Studies on the Neutralization of the Oncogenicity of Friend, Moloney, and Rauscher Viruses

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SUMMARY

Friend, Moloney, and Rauscher (FMR) viruses were incubated with either normal serum or with sera from C57BL/6 mice immunized with syngeneic Friend, Moloney, or Rauscher virus-induced lymphomas. The mixtures were inoculated into newborn BALB/c mice, and the development of lymphomas was followed. The oncogenicity of all 3 viruses was effectively neutralized by each of the antisera. The results suggest that FMR viruses possess similar, if not identical, antigens. A specific rat anti-Gross serum was also active against the 3 viruses, suggesting the existence of a virion coat component antigenically shared by Gross virus and the FMR viruses. Finally, sera from C57BL/6 mice whose autochthonous Moloney sarcoma virus-induced tumors had regressed also neutralized the FMR viruses.

INTRODUCTION

Studies employing the cytotoxicity test (16), immunofluorescence test (7), and transplantation technics (7) have demonstrated that Friend, Moloney, and Rauscher virus-induced lymphoma cells are antigenically indistinguishable from each other, but may be antigenically distinct from tumors induced by Gross virus. Virus neutralization studies, however, have yielded conflicting results concerning both the antigenic relationship among the FMR viruses (4, 10, 18-21), and the antigenic relationship between FMR viruses and Gross virus (5, 8, 15, 21). For example, Fink reported (4) that neutralization tests indicated antigenic similarities between Rauscher and Friend viruses, and between Moloney and Friend viruses, but not between Moloney and Rauscher viruses. By contrast, Steeves and Axelrad (21), employing a spleen focus assay, reported that both Friend and Rauscher viruses can be neutralized by anti-Moloney serum as well as by anti-Friend and anti-Rauscher sera. Although Moloney lymphoma virus was neutralized by murine anti-Rauscher serum (10), murine anti-Friend serum has not been tested. In view of these conflicting results, a complete cross-neutralization study employing FMR viruses and murine anti-FMR sera of known potency was conducted.

Any antigenic relationships between Gross virus and the FMR viruses by virus neutralization tests have been especially difficult to establish. No murine antisera have been reported to neutralize Gross virus, while studies with rabbit anti-Gross serum have yielded conflicting results (8, 15). With the advent of potent and specific rat anti-Gross serum (6), the antigenic relationship between Gross virus and the FMR viruses can be reexamined. This rat anti-Gross serum has recently been reported to neutralize Moloney lymphoma virus (3). Therefore, the neutralizing ability of this rat anti-Gross serum against Friend and Rauscher viruses as well as Moloney virus was studied.

Finally, tumors induced by Moloney sarcoma virus have been shown to be antigenically similar to those induced by FMR viruses, and the oncogenicity of MSV can be neutralized by anti-FMR (1) and anti-Gross sera (3). This suggests an antigenic similarity between Moloney sarcoma virus and the FMR and Gross viruses. In order to corroborate this finding, the ability of anti-Moloney sarcoma serum to neutralize FMR viruses was tested.

MATERIALS AND METHODS

Mice. Adult C57BL/6 and newborn BALB/c mice, less than 72 hours old, were obtained from the production colonies of Microbiological Associates, Inc. and Texas Inbred Mice. W/Fu rats were obtained from Batelle Memorial Institute.

Viruses. Friend and Moloney viruses were obtained from Dr. Moloney. The Friend virus (Lot No. FV-5) was one gram equivalent preparation from spleens of BALB/c mice infected with Friend virus. The Moloney virus (Lot No. MV-2) consisted of a 10-gram equivalent preparation from plasma of Moloney virus-infected BALB/c mice. Rauscher virus (Lot Nos. P-972 and 66-427H) was prepared as a 10% suspension from spleens of BALB/c mice infected with Rauscher virus and was obtained from Dr. Rauscher and Hazelton Laboratories (Falls Church, Va.).

Sera. Rat anti-Gross serum, kindly provided by Dr. L. J. Old, was obtained from inbred W/Fu rats immunized against histocompatible W/Fu lymphomas induced by wild-type Gross virus from C58 mice. The antisemur has been shown to contain specific anti-Gross activity in high titer (6). Specific anti-Moloney, anti-Rauscher, and anti-Friend sera were obtained

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by inoculating adult C57BL/6 mice with 2 doses of X-irradiated (5000 R) transplantable syngeneic Friend, Moloney, or Rauscher lymphoma cells (5–10 × 10^6 cells/mouse), two weeks apart. The mice were bled from the retroorbital sinus 10–14 days after the last immunization. The anti-Moloney sarcoma serum was obtained from the retroorbital sinus of adult C57BL/6 mice whose autochthonous virus-induced Moloney sarcomas had regressed. This antiserum effectively neutralizes the oncogenicity of Moloney sarcoma virus. All sera were pooled and stored at less than −70°C in 5-ml aliquots. The same pools of antisera were used in all studies.

The Indirect Fluorescent Antibody Technic. The technic developed by Möller (13) using suspensions of viable cells was employed, as previously described in detail (2). Generally, the test involved viable target cells reacted with antiserum and fluorescein-conjugated goat anti-mouse globulin (Hyland). All samples were read blind. Cells exhibiting bright green granular or sectorial fluorescence, or any other staining pattern except that characteristic of dead cells, were considered positive; 100–180 viable cells per sample were examined, and the result was expressed as the number of stained viable cells × 100, divided by the total number of viable cells examined. Each test included a negative control (normal serum) and a positive control (specific antiserum with known activity).

Virus Neutralization. Graded ten-fold dilutions of Friend, Moloney, or Rauscher viruses were mixed with an equal volume of normal or immune serum. The mixtures were thoroughly stirred, incubated at room temperature for 90 minutes, and inoculated into newborn BALB/c mice.

RESULTS

Potency of Anti-FMR and Anti-Moloney Sarcoma Sera as Measured by the Indirect Fluorescent Antibody Technic. Prior to their use in cross-neutralization experiments, antisera were tested for specific activity by immunofluorescence. Serially diluted sera from C57BL/6 mice immunized with syngeneic lymphomas induced by Friend, Moloney, or Rauscher viruses were tested by the indirect fluorescent antibody technic against syngeneic lymphomas induced by the same viral agent. The rat anti-Gross serum was tested against E δ G-2, a Gross virus-induced lymphoma of C57BL/6 origin. In addition, sera from C57BL/6 mice whose autochthonous Moloney sarcoma virus-induced tumors had regressed, were tested by immunofluorescence against Moloney lymphoma cells. Table 1 reveals that each antiserum reacted strongly against the specific target cell tested, but not against a nonspecific chemically induced lymphoma.

Neutralization of Rauscher Virus. A 10⁻¹, 10⁻², or 10⁻³ dilution of Rauscher virus was incubated with undiluted normal C57BL/6 mouse serum, with undiluted C57BL/6 anti-FMR, or with anti-Moloney sarcoma serum. A 1:2 dilution of normal rat serum or rat anti-Gross serum was also tested. The mixtures were inoculated s.c. into newborn BALB/c mice (0.1 ml/mouse). The mice were observed on alternate days for splenomegaly and mortality. All mice with splenomegaly ultimately died. The results are presented in Table 2. All antisera directed against FMR, Gross, and Moloney sarcoma ef-

### Table 1

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<th>Serum</th>
<th>Serum dilution in HBSS</th>
<th>Percent staining*</th>
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<tbody>
<tr>
<td>Anti-Friend</td>
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<td></td>
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<td></td>
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<td></td>
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<td>Anti-Rauscher</td>
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<td></td>
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<tr>
<td>Anti-Moloney sarcoma</td>
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<tr>
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<tr>
<td>Rat anti-Gross</td>
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<tr>
<td>Normal rat serum</td>
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Activity of anti-FMR, anti-Gross, and anti-Moloney sarcoma sera by the indirect fluorescent antibody technic. HBSS, Hank's balanced salt solution.

a C57BL/6 anti-Friend, anti-Rauscher, and anti-Moloney lymphoma sera were tested against transplantable syngeneic Friend, Rauscher, and Moloney lymphoma cells, respectively. Anti-Moloney sarcoma serum was tested against a Moloney lymphoma of BALB/c origin, as in previous studies (1). Rat anti-Gross and normal rat serum were tested against a Gross virus-induced lymphoma of C57BL/6 origin. All antisera, as well as normal mouse or rat serum, stained <5% of EL-4, a chemically induced lymphoma of C57BL/6 origin.

b Normal C57BL/6 serum stained 9, 6, and 15% of Friend, Rauscher, and Moloney lymphoma cells, respectively.

Effectively neutralized the oncogenicity of Rauscher virus, as evidenced by decreased incidence of splenomegaly. Cumulatively, 36/44 mice inoculated with Rauscher virus and normal mouse or rat serum developed splenomegaly, in contrast to 2/20, 0/18, 3/21, 0/15, and 0/16 mice inoculated with Rauscher virus and anti-Rauscher, anti-Friend, anti-Moloney, anti-Gross, and anti-Moloney sarcoma serum, respectively. Normal W/Fu rat serum had no neutralizing activity against Rauscher virus.

Neutralization of Friend Virus. Graded dilutions of Friend virus were incubated with normal mouse serum, anti-FMR or anti-Moloney sarcoma serum, or with a 1:2 dilution of rat anti-Gross serum. The mixtures were inoculated s.c. into newborn
Neutralization of the oncogenicity of Rauscher virus. Data above the space were obtained with RV pool No. P-972 over an observation period of 325 days; data below the space were obtained with RV pool No. 66-427H, with an observation period of 150 days. The mean latency period, in days to splenomegaly detection, is in parentheses.

a No. of mice developing splenomegaly/No. of mice inoculated.

Neutralization of Moloney Lymphoma Virus. A 10⁻¹ or 10⁻² dilution of Moloney lymphoma virus was incubated with the above anti-FMR, anti-Moloney sarcoma, and anti-Gross sera and inoculated s.c. into newborn BALB/c mice. The mice were observed daily for mortality and three times weekly for splenomegaly. All mice developing enlarged spleens ultimately died. The results are presented in Table 3. All 5 antisera neutralized Friend virus. Over an observation period of 315 days, 23/32 mice inoculated with Friend virus and normal serum developed splenomegaly and died. By contrast, no mouse (0/80) inoculated with Friend virus after incubation with any of the antisera developed splenomegaly.

Transplantation studies (7) as well as serologic tests (7, 16) based on antibody reactions with cells (i.e., cytotoxicity and immunofluorescence), have demonstrated that murine FMR lymphoma cells are antigenically indistinguishable from each other, but may be antigenically distinct from lymphomas induced by Gross virus. Accordingly, Old et al. (16) have suggested that lymphomas induced by Friend, Moloney, and Rauscher viruses share a common or related antigenic system, designated as FMR. Whether the antigens detectable on FMR lymphoma cells are cellular or virion is not known, since these cells release oncogenic virus (11). It cannot, therefore, be assumed that the antigenic relationship among FMR lymphoma cells, as detected by transplantation and serologic studies, necessarily reflects the antigenic relationship among the FMR viruses.

To determine whether Friend, Moloney, and Rauscher viruses are indeed antigenically similar, complete cross-neutralization tests, employing FMR viruses and specific anti-FMR sera, were conducted. Antisera were prepared in C57BL/6 mice against syngeneic FMR lymphoma cells and were shown to be potent against homologous virus-induced lymphomas (by immunofluorescence) and against homologous viruses (by neutralization). The cross-neutralization tests demonstrated that the oncogenicity of all three FMR viruses was effectively neutralized by each anti-FMR serum. These results suggest that Friend, Moloney, and Rauscher viruses possess similar, if not identical, antigens.

The results obtained are consistent with some previous studies (10, 15, 21), but not with others (4, 18, 20). The previously reported studies, however, often did not involve neutralization of all three FMR viruses or employed antisera of unknown potency. For example, Sleeves and Axelrad (21), using a spleen focus assay and murine anti-FMR sera, demonstrated that Friend and Rauscher viruses are antigenically similar, if not identical. However, the spleen focus assay is not applicable to Moloney virus, which, therefore, could not be studied. Fink et al. (4) showed that BALB/c mice pretreated with formalin-inactivated Friend virus became resistant to both Rauscher and Moloney viruses, whereas those pretreated with inactivated Moloney lymphoma virus were resistant to Moloney and Friend
viruses but not to Rauscher virus. The latter result, suggesting that Moloney and Rauscher viruses are not antigenically similar, is inconsistent with the findings of the present study. It is, of course, possible that the strain of mouse employed for immunization may affect the results obtained. Fink used BALB/c mice, whereas we used C57BL/6 mice. The relative immunization may affect the results obtained. Fink used guinea pigs, is inconsistent with the findings of the present study. It is, of course, possible that the strain of mouse employed for immunization may affect the results obtained. Fink used BALB/c mice, whereas we used C57BL/6 mice. The relative resistance to viral leukemogenesis of C57BL/6 mice as compared with BALB/c mice (11, 15, 18), as well as the relative ease of induction of transplantation resistance in C57BL/6 mice in the FMR systems (7, 11, 12), suggests that C57BL/6 mice may either recognize FMR antigenic components not recognized by BALB/c mice or may react more vigorously against them.

Virus neutralization studies aimed at determining the antigenic relationship between FMR and Gross viruses have yielded conflicting results (3, 8, 15, 21). One rabbit anti-Gross passage A serum neutralized Moloney lymphoma virus (8), whereas another failed to neutralize either Moloney lymphoma virus or Friend virus (15). Murine anti-Gross passage A serum failed to neutralize Rauscher or Friend viruses by the spleen focus assay (21). The failure of the latter must be qualified by the absence of any information about the potency of the antiserum against Gross virus, especially in view of the fact that no mouse anti-Gross passage A serum has been demonstrated to neutralize Gross virus oncogenicity.

The advent of specific rat anti-Gross serum of high potency has led to a reexamination of this problem. This antiserum was prepared in W/Fu inbred or W/Fu F, hybrid rats immunized against histocompatible W/Fu leukemias induced by wild-type Gross virus from C58 mice. The rat anti-Gross serum employed in this study has been extensively studied for the specificity of its activity (6). The antiserum contained cytotoxic antibody in very high titer (≥1:512) against G+ cells, neutralizing antibody against passage A virus, and precipitating antibody to ether-treated (but not intact) Gross and FMR viruses. The cytotoxic rat antibody could not be absorbed by normal tissue from various strains of mice, including BALB/c. Passive transfer of the rat anti-Gross serum has also been reported to protect mice against transplants of histocompatible G+ leukemia (17).

The data presented in this study show that the rat anti-Gross serum, when used in very high concentration (i.e., 1:2 dilution), strongly neutralized the oncogenicity of all three FMR viruses. Normal rat serum failed to neutralize Rauscher virus in this study and Moloney sarcoma virus in a previous study (3). The results suggest that Gross virus shares a virion antigenic coat component with the Friend, Moloney, and Rauscher viruses. These findings confirm the previously reported neutralizing activity of this antiserum against Moloney lymphoma virus (3) and Rauscher virus (6). The failure of rabbit anti-Gross serum to neutralize all FMR viruses, as well as the failure of murine anti-Gross serum to neutralize FMR viruses and Gross virus, may be attributed either to a lesser potency of the antiserum or to the possibility that the rat can recognize and react against some of antigenic components common to Gross virus and the FMR viruses not recognized by the mouse or rabbit.

Finally, the antigenic relationship between FMR viruses and Moloney sarcoma virus was also studied. Sera from mice whose autochthonous Moloney sarcomas had regressed neutralized all 3 FMR viruses, suggesting an antigenic similarity between Moloney sarcoma virus and the FMR viruses. This finding is consistent with the observations that tumors induced by Moloney sarcoma virus are antigenically similar to those induced by the FMR viruses, and that Moloney sarcoma virus can be neutralized by anti-FMR sera (1).

Since the FMR viruses were originally derived from BALB/c mice, and could conceivably have carried with them BALB/c isoantigenic material, the neutralization reactions of the rat anti-Gross serum and, even more likely, of the C57BL/6 anti-FMR sera, may reflect a reaction against BALB/c isoantigens. This possibility is rendered unlikely by the following observations: (a) The cytotoxic activity of the rat anti-Gross serum is not absorbed by normal BALB/c tissue (6). (b) The rat anti-Gross serum reacts against a BALB/c Moloney lymphoma, but not against a mineral oil BALB/c tumor by immunofluorescence (3). (c) The C57BL/6 anti-FMR sera do not react with lymphoid cells of BALB/c origin by immunofluorescence.

The antigenic relationship among Friend, Moloney, and Rauscher viruses derived from these studies should be qualified by the fact that antisera employed was prepared against lymphoma cells which, in addition to releasing oncogenic virus, may possess tumor-specific antigens. Although there is no convincing evidence that FMR lymphoma cells possess tumor-specific antigens, the possibility cannot be ruled out in the absence of virus-free tumors. If, however, the antisera contained antibody specific for cellular antigens, such antibody would only neutralize virus if the virus incidentally, but invariably, acquired the antigen from the cell during the process of viral maturation and release. Finally, to reconcile the ability of rat anti-Gross serum to neutralize FMR viruses, with the inability of Gross lymphomas to induce resistance to the transplantation of FMR lymphomas and vice versa in mice (7, 9), such transplantation studies in rats might be appropriate.

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REFERENCES

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