Proc. Natl. Acad. Sci. USA
Vol. 76, No. 11, pp. 5809–5812, November 1979
Genetics

Fv-2 locus controls expression of Friend spleen focus-forming virus-specific sequences in normal and infected mice

(RNA tumor viruses/erythroleukemia/gene regulation/virus susceptibility/congeneic mice)

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Communicated by Charlotte Friend, August 13, 1979

ABSTRACT **We have recently demonstrated that normal hemopoietic cells express RNA sequences that are homologous to sequences specific for the Friend erythroleukemia virus genome [Bernstein, A., Gamble, C., Penrose, D. & Mak, T. W. (1979) *Proc. Natl. Acad. Sci. USA* 76, 4455–4459]. In this communication, we report that the *Fv-2* locus, the major genetic determinant controlling host susceptibility to erythroleukemia induction by Friend leukemia virus, also controls the expression of endogenous sequences related to the replication-defective component of Friend leukemia virus, Friend spleen focus-forming virus (SFFV), in normal uninfected mice. Two independent congeneic pairs of mice [C57BL/6 (B6) and B6.S; B6 and B6.C(H-7^b)], differing only in a small region of the mouse genome including the *Fv-2* locus, were used for this purpose. In both cases, molecular hybridization analysis indicated that the presence of SFFV-related RNA sequences in normal mice was associated with the *Fv-2^s* allele: bone marrow or spleen cellular RNA from *Fv-2^{rr}* B6 mice contained no detectable SFFV-related sequences, whereas their congeneic *Fv-2^{ss}* pairs contained relatively high levels of these RNA sequences. The absence of these RNA sequences in *Fv-2^{rr}* mice was not due to deletion of these sequences from the DNA of *Fv-2^{rr}* mice. Repopulation of lethally irradiated *Fv-2^{rr}* mice with syngeneic *Fv-2^{rr}* bone marrow cells did not lead to any increase in the levels of these SFFV-related RNA sequences, suggesting that the expression of these sequences is still reduced or inhibited in actively cycling *Fv-2^{rr}* hemopoietic cells. Infection with Friend leukemia virus resulted in the appearance of high levels of RNA homologous to SFFV-specific sequences in the leukemic spleens of B6.S (*Fv-2^{ss}*) mice, whereas these cellular RNA sequences could not be detected in the spleens of Friend virus-infected B6 (*Fv-2^{rr}*) mice. The demonstration that the same gene locus controls both the expression of exogenous SFFV-specific sequences and erythroleukemia induction by Friend leukemia virus suggests that these sequences may be necessary for erythroleukemic transformation. In addition, the finding that the *Fv-2* gene locus controls the expression of endogenous SFFV-related sequences suggests that these sequences may also be involved in normal hemopoiesis.**

The genomes of a number of avian and murine sarcoma viruses contain unique sequences involved in the rapid malignant transformation of fibroblasts (1–3). These sarcoma virus-specific sequences have been found to be present in normal host cell DNA (4, 5). In addition, sequences related to the *src* gene of Rous sarcoma virus appear to be transcribed (6) and translated (7, 8) in normal chicken embryo fibroblasts, although a role for these sequences in normal cell function has not yet been identified. The genome of the replication-defective component of the polycythemic strain of Friend leukemia virus (FLV), Friend spleen focus-forming virus (SFFV), has also been shown to contain specific sequences (9–11). These sequences are also present in normal mouse cell DNA (10) and are transcribed into RNA in a number of different adult mouse strains (10). Moreover, their expression is largely restricted to cells of the hemopoietic tissues in uninfected adult mice (10).

To gain further insight into the role of these SFFV-related sequences in normal cells, we have attempted to identify and characterize host gene loci that regulate the expression of these endogenous sequences. The *Fv-2* locus of the mouse (12), which is situated on chromosome 9, exerts a major effect on the response of mice to erythroleukemia induction by FLV (13). This gene locus has two alleles—a dominant sensitivity allele and a recessive resistance allele. It has been shown that *Fv-2* also controls the replication of the replication-defective SFFV component of FLV, whereas replication of and lymphatic leukemia induction by lymphatic leukemia-inducing viruses are not affected (14). Although the mechanism of action of the *Fv-2* locus is not known, the demonstration that *Fv-2* specifically influences the transformation process by FLV suggests that this locus may somehow regulate the expression of virus-specific sequences in infected mice. In this study, we have utilized a purified DNA probe, complementary to specific sequences on the Friend SFFV genome, to demonstrate that the *Fv-2* locus controls the expression of both endogenous and exogenous SFFV-specific sequences in normal and infected adult mice.

MATERIALS AND METHODS

Mice. Two pairs of mice, congeneic at the *Fv-2* locus, were used. Strain B6.S, congeneic with C57BL/6 (B6) mice, was obtained by serial intercrosses and backcrosses with the Friend virus-sensitive SIM strain (15). *B6* mice are *Fv-2^{rr}* whereas B6.S mice are *Fv-2^{ss}*. These mice were bred at the University of Toronto Animal Colony, Toronto, Canada. Strain B6.C(H-7^b) was made by substitution of the BALB/c *H-7* histocompatibility locus onto the B6 genetic background (16). Because *Fv-2* and *H-7* are closely linked, B6.C(H-7^b) also carries the *Fv-2^{ss}* alleles of BALB/c mice. This latter pair of congeneic mice were purchased from the Jackson Laboratory, as were C57BL/10 mice.

Extraction of Cellular RNA. Total cellular RNA was extracted from spleen and bone marrow cells by using described methods (10, 17).

Extraction of Spleen Cell DNA. Total spleen cell DNA was extracted from a single-cell suspension by a modified procedure described by Varmus *et al.* (18) and was prepared for DNA–DNA hybridization as described (10).

Abbreviations: FLV, Friend leukemia virus; SFFV, spleen focus-forming virus; B6, C57BL/6 (*Fv-2^{rr}*) mice; B6.S and B6.C, *Fv-2^{ss}* congeneic partners of B6(*Fv-2^{rr}*); C_rt, concentration of RNA in moles of nucleotides per liter × time in sec; C_0t, concentration of DNA in moles of nucleotides per liter × time in sec; $cDNA_{sff}$, purified single-stranded DNA complementary to specific sequences on the SFFV genome.

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Preparation of cDNA Specific for SFFV Genome ($cDNA_{sff}$). The preparation of $cDNA_{sff}$ has been described (9). A detailed description of the properties and distribution of $cDNA_{sff}$ has also been described (9, 10).

Hybridization Procedures. Molecular hybridization of cellular RNA and DNA to $cDNA_{sff}$ was performed as described (9, 10).

Virus. Virus was obtained from NIH/3T3 cells chronically infected with the polycythemic strain of FLV (17).

RESULTS

Expression of SFFV-Related RNA Sequences in *Fv-2* Congeneic Mice. To determine whether the *Fv-2* locus controls expression of SFFV-related RNA sequences in adult mice, RNA was extracted from the spleen and bone marrow cells of the *Fv-2* congeneic mouse strains B6 and B6.S and hybridized to $cDNA_{sff}$. As shown in Fig. 1, the homozygous $Fv\text{-}2^{ss}$ strain B6.S expressed high levels of SFFV-related RNA sequences in cells from the bone marrow and spleen, comparable to that observed in other $Fv\text{-}2^{ss}$ mice (10). The final extent of hybridization from experiment to experiment was between 40 and 60%. In contrast, no RNA sequences homologous to $cDNA_{sff}$ could be detected in bone marrow or spleen cells from congeneic $Fv\text{-}2^{rr}$ B6 mice.

As a further test of the hypothesis that the *Fv-2* locus controls the expression of endogenous SFFV-specific sequences, we next examined another pair of mice that were originally constructed to be congeneic at the *H-7* locus and subsequently shown to be congeneic also at the *Fv-2* locus (16). Total cellular RNA was extracted from spleen cells from the *Fv-2* congeneic mice, B6 and B6.C(H-7^b), and hybridized with $cDNA_{sff}$. As shown in Fig. 2, $Fv\text{-}2^{ss}$ B6.C(H-7^b) mice expressed high levels of RNA sequences related to $cDNA_{sff}$ in their spleen cells whereas the cellular RNA from spleen cells of $Fv\text{-}2^{rr}$ B6 mice did not contain any detectable sequences homologous to $cDNA_{sff}$. Fig. 2 also demonstrates that another $Fv\text{-}2^{rr}$ mouse strain, C57BL/10, was also found to contain no detectable SFFV-related RNA sequences in its spleen cells.

Presence of SFFV-Related Sequences in the DNA of B6 and B6.S Mice. Because no cellular RNA sequences homologous to $cDNA_{sff}$ were detected in $Fv\text{-}2^{rr}$ B6 mice, it was of interest to determine if these sequences were present in the DNA of B6 mice. Cellular DNA was extracted from spleen cells of the congeneic mice, B6 and B6.S, and hybridized to $cDNA_{sff}$.

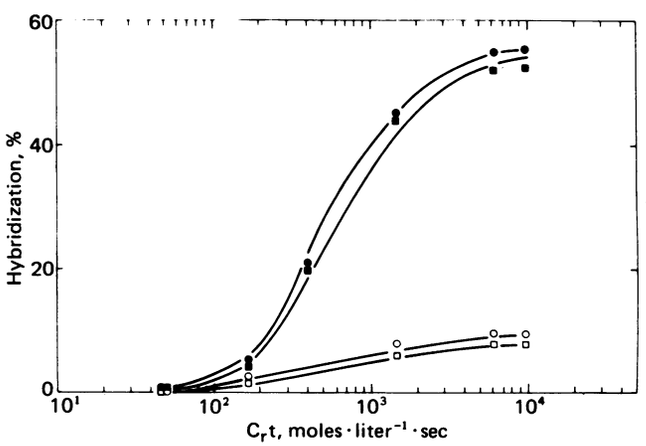

FIG. 1. Expression of SFFV-specific RNA sequences in bone marrow and spleen cells from congeneic B6 ($Fv\text{-}2^{rr}$) and B6.S ($Fv\text{-}2^{ss}$) mice. Total cellular RNA was extracted from uninfected bone marrow and spleen cells of B6 and B6.S mice and hybridized to 500 cpm of $cDNA_{sff}$. ●, B6.S spleen RNA; ■, B6.S bone marrow RNA; ○, B6 spleen RNA; □, B6 bone marrow RNA.

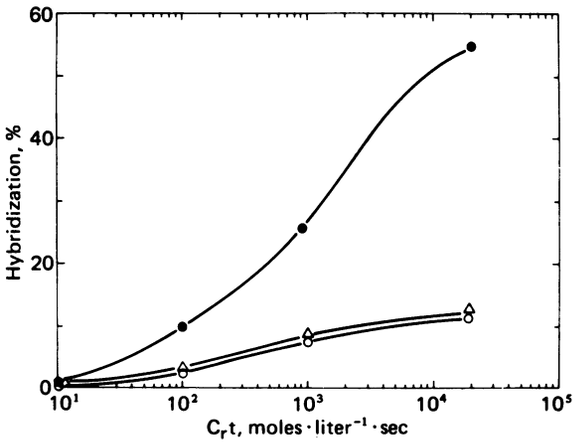

FIG. 2. Expression of SFFV-specific RNA sequences in spleen cells from C57BL/10 mice and congeneic B6 ($Fv\text{-}2^{rr}$) and B6.C(H-7^b)($Fv\text{-}2^{ss}$) mice. Total cellular RNA was extracted from uninfected spleen cells from B6 (○), B6.C(H-7^b) (●), and C57BL/10 (△) mice and hybridized to 500 cpm of $cDNA_{sff}$.

The results in Fig. 3 indicate that the kinetics and maximum percentage of hybridization of $cDNA_{sff}$ to DNA from $Fv\text{-}2^{rr}$ B6 mice were similar to that observed with DNA from $Fv\text{-}2^{ss}$ B6.S mice, indicating that both strains of mice contain the same level of sequences homologous to $cDNA_{sff}$. These data indicate that the absence of SFFV-related RNA sequences from the spleen and marrow cells of Fv-2^{rr} B6 mice was not due to deletion of these sequences from the B6 genome.

Absence of SFFV-Related RNA Sequences in Cycling B6 Hemopoietic Cells. The results presented above, demonstrating that the DNA but not RNA of $Fv\text{-}2^{rr}$ mice contain SFFV-related sequences, suggest that the *Fv-2* locus controls the steady-state levels of these cellular RNA sequences in hemopoietic cells of adult mice. Axelrad *et al.* (19), using *Fv-2* congeneic mice, have reported that the *Fv-2* locus is involved in the control of the cycling state of erythroid progenitor cells. To determine whether SFFV-related RNA sequences could be observed in hemopoietic cell populations from $Fv\text{-}2^{rr}$ mice that were in active cell cycle, B6 bone marrow cells were injected into lethally irradiated B6 hosts and cellular RNA from the regenerating spleens of these irradiated B6 hosts was examined for the presence of SFFV-related RNA sequences 3 and 8 days after repopulation with B6 bone marrow cells. As shown in Fig. 4, RNA from spleen cells of B6 mice 3 and 8 days after irradiation and repopulation with B6 bone marrow cells did not hybridize

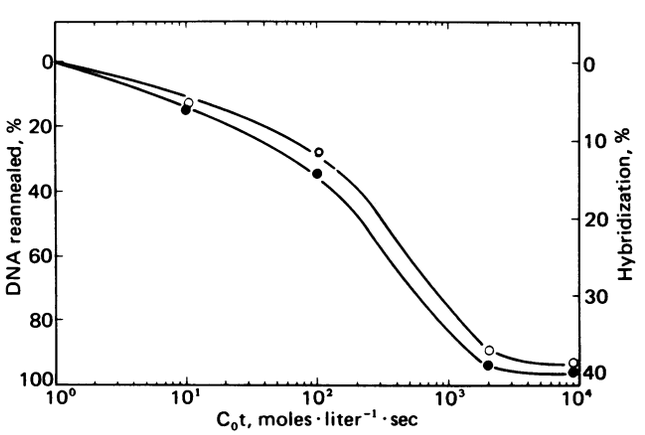

FIG. 3. Hybridization of $cDNA_{sff}$ to DNA of congeneic B6 and B6.S mice. DNA was extracted from spleen cells of uninfected B6 (○) and B6. S (●) mice and prepared for hybridization.

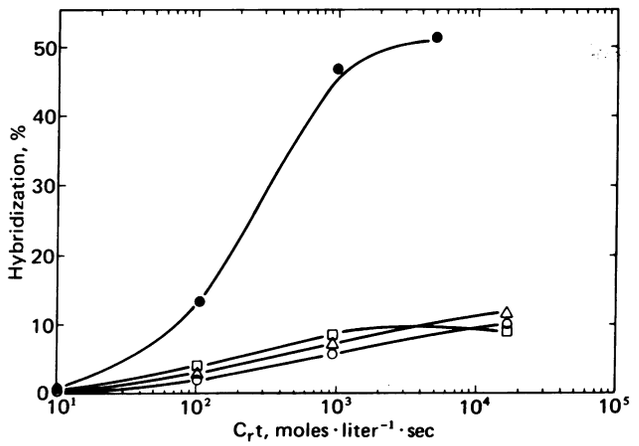

FIG. 4. Hybridization of $cDNA_{sff}$ to RNA from spleen cells of B6 mice 3 and 8 days after irradiation and repopulation with B6 marrow cells. B6 mice were irradiated with 950 rads (1 rad = 0.01 J/kg) and then injected with a single-cell suspension of 1×10^7 B6 bone marrow cells. After 3 (△) and 8 (□) days, spleen cellular RNA was extracted and hybridized to 500 cpm of $cDNA_{sff}$. As controls, spleen cellular RNA from B6 (○) and B6.S (●) mice was also hybridized to $cDNA_{sff}$.

to $cDNA_{sff}$. These results indicate that altering the cycle status of B6 *Fv-2*^{rr} hemopoietic cells did not result in detectable levels of SFFV-related RNA sequences.

Expression of SFFV-Specific Sequences in Friend SFFV-Infected B6 and B6.S Mice. The above observations indicate that the *Fv-2* locus controls the expression of endogenous SFFV-related RNA sequences in uninfected mice. This observation, together with the finding that the induction of erythroleukemia is greatly reduced in *Fv-2*^{rr} mice compared to their partially or fully congeneic *Fv-2*^{ss} pairs (14), suggests that the *Fv-2* locus may control the expression of exogenous SFFV viral RNA. To test this hypothesis, cellular RNA was extracted from spleen cells of B6 and B6.S mice infected with high-titer FLV and hybridized to $cDNA_{sff}$. As shown in Fig. 5, spleen cellular RNA from FLV-infected B6.S mice contained high levels of SFFV-specific sequences ($C_rt_{1/2} \approx 60$), characteristic of spleens from mice infected with the polycythemic strain of FLV (10), whereas spleen cellular RNA from the resistant B6 mice infected with virus containing a high titer of SFFV contained low levels of these sequences. A summary of this data, as well as that shown in Figs. 1 and 2, is presented in Table 1.

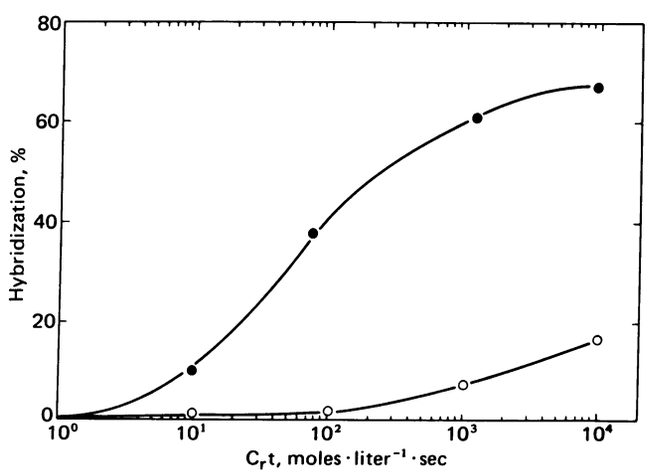

FIG. 5. Hybridization of $cDNA_{sff}$ to spleen cellular RNA from SFFV-infected B6 and B6.S mice. B6 and B6.S mice were infected with 2×10^3 focus-forming units of a NB-tropic stock of Friend virus complex harvested from NIH/3T3 cells (16). Eight days later, their spleens were removed and RNA was extracted. ○, B6; ●, B6.S.

Table 1. Levels of SFFV-specific RNA sequences in infected and uninfected B6 (*Fv-2*^{rr}) and B6.S (*Fv-2*^{ss}) mice

Source of RNA	$C_rt_{1/2}$ B6	$C_rt_{1/2}$ B6.S
Uninfected		
Marrow	$>10^4$	500
Spleen	$>10^4$	500
Infected spleen	$>10^4$	60

$C_rt_{1/2}$ values, the C_rt values necessary for 50% hybridization, were calculated from the data in Figs. 1 and 5.

DISCUSSION

The results presented in this report indicate that the *Fv-2* locus of the mouse, a major genetic determinant governing erythroleukemia induction by Friend virus, controls the expression of SFFV-related sequences in the bone marrow and spleen cell RNA of uninfected mice. In addition, data is also presented indicating that the *Fv-2* locus also controls the expression of SFFV-specific RNA sequences in the hemopoietic tissues of mice infected with FLV. These observations suggest that the *Fv-2* locus plays a regulatory role in both uninfected and infected mice.

Although it is likely that rapidly transforming leukemia viruses such as Friend erythroleukemia virus bear specific genetic determinants for transformation, direct evidence that SFFV-specific sequences are involved in leukemia transformation is not available. However, the present findings, demonstrating that the *Fv-2* locus controls both the expression of SFFV-specific sequences in FLV-infected mice as well as susceptibility to erythroleukemia induction by Friend SFFV, suggest that the expression of these sequences may indeed be involved in the induction of erythroleukemia by the polycythemic strain of FLV.

Our results indicate that the *Fv-2* locus also affects endogenous SFFV-related sequences in uninfected mice. The finding that hemopoietic cells from homozygous *Fv-2*^{rr} mice contain no detectable SFFV-related RNA sequences could be the result of deletion of these sequences from the DNA of these mice. However, because DNA from both B6 (*Fv-2*^{rr}) and B6.S (*Fv-2*^{ss}) mice contained these SFFV-related sequences, we conclude that the *Fv-2* locus does not specify these DNA sequences but regulates their expression.

The conclusion that *Fv-2* is involved in the regulation of gene expression in normal uninfected cells is strengthened by two additional observations: First, Blank *et al.* (20) were unable to observe a fully congeneic *Fv-2*^{rr} mouse strain on a DBA/2 genetic background and have therefore suggested (21) that the presence of at least one dominant *Fv-2*^{s} allele may be required for normal embryological development in this mouse strain. Second, Axelrad *et al.* (19) have shown, using *Fv-2* congeneic mice, that the *Fv-2* locus is involved in the control of proliferation of erythroid progenitor cells in these mice. Thus, if the major effect of *Fv-2* is to regulate the expression of endogenous SFFV-related sequences, then these findings suggest that the expression of these cellular sequences may be necessary for both embryological development and adult hemopoiesis in *Fv-2*^{s} mice.

The observation that the *Fv-2* locus controls the expression of endogenous SFFV-related RNA sequences is also consistent with recent observations by Risser (22) who has demonstrated, using an alloantiserum raised against SFFV-infected spleen

cells, that the *Fv-2* locus also regulates the presence of a SFFV-specified cell surface antigen on normal hemopoietic cells. This observation, together with the finding that the tissue distribution of both this surface antigen (22) and SFFV-related sequences (10) in normal uninfected mice follows a similar pattern, suggests that they may be related gene products.

Infection of susceptible mice with the polycythemic strain of FLV leads to the rapid appearance of large numbers of erythroid progenitor cells that can proliferate and differentiate in the absence of erythropoietin (23). Although the appearance of these erythropoietin-independent progenitor cells has been considered to be characteristic of this disease, recent observations have suggested that normal fetal hemopoietic cells can also undergo extensive erythroid differentiation in the absence of added erythropoietin (24). In addition, FLV-infected hemopoietic cells are still subject to the regulatory influences of the *steel* locus (25), a major host locus governing the proliferation of normal hemopoietic stem cells (26). These results suggest that infection with the polycythemic strain of FLV may result in the amplification of existing genetic mechanisms of erythropoiesis. This conclusion is consistent with our previous findings that normal hemopoietic cells selectively express SFFV-related RNA sequences at a level approximately 1/50th of that observed in SFFV-infected leukemic spleen cells (10). The present findings demonstrating that the expression of these sequences is regulated by the host *Fv-2* gene locus in both normal and infected animals further support the hypothesis that these sequences are involved in normal as well as leukemic hemopoiesis.

We thank D. Penrose, V. Crichley, G. Cheong, and H. Van der Gaag for their excellent technical assistance and Dr. R. Risser for discussions during the course of this work. This work is supported by grants from the National Cancer Institute of Canada and the Medical Research Council of Canada.

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