Activation of Guinea Pig Herpesvirus Antigen in Leukemic Lymphoblasts of Guinea Pig

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Herpesvirus (GPHV) antigen is either present in very small amounts, or absent in leukemic lymphoblasts taken directly from strain 2 guinea pigs. However, after maintenance in tissue culture for 72 hr, almost 100% of these lymphoblasts contained GPHV antigen. The expression of GPHV antigen could be demonstrated by indirect immunofluorescent technique as well as by the direct 125I-labeled antibody technique. However, infectious virus or virus capsids could not be detected in these cells either by infectivity tests or electron microscopy.

Recently, we isolated a guinea pig herpesvirus (GPHV) from leukemic lymphoblasts (7). GPHV is different from known herpesviruses of guinea pig, including cytomegaloviruses (4, 7). However, it was difficult to ascertain the role of GPHV in guinea pig leukemia, since GPHV was isolated from both normal and leukemic strain 2 guinea pigs (4), and no clear-cut transformation of lymphocytes in vitro by GPHV was obtained (3). We report here that GPHV antigen can be activated in leukemic lymphoblasts after in vitro cultivation of the cells. However, no infectious virus was demonstrable in these cultures. These results indicate that at least a portion of virus genome is resident in leukemic cells.

MATERIALS AND METHODS

Initially, leukemic tissues and strain 2 guinea pigs were obtained from S. Opler, Stanford University. Leukemia was maintained by serial inoculation of leukemic tissues in strain 2 guinea pigs. Leukemic lymphoblasts were obtained from peripheral blood, spleen, or lymph nodes and used immediately or kept frozen at −85 C in a medium containing 10% dimethyl sulfoxide. Cells treated with dimethyl sulfoxide were used within 2 to 4 weeks, and 80 to 85% of the cells were viable. Normal lymphoblasts were pooled from spleen and lymph nodes of normal strain 2 guinea pigs.

GPHV was cloned three times in secondary rabbit kidney cells, and the antiserum against GPHV was prepared in rabbits using GPHV-infected rabbit kidney cells mixed with complete Freund adjuvant. Rabbits were immunized twice a week for 5 weeks. Serum from each rabbit was tested for the presence of anti-GPHV antibodies. The serum of rabbit no. 3 had a titer of 1:640 by indirect immunofluorescence test and 1:80 by neutralization assay (i.e., 80% reduction in number of plaques [1]), and was exclusively used in these experiments. Neither preimmune nor normal rabbit serum had any antibodies against GPHV by either test.

In a typical experiment, both leukemic and normal lymphoblasts were suspended in Eagle medium containing glutamine (0.0292%), inactivated fetal calf serum (10%), and Tryptose phosphate broth (10%). Samples of cells were tested at different times for the viability, expression of GPHV antigen by immunofluorescence, presence of virus structural components by electron microscopy, and infectious virus by infectivity assay. Cell viability was determined by eosin exclusion techniques (8).

RESULTS

Indirect immunofluorescence techniques were used to assay for GPHV antigens (9). The results of a typical experiment are presented in Table 1. Fresh leukemic lymphoblasts did not contain significant amounts of GPHV antigen. However, one or two bright specks of fluorescent patches were always present on the cell membrane when anti-GPHV rabbit serum was used. No such fluorescent specks were found when preimmune rabbit serum was used. These specks could represent some specific GPHV antigen. However, the amount of fluorescence was much less than the bright fluorescence induced by in vitro cultivation of lymphoblasts.

After 16 hr in vitro, bright immunofluorescent cells appeared when tested against anti-GPHV rabbit serum (Fig. 1). Such cells were not stained with preimmune rabbit serum, and dead cells appeared to have a dull fluorescence when tested against preimmune rabbit serum. The number of bright immunofluorescent cells increased with the incubation period, as did the percentage of dead cells. However, all the dead cells were not positive
TABLE 1. **Expression of GPHV antigen in leukemic lymphoblasts of guinea pig**

<table>
<thead>
<tr>
<th>Days of incubation</th>
<th>Viability of cells (%)</th>
<th>Immunofluorescence</th>
<th>Presence of GPHV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Positive (%)</td>
<td>Brightness</td>
</tr>
<tr>
<td>0</td>
<td>90</td>
<td>0 -</td>
<td>NV* NV</td>
</tr>
<tr>
<td>1</td>
<td>68</td>
<td>50 ++</td>
<td>NV NV</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>100 +++++</td>
<td>NT NT</td>
</tr>
<tr>
<td>3</td>
<td>0.5</td>
<td>100 +++++</td>
<td>NT NV</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>100 ++</td>
<td>NT NV</td>
</tr>
</tbody>
</table>

*Fresh leukemic lymphoblasts were placed in modified Eagle medium (10^6 cells/ml) containing glutamine (0.0292%), Tryptose phosphate broth (10%), and inactivated fetal calf serum (10%), and incubated at 37°C in an incubator containing CO2 (5%). Samples of cells were removed at intervals and tested for viability and immunofluorescence. Samples were also tested for virus structures in an electron microscope and for infectious virus on secondary rabbit kidney and guinea pig embryo cells.

† Brightness was determined on a scale of 1+ to 4+.

*NV, No virus; NT, not tested.

Fig. 1. **Expression of GPHV antigen in leukemic lymphoblasts in tissue culture.** Leukemic lymphoblasts were incubated in modified Eagle medium containing Tryptose phosphate broth (10%) and inactivated fetal calf serum (10%) for 16 hr (A, B) or 48 hr (C, D), and tested for the presence of GPHV antigen by indirect immunofluorescent technique. A, 16-hr post incubation, photographed in bright light. B, Same field in ultraviolet light. C, 48-hr post incubation, photographed in bright light. D, Same field in ultraviolet light (×125).
elucidation of GPHV antigen in fresh lymphoblasts. Finally, lymphoblasts from five normal guinea pigs were prepared from spleen and mesenteric lymph nodes in the same way and tested individually. All these lymphoblasts were found to be negative for the expression of GPHV antigen.

Next, we wanted to find out if GPHV antigen could also be demonstrated by using $^{125}$I-labeled gamma globulins. Accordingly, gamma globulins were isolated from preimmune and GPHV-immunized rabbit serum and labeled with $^{125}$I to the same specific activity (200,000 counts per min per $\mu$g of protein) (5). Leukemic lymphoblasts were incubated with $^{125}$I-labeled normal and immune globulins for 30 min at 37 C. Lymphoblasts were subsequently layered over a 5% sucrose solution (w:v) containing 0.1 M NaCl and 0.01 M tris(hydroxymethyl)aminomethane (pH 7.4), and centrifuged at 900 x g for 15 min at 4 C. The pelleted cells were suspended in phosphate-buffered saline, a sample was used for determining the number of cells, and another sample was dissolved in NCS-solubilizer (Amersham/Searle) and counted in a liquid scintillation counter. The results (Fig. 2) show that leukemic lymphoblasts, at 48 hr of culture, bound the maximal amount of immune globulins; there was no significant increase of binding nonimmune globulins. The ratio of binding (immune/normal) of immunoglobulins was 4.5 at 48 hr.

DISCUSSION

Two conclusions may be drawn from this study. First, GPHV is not expressed in leukemic lymphoblasts in vivo but becomes derepressed in tissue culture. GPHV, in this respect, follows the same pattern as herpesvirus samirii (10), and to some extent, EB virus in Burkitt lymphoma cells (2, 8). Secondly, the GPHV genome is present in all leukemic lymphoblasts and can be expressed without the production of an infectious virus. The cells in which the virus genome is expressed do not synthesize deoxyribonucleic acid and will die soon. This would explain why numerous attempts to establish continuous cell lines from leukemic lymphoblasts of guinea pigs have failed (Nayak, unpublished data). We have yet to find a culture condition which would keep the virus genome repressed in vitro. The experiments reported here do not distinguish between a partial and a complete virus genome in leukemic lymphoblasts. Since infectious GPHV can be recovered from a small number of normal as well as leukemic lymphoblasts (4, 7), it is likely that the majority of the lymphoblasts contain a partial virus genome, and infectious virus is maintained by a chronic infec-

![Fig. 2. Binding of $^{125}$I-labeled immune globulins to leukemic lymphoblasts. Immune globulins from normal and immunized rabbit serum was labeled with $^{125}$I to the same specific activity (200,000 counts per min per $\mu$g). Samples of leukemic lymphoblasts (2 X 10$^6$ cells/0.2 ml) were incubated with 10$^6$ counts per min of labeled globulins. Normal rabbit gamma globulins (50 $\mu$g) were added to each of the samples. The cells and labeled gamma globulins were incubated for 30 min at 37 C with constant mixing. At the end of incubation, the entire sample (0.5 ml) was layered over 5% sucrose containing 0.1 M NaCl and 0.01 M tris(hydroxymethyl)-aminomethane-hydrochloride (pH 7.4), and centrifuged at 900 x g for 15 min at 4 C. The pelleted cells were suspended in phosphate-buffered saline, and samples were used for determining the number of cells and for counting in a liquid scintillation counter.](http://jvi.asm.org/)

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co-carcinogen for the activation of C-type viruses; however, leukemic lymphoblasts or the leukemic plasma do not contain any matured C-type virus. Only virus-like particles budding from endoplasmic reticulum into the cysternae can be seen (7). Tissues of leukemic guinea pigs (peripheral lymphoblasts, lymph node, tumors, spleen, liver) were tested for GS-3 antigen, the common mammalian group-specific antigen, by complement-fixation and only trace amounts were found (Nayak, unpublished data). However, it is still possible that an unknown C-type virus with a different GS antigen is present in leukemic guinea pigs, or that the GS-3 antigen is either not expressed or is expressed in small amounts that cannot be detected by a standard complement-fixation test. The presence of GPHV genome (partial or complete) in almost 100% of leukemic cells, in repressed form in leukemic animals, excluded the possibility that GPHV is acquired during tissue culture or from a few chronically infected cells. The nature and the status of the GPHV genome in leukemic lymphoblasts and its possible oncogenic role in guinea pig leukemia are now being studied.

**ADDENDUM IN PROOF**

Recently, we have been able to activate an oncornavirus type having the morphological and biochemical characteristics of a C-type RNA virus after bromodeoxyuridine and iododeoxyuridine treatment of cultured cells from leukemic and normal guinea pigs.

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**LITERATURE CITED**