Key words: SSPE virus/neural cells/neurovirulence

Growth of Defective Subacute Sclerosing Panencephalitis Viruses in Human Neural Cells and Their Neurovirulence in Mice

By M. SAKAGUCHI,* Y. YOSHIKAWA, K. YAMANOUCHI AND K. TAKEDA

Laboratory Animal Research Center, Institute of Medical Science, University of Tokyo, Shirokanedai, Minato-ku, Tokyo 108 and 1Department of Neurological Virus Disease, Institute for Virus Research, Kyoto University, Shogoin-Kawara-machi, Kyoto 606, Japan

(Accepted 17 October 1984)

SUMMARY

A defective subacute sclerosing panencephalitis (SSPE) virus which had been passaged in human embryonic lung cells was transferred to cultures of three neural cell types: neuroblastoma, oligodendroglioma and glioblastoma. The growth characteristics of the virus in these cells were essentially similar to those in non-neural cells. On the other hand, a marked difference in neurovirulence was noticed for the virus grown in neural cells when examined by intracerebral inoculation into mice. The virus passaged in neuroblastoma and oligodendroglioma cells showed high neurovirulence, inducing an acute encephalitis, whereas the virus passaged in human embryonic lung cells and that in glioblastoma cells did not show neurovirulence. These results suggest that the virus recovered its neurovirulence after passages in certain human neural cells.

Subacute sclerosing panencephalitis (SSPE) viruses have been isolated by co-cultivation of the patient's brain cells with susceptible cells such as Vero and human embryonic lung (HEL) cells (Fraser & Martin, 1978; Wechsler & Meissner, 1982). Most of the isolated SSPE viruses are defective in terms of production of cell-free infectious virus. On the other hand, a few strains of SSPE virus produce cell-free infectious virus after passage of the virus in vitro (Fraser & Martin, 1978). It is known that several strains of defective SSPE virus induce severe neurological disease in various animals, but productive SSPE viruses lack neurovirulence (ter Meulen et al., 1973; Thormar et al., 1978; Yamanouchi, 1980). Therefore, it is considered that a productive SSPE virus has lost this characteristic through the process of virus isolation or during the passage of the virus in non-neural cells.

The Biken strain of defective SSPE virus shows neurovirulence in mice and hamsters at low passage levels (Thormar et al., 1978; Ohuchi et al., 1981), but its neurovirulence in hamsters has been found to be decreased after passage for more than 100 generations in HEL cells (S. Ueda, personal communication). In this study, defective SSPE virus which had lost its neurovirulence during passages in HEL cells was transferred to three human neural cell lines. The virus regained its neurovirulence in mice after passage in these neural cells.

The Biken strain of SSPE virus was isolated from an SSPE patient by co-cultivation of brain cells with susceptible cells such as Vero and human embryonic lung (HEL) cells (Ueda et al., 1975). The stock virus has been passaged for 170 generations in HEL cells and lacked neurovirulence in mice. The virus was transferred from HEL cells to the following three neural cell lines: IMR-32 neuroblastoma (IMR), KG-1 oligodendroglioma (KG) and 118-MGC glioblastoma (MGC) (Sakaguchi et al., 1984). A monolayer of HEL cells persistently infected with the Biken strain (HEL-Biken) was trypsinized when cytopathic effect (c.p.e.) occupied nearly the whole area of the cell sheet, and the cell suspension was inoculated onto a monolayer of neural cells. Since HEL cells were found to be more sensitive to mitomycin C than the neural cells in a preliminary experiment, the cultures were maintained in a medium containing 5 μg/ml mitomycin C (Sigma) to eliminate surviving HEL cells. After the whole area of the cell sheet was involved in viral c.p.e., the cells were trypsinized and further inoculated.
Table 1. Recognition of proteins of measles and SSPE viruses by monoclonal antibodies

<table>
<thead>
<tr>
<th>Virus strain</th>
<th>Cell type</th>
<th>Localization of antigen detected by monoclonal antibodies*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Measles</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Mice were immunized with the Edmonston strain of measles virus, and their spleens were used to make hybridomas. Specific antibody-producing hybridoma clones were detected by Western blotting and immunoperoxidase techniques. Monoclonal antibodies were prepared as ascitic fluid.
† C, Cytoplasm; N, nucleus.

onto a monolayer of neural cells in the presence of mitomycin C as mentioned above. This passage procedure was repeated ten times to eliminate surviving HEL cells. As a result, persistent infections with the Biken strain were established in IMR, KG and MGC cells and designated as IMR-Biken, KG-Biken and MGC-Biken, respectively.

The virus grown in these neural cells and that in HEL cells were examined for the production of cell-free virus, c.p.e. and haemadsorption in non-neural HEL cells and the three neural cell types. In all the cells, extensive syncytial c.p.e. and a haemadsorption reaction were observed, whereas cell-free virus was produced irregularly at a minimal level. Thus, no difference was found in virus-cell interactions between non-neural and neural cells. Similar attempts to transfer a defective SSPE virus to primary cultures of hamster central nervous system (CNS) cells were reported by Sheppard et al. (1975), but no changes in virus-cell interactions were observed after transfer to hamster CNS cells except for production of a very low level of infectious virus.

Monoclonal anti-measles virus antibodies directed to nucleocapsid-associated phosphorylated (P) and nucleocapsid (N) proteins, and two each to haemagglutinin (H), fusion (F) and matrix or membrane (M) proteins, were used as probes to analyse viral proteins by the indirect immunofluorescent (IF) test (Table 1). Staining of Vero cells infected with the Edmonston strain of measles virus or the Mantooth strain, a productive SSPE virus, showed that all the monoclonal antibodies reacted with their respective viral proteins. In infection with the Biken strain, the H, P and N proteins were detected similarly, but the F and M proteins were not recognized in any of the cells. It is not known whether the F protein is absent or the monoclonal anti-F protein antibodies to measles virus are directed to epitopes which were not present in the F protein of the Biken strain. The former possibility seems to be unlikely, since extensive syncytia were induced by this virus. The failure to detect the M protein by monoclonal antibodies is compatible with previous findings which indicate an absence of M protein by polypeptide analysis (Lin & Thormar, 1980; Choppin et al., 1981).

To examine neurovirulence of the Biken strain grown in HEL cells and the three neural cells, 3-week-old mice of inbred strain DDD were intracerebrally inoculated with 0.025 ml of a suspension of virus-infected cells. When the animals became moribund or at 40 days after virus inoculation, the brains were removed and divided into two portions by transverse cutting; one part was used for histological examination and IF testing and the other part for virus recovery. As summarized in Table 2, different types of lesions were found in the CNS.

Five mice inoculated with the HEL-Biken cells showed no sign of illness during 40 days of observation. Virus antigen was not detected by the IF test and no histological lesion was observed in the brain. In addition, virus was not recovered from the brain, and virus-neutralizing antibody was not detected in the serum.

The IMR-Biken cells showed high neurovirulence in terms of clinical signs and histopathological changes. All five mice inoculated with this virus developed an acute
Short communication

Fig. 1. (a) The brain of a mouse infected with the IMR-Biken strain. Perivascular cuffing with mononuclear cells and proliferation of glial cells (haematoxylin and eosin, ×32). (b) Virus-specific antigens in the brain of a mouse infected with the IMR-Biken strain. Fluorescence-positive cells were observed in the thalamus (×80).

Table 2. Neurovirulence of SSPE viruses in 3 week-old mice

<table>
<thead>
<tr>
<th>Inoculum</th>
<th>P.f.u./mouse</th>
<th>Morbidity</th>
<th>Fatality (MTD)*</th>
<th>Histopathological changes in the CNS</th>
</tr>
</thead>
<tbody>
<tr>
<td>HEL-Biken</td>
<td>2.2 × 10³</td>
<td>0/5</td>
<td>0/5</td>
<td>Perivascular cuffing of mononuclear cells and proliferation of glial cells. Nerve cell degeneration.</td>
</tr>
<tr>
<td>IMR-Biken</td>
<td>2.0 × 10³</td>
<td>5/5</td>
<td>5/5</td>
<td>Perivascular cuffing of mononuclear cells and proliferation of glial cells. Formation of the spongy state in the thalamus.</td>
</tr>
<tr>
<td>KG-Biken</td>
<td>1.2 × 10³</td>
<td>5/5</td>
<td>4/5</td>
<td>Slight perivascular infiltration of inflammatory cells.</td>
</tr>
<tr>
<td>MGC-Biken</td>
<td>6.4 × 10³</td>
<td>5/5</td>
<td>0/5</td>
<td></td>
</tr>
</tbody>
</table>

* MTD, Mean time of death (days).

encephalitis; hypersensitivity was noticed 1 week post-inoculation and subsequently neurological signs such as paralysis and coma were developed. The animals died at a mean death time of 13 days. Histopathological examinations showed perivascular infiltration of mononuclear cells around the lateral ventricle (Fig. 1a) and degeneration of nerve cells in the entire CNS. Viral antigens were detected widely in the CNS by the IF test (Fig. 1b) and the virus was recovered by co-cultivation of the brain cells with Vero cells. A low level of neutralizing antibody was also demonstrated.

All five mice inoculated with the KG-Biken cells showed hypersensitivity at 1 week, and four of them developed neurological signs similar to the mice that received IMR-Biken and became moribund. The remaining one developed neurological signs, but survived for 40 days. Histopathological changes in the moribund mice were similar to those observed in mice
inoculated with IMR-Biken except for mild spongy degeneration in the thalamus (Fig. 2a). A mild inflammatory reaction was observed in a surviving mouse. Viral antigens were detected widely in the CNS (Fig. 2b) and cell-associated virus was recovered from the brain. A low level of neutralizing antibody was also demonstrated.

Five mice inoculated with the MGC-Biken showed hypersensitivity, but developed neither coma nor paralysis. Histopathologically, only mild perivascular cuffing of inflammatory cells was demonstrated in some mice. Virus was not recovered from the brain and neutralizing antibody was not detected in the serum.

The Biken strain of SSPE was reported to be neurovirulent in weanling mice (Ohuchi et al., 1981). However, the virus employed in the present study was found to have lost its neurovirulence during more than 200 passages in HEL cells. After cultivation in human neural cells, marked changes in neurovirulence were observed. The virus grown in IMR and KG cells showed high neurovirulence, inducing an acute encephalitis with histological lesions consisting of inflammatory cell infiltration and degeneration of nerve cell. The virus grown in MGC cells showed very low neurovirulence. Such changes in neurovirulence after passage in different cells have been reported for canine distemper and rabies viruses. After adaptation of canine distemper virus to the same three human neural cells types used in this study, increased neurovirulence in mice was observed (Yoshikawa et al., 1983). Attenuated rabies virus was reported to show increased neurovirulence after a few passages in neuroblastoma cells, and it was speculated that randomly occurring virulent virus mutants replicated selectively in neuroblastoma cells (Clark, 1978, 1980).

The mechanisms for the increase in neurovirulence of the Biken strain after passage in neural cells are not well understood at the moment. The increase in neurovirulence appeared to occur rapidly and remain stable during passage in IMR cells, since the increased neurovirulence was
found after a single passage as well as after 23 passages (data not shown). Co-cultivation of the HEL-Biken and IMR cells at the first passage caused the development of c.p.e. in a similar pattern to that in HEL cells. Moreover, the infectivity titre of the virus measured in IMR cells by infectious centre assay was similar to that measured in HEL cells (data not shown). These findings indicate that the efficiency of infection is similar for both IMR and HEL cells, and tend to exclude the possibility of selective replication of viruses with a high tropism for IMR cells. Further studies to assess the involvement of virus mutation are needed.

This study was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, and by a Research Grant from the Intractable Disease Division, Public Health Bureau, Ministry of Health and Welfare.

REFERENCES


(Received 18 July 1984)