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日本熱帯医学会雑誌、昭和54年3月30日, 1030号.
SUSCEPTIBILITY OF VARIOUS CELL LINES TO RABIES VIRUS

KUMATO MIFUNE1,3, YOSHIHIRO MAKINO1 AND KAZUAKI MANNEN2

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Abstract: Twelve established cell lines including 7 mouse-derived cell lines and primary chick embryo fibroblast cells were examined for their susceptibility to rabies virus. The results indicated that, in addition to CER cells and BHK-21 cells, murine neuroblastoma cells and human neuroblastoma cells (SYM cells) are highly permissive to infection with both fixed and field rabies strains and suggested the usefulness of these neuronal cell lines in various immunologic aspects of rabies studies in which histocompatibility requirements play a significant role.

INTRODUCTION

Rabies virus has been successfully propagated in many tissue culture systems in the past (Kissling, 1958; Wiktor et al., 1964; Kondo, 1965; Diamond, 1967; Hummeler et al., 1967; Sedwick and Wiktor, 1967; Wiktor et al., 1969). These include primary cell cultures such as hamster kidney cells and chick embryo fibroblast cells, and human diploid cells which have been used mainly for the purpose of vaccine production (Kissling, 1958; Wiktor et al., 1964; Kondo, 1965; Wiktor et al., 1969; Grandien, 1977). Of established cell lines, BHK-21 cells and their sublines have been used by several investigators for the production of high yields of virus (Sokol et al., 1968, 1969; Schneider et al., 1971; Cox et al., 1977; Kawai and Matsumoto, 1977). CER cells, recently established by Dr. Motohashi, Japan, have also been demonstrated to be highly permissive to rabies virus infection and to have a potential for isolation of field strains of rabies virus (Smith et al., 1977, 1978).

One aspect of the search for susceptible cells in tissue culture systems is to develop appropriate tissue culture cells for use in in vitro tests of immunological responsiveness to rabies in laboratory animals and in human. In these sorts of studies, it has been demonstrated that a histocompatible system (H-2 in the mouse) is required for the full expression of the interaction between immune lymphocytes and the virus-infected target cells (Doherty and Zinkernagel, 1975). The present study was initiated to examine the susceptibility of mouse-derived cell lines to rabies virus, with the ultimate goal of studying the immunological responses to the virus in mice. In addition, some commonly used cell types derived from other animals were simultaneously tested for permissiveness to the virus.

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MATERIALS AND METHODS

Virus: Three strains of rabies virus were used. The HEP-Flury strain of rabies virus was supplied by Dr. Kondo, National Institute of Health, Tokyo, Japan, and used at passage levels of 106 to 111 in chick embryo fibroblast cells. HEP-Flury of 2nd to 3rd passage level in CER cells was used in some experiments. The mouse brain-adapted CVS strain was used as a 10 per cent infected mouse brain suspension at the 56th passage level. In some experiments, the CVS strain, plaque-cloned in Vero cells (Buckley and Tignor, 1975) obtained from Yale Arbovirus Research Unit (YARU), Yale University, was used after one additional passage in murine neuroblastoma cells. The 1088 rabies strain, originally isolated from a woodchuck by the Center for Disease Control, U.S.A., was obtained from YARU and used at the 3rd passage level in suckling mouse brain.

Cells and culture media: Seven mouse-derived cell lines and 6 cell cultures derived from other animals (5 continuous lines and primary chick embryo fibroblasts) were tested for their susceptibility to rabies virus. Growth media for these cells were shown in Table 1. After virus infection, the percentage of heat-inactivated fetal calf serum was reduced to 2 or 5 per cent.

<table>
<thead>
<tr>
<th>Cell</th>
<th>Mouse-derived</th>
<th>Growth medium</th>
<th>Other animal-derived</th>
<th>Growth medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>L</td>
<td>Eagle MEM + 10% FCS</td>
<td>Vero</td>
<td>Eagle MEM + 10% FCS</td>
<td></td>
</tr>
<tr>
<td>L 1210</td>
<td>RPMI 1640 + 20% FCS</td>
<td>BSC-1</td>
<td>Eagle MEM + 10% FCS</td>
<td></td>
</tr>
<tr>
<td>L 5178Y</td>
<td>RPMI 1640 + 20% FCS</td>
<td>BHK-21</td>
<td>Eagle MEM-Hanks salts + 10% FCS</td>
<td></td>
</tr>
<tr>
<td>Neuroblastoma (N-18)</td>
<td>Dulbecco's modified Eagle MEM + 10% FCS</td>
<td>CER</td>
<td>Eagle MEM + 10% FCS</td>
<td></td>
</tr>
<tr>
<td>SR-CDF1-DBT (glioma)</td>
<td>Eagle MEM + 10% FCS</td>
<td>SYM (Human neuroblastoma)</td>
<td>1/2 Eagle MEM + 1/2 RPMI 1640 + 20% FCS</td>
<td></td>
</tr>
<tr>
<td>Mastocytoma (P-815)</td>
<td>Eagle MEM + 10% FCS</td>
<td>CEF (d)</td>
<td>Eagle MEM-Hanks salts + 10% FCS</td>
<td></td>
</tr>
<tr>
<td>Ehrlich ascites tumor</td>
<td>Eagle MEM + 10% FCS</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a) Heat-inactivated fetal calf serum. All culture media contained antibiotics.
b) Established by Dr. T. Kumanishi (1967), Tokyo University, Japan.
c) Established by Dr. M. Sekiguchi (personal communication), Tokyo University, Japan.
d) Primary chick embryo fibroblast cells.

Parameters of susceptibility: Infection of the cells with virus was carried out in the presence of 50 μg/ml of DEAE-dextran for 1 h at 37 °C. Infected cultures were then refed with maintenance media or overlay media and incubated at 37 °C. At least 2 tests using the following parameters were performed to examine permissiveness.
a) **Fluorescent antibody staining.** The cells capable of adhering to glass were cultured in 4-chamber Lab-Tek chamber slides (Lab-Tek Products, Miles Laboratories, Naperville, Ill.) and infected at a multiplicity of infection (MOI) of approximately 0.1 focus forming unit (FFU)/cell and incubated in a 5% CO₂ incubator. Other cells not capable of adhering to glass were packed by low speed centrifugation and infected at an MOI of 0.1 FFU/cell. The cells were then transferred to test tubes at appropriate cell densities and incubated.

On days 1, 2, 3 and 4 after infection, these cells were examined for cytoplasmic fluorescent antigens using anti-rabies virus FITC-labelled goat serum. The percentage of cells containing specific cytoplasmic fluorescent antigens was determined by examining at least 300 cells. The amount of cytoplasmic fluorescent antigens in the cells was estimated as large, intermediate and minute, respectively, depending on the relative size and distribution of inclusions in the cells.

b) **Cytopathic effect.** Monolayers in culture flasks and suspension cultures in test tubes, both infected with virus at a MOI of 0.1 FFU/cell, were observed for visible cytopathic effect under an inverted microscope for 8 days.

c) **Plaque formation.** The ability of some cells to support plaque formation was also tested in certain cell lines. The procedures were essentially the same as those described by Yoshino et al. (1966) except that calf serum and agar in the overlay were replaced by heat-inactivated fetal calf serum and 2% Sephadex G-200 (Schneider, 1973), respectively. Infected cultures were observed for plaques for 8 days.

d) **Hemadsorption-negative (HAD-) plaque formation.** This was done as described by Makino and Mifune (1975) for dengue virus assay.

e) **Persistent infection.** To examine if the percentage of fluorescent antigen-positive cells increased during subcultures, the cultures infected with virus at a MOI of 0.1 FFU/cell were refed with growth medium and subcultured 4 times at an interval of 5 to 7 days with a split ratio of 1 to 4. At the time of subculture, the percentage of fluorescent antigen-positive cells in a culture was determined as described before. The cultures which contained no cells with cytoplasmic fluorescent antigens at the 2nd and the 4th subculture levels were considered to be “cured”.

f) **Virus yield.** Monolayers of susceptible cells in culture flasks were infected with virus at a MOI of 0.1 FFU/cell and incubated for 4 days. The cultures were frozen and thawed 3 times and the supernatant was assayed for infectivity after low speed centrifugation. Infectivity of the virus was assayed in CER cells by the fluorescent focus formation method using 4-chamber Lab-Tek chamber slides as described by Smith et al. (1977).

**RESULTS**

Of seven mouse-derived cell lines tested, neuroblastoma cells were most sensitive to all strains of rabies virus tested. By fluorescent antibody staining, almost all of the N18 cells contained large, widely spread fluorescent antigens by day 4 after infection even with a field rabies strain (1088), and persistent infection could be readily established. In contrast, SR–CDF1–DBT cells and Ehrlich ascites tumor cells were sensitive only to the tissue culture-passaged, attenuated HEP-Flury strain.
of rabies virus. Susceptibility of other cell lines derived from the mouse was found to be very low (Table 2).

| Cell lines derived from other animals appeared, in general, to be more permissive to rabies virus infection than were the mouse-derived cells, and it was difficult to compare the susceptibility to rabies virus among these cell lines. CER and SYM cells, however, had the capacity to replicate the 1088 strain to almost the same degree as the fixed rabies strains as determined by fluorescent antibody staining, and the infection of CER cells could be successfully maintained in serial subcultures (Table 3). Vero and BSC-1 cells were sensitive only to fixed rabies strains (CVS and HEP-Flury strains) by fluorescent antibody staining but produced lower yields of the virus as |

Table 2  Susceptibility of mouse-derived cell lines to rabies virus as determined by fluorescent antibody staining, CPE induction and ability to support virus growth during subculture

<table>
<thead>
<tr>
<th>Virus strain</th>
<th>1088</th>
<th>CVS (mouse brain-passaged)</th>
<th>HEP-Flury</th>
</tr>
</thead>
<tbody>
<tr>
<td>L</td>
<td>FA\textsuperscript{a)}</td>
<td>0–2\textsuperscript{b)} (M)\textsuperscript{c)}</td>
<td>0–2 (M)</td>
</tr>
<tr>
<td></td>
<td>CPE\textsuperscript{d)}</td>
<td>cure</td>
<td>cure</td>
</tr>
<tr>
<td></td>
<td>Persistent infection</td>
<td>cure</td>
<td>cure</td>
</tr>
<tr>
<td>L 1210</td>
<td>FA</td>
<td>20–30 (M)</td>
<td>20–30 (M)</td>
</tr>
<tr>
<td></td>
<td>CPE</td>
<td>cure</td>
<td>cure</td>
</tr>
<tr>
<td>L 5178 Y</td>
<td>FA</td>
<td>10–20 (M)</td>
<td>10–20 (M)</td>
</tr>
<tr>
<td>Neuroblastoma</td>
<td>CPE</td>
<td>95–100 (L)\textsuperscript{e)}</td>
<td>95–100 (L)</td>
</tr>
<tr>
<td>(N–18)</td>
<td>Persistent infection</td>
<td>yes</td>
<td>±</td>
</tr>
<tr>
<td>SR–CDF1–DBT (glioma)</td>
<td>FA</td>
<td>N.D.\textsuperscript{f)}</td>
<td>0–2 (M)</td>
</tr>
<tr>
<td></td>
<td>CPE</td>
<td>cure</td>
<td>±</td>
</tr>
<tr>
<td>Mastocytoma (P–815)</td>
<td>FA</td>
<td>0–0.1 (M)</td>
<td>0–0.1 (M)</td>
</tr>
<tr>
<td></td>
<td>CPE</td>
<td>cure</td>
<td>cure</td>
</tr>
<tr>
<td>Ehrlich ascites tumor</td>
<td>FA</td>
<td>N.D.</td>
<td>0–2 (M)</td>
</tr>
<tr>
<td></td>
<td>CPE</td>
<td>cure</td>
<td>cure</td>
</tr>
</tbody>
</table>

\textsuperscript{a) Fluorescent antibody staining.}  
\textsuperscript{b) Per cent of fluorescent antigen containing cells on day 4 after infection in 3 experiments.}  
\textsuperscript{c) Minute, pinpoint like antigen.}  
\textsuperscript{d) Cytopathic effect.}  
\textsuperscript{e) Large, widely spread antigen masses in the cells.}  
\textsuperscript{f) Intermediate antigen.}  
\textsuperscript{g) Not done.}
Table 3 Susceptibility of non-mouse-derived cell lines to rabies virus as determined by fluorescent antibody staining, CPE induction, plaque and HAD– plaque formation and ability to support virus growth during subculture

<table>
<thead>
<tr>
<th>Cell</th>
<th>Virus strain</th>
<th>Virus strain</th>
<th>HEP-Flury</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1088</td>
<td>CVS (mouse brain-passaged)</td>
<td>HEP-Flury</td>
</tr>
<tr>
<td>Vero</td>
<td>FA (I)</td>
<td>80–90 (I)</td>
<td>80–90 (I)</td>
</tr>
<tr>
<td>CPE</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Plaque</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Persistent infection</td>
<td>cure</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>BSC-1</td>
<td>FA (M)</td>
<td>50–75 (I)</td>
<td>50–75 (I)</td>
</tr>
<tr>
<td>CPE</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>HAD– plaque</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>BHK-21</td>
<td>FA (I)</td>
<td>95–100 (L)</td>
<td>95–100 (L)</td>
</tr>
<tr>
<td>CPE</td>
<td>–</td>
<td>+</td>
<td>±</td>
</tr>
<tr>
<td>Persistent infection</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>CER</td>
<td>FA (I)</td>
<td>95–100 (L)</td>
<td>95–100 (L)</td>
</tr>
<tr>
<td>CPE</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Plaque</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>HAD– plaque</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Persistent infection</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>SYM (Human neuroblastoma)</td>
<td>FA (I)</td>
<td>95–100 (L)</td>
<td>95–100 (L)</td>
</tr>
<tr>
<td>CPE</td>
<td>–</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>CEF (f)</td>
<td>FA (I)</td>
<td>95–100 (L)</td>
<td>95–100 (L)</td>
</tr>
<tr>
<td>CPE</td>
<td>N.D. (g)</td>
<td>–</td>
<td>±</td>
</tr>
<tr>
<td>Plaque</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
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a) Fluorescent antibody staining.
b) Percent of fluorescent antigen containing cells on day 4 after infection in 3 experiments.
c) Minute, pinpoint like antigen.
d) Intermediate antigen.
e) Large, widely spread antigen masses in the cells.
f) Primary chick embryo fibroblast cells.
g) Not done.

compared with those of CER and BHK–21 cells (Table 4). BSC–1 cells were shown to be useful for the infectivity assay of fixed strains of rabies virus. Vero cells and CER cells permitted plaque formation by the CVS strain of rabies virus.

Virus yields from a limited number of susceptible cell lines are shown in Table 4. In general, tissue culture-passaged rabies virus grew to higher titers than did mouse brain-passaged rabies virus. Among these cell lines, murine neuroblastoma cells and CER cells supported replication of tissue culture-passaged CVS rabies to the highest titers. The highest yields of HEP-Flury virus were obtained in CER cells and CEF cells.
Table 4  Rabies virus yields from susceptible cell lines

<table>
<thead>
<tr>
<th>Cell</th>
<th>Virus strain</th>
<th>CVS (mouse brain-passaged)</th>
<th>CVS (plaque-purified murine neuroblastoma-passaged)</th>
<th>HEP-Flury</th>
</tr>
</thead>
<tbody>
<tr>
<td>Murine neuroblastoma (N-18)</td>
<td>5.3 b)</td>
<td>8.0</td>
<td>5.9</td>
<td></td>
</tr>
<tr>
<td>Vero</td>
<td>3.6</td>
<td>6.4</td>
<td>5.8</td>
<td></td>
</tr>
<tr>
<td>BSC-1</td>
<td>3.2</td>
<td>N.D. c)</td>
<td>5.0</td>
<td></td>
</tr>
<tr>
<td>BHK-21</td>
<td>5.0</td>
<td>7.0</td>
<td>6.3</td>
<td></td>
</tr>
<tr>
<td>SYM (Human neuroblastoma)</td>
<td>5.5</td>
<td>7.2</td>
<td>N.D.</td>
<td></td>
</tr>
<tr>
<td>CER</td>
<td>5.7</td>
<td>7.7</td>
<td>7.3</td>
<td></td>
</tr>
<tr>
<td>CEF d)</td>
<td>N.D.</td>
<td>N.D.</td>
<td>7.3</td>
<td></td>
</tr>
</tbody>
</table>

a) Monolayers in culture flasks were infected with virus at an MOI of 0.1 FFU/cell and incubated at 37 C for 4 days. The cultures were frozen and thawed 3 times and supernatant fluid was assayed for infectivity after low speed centrifugation.
b) \( \log_{10} \) FFU/ml.
c) Not done.
d) Primary chick embryo fibroblast cells.

**DISCUSSION**

Murine neuroblastoma cells, which were derived from an A strain mouse and which express some neuronal characteristics in culture, were demonstrated to have the greatest susceptibility to rabies of the mouse-derived cell lines tested in this study. Since these cells permit expression of antigen on the surface after infection with both fixed and street rabies virus, they have been recently chosen as target cells by us (Mifune and Tignor, in press) and others (Wiktor et al., 1977) in the in vitro studies of immunological responses of rabies in mice. In addition, these cells have potential for isolation of field rabies strains (Smith et al., 1978) and provide a system in which to examine the interaction between field rabies strains and neuronal cells.

The susceptibility of L cells and mastocytoma cells derived from C3H mouse and DBA mouse, respectively, was shown to be very low. The high susceptibility of CER cells to rabies virus including field strains was first described by Smith et al. (1977). In this study, further and comparative observations were made with some other cell lines regarding their susceptibility to the virus. The results showed that, in addition to CER and BHK-21 cells, SYM cells derived from a human neuroblastoma are highly susceptible to both fixed and field rabies strains and might be a useful cell line for the immunological studies of human rabies which might require a histocompatible system in the assays.

**ACKNOWLEDGMENTS**

A part of the present study was undertaken in collaborations with Drs. A. L. Smith, G. Tignor and R. E. Shope of Yale Arbovirus Research Unit, Yale University.
The authors wish to express sincere thanks for their help and suggestions. The authors also acknowledge the supply of SR–CDFI–DBT cells and SYM cells from Dr. Aoyama, Institute for Medical Science, Tokyo University and support by the research grant No. 248169 from the Ministry of Education, Science and Culture of Japan.

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狂犬病ウイルスに対する種々細胞株の感受性

三舟求真人¹ ³・牧野 芳夫¹・万年 和明²

7株のマウス由来組織培養細胞株を含む12の細胞株と初代経胎児細胞について、狂犬病ウイルスに
に対する感受性を検討した結果、これまで報告のあったCER細胞ならびにBHK-21細胞の他に、マ
ウス神経芽細胞腫およびヒト神経芽細胞腫（SYM細胞）由来の細胞が、狂犬病ウイルスの固定毒な
らびに衛生上に高い感受性を示し、マウスおよびヒトにおける狂犬病の研究、特に、組織適合抗原の
適合性が重要な免疫学的研究に、これらの細胞が非常に有用であることが示唆された。

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³ 文献請求先：大阪医科大学微生物学講座（昭和55年以降）