An Animal Model for DNA-RNA Virus Interaction Based upon Virological and Histological Findings

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Summary

The presence of endogenous oncornavirus and herpesvirus in guinea pigs has been established. The oncornavirus apparently is present in all guinea pigs but is expressed only under certain conditions. Expression of the latent herpesvirus is generally age and strain dependent as is the development of spontaneous guinea pig leukemia. Following special laboratory manipulation, expression of both virus types was accomplished in vitro. Studies of the role played by these two virus types in the development of neoplastic disease in guinea pigs revealed that, in the presence of the endogenous oncornavirus, a superinfection with herpesvirus led to the development of self-limited lymphoproliferative changes. Together with the studies reported by other investigators, it appears that interaction between the DNA and RNA viruses may play an important role in the natural occurrence of viral oncogenesis. Guinea pigs provide an intriguing animal model for the study of herpescivirus and oncornavirus interaction both in vivo and in vitro.

Introduction

In 1954 Congdon and Lorenz described an acute lymphoblastic leukemia, L6C, in an inbred strain 2 guinea pig over 1 year old (1). This leukemia can be transmitted serially in strain 2 or F hybrid guinea pigs by i.p. inoculation of blood or spleen cells obtained from diseased animals. The disease is characterized by large numbers of lymphoblasts in the peripheral blood and extensive infiltration of normal tissues by leukemic cells.

In searching for a viral etiology for the L6C guinea pig leukemia, Nadel et al. (11) and Opler (13) reported the observation of oncornavirus particles in the cytoplasm of cells obtained from leukemic guinea pigs. Subsequently, similar virus particles were found in leukemic guinea pigs but not in tissues of normal guinea pigs without the disease (3). However, recently we found that the guinea pig oncornavirus could be activated in cultured cells derived from normal as well as leukemic guinea pigs following treatment with 5-bromo-2'-deoxyuridine (6), and this finding has since been confirmed by other laboratories (2, 12, 15). Moreover, a herpesvirus that apparently occurs in a latent state has been consistently isolated from cultures derived from tissues of strain 2 guinea pigs with or without leukemia (9, 10).

Detection of Herpesvirus and Oncornavirus in Guinea Pigs

Guinea pigs, particularly older inbred strains including strain 2, showed widespread herpesvirus infection (10). When serial blood samples taken from inbred or strain 2 Hartley hybrid guinea pigs were examined for evidence of virus infection by cocultivation, it was noted that the initial appearance of herpesvirus infection in these guinea pigs occurred as they reached 5 to 6 months of age, and almost all were infected when they reached 10 to 12 months or older (7).

One of the most interesting features of the guinea pig herpesvirus is its relationship to host cells in vivo and in vitro. Leukocytes or tissue cells taken directly from infected guinea pigs showed no intracellular viral antigens by immunofluorescence, no intranuclear inclusions by light microscopy, and no virus particles by electron microscopy (10). Nevertheless, the presence of the viral genome was established by demonstrating the presence of virus-induced inclusions and herpesvirus particles following cultivation and cocultivation of the infected tissues.

Since oncornavirus particles were not found in the tissues of normal adult guinea pigs (3), attempts were made to induce the virus in cultured cells derived from the adult tissues. It was found that guinea pig oncornavirus could be induced in all primary cell cultures and passaged cell lines derived from guinea pigs as long as they were exposed to 5-bromo-2'-deoxyuridine (6, 8). Regardless of tissue origin, guinea pig strain, or age of the animals, large aggregates of extracellular virus particles were observed.

Histopathology of Lymphoid Tissues from Guinea Pigs Experimentally Infected with Herpesvirus

As mentioned above, most strain 2 or hybrid guinea pigs showed herpesvirus infection by the age of 10 to 12 months without any evidence of clinical disease nor were any histopathological changes detected. However, young guinea
pigs experimentally inoculated with the herpesvirus suspension showed significant changes in the lymphoid tissues. An intense reactive lymphoid hyperplasia was observed in the spleen and lymph nodes of many guinea pigs inoculated with the guinea pig herpesvirus. Other tissues, including salivary gland, lung, and kidney, were generally normal. As shown in Fig. 1, the germinal centers of the lymph node were enlarged and active and contained many immature, actively dividing lymphoid cells and histiocytes. The sinuses were filled with mature lymphoid cells and active histiocytes. Similar histological findings were observed in the spleen, the lymphoid follicles being enlarged and containing many immature lymphoid cells.

Comparison of Virus Titer and Histopathology of Lymphoid Tissues of Guinea Pigs Infected with Herpesvirus

To determine whether the presence of hyperplasia in the lymphoid tissues was a reflection of high virus titer in the spleen at the time of sacrifice, virus infectivity titers and histopathology of 4 groups of guinea pigs were compared. Table 1 lists examples of each group. In the naturally infected animals with a virus titer of 4 logs/ml of packed spleen cells, there was no evidence of hyperplasia. Animals inoculated with herpesvirus suspensions by various routes, including i.p., i.c., or s.c., revealed a high degree of hyperplasia, although the virus titers were comparable to those obtained from the naturally infected ones. On the other hand, animals given injections of 100-times concentrated UV-inactivated guinea pig herpesvirus suspension did not show any changes in their spleens. Furthermore, guinea pigs inoculated 1 or more times with spleen cell suspensions containing latent herpesvirus, as determined by cocultivation, did not develop hyperplasia although the virus titer was in the same range, i.e., 4 logs of infectious virus.

Histopathology of Lymphoid Tissues following Inoculation with Guinea Pig Oncornavirus and/or Guinea Pig Herpesvirus

In light of the histopathological changes resulting from inoculation with guinea pig herpesvirus, and since all guinea pigs are endogenously infected with guinea pig oncornavirus (8), attempts were made to investigate and compare the effect of these 2 viruses in guinea pigs when inoculated either alone or in combination (Table 2). In a total of 11 guinea pigs inoculated with guinea pig oncornavirus alone, evidence of hyperplasia in spleen and lymph node was observed in only 2 of the animals examined. Hyperplasia was observed in the spleen of 15 of 21 animals that received guinea pig herpesvirus alone and 14 of 20 animals that received both viruses, while manifestations of hyperplasia in the lymph nodes were noted in 15 of 20 animals given herpesvirus alone and 21 of 22 animals given both viruses. Application of statistical testing procedures to these data indicated that the differences between inoculation with herpesvirus alone or in combination with oncornavirus were insignificant. However, when comparing oncornavirus alone with either of the other 2 inocula the differences were statistically significant (p < 0.01 or lower). The

Table 1
Examples of virus titer and histopathology of spleens from guinea pigs infected with herpesvirus

<table>
<thead>
<tr>
<th>Guinea pig</th>
<th>Route of infection</th>
<th>Material inoculated</th>
<th>Age at time sacrificed</th>
<th>Virus titer log 50% tissue culture infectious dose/ml packed spleen cells</th>
<th>Histopathology of lymphoid follicles of spleen</th>
</tr>
</thead>
<tbody>
<tr>
<td>AD002</td>
<td>Natural</td>
<td>None</td>
<td>10 mo.</td>
<td>4.3</td>
<td>Normal</td>
</tr>
<tr>
<td>AD123</td>
<td>Natural</td>
<td>None</td>
<td>5 mo.</td>
<td>4.0</td>
<td>Normal</td>
</tr>
<tr>
<td>AD218</td>
<td>i.c.</td>
<td>Infectious virus</td>
<td>3 wk</td>
<td>4.0</td>
<td>Hyperplastic</td>
</tr>
<tr>
<td>AD240</td>
<td>s.c.</td>
<td>Infectious virus</td>
<td>6 wk</td>
<td>5.2</td>
<td>Hyperplastic</td>
</tr>
<tr>
<td>AD246</td>
<td>s.c.</td>
<td>UV-inactivated virus (100 x concentration)</td>
<td>3 wk</td>
<td>1.0</td>
<td>Normal</td>
</tr>
<tr>
<td>AD248</td>
<td>s.c.</td>
<td>UV-inactivated virus (100 x concentration)</td>
<td>6 wk</td>
<td>1.0</td>
<td>Normal</td>
</tr>
<tr>
<td>K0 52</td>
<td>i.p.</td>
<td>Infected spleen cell suspension (1 x)</td>
<td>2 mo.</td>
<td>4.3</td>
<td>Normal</td>
</tr>
<tr>
<td>K0 001</td>
<td>i.p.</td>
<td>Same (4 x)</td>
<td>4 mo.</td>
<td>+ a</td>
<td>Normal</td>
</tr>
<tr>
<td>K0 002</td>
<td>i.p.</td>
<td>Same (6 x)</td>
<td>6 mo.</td>
<td>4.0</td>
<td>Focal hyperplastic</td>
</tr>
</tbody>
</table>

* Animal found dead.

The abbreviation used is: i.c., intracardiac.
G. D. Hsiung and A. H. McTighe

**Table 2**

Comparison of lymphoid tissues of guinea pigs inoculated with guinea pig oncornavirus and/or guinea pig herpesvirus

<table>
<thead>
<tr>
<th>Material inoculated</th>
<th>No. positive/no. examined</th>
<th>% positive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Spleen</td>
<td>Lymph node</td>
</tr>
<tr>
<td>Oncornavirus alone</td>
<td>2/11</td>
<td>18.1*</td>
</tr>
<tr>
<td>Herpesvirus alone</td>
<td>15/21</td>
<td>71.4*</td>
</tr>
<tr>
<td>Both herpes and oncornavi-</td>
<td>14/20</td>
<td>66.7*</td>
</tr>
<tr>
<td>rous together</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\* Footnote a vs. Footnote b: spleen, t = 2.87, p < 0.01; lymph node, t = 3.04, p < 0.01. Footnote a vs. Footnote c: spleen, t = 2.76, p < 0.01; lymph node, t = 4.55, p < 0.001. Footnote b vs. Footnote c: spleen, t = 0.10, p < 0.95; lymph node, t = 1.89, p < 0.06.

critical event for the development of hyperplasia appears to require the replication of exogenous herpesvirus, since animals receiving UV-inactivated virus did not show any sign of hyperplasia. No real evidence of leukemia was observed in any of the inoculated guinea pigs. While not actually neoplastic, the hyperplasia of spleen and lymph nodes as a result of inoculation with the infectious herpesvirus suspension was characterized by the proliferation of immature lymphoid cells. Similar proliferation of lymphoid cells by other herpesviruses has been demonstrated. For example, Epstein-Barr virus can induce the disorderly, although self-limited, hyperplasia of lymphoid tissues as seen in infectious mononucleosis. As reported previously (8), all guinea pig cells are endogenously infected with guinea pig oncornavirus; therefore, it is possible that the exogenous herpesvirus infection activated the endogenous oncornavirus, resulting in the production of hyperplasia in the lymphoid tissues. Evidence of interaction between Marek's disease herpesvirus and avian leukemia virus has been reported (4, 5, 14), as has the observation that tumor cells derived from Burkitt's lymphoma, with which EB herpesvirus has been associated, apparently contain a RNA related to the RNA of murine leukemia virus (16). It appears that interaction between the DNA and RNA viruses may play an important role in the natural occurrence of viral oncogenesis.

**Acknowledgments**

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**References**

Fig. 1. Histological sections of lymph nodes. a and b, noninfected lymph node; c and d, lymph node obtained from an animal inoculated with herpesvirus suspension showing an enlarged germinal center containing immature lymphoid cells. H & E, a and c, × 100; b and d, × 430.
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