Preparation of Immune Rabbit Sera with Neutralizing Activity against Human Cytomegalovirus and Varicella-zoster Virus

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The classification of herpesviruses into two groups has been proposed (Melnick et al. 1964) based upon the tendency of the virus either to retain infectivity following liberation from host cells (Group A) or to remain intimately associated with host cells (Group B). In the case of primate herpesviruses, Group A strains include such viruses as herpes simplex of man and herpes-B and SA8 of monkeys, while Group B strains include cytomegalovirus of man and monkeys and varicella-zoster virus of man.

The preparation of immune sera in non-primates with neutralizing activity against human or simian Group A herpesviruses can be achieved readily, whereas the preparation of similar sera against Group B strains had proven difficult (Weller & Rowe, 1964). Immune sera have been prepared in sub-human primates which show neutralizing activity and fluorescent antibody activity against human cytomegalovirus and varicella-zoster virus respectively (Plummer & Benyesh-Melnick, 1964; Schmidt et al. 1965; Graham, Minamishima & Benyesh-Melnick, 1969), and immune sera with complement-fixing and fluorescent antibody activity against varicella-zoster virus have been prepared in guinea-pigs (Kissling, Casey & Palmer, 1968).

We recently reported (Ablashi et al. 1969; Hampar et al. 1969) the preparation of immune rabbit sera with neutralizing activity against strains of simian cytomegalovirus, and demonstrated the efficacy of late IgG antibodies for differentiating cross-reacting strains. The potential usefulness of non-primate immune sera for typing simian Group B herpesviruses prompted attempts to produce neutralizing sera in rabbits against human cytomegalovirus and varicella-zoster virus.

Human fibroblast (WI-38) cells were employed for the propagation and assay of varicella-zoster virus and human cytomegalovirus (Ablashi et al. 1969). The cells were grown in Eagle’s basal medium (BME) with 10% heat-inactivated foetal calf serum, penicillin and streptomycin, and were maintained with BME containing 2% foetal calf serum and antibiotics. The medium used for virus propagation was Eagle’s minimal essential medium (MEM) with 2% foetal calf serum and antibiotics.

Varicella-zoster virus, strain 700-A, isolated from a case of chickenpox was obtained from Dr A. Vargosko. When approximately 80% of the cells showed cytopathic effects (CPE) the cell sheets were harvested by scraping, and were pelleted by centrifugation at 2000 rev./min. for 10 min. Preparation of ‘cell-free’ virus working pools (Caunt & Taylor-Robinson, 1964) for use in neutralization tests was essentially as described by Brunell (1967). This involved resuspension of the cells in Hanks’ balanced salt solution, followed by sonication for 30 sec. at 20 watts output (Branson Sonifier Model W-185), and centrifugation at 2000 rev./min. for 10 min. The supernatant fluids were mixed with equal volumes of 70% (w/v) sorbitol (Weller & Hanshaw, 1962) and portions were stored at −70°C. The virus titred approximately 10^4 p.f.u./ml.

Human cytomegalovirus, strain AD-169, was obtained from Dr R. J. Hildebrandt, and working pools were prepared as described previously for simian cytomegalovirus (Ablashi et al. 1969). The classification of this virus as human cytomegalovirus was confirmed in this
laboratory using procedures described previously (Ablashi et al. 1969), which included demonstrating its ability to grow readily in human WI-38 cells but not in primary African green monkey kidney cells (Weller & Rowe, 1964).

The human cytomegalovirus and varicella-zoster virus working pools were tested and found free of contamination by bacteria, fungi, or pleuropneumonia-like organisms.

Immunization of 6- to 8-week-old albino rabbits (strain, New Zealand White) with varicella-zoster virus and human cytomegalovirus was as described previously for simian cytomegalovirus (Ablashi et al. 1969). The animals received six weekly injections of 1.0 ml. of freshly prepared virus by the intravenous route and 2.5 ml. of a mixture of two parts virus and one part Freund's incomplete adjuvant by the intramuscular route. Sera collected 10 days after the last injection were filtered through a 0.22 µm Millipore filter and were stored at −20°C. Sera from individual animals were not pooled.

Table 1. Neutralization titres of rabbit antibodies prepared against varicella-zoster virus and human cytomegalovirus

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Neutralization titres vs. homologous virus*</th>
<th>No Complement†</th>
<th>Complement‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Varicella-Zoster (Strain 700-A)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum No. 660</td>
<td>512</td>
<td>1024</td>
<td></td>
</tr>
<tr>
<td>Serum No. 661</td>
<td>256</td>
<td>512</td>
<td></td>
</tr>
<tr>
<td>Cytomegalovirus (Strain AD-169)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Serum No. 678</td>
<td>256</td>
<td>512</td>
<td></td>
</tr>
<tr>
<td>Serum No. 688</td>
<td>256</td>
<td>256</td>
<td></td>
</tr>
<tr>
<td>Serum No. 689</td>
<td>128</td>
<td>256</td>
<td></td>
</tr>
</tbody>
</table>

* Reciprocal of highest antibody dilution (twofold) which resulted in 50% or more plaque reduction. The final antibody dilution in each reaction mixture was 2.5 times the values shown. Preimmunization sera showed no neutralizing activity when tested at a 1:4 dilution in the presence of complement.
† Heat-inactivated guinea pig serum added to the reaction mixtures.
‡ Guinea-pig serum added to the reaction mixtures as a source of complement.

Antibody neutralization tests against varicella-zoster and human cytomegalovirus were carried out using monolayer cultures of WI-38 cells grown in 30 ml. plastic flasks as described previously (Ablashi et al. 1969). The sera were heat-inactivated at 56°C for 30 min. prior to use. Guinea-pig serum was added to the reaction mixtures to yield a final concentration of 10 haemolytic units 50% of complement. Heat-inactivated guinea-pig serum was added to parallel tubes as controls. Antibody neutralization titres were expressed as the reciprocal of the highest dilution (twofold) added to the reaction mixtures which caused a plaque reduction of 50% or greater.

Table 1 shows that relatively high titred neutralizing antibodies could be prepared in rabbits against varicella-zoster virus and human cytomegalovirus. The presence of complement did not significantly enhance the neutralization titres, similar to previously reported findings with hyperimmune rabbit sera prepared against herpes simplex (Hampar et al. 1968), SA8 (Stevens et al. 1968), and simian cytomegalovirus (Ablashi et al. 1969).

As reported previously (Ablashi et al. 1969) for rabbit antisera prepared against simian cytomegalovirus, the presence of anti-WI-38 cell antibodies did not affect the neutralization titres of the antisera prepared against varicella-zoster virus or human cytomegalovirus. This was shown in two ways. First, absorption of antisera with control WI-38 cells to a point where complement-fixing and fluorescent antibody activity against WI-38 cells was negative did not reduce the neutralization titres against varicella-zoster virus or human cytomegalovirus.
Second, rabbit antisera prepared against herpes simplex, SA 8, and simian cytomegalovirus grown in WI-38 cells did not neutralize varicella-zoster or human cytomegalovirus, although all of these antisera were prepared in the same manner, and they all contained antibodies to WI-38 cells.

Immune sera against varicella-zoster virus were also prepared in guinea pigs using the immunization procedures described by Kissling et al. (1968). The neutralization titres of these guinea pig sera in the absence of complement were four-fold less than those obtained with rabbit antisera. In the presence of complement, the guinea pig and rabbit sera showed comparable titres, suggesting qualitative differences in the antibodies produced by these two species under the conditions used for immunization. Neutralizing antisera have also been successfully prepared in rabbits against human cytomegalovirus strains other than AD-169, and these are being used presently for studying antigenic relationships between strains of human cytomegalovirus.

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REFERENCES  

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