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Dynamics of Lymphocytic Subpopulations in Friend Leukemia Virus-induced Leukemia[1]

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ABSTRACT

The *in vivo* roles of the immunosurveillance mechanism of the host against leukemia induced by Friend leukemia virus (FLV) were examined. The significance of T-cells in host defense against FLV-induced leukemia was indicated by the fact that thymus-deprived C57BL/6N-*nu/nu* mice were sensitive to FLV, although normal C57BL/6N mice were, as already reported by many authors, resistant to FLV. In relation to the role of T-cells on the onset of FLV-induced leukemia, the population dynamics of the lymphocytic subpopulations of the systemic lymphoid organs after FLV injection in FLV-resistant C57BL/6N mice were examined in comparison with the dynamics in FLV-sensitive strains, C57BL/6N-*nu/nu* mice and normal C3H/HeN mice. In this system, Lyt-1$^+$2$^-$ helper T-cells in the spleen of FLV-resistant C57BL/6N mice increased in number after FLV injection. The number of immunoglobulin positive cells did not remarkably change in FLV-resistant C57BL/6N mice after FLV injection, whereas the number increased in the lymph node of FLV-sensitive C3H/HeN mice. The results indicated that a major contribution to the relative susceptibility and resistance of the host to FLV was controlled by the capacity to mobilize T-cells to the spleen in an early stage of disease, although the interaction of these T-cells with other immune cells may play an important role in mediating host resistance to FLV-induced disease.

INTRODUCTION

The T-cell-mediated immunosurveillance mechanism appears to play a major role in host defense against antigenically foreign cells, such as allografts and tumors. Passive transfer of immune lymphoid cells can often accelerate allograft rejection (1, 2) or protect the host specifically against challenge with neoplastic cells (3, 4). There is also some evidence in mice suggesting that T-cells may control the incidence of certain virus-induced or radiation-induced tumors (5–7).

FLV[3] is a type C retrovirus that induces rapid multistage leukemia in mice (8, 9), and FLV-induced leukemia is a very good model for use in investigations of host defense. Many strains of immunologically mature adult mice are susceptible to FLV-induced leukemia (10), whereas some strains, such as C57BL/6, C57BL/10, and B10.D2 mice, are resistant to FLV (11). Previous studies have demonstrated that several genes, *Fv-2* (12, 13), *W* (14), *Steel* (15), and *H-2* (16), control susceptibility to FLV-induced leukemia. We have reported a correlation between the *H-2* haplotype of the host and susceptibility to FLV (11), but the role of the host immune response to FLV is still uncertain.

In the responses of mice to leukemia and sarcoma virus-induced tumors, several different cell-mediated immune mechanisms have been demonstrated *in vitro* (17–19), but the *in vivo* roles and relative importances of these mechanisms are unknown. In the present study, first of all, the importance of T-cell function in protection of host against FLV is identified using FLV-resistant normal C57BL/6N mice and syngeneic thymus-deprived nude mice. And thereafter, the paper describes the dynamics of lymphocytic subpopulations in the systemic lymphoid organs of FLV-resistant C57BL/6N, FLV-sensitive C57BL/6N-*nu/nu* mice, and FLV-sensitive C3H/HeN mice after FLV inoculation in order to investigate the role of the specific cell-mediated immune response in this disease.

MATERIALS AND METHODS

Mice. C57BL/6N and C3H/HeN mice were obtained from Charles River Japan, Inc. (Tokyo, Japan). C57BL/6N-*nu/nu* mice were from Japan Clea Co. (Tokyo, Japan). All animals were 9-wk-old female specific-pathogen-free mice. They were housed 5 per cage and given radiologically sterilized laboratory pellets and tap water *ad libitum*. All animals were fed under specific-pathogen-free conditions.

Virus and Virus Infection. FLV was obtained from Dr. Hirashima, National Institute of Radiological Sciences, in Chiba, Japan, which was originally derived from Dr. C. Friend (20). A FLV complex was obtained from the supernatant fluid of homogenized spleen taken from 6- to 12-wk-old female C3H/HeJ mice that had been serially infected with FLV. The leukemogenicity of the virus was well maintained and was confirmed by both observation of splenomegaly and hematological assays. The spleens of FLV-infected mice weighing 2 to 3 g were placed in 0.9% NaCl solution aseptically, diluted to 10% weight per volume, and homogenized for 2 min at moderate speed in a Polytron (Kinematica, Luzern, Switzerland). The homogenate was centrifuged at 3500 rpm for 20 min, and the supernatant fluid was filtrated successively through Millipore filters of 0.45- and 0.22-nm mesh. The resulting clear filtrate (0.2 ml) was injected into the peritoneal cavity of 9-wk-old mice.

Hematological Assays. For examination of the role of T-cells in surveillance of the host against tumors, the leukemogenicities of FLV in C57BL/6N-+/+ and -*nu/nu* mice were compared by hematological assays of peripheral blood from the tail vein. Hematocrit values, nucleated cell counts, and peripheral blood smears stained with May-Grünwald Giemsa stain and benzidine stain for hemoglobin by Ralph's method were examined once a week until the time of sacrifice. Histological sections of the spleen and bone marrow of the sacrificed animal were stained with hematoxylin and eosin.

Cell Suspensions. FLV was injected i.p. into 9-wk-old C57BL/6N-+/+, C57BL/6N-*nu/nu*, and C3H/HeN mice. The animals were sacrificed at various times thereafter to measure total amounts of Thy-1$^+$, Lyt-1$^+$, Lyt-2$^+$, and IgG-positive cells in various lymphoid organs. Cell suspensions were prepared from the inguinal lymph node, spleen, and thymus by teasing the organs with two forceps in ice-cold RPMI 1640 medium (Nissui Seiyaku Co., Tokyo, Japan). Bone marrow cells were recovered by flushing the femurs with cold RPMI 1640. The cells were washed 3 times with RPMI 1640 and once with PBS containing 10% normal calf serum, and their viability was determined by staining them with eosin.

Cell Surface Staining. Cell suspensions were centrifugated at 800 rpm for 10 min with Cytospin (Schandon, Runcorn, Cheshire, England), and cells attached to the slides were fixed with acetone for 10 s and dried. The slides were immersed in absolute methanol containing 0.3% H_2O_2 for 30 min to abolish endogenous peroxidase activity and then washed with three changes of PBS. Then biotin-conjugated primary antibodies were added to the slides. The monoclonal antibodies used for immunoperoxidase staining were biotin-conjugated anti-Thy-1.2, from Becton Dickinson Monoclonal Center, Inc. (Mountain View, CA), and anti-Lyt-1.1, anti-Lyt-1.2, anti-Lyt-2.1, and anti-Lyt-2.2, from Meiji Institute of Health Science (Tokyo, Japan). Antibody dilutions of 1:100 in PBS were used. As negative controls, the primary antibodies

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[3] The abbreviations used are: FLV, Friend leukemia virus; PBS, phosphate-buffered saline (0.1 M NaCl:0.01 mM Na_2HPO_4:0.003 M KH_2PO_4, pH 7.4); MSV, murine sarcoma virus.

were replaced by PBS. After incubations at room temperature for 2 h, the slides were washed for 15 min with three changes of PBS and shaken to remove excess buffer. The slides were then treated with avidin-biotin-peroxidase complex (Vector Laboratories, Inc., Burlingame, CA) for 30 min, washed as described above, and treated with a filtered, freshly prepared solution of 3,3′-diaminobenzidine-tetrahydrochloride in 0.05 M Tris buffer, pH 7.6, containing 0.03% H_2O_2. The slides were rinsed with Tris buffer, washed in tap water, dehydrated, and mounted in Permount (Fisher Scientific Co., Fair Lawn, NJ). Some preparations were stained for detection of IgG-positive cells by Si-Mung's avidin-biotin-peroxidase complex method (21). Primary rabbit anti-mouse IgG antibody was obtained from Miles Scientific (Naperville, IL).

Lymphocytic Subpopulations in Lymphoid Organs. From the measurement of the weight (*W*) of the lymph node, spleen and thymus in mg, and the body weight of mice (*BW*) in g, the normalized value [*W/BW*] was calculated for the correction of the individual variation of mice in size. The percentage of positive cells (*P*) in each organ was measured. It has been confirmed that the size of the individual cell has not been so variable in normal lymphoid organs. Therefore, the amount of the positive cells was estimated by the value [$W/BW \times P$]. This value reflected the amount of positive cells in each organ of the individual mouse. Unless otherwise noted, values were obtained on pooled samples from three mice in each experimental group and are expressed as percentages of the values obtained on the same day from pooled samples from three control mice treated with the same volume (0.2 ml) of 0.9% NaCl solution only. The percentage (% of control) was calculated from the formula

$$\%\ \text{of control} = \frac{\text{test } [W/BW \times P]}{\text{control } [W/BW \times P]} \times 100$$

In the bone marrow, the percentage (% of control) was estimated from the ratio of the value [*P/BW*] of the experimental mice to that of the control mice. The amounts of Lyt-1$^+$ and Lyt-2$^+$ cells in the lymph node and spleen were represented as values for [$W/BW \times P$] at each time.

RESULTS

FLV-induced Leukemia in C57BL/6N-+/+ and -*nu/nu*. Normal C57BL/6N mice were resistant to FLV. They showed no remarkable change in hematocrit value or in the nucleated blood cell count of peripheral blood after FLV injection. Table 1 shows a comparison of chronological changes of the hematocrit values, nucleated blood cell counts of peripheral blood, and weights of the spleen in C57BL/6N-+/+ and in C57BL/6N-*nu/nu* mice after FLV injection. All the C57BL/6N-*nu/nu* mice developed leukemia within 8 wk, whereas all the C57BL/6N-+/+ mice were resistant to FLV. When leukemia progressed, C57BL/6N-*nu/nu* mice showed erythroblastosis with marked splenomegaly and marked increase in number of nucleated cells of the peripheral blood. Morphological examination revealed that the nucleated cells of the peripheral blood included the erythroid blastic cells with various degrees of maturation. The cytoplasm of some of these blastic cells was positively stained with benzidine stain (Fig. 1). They were erythroid progenitor cells and erythroblasts. The spleen (Fig. 2) and bone marrow (Fig. 3) were filled with the immature blastic cells that partly showed various degrees of maturation to the erythroblast. Fig. 4 shows that most of the splenic cells were positively stained with benzidine stain. These blastic cells were similar to those of FLV-induced leukemia-developing C3H/HeN mice. The C57BL/6N-*nu/nu* mice all died with FLV-induced leukemia within 15 wk after FLV injection; namely, they were FLV sensitive.

Table 1 *Effect of FLV inoculation upon C57BL/6N-+/+ mice and C57BL/6N-nu/nu mice*

Chronological changes of the hematocrit, the WBC count, and weight of the spleen in C57BL/6N-+/+ and *nu/nu* mice after FLV injection are shown. Pooled materials from three animals per group were used, except for the values for C57BL/6N-*nu/nu* mice 4 and 10 wk after FLV injection which were for a single mouse because most of the animals had died.

Parameter	Preinjection	Wk after injection 1	4	10
C57BL/6N-+/+ (R)[a]				
Hematocrit (%)	53.3 ± 0.6[b]	54.3 ± 0.6	54.0 ± 1.7	56.0 ± 1.7
Nucleated cell counts (10^3/mm^3)	11.2 ± 0.9	5.7 ± 1.4	12.1 ± 1.5	13.1 ± 1.6
Spleen weight (mg)	79.9 ± 8.3	84.0 ± 5.3	102.2 ± 7.2	108.7 ± 1.4
C57BL/6N-*nu/nu* (S)				
Hematocrit (%)	53.7 ± 0.6	54.3 ± 0.6	72.0	71.0
Nucleated cell counts (10^3/mm^3)	10.2 ± 0.3	20.1 ± 0.7	23.7	640.0
Spleen weight (mg)	74.6 ± 12.8	101.0 ± 21.5	662.0	3004.4

[a] (R), resistant to FLV; (S), sensitive to FLV.
[b] Mean ± SD.

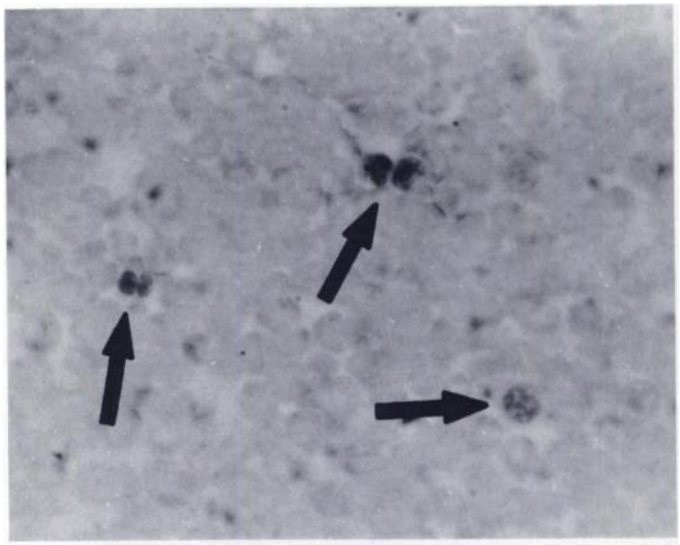

Fig. 1. Blastic cells in the peripheral blood of a C57BL/6N-*nu/nu* mouse 10 wk after FLV injection. The cytoplasm of the blastic cells is positively stained with benzidine stain (*arrows*). Benzidine stain, × 350. Nucleated cells of the peripheral blood of nontreated C57BL/6N-*nu/nu* mice have contained myeloid cells (about 70%) and lymphoid cells (about 30%) but no erythroid cells. Therefore, the appearance of the erythroid blastic cells in the peripheral blood is very important for the diagnosis of FLV-induced leukemia.

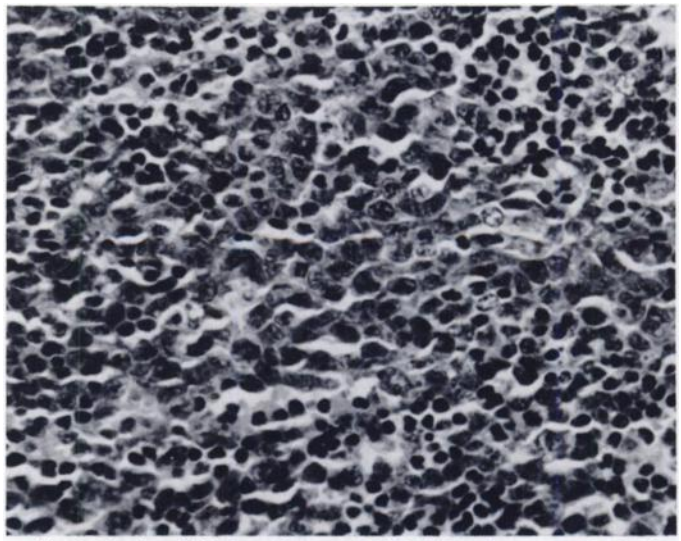

Fig. 2. Blastic cells in the spleen of a C57BL/6N-*nu/nu* mouse 10 wk after FLV injection. More than 80% of the nucleated cells are erythroid blastic cells, and about one-third of them are mature erythroblasts. H & E, × 200.

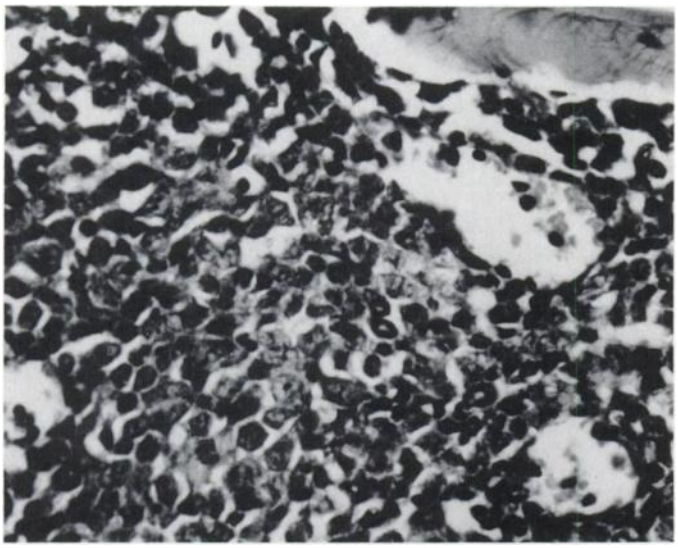

Fig. 3. Blastic cells of the bone marrow of a C57BL/6N-*nu/nu* mouse 10 wk after FLV injection. About 70% of the medullary cord is filled with erythroid blastic cells with various degrees of maturation. H & E, × 200.

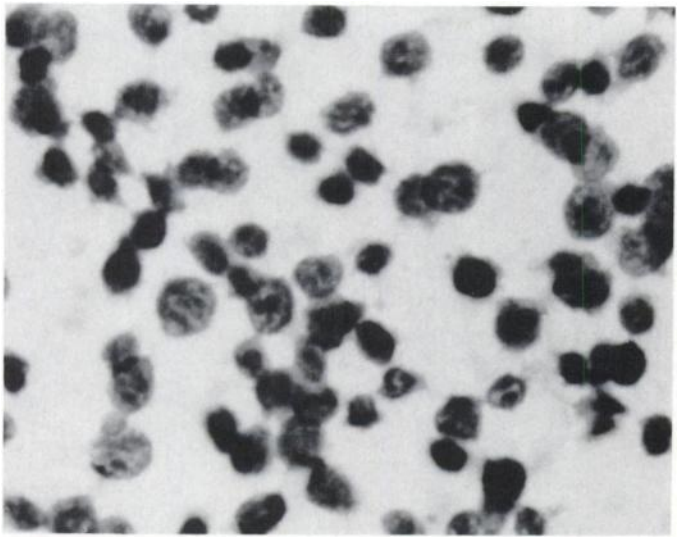

Fig. 4. Splenic cells of a C57BL/6N-*nu/nu* mouse 10 wk after FLV injection. Most of the cells are positively stained with benzidine stain. Benzidine stain, × 500.

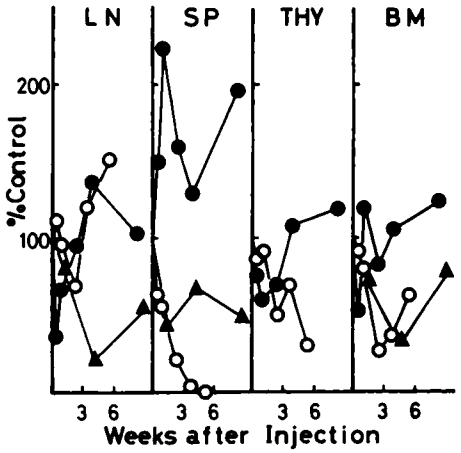

Fig. 5. Effects of FLV injection on the distribution of Thy-1$^+$ cells from various lymphoid organs of C57BL/6N-+/+, C57BL/6N-*nu/nu*, and C3H/HeN mice. Chronological changes in the numbers of Thy-1$^+$ cells in the lymph node, spleen, thymus, and bone marrow of mice after FLV injection are shown. Values are shown as percentage of those on the same day in control mice treated with saline only. Pooled samples from three animals per group were used for each determination. For simplicity, the proportions of Thy-1$^+$ cells and the weights of organs are not shown, but the SDs of the mean values were below 10% in every case. Note that splenic Thy-1$^+$ cells of C57BL/6N mice showed quite different dynamics from those of C3H/HeN mice. The data in the thymus of C57BL/6N-*nu/nu* mice are not shown, because *nu/nu* mice are thymus deprived. *LN*, lymph node; *SP*, spleen; *THY*, thymus; *BM*, bone marrow; ●, Thy-1$^+$ cell population of C57BL/6N mice; ▲, Thy-1$^+$ cell population of C57BL/6N-*nu/nu* mice; ○, Thy-1$^+$ cell population of C3H/HeN mice.

Effects of FLV on the Distribution of Thy-1$^+$ Cells in Various Lymphoid Organs. As shown in Fig. 5, FLV-resistant C57BL/6N mice showed a slight decrease in Thy-1$^+$ cells in the lymph node, thymus, and bone marrow 1 wk after FLV injection. Maximal cell depletion (about 30% of the control value) was observed in the lymph node in Wk 1 after the injection, but by Wk 2 to 3, the Thy-1$^+$ cell population of the lymph node had recovered and then increased to 150% of the control in Wk 5, returning to the normal level and increasing a little by wk 8. In the thymus and bone marrow, the Thy-1$^+$ cells decreased to about 50% of the control number 1 wk after the injection and then gradually returned to the normal level by Wk 8. In contrast, the Thy-1$^+$ cells of the spleen increased in number from Day 2, reaching an average of 220% of the control cell number by Wk 1 after FLV injection, then decreasing to about 120%, and then increasing again to about 200% of the control cell number by Wk 8. Thus the effect of FLV on Thy-1$^+$ cells in the spleen was different from those observed in the lymph node, thymus, and bone marrow.

In C57BL/6N-*nu/nu* mice, the number of Thy-1$^+$ cells accounted for less than 5% of the total nucleated cells in each organ. As shown in Fig. 5, injection of FLV into FLV-sensitive C57BL/6N-*nu/nu* mice resulted in the decrease of Thy-1$^+$ cells in the lymph node, spleen, and bone marrow. The Thy-1$^+$ cell population had partially recovered in Wk 10, but all mice died within 15 wk without complete recovery to the normal level in any organ.

The effects of FLV on the Thy-1$^+$ cell population of the FLV-sensitive mouse strain, C3H/HeN, are also shown in Fig. 5. In the lymph node, the Thy-1$^+$ cells decreased to 70% of the control number by Wk 2 and then increased to 180% of the control numbers in Wk 5. In contrast, the numbers of Thy-1$^+$ cells in the spleen, thymus, and bone marrow gradually decreased. The decrease was greatest in the spleen, where the number of Thy-1$^+$ cells decreased more than 95% by 4 to 5 wk after FLV injection. All the C3H/HeN mice given FLV died within 6 wk in this experiment.

Effects of FLV on the Distributions of Lyt-1$^+$ and Lyt-2$^+$ Cells in Various Lymphoid Organs. Table 2 shows the chronological changes of the amounts of Lyt-1$^+$ and Lyt-2$^+$ cells in the lymph node and the spleen of normal strains, C57BL/6N and C3H/HeN mice, before and 1 and 4 wk after FLV injection.

The normal amount of Lyt-1$^+$ cells of the lymph node was almost twice as much in C57BL/6N mice as in C3H/HeN mice. FLV injection induced a decrease in the Lyt-1$^+$ cell population of C57BL/6N and C3H/HeN mice to about 50 to 60% of the normal value in Wk 1 and then recovery to almost the normal value by Wk 4. In C57BL/6N mice, the amount of Lyt-2$^+$ cells decreased to 40% of the normal value in Wk 1 and recovered to 70% of the normal value by Wk 4. The amount of Lyt-2$^+$ cells of C57BL/6N mice changed almost in parallel with the amount of Lyt-1$^+$ cells. Namely, the Lyt-1$^+$2$^-$ cell population showed little change after FLV injection. The absolute value of the Lyt-2$^+$ cell population of C3H/HeN mice in the data, unlike that of C57BL/6N mice, showed only a slight decrease. Namely, the Lyt-1$^+$2$^-$ cell population decreased in Wk 1 and then returned to the normal value by 4 wk after the injection.

Table 2 *Early effects of FLV injection on the distribution of Lyt-1⁺ cells and Lyt-2⁺ cells in various lymphoid organs of C57BL/6N and C3H/HeN mice*

Chronological changes (0 to 4 wk) in numbers of Lyt-1⁺ and Lyt-2⁺ cells in the lymph node and the spleen of C57BL/6N and C3H/HeN mice are shown. Values are expressed as ratios of amounts of cells to body weight and were calculated as weight of organ (mg)/body weight (g) × percentage of positive cells (%). Samples from one mouse or pooled samples from two to three animals per group were used. For simplicity, only mean values are shown, but the SDs of the mean values were below 10% in every case.

Positive cells	Preinjection	Wk after injection 1	4
C57BL/6N			
Lymph node Lyt-1⁺	0.104	0.062	0.091
Lymph node Lyt-2⁺	0.055	0.021	0.040
Spleen Lyt-1⁺	0.684	1.480	1.219
Spleen Lyt-2⁺	0.441	0.551	0.319
C3H/HeN			
Lymph node Lyt-1⁺	0.064	0.033	0.061
Lymph node Lyt-2⁺	0.018	0.011	0.012
Spleen Lyt-1⁺	0.683	0.654	0
Spleen Lyt-2⁺	0.364	0	0

The amount of Lyt-1⁺ cells in the spleen before treatment was almost the same in C57BL/6N mice as in C3H/HeN mice. The amounts of Lyt-2⁺ cells in the spleens of untreated C57BL/6N and C3H/HeN mice were also similar. Effects of FLV inoculation upon the changes of the Lyt-1⁺ cell population were similar to those upon the Thy-1⁺ cell population. In the spleen of the C57BL/6N mice, the Lyt-1⁺ cell population increased in Wk 1 and then slightly decreased by Wk 4, whereas in C3H/HeN mice Lyt-1⁺ cells gradually decreased to zero by 4 wk after the injection. The Lyt-2⁺ cells of C57BL/6N mice increased a little in Wk 1 and reduced to 70% of the normal value by Wk 4. Namely, the Lyt-1⁺2⁻ cell population increased in Wk 1, and then the Lyt-1⁺2⁺ cell population decreased from Wk 1 to 4 after the injection.

In contrast, the Lyt-2⁺ cell population of the spleen of C3H/HeN mice decreased to zero in Wk 1. Namely, the Lyt-1⁺2⁺ cell population decreased first in Wk 1, and then the Lyt-1⁺2⁻ cell population decreased from Wk 1 to 4 after the injection.

Effects of FLV on the Distribution of IgG-positive Cells in Various Lymphoid Organs. In FLV-resistant C57BL/6N-+/+ mice, FLV injection had no remarkable effect on the number of IgG-positive cells in the spleen or bone marrow. In the lymph node, the number of IgG-positive cells decreased to 50% of the normal value in Wk 1 after the injection, then increased to 180% of the control value by Wk 4, and recovered to the normal value by Wk 8 (Fig. 6).

The IgG-positive cell population in the lymph node of FLV-sensitive C3H/HeN mice continued to increase until the time of death to more than 400% of the control value. In contrast to the distribution in the lymph node, in the spleen, the increase in IgG-positive cells occurred in WK 1, followed by their decrease to 10% of the control value by Wk 4 after the injection, and then they recovered to the normal cell number by Wk 6. In the bone marrow, IgG-positive cells decreased to 40% of the control value in Wk 1 after the injection and showed no subsequent recovery (Fig. 6).

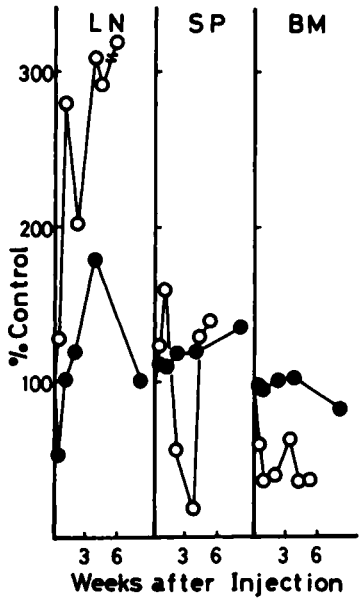

Fig. 6. Effects of FLV injection on the distribution of IgG-positive cells in various lymphoid organs of C57BL/6N and C3H/HeN mice. Experimental conditions were similar to those for Fig. 5. Results are expressed as percentages of those for the same cell population on the same day in control mice treated with saline only. Note the marked increase of the IgG-positive cell population in FLV-sensitive C3H/HeN mice with little change in that in C57BL/6N mice in the lymph node. *LN*, lymph node; *SP*, spleen; *BM*, bone marrow; ●, IgG-positive cell population of C57BL/6N mice; ○, IgG-positive cell population of C3H/HeN mice.

DISCUSSION

Suppression of the immune system of mice by treatment with antilymphocyte serum or neonatal thymectomy is known to increase the incidence of tumors induced by RNA or DNA viruses (22). A possible explanation for the stronger association of T-cells with surveillance against virus-induced tumors is that initial sensitization of the host to viral antigens promotes a stronger cellular response to subsequently developing tumors expressing viral antigens on their cell surface. Congenitally thymus-deprived *nu/nu* mice provide a convenient test system for analyzing the role of T-cells in surveillance against tumors. The present results demonstrate that thymus-deprived *nu/nu* mice were sensitive to FLV-induced leukemia as well as to previously demonstrated virus-induced solid tumor (5), whereas syngeneic normal +/+ mice did not develop leukemia. Susceptibility to FLV-induced leukemia is known to be influenced by many variable factors, including the virus strain (23), initial viral dose (12, 24), and several host genes (10, 12–16). In this work, the experimental conditions of *nu/nu* mice were identical with those of +/+ mice, and so the difference in the responses of the two was ascribable to T-cells.

Chesebro and Wehrly (25) reported that θ-positive cells appear in the spleen of mice during spontaneous recovery from FLV-induced splenomegaly and that these may be specifically cytotoxic to FLV-induced leukemia cells *in vitro*. A very close correlation was also found between the occurrence of specific cytotoxic effector cells in the spleen and recovery from splenomegaly (25). As shown in Fig. 5, a comparison of the kinetics of Thy-1⁺ cells in FLV-resistant and -sensitive mice indicated that capacity to mobilize Thy-1⁺ cells to the spleen was a major factor controlling the susceptibility to FLV-induced leukemia. The total amounts of Thy-1⁺ cells in various lymphoid organs did not change in Wk 1 to 4 after FLV injection but increased in Wk 4 to 8. Therefore, mobilization of Thy-1⁺ cells may occur in an early stage, and their proliferation in a later stage.

The review of Wheelock and Robinson (7) cited several reports presenting presumptive evidence for the role of cytolytic T-cells in the rejection of MSV-induced tumor. The reviewed reports demonstrate that splenic T-cells from mice with regressing tumors express viral antigen-specific cytolytic activity augmented by *in vitro* exposure to viral antigen (2, 26), that highly cytolytic T-cells can be isolated from regressing tumors (27), that T-cell depletion of mice abrogates tumor rejection, and

that infusion of T-cell-depleted mice with virus-immune T-cells restores their tumor rejection capability (28). Recent evidence suggests that, whereas rejection of transplanted Moloney leukemia virus antigen-positive lymphoma cells requires the participation of Lyt-23$^+$ cytolytic T-cells, the protection of mice from MSV-induced tumors requires only the presence of Lyt-1$^+$ helper T-cellls (29). The explanation for this observation is that the Lyt-1$^+$ T-cells provoke a delayed type hypersensitivity reaction at the site of tumor growth.

Delayed type hypersensitivity reactions in an area of tumor growth have also been shown to promote tumor regression or rejection (2, 30) via an interaction between Lyt-1$^+$ T-cells and macrophages (31). This finding is consistent with an observation that peritoneal MSV tumor-associated macrophages from mice bearing regressing MSV tumors have nonspecific cytolytic or cytostatic effects on MSV-induced tumor cells *in vitro* (32–34). Therefore, tumor regression of MSV-induced tumors results from antigen-specific and -nonspecific cytotoxic effects of T-cells and macrophages. Natural killer cell-mediated cytotoxicity may be involved, but only in regression of tumors induced in certain mouse strains (35). Unlike the MSV system, FLV-induced leukemia is not a solid tumor and may evoke the immune response of the host due to the systemic circulation of the tumor cells. The present results indicate that Lyt-1$^+$2$^-$ helper T-cells of the spleen of the FLV-resistant mice may be the main factor controlling the resistance in this FLV system. Further studies on the roles of macrophages, natural killer cells, or other effector cells in this system are necessary.

The *in vivo* roles of the immunosurveillance mechanism of the host must be clarified by experiments involving *in vivo* transfer of fractionated effector cell populations, antibodies, or inhibitory factors to tumor-bearing animals. This has been carried out to some extent in the MSV system, and results have suggested that T-cells are effective against tumor challenge (27, 28, 36, 37). In the case of FLV-induced leukemia, Chesebro and Wehrly (38) reported that passively transferred antiserum is effective against a small number of early (1 to 3 day) FLV spleen colonies but has little effect on recovery from leukemic splenomegaly in this system. Further, Collins and coworkers have reported that mice challenged with a leukemogenic dose of FLV can be protected against the development of disease by subsequent passive therapy with heterologous antisera raised against disrupted virus or the purified major viral envelope glycoprotein with a molecular weight of 71,000 (39, 40). The present experiments showed that, in FLV-resistant C57BL/6N mice, the IgG-positive cell population in various lymphoid organs did not increase, unlike that in FLV-sensitive C3H/HeN mice. Therefore, the humoral factor such as noncytolytic blocking antibody may have played a possible role, especially in FLV-sensitive mice. But the dynamics of the IgG-positive cells did not directly control the susceptibility to FLV-induced leukemia. Regarding the immunologically functional cells of the host, Chesebro *et al.* have reported previously that the effector cells from anti-FLV spleens are T-cells that function *in vitro* without assistance or inhibition by B-cells or macrophages (25). We did not measure the titers of the anti-viral antibody, anti-tumor cell antibody, blocking antibody, and other humoral factors in the serum, nor demonstrate functional changes of each type of cells, including their interactions with other effector cells. Senn and Papoian (41) have suggested that antisera directed against FLV act upon activated T-cells by enhancing the endogenous production and/or release of interleukin 2. Although the interaction between T-cells and other cells may be important for the resistance to FLV-induced leukemia, kinetics of T-cells seems to be the major factor controlling the resistance in the natural course of the disease.

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