Serological Relationships among Viruses of the Herpes Group

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Serological relationships between members of the herpes group of viruses have been reported previously on the basis of neutralization tests by Sabin (1) and by Burnet et al. (2) who showed neutralization of herpes simplex virus by convalescent sera from monkeys infected with herpes virus B. Complement fixation tests have also been claimed to show a relation between herpes simplex and pseudorabies viruses. (3) On the other hand, Kaplan & Vatter (4) reported no cross neutralization between herpes simplex and pseudorabies viruses. In this note we report the results obtained in cross-neutralization and gel immunodiffusion tests with herpes simplex, herpes B and pseudorabies viruses using a rabbit antiserum to herpes infected RK 13 cells (5) and an antiserum to B virus (obtained by inoculating a naturally infected monkey with B virus, grown in monkey kidney cells).

Herpes simplex virus (strain HFEM) was grown in BHK 21 cells (6) or RK 13 cells (7) as previously described (5). Cell extracts for antigens in immunodiffusion tests were obtained by high multiplicity infection of BHK 21 cells in suspension followed by ultrasonic disruption of the cells in 0.05 M-NaCl 12 hr after infection at a cell concentration of 10^6 cells/ml. This extract was centrifuged at 100,000 g before use (5). Antiserum to herpes infected RK 13 cells was prepared by eight monthly injections of 200 mg. freeze-dried cell extract, the first inoculation being of formalin inactivated material. This antiserum gave no reaction with uninfected BHK 21 or RK 13 cells. Pseudorabies virus and cell extracts were obtained in a similar fashion. A strain of B virus was grown using the same methods. Virus for neutralization tests was released from infected cells by freezing and thawing. Cell extract for immunodiffusion tests was obtained by high multiplicity infection of cell monolayers and the cells were disintegrated in a TenBroek grinder. The cell extract was centrifuged at low speed only.

Kinetic neutralization tests were done at room temperature by incubation of 10^6 plaque forming units virus with dilutions of antiserum. Surviving virus in the first hour was assayed at 10 min. intervals by the suspension assay method of Russell (8) after further diluting the mixture 1/100 and 1/1000. The results (Table 1) are remarkable in showing significant neutralization of B virus by anti-herpes serum in contrast to previous results. The herpes antiserum was, of course, more potent than those previously tested, but it should be noted that we have also observed significant neutralization of B virus by 2/6 convalescent sera from herpetic subjects. Neither B virus nor herpes virus was neutralized by pre-immune sera from rabbits subsequently immunized with herpes infected RK cells. No significant neutralization of pseudorabies virus was observed with either antiserum.

Agar immunodiffusion tests were done as previously described (5). Antiserum to herpes infected RK cells gives three lines with extract of B virus infected cells. These three precipitins showed reactions of identity with antigens in herpes infected cells. The same antiserum also gave a precipitin band with pseudorabies infected cells showing a reaction of identity with precipitins in both herpes and B virus infected cells. Antiserum to B virus gave three precipitin bands with B virus infected cell extract, one of
which is identical to precipitins obtained with extracts of herpes and pseudorabies infected cells.

There may therefore be a group antigen common to these three members of the herpes virus group. The neutralization tests suggest a common virus structural antigen between herpes and B virus particles, but of course we cannot be sure that this is identical with one of the precipitin bands of the gel diffusion test. The group antigen

<table>
<thead>
<tr>
<th>Virus</th>
<th>k value obtained in neutralization with</th>
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<tbody>
<tr>
<td></td>
<td>anti-herpes</td>
</tr>
<tr>
<td>Herpes simplex</td>
<td>22</td>
</tr>
<tr>
<td>B</td>
<td>0.5</td>
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<tr>
<td>Pseudorabies</td>
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may be a structural antigen but if so is not susceptible to neutralizing antibody because, for example, it may be buried deep in the particles. Cells infected with herpes simplex and herpes B viruses seem to share several antigens, and these two viruses therefore seem to be more closely related to each other than either is to pseudorabies virus. Results of this kind should always be interpreted with care; nevertheless, the neutralization results appear to confirm the separate identity of each of the three virus stocks used. The immunodiffusion results could be occasioned by contamination of either test or immunizing antigen. However, it should be noted that the common antigen between herpes and pseudorabies virus is not the most pronounced herpes antigen, as might be expected were this a carried over contaminant. The chances of sporadic contamination are in any event minimal since we have obtained the same result using sera from three rabbits, each of which was immunized with completely separate batches of antigen. Finally, herpes B virus has been maintained in complete isolation from the other viruses. There remains the possibility that by pure random chance large DNA molecules of the size present in these viruses will specify common proteins even where there are no other grounds for grouping the viruses together. To test this we intend to prepare antiserum to a pox virus using the same methods as those used for the herpes antiserum. This antiserum could be used to test for reacting antigens in extracts of herpes group virus infected cells.

We are also attempting to prepare an antiserum to RK cells infected with pseudorabies virus to examine the reciprocity of the herpes simplex/pseudorabies reaction.

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Short communications

REFERENCES


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