ANTIGENIC PROPERTIES OF CULTURED TUMOR CELL LINES DERIVED FROM SPLEENS OF FRIEND VIRUS-INFECTED BALB/c AND BALB/c-*H-2*^{b} MICE*

By HERBERT A. FREEDMAN,‡ FRANK LILLY, and RICHARD A. STEEVES

(From the Departments of Genetics and of Developmental Biology and Cancer, Albert Einstein College of Medicine, Bronx, N. Y. 10461)

An important factor determining the pathological consequences in vivo of infection with murine leukemia viruses (MuLV)[1] is that segment of chromosome 17 bearing the major histocompatibility complex of mice, the histocompatibility-2 (*H-2*) system (1). The particular event of the leukemogenic process directly influenced by *H-2* type has yet to be identified, but since *H-2* has no demonstrable effect on the susceptibility of cultured cells to infection by MuLV (2), this event is not at the level of cellular susceptibility to virus per se.

We have recently examined tumors induced by the Friend erythroleukemia virus complex (FV) in mice of two highly congenic strains differing with respect to the *H-2* chromosomal region: BALB/c (*H-2*^{d}) and BALB/c-*H-2*^{b} (BALB.B). These tumors were maintained by subcutaneous transplantation in syngeneic hosts for several transfers and then adapted to growth in culture. We have already reported on some aspects of our findings with these tumor cell lines, i.e., production of infectious FV and growth characteristics in syngeneic hosts. Essentially, cells of the BALB/c tumor line, which readily produce tumors in vivo, ceased producing detectable levels of virus after a relatively brief residence in culture. On the other hand, BALB.B tumor cells, which grow poorly in vivo, have continued to yield complete, infectious FV throughout their 2-yr culture history (3).

Each of the contrasting findings with these two cell lines, the marked difference in growth in vivo and the production or nonproduction of infectious virus, might be a reflection of important antigenic differences between these lines. For this reason, we have examined the expression on these cells of virus-associated

* Supported by contract NO 1 CP NCI 33249 from the Virus-Cancer Program of the National Cancer Institute.

‡ Special Fellow of the Leukemia Society of America.

[1] *Abbreviations used in this paper:* BALB.B, BALB/c-H-2^b, congenic partner to BALB/c (H-2^d); CSA, cell surface antigen; FMR, CSA induced by Friend, Moloney, and Rauscher leukemia viruses; FV, Friend virus complex; HFL/b-sc, solid subcutaneous tumor derived from FV-infected BALB.B spleen fragments; HFL/b-tc, HFL/b-sc grown in tissue culture; HFL/d-sc, solid subcutaneous tumor derived from FV-infected BALB/c spleen fragments; HFL/d-tc, HFL/d-sc grown in tissue culture; LLV-F, lymphatic leukemia virus isolated from FV; MEM-Earle's, minimum essential medium (Eagle) with Earle's salts; MuLV, murine leukemia virus; PBS, phosphate-buffered saline; SFFV, spleen focus-forming virus; VEA, virus envelope antigen.

antigens. Our findings indicate that both cell lines bear tumor-associated transplantation antigens capable of inducing transplantation immunity in syngeneic hosts. There was, however, no simple relationship between these transplantation antigens and a particular viral antigen.

Materials and Methods

Mice. All mice were from our own colony of highly inbred mouse strains. The congenic mouse strain, BALB.B, was derived from the (BALB/c × C57BL/10)F_1 cross. 13 consecutive generations of backcrosses to BALB/c were performed, with selection in each generation for the *H-2*^{b} haplotype of the C57BL/10 parent. The strain was then inbred. Animals were usually 6–10-wk old at the time of inoculation.

Viruses. The variant N→NB-tropic strain of FV was used throughout these experiments (4). This strain is equally infectious in both DBA/2 (*Fv-1*^{n}) and BALB/c (*Fv-1*^{b}) mice. Virus preparations were made from the greatly enlarged spleens of infected BALB/c mice. 2 wk after infection the spleens were homogenized in nine times their weight of cold phosphate-buffered saline (PBS), the homogenate centrifuged slowly at 4°C to remove large particulate matter, and the supernate recentrifuged for 4 min at 7,000 *g*. The resulting supernate served as the basic virus preparation and was stored in small ampules at −70°C.

N→NB-tropic Friend lymphatic leukemia virus (LLV-F) was derived from N-tropic FV by passage into newborn C57BL (*Fv-1*^{b}) mice followed by blind passage in weanling Ha/ICR swiss mice (5). Conversion to NB-tropism was afforded by five serial passages in BALB/c mice. Stocks of LLV-F were prepared as 20% perfusates of leukemic BALB/c spleens and were free of detectable spleen focus-forming activity.

Enlarged spleens were pooled, perfused with McCoy's medium containing 3% fetal calf serum, the perfusate clarified at 2400 *g* for 10 min and stored at −196°C. Stocks of LLV-F were titrated for their helper activity for the defective spleen focus-forming component (SFFV) of FV by the method of Steeves et al. (6) and showed 8 × 10^4 helper U per ml.

Tumors. Tumors of FV-transformed cells were established by the method of Buffet and Furth (7) and Friend and Haddad (8). This procedure involved subcutaneous transplantation to syngeneic hosts of washed fragments of the greatly enlarged spleens of mice infected 25–28 days earlier with N→NB-tropic FV. Tumors of both BALB/c and BALB.B mice were established and passed serially in their respective syngeneic hosts. Tumors of BALB/c origin are referred to as HFL/d-sc; "d" refers to the *H-2* type of the mouse from which this tumor was derived; "sc" refers to subcutaneous passage. Similarly, tumors of BALB.B origin are called HFL/b-sc.

Tissue Culture. All cell lines grown in culture were established from their respective subcutaneous solid tumors. These lines consisted of nonadherent cells maintained in suspension culture by planting 5-ml samples containing 5 × 10^4 cells/ml in T-30 flasks and subculturing approximately 5 days later when the cell concentration reached 5 × 10^6 cells/ml. The growth medium used throughout was Eagle's minimum essential medium with Earle's base (MEM-Earle's) plus 0.58 mg/ml L-glutamine, 100 U/ml penicillin and 100 mcg/ml streptomycin supplemented with 20% fetal calf serum. Cell lines originating from BALB/c tumors are called HFL/d-tc, those of BALB.B origin, HFL/b-tc. These cell lines have been in continuous serial culture for at least 2 yr.

Virus Neutralization Assay. Immune sera were tested for FV-neutralizing activity by the method of Steeves and Axelrad (9). Diluted sera and virus were combined at time zero and incubated at 37°C. Samples were removed from the reaction tubes at 10- or 15-min intervals for up to 40 min, appropriately diluted in ice-cold PBS, and immediately assayed in groups of seven BALB/c mice for residual virus capable of producing spleen foci. Antisera potencies were expressed in terms of the inactivation constant, K, determined from the equation $K = (D/t)\ \mathrm{Log}_e\ (V_o/V_t)$, where D = dilution of antiserum, V_o = virus titer at time zero, and t = virus titer at time t. Neutralization of LLV by immune sera was tested by the method of Steeves et al. (6).

Antisera. Antiserum specific for the alloantigen H-2K.31 was prepared by hyperimmunization of A/J mice with cells of the methylcholanthrene-induced BALB/c sarcoma, Meth A. Anti-FV serum, containing both cytotoxic (anti-FMR) and virus-neutralizing (anti-virus envelope antigen [VEA]) activity, was prepared by hyperimmunization of BALB/c or BALB.B mice with N→NB-tropic FV grown in syngeneic mice. Anti-HFL/d-tc was prepared by hyperimmunization of BALB/c

mice with HFL/d-tc cells after regression of HFL/d-tc-produced subcutaneous tumors. Assays of these sera for cytotoxic activity were performed in the presence of guinea pig complement using appropriate ^{51}Cr-labeled target cells (10, 11).

Absorption of Anti-FV. For the quantitative absorption of anti-FV serum, cells were harvested, washed five times in medium 199, and resuspended to a convenient volume. The number of viable cells, determined by exclusion of trypan blue (0.4% in normal saline), was >90%. Serial twofold dilutions of viable cells were mixed with antiserum appropriately diluted with medium 199 and incubated at 37°C with shaking for 1 h, followed by incubation at 4°C with shaking for an additional hour. The suspensions were then centrifuged at 300 *g* for 15 min and the resultant supernate assayed in the presence of guinea pig complement for residual cytotoxic activity toward ^{51}Cr-labeled BALB/c spleen cells infected 7–11 days earlier with N→NB tropic FV.

Results

Induction of Transplantation Immunity. Cultured cells of both the HFL/d-tc and HFL/b-tc lines induced tumors when inoculated into their respective syngeneic hosts. These tumors often grew only transiently and then regressed. In both cases regressor mice showed immunity to the growth of a second inoculation of the same cells. Thus these cells appeared to possess tumor-associated transplantation antigens.

The growth and regression of HFL/b-tc cells in BALB.B mice was noted in a previous article (3). Inoculation of fewer than 10^8 viable cells subcutaneously never resulted in tumor growth in normal recipients. BALB.B mice injected subcutaneously with 10^8 HFL/b-tc cells did show tumor growth, followed by regression. 6 mo later four of these mice and four normal controls received subcutaneous solid tumor implantations (strips about 2-cm long and 1-mm in diameter) of the HFL/b-sc tumor, from which the HFL/b-tc cell line originated. Tumor growth occurred only in the four normal control mice, whereas the animals previously exposed to HFL/b-tc cells showed no detectable tumor development.

HFL/d-tc cells, tested through passage 100 in culture, also showed a marked capacity for inducing tumor immunity in syngeneic BALB/c hosts, although to a considerably lesser degree than that observed in BALB.B mice inoculated with HFL/b-tc cells. Subcutaneous inoculation of 10^6 HFL/d-tc cells in normal syngeneic hosts resulted in the development of a palpable tumor, 0.5–1.0 cm in diameter, within 7–10 days. Table I shows that more than half of these mice appeared tumor-free 18–21 days later.

All regressor mice within each group noted in Table I were rechallenged

TABLE I

Regression of Tumors Resulting from Subcutaneous Inoculation of 10^6 HFL/d-tc Cells in BALB/c mice

HFL/d-tc passage number in culture	Number of tumors regressing/number of tumors
9	4/6
13	9/9
21	5/7
22	6/7
24	7/9

subcutaneously, at least 16 days after tumor regression, with 10^6 viable HFL/d-tc cells. None of these mice showed detectable tumor development, although 0.5–1-cm tumors developed at least transiently in all normal control mice similarly inoculated. Thereafter it was possible to hyperimmunize these regressor mice with progressively increasing doses of HFL/d-tc cells (see below).

Since both HFL/b and HFL/d cells originated from tumors induced in vivo with FV, they might both be expected to show FV-associated cell surface specificities which could function as transplantation antigens. This is particularly likely in the case of HFL/b-tc cells, since these cells produce complete, infectious FV and release it into the culture medium. HFL/d-tc cells, which produce no detectable levels of infectious FV, nevertheless possess the complete FV genome (3), the partial expression of which could result in the production of a transplantation antigen.

Viral Antigen Expression: Cell Surface Antigen (FMR). Mice immunized against infectious FV produce two populations of antibodies in their sera: (*a*) virus-neutralizing antibodies specific for VEA on the surfaces of both productively infected cells and intact progeny virus particles, and (*b*) cytotoxic antibodies to the cell surface antigen (CSA) present on virus-infected cells, but not present in antibody-accessible form on the surface of intact virus particles (12). These two antibody activities are properties of two separable populations of antibodies that recognize different virus-coded polypeptides (13, 14). The CSA specificity induced by FV cross-reacts with CSA specificities similarly induced by Moloney and Rauscher viruses, and has therefore been termed FMR (15).

After regression of HFL/d-tc-produced solid tumors in BALB/c mice, hyperimmunization with HFL/d-tc cells did not induce the production of antibodies detectable in the complement-dependent cytotoxic assay, using guinea pig serum as a complement source. Fig. 1 illustrates that sera from such animals failed to show cytotoxic activity toward either the homologous immunogen, cultured HFL/d-tc cells, or spleen cells from FV-infected BALB/c mice. When HFL/d-tc cells taken at the 13th and 15th passages in vitro were tested directly for sensitivity to complement-mediated cytolysis by a known anti-FMR serum, these cells were completely resistant. Moreover, mechanically dispersed cells from a primary solid tumor derived by the subcutaneous implantation of HFL/d-tc cells harvested at the 13th passage in vitro were also resistant to cytotoxic lysis by anti-FMR serum. In contrast, primary FV-infected syngeneic spleen

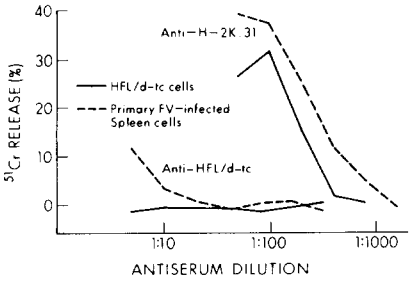

FIG. 1. Absence of cytotoxic activity in hyperimmune BALB/c anti-HFL/d-tc serum tested on HFL/d-tc cells and on BALB/c FV-infected spleen cells. Control serum, A anti-Meth A (anti-H-2K.31).

cells exhibited characteristic immune cytolysis by anti-FMR serum. Both infected spleen cells and HFL/d-tc cells were lysed by anti-H-2K.31 serum.

The absence of the FMR antigenic specificity on the surface of nonproducer HFL/d-tc cells was confirmed by comparison of the quantitative absorption of anti-FMR serum by normal syngeneic spleen cells, primary FV-infected syngeneic spleen cells, and HFL/d-tc cells. Fig. 2 shows that the amount of anti-FMR absorbed by as few as 2×10^5 infected spleen cells was appreciable and increased with increasing cell number. However, absorption with as many as 6×10^7 nonproducer HFL/d-tc cells did not decrease the cytotoxic activity of the anti-FMR serum. This was true for normal syngeneic spleen cells, as well.

HFL/d-tc cells passaged intraperitoneally in FV-infected syngeneic hosts were harvested, washed three times in PBS, and assayed for sensitivity to complement-mediated cytolysis by anti-FMR and anti-H-2K.31 sera. Fig. 3 shows that these ascitic HFL/d cells were completely resistant to lysis by anti-FMR serum, whereas cytolysis by anti-H-2K.31 serum was readily demonstrable. Thus HFL/d cells were resistant to immune cytolysis by anti-FMR serum even when resident in viremic hosts. Cells from the enlarged spleens of these viremic, autochthonous hosts lysed readily with both anti-FMR and anti-H-2K.31 sera, as did spleen cells from syngeneic, FV-infected hosts.

Unlike HFL/d-tc cells, FV-producer HFL/b-sc and HFL/b-tc cells of BALB.B origin did exhibit the cell surface antigenic specificity, FMR. In the present study, this antigen was demonstrated by comparing the quantitative absorption of cytotoxic anti-FMR serum with viable HFL/b-sc, HFL/b-tc, and HFL/d-tc cells. Parallel absorption with normal and primary FV-infected BALB/c spleen cells served as controls. It is apparent from the data illustrated in Fig. 4 that FV-producer HFL/b-sc, HFL/b-tc, and primary infected spleen cells all absorbed appreciable amounts of anti-FMR activity when as few as 3×10^5 cells were used. Again, nonproducer HFL/d-tc cells did not absorb anti-FMR activity, even at much higher cell concentrations.

From these studies we conclude that expression of FMR antigen correlates with the virus-producer status of the HFL/b and HFL/d cell lines. HFL/d cells express no FMR antigen, and therefore the transplantation antigen demonstrated on these cells is not FMR. It is possible, however, that the higher level of

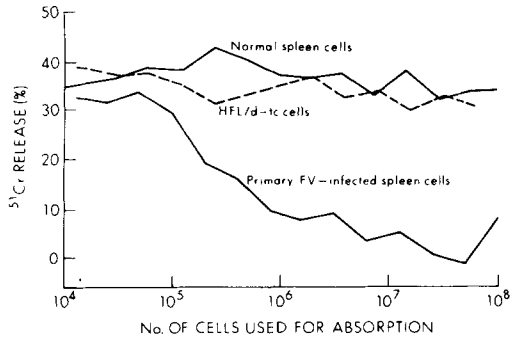

FIG. 2. Quantitative absorption of the cytotoxic (anti-FMR) activity of BALB/c anti-FV antiserum by: (*a*) normal BALB/c spleen cells, (*b*) cultured nonproducer BALB/c tumor cells (HFL/d-tc), and (*c*) primary FV-infected BALB/c spleen cells.

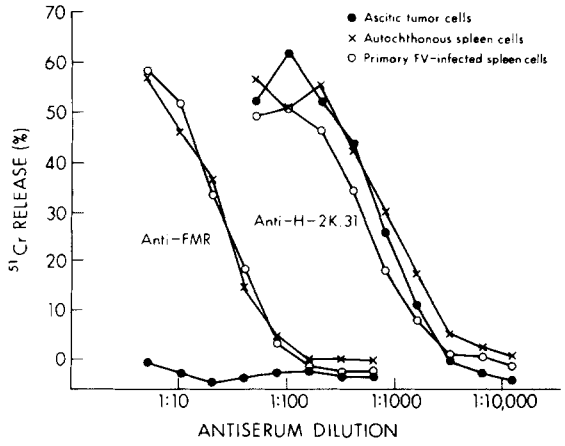

FIG. 3. Cytotoxic assay of BALB/c anti-FV (anti-FMR) and of A anti-Meth A (anti-H-2K.31) antisera on HFL/d-tc cells grown in ascitic form in a FV-infected BALB/c mouse, on spleen cells from the autochthonous host, and on FV-infected spleen cells from a control BALB/c mouse.

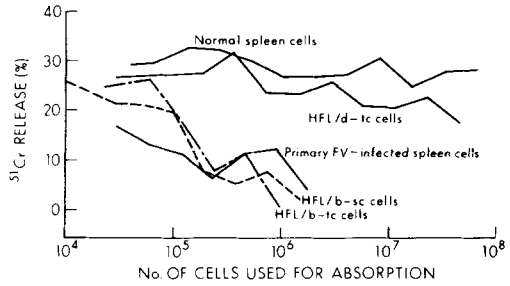

FIG. 4. Quantitative assay for the capacity to absorb the cytotoxic activity of BALB/c anti-FV serum by: (*a*) normal BALB/c spleen cells, (*b*) cultured nonproducer BALB/c tumor cells (HFL/d-tc), (*c*) primary FV-infected BALB/c spleen cells, (*d*) subcutaneously grown BALB.B tumor cells (HFL/b-sc), and (*e*) cultured producer BALB.B tumor cells (HFL/b-tc).

immunogenicity of HFL/b cells by comparison with HFL/d cells is due to the presence of the FMR antigen.

Viral Antigen Expression: Virus Envelope Antigen (VEA). After regression of HFL/d-tc-produced solid tumors, BALB/c mice challenged with a high dose of FV failed to develop splenomegaly. Normal control mice inoculated in parallel with the same preparation of FV showed rapid and marked splenomegaly. Furthermore, although sera from BALB/c mice hyperimmunized with HFL/d-tc cells did not show cytotoxic activity, these sera did neutralize the spleen focus-forming activity of FV and the helper activity of LLV-F. The kinetics of FV-neutralization by this hyperimmune serum (BALB/c anti-HFL/d-tc) are shown in Fig. 5, indicating a K value of 1.45 ± 0.19. This demonstration of high titer virus-neutralizing antibody suggests that FV-nonproducer, FMR-negative HFL/d-tc cells express Friend VEA on their surfaces.

Additional evidence for the presence of VEA on the surfaces of HFL/d-tc cells was obtained by absorption studies. Hyperimmune serum raised against FV was

absorbed in parallel with equal numbers of viable HFL/d-tc cells, normal syngeneic spleen cells, and primary FV-infected spleen cells. The neutralization of FV by these absorbed sera is shown in Fig. 6. The results demonstrate that nonproducer HFL/d-tc cells absorb virus-neutralizing antibody as effectively as primary infected syngeneic spleen cells.

HFL/b-tc cells also bear VEA specificities, as expected from the fact that they produce complete, infectious FV. BALB.B mice immunized with HFL/b-tc cells show marked virus-neutralizing activity in their sera in addition to significant levels of cytotoxic anti-FMR activity. Furthermore, HFL/b-tc cells, but not normal BALB.B spleen cells, efficiently absorb virus-neutralizing activity from anti-FV antiserum.

Loss of VEA. Around the 125th serial passage in culture, HFL/d-tc cells began to show a much reduced capacity for inducing transplantation immunity in vivo. The incidence of rejection of HFL/d-tc cells in syngeneic hosts was markedly reduced. No more than 20% of all BALB/c animals receiving HFL/d-tc cells intraperitoneally showed tumor regression, and the incidence of regression was near zero when HFL/d-tc cells were inoculated subcutaneously. Thus, it had become extremely difficult to immunize BALB/c mice with these cells. Even in those few BALB/c animals in which hyperimmunization with HFL/d-tc cells was successful, neither cytotoxic nor virus-neutralizing antibodies could be detected. Furthermore, HFL/d-tc cells could no longer boost the titer of virus-neutralizing antibody in BALB/c mice previously hyperimmunized against VEA or absorb the virus-neutralizing activity of hyperimmune anti-VEA serum. It therefore appears that these HFL/d-tc cells no longer expressed VEA on their surfaces.

Previous studies, conducted during the period of time in which HFL/d-tc cells were VEA-positive, indicated that, although no infectious virus was produced, these cells contained the genomes of both SFFV and its helper virus (3). The subsequent loss of VEA expression by HFL/d-tc cells, reported above, did not, however, reflect loss of one or both of these viral genomes. This was evidenced by the recovery of infectious NB-tropic FV, i.e., SFFV plus its original helper virus, from VEA-negative HFL/d-tc cells in the absence of exogenously added helper virus. Recovery of FV was accomplished either by intraperitoneal passage in syngeneic hosts (3) or by treatment in culture with 200 μg/ml 5-iodo-2′-deoxyuridine for 24 h. Since the tropism of the FV complex is determined by its

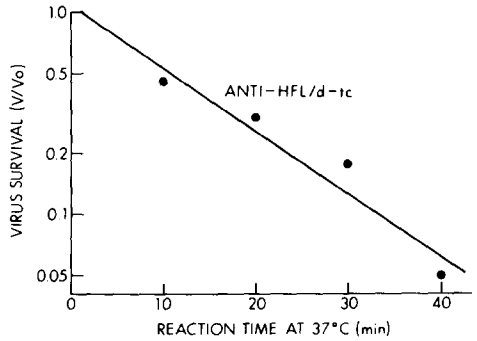

FIG. 5. Kinetics of neutralization of FV by hyperimmune BALB/c anti-HFL/d-tc serum, tested at 1:20 dilution.

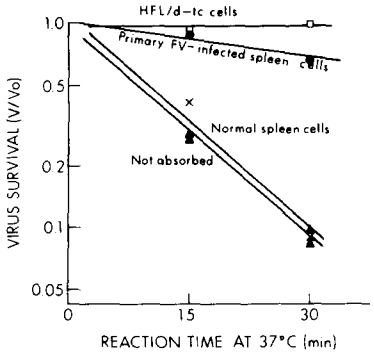

FIG. 6. Neutralization of FV by BALB/c anti-FV (1:20 dilution) absorbed with: (*a*) cultured nonproducer BALB/c tumor cells (HFL/d-tc), (*b*) primary FV-infected BALB/c spleen cells, and (*c*) normal BALB/c spleen cells.

helper virus component and since NB-tropic viruses are not known to occur naturally, the recovery of NB-tropic FV would indicate the presence within these nonproducer HFL/d-tc cells of the original helper virus genome. Therefore, the loss of VEA expression by these cells reflects a further repression of these viral genomes rather than their loss.

Although HFL/d-tc cells had become both FMR- and VEA-negative, it was nonetheless possible, though considerably more difficult than before, to induce transplantation immunity against them in syngeneic BALB/c mice. Moreover, both BALB/c and BALB.B mice immunized with VEA-negative HFL/d-tc cells were resistant to the disease syndrome resulting from FV inoculation. These observations suggest the presence of a virus-induced surface antigen other than VEA or FMR on these cells.

Discussion

Both HFL/b and HFL/d cultured, FV-induced tumor cells have demonstrated their capacity to induce transplantation immunity in their respective syngeneic hosts. Thus both cell lines possess new, tumor-associated transplantation antigens. This induction of transplantation immunity was accomplished much more readily in the HFL/b-BALB.B system than in the HFL/d-BALB/c system. It will be important to determine if this difference is due to (*a*) greater immunogenicity of the HFL/b tumor cells, (*b*) greater immune responsiveness of BALB.B mice, or (*c*) both.

We have observed a correlation betwen the expression of FV-associated antigens and the induction of transplantation immunity. HFL/b-tc cells produce complete, infectious FV which is released into the culture medium, and their cell surfaces bear both VEA and FMR antigenic specificities. Either or both of these specificities might act as transplantation antigens. HFL/d-tc cells, on the other hand, make no detectable level of infectious FV and do not express the FMR antigen. Thus, the absence of an important viral antigen correlates with the lower capacity of HFL/d-tc cells to induce transplantation immunity. Furthermore, HFL/d-tc cells, which expressed Friend VEA for more than a 100 passages in culture, ceased to express this antigen at a point in time that

coincided with the observation of a marked further decrease in the capacity of the cells to induce transplantation immunity in syngeneic hosts. These facts make a circumstantial case for the interpretation that HFL/b cells are more immunogenic than HFL/d cells because they express more viral antigens.

There is no evidence from the present experiments directly implicating the relative immune responsiveness of BALB/c vs. BALB.B mice to FV-associated tumor antigens. However, in unpublished studies in our laboratory, killer cells specific for FV-infected target cells have been detected among the peritoneal exudate population from BALB.B mice immunized with HFL/b-tc cells. Such killer cells have not been detected to date in BALB/c mice immunized with HFL/d-tc cells. This finding might reflect a difference betwen BALB.B and BALB/c mice in specific immune responsiveness to a particular cell surface antigen.

Previous studies in our laboratory (16) have shown that the amount of FMR antigen activity detectable on the surface of spleen cells from FV-infected mice decreases markedly in the late stages of the disease syndrome in BALB/c but not BALB.B mice. The present studies indicate that this phenomenon is also reflected in the persistent presence or absence of FMR antigen expression in cultured tumor cell lines derived from these same mouse strains. Since the presence or absence of FMR antigen correlates with the FV-producer or nonproducer status of these cell lines, it is possible to pose the question: does the suppression of viral cell surface antigens in $H\text{-}2^d$ mice, by whatever means, have a causal relation to the observed nonproducer status of the HFL/d-tc tumor cells? Our studies, so far, offer no answer to this question.

In addition to HFL/d-tc and HFL/b-tc, two other FV-induced tumor cell lines, one of BALB/c and one of BALB.B origin, have been examined. Although the BALB/c line, like HFL/d-tc, has become FV-nonproducer in culture and the BALB.B line, like HFL/b-tc, has retained its FV-producer phenotype for over a year in culture, it is not yet possible to infer on statistical grounds that the difference in *H-2* genotype is responsible for these phenotypic differences. However, since these findings with tumor cell lines grown in vitro are consistent with our earlier findings in vivo (16), the hypothesis that the *H-2* difference is a major causal factor seems reasonable. These earlier studies in vivo further indicated that antigen H-2K.31, like FMR, also showed a markedly decreased expression on FV-infected BALB/c spleen cells in the late stages of the disease syndrome. This finding may suggest some molecular interaction between the *H-2* molecule and virus-associated molecules similar to the interaction suggested by recent studies with lymphocytic choriomeningitis virus (17).

Nonproducer HFL/d-tc cells do not express FMR, but the presence of Friend VEA on the surface of these cells was demonstrated by: (*a*) the presence of virus-neutralizing activity in the sera of mice immunized with syngeneic HFL/d-tc; and (*b*) the absorption of virus-neutralizing activity from hyperimmune anti-FV serum by HFL/d-tc cells. This was further confirmed by the finding of relatively high levels of the MuLV envelope glycoprotein, gp69/71, in nonproducer HFL/d-tc cell preparations (M. Strand and J. T. August, personal communication).

FV represents a complex consisting of a replication-defective, erythroleukemia-inducing virus, SFFV, together with its helper virus. Since it has been

shown that the major VEA (gp69/71) of both SFFV and helper virus particles is governed by the helper virus genome (18), the presence of Friend VEA on the surface of HFL/d-tc cells suggests that these nonproducer cells possess all or part of the helper virus genome. Thus, in the present study, antibodies raised against VEA-positive nonproducer HFL/d-tc cells neutralized both FV complex and helper virus (LLV-F) isolated from the FV complex.

Moreover, the presence of VEA on the surfaces of nonproducer HFL/d-tc cells provides a useful tool. Previously, it has been difficult to raise an antiserum exhibiting virus-neutralizing (anti-VEA) activity without cytotoxic (anti-FMR) activity as well. However, hyperimmunization of BALB/c mice with syngeneic VEA-positive nonproducer HFL/d-tc cells results in the production of virus-neutralizing antiserum devoid of cytotoxic activity. Furthermore, it is possible, using VEA-positive, FMR-negative HFL/d-tc cells, to absorb out virus-neutralizing activity leaving intact the cytotoxic activity of hyperimmune serum raised against the FV complex.

Around the 125th serial passage in culture the HFL/d-tc cell line appeared to have undergone a marked alteration in its antigenic characteristics. This change was evidenced by the observations that: (*a*) the incidence of tumor regression of HFL/d-tc cells in syngeneic hosts was markedly reduced, and thus immunization of syngeneic mice with HFL/d-tc cells became difficult; (*b*) even when hyperimmunization of BALB/c mice with syngeneic HFL/d-tc cells was successful, no virus-neutralizing activity could be detected in the serum of immune mice; and (*c*) HFL/d-tc cells could no longer either boost the titer of virus-neutralizing antibody in BALB/c mice previously hyperimmunized against VEA or absorb the virus neutralizing activity of hyperimmune anti-VEA serum. In addition, HFL/d-tc cells, tested after the 160th serial passage in culture, were negative for the viral envelope glycoprotein, gp69/71 (M. Strand and J. T. August, personal communication).

Thus the HFL/d-tc cell line has gone through three distinct phases in its 2-yr passage history. During its very early passages, it produced small but demonstrable amounts of complete FV (3); after about 14 passages in culture, it ceased making any detectable level of infectious virus, and was found to be phenotypically FMR-negative, VEA-positive, and gp69/71-positive. More than 1 yr later (125 serial passages in culture) it changed again, becoming VEA-negative and gp69/71-negative.

Summary

BALB/c-*H-2*^{b} (BALB.B) mice are less susceptible to the Friend virus (FV) disease syndrome than congenic BALB/c (*H-2*^{d}) mice, and spleen cells from FV-infected BALB.B mice are markedly less tumorigenic on transplantation to syngeneic hosts than those from FV-infected BALB/c mice. For these reasons we investigated the expression of FV-associated cell surface antigens on cultured, FV-transformed cell lines of BALB.B and BALB/c origin.

Both cell lines induced transplantation immunity in syngeneic hosts toward further implantations of the same tumor, BALB.B cells being significantly more potent in this respect than BALB/c cells. BALB.B tumor cells, which produce complete, infectious FV, expressed both the cell surface antigen, FMR (corre-

sponding to the cytotoxic antibodies in anti-FV antisera), and virus envelope antigen (VEA, corresponding to the virus-neutralizing antibodies in the anti-FV antisera). BALB/c tumor cells, on the other hand, which are FV-nonproducers, expressed no FMR antigen, but did express VEA on their surfaces for at least 100 passages in culture. These cells could induce FV-neutralizing but not cytotoxic anti-FMR antibodies when used to immunize syngeneic hosts. The absence of FMR antigen may be the basis for the reduced capacity of BALB/c tumor cells, by comparison with BALB.B tumor cells, to induce transplantation immunity.

After about the 125th serial transfer in culture, BALB/c tumor cells spontaneously ceased to express VEA and simultaneously became very weak inducers of transplantation immunity in BALB/c hosts. This loss of VEA did not stem from the loss of either the spleen focus-forming virus or the helper virus genomes from these cells, since both viruses could still be recovered from the cell line.

We thank Ms. Harriel Martinez for her assistance in the breeding and maintenance of our colony of highly inbred mouse strains, Mr. Philippe Roe for assistance with culturing of cell lines, and Ms. Ida Shapiro for her assistance in the completion of this manuscript.

Received for publication 7 August 1975.

References

1. Lilly, F., E. A. Boyse, and L. J. Old. 1964. Genetic basis of susceptibility to viral leukemogenesis. *Lancet.* 2:1207.
2. Pincus, T., J. W. Hartley, and W. P. Rowe. 1971. A major genetic locus affecting resistance to infection with murine leukemia viruses. I. Tissue culture studies of naturally occurring viruses. *J. Exp. Med.* 133:1219.
3. Freedman, H. A., and F. Lilly. 1975. Properties of cell lines derived from tumors induced by Friend virus in BALB/c and BALB/c-*H-2*^{b} mice. *J. Exp. Med.* 142:212.
4. Lilly, F. 1967. Susceptibility to two strains of Friend leukemia virus in mice. *Science (Wash. D. C.).* 155:461.
5. Steeves, R. A., R. J. Eckner, M. Bennet, and E. A. Mirand. 1971. Isolation and characterization of a lymphatic leukemia virus in the Friend virus complex. *J. Natl. Cancer Inst.* 46:1209.
6. Steeves, R. A., R. J. Eckner, E. A. Mirand, and R. L. Priore. 1971. Rapid assay of murine leukemia virus helper activity for Friend spleen focus-forming virus. *J. Natl. Cancer Inst.* 45:1219.
7. Buffett, R. F., and J. Furth. 1959. A transplantable reticulum cell sarcoma variant of Friend's viral leukemia. *Cancer Res.* 19:1063.
8. Friend, C., and J. Haddad. 1959. Local tumor formation with transplants of spleen or liver of mice with a virus-induced leukemia. *Proc. Am. Assoc. Cancer Res.* 3:1. (Abstr.)
9. Steeves, R. A., and A. A. Axelrad. 1967. Neutralization kinetics of Friend and Rauscher leukemia viruses studied with the spleen focus assay method. *Int. J. Cancer* 2:235.
10. Wigzell, H. 1965. Quantitative titration of mouse *H-2* antibodies using Cr^{51} labeled target cells. *Transplantation (Baltimore).* 3:423.
11. Lilly, F., and S. G. Nathenson. 1969. Studies on the FMR antigen. *Transplant. Proc.* 1:85.

12. Friedman, M., F. Lilly, and S. G. Nathenson. 1974. Cell surface antigen induced by Friend virus is also in the virion. *J. Virol.* **14**:1126.
13. Steeves, R. A. 1968. Cellular antigen of Friend virus-induced leukemias. *Cancer Res.* **28**:338.
14. Lilly, F., and R. A. Steeves. 1974. Antigens of murine leukemia viruses. *Biochim. Biophys. Acta* **355**:105.
15. Old, L. J., and E. A. Boyse. 1965. Antigens of tumors and leukemias induced by viruses. *Fed. Proc.* **24**:1009.
16. Lilly, F. 1972. Antigen expression on spleen cells of Friend virus-infected mice. *In* RNA Viruses and Host Genome in Oncogenesis. P. Emmelot and P. Bentvelzen, editors. North-Holland Publishing Co., Amsterdam, The Netherlands. 229.
17. Zinkernagel, R. M., and P. C. Doherty. 1975. *H-2* compatibility requirements for T-cell-mediated lysis of target cells infected with lymphocytic choriomeningitis virus. *J. Exp. Med.* **141**:1427.
18. Eckner, R. J., and R. A. Steeves. 1972. A classification of the murine leukemia viruses: neutralization of pseudotypes of Friend spleen focus-forming virus by type-specific murine antisera. *J. Exp. Med.* **136**:832.