The Effect of Antineuraminidase Antibody on the Elution of Influenza Virus from Cells

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SUMMARY

Antibody directed against the neuraminidase of an influenza virus did not prevent attachment of the virus to red cells, but strongly inhibited elution of the virus from these cells. The virus used in these experiments had approximately 500 enzyme antigenic sites on the surface of each particle. In the presence of sufficient antibody to combine with all of these sites there was no measurable elution of virus from cells. In the presence of smaller amounts of antibody, the virus eluted slowly.

INTRODUCTION

Three kinds of surface antigens occur on particles of influenza viruses. These are the haemagglutinin of the virus, the viral neuraminidase and a host-specific antigen which is composed mainly of carbohydrate (Haukenes, Harboe & Mortensson-Egnund, 1965; Laver & Webster, 1966). Antibody to the haemagglutinin inhibited haemagglutination by the virus and neutralized its infectivity. Antibody to the neuraminidase, which neither inhibited haemagglutination by a number of strains of influenza virus nor prevented these viruses from infecting susceptible cells, did prevent infected cells from releasing detectable levels of haemagglutinin (Seto & Rott, 1966; Webster & Laver, 1967, 1968). Antibody to the viral neuraminidase did not reduce the number of plaques formed by some strains of influenza virus in tissue cultures, but caused a marked reduction in plaque size (Jahiel & Kilbourne, 1966).

This paper reports on measurements made on the rate of elution of a recombinant influenza virus (x-7) from red cells in the presence of antineuraminidase antibody. This virus was chosen because it was known to elute from red cells very rapidly at 35° (Laver & Kilbourne, 1966), it possessed a known number (500) of enzyme antigenic sites (Webster & Laver, 1968), and antibody to the neuraminidase of the virus, containing a known concentration of antibody molecules, had been prepared (Webster & Laver, 1968).

METHODS

Virus. A recombinant influenza virus, x-7, possessing type A o (NWS) haemagglutinin and type A 2 (R157+) neuraminidase (Laver & Kilbourne, 1966) was used. The virus was grown in the allantoic sac of 11-day-old chick embryos and partially purified by one cycle of adsorption to and elution from chick erythrocytes.

Assay of haemagglutinin. Haemagglutinin was titrated in 0.25 ml. volumes of
saline in plastic trays using 0.5% red cells; the end-point of the titration was read by interpolation between complete agglutination and no agglutination. One haemagglutinin (HA) unit, defined as that amount of virus giving partial agglutination (15% of dimers) with 0.25 ml of 0.5% chick erythrocytes, corresponds to about 6 x 10^6 virus particles (Fazekas de St Groth & Webster, 1966).

**Estimation of neuraminidase activity.** Samples (0.1 ml) to be tested for neuraminidase activity were incubated at 35° for 30 min. with 0.1 ml of buffered fetuin solution (1.25 mg in 0.1 M-sodium phosphate, pH 5.9). Liberated N-acetyl neuraminic acid was assayed by the method of Warren (1959) except that the colour was extracted into n-butanol containing 5% (v/v) concentrated hydrochloric acid (Aminoff, 1961). Fetuin was prepared by the method of Graham (1961).

**Preparation of antineuraminidase antibody.** Antibody of known concentration directed specifically against type A2 influenza virus neuraminidase was prepared as described in detail elsewhere (Webster & Laver, 1968). The preparation used in these experiments contained approximately 7.5 x 10^14 antibody molecules per ml.

**Elution of virus from red cells.** Samples of X-7 influenza virus containing 10^{4.3} HA units of virus suspended in saline (12 ml) were mixed with different amounts of antineuraminidase antibody. The mixtures were titrated for haemagglutinin activity, assayed for neuraminidase activity using fetuin as substrate, and then chilled to 0°. Washed chick red cells (1.0 ml) were then added, the mixtures were well stirred and kept at 0° for 30 min. They were then transferred to 35° and samples (0.5 ml) were removed at intervals, centrifuged immediately to remove red cells and the supernatant fluids were titrated for haemagglutinin activity. All samples were subjected to ultrasonic vibrations (MSE oscillator at maximum output for 1 min. at 0°) before titration.

**RESULTS**

**Effects of antineuraminidase on X-7 virus**

Antibody to type A2 neuraminidase was mixed with the recombinant virus, X-7, which possessed A0 haemagglutinin and A2 neuraminidase. The antibody neither inhibited haemagglutination by this virus nor prevented its complete adsorption to red cells. The antibody inhibited the neuraminidase activity of this virus for a soluble substrate (Table 1) and greatly reduced the rate of elution of the virus from red cells (Fig. 1). In the control experiment, where antibody was not added, 60% of the virus adsorbed to the cells was released after 1 hr at 35°. In the presence of low concentrations of antineuraminidase antibody the rate of elution of the virus was reduced considerably, and at the highest antineuraminidase concentration (6.4 x 10^{12} antibody molecules/ml) none of the adsorbed virus was released from the cells after 1 hr at 35°. This virus, which remained adsorbed to the cells in the presence of antineuraminidase antibody, eluted rapidly and completely after the addition of bacterial *Vibrio cholerae* neuraminidase. Ada, Lind & Laver (1963) had previously shown that *V. cholerae* neuraminidase was not related immunologically to the viral enzyme.

It was possible to estimate approximately how much of the enzyme on the surface of the virus had to be neutralized by antibody before elution of the virus from cells was inhibited completely. The antineuraminidase preparation used in these experiments contained 7.5 x 10^{14} antibody molecules/ml and the surface of each particle of X-7 virus was found previously to possess approximately 500 enzyme antigenic sites.
Antineuraminidase and elution of virus

Table I. Haemagglutination and neuraminidase activity in mixtures of X-7 virus and antineuraminidase antibody

<table>
<thead>
<tr>
<th>Concentration of antineuraminidase (molecules/ml.)</th>
<th>Haemagglutinin titres of virus + antibody mixtures (log. HA units/ml.)</th>
<th>Percentage inhibition of X-7 neuraminidase</th>
<th>Percentage of virus adsorbed to red cells*</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>3.30</td>
<td>0</td>
<td>&gt; 99</td>
</tr>
<tr>
<td>1.6 × 10^{12}</td>
<td>1.6 × 10^{12}</td>
<td>53</td>
<td>&gt; 99</td>
</tr>
<tr>
<td>3.2 × 10^{12}</td>
<td>3.30</td>
<td>3.30</td>
<td>&gt; 76</td>
</tr>
<tr>
<td>6.4 × 10^{12}</td>
<td>3.30</td>
<td>3.30</td>
<td>&gt; 97</td>
</tr>
</tbody>
</table>

* 1.0 ml. of washed chick red cells were added to 12 ml. of virus + antibody mixture. Cells pre-treated with bacterial (Vibrio cholerae) neuraminidase did not remove any virus from the mixtures.

![Graph](image)

Fig. 1. Rate of elution of X-7 virus from red cells in the presence of saline (O—O), 0.025 ml. (■—■), 0.05 ml. (△—△) and 0.1 ml. (▲—▲) of antineuraminidase antibody.

(Webster & Laver, 1968). When antineuraminidase at a concentration of 6.4 × 10^{12} antibody molecules/ml. was added to X-7 virus at a concentration of 10^{3.80} HA units/ml., 97% of the viral neuraminidase was inhibited (Table I) and the virus did not elute at all from red cells under the conditions used (Fig. 1).

Fazekas de St Groth & Webster (1966) estimated that one haemagglutinin unit corresponds to about 6 × 10^{6} virus particles, therefore the concentration of virus in the above experiment was approximately 1.2 × 10^{10} particles/ml. Thus, at the highest
concentration of antineuraminidase it was estimated that there were 537 antibody molecules for every virus particle, which was just sufficient antibody to combine with all of the 500 enzyme antigenic sites present on the surface of the virus.

DISCUSSION

The results reported in this paper show that antibody directed against the neuraminidase of an influenza virus reduced the rate at which the virus eluted from cells. This is not a surprising finding since it is well known that influenza viruses whose neuraminidase has been destroyed, e.g. by heating, will not elute at all from cells, and Webster & Laver (1968) showed that the rate of elution of influenza virus depends on the amount of neuraminidase present in the virus particles. The present observations suggest that all of the enzyme antigenic sites on the surface of the virus must be neutralized by antibody before elution of the virus from cells ceases. The number of antibody molecules per virus particle (537) which were required for complete enzyme inhibition was in remarkable agreement with the number of enzyme antigenic sites (500) estimated to occur on the surface of the virus. This close agreement may be fortuitous, however, since considerable errors were possible, particularly in the haemagglutinin titrations and hence in the estimate of the number of virus particles taken.

The role of the viral neuraminidase during influenza virus infections is unknown, but it has been suggested that this enzyme is needed either to facilitate the entry of the virus into susceptible cells, to enable progeny virus to be released from cells, or to assist the virus to spread through infected tissues (Hirst, 1959). It has been shown that antineuraminidase will not prevent at least some influenza viruses from attaching to cells and subsequently infecting susceptible cells, so that it is unlikely that the viral enzyme is involved in the process of infection. Our results provide additional support for the suggestion already made (Seto & Rott, 1966) that the main function of influenza virus neuraminidase is to facilitate the release of the virus from cells. Thus antineuraminidase, by inhibiting this release may play an important part in the immunological defences of the body against influenza virus infections.

Influenza viruses undergo extensive antigenic variation (immunological or antigenic drift; Burnet, 1955) but in the past variation of the haemagglutinin antigen only has been considered. Paniker (1968) has shown that antigenic drift also occurs in the viral neuraminidase, and the recent findings of the effects of antineuraminidase on influenza viruses suggest that variation in the enzyme antigen may also be important in the epidemiology of influenza.

REFERENCES


Antineuraminidase and elution of virus


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