MECHANISMS OF GENETIC RESISTANCE TO FRIEND VIRUS LEUKEMIA IN MICE

IV. Identification of a Gene (*Fv-3*) Regulating Immunosuppression In Vitro, and its Distinction from *Fv-2* and Genes Regulating Marrow Allograft Reactivity*

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Infection of susceptible adult mice with Friend virus complex (FV),[1] consisting of spleen focus-forming virus (SFFV) and lymphatic leukemia virus (LLV), results in a rapidly progressing erythroleukemia (1) and a profound immunosuppression (2). Several genes are involved in the regulation of host responses to FV. Genes linked to *H-2*, the major histocompatibility complex of the species, appear to be involved in interactions between immunocompetent cells with virus-infected cells (3) and are involved in recovery from the active infection with FV (4). Physical association between FV and *H-2* molecules have been detected (5). The Friend virus-1 (*Fv-1*) gene controls resistance to LLV (6), the helper virus for SFFV. Resistance to SFFV, which induces erythroleukemia seen grossly as splenic foci, is controlled by the Friend virus-2 (*Fv-2*) gene. Susceptibility to spleen focus formation is dominant over resistance (6). Mice of a narrow ancestral group of strains (7), which include C57BL, C57L, and C58, are resistant to the erythropoietic effects of SFFV, i.e., are $Fv\text{-}2^{rr}$, and are also relatively resistant to the immunosuppressive effects of FV (8).

Marrow-dependent (M) cells appear to mediate the genetically determined resistance of C57BL/6 mice to both the erythropoietic and immunosuppressive effects of FV (9, 10). M cells are the effector cells responsible for the rejection of allogeneic or incompatible parental-strain hemopoietic cell grafts (11). The cells are so named because continuous irradiation of the marrow microenviron-

* Research supported by grants CA 15369 and CA 15848 from the National Institutes of Health.

‡ Recipient of a fellowship from The Medical Foundation, Inc., Boston, Mass.

§ Recipient of Research Career Development Award CA 70879 from the National Institutes of Health.

[1] *Abbreviations used in this paper:* B, bursa equivalent-dependent; Con A, Concanavalin A; FFU, focus forming units; FUdR, 5-fluoro-2-deoxyuridine; FV, Friend virus complex; Fv-1, Friend virus-1; Fv-2, Friend virus-2; Fv-3, Friend virus-3; Hh, Hemopoietic histocompatibility; Ir, immune response; IUdR, 5-iodo-2-deoxyuridine-^{125}I; LLV, Lymphatic leukemia virus; SFFV, spleen focus forming virus; T, thymus dependent; TCA, trichloroacetic acid.

ment with beta particles emitted by the bone-seeking isotope, ^{89}Sr, abrogates the ability of irradiated mice to reject marrow cell allografts without altering thymus-dependent (T) or bursa equivalent-dependent (B) cell functions (12). C57BL/6 mice treated with ^{89}Sr and later infected with FV develop erythroleukemia and are immunosuppressed (9). The genetic resistance or susceptibility to FV induced immunosuppression in vivo appears to be reflected in an in vitro model involving stimulation of lymphocytes with mitogens in the presence or absence of FV (10). For example, spleen cells from C57BL/6 mice which are resistant to FV in vitro are rendered susceptible by prior treatment of donors with ^{89}Sr to eliminate M cells. Further analysis of the model indicated that inhibition of lymphocyte mitogenesis produced by FV in vitro is mediated via T suppressor cells. M cells regulate the number and/or function of such T-suppressor cells in the body (13). In this paper, we have performed genetic experiments to determine if the same or different genes control resistance to immunosuppression by FV in vitro and spleen focus formation or marrow allograft reactivity in vivo. We have identified a gene, *Fv-3*, which segregates independently from *Fv-2* but which is also dominant for susceptibility. The ability to reject marrow cell allografts in one donor-host combination was also not controlled by this gene. In a forthcoming paper, a genetic analysis will be presented which indicates that *Fv-3* controls resistance to FV induced immunosuppression in vitro and in vivo.[2]

Materials and Methods

Mice. Inbred DBA/2, B10.D2, and C57BL/6 (B6) mice were purchased from The Jackson Laboratory, Bar Harbor, Maine. 129/Rr (129) mice were bred in our animal facility. Mice of the parental strain were mated to produce DBA/2 × B10.D2 and 129 × B6 F_1 hybrid mice (with both strains in crosses as males or females). F_2 progeny were produced by intercrossing the F_1 mice. (DBA/2 × B10.D2)F_2 mice were backcrossed to DBA/2 or to B10.D2 mice (with males and females of each members of the matings). Mice of both sexes were tested when 8- to 12-wks of age. Sex of the individual mice or of the parents producing the offspring did not affect the results obtained.

Virus. The Mirand strain of NB-tropic FV (14) was used. The virus is maintained by serial in vivo passages in BALB/c mice and virus stocks are prepared as 20% wt/vol cell-free extracts of leukemic spleens. The spleen focus assay is used to titrate the virus and the titer is expressed in focus-forming units (FFU), where 1 FFU results in the appearance of 1 spleen focus 8–10 days after intravenous injection of the virus into susceptible hosts (15). To type for *Fv-2*, mice were infected with 50 FFU of FV intravenously. The spleens were removed 9 days later and fixed in Bouin's fluid. The mice whose spleens were devoid of any foci were designated $Fv\text{-}2^{rr}$, whereas the mice whose spleens contained foci were either $Fv\text{-}2^{rs}$ or $Fv\text{-}2^{ss}$ and were termed "susceptible". Progeny testing of mice to distinguish between $Fv\text{-}2^{ss}$ and $Fv\text{-}2^{rs}$ mice among "susceptible" population was not performed.

Assay for Immunosuppression by FV. The procedure for preparing lymphoid cell suspensions, culturing cells with mitogens, and infecting the cultures with graded doses of FV have been described in detail (10). Spleen and thymus cells were harvested and cultured in RPMI 1640 medium (Grand Island Biological Co., Grand Island, N.Y.) containing 10% fetal calf serum. Spleen cells were washed once and were cultured in wells of Microtest II plates (Falcon Plastics, Division of BioQuest, Oxnard, Calif.). Each well contained 0.2-ml vol with 10^6 spleen cells. Triplicate samples contained (*a*) 0.5 μg Concanavalin A (Con A) (Calbiochem, San Diego,

[2] V. Kumar, P. Resnick, J. W. Eastcott, and M. Bennett. 1977. Mechanism of genetic resistance to Friend virus leukemia in mice. V. Relevance of Fv-3 gene in the regulation of in vivo immunosuppression. Manuscript submitted for publication.

Calif.), (*b*) FV, 100–500 FFU (*c*) Con A and FV, or (*d*) neither Con A nor FV. In each experiment, three doses of FV were tested.

Thymus cells were washed three times and cultured in 12 × 75 mm plastic test tubes (Falcon Plastics). Preliminary experiments indicated that thymus cells responded to Con A much better in tube cultures than in Microtest II plates. Each tube contained 3-ml vol and 6×10^6 thymus cells. Triplicate samples contained (*a*) Con A, 2 μg/ml, (*b*) FV, 300 FFU/ml, (*c*) Con A plus FV, or (*d*) neither Con A nor FV.

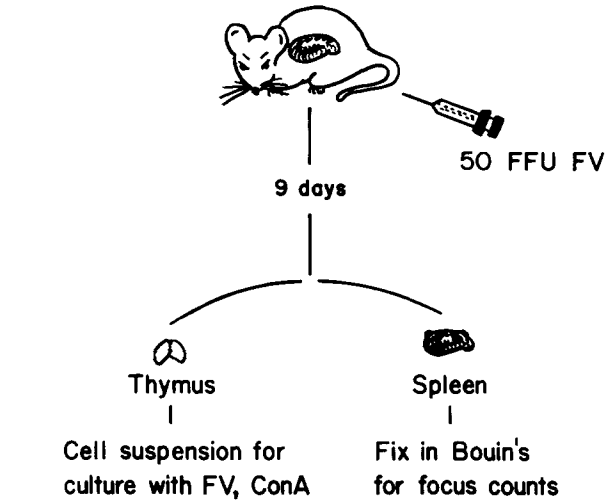
[^{3}H]thymidine (New England Nuclear, Boston, Mass.) was added to each well of Microtest II plates (0.5 μCi) or to each test tube (1 μCi) 48 h after initiation of the culture. The cultures were harvested 18 h later for liquid scintillation counting. A MASH II automatic harvester was used to collect trichloroacetic acid (TCA) precipitable material when Microtest II plates were used. The test tube cultures were harvested by washing the tubes three times with 5 ml of phosphate-buffered saline and three times with 5 ml of 5% cold TCA. After the final wash, a mixture of 0.5 ml methanol and 1.5 ml alkali (protosol, New England Nuclear) was added to each pellet. The tubes were incubated in a water bath at 56°C for 1 h to dissolve the pellet. The content of each tube was then processed for liquid scintillation counting.

The results are expressed as Δ blastogenesis, which represents mean [^{3}H]thymidine incorporation (counts per minute, cpm), in appropriate cultures with Con A minus cultures without Con A, or groups (*a*)–(*d*) and (*c*)–(*b*) described above. FV alone did not significantly affect incorporation values of [^{3}H]thymidine. Therefore, FV-induced changes in Δ blastogenesis reflected changes in response to Con A and not to proliferation of unstimulated cultures. The percentage (%) suppression of Δ blastogenesis by FV was calculated by the formula, % suppression = mean Δ blastogenesis Control-FV/Control × 100, where control is the uninfected culture.

Assay for Marrow Allograft Rejection. The procedure has been described in detail elsewhere (16). The recipients in these experiments were F_2 progeny of reciprocal (DBA/2 × B10.D2)F_1 intercross matings. These mice are *H-2*^{d}. The donors of the marrow cells were C57BL/6 mice (*H-2*^{b}). The ability to reject *H-2*^{b} marrow cells is under genetic control of two or more dominant genes which segregate independently from *H-2* (17). Recipient mice were exposed to 800 rads of total-body irradiation in a small animal irradiator with two ^{137}Cs sources (Gamma Cell 400, Atomic Energy of Canada, Ltd., Toronto). Each mouse was infused intravenously with 4×10^6 bone marrow cells in a vol of 0.5 ml within 2 h of irradiation. Proliferation of the donor-derived cells in the spleens of recipient mice was assessed by measuring the incorporation of 5-iodo-2′-deoxyuridine-^{125}I (IUdR), a specific DNA precursor and thymidine analogue. The mice were each injected with 10^{-7} M 5-fluoro-2′-deoxyuridine (FUdR) i.p. in a vol of 0.1 ml 1 h before the i.p. injection of 0.5 μCi IUdR. FUdR inhibits endogenous thymidylate synthesis and enhances IUdR uptake, under these conditions. Spleens were removed 2 h after isotope injection, were soaked in 70% ethanol for 3 days to remove ^{125}I not incorporated into DNA, and the ^{125}I radioactivity was measured in a crystal scintillation counter (Packard Instrument Co., Inc., Downers Grove, Ill.). DBA/2 and B10.D2 mice have been reported to reject 1×10^6 *H-2*^{b} bone marrow cells (18). However, in preliminary experiments with 2×10^6 and 4×10^6 B6 (*H-2*^{b}) bone marrow cells, we found that DBA/2 mice were unable to reject 4×10^6 B6 bone marrow cells while B10.D2 mice could reject them. With this inoculum size of bone marrow cells, we could clearly classify the DBA/2 mice as "poor responders" and B10.D2 mice as "good responders" to B6 bone marrow grafts. Therefore, 4×10^6 B6 marrow cells were used to type the (DBA/2 × B10.D2)F_2 mice.

Experimental Protocols. To type individual mice both for *Fv-2* and susceptibility of their lymphocytes to suppression by FV in vitro, two protocols were used (Fig. 1): (i) (B6 × 129)F_2 mice were infected with 50 FFU of N-B tropic FV intravenously; 9 days later mice were killed and the spleen and thymus from each mouse was removed. Spleens were fixed individually in Bouin's fluid to detect presence or absence of foci and thymus cells were cultured (after three washes with medium) with and without Con A and/or FV, to detect degree of immunosuppression by FV. In preliminary experiments we observed that washed thymus cells from mice infected 9 days earlier with FV responded normally to Con A and that there was no difference in their

FIG. 1. Experimental protocols for testing individual mice for susceptibility to FV-induced spleen focus formation in vivo and suppression of lymphocyte mitogenesis in vitro. A. (129 × B6)F_2 mice. B. (DBA/2 × B10.D2)F_2, (DBA/2 × B10.D2)F_1 × DBA/2, and (DBA/2 × B10.D2)F_1 × B10.D2 mice. Some of these mice were typed for susceptibility to suppression of lymphocyte mitogenesis and ability to reject C57BL/6 bone marrow cells.



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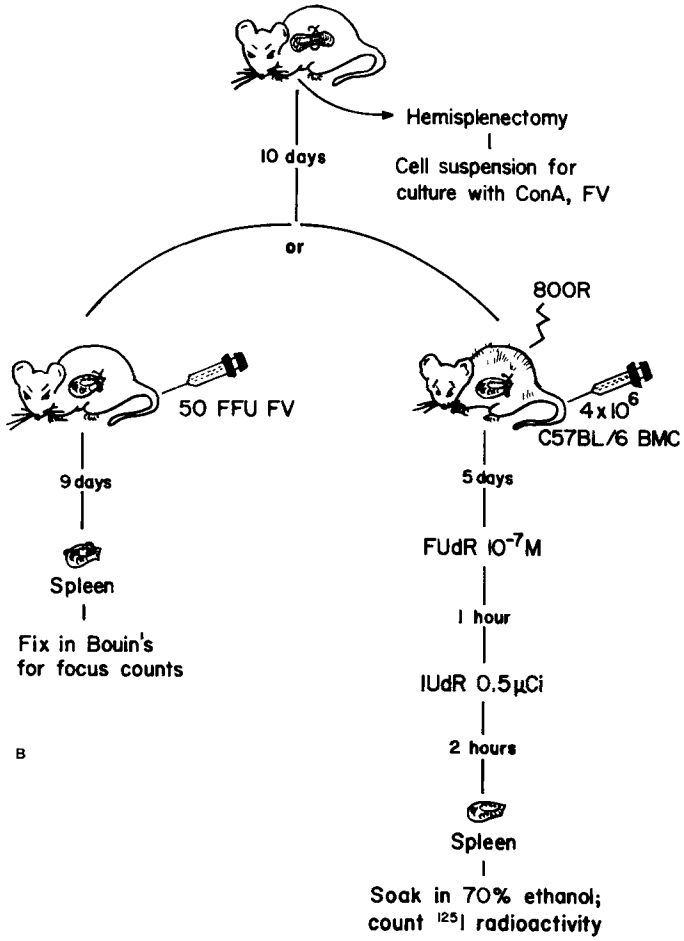

B

TABLE I

Suppression of Con A Induced Mitogenesis of Resistant and Susceptible Parental Strains and their F_1 Hybrids by FV In Vitro

Exp.	Mouse strain	Lymphoid cell* source	Δ Blastogenesis‡		Suppression
			No FV	+FV§	
			cpm ± SE		%
1.	B10.D2n	Spleen	23,456 ± 1970	20,571 ± 2152	13
	DBA/2	Spleen	41,673 ± 1123	19,662 ± 2012	53
	(DBA/2 × B10.D2)F_1	Spleen	30,259 ± 750	17,015 ± 1411	44
2.	C57BL/6	Thymus	17,566 ± 1719	18,460 ± 3125	−5
	129	Thymus	32,971 ± 2005	10,523 ± 975	69
	(129 × C57BL/6)F_1	Thymus	35,721 ± 4123	9,923 ± 575	73

* 1×10^6 spleen cells per well and 6×10^6 thymus cells per tube were cultured as described in Materials and Methods.

‡ Δ blastogenesis, (cpm with mitogen-cpm without mitogen). In groups with FV, Δ blastogenesis, (cpm + mitogen + FV − cpm + FV).

§ The final concentration of FV in spleen cell cultures was 300 FFU/well and 300 FFU/ml in thymus cell cultures.

susceptibility to FV in vitro as compared to thymus cells from uninfected mice (Fig. 1 A). (ii) Despite the fact that thymocytes from FV infected mice behaved normally in vitro (above), it was considered desirable to use lymphocytes from uninfected mice and then infect the mice for *Fv-2* typing. Progeny of (B10.D2 × DBA/2)F_1 × F_1, DBA/2, and B10.D2 matings were anesthetized with chloral hydrate and a hemisplenectomy was performed. A suture was tied in the middle of the spleen, with care taken not to occlude blood vessels in the hilus. There was minimal bleeding after the spleen proximal to the suture was severed. There was a 5% operative mortality rate. The one-half spleen removed was used to make a cell suspension for culture with Con A and/or FV. 10 days after surgery, each mouse was infected with 50 FFU of FV. 9 days after infection, the residual spleen was removed and fixed in Bouin's fluid for focus counting. Although there were some adhesions on the surface of the spleen, the foci were easily detectable (Fig. 1 B).

A protocol similar to (ii) was used to type mice for ability to reject marrow allografts (Fig. 1 B). 10 days after hemisplenectomy and spleen cell culture, individual mice were irradiated (800 rads) and infused with 4×10^6 C57BL/6 bone marrow cells. IUdR uptake (%) was measured 5 days after cell transfer. There was a 15% incidence of mortality after irradiation.

Results

Dominance for Susceptibility to Immunosuppression by FV In Vitro. Spleen cells or thymus cells from adult untreated mice were cultured in the presence or absence of FV and Con A. The donors were DBA/2, B10.D2, (DBA/2 × B10.D2)F_1, 129, B6, and (129 × B6)F_1 mice. Cells were pooled from two or three donors. T cells from mice genetically resistant to FV-induced leukemia, i.e., B10.D2 and B6, were relatively resistant to suppressive effects of FV (Table I and reference 10), whereas cells from susceptible DBA/2 and 129 mice were quite susceptible. Both (DBA/2 × B10.D2)F_1 and (129 × B6)F_1 spleen cells were also susceptible (Table I), indicating that the gene(s) for susceptibility were dominant over the gene(s) for resistance. In these, as in other experiments (10), the lack of importance of *H-2* type was apparent, since DBA/2 and B10.D2 mice are *H-2*^{d} and 129 and B6 mice are *H-2*^{b}.

A Single Gene Controls Resistance to Immunosuppression by FV In Vitro. Parental-strain 129 and B6 mice and 30 (129 × B6)F_2 mice were

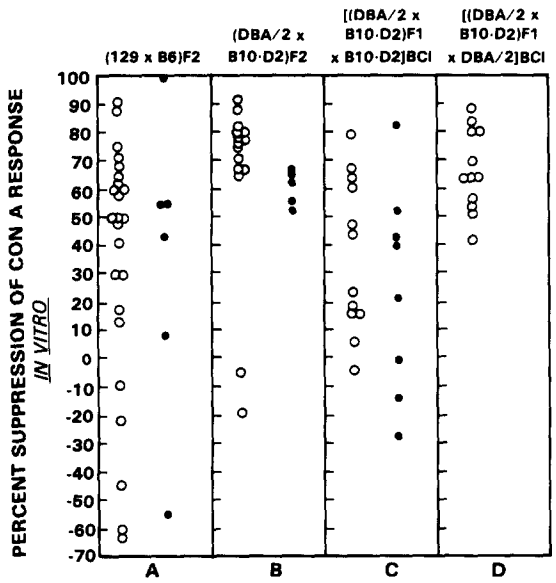

FIG. 2. Typing of individual intercross and backcross mice for susceptibility to spleen focus formation in vivo and suppression of Con A induced lymphocyte mitogenesis by FV in vitro. Mice positive for spleen foci (○); negative for foci (●). The mean percent suppression of Con A response (±SE), for the parental strains was: 129 = 46.6 ± 28.4, B6 = −26.6 ± 7.6, DBA/2 = 68.7 ± 21.7, B10.D2 = 2 ± 13.9. Details of experimental protocol and criteria for phenotypic classification in vitro given in the text. Result: no correlation seen between susceptibility to spleen focus formation and susceptibility to suppression of the Con A response.

individually tested for resistance to FV in vitro, with the thymus cell cultures. In this experiment, 129 thymus cells were suppressed 10–70% while B6 thymus cells were actually "stimulated" by the addition of FV to the cultures (Fig. 2). With these criteria 6/30 of the (129 × B6)F_2 progeny had thymocytes resistant to FV while 24/30 had susceptible thymocytes (Table II and Fig. 2A). A Chi-square analysis indicates that this finding is most consistent with the hypothesis that a single gene dominant for susceptibility controls the response to FV in vitro. A two-gene hypothesis was also tested but did not fit the data well.

A similar analysis was performed utilizing spleen cells from parental-strain DBA/2 and B10.D2 mice, as well as (DBA/2 × B10.D2)F_2, [(DBA/2 × B10.D2)F_1 × DBA/2] BC1 and [(DBA/2 × B10.D2)F_1 × B10.D2] BC1 mice. In these experiments the range of suppression of Con A response of the DBA/2 parent with 300 FFU of FV in vitro varied from 40 to 80% while that of the B10.D2 parent varied from −20 to 20%. These criteria were therefore used to judge the phenotypes of individual intercross mice. Spleen cells of 54 (DBA/2 × B10.D2)F_2 mice were tested: 37/54 were susceptible, 14/54 were resistant and 3/54 had indeterminant values (20–40% suppression [Table II]). A Chi-square analysis again supported the one-gene hypothesis and not a two-gene hypothesis (Table II). Some of these mice were also tested for *Fv-2* and the values are given in Fig. 2B.

20 individual [(DBA/2 × B10.D2)F_1 × B10.D2] BC1 mice were typed for susceptibility to FV in vitro. With the above mentioned criteria for judging the

TABLE II

Observed and Expected Numbers of Mice Resistant or Susceptible to FV In Vitro, if a Single Dominant Gene were Responsible for Susceptibility

Mating type	Number typed	Resistant		Susceptible		χ^2	P*
		Expected	Observed	Expected	Observed		
1. (129 × B6)F_2	30	7.5	6	22.5	24	0.4361	>0.1
2. (DBA/2 × B10.D2)F_2‡	54	13.5	14	40.5	37	0.1633	>0.1
3. [(DBA/2 × B10.D2) × B10.D2]‡	20	10	8	10	9	0.4285	>0.1
4. [(DBA/2 × B10.D2) × DBA/2]	12	0	0	12	12	0	

* Data from all the crosses were significantly different ($P < 0.005$) from the expected numbers if two genes were controlling susceptibility. [χ^2 values = 9.680, 39.1229, 11.5714 for mating type 1, 2, and 3, respectively.)

‡ Three mice in each group were indeterminate (see text for criteria of phenotypic classification).

phenotype of the backcross mice, it was found that three mice fell within the range of 20–40% (22, 22, and 34%) suppression of Con A response, i.e., in between the ranges of the parental types, and were therefore considered "indeterminate" for the purpose of Chi-square analysis. Of the remaining 17, 8 could be clearly classified as resistant to suppression in vitro (suppression <20%) and 9 were classified as susceptible (>40% suppression). A Chi-square analysis supports the one gene hypothesis suggested by analysis of the previous two crosses (Table II, Fig. 2 C).

If a single dominant gene were controlling susceptibility to suppression in vitro, it would be expected that 100% of the [(DBA/2 × B10.D2)F_1 × DBA/2] BC1 mice would be susceptible to FV-induced suppression in vitro. Analysis of 12 DBA/2 backcross mice showed that each mouse was susceptible to suppression (>40%) in vitro (Fig. 2 D, Table II).

Independent Segregation of the Gene Controlling Susceptibility to Immunosuppression by FV In Vitro and Fv-2. The data in Fig. 2 graphically indicates that *Fv-2* type does not correlate with the degree of suppression of mitogenic responses of lymphocytes by FV. Of 30 (129 × B6)F_2 mice tested, 6 were resistant to focus-formation in vivo, i.e., were $Fv\text{-}2^{rr}$, whereas 24 were susceptible (Fig. 2 A). Among the six $Fv\text{-}2^{rr}$ F_2 mice, five had thymocytes mildly to profoundly suppressed by FV and one had thymocytes resistant to FV in vitro. 5 of 24 mice susceptible to focus-formation in vivo, i.e., $Fv\text{-}2^{ss}$ or $Fv\text{-}2^{rs}$, had thymocytes resistant to suppression by FV in vitro. The other 19 had thymocytes susceptible to FV in vitro (Fig. 2 A). A Chi-square analysis of the data supports the hypothesis that the genes are not linked ($\chi^2 = 0.8146$ $P > 0.1$).

In a similar analysis of 20 (DBA/2 × B10.D2)F_2 mice, there was no correlation between resistance to focus-formation in vivo and to suppression of mitogenesis in vitro (Fig. 2 B). All $Fv\text{-}2^{rr}$ mice had spleen cells susceptible to FV in vitro and 2/15 mice susceptible to focus-formation in vivo had spleen cells resistant to FV in vitro. The fraction (2/20) of these mice with resistant spleen cells was low, but subsequent experiments with larger numbers of spleen cell suspension from individual F_2 mice resulted in a larger fraction, i.e., 14/54 (Table II). The data support the hypothesis that *Fv-2* and the gene for resistance to FV in vitro segregate independently ($\chi^2 = 2.75$ $P > 0.1$).

Of the 20 [(DBA/2 × B10.D2)F_1 × B10.D2] BC1 mice tested, 3 had spleen cells of "indeterminant" susceptibility to FV in vitro (Fig. 2 C, Table II). The data, as analyzed by a Chi-square formula, indicate that *Fv-2* and the gene for

susceptibility segregate independently ($\chi^2 = 3.00$ $P > 0.1$). There were eight *Fv-2*^{rr} mice and spleen cells from three were susceptible to FV in vitro.

All 12 [(DBA/2 × B10.D2)F_1 × DBA/2] BC1 mice were susceptible to focus-formation in vivo and their spleen cells were susceptible to FV in vitro (Fig. 2 D). The data confirm the finding that susceptibility is dominant over resistance to FV in vitro.

Different Genes Control Ability to Reject Marrow Cell Allografts and Susceptibility to FV In Vitro. Mice of the C57BL background, including B6, B10, and B10 congenic strains, are able to reject marrow cell allografts of several *H-2* types (19) and their lymphocytes are resistant to FV in vitro (10). The immune response (*Ir*)-like genes controlling this ability are multiple (two or more) and "good responder" status is dominant over "poor responder" status (19). Despite these differences, we decided to test the hypothesis that one of such genes is linked to or identical with the gene determining resistance of lymphocytes to FV in vitro. Therefore, individual (DBA/2 × B10.D2)F_2 mice were hemisplenectomized and their spleen cells were tested for resistance to FV in vitro (Fig. 1). 10 days later, the mice were irradiated (800 rads) and challenged with inocula of 4×10^6 B6 (*H-2*^{b}) bone marrow cells. Previously hemisplenectomized DBA/2 "poor responder" and B10.D2 "good responder" were irradiated and challenged with B6 marrow cells as controls for classifying the F_2 progeny mice. The geometric mean (95% confidence limits) IUdR uptake (%) values in spleens of DBA/2 and B10.D2 recipients, respectively, were 0.40 (0.17–0.92) and 0.04 (0.02–0.06).

The data in Fig. 3 depicts the relationship between responder status to B6 marrow grafts and susceptibility of spleen cells to FV in vitro. 16 of the 20 mice were "good responders" to B6 marrow cell allografts and 4/20 were "poor responders", as judged by splenic IUdR uptake (%) values 5 days after cell transfer. The degree of suppression of response of the spleen cells to Con A ranged from 3 to 76% when taken from the 16 "good responder" mice (Fig. 3), indicating a lack of correlation between ability to reject allogeneic marrow cells and resistance to FV in vitro. The percent suppression values of the spleen cell cultures from "poor responder" mice were within the same range as spleen cell cultures of the "good responder" mice.

Discussion

Friend leukemia virus complex causes erythroleukemia and profound immunosuppression in susceptible strains of mice. We have previously described that mitogenic response of normal T and B cells from various lymphoid organs of mice susceptible to FV leukemia is suppressed by addition of FV in vitro (10). Suppression of the mitogenic response is mediated by T-suppressor cells and is under genetic control (13). Lymphoid cells from mice resistant to FV leukemia are refractory to suppressive effects of FV in vitro. However, such resistance is lost when M cells are eliminated by treatment of mice with ^{89}Sr (10). This and other evidence led us to postulate that M cells serve to regulate the number and/or functions of T-suppressor cells. In mice with lymphocyte populations genetically resistant to FV in vitro, the number of suppressor cells is maintained at a low level, thus affording them protection from immuno-

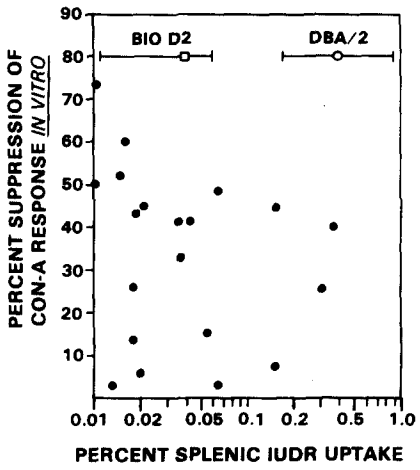

FIG. 3. Typing of individual (DBA/2 × B10.D2)F_2 mice for ability to reject C57BL/6 marrow allografts and susceptibility to suppression of lymphocyte mitogenesis. Result: no correlation exists between susceptibility to suppression of lymphocyte mitogenesis by FV and marrow allograft reactivity.

suppression in vitro (13). These studies led to two important questions: (*a*) what is the nature and number of genes which regulate the suppressor cell number and/or functions and thus susceptibility to suppression in vitro? (*b*) Do the genes which regulate the T-suppressor cells detected in vitro also function similarly in vivo? We have attempted to answer the first of these two questions in the present paper.

The first known gene we considered that may regulate susceptibility to FV in vitro was *Fv-2* (6). Until the present genetic analysis was performed, all evidence indicated that inbred mice typed as *Fv-2*^{rr} also had lymphocytes resistant to FV in vitro (10). Susceptibility to focus-formation and to immunosuppression in vitro is dominant over resistance (6 and Table I). Finally, treatment of genetically resistant adult C57BL/6 mice with ^{89}Sr to deplete M cells abolished the resistance to the malignant erythropoietic effects (a function of *Fv-2*) and to the immunosuppressive effects (both in vivo and in vitro) of FV (9, 10). However, the analysis presented in Fig. 2 clearly indicates that *Fv-2* and the gene controlling susceptibility to immunosuppression by FV in vitro segregate independently. In every cross, some mice susceptible to spleen focus formation had lymphocytes resistant to FV in vitro and mice resistant to spleen focus formation had lymphocytes susceptible to FV in vitro. This was true whether spleen or thymus cells were tested.

The observed and expected numbers of mice susceptible in vitro to immunosuppression if a single gene dominant for suppression were involved is presented in Table II. Notwithstanding the three indeterminants in the group of (DBA/2 × B10.D2)F_1 × B10.D2 backcross mice, the data best fits the single gene probability. Of the total number of 116 mice tested, the number of mice which could not be classified clearly as resistant or susceptible, i.e., "indeterminants" was 5 or 4%. We do not believe that this small percent significantly affects our conclusions.

The *Ir*-like genes which control relative ability to reject marrow cell allografts (17) could possibly have regulated the resistance to FV immunosuppression in vitro. The *Ir*-like genes are multiple (two or more) and resistance is dominant over susceptibility (17), in contrast to the gene regulating resistance to FV in vitro. However, treatment of adult mice with ^{89}Sr abrogates both the *Ir*-like gene functions (11) as well as resistance to immunosuppression by FV (9, 10), indicating that M cells are involved in both types of gene functions. The *Ir*-like genes detected so far are not linked to *H-2* (17) and mice of the C57BL genetic background are "good responders" to most types of immunogenic marrow cell grafts (19) and are resistant to FV in vitro. However, the data in Fig. 3 indicates that the ability of *H-2*^{d} (DBA/2 × B10.D2)F_2 mice to reject *H-2*^{b} C57BL/6 marrow cell grafts did not correlate with the resistance of their spleen cells to FV in vitro. However, this statement is subject to qualification since the ability to reject marrow grafts is determinant specific (20) and therefore the *Ir*-like genes studied by us were specific for the *H-2*^{b} test graft used by us. In view of this, the only definitive statement that can be made from our data is that *Ir*-like genes regulating ability to reject *H-2*^{b} marrow cell grafts do not control the resistance to immunosuppression by FV in vitro.

The gene is not X-linked as sex of the individual mice test or sex of the parents in the matings had no effect on resistance or susceptibility to FV in vitro (data not shown).

In summary, a single autosomal gene appears to regulate the in vitro susceptibility of lymphocytes to the suppressive effects of FV. This gene most likely functions on the M cell T-suppressor cell relationship (13). Because this gene also regulates the immunosuppressive effects of FV in vivo,[2] we propose that this gene be called *Fv-3*. Inbred strains tested by us and classified as *Fv-3*^{rr} include C57BL/6, C57BL/10, and B10 congenic mice, C58 and HTG. *Fv-3*^{ss} strains include 129, DBA/2, BALB/c, CBA, SIM, SIM.R, C3H, SJL, NZB, NZW, and A. Non-inbred Swiss mice of various types are also *Fv-3*^{ss}.

How might *Fv-3* govern M cell T-suppressor cell interaction? The answers to this question are not provided by this study, but two possible mechanisms may be suggested. First, *Fv-3* could affect the antigenicity of suppressor cells such that they are recognized and eliminated by M cells. Alternately, *Fv-3* may affect the ability of M cells to recognize and/or interact with T suppressor cells. The former of these two possibilities would imply that only mice with the genotype *Fv-3*^{rr} would express the antigens on suppressor cells which can be recognized by M cells. This appears to be a more appealing possibility since it is already known that homozygosity is a requirement for optimal expression of hemopoietic histocompatibility (Hh) antigens associated with the *H-2* region (21). Hh antigens are normally expressed on marrow stem cells and are the antigens known to be recognized by M cells (19).

There are other possible gene functions of the C57BL background that deserve investigating for a possible relationship to *Fv-3*. The resistance to infection with *Listeria monocytogenes*, a facultative intracellular bacterium, is under genetic control, and C57BL/6 mice are resistant (22). Moreover, treatment of adult mice with ^{89}Sr to deplete M cells weakens the resistance to this bacterium (23). Herpes simplex-1 virus resistance is also under genetic control

and C57BL/6 mice are resistant while A strain mice are susceptible (24). It is therefore conceivable that *Fv-3* functions in resistance to nononcogenic infections as well as in resistance to FV.

Summary

Friend leukemia virus (FV) suppresses the proliferative response of normal lymphocytes to mitogens. The in vitro suppressive effect of FV on lymphocyte mitogenesis is mediated by T-suppressor cells and is under host genetic control. Lymphocytes from strains of mice of the C57BL background (e.g., C57BL/6) are resistant while cells from other strains (e.g., 129 and DBA/2) are susceptible. Genetic analyses utilizing resistant and susceptible parental strains, their F_1, intercross and backcross progeny indicated that susceptibility to in vitro suppression is regulated by a single autosomal gene, dominant for susceptibility to suppression. This gene, which is not linked to the *H-2* complex, segregated independently of the *Fv-2* gene which controls resistance to spleen focus formation in vivo. The gene is also unlinked to the *Ir*-like genes which regulate the ability of $H\text{-}2^d$ mice to reject $H\text{-}2^b$ bone marrow grafts. The gene is therefore designated as *Fv-3*. *Fv-3* may mediate its effect by regulating the numbers and/or functions of T-suppressor cells.

We thank Dr. Fred Moolton for critically reviewing the manuscript, Kristine Eckner for the illustraions, and Merla Higgins for secretarial assistance.

Received for publication 21 September 1977.

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