Specific Serum Neutralization of the Reactivating Activity of Influenza A2 Viruses

(Accepted 25 September 1966)

Human influenza A2 viruses have been shown to be capable of reactivating ultraviolet-inactivated strains of influenza A viruses of animal origin (1). Reactivation of classical fowl plague virus by strain A2/Singapore/1/57 gives rise to recombinants sharing properties from each of the parents. Subsequent work with this system revealed that when monolayers of chick embryo fibroblasts inoculated with a given amount of ultraviolet-inactivated fowl plague virus are superinfected with varying doses of living influenza A2, the number of reactivated plaques varies directly with the dose of A2 virus. This provides a simple technique for the quantitative assay of the reactivating capacity of influenza A2 and for the study of neutralization of this capacity by antibody. This constitutes the subject of the present report.

Reactivation experiments were conducted essentially as described by Tumova & Pereira (1). Monolayers of chick embryo fibroblasts in Petri dishes (approximately $2 \times 10^7$ cells per dish) were inoculated with $5 \times 10^6$ doses (EID 50) of u.v.-inactivated fowl plague virus together with influenza A2 virus in a standard dose previously determined to induce the formation of 50 to 100 reactivated plaques. All dilutions were made in Gey's solution and the inoculum volume was 1 ml. per dish. After adsorption for 1 hr at $37^\circ$ the inoculum was removed and the cells covered with agar containing overlay as described by Porterfield (2). In neutralization experiments, serum + virus mixtures containing 30 times the standard dose of influenza A2 in a 1/500 dilution of periodate-treated (3) homologous or heterologous ferret antiserum were kept at room temperature for varying periods at the end of which 0·1 ml. aliquots were transferred to 3 ml. of a dilution of fowl plague virus containing $5 \times 10^6$ u.v.-inactivated doses and immediately plated. After adsorption for 1 hr, removal of inoculum and addition of overlay, the cultures were incubated at $37^\circ$ for 3 days and the number of plaques counted. Control cultures inoculated with the standard doses of A2 or u.v.-treated fowl plague alone were included in each test and showed no plaques.

The dose of each strain of A2 virus to be used in neutralization experiments was determined by plating mixtures containing the standard amount of u.v.-treated fowl plague virus with different dilutions of the A2 virus. Results obtained with strain A2/Singapore/1/57 are shown in Fig. 1, where it is seen that the number of plaques varies more or less directly with A2 virus concentration within the dose range tested. Closely similar results were obtained with strains A2/Netherlands/65/63 and A2/England/12/64.

Neutralization of the reactivating capacity of A2/Singapore/1/57 by homologous and heterologous A2 antisera is shown in Fig. 2 where the residual reactivating activity $R_t/R_0$ is plotted against reaction time in minutes. Accepting that these results are consistent with a first order reaction, inactivation constants were estimated applying the formula $R_t/R_0 = e^{-kt}$, where $R_0 =$ number of plaques obtained in
the absence of antiserum, $R_t$ = number of plaques obtained with A2 virus in contact with serum for $t$ min., $e$ = base of natural logarithms, $k$ = inactivation constant and $C$ = serum concentration.

Fig. 1. Number of plaques in cultures incubated for 3 days at 37°C after inoculation with $5 \times 10^4$ u.v.-inactivated EID50 of fowl plague virus and varying doses of A2/Singapore/1/57.

Fig. 2. Residual reactivating activity of A2/Singapore/1/57 after varying times of exposure to 1/500 dilutions of homologous and heterologous antisera. Each point is estimated from the average number of plaques in 2 comparable cultures. O, A2/Sing./1/57; x, A2 Ned./65/63; Δ, A2/Eng./12/64.

Table 1. Rate constants of neutralization of the reactivating activity of influenza A2 strains by homologous and heterologous antisera

<table>
<thead>
<tr>
<th>Antisera</th>
<th>A2/Singapore/1/57</th>
<th>A2/Netherlands/65/63</th>
<th>A2/England/12/64</th>
</tr>
</thead>
<tbody>
<tr>
<td>A2/Singapore/1/57</td>
<td>115.4</td>
<td>30.7</td>
<td>16.8</td>
</tr>
<tr>
<td>A2/Netherlands/65/63</td>
<td>49.1</td>
<td>58.4</td>
<td>9.5</td>
</tr>
<tr>
<td>A2/England/12/64</td>
<td>3.8</td>
<td>7.8</td>
<td>88.7</td>
</tr>
</tbody>
</table>

Figures represent average $k$ values ($R_t/R_o = e^{-ktc}$) estimated from at least three points in each serum+virus combination.

Table 1 shows the inactivation constants when each of 3 different A2 strains were tested against homologous and heterologous antisera. The $k$ values were always higher in homologous than in heterologous serum+virus combinations, indicating specific neutralization. A comparison of the same strains by haemagglutination inhibition and by strain specific complement fixation (4) revealed antigenic relationships consistent with the data shown in Table 1. The method described may therefore provide an additional technique for the study of antigenic relationships among influenza A viruses.
Short communications

We are pleased to acknowledge the able technical assistance provided by Mr G. Hewlett.

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REFERENCES


(Received 17 September 1966)