Differential Neurooncogenicity of Strains of JC Virus, a Human Polyoma Virus, in Newborn Syrian Hamsters

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SUMMARY

The neurooncogenicity of three recently isolated strains of the human polyoma virus, JC virus, was determined by intracerebral inoculation of newborn Syrian golden hamsters. All three strains produced malignant brain tumors in a majority of inoculated animals during a 6.5-month observation period. The results obtained with the MAD-2 strain, 19 of 20 animals with cerebellar medulloblastomas and 0 of 20 animals with pineal gland tumors, were quite similar to those observed previously with the prototypic strain of JC virus, MAD-1. Inoculation of the MAD-4 strain, however, resulted in 10 of 22 animals with pineal gland tumors and only 10 of 22 animals with tumors in the cerebellum. The MAD-3 strain was neurooncogenic, but too few animals lived to be weaned to provide significant additional information. The basis for the apparent predilection of the MAD-4 strain for the pineal gland is unknown. Two hamsters in the experiment developed extracranial neuroblastomas.

INTRODUCTION

JCV, a human polyoma virus, is the virus most commonly associated with the rare demyelinating disease, progressive multifocal leukoencephalopathy (4). The 1st isolation of JCV, in 1971, was from diseased brain tissue of a patient with this disease (3). Polyoma viruses are oncogenic DNA viruses. A frequent histological finding in old lesions of progressive multifocal leukoencephalopathy is enlarged astrocytes, with bizarre nuclei, that resemble malignant astrocytes in glioblastomas (10). The original JCV isolate is extremely neurooncogenic in newborn Syrian hamsters (9). Eighty-three % of inoculated hamsters developed malignant gliomas within 6 months. Most of the gliomas were medulloblastomas, glioblastomas, or unclassified primitive tumors. Such tumors were not previously reported to be induced in vivo by polyoma viruses.

In discussion of these findings, a frequent question was whether the observed neurooncogenicity was a characteristic of JCV or a peculiarity of the 1st isolate. The opportunity to answer this question came when viruses serologically indistinguishable from the original JCV were isolated from brain tissues of each of 4 additional cases of progressive multifocal leukoencephalopathy (4). The new isolates are designated the MAD-2, MAD-3, MAD-4, and MAD-5 strains of JCV; the original isolate, which is now the prototypic strain of JCV, is the MAD-1 strain. The neurooncogenicity of 3 of the new strains was determined by i.c. inoculation of newborn hamsters.

MATERIALS AND METHODS

Pregnant Syrian golden hamsters (LVG:LAK, outbred strain) were purchased from Lakeview Hamster Colony, Newfield, N. J. They were fed Purina mouse and rat diet supplemented with a piece of vegetable daily (alternately, carrot and potato) and water ad libitum. Newborn hamsters (less than 24 hr old) were inoculated in the right cerebral hemisphere with 0.02 ml of virus or control material. Each virus inoculum contained 2560 hemagglutinating units of purified virus that had been grown in primary human fetal glial cells (3). Control inoculum consisted of material prepared similarly from uninfected cultures of glial cells. All inocula were in Hanks’ solution supplemented with 0.01% bovine albumin.

After weaning, the animals were caged individually and observed daily for signs of central nervous system disease. Animals with signs of disease were exsanguinated under ether anesthesia. The sera were collected and tested for the presence of antibodies against JCV and T-antigen. The hemagglutination-inhibition test (2) was used to measure antibodies against viral coat protein; titers are the reciprocal of the highest serum dilution that inhibited hemagglutination completely. Antibody against T-antigen was detected by indirect immunofluorescent staining (4) of the JCV-induced hamster tumor cell line, HJC-15 (9). This test was scored as positive or negative. Fluorescein-conjugated anti-hamster γ-globulin was purchased from Antibodies, Inc., Davis, Calif.

A complete autopsy was performed on every animal. The internal organs were examined grossly but were not fixed for histological examination if of normal appearance. Prior to brain removal, the calvarium of each animal was deflected in 1 piece and examined for adherent tumor tissue. If tumor was present, 1 or more pieces were processed for light microscopy; sections were stained with hematoxylin.
and eosin and with reticulum stain. All brains were removed and examined grossly and microscopically for presence of tumors. Samples from selected tumors detected by gross examination were taken for tissue culture attempts. Each brain was fixed by immersion in 10% neutral formalin, cut into 6 to 8 pieces, and embedded in paraffin. Eight-μm sections were cut by the conventional method and stained with hematoxylin and eosin. One section from each piece was examined microscopically.

For growth in vitro, a piece of the tumor was minced finely with scissors and added to 2 ml of growth medium (Eagle’s minimum essential medium with Earle’s salts supplemented with nonessential amino acids, L-glutamine, 10% heated fetal calf serum, penicillin, and streptomycin) in a 25-sq cm plastic flask. Following attachment and outgrowth of cells, the cultures were kept in 5 ml of medium. For detection of T-antigen, tumor cells were grown on glass coverslips in Leighton tubes, then fixed in acetone for 5 min, and stained by the indirect immunofluorescent technique with a hamster anti-JCV T-antiserum (a pool of sera from hamsters bearing JCV-induced tumors).

RESULTS AND DISCUSSION

Thirty-two, 27, 33, and 13 newborn hamsters were inoculated i.c. with MAD-2, MAD-3, MAD-4, and control material, respectively. Cannibalism and an accident that resulted in inability to identify 8 animals reduced the number in each group to 20, 2, 22, and 6, respectively. The 1st deaths due to brain tumors occurred 4 months after inoculation. Most virus-inoculated animals died or were sacrificed after showing signs of disease between the 21st and 25th week postinoculation. The remaining virus-inoculated animals (4 in the MAD-2 group and 1 in the MAD-4 group) were sacrificed at 6.5 months postinoculation. The 6 control animals were sacrificed at 8 months postinoculation.

Twelve virus-inoculated animals were found dead. However, sera were obtained from 13, 2, and 17 animals inoculated with the MAD-2, MAD-3, and MAD-4 strains, respectively; of these sera, 9, 1, and 16, respectively, contained antibodies against JCV T-antigen. The T-antigen is not a structural component of the virus; therefore, it was not present in the inoculum. T-antigen is produced in cells during viral multiplication and in cells transformed by the virus. Twenty-four of the 26 hamsters with T-antibody had tumors; 4 of the 24 were detected only by microscopic examination. The 2 hamsters with anti-JCV T-antibody in which tumors were not detected had been inoculated with MAD-4 virus. All of the sera, except 1 from a MAD-4-inoculated animal, had antibodies against JCV coat protein with titers ranging from 80 to 1280. These titers represent an antibody response to the virus inoculum. The 6 sera from control animals did not contain antibodies against either antigen.

No tumors were found in the control hamsters. The number and distribution of tumors in the virus-inoculated groups are shown in Table 1. All of the animals that were inoculated with the MAD-3 strain of JCV and survived to be weaned developed brain tumors; however, because there were only 2 hamsters in this group, the only conclusion to be drawn is that this strain is neurooncogenic. Every hamster inoculated with the MAD-2 strain developed tumors also. Many animals had tumors in 2 locations and 1 had tumors in 3 sites, cerebellum, deep cerebrum, and lateral ventricle. Ninety-five % had medulloblastomas of the cerebellum (Fig. 1). Malignant tumors in the deep cerebrum were 2nd in frequency, an occasional ependymoma was found in the ventricles, and no tumors were observed arising from the pineal gland. This distribution of tumors parallels closely the results obtained when newborn hamsters were inoculated i.c. with the MAD-1 strain of JCV (6, 9, 11). In these MAD-1-inoculated animals, the vast majority of tumors were medulloblastomas in the cerebellum; thalamic gliomas were next in frequency; and a few ependymomas and 1 pineocytoma were also found in over 60 inoculated animals held for 6 months.

Unexpectedly, the MAD-4 strain gave quite different results. Ninety-one % of MAD-4-inoculated animals had tumors; again many of these animals had tumors in 2 locations and 3 animals had tumors at 3 sites, but the incidence of tumors in the various locations was distinctly different from that found in the hamsters inoculated with the MAD-2 strain. Compared to the MAD-2 group, the MAD-4 group had a strikingly higher incidence of tumors arising from the pineal gland, a lesser incidence of tumors in the cerebellum, and no tumors in the olfactory-frontal region. Forty-five % of the animals developed gross tumors of the pineal gland (Fig. 2), identified as pineocytomas by microscopy. Separate studies have been performed on certain of these tumors by electron microscopy (8), and enzymatic analyses

<table>
<thead>
<tr>
<th>Strain of JCV</th>
<th>No. with tumors/ no. in group</th>
<th>Intracranial</th>
<th>Olfactory-frontal area</th>
<th>Ventricular</th>
<th>Pineal</th>
<th>Extracranial</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAD-2</td>
<td>20/20</td>
<td>19</td>
<td>6</td>
<td>4</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>MAD-3</td>
<td>2/2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>MAD-4</td>
<td>20/22</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td>1</td>
<td>10</td>
</tr>
</tbody>
</table>

* Tumors were identified by gross and microscopic examination or by microscopy alone.
have shown that these tumors contain hydroxyindole-O-methyltransferase (5), an enzyme practically restricted to the pineal gland. JCV is the 1st agent, viral or chemical, reported to induce tumors of the pineal gland in any experimental animal; the MAD-4 strain appears to do so with a fair degree of regularity in the newborn hamster.

Attempts were made to grow 3 of the pineal tumors in tissue culture, but these were unsuccessful. Tumor cell lines have been established from 2 cerebellar tumors induced by the MAD-2 strain and a deep cerebral tumor induced by the MAD-4 strain. The cultured tumor cells contain the intranuclear JCV T-antigen.

An additional finding in this experiment, not seen previously after i.c. inoculation, was the presence of extracranial tumors in 2 hamsters. One animal inoculated with MAD-2 virus developed a large abdominal tumor in the mesentery in addition to a cerebellar tumor. One animal in the MAD-4 group also had a large abdominal tumor and a few small nodules in the wall of the ileum. No brain tumor was observed in this animal. The histogenesis of the malignant tumors in the ileum was not determined by microscopy. A tissue culture cell line has been established from 1 of these tumors, however, and is available for future study. The 2 abdominal tumors have been identified as neuroblastomas. Neuroblastomas are among the most common tumors in children. JCV is the 1st virus reported to induce this type of tumor in an experimental animal (7). It is effective with i.o., combined s.c. and i.p., and i.c. inoculations.

The basis for the differences in neurooncogenicity between the MAD-2 and MAD-4 strains of JCV is not known. No differences have been observed in their other biological characteristics, and they are indistinguishable antigenically (4). Initial comparison of virion DNA extracted from MAD-4 with that from MAD-1 and MAD-2 strains has shown that the MAD-4 DNA was more homogeneous in size; however, the restriction endonuclease fragmentation patterns obtained after digestion with Hpa I (restriction endonuclease I from Hemophilus parainfluenzae), Eco RI (restriction endonuclease from Escherichia coli carrying resistance transfer factor I), or Hin III (restriction endonuclease III from Hemophilus influenzae) revealed no differences between these strains (1).

REFERENCES

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