FRIEND ERYTHROLEUKEMIA ANTIGEN
A Viral Antigen Specified by Spleen Focus-Forming Virus
and Differentiation Antigen Controlled by the \textit{Fv}-2 Locus*

BY REX RISSE

From the McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, Wisconsin 53706

Since its isolation in 1957 by C. Friend (1), the Friend virus complex (FV)\(^1\) has provided tumor virologists and immunologists with a useful system for study of the pathogenetic mechanisms that regulate murine leukemia (2). The biphasic nature of Friend disease, consisting of a rapid, frequently fatal, erythroblastic leukemia followed in survivors by a later lymphocytic neoplasm (3), led to the identification of two viral genomes in FV (4). The early erythroleukemic phase of FV disease is effected by a defective virus genome, termed spleen focus-forming virus (SFFV) because it can be assayed by the induction of splenic foci of proerythroblast proliferation 9 d postinfection (5). The later lymphocytic phase of FV disease is effected by a nondefective helper virus, now most commonly referred to as Friend-murine leukemia virus (F-MuLV) (6). Genetic studies of the susceptibility of mice to FV established that the two phases of FV disease are controlled by at least two independent loci (7-13). The \textit{Fv}-1 locus, which has \textit{n}-alleles that permit replication of \textit{N}-tropic but restrict replication of \textit{B}-tropic MuLV or \textit{b}-alleles that act conversely, affects both phases of FV disease when \textit{N}-tropic or \textit{B}-tropic MuLV strains are used as helper viruses (12-14). The \textit{Fv}-2 locus, at which sensitivity alleles are dominant to resistance alleles (11), affects only focus formation by SFFV. The mode of action of \textit{Fv}-2 is not yet clear; effects have been observed on SFFV replication (15) and on the proliferative status of erythroid target cells (16).

Serologic studies of FV first demonstrated the utility of mouse typing serum in the analysis of murine leukemias (17, 18). Antibody raised in C57BL/6 (B6) mice to syngeneic FV-induced lymphocytic tumors, or in BALB/c mice to \textit{N}-tropic FV, did not cross-react with spontaneous AKR lymphomas or Gross MuLV lymphomas, whereas the sera did cross-react with lymphomas induced by the Rauscher or Moloney MuLV strains (17, 18), hence, the term Friend-Moloney-Rauscher (FMR) antigen. A similar cross-reactivity among the FMR viruses was found by transplantation tests (19). Proteins assigned to the \textit{gag-} or \textit{env-} gene regions have been implicated as FMR-bearing molecules (20, 21); it is hoped that absorption of FMR typing antibody (18) with purified viral proteins will resolve this apparent discrepancy. Serologic identification of cell-surface antigens exclusively associated with SFFV has not yet succeeded,

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1 Abbreviations used in this paper: B6, C57BL/6; FE, Friend erythroleukemia; FEC, Friend erythroleukemic spleen cells; FFU, focus-forming units; FMR, Friend-Moloney-Rauscher; F-MuLV, Friend-murine leukemia virus; FV, Friend virus complex; MCF, mink cell focus-inducing; SFFV, spleen focus-forming virus.
though studies of cell-mediated cytotoxicity to tissue culture cells infected with SFFV or F-MuLV suggest such antigens exist (22). Furthermore, studies using heteroantibody indicate that SFFV shares antigenic determinants with env gene products of recombinant mink cell focus-inducing (MCF) FMR MuLV strains (23, 24).

Our recent serologic detection of a viral tumor antigen specified by the Abelson MuLV defective transforming genome (25), and the similarities of SFFV to the defective Abelson genome in terms of genome structure (6, 26), rapid oncogenic action (5, 27), and genetic control of susceptibility (11, 28) prompted us to attempt a similar analysis of SFFV. In so doing, we have been cognizant of the fact that Abelson antigen also occurs as a differentiation antigen on bone marrow of uninfected susceptible mice (25); indeed, mice that normally express this determinant do not make cytotoxic anti-Abelson antigen antibodies after immunization with Abelson MuLV tumor cells (unpublished observations). If the putative SFFV antigen were also a differentiation antigen of susceptible mice, then cytotoxic antibody to SFFV determinants might be expected only in Fv-2"/" mice.

Methods and Materials

Mice. Mice (BALB/cN, C57BL/6N, or NFS/N) were bred in our colony. The partially congeneric D2.RB strain (87.5% DBA/2, but carrying the Fv-2"/" gene of C57BL/6) (29) was obtained from Dr. F. Lilly and Dr. R. Steeves, Albert Einstein College of Medicine, N. Y. The congenic B6.S strain (B6-genetic background carrying the Fv-2"/" gene of SIM) (9) was obtained from Dr. A. Axelrad, University of Toronto, Ontario. All other mice were purchased from The Jackson Laboratory, Bar Harbor, Maine.

Cell Lines and Viruses. The ecotropic F-MuLV strain 201 (30) and dual-tropic Fr-MCF-1 MuLV strain (23) were obtained from Dr. D. Troxler, National Cancer Institute, Bethesda, Md. Dr. Troxler also provided the SFFV-nonproducer cells; SFFV-NRK C1 1, SFFV-NIH 3T3, and SFFV-FRE (31). All other tissue culture cell lines and viruses were originally from the collection of Dr. J. Hartley and Dr. W. P. Rowe, National Institute of Allergy and Infectious Diseases, Bethesda, Md., and have been maintained in this laboratory as described in earlier publications (32, 33). Ecotropic MuLV were propagated on SC-1 cells and titered by the XC-plaque test (34); dual-tropic MuLV were propagated in SC-1 cells and titered by fluorescent-antibody methods (35); xenotropic MuLV were propagated in mink cells (CCL64) and titered by fluorescent-antibody methods (35). Tissue culture cells were harvested for absorption tests according to published procedures (36).

The animal passage strains of FV were obtained from Dr. F. Lilly, Albert Einstein College of Medicine, N. Y. NB-tropic and B-tropic FV strains were propagated and titered in BALB/c mice, and N-tropic FV was propagated and titered in DBA/2 mice as described in earlier publications (5, 10, 14). Rauscher virus complex was provided by Dr. R. Steeves.

Tumors and Target Cells. The transplantable tumors RADA1, AKSL2, ASLI, Ec~G2, ERLD, Meth A, RL51, MOPC-70A, and BALB RV1 were obtained from Dr. E. Stoeckert and Dr. L. J. Old, Memorial Sloan-Kettering Cancer Center, N. Y.; the origin and maintenance of these tumors has been described (37). The B6 F-MuLV lymphoma FBL-3 and the B6 R-MuLV lymphoma RBL-5 were obtained from Dr. A. Fefef, University of Washington, Seattle, Wash. (19). The derivation and maintenance of the B6 Abelson MuLV lymphomas B6T1 and B6T3 were described in a previous publication from this laboratory (25). The transplantable A-MuLV lymphoma, CT1, was derived from the pooled lymphomatous tissue of the paravertebral region and enlarged lymph nodes of a 2½-mo-old female BALB/c mouse injected 50 d earlier with 10^3.5 focus-forming units (FFU) of cloned Abelson MuLV. The tumor is maintained by ascites transfer of 10^7 cells/week in irradiated BALB/c mice.

Tumors of the B6FR series were induced in B6 mice by intraperitoneal injection of 10^3.5 FFU of NB-tropic FV into neonates. The latent period for tumor appearance ranged from 4½ to 7½ mo, and each tumor was diagnosed as splenic lymphoma. None of these tumors, established from lymphomatous spleens, produced SFFV, though all tumors produced high titers of F-
Friend erythroleukemic spleen cells were obtained by perfusion with medium 199 of the massively enlarged spleens of mice infected 10–12 d earlier with 10^3.5 FFU of the appropriate FV strain. Cells obtained in this manner were 85–95% viable as judged by trypan blue exclusion. The target Friend erythroleukemic spleen cells (FEC) for the cytotoxic assay were obtained from the spleens of (C3H/HeJ × B6)F1 mice infected 10–12 d earlier with 10^16 FFU of NB-tropic FV.

Antisera. BALB/c anti-FV serum was prepared by intraperitoneal injection into 10-wk-old female BALB/c mice of 10^2 FFU of N-tropic FV followed by injection of increasing doses of NB-tropic FV (17). B6 anti-FV serum was obtained after six intravenous injections of 10^16 FFU of NB-tropic FV into female B6 mice at bimonthly intervals. B6 anti-RBL-5 serum was prepared by hyperimmunization of 10-wk-old B6 mice with living RBL-5 cells according to the immunization schedule detailed in our previous publication (25). Mice were bled from the tail vein in alternative weeks, and serum from each bleeding was pooled. Mice were reimmunized 3–4 d after bleeding.

Cytotoxic and Absorption Tests. Direct cytotoxic tests were performed, as previously described (25, 37), by incubation of equal volumes (50 μl) of 2.5 × 10^6 FEC, diluted antiserum and appropriately diluted rabbit serum (C source) at 37°C for 45 min. Viability counts were made in the presence of trypan blue. Preselected rabbit serum was used at a dilution of 1:5 or 1:7 in all tests with transplantable lymphoma cells (C control gave 10% lysis) and at a dilution of 1:15 in all tests with FEC (C control gave 13% lysis).

Antibodies that react with helper F-MuLV determinants were removed from B6 anti-FV serum by absorption of 2 vol of antiserum, four- to sixfold more concentrated than that which gave 50% FEC lysis (generally a dilution of 1/20–1/40) with 1 vol of packed B6FRδ2 cells at 4°C for 30 min. Cells were removed by centrifugation at 1,000 g for 10 min, and this FMR-preabsorbed antiserum was then used for typing of Friend Erythroleukemia antigen by a second absorption test. B6FRδ2-preabsorbed antiserum (50 μl) was absorbed a second time with a range of counted numbers of cells for 30 min at 4°C, and the residual cytotoxic activity for FEC was determined in direct cytotoxic tests. Cytotoxicity is calculated according to the formula: (absorbed (percent lysis) − C)/100 − C.

Results

Sero logical Reactivities of Anti-FV Antisera. The Fv-2 resistance gene of B6 mice absolutely inhibits the induction of erythroleukemic colonies by FV (11), but only partially inhibits SFFV replication (15). Thus, it follows that cytotoxic antibodies to SFFV-specific antigens might be produced in B6 mice. After hyperimunization with NB-tropic FV, B6 mice produced antibodies cytotoxic for (C3H/He × B6)F1 FEC and for cells of the B6 F-MuLV lymphoma B6FRδ2 (Fig. 1). The sensitivity of B6FRδ2 cells, as well as cells from several other primary or transplanted F-MuLV lymphomas, to lysis by B6 anti-FV serum was less than that of FEC. To distinguish a quantitative difference in the amount of antigen expressed by these two target cells from a qualitative difference in the determinants expressed, absorption experiments were carried out.

Absorption of B6 anti-FV serum with B6FRδ2 cells completely removed cytotoxic activity for B6FRδ2 but not FEC (Fig. 1). The reactivity of B6 anti-FV serum for B6FRδ2 but not FEC, was also removed by absorption with SC-1 tissue culture cells productively infected with NB-tropic F-MuLV (Fig. 1). Absorption with FEC, on the other hand, removed all cytotoxic activity for both target cells (Fig. 1). To exclude the possibility that these results were due solely to the greater sensitivity of FEC to
Figure 1. Direct cytotoxic tests of B6 anti-FV serum on FEC and B6FRd2 cells. Antiserum diluted 1/10 was absorbed with an equal volume of packed cells and then titered on the two target cells. FEC express an antigen(s) not expressed on B6FRd2 cells. Test cells: FEC; □ B6FRd2.

Table I

| Antiserum              | No. mice immunized | Serum dilution* | Cytotoxicity for FEC after B6FRd2 absorption‡ | FE antigen reactivity |
|------------------------|--------------------|-----------------|---------------------------------------------|----------------------|
| BALB/c anti-FV         | 10                 | 1/120           | 18                                          | -                    |
| B6 anti-RBL-5          | 5                  | 1/40            | 12                                          | -                    |
| B6 anti-FV No. 1       | 6                  | 1/160           | 40                                          | +                    |
| B6 anti-FV No. 2       | 18                 | 1/200           | 55                                          | +                    |

* The dilution of antiserum that resulted in 50% lysis of FEC in direct cytotoxic tests.
‡ BALB/c anti-FV serum, diluted 1/20; B6 anti-RBL-5 serum, diluted 1/10; and B6 anti-FV sera, diluted 1/20, were each absorbed with 1/2 vol of packed B6FRd2 cells, and the residual cytotoxicity for FEC was then determined in direct cytotoxic tests. Control registered 13% lysis.

Earlier serologic studies of the FMR antigen(s) indicated that the antigens detected on lymphoma and erythroleukemia cells were entirely cross-reactive (17–21). Those studies differ from the present one in two important aspects: (a) anti-FV sera, which might be expected to recognize SFFV antigens, were prepared in Fe-2s mice, primarily BALB/c (17, 20); and (b) when antisera were prepared in B6 mice, FMR MuLV lymphomas, which lack SFFV, were used for immunization (18, 19). Therefore, we have tested two antisera, representative of earlier FMR-typing sera (17, 18), in our test system. The data in Table I indicate that antibody cytotoxic for FEC can be completely removed from B6 anti-RBL-5 (a Rauscher MuLV lymphoma) or BALB/
FRIEND ERYTHROLEUKEMIA ANTIGEN

FIG. 2. Quantitative absorption tests of B6 anti-FV serum. 50 μl antiserum, diluted 1/30, was absorbed with increasing numbers of the indicated cells, and the cytotoxicity remaining for FEC was then determined in direct cytotoxic tests. FEC and SFFV-NIH absorb all cytotoxic activity for FEC, whereas FMR cell lines show only partial absorption.

c anti-FV antisera by absorption with B6FRd2; these results are thus consistent with previous studies (17–19). However, sera from two groups of FV-hyperimmunized B6 mice contain antibody cytotoxic for FEC that cannot be removed by absorption with B6FRd2. It should be noted that absorptions of B6 or BALB/c anti-FV antisera were performed at dilutions of 1/30 or 1/20, respectively, so that results from Table I might be compared to results from earlier studies (20, 21). However, later sera from 2 of the 10 individual BALB/c mice tested showed a residual cytotoxic activity specific for FEC when sera were absorbed at 1/7. This low titer antibody does not have the alloantigen reactivity described below and has not been further analyzed.

The results presented thus far indicate that two cytotoxic antibodies are present in B6 anti-FV serum; one directed to determinants shared by FEC and B6FRd2, and the other directed to determinants expressed only on FEC.

Analysis of B6 Anti-FV Serum by Absorption with Primary and Transplantable Mouse Tumors: Serological Definition of Friend Erythroleukemia (FE) Antigen. The serological reactivities of B6 anti-FV antiserum were analyzed by quantitative absorption tests with a number of tumors. Cells from a spontaneous AKR thymoma absorbed no cytotoxic activity, whereas absorption with FMR lymphoma cells or tissue culture cells producing F-MuLV removed 40–50% of the cytotoxic activity for FEC (Fig. 2). To exclude the possibility that the partial absorption shown by FMR tumors reflected a lower concentration of antigen expressed on these tumors compared with FEC, a second quantitative absorption experiment was performed with a number of FMR lymphomas using B6FRd2-preabsorbed B6 anti-FV antiserum. The results of this analysis, presented in Table II and Fig. 3, indicate that a second absorption of B6FRd2-preabsorbed serum with FMR lymphomas did not further reduce cytotoxicity for FEC. Erythroleukemias induced by SFFV, whether of FV or Rauscher virus origin, completely absorbed cytotoxic activity for FEC. This consistent pattern of reactivity suggests that a single antigenic system is detected by the cytotoxic activity of B6FRd2-
**Table II**

*FE Antigen Phenotypes of FMR MuLV-Induced Tumors*

| Tumor     | Strain of origin | Virus used for induction | SFFV produced* | FE antigen cytotoxicity† |
|-----------|------------------|--------------------------|----------------|-------------------------|
| Lymphomas |                  |                          |                |                         |
| CT1       | BALB/c           | Abelson virus            | ND             | 50/55                   |
| B6FR1     | B6               | NB-tropic FV             | 0              | 35/39                   |
| B6FR2     | B6               | NB-tropic FV             | 0              | 50/45                   |
| B6FR3     | B6               | NB-tropic FV             | 0              | 47/45                   |
| Primary   | B6               | NB-tropic FV             | 0              | 56/66                   |
| FBL-3     | B6               | NB-tropic FV             | 0              | 42/43                   |
| Primary   | B6               | M-MuLV                   | ND             | 49/39                   |
| Primary   | B6               | M-MuLV                   | ND             | 52/55                   |
| B6Mo3     | B6               | M-MuLV                   | ND             | 41/45                   |
| RBL-5     | B6               | Rauscher virus           | 0              | 61/55                   |
| B6T1      | B6               | Abelson virus            | ND             | 54/55                   |
| B6T3      | B6               | Abelson virus            | ND             | 65/55                   |
| Primary   | SWR              | Cloned F-MuLV            | 0              | 35/43                   |
| Primary   | SWR              | Cloned F-MuLV            | 0              | 39/66                   |
| Primary   | SWR              | F-MuLV clone 201         | 0              | 49/66                   |
| Primary   | SWR              | F-MuLV clone 201         | 0              | 54/66                   |
| Erythroleukemias |   |                          |                |                         |
| Primary   | A/J              | B-tropic FV              | ≥10⁴           | 4/90†                   |
| Primary   | BALB             | B-tropic FV              | ≥10⁴           | 10/90                   |
| Primary   | DBA/2            | N-tropic FV              | ≥10⁴           | 10/90                   |
| Primary   | SWR              | N-tropic FV              | ≥10⁴           | 0/90                    |
| Primary   | A/J              | NB-tropic FV             | ≥10⁴           | 0/90                    |
| Primary   | BALB/c           | NB-tropic FV             | ≥10⁴           | 0/90                    |
| Primary   | (BALB/c × B6)F₁ | NB-tropic FV             | ≥10⁴           | 0/90                    |
| Primary   | (C3H × B6)F₁     | NB-tropic FV             | ≥10⁴           | 0/90                    |
| Primary   | SWR              | NB-tropic FV             | ≥10⁴           | 0/90                    |
| Primary   | BALB/c           | Rauscher virus           | ND             | 0/90                    |
| Primary   | (C3H × B6)F₁     | Rauscher virus           | ND             | 0/90                    |

* When tumors were harvested for serological analysis, a 10% wt/vol extract was prepared (11) and titered for SFFV (5). Units are in spleen foci/ml of extract.

† The expression of FE antigen was determined by quantitative absorption tests of FE typing serum (B6FRd2 preabsorbed B6 anti-FV antiserum) with counted numbers of cells of the indicated tumor. The cytotoxic activity remaining for FEC after absorption of FE typing serum (50 μl) with 2 × 10⁷ cells of the indicated tumor (test %) is to be compared with the cytotoxic activity of FE typing serum without a second absorption as determined in the same test (control %). No FMR lymphoma removes cytotoxic antibody from FE typing serum.

§ As it was anticipated that FV erythroleukemias would express FMR antigen (17), unabsorbed B6 anti-FV serum (50 μl), diluted from 1/20 to 1/40, was absorbed with 2 × 10⁷ erythroleukemia cells and the residual cytotoxic activity for FEC determined. FV or Rauscher virus erythroleukemias remove all cytotoxic antibody for FEC from B6 anti-FV serum. We shall refer to the antigen detected by this test system as FE antigen to clearly distinguish it from other FV-related determinants.

We have examined the expression of FE antigen on a number of transplantable tumors of the mouse by quantitative absorption tests (Table III). None of these tumors remove cytotoxic activity from FE typing serum, i.e. B6FRd2 preabsorbed B6 anti-FV antiserum; therefore they do not express this antigen. From the known antigenic phenotypes of this panel of leukemia cells, we conclude that FE antigen is serologically unrelated to the previously defined cell surface alloantigens or MuLV-related antigens.
Table III
FE Antigen Phenotypes of Transplanted Tumors of the Mouse

| Tumor            | Strain of origin | Tumor description                  | FE antigen cytotoxicity |
|------------------|------------------|------------------------------------|------------------------|
| ASL1             | A/J              | Spontaneous lymphoma               | 45/45                  |
| RADA1            | A/J              | X-ray lymphoma                     | 51/45                  |
| Primary          | AKR              | Spontaneous lymphoma               | 33/39                  |
| Primary          | AKR              | Spontaneous lymphoma               | 67/55                  |
| AKSL2            | AKR              | Spontaneous lymphoma               | 52/55                  |
| Meth A           | BALB/c           | Methylcholangiathrene sarcoma      | 37/39                  |
| MOPC-70A         | BALB/c           | Mineral oil plasmacytoma           | 35/46                  |
| RL21             | BALB/c           | X-ray lymphoma                     | 37/45                  |
| BALB RV1         | BALB/c           | Rad LV lymphoma                    | 45/45                  |
| EL4              | B6               | DMBA lymphoma                      | 46/45                  |
| EFG2             | B6               | Gross MuLV lymphoma                | 33/45                  |
| ERLD             | B6               | X-ray lymphoma                     | 39/46                  |
| Primary          | SJL              | Reticulum cell sarcoma             | 38/45                  |
| Primary          | SJL              | Reticulum cell sarcoma             | 38/45                  |
| Primary          | SJL              | Reticulum cell sarcoma             | 48/58                  |

* See footnote of Table II for methods. None of this panel of transplanted or primary mouse tumors expresses FE antigen.

In view of the syngeneic typing system, i.e., B6 antiserum on (C3H × B6)F1 cells, and lack of serological cross-reactivity between FMR typing serum and naturally occurring MuLV (17, 18), this result is not surprising.

Expression of FE Antigen on MuLV-Infected Tissue Culture Cells. The ability of MuLV strains to induce FE antigen expression on tissue culture cells was determined by absorption of FE typing serum with productively infected cells (36). Endogenous ecotropic or xenotropic MuLV strains do not induce the appearance of this determinant (Table IV). Antigens induced by the two dualtropic viruses MCF 247 and Fr MCF-1, which were isolated from a preleukemic AKR thymus (38) and a F-MuLV lymphoma (23), respectively, do not react with FE typing serum. The failure of endogenous ecotropic, xenotropic, or MCF-recombinant MuLVS (23, 38) to absorb
**Table IV**

*Induction of FE Antigen by Infection with MuLV In Vitro*

| MuLV strain       | Virus titer* | FE antigen cytotoxicity† |
|-------------------|--------------|--------------------------|
|                   | Infectious U/ml | Test %/control %          |
| **Ecotropic MuLV**|              |                          |
| Uninfected SC-1   | 0            | 39/45                    |
| AKR-L1            | 6.4          | 57/55                    |
| WN1802 N          | 5.8          | 61/55                    |
| DBA/2 N-tropic    | 6.0          | 41/45                    |
| B6 N-tropic       | 5.7          | 43/45                    |
| M-MuLV            | 5.6          | 63/55                    |
| F-MuLV            | 6.4          | 61/55                    |
| **Xenotropic MuLV**|              |                          |
| Uninfected Mink   | 0            | 44/51                    |
| NZB-IU            | 5.2          | 29/35                    |
| BALB-IU           | 5.6          | 45/35                    |
| DBA-IU            | 4.8          | 56/51                    |
| **Dualtropic MuLV**|              |                          |
| MCF 247           | 5.3          | 33/39                    |
| Fr MCF-1          | 5.2          | 49/45                    |
| **SFFV nonproducer cells** | |                          |
| Uninfected NIH 3T3| ND           | 57/51                    |
| SFFV-NIH          | 0            | 0/51                     |
| Uninfected NRK    | ND           | 51/51                    |
| SFFV-NRK          | 0            | 6/51                     |
| SFFV-FRE          | 0            | 0/51                     |

* At the time cells were harvested for absorption, supernatant fluids were collected and titered for ecotropic MuLV (34), xenotropic MuLV (35), dualtropic MuLV (35), or SFFV (5).

† See footnote| of Table II. SFFV nonproducer cells but not ecotropic, xenotropic, or dualtropic MuLV-infected cells express FE antigen.

the cytotoxic reactivity of FE typing serum establishes the viral specificity of this antigenic system.

Recently, mouse and rat nonproducer cell lines have been established after FV infection of cells in vitro (31, 39). Biological and biochemical studies have demonstrated that these lines carry only the SFFV genome and morphologically resemble the parental cell lines from which they were established (31, 39). Results from quantitative absorption experiments indicate that the nonproducer cell SFFV-NRK expresses approximately the same quantity of FE antigen as FEC cells (Fig. 3). Similar results were observed with the other two SFFV nonproducer cell lines (Table IV). It should be noted that SFFV-NIH also apparently expresses the FMR determinant detected by unabsorbed B6 anti-FV serum because absorption of B6 anti-FV antiserum with SFFV-NIH removed all cytotoxic activity for FEC (Fig. 2). A similar specification of FMR antigen(s) by the Abelson defective genome was observed in studies of Abelson MuLV nonproducer cells (25). The serologic evidence presented so far defines a previously unrecognized viral cell-surface antigen of mouse leukemia, FE antigen. Expression of FE antigen is limited to erythroleukemias induced by, or cell lines infected with SFFV.

Expression of FE Antigen on Tissues of Uninfected Mice. The appearance of FE antigen in hematopoietic tissues of uninfected mice was determined by quantitative absorption
tests with FE typing serum. Tissues of B6 and C57L mice uniformly typed negative for FE antigen expression. Bone marrow and spleen of BALB/c, DBA/2, SWR, and AKR mice were clearly positive; thymus, peripheral blood, and lymph node of these strains were negative (Fig. 4). BALB/c and SWR, but not B6 mice, express FE on fetal liver; the absorptive capacity of this tissue is about threefold greater than that of bone marrow (Fig. 4). Adult liver or kidney was negative in these three strains. Direct cytotoxic tests with FE typing serum did not detect significant lysis of BALB/c or SWR fetal liver, bone marrow, spleen, thymus, or lymph node cells. The absorptive capacity of fetal liver from uninfected FE-positive mice is ~3% that of FEC; the absorptive capacity of bone marrow is ~1% that of FEC; and positive spleen cells from uninfected mice have ~0.2% the absorptive capacity of FEC. Thus, results from quantitative absorption experiments and direct cytotoxic tests are consistent with the expression of FE by a minor cell population present in bone marrow, fetal liver, and spleen. Because cell-surface expression of FE in uninfected mice is a characteristic of only certain differentiated tissues, it is appropriate to also describe this determinant as a differentiation antigen.

Genetic Control of FE Antigen in Uninfected Mice: Concordance of Antigen Expression with Inheritance of the Fv-2<sup>s</sup> Virus Sensitivity Allele. We have typed bone marrow of several inbred strains for expression of FE by quantitative absorption tests. Only mice of the C57-C58 family were negative; all other inbred strains express FE antigen on bone marrow (Table V). This strain distribution profile is reminiscent of that observed for the Fv-2 locus (40). Therefore, we have tested BALB/c × B6 recombinant inbred strains, i.e., the seven strains generated by Bailey (41) by inbreeding BALB/c × B6 F<sub>2</sub> mice, for expression of FE antigen. Again, expression of FE antigen was concordant with inheritance of the Fv-2<sup>s</sup> allele (Table V) (42).

Four congenic strains that have substituted Fv-2 regions were tested for expression of FE antigen. The strains B10.C(47N) and B6.C-H-7<sup>b</sup> were made by substitution of the BALB/c H-7 histocompatibility locus onto the respective C57BL/10 or B6 genetic backgrounds (43, 44). Because H-7 is tightly linked to Fv-2, these strains also carry the
Expression of FE Antigen on Bone Marrow from Inbred, Recombinant Inbred, and Congenic Mouse Strains

| Inbred Strains          | $Fv-2^s$ strains | $Fv-2^r$ strains |
|-------------------------|-----------------|-----------------|
| A/J                     | 0/35           | DBA/2           |
| AKR                     | 0/56           | NZB             |
| BALB/c                  | 0/52           | NFS             |
| CBA/J                   | 8/66           | SJL             |
| CE/J                    | 0/35           | SWR             |
| C3H/HeJ                 | 1/35           | 129             |

| Recombinant Inbred Strains |
|----------------------------|
| CXB D                     | 8/52           |
| CXB E                     | 5/32           |
| CXB G                     | 7/52           |
| CXB J                     | 7/45           |
| CXB K                     | 8/32           |

| Congenic Strains          |
|----------------------------|
| B10.C(47N)                | 0/58           |
| B6.C-H-7.7$^+$            | 0/58           |
| B6.S                      | 1/42           |

* The numbers indicate the (test %/control %) cytotoxicity for FE antigen as described in footnote$^+$ of Table II. All $Fv-2^s$ strains express FE antigen, but no $Fv-2^r$ strain does.

$^+$ Typed in this laboratory.

$Fv-2$ allele of BALB/c (8, D. Bailey and W. Rowe, personal communication). The congenic strain B6.S was derived by substitution of the $Fv-2^s$ allele of B6 with the $Fv-2^r$ allele of SIM (9). The partially congenic D2.RB strain was constructed by three backcrosses to DBA from B6 with selection for $Fv-U$ heterozygosity at each generation (29). Mice then were interbred, and $Fv-2^s$ homozygotes used to establish the D2.RB line, which is in its 14th generation of inbreeding; D2.RB is $\approx$87.5% DBA genetic background (R. Steeves, personal communication). Data from quantitative absorption tests of FE-antigen expression on bone marrow of parental and congenic strains, presented in Fig. 5, indicate that substitution of the $Fv-2$ region of B6 or C57BL/10 with that of BALB/c or SIM results in FE antigen expression; conversely, substitution of the $Fv-2$ region of DBA/2 with that of B6 results in the loss of expression. Participation of the H-7-histocompatibility antigens in this serological typing system is excluded because B6 and DBA mice do not differ at this locus (40).

Discussion

The FE antigenic system identifies the second differentiation alloantigen that has been specifically associated with the defective transforming genome of a rapidly oncogenic MuLV. Absorption tests with FE typing serum establish that this immunogenetic system does not cross-react with previously described cell-surface antigens of mouse leukemia (37), induced by viral or cellular genes. The consistent appearance of FE in FV and Rauscher virus erythroleukemias and its absence from lymphomas of diverse origin support the designation of this determinant as an antigen of virally induced erythroleukemic differentiation. However, FE is not limited to erythroleukemias, but also appears on fibroblastoid cells carrying the SFFV genome, and on
absorption experiments with B6 anti-FV antiserum indicated that SFFV nonproducer cells and FMR lymphoma cells share antigenic determinants. An analogous expression of FMR determinant(s) on Abelson virus nonproducer cells was noted in a previous study (25). As both defective virus genomes share some gag but not env gene products with their respective helper viruses, proteins encoded by the gag region are logical candidates for FMR-bearing molecules. Previous work suggested that this might be the case (20), however, more recent studies indicate env gene-products bear the FMR determinants (21). The recent observation that FMR MuLV are more efficient for oncogenesis by, but not replication of, FMR-derived defective virus genomes (45, 46; Dr. D. Troxler, personal communication) may suggest the interaction of related molecular determinants in leukemogenesis by this family of MuLV. Further serological and biochemical analysis of the FMR antigenic system, which identifies such determinants, may clarify this point.

A conclusion consistent with the appearance of FE antigen on non-transformed SFFV-infected fibroblasts is that cell-surface expression of FE is programmed by SFFV rather than erythroleukemic differentiation per se. The simplest explanation for such a viral program is that SFFV encodes FE antigen, a proposition that can be substantiated only by biochemical identification of FE antigen. Accumulating evidence points to the recombinational origin of SFFV (24, 31, 39, 47), and other pathogenic MuLVs (38, 48, 49); thus, it is possible that SFFV arose by recombination between exogenous helper MuLV and the cellular sequences encoding FE antigen. The initial appearance of erythroleukemia-inducing activity in FV after passage through Swiss mice (1), and in Rauscher virus after passage through BALB/c mice (50) is consistent with this hypothesis, because these two strains express FE antigen on uninfected hematopoietic tissue. Results from molecular hybridization experiments indicate that SFFV-specific nucleotide sequences can be detected in the DNA of uninfected mice; the appearance of these sequences in RNA shows a differentiative
program similar to that described for FE antigen (Bernstein and Mak, personal communication). It will be of interest to determine if the presence of such sequences in DNA or in RNA also shows genetic polymorphism.

The alternative explanation that FE antigen is universally encoded in mice and rats and induced by SFFV functions, has a precedent in the TL antigenic system (51). TL antigen is a thymocyte differentiation antigen in some strains of mice; in other strains, which do not normally express this determinant, TL is an antigen of preleukemic and leukemic differentiation (37, 51). Only TL− mice produce TL antibody (37). In these regards, our observations on FE antigen parallel those on TL; however, no TL-inducing virus has yet been identified (37).

The detection of FE antigen on a minor cell population present in bone marrow, fetal liver, and spleen suggests that this determinant is an alloantigen of primitive hematopoietic differentiation. Preliminary experiments indicate that FE antigen is expressed on some hematopoietic colony-forming cells. Because the active organs of erythropoiesis in the mouse are fetal liver, bone marrow, and spleen, and because expression of FE antigen shows a similar pattern of expression, it may also be expressed on primitive cells committed to erythroid differentiation. It is worth noting that the immunogenetic system that regulates transplantation of spleen colony-forming cells has also been implicated in resistance to FV erythroleukemia (52). Perhaps the FE antigen with its dual appearance on SFFV cells and hematopoietic colony-forming cells, is an antigen recognized by this unusual immunogenetic system.

Relevant to a discussion of the normal differentiative functions of the FE antigen is the observation that FE antigen appears to be controlled by the Fv-2 locus. Though all tests demonstrated cosegregation of FE with Fv-2s, it is possible that FE is controlled by a gene in the Fv-2 region and not the susceptibility factor itself. Only more extensive genetic tests can clarify this point. The studies of Steeves et al. on the partially congenic D2.RB strain indicated that Fv-2 has an inhibitory effect on SFFV replication but not on virus infection (15). Blank and Lilly went on to make the surprising observation that the fully congenic DBA-Fv-2−/− genotype is apparently lethal in utero because no Fv-2−/− mice were recovered when 14th generation DBA-Fv-2−/− heterozygotes were intermated (53). The results of Axelrad et al., using B6 and B6.S strains, indicated that the primary effect of Fv-2 may be physiologic control of erythroid progenitor cell proliferation(16). If Fv-2s determines the presence of a cell-surface antigen as our results indicate, and if such an antigen were a necessary signal for hematopoietic proliferation and differentiation in certain genetic backgrounds, then genetic removal of such a determinant would half normal development. The unconventional inheritance patterns of the Gix antigen, an MuLV-related cell-surface structure, could be similarly interpreted to result from unfavorable cell-surface interactions in normal development (54, 55). These two immunogenetic systems—Fv-2-controlled FE antigen and Gv-1-controlled Gix antigen—provide evidence for a physiologic role of MuLV-related cell-surface antigens in normal development.

A possible role for FE antigen is that of a cell-surface signal or receptor involved in proliferation and differentiation of the erythroid pathway, perhaps as a critical determinant of the hematopoietic inductive microenvironment (56). The control of FE antigen by SFFV or by Fv-2s would thus reflect the fact that both viral and cellular genes control cell proliferation through a common mechanism. The observations of Axelrad et al. (16) that erythroid burst-forming cells are more rapidly dividing
in B6.S than in B6 mice (and thus more likely to be transformed), might lend support to this idea. Alternatively, FE antigen might function as a tissue-specific self-recognition structure. In such a case, the failure of SFFV-infected cells to differentiate into erythroblastic colonies in Fv-2/R mice may result from a failure of self-recognition. The C57–C58 family of mice have apparently evolved alternative structures to effect the same result.

The FE antigenic system appears quite similar to that described for Abelson antigen (25). Each antigen is specified by a defective transforming genome of MuLV, though the tissue specificity of these two viruses is quite different. Each antigen is also a normal differentiation alloantigen of bone marrow, fetal liver, and spleen in uninfected mice of the strain from which the defective virus was first isolated. Each antigen is apparently controlled by a dominant sensitivity locus, which also appears to govern oncogenesis by the defective virus (Table V, R. Risser, unpublished observations), and each antigen is expressed on spleen colony-forming cells of the bone marrow (R. Risser, unpublished observations). The detailed parallels of FE and Abelson antigens encourage the belief that these similarities reflect general principles of murine leukemogenesis.

Summary

Serum from C57BL/6 (B6) mice hyperimmunized with NB-tropic Friend virus complex (FV) was cytotoxic for FV-induced erythroleukemic spleen cells and B6 Friend-murine leukemia virus (F-MuLV) lymphoma cells. Cytotoxic activity for erythroleukemia cells remained after repeated absorption of B6 anti-FV antiserum with Friend-Moloney-Rauscher MuLV lymphoma cells but was removed by absorption with erythroleukemia cells induced by FV or Rauscher virus. This serologic test system identified a previously unrecognized cell-surface antigen of mouse leukemia, designated Friend Erythroleukemia (FE) antigen to signify its appearance as a determinant of virally induced erythroleukemic differentiation. FE antigen was not detected on 15 transplanted or primary hematopoietic neoplasms, nor was it detected on cells infected with ecotropic, xenotropic, or dualtropic MuLV isolates in tissue culture. Two spleen focus-forming virus (SFFV) nonproducer cells of rats and one of mice express FE antigen in amounts comparable to primary erythroleukemia cells.

Absorption tests with FE typing serum indicated that FE antigen was expressed on bone marrow and spleen but not thymus, lymph node, or peripheral blood of uninfected AKR, BALB/c, DBA, and SWR mice; all five tissues from B6 and C57L were negative. Quantitative absorption tests indicated that the expression of FE antigen, though much lower than on erythroleukemic cells, was greatest on fetal liver, less on bone marrow, and lowest on spleen from BALB and SWR mice. Treatment of BALB/c or SWR fetal liver, bone marrow, spleen, thymus, or lymph node cells with FE typing serum did not result in significant lysis. These observations are consistent with the interpretation that FE antigen is expressed by a minor cell population present in fetal liver, bone marrow, and spleen. Expression of FE antigen, determined by absorption with bone marrow cells, cosegregated with inheritance of the Fv-2S allele in the 17 inbred, 7 recombinant inbred, and 4 congenic mouse strains tested. In summary, the FE antigenic system identifies a cell-surface determinant that has the properties of a SFFV-specified antigen and hematopoietic differentiation alloantigen controlled by the Fv-2 locus. The similarity of FE antigen to Abelson antigen may provide insight into the pathogenic properties of defective transforming MuLV.
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