Enhancement of Friend Leukemia Virus Infection in Mice by Guaroa Virus: Quantitation and Action of Various Oncogenic and Nononcogenic Viruses

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Previous studies have indicated that Guaroa virus (GV), an arbovirus, enhanced the replication of Friend leukemia virus (FLV) in mice. To study further the interaction of GV and FLV, different levels of FLV activity were inoculated into mice. At a level of activity capable of producing limited splenic response, coinfection with GV resulted in a marked increase in spleen-foci activity, mean spleen weight, and amount of infectious virus recoverable from plasma and spleen of dually infected mice. Various oncogenic and nononcogenic viruses were also tested for their ability to enhance FLV infection.

The in vivo interaction of leukemogenic and nonleukemogenic viruses was studied. This interaction may result in an inhibition of leukemia virus replication, as demonstrated with Sendai virus (11, 12) and lymphocytic choriomeningitis virus (13), or the leukemia virus may enhance the nonleukemogenic virus as demonstrated with mouse hepatitis virus and murine leukemia virus (3), and murine sarcoma virus by murine leukemia virus (2). In contrast, leukemia virus replication may be enhanced without apparent nonleukemogenic virus replication as reported with Guaroa virus (GV; 10), an arbovirus of the Bunyamwera group; with Newcastle disease virus; and lactic dehydrogenase-elevating virus (9).

The mechanism by which a nonleukemogenic virus inhibits replication of leukemia viruses is thought to be mediated through interferon stimulation (4, 5); although the mechanism by which a nonleukemogenic virus enhances the replication of leukemia viruses is unproven, the enhancement may be due to an increase in the number of leukemia virus target cells in the spleen (9).

This report concerns the quantitative analyses of the enhancement of Friend leukemia virus (FLV), leukemogenesis by GV, and additional studies of various oncogenic and nononcogenic viruses as to their ability to enhance FLV leukemogenesis.

MATERIALS AND METHODS

Mice. Male, 6- to 8-week-old BALB/cCr mice were obtained from the colony at Microbiological Associates, Inc., Walkersville, Md. The mice were maintained in plastic cages, five mice per cage, and fed Purina laboratory chow and water ad libitum.

Viruses. A standard plasma pool of FLV, having a titer of 10^8 50% spleen-enlarging doses (SED50) per 0.1 ml, was employed throughout these experiments. A sample of FLV, diluted in phosphate-buffered saline (PBS) at pH 7.2 to contain 20 and 200 SED50 in 0.2 ml, was inoculated intraperitoneally (ip) into test mice.

GV was prepared as described previously (4). As inoculum, 0.2 ml of an appropriately diluted virus, 4 × 10^4 suckling mouse lethal doses (SMLD50), was inoculated ip into adult BALB/cCr mice.

Various other viruses were included to test their ability to enhance the leukemogenesis of FLV: Rous sarcoma virus (RSV, Schmidt-Ruppin), herpesvirus (HS-8), vaccinia (IHD), influenza (PR-8), and vesicular stomatitis virus (VSV).

Spleen focus assay. The macroscopic spleen focus assay for FLV was performed by using the procedure of Pujiznik and Sachs (7). After fixation, foci observed on the surface of infected spleens were counted. The total number of foci from five mice, 7 and 14 days after infection, was counted.

Mean spleen weight. The mean weight of spleens harvested from five donor mice at 7 and 14 days after infection was recorded to the nearest milligram.

Bioassay of FLV infected plasma and spleen extract. In determining the titer of FLV in plasma and spleen from infected mice, the bioassay procedure of Chirigos et al. (1) was used. In the bioassay of plasma from infected donors, blood was collected by anesthetizing mice with ether, severing the brachial artery, and collecting the blood in 0.153 M potassium citrate. The plasma was separated by centrifugation at 3,000 rev/min in an International PR-6 centrifuge for 20 min, collected, and pooled. A 10% (w/v) extract of donor FLV-infected spleens was prepared in PBS by
using a Virtis homogenizer. The extract was cleared at 3,000 rev/min as above and the supernatant collected. Serial 10-fold dilutions of plasma and spleen extracts were made in PBS. Each of five mice was inoculated ip with 0.2 ml per dilution of donor plasma or spleen extract and held for 14 days. The donor plasma and spleen extracts were then assayed for the amount of infectious FLV that could be recovered. The mice were killed by cervical dislocation, and the spleens removed and weighed to the nearest milligram on a Sauter-Toppan balance. Spleens having a weight of 180 mg or greater were considered as positive in the Reed and Muench determination (8) of SED50 for FLV leukemogenesis.

RESULTS

Comparison of concentration of FLV for leukemia induction. Following the procedure of Turner et al. (10), mice were conditioned with GV 3 days prior to FLV inoculation (Table 1). In co-infected mice which received the higher concentration of FLV (200 SED50), there was a greater than twofold increase in the number of foci and a 24 mg increase (P < 0.05) in mean spleen weight over FLV-infected controls; however, there was a decreased amount of recoverable virus from both plasma and spleen when compared to FLV-inoculated controls. If one log less virus was used (20 SED50), the enhanced activity of FLV became more apparent. The number of foci formed is nearly ninefold (P < 0.01) that of the control; the mean spleen weight increased 34 mg (P < 0.05), and the amount of recoverable virus from plasma and spleen was markedly increased. In plasma preparations of FLV alone, recoverable virus was barely detectable, whereas in co-infected mice nearly 75-fold (P < 0.01) increase of infectious virus was recovered. In

### Table 1. Enhanced activity of Friend leukemia virus (FLV) by Guaroa virus (GV) at 7 days post-inoculation

| Virus concn | Donor mice sacrificed day 7 |
|-------------|-----------------------------|
|             | Spleen foci | Mean spleen wt | Bioassay |
| GV 4 × 10^6 | 0 | 129 | |
| FLV 2 × 10^6 | >160 | 156 | 2.83 3.22 |
| GV 4 × 10^6 | 74 | 132 | 3.00 3.75 |
| FLV 2 × 10^4 | 25 | 100 | 0.08 1.44 |
| GV 4 × 10^4 | 217 | 134 | 1.95 2.15 |

* Titer of recoverable virus expressed as ED50/0.2 ml.

### Table 2. Enhanced activity of Friend leukemia virus (FLV) by Guaroa virus (GV) 14 days post-inoculation

| Virus concn | Donor mice sacrificed day 14 |
|-------------|-----------------------------|
|             | Spleen foci | Mean spleen wt | Bioassay |
| GV          | 116 | 4.30 |
| FLV         | 426 | 3.83 |
| GV 4 × 10^6 | 301 | 3.17 |
| FLV 2 × 10^6 | 172 | 3.17 |
| GV 4 × 10^4 | 299 | 4.00 |

* Titer of recoverable virus expressed as ED50/0.2 ml. 

spleen preparation, a fivefold increase of recoverable virus was noted (P < 0.01).

By the 14th day spleen foci determinations were no longer sensitive, as foci were confluent and uncountable due to the infection. However, differences were still observed in mice receiving the lower dose of FLV (20 SED50) in two of the three parameters examined (Table 2). An increase in mean spleen weight of 127 mg (P < 0.01) and in recoverable virus from spleen extracts of nearly sevenfold (P < 0.01) was seen in co-infected mice when compared to FLV alone.

Comparison of viruses to GV for the enhancement of FLV leukemogenesis. By using the low dose (20 SED50) for FLV infection, various viruses were tested for their capacity to enhance FLV activity. From the previous data, the most significant information could be obtained from spleen foci counts that were enumerated at 7 days, from the mean spleen weight data that was collected at 14 days, and from the bioassay of plasma (7 days) and spleen (14 days). With each virus, a comparison is made with GV for determining the enhancement of FLV leukemogenesis (Table 3).

Although a significant increase in spleen foci was observed with VSV and vaccinia, neither virus approached GV in increased infectivity as determined by spleen focus analysis. With spleen weight analysis, VSV produced a significant increase in spleen weight greater than GV. In the bioassay of plasma and spleen from co-infected mice, the amount of recoverable FLV was not significantly (P > 0.10) greater when compared with GV. Both VSV and vaccinia were capable of increasing recoverable virus from plasma; when compared with the FLV control, the differences expressed between GV and these viruses were not
significant, and neither of the viruses exhibited increased ability to enhance infection when harvested from spleens of co-infected mice. Although VSV gave the best indication of the other viruses tested as being able to enhance FLV leukemogenesis, it did not enhance leukemogenesis in the test parameters to the same degree as observed for GV. Of the other viruses tested, vaccinia and RSV each showed an ability to increase infectivity of FLV, but none approached GV in increasing leukemogenesis. Except for increasing the amount of FLV recovered from the plasma, herpesvirus decreased leukemogenic activity. Influenza caused an increase in spleen weight, but none of the other test systems were affected by influenza.

None of the viruses tested had the ability to induce spleen foci or cause an increased spleen weight when given to the mice without FLV.

**DISCUSSION**

The interaction of viruses plays an important role in infection or disease processes in an animal. Perhaps interaction plays a more important role than single virus in the study of natural infections. There are many reactions that could trigger a latent or subliminal infection and cause a disease to become manifest. This study was concerned with the ability of an unrelated virus causing an enhancement of leukemia. FLV was chosen for its short latent period in the observance of infection. GV was previously shown (9) to enhance the leukemia response of FLV and Rauscher leukemia virus (RLV). The ability of virus to enhance the leukemic response was found to be related to the amount of input leukemia virus. If too much leukemia virus was administered, an overwhelming response was observed, which tended to obliterate the interaction of another virus. If, however, a quantity of leukemia virus was administered that, by itself, did not induce an early and overwhelming disease but rather gave a diminished but interpretable response, the effect of coinfection could be measured. When a concentration of FLV, sufficient to cause only a minimal response was used, prior co-infection with GV increased the number of spleen foci nearly ninefold, increased the mean weight of spleens harvested at 7 and 14 days, and greatly increased the amount of recoverable virus from the plasma at 7 days and from the spleen at 14 days. An early viremia would account for the amount of virus recovered from the plasma, and the later increases in virus concentrations, such as in the spleen, could be attributed to infectious viruses being absorbed by internal organs. GV by itself is refractory in the adult mouse (5), and there was no evidence of GV being present in plasma or spleen preparations. The action of GV is thought to be due to increased numbers of spleen cells susceptible to FLV infection.

Since GV is an arbovirus with the size and ribonucleic acid content similar to leukemia viruses, different other viruses were tested to see whether they also enhanced FLV infection. Although VSV somewhat increased the response of FLV infection, the degree of response was not as great as noted with GV. The other viruses, vaccinia, RSV, influenza, and herpesvirus, had little effect on enhancing leukemia. None of the viruses tested had the ability to increase the splenic response by itself.

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