Studies with Antibody to the Purified Haemagglutinin of an Influenza Ao Virus

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SUMMARY

Antiserum was prepared in rabbits against purified haemagglutinin obtained from AO/BEL virus. The antiserum had potent virus-neutralizing and haemagglutination-inhibiting activity, but was free of antibody to AO/BEL neuraminidase or to influenza A type-specific ribonucleoprotein. In immunodiffusion tests, the anti-haemagglutinin serum gave precipitin reactions with all Ao and A1 virus strains tested but did not react with human A2 viruses or avian or porcine influenza A viruses. The studies suggested a close immunological relationship between the haemagglutinins of Ao and A1 influenza viruses which was not revealed by haemagglutination-inhibition tests.

INTRODUCTION

Schild & Pereira (1969) have described the identification by immunoprecipitin techniques in agar of two structural proteins of the influenza A2 virus, namely the neuraminidase and the ribonucleoprotein. The methods employed involved the use of sodium dodecyl sulphate to disrupt the influenza virus particles and to liberate the antigenic components. However, no precipitin line corresponding to the haemagglutinin of A2 virus could be detected in these tests, probably because A2 haemagglutinin was denatured in the presence of the detergent. The present report describes immunoprecipitin studies with the haemagglutinins of influenza A virus strains using antiserum prepared against purified haemagglutinin of AO/BEL virus. The latter virus was selected for study since its haemagglutinin retains biological activity after treatment with sodium dodecyl sulphate and can be obtained in highly purified form (Laver, 1964; Laver & Valentine, 1969). It was of interest to re-examine the immunological specificity of the haemagglutinin of an influenza A virus by a method other than the conventional haemagglutination-inhibition test.

METHODS

Virus strains. AO/BEL/1943 and X7 were virus strains kindly provided by Dr W. G. Laver, John Curtin School of Medical Research, Canberra, Australia. X7 is a recombinant of NWS and A2 (R15+) virus known to contain haemagglutinin derived from the NWS parent and neuraminidase derived from the A2 parent (Laver & Kilbourne, 1966). AO/BEL-A2 was a recombinant virus known to contain haemagglutinin derived from its AO/BEL parent and neuraminidase derived from its A2/SINGAPORE/1/57 parent (Schild & McCahon, 1970). Fowl plague virus was the DUTCH strain. Other influenza A virus strains were from the collection of the World Influenza Centre, London.
\textbf{Virus purification and concentration.} Suspensions of purified, concentrated influenza viruses were prepared as described previously (Laver & Kilbourne, 1966; Schild & Pereira, 1969).

\textbf{Immunological techniques.} Immunoprecipitation: The micro-technique (Crowle, 1958) has been described by Schild & Pereira (1969). For immunodiffusion studies, virus concentrates were disrupted by the addition of sodium dodecyl sulphate or Nonidet-P40 at a final concentration of 1\%. A convenient procedure which gave reproducible results in immunodiffusion tests was to add detergent as 10\% aqueous solution to the virus concentrates in the wells in the agar gel. Immunoelectrophoretic detection of influenza virus antigens was carried out by separating the virus proteins on cellulose acetate strips by electrophoresis and transferring sections of the strip to the surface of agar gel (Schild & Pereira, 1969).

Haemagglutinin estimations and measurements of haemagglutination-inhibiting antibody were made by standard methods (W.H.O. Expert Committee on Influenza, 1953). Estimations of neuraminidase activity and of neuraminidase-inhibiting activity were made by a modification of Warren’s (1959) technique (Webster & Pereira, 1968). The neuraminidase-inhibition activity of a serum was expressed as the reciprocal of the serum dilution which produced 50\% inhibition of enzyme activity after incubation at 4\°C for 18 hr with 1 to 2 units of enzyme.

Virus neutralization tests were made in monolayer cultures of rhesus monkey kidney cells (Schild & Stuart-Harris, 1965).

\textbf{Preparation of antisera.} Antivirus sera were prepared in rabbits using suspensions of purified u.v.-inactivated influenza virus preparations with Freund’s incomplete adjuvant (Webster & Laver, 1967). Post-infection antisera against influenza virus strains were prepared in ferrets for viruses of human origin or in chickens for avian viruses.

\textbf{Preparation of purified A0/BEL haemagglutinin and anti-haemagglutinin antiserum.} Purified haemagglutinin from A0/BEL virus was prepared by disrupting the virus with sodium dodecyl sulphate and separating the components by electrophoresis on cellulose acetate (Laver, 1963). A stock of purified haemagglutinin was obtained from 15 mg. purified A0/BEL virus and made up to a volume of 10 ml. in phosphate buffered saline pH 7.2. The detergent was removed by precipitation by adding 1 drop of saturated aqueous KCl solution and standing at 0\°C for 1 hr; the precipitated detergent was then removed by low-speed centrifugation at 0\°C to 2\°C. The resulting solution contained $5 \times 10^4$ haemagglutinin units/ml and 0.4 mg./ml. of protein (Lowry et al. 1951). Rabbits were immunized with three serial doses of 1 ml. of haemagglutinin preparation at 3-week intervals. The first immunizing doses were given intramuscularly using antigen mixed with Freund’s complete adjuvant. Subsequent doses were given intravenously without adjuvant. Serum samples were taken 3, 6 and 12 weeks after commencing immunization.

\textbf{RESULTS}

\textit{Specificity of antihaemagglutinin antiserum}

Antiserum prepared against purified A0/BEL haemagglutinin contained high activities of haemagglutinin-inhibiting antibody in tests with A0/BEL virus suspensions. The inhibition activities of the antisera collected at 3, 6 and 12 weeks after commencement of immunization were 5, 120, 12,800 and 51,200 respectively. To test for the presence of antibody to influenza A0 neuraminidase, the sera were studied in enzyme inhibition tests. When suspensions of untreated A0/BEL virus were used as a source of enzyme in these tests, low levels of neuraminidase-inhibition activity were detected (Table 1). However, in tests with pronase-treated virus, inhibition of enzyme activity could not be detected. As a control, a recombinant
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virus of AO/BEL and A2/SINGAPORE/1/57 (AO/BEL-A2) containing haemagglutinin identical to that of AO/BEL and neuraminidase identical to that of A2/SINGAPORE/1/57 was used. Although it contained A2 neuraminidase, which is immunologically unrelated to the Ao enzyme (Schild & Newman, 1969), the enzymic activity of this virus, like that of AO/BEL, was slightly inhibited by the antihaemagglutinin antisera when intact virus was used as a source of enzyme, but not when pronase-treated virus was used. This suggested that inhibition was due to antibody to Ao haemagglutinin-producing steric hindrance of access of substrate to the enzyme, as noted by Easterday et al. (1969), rather than to antineuraminidase antibody. Pronase treatment destroys virus haemagglutinin (Seto, Drzeniek & Rott, 1966; Biddle, 1968), and would thus be expected to abolish steric hindrance caused by antihaemagglutinin antibody.

Table 1. Reactions of anti-AO/BEL haemagglutinin antiserum*

<table>
<thead>
<tr>
<th>Virus strain</th>
<th>Haemagglutination-inhibition activity (serum RDE-treated)</th>
<th>Virus-neutralizing antibody activities†</th>
<th>Neuraminidase-inhibiting antibody activities with enzyme in form of:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>51,200</td>
<td>12,800†</td>
<td>40</td>
</tr>
<tr>
<td>AO/BEL</td>
<td></td>
<td></td>
<td>&lt;10</td>
</tr>
<tr>
<td>AO/BEL-A2/SINGAPORE/1/57</td>
<td>25,600</td>
<td>6,400</td>
<td>35</td>
</tr>
<tr>
<td>recombinant</td>
<td></td>
<td></td>
<td>&lt;10</td>
</tr>
<tr>
<td>AO/PR8</td>
<td>3,200</td>
<td>640</td>
<td>-</td>
</tr>
<tr>
<td>A1/FM1</td>
<td>&lt; 40</td>
<td>32</td>
<td>-</td>
</tr>
<tr>
<td>A2/SINGAPORE/1/57</td>
<td>64†</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>A2/ENGLAND/76/66</td>
<td>&lt; 40</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>A2/HONG KONG/1/68</td>
<td>&lt; 40</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Fowl plague virus</td>
<td>&lt; 40</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>A/Swine/CAMBRIDGE/39</td>
<td>64†</td>
<td>&lt;10</td>
<td>-</td>
</tr>
</tbody>
</table>

* Antiserum obtained 12 weeks after commencement of immunization.
† Reciprocal of serum dilution giving 50% or greater inhibition of haemadsorption compared with control cultures.
‡ The low inhibition activities recorded with A2/SINGAPORE and A/Swine are probably due to residual non-specific inhibitors. These viruses gave similar inhibition activities with normal rabbit serum treated with RDE.
—, not tested.

Antibody to influenza A ribonucleoprotein could not be detected (complement-fixing activity < 20) in the anti-haemagglutinin antisera in complement-fixation tests with influenza A ribonucleoprotein (soluble) antigen derived from AO/PR8 or A2/SINGAPORE/1/57.

The anti-haemagglutinin antisera were tested (Table 1) in virus neutralization tests against AO/BEL and a number of other influenza A virus strains. High virus-neutralizing antibody activities were detected in tests with AO/BEL. The neutralization activities obtained with AO/PR8 and A1/FM1 were considerably lower than those obtained with the homologous virus, whilst no neutralizing activity was detected in tests with A2 virus strains, fowl plague virus or Swine/CAMBRIDGE/39 virus.

Immunoprecipitin studies

The reactions of the antihaemagglutinin sera were studied in immunoelectrophoresis experiments in which the proteins of AO/BEL virus were separated by electrophoresis on cellulose acetate strips which were then placed parallel to strips of filter paper soaked in antiserum on the surface of 1 mm. layers of agar. The results are shown in Fig. 1. The antihaemagglutinin antiserum produced precipitin lines which corresponded closely to the
positions of the protein bands \((1(a)\) and \((b))\), from which haemagglutinin activity could be recovered. The precipitin line seemed to be continuous for bands \(1(a)\) and \(1(b)\), suggesting that both bands contained immunologically closely related antigens. Since the separation of the proteins from \(1(a)\) and \(1(b)\) was incomplete, immunological comparisons of \(1(a)\) and \(1(b)\) by the preparation of antisera against each fraction was not attempted. However, no evidence of immunological differences between the haemagglutinins contained in bands \(1(a)\) and \(1(b)\) was obtained from haemagglutination-inhibition tests. Haemagglutinins in eluates from the two bands were inhibited to similar titres by post-infection ferret serum to \(A_0/BEL\).

![Immunodiffusion](image)

**Fig. 1.** Stained portion of cellulose acetate paper strip containing the electrophoretically separated proteins of \(A_0/BEL\) virus concentrates disrupted by sodium dodecyl sulphate (1%). Haemagglutinin assays were made on protein eluted from unstained sections of strip. Haemagglutinin activity was found associated with bands \(1(a)\) and \(1(b)\). Immunological studies indicated that band 3 contained ribonucleoprotein. The position of precipitin lines produced by anti-\(A_0\) haemagglutinin serum corresponded with bands \(1(a)\) and \(1(b)\).

When antiserum against influenza A ribonucleoprotein was used a precipitin line was formed which corresponded in position to the protein band which moved fastest towards the anode (band 3). In previous studies with influenza A2 viruses (Schild & Pereira, 1969), the fast moving band during cellulose acetate electrophoresis was similarly identified as containing the ribonucleoprotein antigen.

In double immunodiffusion techniques, both ‘early’ and ‘late’ antihaemagglutinin sera produced a single, well defined precipitin line when tested against disrupted \(A_0/BEL\) virus (Fig. 2). When purified haemagglutinin was used as antigen, the reaction was more diffuse...
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and showed two, or occasionally three, close precipitin lines which merged with the single precipitin line produced by disrupted AO/BEL virus. A possible interpretation of the more diffuse reaction given by the purified haemagglutinin in contrast to disrupted BEL virus is that antigenic determinant groups were exposed in the purified haemagglutinin which were not exposed on haemagglutinin freshly released from virus particles by the addition of detergent. Alternatively, it is possible that in purified preparations the haemagglutinin subunits fragment into a number of immunologically active components, but this is unlikely since the haemagglutinin activity was stable on storage, suggesting that no breakdown of subunits occurred.

The reaction of AO/BEL virus was compared with that of an AO-A2 recombinant virus known to contain haemagglutinin derived from its AO/BEL parent and neuraminidase derived from its A2/SINGAPORE/1/57 parent (Schild & McCallon, 1970). In tests with anti-AO haemagglutinin, the AO-A2 recombinant gave a single precipitin line which was continuous with the line given by AO/BEL virus (Fig. 3) whilst with antiserum against purified A2 neuraminidase only the A2 parent recombinant gave precipitin reactions. In tests using a mixture of antisera containing anti-AO haemagglutinin and anti-purified A2 neuraminidase the AO-A2 recombinant gave precipitin lines corresponding to both AO haemagglutinin and A2 neuraminidase.

When the reactions of antiserum prepared against concentrated, purified AO/BEL virus were studied in similar tests, three precipitin lines were detected with AO/BEL virus (Fig. 5), in contrast to the single precipitin line given by anti-AO-haemagglutinin antiserum. One of the three precipitin lines (R, Fig. 5) was identified as corresponding to influenza A ribonucleoprotein antigen since it was continuous with the single line given by antiserum against influenza A ribonucleoprotein. The precipitin line nearest to the well containing antiserum (H) was identified as corresponding to AO haemagglutinin, since it was continuous with the single line given by purified AO haemagglutinin. The remaining precipitin line (N) given by AO/BEL virus probably corresponded with AO/BEL neuraminidase; the AO/BEL antiserum contained high activity of neuraminidase-inhibiting antibody.

The precipitin reactions of the anti-AO haemagglutinin serum strongly suggested that the antiserum was specific for virus haemagglutinin. However, these findings cannot be interpreted as proof that bands 1(a) and 1(b) of AO/BEL virus (Fig. 1) contained only haemagglutinin. It is possible that other virus components were present which had lost their immunological properties as a result of exposure to sodium dodecyl sulphate.

In further studies the spectrum of precipitin reactions of the anti-AO haemagglutinin antisera was investigated against a collection of influenza A viruses including examples of human AO, A1 and A2 virus strains and influenza A viruses of swine and avian origin. All AO and A1 viruses used in the tests, including AO/PR8, AO/NWS, A1/FM1 and A1/NETHERLANDS/1/56, gave precipitin lines with this antiserum. With the ‘early’ anti-AO haemagglutinin antiserum these reactions were strong for AO viruses and relatively weak A1 viruses. However, with the ‘late’ antiserum, both AO and A1 viruses gave well-defined precipitin reactions. The precipitin line formed by any of the different human AO and A1 viruses showed incomplete fusion with the line produced by AO/BEL, as indicated by ‘spur’ formation at the intersection of the lines formed from adjacent wells. There was no evidence of the precipitin lines crossing over. Furthermore, similar reactions of partial identity were demonstrated when any two of the AO and A1 viruses were placed in adjacent wells (Fig 6, 7). No precipitin reactions were detected with three A2 strains, A2/SINGAPORE/1/57 and A2/ENGLAND/12/64 and A2/HONG KONG/1/68 or with Swine/CAMBRIDGE/39 and Fowl plague virus. The failure of these viruses to react in precipitin tests might be due to immuno-
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logical differences between their haemagglutinins and that of A0/BEL or, alternatively, it might be their haemagglutinins were rendered immunologically inactive by sodium dodecyl sulphate. However, these virus strains also failed to react with the antihaemagglutinin serum in tests in which they were disrupted with a non-ionic detergent Nonidet-P-40 (Shell Ltd, London, 1% aqueous solution) was used because unlike sodium dodecyl sulphate, it does not destroy the haemagglutinating activity of influenza virus. The failure to react thus appeared to be due to lack of immunological similarities between their haemagglutinins and that of A0/BEL, since A0/BEL virus itself, after disruption with Nonidet-P-40, gave a well-defined precipitin line with antihaemagglutinin antiserum. These results are summarised in Table 2.

Table 2 shows the cross-reactions between the virus strains in haemagglutination-inhibition tests with post-infection ferret sera and antihaemagglutinin rabbit sera. The reactions of ferret sera against A0/BEL and A1/FM1 were highly specific. No cross-reactions were detected between A0 and A1 viruses, and anti-A0/BEL antiserum failed to react with the other A0 viruses. This latter finding was not surprising since the homologous inhibition activity of the A0/BEL antiserum was low, and a considerable degree of antigenic variation is known to exist amongst the A0 viruses. A0/BEL/42 was isolated several years after the other examples of A0 viruses used in these tests. Tests with antihaemagglutinin rabbit antiserum similarly failed to show relationships between the A0 and A1 viruses, in spite of their high homologous inhibition activities. These sera, however, did indicate cross-reactions between A0/BEL and the other A0 viruses used. In view of the apparent lack of cross-reaction between A0/BEL and the A1 virus strains in haemagglutination-inhibition tests, the apparently close relationships between their haemagglutinins in immunodiffusion tests was surprising.

Legend to Fig. 2 to 7

Fig. 2 to 7. Stained slides showing double immunodiffusion reactions of influenza viruses. Antisera: throughout the figures, wells marked a-h contained rabbit antiserum prepared against the purified haemagglutinin of A0/BEL virus, wells marked a-n contained rabbit antiserum purified A2 neuraminidase and those marked a-BEL(V) contained antiserum prepared against a suspension of concentrated, purified A0/BEL virus. The sera were used undiluted. Antigens: wells containing virus concentrates are marked with the virus strains. Abbreviations are as follows: A1/NED, A1/NETHERLANDS/1/56; A2/57, A2/SINGAPORE/1/57; A2/64, A2/ENGLAND/12/64; A2/66, A2/ENGLAND/76/66; B/5/66, B/ENGLAND/5/66; FPV, Fowl plague virus; A/sw, A/Swine/CAMBRIDGE/39; REC indicates a recombinant of A0/BEL and A2/SINGAPORE/1/57.

Fig. 2. Precipitin reactions of purified A0 haemagglutinin and disrupted A0/BEL virus in tests with anti-A0 haemagglutinin serum. The disrupted virus gave a single precipitin line which was continuous with a line given by purified A0 haemagglutinin.

Fig. 3. Reactions of anti-A0 haemagglutinin serum with A0/BEL virus, A2/SINGAPORE/1/57 and an A0-A2 recombinant. The recombinant virus and A0/BEL gave identical precipitin reactions. The A2 and swine viruses showed no reactions.

Fig. 4. Reactions of a mixture of anti-A0 haemagglutinin and anti-A2 neuraminidase sera. The A0-A2 recombinant gave well-separated precipitin lines corresponding to A0 haemagglutinin (H) and A2 neuraminidase (N).

Fig. 5. Reactions of antisera against A0/BEL virus. Three precipitin lines were demonstrated with disrupted A0/BEL virus. The innermost line (H), identified as A0 haemagglutinin, and the outermost line (R) were continuous with a single line given by A2/SINGAPORE virus, and corresponded to influenza A ribonucleoprotein. The third line (N) given by A0/BEL virus probably corresponded with A0/BEL neuraminidase.

Fig. 6, 7. Reactions of A0 anti-A0 haemagglutinin serum against various A0 and A1 virus strains. All A0 and A1 viruses showed precipitin lines in partial identity with the line given by A0/BEL virus. The human A2 viruses, swine virus and fowl plague virus showed no reactions.
Table 2. Comparison of double immunodiffusion and haemagglutination-inhibition reactions of anti-BEL antisera

<table>
<thead>
<tr>
<th>Antigens*</th>
<th>Rabbit antisera against purified A0/BEL haemagglutinin</th>
<th>Post-infection ferret antisera A0/BEL A1/FMI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Early serum‡</td>
<td>Late serum</td>
</tr>
<tr>
<td>A0/BEL (Purified HA)</td>
<td>10,240 ++ . 25,600 ++ .</td>
<td>320 &lt; 10 .</td>
</tr>
<tr>
<td>A0/BEL virus</td>
<td>5,120 ++ . 51,200 ++ .</td>
<td>640 &lt; 10 .</td>
</tr>
<tr>
<td>A0/BEL-A2/SINGAPORE recombinant</td>
<td>5,120 ++ i 25,600 ++ i</td>
<td>640 NT</td>
</tr>
<tr>
<td>A0/NWS</td>
<td>256 + pi 1,600 ++ pi &lt; 10 NT</td>
<td></td>
</tr>
<tr>
<td>X7 (NWS-A2 recombinant)</td>
<td>128 + pi 1,600 ++ pi &lt; 10 NT</td>
<td></td>
</tr>
<tr>
<td>A0/PR8 (1933)</td>
<td>960 + pi 3,200 ++ pi &lt; 10 &lt; 10</td>
<td></td>
</tr>
<tr>
<td>A1/FMI (1946)</td>
<td>&lt; 40 + pi &lt; 40 ++ pi &lt; 10 640</td>
<td></td>
</tr>
<tr>
<td>A1/NETHERLANDS/1/56</td>
<td>&lt; 40 + pi &lt; 40 ++ pi &lt; 10 120</td>
<td></td>
</tr>
<tr>
<td>A2/SINGAPORE/1/57</td>
<td>64 o . 64 o . &lt; 10 &lt; 10</td>
<td></td>
</tr>
<tr>
<td>A2/ENGLAND/12/64</td>
<td>&lt; 40 o . &lt; 40 o . &lt; 10 &lt; 10</td>
<td></td>
</tr>
<tr>
<td>A2/HONG KONG/1/68</td>
<td>&lt; 40 o . &lt; 40 o . &lt; 10 &lt; 10</td>
<td></td>
</tr>
<tr>
<td>Fowl plague virus</td>
<td>&lt; 40 o . &lt; 40 o . &lt; 10 &lt; 10</td>
<td></td>
</tr>
<tr>
<td>A2/Swine/CAMBRIDGE/39</td>
<td>64 o . 64 o . &lt; 10 &lt; 10</td>
<td></td>
</tr>
</tbody>
</table>

* Concentrates of virus were disrupted by the addition of 1% sodium dodecyl sulphate for use in immuno-precipitin tests or by 1% Nonidet-P40.
‡ ‘Early’ and ‘late’ antisera were obtained 3 weeks and 12 weeks respectively after the commencement of immunization with BEL-HA + Freund’s complete adjuvant.
HI: Results of haemagglutination-inhibition tests with RDE-treated antisera. Haemagglutination-inhibition antibody activities expressed as the reciprocal of the serum dilution inhibiting haemagglutination by 4 HA units of virus.
IP: Results of immunoprecipitin tests using undiluted sera. + +, strong precipitin reaction; + , moderate to weak precipitin reactions, pi, reactions of partial identity with precipitin line given by disrupted A0/BEL virus; i, reactions of identity with that produced by A0/BEL. O, no clear evidence of a precipitin reaction.

DISCUSSION

The present study suggests that the immunoprecipitation methods used, which were simple to carry out and gave reproducible results, are useful for immunological comparison of the haemagglutinin antigens of different influenza A viruses, as well as for the neuraminidase and ribonucleoprotein antigens, as previously described (Schild & Pereira, 1969). The interpretation of the results of haemagglutination-inhibition tests, which are widely used for the routine identification of influenza virus isolates, are difficult because of the existence of poorly understood phenomena, such as differences in the avidity of the virus strain under test for antibody, or its susceptibility to inhibition by normal serum inhibitors, or the existence of antihemagglutinin antibodies of different avidities (Webster, 1968). Also, in tests with sera from animals immunized with influenza antibody formed against the influenza virus, ‘host’ components may contribute to the ability of the serum to inhibit haemagglutination (Haukenes, Harboe & Mortensson-Egnund, 1966). Precipitin tests with antisera prepared specifically against haemagglutinin preparations are not subject to these disadvantages and may provide information on the serological relationships between influenza virus haemagglutinations additional to that obtained in haemagglutination-inhibition tests.

In the present study, immunoprecipitin tests with antisera prepared against purified haemagglutinin revealed a closer relationship between human A0 and A1 viruses than would be expected by their behaviour in haemagglutination-inhibition tests. It is of interest that the neuraminidases of human A0 and A1 viruses are also immunologically related (Paniker
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1968; Schild & Newman, 1969), but distinct from that of A2 viruses. These findings thus raise doubts about the validity of the immunological basis for classifying A0 and A1 viruses in two separate subtypes. However, both tests indicated a lack of serological relationship between the haemagglutinins of A0/BEL and A2 viruses or the examples of porcine and avian influenza A virus studied. A possible explanation for these differences in the specificities of the two tests is that different parts of the haemagglutinin sub-unit of the virus, shown by Laver & Valentine (1969) to be contained in one of the two types of surface structure present in the virus envelope, might contain at least two antigenic determinants. The results of precipitin tests presumably reflect the immunological specificity of the whole of the haemagglutinin subunit, while the results of haemagglutination-inhibition tests probably reflect the existence of antibody reacting with that part of the haemagglutinin sub-unit which is responsible for attachment to erythrocytes. Since, presumably, it is the distal portion of the haemagglutinin ‘spike’ which is responsible for this function, it may be hypothesized that, while the haemagglutinin subunits of A0 and A1 influenza viruses contain erythrocyte attachment sites which are immunologically unrelated, there are portions of the haemagglutinin subunits which carry similar immunological determinants for these two subtypes of influenza virus. Confirmation of this hypothesis must await the further fractionation of the haemagglutinin subunits and the biochemical and immunological comparison of such fractions for different subtypes of virus.

A number of previous studies have suggested the existence of serological relationships between haemagglutinins of different subtypes of influenza A virus which appeared to be unrelated on the basis of lack of cross-reaction in haemagglutination-inhibition tests with animal antisera. The studies of Davenport, Hennessy & Francis (1953) on the patterns of age distribution of antibody in human sera to successively occurring subtypes of influenza A viruses led to the concept of ‘original antigenic sin’ and those of Jensen et al. (1956) on the absorption of antibody by virus from immune human and ferret sera have indicated the existence of immunological relationships between the haemagglutinins of human A0 and A1 virus strains. In addition, Schulman & Kilbourne (1965) demonstrated the existence of cross-immunity in mice infected with influenza A virus strains which by haemagglutination-inhibition tests appeared to be unrelated. Alternative methods of comparing the antigens of the influenza virus, such as the immunodiffusion techniques described here, may aid the interpretation of such phenomena.

I am grateful to Mr R. Newman and Miss Susan Piddington for excellent technical assistance, to Mr R. Bowlby for photography and to Dr D. Breeze of Evans Medical Ltd, Liverpool, for the provision of bulk amounts of certain A2 viruses. I wish to thank Dr H. G. Pereira for his helpful comments on the manuscript.

REFERENCES


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