H-2D (Rfv-1) GENE INFLUENCE ON RECOVERY FROM FRIEND VIRUS LEUKEMIA IS MEDIATED BY NONLEUKEMIC CELLS OF THE SPLEEN AND BONE MARROW

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Friend leukemia virus complex (FV)[1] induces a rapidly progressive erythroleukemia in young or adult mice. FV-induced leukemic splenomegaly occurs 7–14 d after virus inoculation and persists until death (30–120 d) in susceptible mice. In certain mouse strains, recovery from leukemia occurs 14–28 d after virus inoculation and confers long-lasting protection against repeated FV challenge or relapse of leukemia. Several individual genes responsible for resistance/susceptibility to FV leukemia have been described (1). The mouse major histocompatibility complex (H-2) strongly influences recovery from established leukemia (2, 3). At high virus doses (1,500 focus-forming units [FFU]), H-$2^{b/b}$ mice have a high incidence of recovery, whereas H-$2^{a/b}$ and H-$2^{a/a}$ mice have a lower incidence of recovery (4). However, when a lower virus dose (15 FFU) is given, H-$2^{b/b}$ and H-$2^{a/b}$ have a high incidence of recovery as compared to H-$2^{a/a}$ mice (4). The differences in recovery seen among these H-2 genotypes are due to a gene (Rfv-1) located in the H-2D subregion (3). Viral replication and neoplastic transformation, as measured by spleen focus formation, are quite similar in H-$2^{b/b}$ and H-$2^{a/b}$ mice (3). The H-2 influence on recovery from FV-induced leukemia seems to be associated with later events of FV infection such as the rejection of FV transformed cells. The actual mechanism of rejection is unclear; however, both anti-FV antibodies and anti-FV cytotoxic T lymphocytes (CTL) are probably required (5, 6).

Our studies were carried out to identify the organs and cell type(s) responsible for the H-2D (Rfv-1) influence on recovery from FV leukemia. The results indicated that recovery was not dependent on the H-2 genotype of the leukemia cells, but instead seemed to be dependent on effector mechanisms developed by nonleukemic cells of the spleen and bone marrow from high recovery H-$2^{b/b}$ mice.

Materials and Methods

Animals. C57BL/10Sn (H-$2^{b/b}$), B10.A (H-$2^{a/a}$), A.BY (H-$2^{b/b}$), and A/WySN (H-$2^{a/a}$) mice were purchased from The Jackson Laboratory, Bar Harbor, Maine. F_1 hybrids were bred at the Rocky Mountain Laboratory, Hamilton, Mont. Mice used in all experiments were between 2 and 4 mo of age. Mice within experimental groups were sex and age matched.

[1] *Abbreviations used in this paper:* CTL, cytotoxic T lymphocyte(s); FFU, focus-forming units; FV Friend leukemia virus complex; FV-B B-tropic strain of Friend virus; Hh, hemopoietic histocompatibility; NK, natural killer.

Virus. The B-tropic strain of Friend virus (FV-B) was obtained from Dr. Frank Lilly, Department of Genetics, Albert Einstein School of Medicine, Bronx, N. Y. The virus stocks were prepared in (C57BL/10Sn × A.BY)F_1 mice and assayed as previously described (4).

Cell Culture. Origin of and method of in vitro growth of Y57 (H-$2^{b/b}$), YA97 (H-$2^{a/b}$) and AA41 (H-$2^{a/a}$) FV-induced erythroleukemia cell lines were previously described (7, 8). For tumor challenge experiments, clone 101 of Y57 was used. Clone 101 was obtained by limiting-dilution cloning in vitro and produced both the defective and helper components of FV. Furthermore, it grew rapidly in vivo after intravenous inoculation.

Recovery Experiments. Mice were injected with FV and followed for recovery from palpable splenomegaly as previously described (3).

Irradiation Chimeras. Recipient mice were given 900 rad of whole body x-irradiation from a Maximar type III 250 kV deep therapy x-ray unit (General Electric Co., Medical Systems Div., Milwaukee, Wisc.) delivering 32 rad/min at 52 cm from the mid-plane of the mouse. Approximately 4–6 h postirradiation, the mice were reconstituted with 10×10^6–15×10^6 spleen and 5×10^6 bone marrow cells. These chimeric mice were then rested for 60–90 d before use in recovery experiments.

Neonatal Tolerant Donor Mice. Neonatal (<24 h of age) (C57BL/10Sn × A.BY)F_1 or (B10.A × A/WySn)F_1 mice were inoculated intravenously via the retroorbital vein with 10×10^6–15×10^6 spleen and 5×10^6 bone marrow cells from a (B10.A × A/WySn)F_1 donor. 40–60 d after tolerance induction, the mice were tested for tolerance by skin grafting with (B10.A × A.BY)F_1 tail skin grafts. Mice with viable, intact skin grafts 35 d after grafting were considered tolerant.

Fetal Liver Chimeras. Fetal liver cells were obtained from 15-day-old (C57BL/10Sn × A.BY)F_1 fetuses. Recipient mice received 900 rad and were then reconstituted by intravenous inoculation of 20×10^6 viable fetal liver cells. These chimeras were then rested for 120 d before use as experimental mice.

Anti-Thy-1 Complement + (C′) Treatment. A rat anti-mouse Thy-1 hybridoma cell line, clone 31-11 (9), was kindly provided by Dr. Irving Weissman, Stanford University School of Medicine, Palo Alto, Calif. For elimination of T lymphocytes, 10^9 spleen and bone marrow cells were suspended in 15 ml of diluent (phosphate-buffered balanced salt solution with 2% heat inactivated fetal calf serum) mixed with 7 ml of anti-Thy-1 hybridoma tissue culture supernate and incubated at 37°C for 30 min. Cells were washed twice with diluent, resuspended in 40 ml of diluent, and mixed with 3 ml of absorbed rabbit serum as C′ source (5). After a 45-min incubation at 37°C, the cells were washed three times with diluent. This anti-Thy-1 + C′ treatment yielded 60% of the viable starting cell number, eliminated the concanavalin A-induced mitogenic response and anti-FV cytotoxic T lymphocyte activity in vitro, whereas it enriched for lipopolysaccharide-induced mitogenesis. Furthermore, pretreatment of donor (C57BL/10Sn × A.BY)F_1 (H-$2^{b/b}$) spleen cells with anti-Thy-1 monoclonal antibody plus C′ before transfer into (B10.A × A.BY)F_1 (H-$2^{a/b}$) mice prevented a detectable graft versus host reaction.

H-2 Typing. A two-stage microcytotoxicity assay using leukemia cell absorbed rabbit C′ and ^{51}Cr-labeled targets was used as previously described (5). Anti-H-2 sera were as follows: (A/WySn × B10.A)F_1 anti-C57BL/10Sn (H-2^a anti-H-2^b) and National Institutes of Health contract sera D4 (B10.AKM × 129)F_1 anti-B10.A ($(K^kD^a \times K^{bc}D^{bc})$ anti-K^kD^d).

Statistical Methods. Analysis of statistical significance of data was done using the χ^2 test for a 2 × 2 contingency table.

Results

H-2-associated Recovery from Leukemia Is Transferred by Spleen and Bone Marrow Cells. Lethally irradiated H-$2^{b/b}$ mice were reconstituted with spleen and bone marrow cells of the H-$2^{a/b}$ or H-$2^{b/b}$ genotype. 8 wk later these mice were inoculated with FV and followed for recovery from leukemia. Recipients which received H-$2^{a/b}$ cells had a low incidence of recovery, whereas mice reconstituted with H-$2^{b/b}$ cells had a high incidence of recovery (Table I). Thus, the H-2 genotype of the donor spleen and bone marrow cells influenced the incidence of recovery, in that donor cells

TABLE I

Recovery From FV Leukemia after Transfer of Spleen and Bone Marrow Cells into Irradiated Recipients

Donor genotype*	Irradiated recipient genotype	Virus dose‡	Recovered/total§
H-$2^{b/b}$	H-$2^{b/b}$	280	27/38 (71%)
H-$2^{a/b}$	H-$2^{b/b}$	280	2/16 (13%)‖
	Unirradiated control¶ H-$2^{b/b}$	280	15/20 (75%)
	Unirradiated control H-$2^{a/b}$	280	2/10 (20%)
H-$2^{b/b}$	H-$2^{b/b}$	180	14/14 (100%)
H-$2^{a/b}$	H-$2^{b/b}$	180	4/23 (17%)‖
	Unirradiated control H-$2^{b/b}$	180	9/10 (90%)
	Unirradiated control H-$2^{a/b}$	180	2/10 (20%)
H-$2^{a/a}$	H-$2^{a/a}$	25	1/13 (8%)
H-$2^{a/b}$	H-$2^{a/a}$	25	14/20 (70%)‖
	Unirradiated control H-$2^{a/a}$	25	3/10 (30%)
	Unirradiated control H-$2^{a/b}$	25	9/9 (100%)

* Strains of mice used as follows: H-$2^{b/b}$ = (C57BL/10Sn × A.BY)F_1; H-$2^{a/b}$ = (B10.A × A.BY)F_1; H-$2^{a/a}$ = (B10.A × A/WySn)F_1.

‡ FFU of FV-B given intravenously.

§ Number of mice recovering from FV splenomegaly over total number of mice.

‖ $P < 0.001$ for incidence of recovery of chimeras reconstituted with syngeneic spleen and bone marrow cell as compared with chimeras reconstituted with semi-allogeneic spleen and bone marrow cells.

¶ Unirradiated controls were sex- and age-matched mice given virus only.

from the higher recovery phenotype (H-$2^{b/b}$) produced a higher incidence of recovery after adoptive transfer. Because the recipients with the lower recovery incidence had received heterozygous H-$2^{a/b}$ donor cells, it was possible that the low recovery seen could have been a result of incompatibility between H-$2^{a/b}$ donor and H-$2^{b/b}$ recipient. To examine this possibility, donor cells of H-$2^{a/b}$ and H-$2^{a/a}$ genotypes were compared for their influence on recovery after inoculation into lethally irradiated H-$2^{a/a}$ recipients. A lower FV dose (25 FFU) was used to distinguish the higher recovery incidence of H-$2^{a/b}$ mice from that of H-$2^{a/a}$ mice (4). Here, as in the previous experiments, the donor cells from the higher recovery phenotype (in this case H-$2^{a/b}$) produced a higher incidence of recovery after transfer (Table I). Thus, the incompatibility between H-$2^{a/b}$ donor and H-$2^{a/a}$ recipient did not influence the recovery from leukemia.

Some chimeras were also made using fetal liver as donor tissue. H-$2^{b/b}$ fetal liver was transferred to irradiated H-$2^{a/b}$ or H-$2^{b/b}$ recipients. A high incidence of recovery from FV leukemia was seen in both groups (Table II). Because the high recovery phenotype was transferable with H-$2^{b/b}$ fetal liver cells, it would appear that the environment in which these cells matured in vivo did not alter their influence on recovery from leukemia.

In all adoptive transfers, the incidence of recovery from FV-induced splenomegaly was dependent on the genotype of the donor spleen, bone marrow, or fetal liver cells. Because these tissues contained both the primitive erythroid cells susceptible to transformation by FV and cells involved in immunological effector mechanisms of potential importance for recovery from FV leukemia, subsequent experiments were designed to distinguish which of these cell populations accounted for the H-2-associated effect on recovery from FV-induced leukemia.

TABLE II

Recovery From FV Leukemia after Transfer of H-2$^{b/b}$ Fetal Liver Cells into Irradiated Recipients

Fetal liver donor genotype	Irradiated recipient genotype*	Recovered/total‡
H-2$^{b/b}$	H-2$^{a/b}$	25/25 (100%)
H-2$^{b/b}$	H-2$^{b/b}$	21/21 (100%)
	Unirradiated H-2$^{a/b}$ control§	0/8 (0%)
	Unirradiated H-2$^{b/b}$ control§	10/10 (100%)

* Strains of mice used as follows: H-2$^{b/b}$ = (C57BL/10Sn × A.BY)F$_1$ and H-2$^{a/b}$ = (B10.A × A.BY)F$_1$. Mice were irradiated and reconstituted with 20 × 10^6 viable (C57BL/10Sn × A.BY)F$_1$ fetal liver cells as described in Materials and Methods.

‡ Number of mice recovering from FV splenomegaly over total number of experimental mice. All mice were given 800 FFU FV-B intravenously.

§ Unirradiated controls were sex- and age-matched littermates that were given virus only.

TABLE III

Resistance of (B10.A × A.BY)F$_1$ (H-2$^{a/b}$) Mice to Challenge with Congeneic FV-induced Tumors

Tumor*	Genotype	Recipient‡	Recovered/total§
Y57 (clone 101)	H-2$^{b/b}$	Normal	5/32 (16%)
Y57 (clone 101)	H-2$^{b/b}$	FV inoculated	9/9 (100%)‖
YA-97	H-2$^{a/b}$	Normal	2/23 (9%)
YA-97	H-2$^{a/b}$	FV inoculated	8/8 (100%)‖
AA41	H-2$^{a/a}$	Normal	7/34 (20%)
AA41	H-2$^{a/a}$	FV inoculated	14/14 (100%)‖

* Tumors established from FV leukemic spleens from (C57BL/10Sn × A.BY)F$_1$ (H-2$^{b/b}$), (B10.A × A.BY)F$_1$ (H-2$^{a/b}$), and B10.A × A/WySn (H-2$^{a/a}$). Median survival time for tumors as follows: Y57 (clone 101) 16.1 ± 3.9 d, YA-97 14.5 ± .68 d, and AA41 15.8 ± 1.0 d as determined in (B10.A × A.BY)F$_1$ H-2$^{a/b}$ mice given 10^6 viable cells intravenously.

‡ Recipient mice (B10.A × A.BY)F$_1$ H-2$^{a/b}$ received 10^5 or 10^6 Y57 (H-2$^{b/b}$) tumor cells; 10^5 or 10^6 YA-97 (H-2$^{a/b}$) tumor cells; and 10^5, 10^6 or 10^7 AA41 (H-2$^{a/a}$) tumor cells intravenously. Data were pooled for all doses of tumor cells because no significant differences among doses were noted. Recipient FV-inoculated mice were given 280 FFU of FV-B intraperitoneally 10–14 d before inoculation with FV-induced tumors. Normal recipients were age- and sex-matched littermates of FV-inoculated recipients.

§ Recovered mice over total number of mice inoculated.

‖ $P < 0.001$ for incidence of recovery in FV-inoculated mice as compared with normal mice.

Resistance of (B10.A × A.BY)F$_1$ (H-2$^{a/b}$) Mice to Challenge with Congeneic FV-induced Tumors. If the H-2 genotype of the FV leukemia cell determined the incidence of recovery, then leukemia cells of the high-recovery H-2$^{b/b}$ genotype could be more easily rejected than those of the low-recovery H-2$^{a/a}$ genotype. To determine if the H-2 genotype of FV leukemia cells influenced their sensitivity to in vivo rejection mechanisms, heterozygous (B10.A × A.BY)F$_1$ (H-2$^{a/b}$) mice were challenged with cells from three FV erythroleukemia lines derived from congeneic mice differing only for their H-2 genotype. All three tumors were lethal for 80–90% of H-2$^{a/b}$ mice and produced similar mean survival times (Table III). H-2$^{a/b}$mice were inoculated intraperitoneally with a dose of FV previously shown to result in recovery from FV-induced splenomegaly (4). These mice were then challenged with a lethal number of FV-induced leukemia cells 10–14 d after FV inoculation, a time at which these mice

TABLE IV

Recovery From FV Leukemia of Unirradiated (B10.A × A.BY)F_1 Given Neonatally Tolerant Spleen and Bone Marrow Cells

Donor*	Recipient‡	Recovered/total§	*P* value
H-$2^{b/b}$	H-$2^{a/b}$	25/28 (89%)	<0.001
Control‖	H-$2^{a/b}$	3/17 (18%)	
H-$2^{a/a}$	H-$2^{a/b}$	2/14 (14%)	>0.1
Control	H-$2^{a/b}$	2/15 (13%)	

* Strain of mice used as follows: H-$2^{b/b}$ = (C57BL/10 × A.BY)F_1; H-$2^{a/b}$ = (B10.A × A.BY)F_1; and H-$2^{a/a}$ = (B10.A × A/WySn)F_1. Donor mice were made neonatally tolerant as described in Materials and Methods.

‡ Recipient (B10.A × A.BY)F_1 H-$2^{a/b}$ mice were given 280 FFU intravenously; 4–6 h later, the mice received 35 × 10^6–40 × 10^6 viable spleen and bone marrow cells intravenously from tolerant donors.

§ Number of mice recovering from FV splenomegaly over total number of mice. H-2 typing by cytotoxic antibody plus C′ of 10 representative recipient mice revealed >90% of cells in recipient mice were of the H-$2^{a/b}$ genotype.

‖ Sex- and age-matched control mice received only virus.

TABLE V

Presensitization of H-$2^{a/b}$ Mice with H-$2^{b/b}$ FV Leukemia Cells Does Not Increase Incidence of Recovery

Group	Donor of FV leukemia cells*	Recipient‡	Recovered/total§	*P* value‖
I	H-$2^{b/b}$	H-$2^{a/b}$	2/14 (14%)	>0.1
II	H-$2^{a/b}$	H-$2^{a/b}$	1/13 (8%)	>0.1
III	No cells transferred¶	H-$2^{a/b}$	2/10 (20%)	—
IV	H-$2^{b/b}$	H-$2^{b/b}$	12/14 (86%)	<0.01

* Donor mice (C57BL/10Sn × A.BY)F_1 H-$2^{b/b}$ or (B10.A × A.BY)F_1 H-$2^{a/b}$ were given 1,000 FFU FV-B intravenously. 8 d after FV inoculation spleens were removed and single cell suspensions were treated with anti-Thy-1 plus C′ as described in Materials and Methods.

‡ (B10.A × A.BY)F_1 recipient mice were given 40 × 10^6 viable Thy-1-depleted FV leukemic spleen cells from appropriate donors. 4 h later all recipients received 500 FFU of FV-B intravenously.

§ Number of mice recovering from FV splenomegaly over total number of mice.

‖ *P* value as compared to control group III.

¶ Control group of sex- and age-matched (B10.A × A.BY)F_1 H-$2^{a/b}$ mice received only virus.

were presumably rejecting FV-transformed autologous cells. FV-infected H-$2^{a/b}$ mice were capable of rejecting all three congeneic leukemia cell types (Table III). The rejection of FV-induced leukemia cells of the H-$2^{a/a}$, H-$2^{a/b}$, and H-$2^{b/b}$ genotype by FV-infected H-$2^{a/b}$ mice indicated that effector mechanisms responsible for rejection of FV leukemia cells by H-$2^{a/b}$ mice were operative against all three H-2 genotypes of FV leukemia cells. The H-2 genotype of the FV leukemia cell did not influence its susceptibility to in vivo mechanisms mediating the rejection of FV leukemia cells.

Transfer of Resistance to FV-induced Splenomegaly by Neonatally Tolerant H-$2^{b/b}$ Spleen Cells. Because the H-2 type of the erythroleukemia cell lines used in the previous experiments did not influence their rejection, we considered the possibility that nonleukemic spleen and bone marrow cells of differing H-2 types could vary in their influence on recovery from FV leukemia. To study whether potential anti-FV leukemia effector mechanisms in H-$2^{b/b}$ mice were superior to those in H-$2^{a/b}$ or H-$2^{a/a}$ mice and whether these mechanisms were effective against H-$2^{a/b}$ leukemia cells,

cell transfer experiments were performed using unirradiated H-$2^{a/b}$ recipients. After inoculation with FV, unirradiated H-$2^{a/b}$ recipient mice received spleen and bone marrow cells from H-$2^{b/b}$ and H-$2^{a/a}$ mice made neonatally tolerant to H-$2^{a/b}$ antigens to avoid a lethal graft vs. host reaction.

When tolerant H-$2^{b/b}$ (high recovery) spleen and bone marrow cells were injected into H-$2^{a/b}$ mice, these cells were capable of causing increased recovery from FV-induced splenomegaly (Table IV). In a reciprocal experiment, cells from tolerant H-$2^{a/a}$ (low recovery) mice did not mediate recovery from FV-induced splenomegaly in H-$2^{a/b}$ recipients. Because the majority of leukemia cells in the unirradiated recipients were of the H-$2^{a/b}$ genotype (Table IV), these experiments demonstrated that H-$2^{a/b}$ FV leukemia cells were readily rejected in vivo after transfer of spleen and bone marrow cells from H-$2^{b/b}$ mice but not after transfer of spleen and bone marrow cells from H-$2^{a/a}$ mice. These results offered strong evidence in support of the interpretation that H-2-associated recovery effect seen in FV leukemia operated via cells involved in the immune response to the leukemia rather than at the level of the leukemia cells themselves.

Presensitization of H-$2^{a/b}$ Mice with H-$2^{b/b}$ Leukemic Spleen Cells. An alternative explanation for the above results is possible. If erythroleukemia cells of the H-$2^{b/b}$ genotype were more immunogenic than those of the H-$2^{a/b}$ or H-$2^{a/a}$ genotypes, then perhaps FV-transformed cells derived from the donor H-$2^{b/b}$ population could have induced a stronger or more rapid specific immune response to FV leukemia cells of both donor and recipient origin leading to an increased incidence of recovery. To test this possibility, we inoculated H-$2^{a/b}$ mice with H-$2^{b/b}$ or H-$2^{a/b}$ FV leukemic spleen cells and cell-free FV, and the course of leukemia was followed. Because all recipients received a defined dose of cell-free virus, it was possible to ascribe any differences in the incidence of recovery to differences in the H-2 genotype of the sensitizing cell and not to any variation in the amount of cell-associated virus transferred. Donor cell populations were pretreated with an anti-Thy-1 reagent and C′ to eliminate T lymphocytes, which could contribute either to a graft vs. host reaction or to an anti-FV immune response. The results indicated that transfer of 40×10^6 H-$2^{b/b}$ leukemic spleen cells did not result in an increased incidence of recovery from leukemia in H-$2^{a/b}$ mice (Table V). Thus, H-$2^{b/b}$ leukemia cells did not appear to exhibit enhanced immunogenicity leading to recovery from leukemia in this situation. These results also supported our original interpretation that nonleukemic H-$2^{b/b}$ spleen and bone marrow cells were responsible for the increased recovery seen in Table IV.

Discussion

In this paper, the H-2 genotype of nonleukemic effector cells of the spleen and bone marrow appeared to account for the differences in recovery from FV leukemia previously mapped to the H-2D subregion (3, 4). In irradiation chimeras, the H-2 genotype of the donor spleen, bone marrow, and fetal liver cells influenced the incidence of recovery from FV leukemia, whereas the H-2 genotype of the irradiated recipient did not. Heterozygous H-$2^{a/b}$ mice recovering from primary FV leukemia generated effector mechanisms that rejected FV leukemia cells of three different H-2 genotypes with equal efficiency. Furthermore, immunocompetent high-recovery H-$2^{b/b}$ spleen and bone marrow cells led to enhanced recovery from leukemia in H-$2^{a/b}$ mice. This result suggested that H-$2^{a/b}$ leukemia cells were eliminated by effector cells

derived from the transferred H-$2^{b/b}$ donor cells. This observation was somewhat surprising in that the number of transferred H-$2^{b/b}$ cells was small compared to the number of spleen cells in the unirradiated H-$2^{a/b}$ recipient. Presumably, the passive transfer of these cells into unirradiated recipients was effective because of the strong antigenic stimulus provided by the presence of FV-infected cells in these recipients.

Recovery did not appear to be a result of allogeneic H-2-specific graft vs. host reaction because H-$2^{a/b}$ mice that had received neonatally tolerant H-$2^{b/b}$ cells and had recovered from FV leukemia were normal in appearance and exhibited no evidence of graft vs. host disease at >120 d post-cell transfer. The reciprocal cell transfer in which H-$2^{a/a}$ spleen and bone marrow cells tolerant to H-$2^{a/b}$ were given to H-$2^{a/b}$ mice did not increase the incidence of recovery in recipient mice. Because tolerance to H-$2^{a/b}$ was determined by the same criteria in both H-$2^{b/b}$ and H-$2^{a/a}$ donor mice, it was unlikely that an allogeneic anti-H-2 graft vs. leukemia reaction occurred in one chimera but not in the other. Because our results indicated that H-$2^{b/b}$ cells tolerant to H-2^{a} could mediate recovery from FV leukemia, cross-reactivity between H-2^{a} alloantigens and FV-induced antigens could not explain the low recovery incidence previously noted in H-$2^{a/a}$ and H-$2^{a/b}$ mice (4).

Although immunosuppression has been a prominent finding during FV leukemia in many mouse strains (10, 11), we have observed strong humoral immune responses to FV antigens in the F_1 congenic mice used in the present study (12). Humoral immunity in this model appeared to be influenced by a single non-H-2 gene, Rfv-3 (12). The results of the present experiments suggest that immunosuppression of humoral or cellular immune mechanisms was not responsible for the low recovery incidence seen in H-$2^{a/b}$ mice because H-$2^{b/b}$ effector cells were able to develop and function adequately in the H-$2^{a/b}$ splenic environment even after high FV inoculation doses.

The mechanism through which H-2 influences recovery from FV leukemia is unknown. T lymphocytes are required because neonatally thymectomized mice of the high-recovery H-$2^{b/b}$ genotype have a low incidence of recovery (W. J. Britt and B. Chesebro. Manuscript in preparation.). The most consistent finding associated with recovery from FV leukemia is the appearance of anti-FV CTL in recovered mice (6). However, because anti-FV CTL can be demonstrated in both low- and high-recovery strains, a genetically determined inability to produce anti-FV CTL does not explain the difference in the incidence of recovery seen in congeneic mice of differing H-2D (Rfv-1) genotypes (6). A more likely explanation would be a difference in the rate of production of anti-FV CTL in mice of different H-2 genotypes. Mice of the high recovery H-$2^{b/b}$ genotype might generate anti-FV CTL earlier in the course of the disease as compared to low recovery H-$2^{a/b}$ or H-$2^{a/a}$ mice. Thus, a high incidence of recovery from FV leukemia might be the result of an early CTL response at a time when the leukemia cell burden is low and the elimination of leukemia cells can be accomplished. This mechanism would account for the increased incidence of recovery seen in H-$2^{a/b}$ mice given a low dose of FV (4). A lower dose of virus results in a later attainment of maximum splenomegaly and might allow generation of anti-FV CTL in H-$2^{a/b}$ mice at a time when the leukemia cell number is lower.

The H-2 complex has been shown to exert control over the generation of CTL in the H-Y and trinitrophenyl systems (13, 14). Zinkernagel et al. (15) have reported the influence of the H-2D subregion on the in vivo generation of vaccinia and Sendai

virus-specific CTL. In contrast to previously reported immune response (Ir) gene phenomena (16), these virus-specific H-2D region CTL-Ir genes were inherited as recessive traits (15). Because the H-2D (Rfv-1)-associated recovery from FV leukemia was inherited in a recessive or gene-dose fashion (4), it appeared possible that this gene could operate like a CTL-Ir gene in controlling the production of anti-FV CTL. Previously it has been postulated that H-2-linked CTL-Ir genes may operate through the influence of the thymic epithelium on CTL precursors during ontogeny (17). Thus, the thymic epithelium may influence the CTL response by control of H-2 restriction of CTL. Our findings that fetal liver of the H-$2^{b/b}$ genotype produced a high incidence of recovery even after maturation in an irradiated H-$2^{a/b}$ host (Table I) did not support the interpretation that the thymic epithelium had a major influence on the in vivo response to FV leukemia. This observation was, however, consistent with previous data using fetal liver irradiation chimeras in which the Ir gene control of the response to poly(L-tyrosine,L-glutamic acid)-DL-alanine--L-lysine was determined at the level of differentiation of fetal liver and was not influenced by the thymic epithelium present during differentiation (18).

Our results are in contrast with reports which have suggested the H-2 genotype of the FV leukemia cell was important in determining the incidence of recovery from FV (19). One group of investigators noted that FV selectively associated with the H-$2D^b$ gene product (20, 21) and postulated that this association might produce a better target for anti-FV CTL (19). Anti-FV CTL might then more readily eliminate FV leukemia cells of the H-$2D^b$ genotype and lead to an increased incidence of recovery (19). Although H-$2D^b$ expression by leukemia cells may be necessary for maximum CTL activity (22) and maximum recovery, the difference in recovery incidence between H-$2^{b/b}$ and H-$2^{a/b}$ mice, both of which possess an H-$2D^b$ allele suggests that H-$2D^b$ expression by leukemia cells is not sufficient for recovery (3). The present cell-transfer experiments supported this interpretation and appeared to indicate that the association of FV with the H-$2D^b$ gene product on leukemia cells did not explain the influence of the H-2D region on recovery from leukemia.

Natural killer (NK) cells or hemopoietic histocompatibility (Hh) resistance mechanisms may also play a role in recovery from FV leukemia. These mechanisms have been shown to recognize FV leukemia cells, and both appear to be influenced by genes mapping to the H-2D region (23, 24). However, our preliminary data suggest that NK activity does not differ between the high and low recovery F_1 congenic mice (H-$2^{b/b}$, H-$2^{a/b}$, and H-$2^{a/a}$) used in this system (W. J. Britt and B. Chesebro. Manuscript in preparation.). Furthermore, both Hh- and NK-mediated resistance to bone marrow and tumors are only effective against low numbers of cells in the challenge inoculum (25), whereas recovery from the massive splenomegaly of FV leukemia requires elimination of a large number of leukemia cells. Although it appears that anti-FV antibody and natural resistance mechanisms such as NK cells do not account for the influence of H-2 or recovery from FV leukemia, these and other resistance mechanisms could still contribute to the incidence of recovery. In the present system, cytotoxic anti-FV antibody seems to be necessary but not sufficient for recovery (5, 12). Antibody to FV could augment anti-FV CTL activity either by direct cytolysis of FV leukemia cells or by decreasing cell-free FV antigens that could potentially block anti-FV CTL. Thus, the complex process of recovery from FV leukemia probably requires anti-FV antibody and anti-FV CTL operating simulta-

neously. The importance of additional mechanisms of resistance, such as NK cells, to the overall process of recovery is currently being studied.

Summary

H-2D (Rfv-1)-associated control of recovery from FV leukemia was studied in congenic mice. In irradiation chimeras, the high recovery phenotype was transferred by cells of the spleen, bone marrow, and fetal liver. Furthermore, in cell transfers using unirradiated recipients, spleen and bone marrow cells of the high-recovery genotype were able to mediate recovery from leukemia in mice of the low-recovery genotype. Thus, the H-2D (Rfv-1) influence on recovery appeared to operate via nonleukemic cells of the spleen and bone marrow rather than via leukemic cells. The specific nonleukemic cell type(s) involved in recovery remains unknown. However, the mechanism appears to be complex and probably involves both anti-FV antibody and FV-specific cytotoxic T lymphocytes.

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