

INFLUENCE OF THE MURINE MHC (H-2) ON FRIEND LEUKEMIA VIRUS-INDUCED IMMUNOSUPPRESSION

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Avian, murine, feline, and human retroviruses frequently cause immunosuppression in their respective hosts (1, 2). The mechanisms by which immune reactivity is impaired remain speculative. Virus infection often results in immune hyporeactivity, but in some instances inactivated virus causes suppression independently of cellular infection (3, 4). Thus, it appears likely that some viral proteins themselves can directly induce immunosuppression.

The Friend murine leukemia virus complex (FV)¹ causes severe immunosuppression when inoculated into adult or newborn mice (1). FV complex, which is composed of a replication-competent helper virus (F-MuLV) and a replication-defective spleen focus-forming virus (SFFV), causes a rapidly developing erythroleukemia in susceptible strains of adult mice. Early suppression of B lymphocyte immune functions and altered leukocyte migration have been reported (5–7). In some instances, cell-mediated immune functions are also diminished (8, 9). Kumar and Bennet (10) and Kumar et al. (11) developed a murine model for studying genetic resistance to FV-induced immunosuppression. They found that a non-H-2-linked, autosomal gene, *Fv-3*, regulated susceptibility to in vitro and in vivo FV-induced immunosuppression, and proposed that *Fv-3* acted by regulating the numbers or functions of suppressor T cells (12, 13).

Several other mouse genes have been found to affect the course of FV-induced erythroleukemia (14–17 and Table I). In particular, two genes found within the MHC (H-2) influence spontaneous recovery from leukemia, probably by influencing development of virus-specific T cell-mediated immune responses (16–18). The H-2^{b/b} genotype is associated with a high incidence of recovery compared to the H-2^{a/b} and H-2^{a/a} genotypes (17). In addition, a non-H-2-associated gene, *Rfv-3*, acts in a complementary fashion with H-2, and is required for recovery from leukemia (19). In the presence of a low-recovery H-2 genotype, H-2^{a/b} or H-2^{a/a}, the resistant *Rfv-3^{r/s}* genotype is associated with recovery from viremia and production of virus-specific antibodies that neutralize virus and lyse virus-infected cells in the presence of complement (19–21). Nevertheless, these mice die from progressive leukemia. Conversely, low-recovery BALB/c, A/WySn and A.BY mice, which have the *Rfv-3^{s/s}* genotype fail to produce anti-FV

¹ *Abbreviations used in this paper:* AIDS, acquired immunodeficiency syndrome; FV, Friend murine leukemia virus complex; F-MuLV, Friend murine leukemia helper virus; HTLV-III, human T cell leukemia/lymphoma (lymphotropic) virus type III; LAV, lymphadenopathy-associated virus; MCF, mink cell focus-inducing; PBBS, phosphate buffered balanced salts; RAMIgG, rabbit anti-mouse IgG; SFFU, spleen focus-forming unit; SFFV, spleen focus-forming virus.

antibodies, and remain viremic until death (20, 21). Because of the influence of the *Rfv-3* gene on the ability of FV-infected mice to make anti-FV antibodies, it seemed possible that this gene might act by influencing susceptibility to FV-induced immunosuppression. This study was initiated to test this hypothesis. Our results indicate that the *Rfv-3* gene did not influence FV-induced immunosuppression; however, immunosuppression in FV-infected mice was strongly influenced by H-2 genotype. Mice having the H-2^{a/b} genotype were less suppressed than mice having the H-2^{a/a} genotype. This suppression not only appeared to be independent of the occurrence of FV-induced viremia and splenomegaly, but also was independent of recovery from FV leukemia.

Materials and Methods

Animals. C57BL/10SnJ, B10.A/SgSnJ, A.BY/SnJ, A/WySnJ, and BALB/cJ mice were purchased from The Jackson Laboratory, Bar Harbor, ME. F₁ hybrids were bred at the Rocky Mountain Laboratories, Hamilton, MT (16). Mice used in all experiments were between 2 and 6 mo of age. Pertinent information concerning mouse genes relevant to recovery from Friend virus is summarized in Table I.

Virus. The B-tropic strain of FV complex was obtained from Dr. F. Lilly, Albert Einstein School of Medicine, Bronx, NY. Virus stocks used for inoculation were prepared in BALB/cJ mice and assayed as previously described (16).

Animal Inoculations. The virus stock was thawed and diluted in phosphate buffered balanced salt solution (PBBS) containing 2% FCS. Mice were inoculated intravenously with 0.5 ml of diluted virus stock, which contained ~1,000 spleen focus-forming units (SFFU) and 4,000 fluorescent focus-forming units of F-MuLV (22). Mice were immunized by i.p. inoculation of either 0.25 ml of a 10% suspension of washed SRBC or 10 µg of TNP-Ficoll (Biosearch, San Rafael, CA). At appropriate intervals, spleens were removed, dissociated into single-cell suspensions, and assayed for either anti-SRBC or anti-TNP PFC.

Assays. The number of splenic anti-SRBC IgM- and IgG-secreting cells was determined by using the slide monolayer technique of Cunningham and Szenberg (23). Both direct and anti-mouse Ig-facilitated PFC responses were determined. The peak primary IgM

TABLE I
Comparison of H-2 and Rfv-3 Genotypes and Parameters of Recovery From Friend Disease in Various Mouse Strains

Mouse strain	H-2 genotype	Rfv-3 genotype	Production of anti-FV antibody*	Recovery from viremia*	Recovery from leukemic splenomegaly‡
(C57BL/10 × A.BY)F ₁	b/b	r/s	+	+	+
(B10.A × A.BY)F ₁	a/b	r/s	+	+	- or +§
(B10.A × A/WySn)F ₁	a/a	r/s	+	+	-
A.BY	b/b	s/s	-	-	-
A/WySn	a/a	s/s	-	-	-
BALB/c	d/d	s/s	-	-	-

* All mouse strains tested were viremic (~10⁵ XC-PFU/ml, ~10⁴ SFFU/ml) early after FV infection, but those having the *Rfv-3*^{r/s} genotype developed serum anti-FV antibody and began to clear the viremia about 25 d after infection (19-21, 25, and our unpublished data).

‡ All mice in this table developed leukemic splenomegaly (spleen wt 1-4 g) 10-15 d after FV inoculation. Certain strains (see table) recovered spontaneously between 25 and 40 d postinoculation, and spleen weights remained normal thereafter (16, 17).

§ Using a viral dose of 1,000 SFFU, (B10.A × A.BY)F₁ mice did not recover from FV-induced splenomegaly. However, if given 100 or 10 SFFU, a significant number recovered (16, 17).

and IgG PFC responses were previously determined to occur at 4 and 7 d postimmunization, respectively. Therefore, the antigen-specific IgM and IgG PFC responses were determined by inoculating mice with SRBC 4 or 7 d before assay. To determine the PFC responses to a secondary immunization, mice were inoculated with SRBC and FV simultaneously, boosted with SRBC 23 d later, and assayed for antigen-specific IgM and IgG PFC 7 d after the booster immunization. Antigen-specific IgM PFC were defined as those plaques that developed without the addition of rabbit anti-mouse IgG (RAMIgG). The number of antigen-specific IgG-secreting PFC was determined by subtracting the direct PFC response from the facilitated response. The number of splenic anti-TNP IgM-secreting cells was determined by using the slide monolayer technique, except TNP-conjugated SRBC were used (24). Background anti-SRBC and anti-TNP PFC responses in unimmunized normal and FV-infected mice were consistently below 200 PFC/spleen.

Plasma was collected at intervals after FV infection and SRBC immunization, and used to determine circulating virus and anti-SRBC antibody titers. Titers of F-MuLV were determined by plating plasma dilutions onto SC-1 cells and counting infected cell foci visualized by indirect immunofluorescence using F-MuLV-specific MAb 48, as described previously (22).

Plasma samples were divided and tested for anti-SRBC antibodies either directly, or with the addition of RAMIgG, or after treatment with 2-ME. Briefly, 50 μ l of serially diluted plasma, prepared in PBBS containing 2% FCS, was added to the pelleted cells from 25 μ l of a 1% SRBC suspension. Anti-SRBC antibody titers were expressed as the reciprocal of the highest serum dilution that showed a distinct agglutination pattern. Direct titers were determined by incubating plasma samples with SRBC for 4 h at 4°C. Indirect titers were determined by incubating plasma samples with SRBC for 45 min at room temperature, washing three times with PBBS containing 2% FCS, adding 50 μ l of a 1:100 dilution of RAMIgG and incubating for 4 h at 4°C. Some plasma samples were treated with 0.1 M 2-ME at 37°C for 30 min to inactivate IgM antibodies, diluted, added to SRBC, and incubated at 4°C for 4 h.

Statistical Methods. The Student's *t* test for unpaired data was used to determine the level of significance between the means of samples.

Results

Immunosuppression in Rfv-3^{r/s} and Rfv-3^{s/s} Mice. (B10.A \times A.WySn)F₁, Rfv-3^{r/s}, mice were compared with BALB/c Rfv-3^{s/s} mice for their ability to respond immunologically to SRBC. BALB/c mice were used as controls because of their known susceptibility to FV-induced immunosuppression (5), and their lack of a humoral immune response to FV during active infection (20 and Table I). The direct PFC response was assayed at 25 d after FV infection, and mice having the Rfv-3^{r/s} genotype were found to be as suppressed as mice having the Rfv-3^{s/s} genotype (Table II). In both strains, the direct PFC response was decreased by

TABLE II
Effect of Rfv-3 Genotype on FV-induced Immunosuppression

Strain	Rfv-3 genotype	F-MuLV viremia (PFU/ml plasma)	Direct Anti-SRBC PFC/spleen		Percent suppression
			Uninfected	FV-infected	
(B10.A \times A/WySn)F ₁	r/s	$1.2 \times 10^3 \times 1.5$	87,000 $\times 1.10$	300 $\times 1.17$	99.65
BALB/c	s/s	$7.5 \times 10^4 \times 1.4$	110,000 $\times 1.07$	480 $\times 1.26$	99.56

FV-infected mice were immunized with SRBC 21 d after infection. All mice were bled and then sacrificed for analysis of direct anti-SRBC PFC 4 d after SRBC immunization. Viremia was measured as described in Materials and Methods. Values shown are the geometric mean \times SE factor.

>100-fold. Thus, the Rfv-3^{r/s} genotype appeared not to confer resistance to immunosuppression. This finding was surprising, since (B10.A × A.WySn)F₁ Rfv-3^{r/s} mice were capable of responding immunologically to FV, and most had reduced FV viremia by day 25 (Table I).

Comparison of Immunosuppression in H-2-congenic Mice Having the Rfv-3^{r/s} Genotype. Genes within the H-2 complex have a strong influence on FV-induced leukemia. By examining congenic Rfv-3^{r/s} mice that differed only at the H-2 locus, we were able to study the effect of H-2 genotype on the development of FV-induced immunosuppression. We assayed the anti-SRBC immune response of FV-infected mice at 40 d after FV inoculation, when these mice were responding immunologically to FV (Table I). At this time, H-2^{a/a} mice had gross splenomegaly and showed depressed 7-d IgG anti-SRBC PFC responses and lowered IgM anti-SRBC PFC levels at both times tested (4 and 7 d post-SRBC immunization) (Fig. 1). At this same time, H-2^{a/b} mice also had severe splenomegaly, but showed no depression in anti-SRBC PFC of the IgG class, and depression of the IgM PFC response only at 4 d postimmunization, returning to normal by day 7 (Fig. 1). These results indicated that FV-induced immunosuppression was strongly influenced by genes within the H-2 complex. As expected, the homozygous H-2^{b/b} genotype was associated with both recovery from leukemia and lack of immunosuppression (data not shown). However, heterozygous H-2^{a/b} mice, which did not recover from leukemia, nevertheless failed to develop FV-induced suppression of the IgG anti-SRBC response, and had only transient suppression of the IgM response.

Kinetics of FV-induced Immunosuppression. To assess the role of viremia in FV-induced immunosuppression, we studied the immune response of H-2^{a/a} Rfv-3^{r/s} mice at various times after FV infection. Early (13 d) after FV-infection, when all mice were viremic, the SRBC-specific IgG PFC response was found to be depressed (Fig. 2). This response was also found to be suppressed (>1,000-fold) at 25 and 40 d post-FV inoculation, when most mice had eliminated the viremia. In contrast, the 4- and 7-d antigen-specific IgM PFC responses were not significantly suppressed early after FV infection, but were suppressed later during the disease. The 4-d IgM response appeared more suppressed than the 7-d IgM response at both 25 and 40 d after FV.

In contrast to H-2^{a/a} mice, mice having the H-2^{b/b} or H-2^{a/b} genotypes did not have significantly suppressed 7 day IgM or IgG PFC responses early (13 d) or late (25 and 40 d) after FV infection (data not shown). However, the 4-d IgM PFC response was found to be significantly suppressed ($p < 0.001$) in H-2^{a/b} mice, but not in mice having the H-2^{b/b} genotype (data not shown). Thus, the influence of H-2 genotype on FV-induced immunosuppression was observed both early and late during disease. Furthermore, viremia alone was not sufficient for the induction of the depressed response, since mice having the H-2^{b/b} or H-2^{a/b} genotype were viremic during early disease (13 d), but were not immunosuppressed.

Comparison of H-2-congenic Mice Having the Rfv-3^{r/s} Genotype. We studied H-2-congenic mice having the A strain background genes to assess the influence of H-2 genotype on FV-induced immunosuppression in Rfv-3^{r/s} mice. These mice do not produce virus-neutralizing antibody, and remain viremic until death

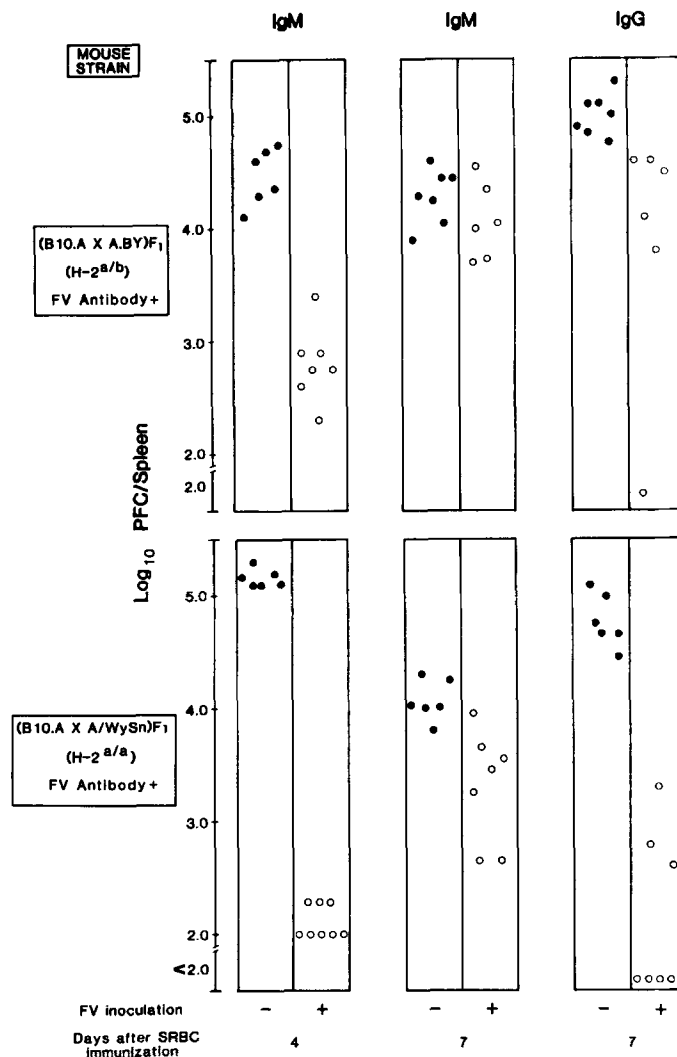


FIGURE 1. SRBC-specific IgM and IgG PFC responses of uninfected (●) and FV-infected (○) (B10.A × A.BY)_{F1}, H-2^{b/b}, and (B10.A × A/WySn)_{F1}, H-2^{a/a} mice. FV-infected mice were immunized with SRBC at either 33 d (for 7-d PFC responses) or 36 d (for 4-d PFC response) after FV infection, and assayed for PFC responses at 40 d post-FV inoculation. Both strains of mice had the Rfv-3^{r/s} genotype and were nonviremic but had severe splenomegaly (>1 g) at this time.

(Table I). A.BY (H-2^{b/b}) and A/WySn (H-2^{a/a}) mice were assayed for anti-SRBC PFC at 40 d after FV infection, at which time both strains of mice had similar levels of viremia (>10⁵ FFU/ml). The IgM PFC responses in FV-infected A/WySn mice were slightly suppressed (less than one order of magnitude) at 4 and 7 d after SRBC immunization (Fig. 3). A.BY mice had a suppressed IgM PFC response at 4 d after SRBC-immunization (<1.5 log₁₀), but not at day 7. The IgG PFC response was suppressed in all H-2^{a/a} mice tested ($p < 0.001$), and the majority of these mice (five out of nine) were >99% suppressed. In contrast,

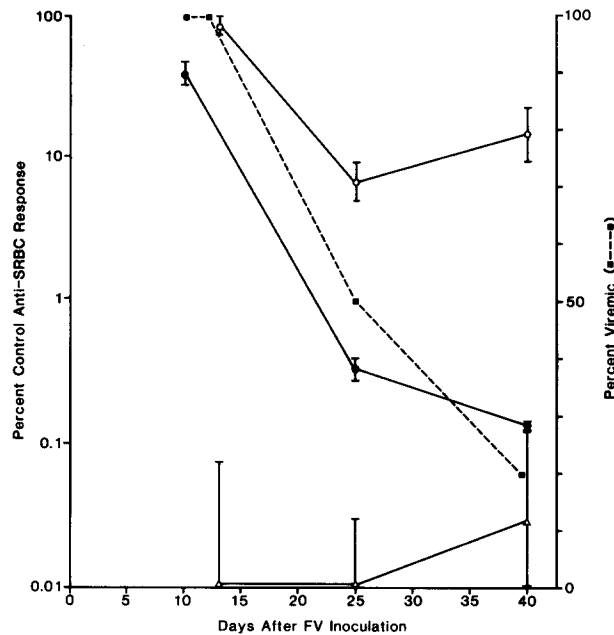


FIGURE 2. SRBC-specific IgM and IgG PFC responses of spleen cells from FV-infected (B10.A \times A/WySn) F_1 , H-2^{a/a} mice were compared to responses of uninfected control mice at various times after FV infection. Mice were immunized with SRBC at either 4 or 7 d before assay. Data were normalized to the control responses. (●) IgM PFC response 4 d after SRBC immunization. (○) IgM PFC response 7 d after SRBC immunization. (Δ) IgG PFC response 7 d after SRBC immunization. Percentage of mice with viremia ($>10^3$ FFU/ml plasma) is also shown (■).

in mice having the H-2^{b/b} genotype, immunosuppression of the IgG response was marginal (i.e., not significant at $p < 0.001$, but significant at $p < 0.05$), and only one animal was $>99\%$ suppressed. Thus, lack of severe suppression of the IgG PFC response correlated with the presence of the H-2^{b/b} genotype, even in the presence of the Rfv-3^{s/s} genotype and other A strain genes.

Analysis of Plasma Anti-SRBC Antibody. Plasma anti-SRBC antibody was measured to determine whether the suppressed PFC responses were spleen-specific phenomena or whether the suppression was systemic. Plasma anti-SRBC antibody titers were reduced in FV-infected H-2^{a/a} mice at 4 and 7 d after SRBC immunization (Table III). The suppressed antibody responses correlated with the reduced PFC responses of these mice. Also, the lack of production of IgG anti-SRBC was evident, since most antibodies from FV-infected mice immunized 7 d previously with SRBC were susceptible to inactivation by treatment with 2-ME, and thus were of the IgM class. In contrast, antibodies obtained on day 7 from control uninfected mice were not susceptible to this treatment. Plasma anti-SRBC antibody titers were performed in conjunction with all PFC assays. In all instances, alterations in the PFC responses were reflected in the plasma antibody titers. Thus, the suppressed immunological responsiveness to SRBC appeared to be not only a local suppression of the PFC response in the spleen, but a systemic suppression of the humoral antibody response.

Suppression of Secondary Immune Response in H-2^{a/a}, Rfv-3^{r/s} Mice. To study

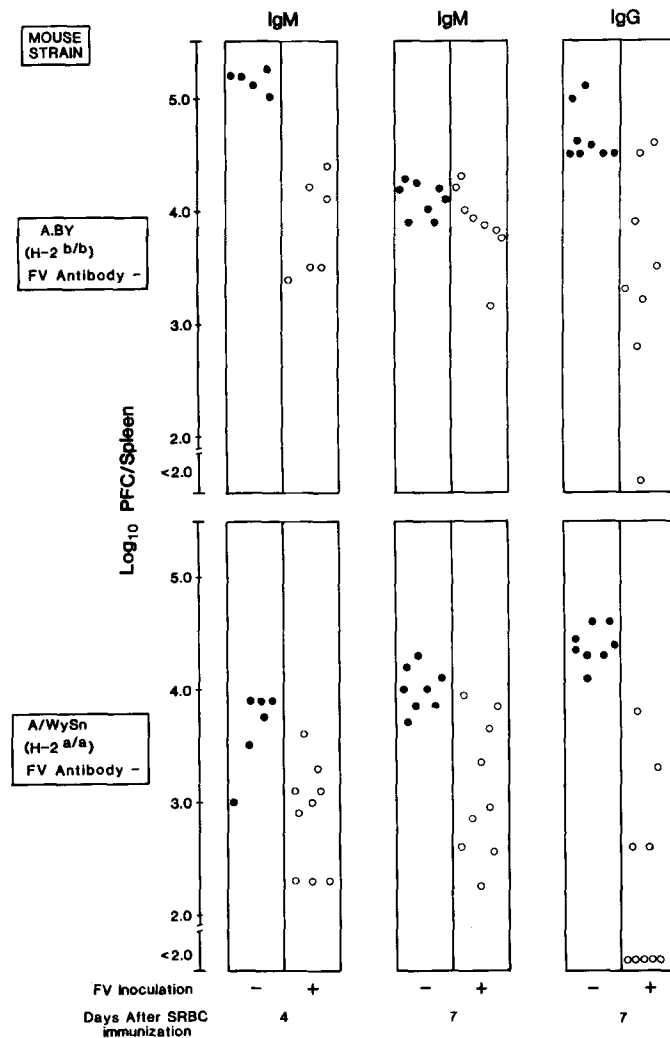


FIGURE 3. SRBC-specific IgM and IgG PFC responses of uninfected (●) and FV-infected (○) A.BY, H-2^{b/b}, and A/WySn, H-2^{a/a} mice. FV-infected mice were immunized with SRBC at either 33 d (for 7-d PFC responses) or 36 d (for 4-d PFC response) after FV infection, and assayed for PFC responses at 40 d post-FV inoculation. Both strains of mice had the Rfv-3^{r/s} genotype and were viremic at this time.

the effect of FV infection on the secondary immune response, we chose to examine H-2^{a/a}, Rfv-3^{r/s} mice, since these mice responded immunologically to FV, but were suppressed to a primary SRBC immunization. These mice developed a normal anti-SRBC IgM PFC response at 7 d after the secondary immunization, but had a depressed IgG response (Fig. 4). The majority of FV-infected mice had no detectable levels of SRBC-specific IgG PFC, and those that did respond had significantly lower levels than uninfected mice. Also, some mice were immunized with SRBC 7 d before infection with FV, boosted 23 d after FV-infection, and assayed for PFC 7 d after the booster immunization (data not shown). These mice developed normal IgM PFC responses, but had suppressed

TABLE III
Plasma Anti-SRBC Titers of FV-Infected and Uninfected H-2^{a/a} Rfv-3^{r/s} Mice

Days after SRBC immunization	FV inoculation	Anti-SRBC titer (log ₂)*		
		Not treated	2-ME	Rabbit anti-mouse Ig
4	-	8.0 ± 0	2.22 ± 0.22	9.4 ± 0.16
	+ [‡]	<u>3.5 ± 0.5[§]</u>	2.22 ± 0.22	<u>6.4 ± 0.37</u>
7	-	10.0 ± 0	10.0 ± 0	13.8 ± 0.13
	+	<u>6.5 ± 0.46</u>	<u>3.25 ± 0.25</u>	<u>9.0 ± 0.38</u>

* Mean ± SE of the anti-SRBC titer of plasmas treated as described in Materials and Methods (8–10 mice/group).

[‡] (B10.A × A/WySn)_{F1} mice were infected with FV, immunized with SRBC 18 or 21 d later, and assayed 25 d after FV infection.

[§] Underscored values are significantly different from uninfected mice at $p < 0.001$.

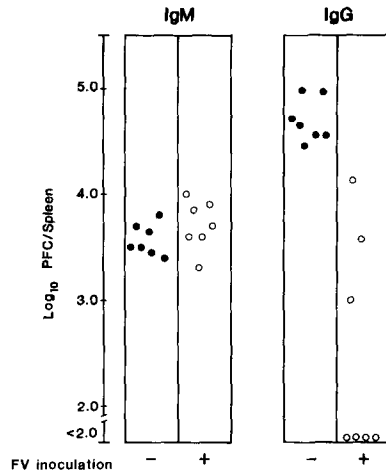


FIGURE 4. Secondary PFC responses of uninfected (●) and FV-infected (○) (B10.A × A/WySn)_{F1}, H-2^{a/a} mice to SRBC. Two groups of seven mice per group were immunized with SRBC, and one group was inoculated with FV simultaneously. 23 d later, they were given a second SRBC immunization, and SRBC-specific IgM and IgG PFC responses were determined at 30 d after FV infection. These mice have the Rfv-3^{r/s} genotype and were nonviremic at this time.

secondary IgG PFC responses. Three of four FV-infected mice had IgG PFC responses suppressed by 90–99%. Therefore, the secondary IgG response appeared to be sensitive to the effects of FV infection, even if the primary SRBC immunization was given before FV infection.

Suppression of PFC Response to a T Cell-independent Antigen in H-2^{a/a}, Rfv-3^{r/s} Mice. To determine whether B lymphocyte function was altered during FV infection, we examined the PFC response of spleen cells from FV-infected mice to the T cell-independent antigen, TNP-Ficoll. (B10.A × A/WySn)_{F1}, H-2^{a/a}, Rfv-3^{r/s} mice failed to produce normal levels of direct TNP PFC when assayed at 25 d after FV infection (Table IV). This response was suppressed by >99%, which indicated that B lymphocyte function was markedly reduced in these FV-infected mice.

TABLE IV
*PFC Response to a T Cell-independent Antigen in FV-infected
 H-2^{a/a}, Rfv-3^{r/s} Mice*

Infection	Direct anti-TNP PFC/spleen*	Percent suppression
None	53,456 \times 1.32	-
FV	448 \times 1.52	99.16 [‡]

FV-infected (B10.A \times A/WySn)F₁ mice were immunized i.p. with 10 μ g of TNP-Ficoll 21 d after infection. All mice were assayed for direct anti-TNP PFC 4 d after immunization with TNP-Ficoll.

* Numbers are the geometric mean \times SE factor of groups of five mice.

[‡] Significantly lower than uninfected mice ($p < 0.001$).

Discussion

Our results show that genes within the H-2 complex influenced FV-induced immunosuppression. The IgG PFC response in mice having the H-2^{a/b} or H-2^{b/b} genotype was not significantly suppressed, whereas in mice having the H-2^{a/a} genotype, this response was suppressed both early (13 d) and late (25 and 40 d) during FV disease. In the case of (B10.A \times A/WySn)F₁, H-2^{a/a} mice, this finding was surprising, since these mice have the Rfv-3^{r/s} genotype and thus they respond immunologically to FV (19, 21). Our data indicated that the Rfv-3 gene alone did not appear to convey resistance to FV-induced immunosuppression.

Only a few studies have been concerned with defining the genetic regulation of FV-induced immunosuppression. Kumar et al. (12, 13) described a gene, *Fv-3*, that maps outside the H-2 complex and regulates susceptibility to FV-induced suppression of lymphocyte mitogenesis in vitro and to PFC responses in vivo. In our previous (18, 19, 21) and present experiments, we did not observe the effect of the *Fv-3* gene. This may have been due to technical differences such as FV dose used, or to genetic differences in the mice tested.

Massive splenomegaly often accompanies FV disease, and a depressed splenic PFC response could be due to alterations in specific splenic functions rather than general systemic immunosuppression. However, several lines of evidence argue against this interpretation. PFC responses were calculated as PFC/spleen and not PFC/10⁶ nucleated cells; therefore, the large number of nucleated leukemic cells would not affect the calculation. Also, plasma anti-SRBC responses were depressed whenever we observed suppressed PFC responses. Thus, we were observing a systemic suppression and not an organ-specific phenomenon. Lastly, mice having the H-2^{a/b} or H-2^{a/a} genotype had similarly enlarged spleens at both 13 and 40 d after FV infection, but only H-2^{a/a} mice had a suppressed IgG PFC response. Therefore, FV-induced splenomegaly itself was not sufficient to cause this immunosuppression. However, it is possible that FV-induced changes in splenic architecture could alter interactions among cells of the immune system. This might explain the transient suppression of the IgM PFC responses in splenomegalic H-2^{a/b} mice.

Similarities are evident between the immunosuppression we observed in (B10.A \times A/WySn)F₁ mice and that found previously in other susceptible strains of mice inoculated with FV. Primary IgM PFC responses and secondary IgG

PFC responses were suppressed in FV-infected BALB/c mice (5, 26, 27). In some instances, serum antibody titers were also shown to be suppressed (5, 26). Our data indicated that the IgM PFC response was not as sensitive as the IgG PFC response to suppression, since the IgM PFC response was only transiently suppressed and returned to normal levels by 7–10 d after immunization (Figs. 1 and 2), whereas both the primary and secondary IgG PFC responses were exquisitely sensitive to FV-induced immunosuppression (Figs. 1 and 4).

The suppression we observed in both Ig classes could be due to altered B or T cell functions, since both IgM and IgG responses to SRBC are T cell-dependent. However, since IgG responses require additional T cell help to make the class switch from IgM to IgG, the increased suppression of IgG PFC might be explained by lack of this T cell function. By examining the immune response of FV-infected mice to a T cell-independent antigen, B cell function could be tested directly. (B10.A \times A/WySn) F_1 , H-2^{a/a} mice failed to generate normal numbers of PFC cells to TNP-Ficoll, which indicated that B lymphocyte function was also altered (Table IV). Therefore, FV-induced immunosuppression appeared to be a complex phenomenon probably involving many different cell types of the immune system. This conclusion is consistent with previous observations (28, 29) that FV can infect and/or transform several different hemopoietic cell types, including B and T lymphocytes, myeloid cells, erythroid cells, and megakaryocytes.

The mechanism(s) of retrovirus-induced immunosuppression is not well understood. Both UV-inactivated retroviruses and retroviral components can be immunosuppressive, suggesting that viral proteins may be responsible for the induction of the suppressed condition. In the feline leukemia virus system, UV-inactivated virus and p15E protein have been shown to cause suppression of the blastogenic response to T cell mitogens, inhibition of inflammatory macrophage accumulation, and abrogation of tumor immunity (3, 4, 30–33). However, our experiments indicated that high titers of F-MuLV helper virus were not solely responsible for the induction or maintenance of the suppressed condition. For instance, (B10.A \times A/WySn) F_1 mice were viremic and suppressed early (13 d) during FV disease, and remained suppressed even after clearing the virus (40 d) (Figs. 1 and 2). Also, only a small percentage of spleen cells from these mice expressed viral proteins or released infectious virus (21, 34, 35). Thus, there must be a mechanism by which the immunosuppressed state was maintained even when levels of infectious virus and viral proteins were reduced by 1,000-fold. Conversely, A.BY mice had very high titers of circulating virus ($>10^5$ FFU/ml), yet they were not severely immunosuppressed (Fig. 3). Thus, in contrast to the feline system, it appears unlikely that circulating F-MuLV helper virus or F-MuLV proteins were the sole cause of the induction or maintenance of the immunosuppression.

The importance of the SFFV component of the FV complex in inducing the immunosuppressed condition is not known. SFFV has the genetic structure of a defective recombinant virus (36), similar to dual-tropic recombinant mink cell focus (MCF)-inducing virus described previously (37, 38). A recent study by Mosier et al. (39) indicated that a MuLV mixture containing MCF viruses was required for induction of an immunosuppressive lymphoproliferative disease.

Therefore, it is possible that SFFV might have a similar effect in the FV system. Furthermore, our FV preparations contained nondefective MCF MuLV, which might have been involved in the immunosuppression observed.

FV-infected (B10.A \times A/WySn) F_1 , H-2^{a/a} mice have interesting analogies to patients with acquired immunodeficiency syndrome (AIDS). (a) In both, a retrovirus infection leads to profound immunosuppression of adult immunocompetent hosts. (b) Virus-specific antibodies are found in both cases (19, 21, 40–42), yet both are immunologically compromised with regard to new immunological challenges (2). (c) Only low levels of infectious virus are found. In the FV system, this is due to the presence of antiviral antibodies (35), and in AIDS, the antiviral humoral response (40–42) might be partially responsible for the low number of infected cells observed. (d) HTLV-III/LAV causes a marked decrease in the number of T helper cells, which results in an abnormal helper/suppressor cell ratio (43, 44). Although we have no data concerning this ratio in (B10.A \times A/WySn) F_1 mice, our data suggested altered T helper cell function. The similarities of the two diseases and the common retroviral etiology warrant further studies concerning the virus-specific and antigen-specific immune responses of this murine model.

Recently, the Laterjet-Duplan strain of radiation leukemia virus was shown (39) to cause profound immunosuppression in mice having the H-2^{b/b} genotype. Conversely, in the FV system, the H-2^{b/b} genotype influences recovery from disease and resistance to immunosuppression (17). Thus, in retrovirus infections, the same H-2 haplotype can have markedly different effects on recovery and/or immunosuppression, depending on the strain of retrovirus involved. By analogy, if there exist multiple retroviruses that cause AIDS (45, 46), it is possible that a single HLA type might have different effects on the susceptibility to different virus strains.

Summary

Friend murine leukemia virus complex (FV)-induced immunosuppression was studied by assaying splenic anti-SRBC PFC responses and plasma antibody titers in mice at various times after FV inoculation. Genes located within the H-2 complex were found to influence resistance to FV-induced immunosuppression. Near normal responses were observed in mice having the H-2^{a/b} or H-2^{b/b} genotype, whereas mice having the H-2^{a/a} genotype were suppressed. This H-2 effect was observed not only in mice having heterozygous C57BL/10 \times A background genes, including Rfv-3^{r/s}, but also was apparent in mice having homozygous A-strain background genes, including Rfv-3^{s/s}. Therefore, the *Rfv-3* gene did not appear to convey resistance to FV-induced immunosuppression. The suppression in susceptible H-2^{a/a} mice was characterized by a partial suppression of the IgM response and a profound suppression of both the primary and secondary IgG responses. Neither splenomegaly nor viremia alone appeared to be sufficient for the induction or maintenance of the immunosuppression. The mechanism of suppression was unclear, but both B lymphocyte and T lymphocyte functions appeared to be altered.

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References

1. Dent, P. B. 1972. Immunodepression by oncogenic viruses. *Prog. Med. Virol.* 14:1.
2. Lane, H. C., and A. S. Fauci. 1985. Immunologic abnormalities in AIDS. *Ann. Rev. Immunol.* 3:477.
3. Olsen, R. G., E. A. Hoover, J. P. Schaller, L. E. Mathes, and L. H. Wolff. 1977. Abrogation of resistance to feline oncornavirus disease by immunization with killed feline leukemia virus. *Cancer Res.* 37:2082.
4. Schaller, J. P., E. A. Hoover, and R. G. Olsen. 1977. Active and passive immunization of cats with inactivated feline oncornavirus. *J. Natl. Cancer Inst.* 59:1441.
5. Ceglowski, W. S., and H. Friedman. 1968. Immunosuppression by leukemia viruses. I. Effect of Friend virus disease virus on cellular and humoral hemolysis responses of mice to a primary immunization with sheep erythrocytes. *J. Immunol.* 101:594.
6. Ceglowski, W. S., and H. Friedman. 1970. Immunosuppression by leukemia viruses. IV. Effect of Friend leukemia virus on antibody-precursors as assessed by cell transfer studies. *J. Immunol.* 105:1406.
7. Friedman, H., and W. S. Ceglowski. 1971. Leukemia virus-induced immunosuppression. VIII. Rapid depression of in vitro leukocyte migration after infection of mice with Friend leukemia virus. *J. Immunol.* 107:1673.
8. Mortensen, R. F., W. S. Ceglowski, and H. Friedman. 1973. Leukemia virus-induced immunosuppression. IX. Depression of delayed hypersensitivity and MIF production after infection of mice with Friend leukemia virus. *J. Immunol.* 111:1810.
9. Mortensen, R. F., W. S. Ceglowski, and H. Friedman. 1974. Leukemia virus-induced immunosuppression. X. Depression of T cell-mediated cytotoxicity after infection of mice with a Friend leukemia virus. *J. Immunol.* 112:2077.
10. Kumar, V., and M. Bennett. 1976. Mechanisms of genetic resistance to Friend virus leukemia in mice. II. Resistance of mitogen-responsive lymphocytes mediated by marrow-dependent cells. *J. Exp. Med.* 143:713.
11. Kumar, V., T. Caruso, and M. Bennett. 1976. Mechanisms of genetic resistance to Friend virus leukemia. III. Susceptibility of mitogen-responsive lymphocytes mediated by T cells. *J. Exp. Med.* 143:728.
12. Kumar, V., L. Goldschmidt, J. W. Eastcott, and M. Bennett. 1978. 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. *J. Exp. Med.* 147:422.
13. Kumar, V., P. Resnick, J. W. Eastcott, and M. Bennett. 1978. Mechanism of genetic resistance to Friend virus leukemia in mice. V. Relevance of Fv-3 gene in the regulation of in vivo immunosuppression. *J. Natl. Cancer Inst.* 61:1117.
14. Odaka, T. 1969. Inheritance of susceptibility to Friend mouse leukemia virus. V. Introduction of a gene responsible for susceptibility in the genetic complement of resistant mice. *J. Virol.* 3:543.
15. Lilly, F. 1970. Fv-2: Identification and location of a second gene governing the spleen focus response to Friend leukemia virus in mice. *J. Natl. Cancer Inst.* 45:163.
16. Chesebro, B., K. Wehrly, and J. Stimpfling. 1974. Host genetic control of recovery from Friend leukemia virus-induced splenomegaly. Mapping of a gene within the major histocompatibility complex. *J. Exp. Med.* 140:1457.

17. Chesebro, B., and K. Wehrly. 1978. Rfv-1 and Rfv-2, two H-2-associated genes that influence recovery from Friend leukemia virus-induced splenomegaly. *J. Immunol.* 120:1081.
18. Britt, W. J., and B. Chesebro. 1983. H-2D control of recovery from Friend virus leukemia: H-2D region influences the kinetics of the T lymphocyte response to Friend virus. *J. Exp. Med.* 157:1736.
19. Chesebro, B., and K. Wehrly. 1979. Identification of a non-H-2 gene (Rfv-3) influencing recovery from viremia and leukemia induced by Friend virus complex. *Proc. Natl. Acad. Sci. USA.* 76:425.
20. Chesebro, B., and K. Wehrly. 1976. Studies on the role of the host immune response in recovery from Friend virus leukemia. I. Antiviral and antileukemia cell antibodies. *J. Exp. Med.* 143:73.
21. Doig, D., and B. Chesebro. 1979. Anti-Friend virus antibody is associated with recovery from viremia and loss of viral leukemia cell-surface antigens in leukemic mice. Identification of Rfv-3 as a gene locus influencing antibody production. *J. Exp. Med.* 150:10.
22. Sitbon, M., J. Nishio, K. Wehrly, D. Lodmell, and B. Chesebro. 1985. Use of a focal immunofluorescence assay on live cells for quantitation of retroviruses: Distinction of host range classes in virus mixtures and biological cloning of dual-tropic murine leukemia viruses. *Virology.* 141:110.
23. Cunningham, A. J., and A. Szenberg. 1968. Further improvements in the plaque technique for detecting single antibody-forming cells. *Immunology.* 14:599.
24. Henry, C. 1980. Hemolytic plaque assays. In *Selected Methods in Cellular Immunology.* B. B. Mishell and S. M. Shiigi, editors. W. H. Freeman and Co., San Francisco, CA. 100.
25. Chesebro, B., M. Bloom, K. Wehrly, and J. Nishio. 1979. Persistence of infectious Friend virus in spleens of mice after spontaneous recovery from virus-induced erythroleukemia. *J. Virol.* 32:832.
26. Salaman, M. H., and N. Wedderburn. 1966. The immunodepressive effect of Friend virus. *Immunology.* 10:445.
27. Wedderburn, N., and M. H. Salaman. 1968. The immunodepressive effect of Friend virus. II. Reduction of splenic haemolysin-producing cells in primary and secondary responses. *Immunology.* 15:439.
28. Chesebro, B., J. Portis, K. Wehrly, and J. Nishio. 1983. Effect of murine host genotype on MCF virus expression, latency, and leukemia cell type of leukemias induced by Friend murine leukemia helper virus. *Virology.* 128:221.
29. Dennis, L. H., and I. Brodsky. 1965. Thrombocytopenia induced by the Friend leukemia virus. *J. Natl. Cancer Inst.* 35:993.
30. Hebebrand, L. C., L. E. Mathes, and R. G. Olsen. 1977. Inhibition of concanavalin A stimulation of feline lymphocytes by inactivated feline leukemia virus. *Cancer Res.* 37:4532.
31. Mathes, L., R. Olsen, L. Hebebrand, E. Hoover, and J. Schaller. 1978. Abrogation of lymphocyte blastogenesis by feline leukemia virus protein. *Nature (Lond.).* 274:687.
32. Mathes, L. E., R. G. Olsen, L. C. Hebebrand, E. A. Hoover, J. P. Schaller, P. W. Adams, and W. S. Nichols. 1979. Immunosuppressive properties of a virion polypeptide, a 15,000-dalton protein, from feline leukemia virus. *Cancer Res.* 39:950.
33. Ciancolo, G. J., T. J. Matthews, D. P. Bolognesi, and R. Synderman. 1980. Macrophage accumulation in mice is inhibited by low molecular weight products from murine leukemia viruses. *J. Immunol.* 124:2900.
34. Doig, D., and B. Chesebro. 1978. Antibody-induced loss of Friend virus leukemia

- cell surface antigens occurs during progression of erythroleukemia in F₁ mice. *J. Exp. Med.* 148:1109.
35. Chesebro, B., K. Wehrly, D. Doig, and J. Nishio. 1979. Antibody-induced modulation of Friend virus cell surface antigens decreases virus production by persistent erythroleukemia cells: Influence of the Rfv-3 gene. *Proc. Natl. Acad. Sci. USA.* 76:5784.
 36. Evans, L., M. Nunn, P. H. Duesberg, D. Troxler, and E. Scolnick. 1980. RNAs of defective and nondefective components of Friend anemia and polycythemia virus strains identified and compared. *Cold Spring Harbor Symp. Quant. Biol.* 44:823.
 37. Fischinger, P. J., S. Nomura, and D. P. Bolognesi. 1975. A novel murine oncornavirus with dual eco- and xenotropic properties. *Proc. Natl. Acad. Sci. USA.* 72:5150.
 38. Hartley, J. W., N. K. Wolford, L. J. Old, and W. P. Rowe. 1977. A new class of leukemia virus associated with development of spontaneous lymphoma. *Proc. Natl. Acad. Sci. USA.* 74:789.
 39. Mosier, D. E., R. A. Yetter, and H. C. Morse. 1985. Retroviral induction of acute lymphoproliferative disease and profound immunosuppression in adult C57BL/6 mice. *J. Exp. Med.* 161:766.
 40. Safai, B., M. G. Sarngadharan, J. E. Groopman, K. Arnett, M. Popovic, A. Sliski, J. Schupbach, and R. C. Gallo. 1984. Seroepidemiological studies of human T-lymphotropic retrovirus type III in acquired immunodeficiency syndrome. *Lancet.* 1:1438.
 41. Weiss, R. A., P. R. Clapham, R. Cheingsong-Popov, A. G. Dalgleish, C. A. Carne, I. V. D. Weller, and R. S. Tedder. 1985. Neutralization of human T-lymphotropic virus type III by sera of AIDS and AIDS-risk patients. *Nature (Lond.).* 316:69.
 42. Robert-Guroff, M., M. Brown, and R. C. Gallo. 1985. HTLV-III-neutralizing antibodies in patients with AIDS and AIDS-related complex. *Nature (Lond.).* 316:72.
 43. Fahey, J. L., H. Prince, M. M. Weaver, J. Groopman, B. Visscher, K. Schwartz, and R. Detels. 1983. Quantitative changes in the T helper or T suppressor/cytotoxic lymphocyte subsets that distinguish acquired immunodeficiency syndromes from other immune subset disorders. *Am. J. Med.* 76:95.
 44. Ammann, A. J., D. Abrams, M. Conant, D. Chudwin, M. Cowan, P. Volberding, B. Lewis, and C. Casavant. 1983. Acquired immune dysfunctions in homosexual men: immunologic profiles. *Clin. Immunol. Immunopathol.* 27:315.
 45. Hahn, B. H., M. A. Gonda, G. M. Shaw, M. Popovic, J. A. Hoxie, R. C. Gallo, and F. Wong-Staal. 1985. Genomic diversity of the acquired immune deficiency syndrome virus HTLV-III: Different viruses exhibit greatest divergence in their envelope genes. *Proc. Natl. Acad. Sci. USA.* 82:4813.
 46. Wong-Staal, F., G. M. Shaw, B. H. Hahn, S. Z. Salahuddin, M. Popovic, P. Markham, R. Redfield, and R. C. Gallo. 1985. Genomic diversity of human T-lymphotropic virus type III (HTLV-III). *Science (Wash. DC).* 229:759.