B6C3F_1$ mice showed spontaneous recovery from the disease and, finally, all of the mice were FLV-resistant. The nucleated cell count increased and a large number of mature erythroblasts appeared in the peripheral blood when polycythemia and splenomegaly occurred (Fig. 3). Although the polycythemic type of Friend leukemia occurred in $B6C3F_1$ mice, at that time, immature blastic cells did not appear in the peripheral blood. As the Ht value recovered to the normal level and splenomegaly regressed, the nucleated cell count recovered to the normal level and the erythroblasts of the peripheral blood disappeared. A few atypical mono-lymphoid cells remained in the peripheral blood even after spontaneous recovery from Friend leukemia.

Fig. 1. Effects of FLV inoculation on changes in the nucleated cell population in inbred mice. The three reactive patterns to FLV are shown. 1a shows changes in C57BL/6 mice, 1b in DBA/2 mice, and 1c in C3H/He mice. Note that mature erythroblasts increase in the later stage in DBA/2 mice, while blastic cells show a monotonous proliferation in C3H/He mice. NCC, nucleated cell count; M (hatched column, straight striped), myeloid cell; A (stippled column), atypical mono-lymphoid cell; L (open column), lymphoid cell; E (hatched column, diagonally striped), erythroblast; B (shaded column), naked blastic cell.

Fig. 2. Peripheral blood smear of CDF_1 mice (May-Grünwald Giemsa's stain, x400, 24 days after FLV injection). Erythroblasts with various degrees of maturation can be seen (IE, immature erythroblast; ME, mature erythroblast; B, naked blastic cell).

Fig. 3. Peripheral blood smear of $B6C3F_1$ mice (May-Grünwald Giemsa's stain, x800, 10 days after FLV injection). Several mature erythroblasts can be observed (ME, mature erythroblast).





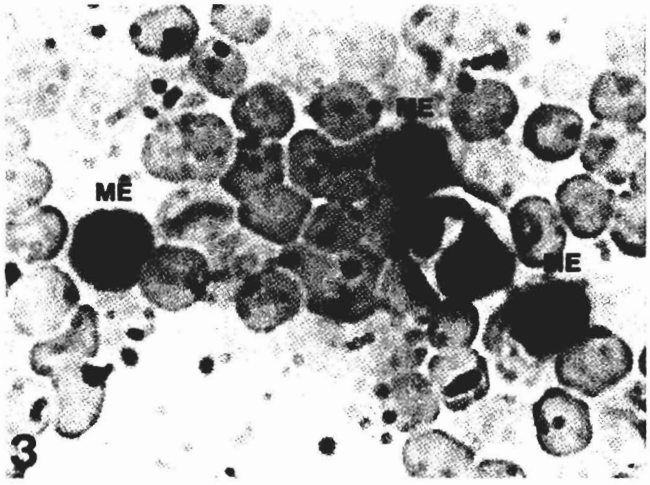


Table 1. Effects of FLV inoculation on chronological changes of Ht value in F_1 mice

MICE Strain	Control	Days after Injection 1 W	3 W	8 W	Sensitivity[a]
BALB/c	62.5 ± 2.1	67.5 ± 0.5	64.5 ± 0.7	+	P
DBA/2	49.5 ± 0.7	55.0 ± 1.4	71.0 ± 2.8	+	P
CDF_1	54.0 ± 0	53.5 ± 0.7	62.5 ± 0.7	+	P
C57BL/6	57.0 ± 0[b]	47.0 ± 0	49.0 ± 0	49.0 + 1.4	R
DBA/2	49.5 ± 0.7	55.0 ± 1.4	71.0 ± 2.8	+[c]	P
BDF_1	49.0 ± 0	51.5 ± 0.7	52.5 ± 0.7	53.0 ± 1.4	R
C57BL/6	57.0 ± 0	47.0 ± 0	49.0 ± 0	55.7 ± 0.1	R
C3H/He	50.7 ± 2.1	34.0 ± 5.7	21.0 ± 0.6	20.0 ± 3.0	A
$B6C3F_1$	53.0 ± 0	55.5 ± 3.5	64.0 ± 0	53.0 ± 1.4	P,R

a) Sensitivity to FLV is described as follows; R, resistant; P, sensitive with polycythemia; P,R, polycythemia with regression; A, sensitive with anemia.

b) Mean ± S.D.

c) All FLV-sensitive mice died before week 8.

Table 2. Effects of FLV inoculation on populations of the nucleated cell components in F_1 mice

Mice strain	Days after Injection	Nucleated cell count ($10^2/mm^3$)	Cellular population (%)[a]				
			M	A	L	E	B
CDF_1	0	176[b]	22(13)	2(1)	152(86)	—	—
	14	76	9(12)	2(3)	62(82)	—	—
	24	2,840	109(4)	90(11)	373(13)	795(28	1,456(52)
	31	745	124(17)	76(10)	377(51)	53(7)	115(15)
BDF_1	0	156	14(9)	—	142(91)	—	—
	14	89	9(10)	—	77(87)	3(3)	—
	21	181	14(8)	4(2)	161(89)	4(2)	—
	56	145	13(9)	2(2)	129(89)	—	—
$B6C3F_1$	0	153	15(10)	—	138(90)	—	—
	10	500	52(10)	—	284(57)	164(33)	—
	17	133	9(7)	6(4)	118(89)	1(1)	—
	28	175	11(6)	5(3)	159(91)	—	—

a) The cellular populations are presented as follows: M, myeloid cell; A, atypical mono-lymphoid cell; L, lymphoid cell; E, erythroblast; B, naked blastic cell.

b) Results are expressed as the mean value of 2 to 3 mice at each time. For simplicity, only mean values are presented, but all mice of the same F_1 showed a similar pattern.

Discussion

There have been several reports concerning susceptibility of inbred mice F_1 and F_2 mice to FLV-induced leukemia (Odaka and Yamamoto, 1962; Lilly and Pincus, 1973). The present data confirmed previous reports that the resistancy of inbred C57BL/6 mice to FLV-induced leukemia is dominantly inherited in F_1 hybrid mice (FLV-resistant & FLV-sensitive) (Lilly and Pincus, 1973). But chronological examination of peripheral blood revealed the different population dynamics of the nucleated cell components of BDF_1 and $B6C3F_1$ mice from those of C57BL/6 mice. These findings suggest the participation of more complicated mechanisms of the host reaction in controlling susceptibility to FLV.

As to the explanation for these findings, it is possible that FLV induces tumors with various degrees of differentiation, according to the difference in the hereditary factors of the host. From a comparison of the patterns of the population dynamics of the nucleated cell components and of the morphological features of the blastic cells in peripheral blood of inbred mice, tumor cells of polycythemic FLV-sensitive mice may be well differentiated and those of anemic mice poorly differentiated (Kitagawa et al., 1983). From this viewpoint, the degree of differentiation of tumor cells of F_1 mice in the present study can be represented as follows: 1) BDF_1 mice, F_1 hybrid mice (DBA/2 mice in which FLV induces well differentiated leukemia & FLV-resistant C57BL/6 mice) show slight erythroblastosis by week 2 to 3. 2) In $B6C3F_1$ mice, F_1 hybrid mice, (C3H/He mice in which FLV induces poorly differentiated leukemia & FLV-resistant C57BL/6 mice) FLV induces well differentiated leukemia with spontaneous regression. In F_1 hybrid mice, FLV induces an intermediate reactive pattern of the two patterns of the parental strains. These findings suggest the possibility that the degree of differentiation of FLV-induced leukemia is controlled by the genetic control of the host.

Several previous reports have indicated that the types of modification of the erythropoiesis patterns depend on the strain of FLV used and that the modulation of the hematopoietic organization by FLV is the result of a complex array of interactions between host genes and the viral genomes (MacDonald et al., 1980; Peschle et al., 1980). Furthermore, the natures of tumorigenic colonies emerging in the late stages of FLV-induced leukemia have been said to differ in the degree of differentiation, according to the difference of the strain of FLV used (Mager et al., 1981; Shibuya and Mak, 1982). These colonies, when derived from mice with different strains of FLV infection, differ in their self-renewing capacities (Mager et al., 1981), their incidence of chromosomal aberrations (Mager et al., 1981) and their responsiveness to induction by erythropoietin (Kluge et al., 1974; Mager et al., 1981). However, Shibuya and Mak (1982) proposed that the induction of early polycythemia or anemia in DBA/2 and CBA mice, infected with the same strain of FLV, was controlled by a single co-dominant locus which was designated as Fv-5. Their findings are consistent with the present data which have been interpreted as the result of the different degrees of differentiation of tumor cells caused by the difference of the host factors and not by the difference of viral strains.

The difference in tumor cell differentiation may be caused by the different system of hematopoietic cell differentiation and different FLV target cells of the host. We have reported that there was considerable correlation between the pattern of FLV-induced leukemia and H-2 haplotype of the host (Kitagawa et al., 1983). Furthermore, T-cells may play an important role in mediating host resistance to FLV-induced leukemia (Kitagawa et al., 1986). It is thus also possible that the immunosurveillance systems permits the proliferation of the selected tumor cell, resulting in the proliferation of different tumor cells in different strains of mice. As to the cause of various differentiation in FLV-induced leukemia, further study is necessary.

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