JAPANESE ENCEPHALITIS VACCINE FROM TISSUE CULTURE

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Since the middle of 1950’s vaccination of human against Japanese encephalitis has been put into practice in Japan, using formalin-inactivated vaccine prepared from infected mouse brains. Extensive efforts have been made by many investigators for preparing more potent vaccine and for eliminating possible contaminating substances, which cause allergic encephalomyelitis, from it.

As reported by Takaku this morning, our institute has developed the purification method of this vaccine from infected mouse brains using two cycles of ultracentrifugation of virus particles. This method gives highly potent highly purified vaccine. As reported by Takaku, the vaccine containe no or little encephalitogenic substances, judged from the results of animal experiment and immunodiffusion analysis for the encephalitogenic basic protein.\(^1\,\)\(^2\) In fact no compatible case of allergic encephalomyelitis resulting from administration of Japanese encephalitis vaccine has been reported, even before ultracentrifuge purified vaccine was introduced.

Fear is still persistent, however, among some of investigators who are considering the rapid increase of the population of vaccinated people in recent years and also the accumulation of antigenic stimuli by possible contaminating encephalitogenic substances, even at the least concentration, in the individuals receiving the vaccine repeatedly year after year.

While we are still concentrating in the improvement of our ultracentrifuge-purified vaccine, we have made our efforts to prepare Japanese encephalitis vaccine from tissue culture, we have tried primary culture of kidney cells from monkeys, pigs and hamsters inoculated various strains of Japanese encephalitis virus.

In this paper, the preliminary results will be reported on the vaccine prepared from a monkey kidney (MK)-adapted strain OH 0566 cultivated on the primary MK cells.

1. Adaptation of the strain OH 0566 on primary MK cells

The strain, OH 0566 was isolated in 1966 in the Virus Laboratory of the Osaka Public Health Institute from the brain of a patient who had died of Japanese encephalitis. We received this strain after 3 intracerebral passages through suckling mice.
Fig. 1
Passage of JE virus, strain OH 0566

Isolated from the brain of Patient who had died of Japanese Encephalitis
(Virus Laboratory, Osaka Public Health Institute)
3 Passages through brains of suckling mice
(Virus Laboratory, Osaka Public Health Institute)
Passages through MK Cells
(Kan-Onji Institute, Research Foundation for Microbial Diseases of Osaka University)

Fig. 2
Characteristic of strain OH 0566

a) pH range of Hemagglutination

<table>
<thead>
<tr>
<th>pH of virus adjusting diluent</th>
<th>OH 0566</th>
<th>Nakayama Yoken</th>
<th>JoGA/#01</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>6.0</td>
<td>6.2</td>
<td>6.4</td>
</tr>
<tr>
<td>Log HA titer</td>
<td>4</td>
<td>3</td>
<td>2</td>
</tr>
</tbody>
</table>
Fig. 1 shows the virus titers during the course of adaptation of this strain on MK cells. After the 39th subculture definite cytopathogenic effect (CPE) on MK cells has appeared and the virus titer has been maintained at the levels over $10^{8.5}$ PFU per ml when measured on chick embryo cell culture.

2. Characteristics of MK-Adapted Strain OH 0566

As shown in Fig. 2, pH optimum and range for the hemagglutination of goose cells by this strain show a similar pattern to the JaGAr#01 strain of Japanese encephalitis virus, which was isolated from mosquitoes in 1959, and different from those for Nakayama-Yoken strain, which has been used for the vaccine production from infected mouse brains.

The differences of antigenic composition are shown in Table 1, in which hemagglutination inhibition among these three strains was tested. All antigens employed were prepared from infected mouse brains. Antisera were prepared in rabbit immunized with brain homogenate of mice infected with corresponding strain of Japanese encephalitis virus.

<table>
<thead>
<tr>
<th>Antiserum</th>
<th>OH 0566</th>
<th>Nakayama-Yoken</th>
<th>JaGAr #01</th>
</tr>
</thead>
<tbody>
<tr>
<td>OH 0566</td>
<td>128</td>
<td>32</td>
<td>128</td>
</tr>
<tr>
<td>Nakayama-Yoken</td>
<td>16</td>
<td>128</td>
<td>32</td>
</tr>
<tr>
<td>JaGAr #01</td>
<td>32</td>
<td>32</td>
<td>128</td>
</tr>
</tbody>
</table>

As shown in this table, the strain OH 0566 possesses some differences in antigenic composition from both Nakayama and JaGAr#01 strains, but is close to the latter strain to a certain extent.

As mentioned before, this strain showed CPE on the primary MK cells. It was shown that the CPE appeared was resulted from the action of Japanese encephalitis virus but not from the action of any other agent which had been contaminated during the passage through the cultures. As shown in Table 2, the CPE developed by this strain was clearly neutralized by the antiserum from rabbit immunized with the supernatant of brain homogenate from mice infected with Japanese encephalitis virus, Nakayama strain.

The CPE developed by this MK-adapted strain OH 0566 is shown in Fig. 3. Fig. 3(a) shows the uninfected primary MK cell culture. Fig. 3(b) through (d) show the increasing degrees of CPE manifested by this strain.

Fig. 4 shows a growth curve obtained by this strain on primary MK cell culture at 37°C. The virus titer was measured on chick embryo cell culture. The multiplicity of infection employed was 0.001.

As shown in this figure, the degree of CPE correlates to some extent to the virus titer in the culture fluid. This makes it feasible to determine the time to harvest the viruses at maximum yield.
Fig. 3. CPE by Japanese Encephalitis Virus. Strain OH 0556, on Primary MK Cells

Fig. 4

Growth of MK adapted strain OH 0566 of JE virus on MK cell

![Graph showing growth of MK adapted strain OH 0566 of JE virus on MK cell](chart.png)
3. Inactivation of the Viruses by Formalin

Fig. 5 shows the inactivation curves of this strain of Japanese encephalitis virus by the action of formalin at a concentration of 1:4,000 at various temperatures.

4. Preparation of the Vaccine

The method employed for the preparation of vaccine from this strain is outlined in Fig. 6. After the completion of the formalin-inactivation, the viruses were concentrated by ultracentrifugation in consideration of the virus titer obtained.

This preliminary preparation of vaccine from tissue culture could protect immunized mice against intracerebral challenge of either homologous strain,
Fig. 6. JE vaccine from tissue culture

Primary culture of kidney cells of cynomolgus monkeys
Washed twice with Hank’s solution
Inoculation of virus (5 ml/Roux bottle)
Incubated at 37°C for 2 hours
Added TC medium 199
Incubated at 33°C
Harvested after CPE appeared
Centrifuged for removal of cell debris
Inactivated with 1:4000 formalin at 4°C
Concentration by ultracentrifugation

Table 3. Comparison of Potencies of Tissue Culture Vaccine Prepared from the OH 0566 Strain by the Direct Mouse Challenge Test

<table>
<thead>
<tr>
<th>Challenge virus</th>
<th>Vaccine</th>
<th>Challenge virus</th>
<th>Vaccine</th>
<th>Challenge virus</th>
<th>Vaccine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OH 0566</td>
<td></td>
<td>JaGar #01</td>
<td></td>
<td>Nakayama</td>
</tr>
<tr>
<td></td>
<td>(52 mouse LD_{50})</td>
<td></td>
<td>(170 mouse LD_{50})</td>
<td></td>
<td>(185 mouse LD_{50})</td>
</tr>
<tr>
<td>Vaccine dilution</td>
<td>4^{0} 4^{1} 4^{2} 4^{3} ED_{50}</td>
<td>Vaccine dilution</td>
<td>4^{0} 4^{1} 4^{2} 4^{3} ED_{50}</td>
<td>Vaccine dilution</td>
<td>4^{0} 4^{1} 4^{2} 4^{3} ED_{50}</td>
</tr>
<tr>
<td>OH 0566 Vaccine*</td>
<td>2/9 2/9 4/8 7/9 1.74</td>
<td>JaGar #01 Vaccine</td>
<td>3/10 6/9 5/9 9/10 1.22</td>
<td>Nakayama Vaccine</td>
<td>7/10 6/10 9/10 8/10 0.37</td>
</tr>
<tr>
<td>Reference Vaccine**</td>
<td>1/10 3/10 8/10 12/12 1.35</td>
<td></td>
<td>4/10 8/10 8/10 10/10 0.46</td>
<td></td>
<td>0/10 1/10 8/10 9/10 1.70</td>
</tr>
</tbody>
</table>

* Tissue culture vaccine.
** Standard vaccine from the National Institute of Health of Japan distributed for 1967 production season.
*** The numerator shows the number of deaths, and the denominator shows the total number in the group.

Table 4. Comparison of Potencies of Tissue Culture Vaccine Prepared from the OH 0566 Strain by the Neutralization Antibody Test by Plaque Reduction

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Challenge virus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OH 0566 (96 PFU/Plate)</td>
</tr>
<tr>
<td>OH 0566 vaccine*</td>
<td>3.2***</td>
</tr>
<tr>
<td>Reference vaccine**</td>
<td>2.3</td>
</tr>
</tbody>
</table>

* Tissue culture vaccine.
** Standard vaccine from the National Institute of Health of Japan distributed for 1967 production season.
*** Expressed as the Log_{4} of the NT. ED_{50}. 
OH 0566 or heterologous but closely-related strain, JaGAr #01, as shown in Table 3. However, it showed the lower ED₅₀ against the challenge with Nakayama strain.

On the other hand, the same vaccine showed high ED₅₀ values against each of these three strains, when measured by the neutralizing antibody test by plaque reduction¹⁻² as shown in Table 4.

The neutralizing antibody test by plaque reduction has been regarded as more accurate method for measuring the immunizing potency of Japanese encephalitis vaccine than the direct mouse challenge method. It might be true within the limit of dealing with a same kind of vaccine preparations. However, it should be reconsidered which method could give more reliable measure on the effectiveness of the vaccine when the different kinds of vaccine, which are different in either the virus strains or the host cells, are compared.

As both the methods gave high ED₅₀ values for this vaccine, at least, against homologous strain, OH 0566 and a similar strain, JaGAr #01, however, the effectiveness of this vaccine can be expected for immunizing humans against wild strains of Japanese encephalitis virus.

A field trial of this vaccine is now in progress in Hokkaido by Dr. Kanamitsu of Sapporo Medical College.

**SUMMARY**

A strain of Japanese encephalitis virus, OH 0566, which was recently isolated from the brain of a patient who had died of Japanese encephalitis, was adapted on primary culture of monkey kidney (MK) cells by serial passages through the culture. This MK-adapted OH 0566 strain develops cytopathogenic effects on MK cells.

Effective vaccine with a similar potency to those from infected mouse brains was prepared by concentrating the formalin-inactivated culture fluid of MK cells inoculated with this strain.

**REFERENCES**