Sensitization of Influenza Virus A2/Singapore by Antineuraminidase

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Influenza virus particles possess two well-known virus-coded surface antigens: the haemagglutinin and the neuraminidase. Both seem to have essential functions in the growth cycle of the virus, haemagglutinin being responsible for the initial union of virus with the cell and neuraminidase being involved in the release of virus progeny from the host cell. Antibodies against purified haemagglutinin neutralize the virus, whereas antineuraminidase antibodies fail to do so (Webster & Laver, 1967). The existence of infectious virus-antibody complexes is now well documented for a variety of viruses (see Majer & Link, 1970). Most of the complexes represent sensitized virus, i.e. can be neutralized by homologous antoglobulin. In this communication we shall present results obtained with antibodies against whole influenza virus particles and with antibodies directed against their subunits.

Hyperimmune rabbit sera against the haemagglutinin of the virus Ao/Bel/1943 and against the neuraminidase of the virus A2/Singapore/1/57 as well as the corresponding virus strains were kindly supplied by Dr Schild, London (Schild, McCahon & Kendal, 1970). The antihaemagglutinin serum was reported to have a haemagglutination inhibition titre of 128,000 and the antineuraminidase serum an enzyme inhibition titre of 3500. Virus stocks were prepared in our laboratory by one intra-allantoic passage in eggs. Antisera to whole virus particles were gained 3 weeks after single intravenous injection of 0.5 ml. infectious allantoic fluids in rabbits. Direct neutralization tests were performed in 11-day-old embryonated eggs using constant amount of virus (about 100 EID50) with twofold serum dilutions. Virus-antiserum mixtures were incubated for 2 hr at 37 °C and then kept for 30 min. at 4 °. Eggs were inoculated intra-allantoically with 0.3 ml. volumes. After 48 hr incubation at 36 ° the allantoic fluids were collected and tested for the presence of haemagglutinin. The results presented in Table 1 show that antihaemagglutinin neutralized the homologous virus, whereas no significant neutralization of the homologous virus was achieved by antineuraminidase and no cross-neutralization was observed whatsoever.

In indirect neutralization assay, antiglobulin (anti-rabbit serum ‘Behringwerke AG’, Marburg-Lahn, Germany) was added in a dilution of 1:10 to the virus-antibody mixtures preincubated at 37 °. The samples were then kept at 4 ° for 30 min. prior to the inoculation of eggs. (In the direct neutralization test, the antiglobulin was substituted by PBS.) The antiglobulin had no inactivating effect on the viruses themselves. The results are summarized in Table 1. No significant enhancement of the neutralization by the antihaemagglutinin was observed after incubation with antiglobulin. The neutralization enhancement index (NEI) representing the ratio of the antisemum titre in the indirect neutralization test over the titre in the direct test was 2. On the other hand, A2/Singapore virus was sensitized by the antineuraminidase to a dilution of 1:1280 (NEI = 128). This finding offers the possibility to study the neuraminidase-antineuraminidase interaction in a neutralization test in addition to the enzyme-inhibition test and the immunoprecipitin test (Schild & Pereira, 1969). Antisera prepared by immunization with intact viruses were shown to have no sensitizing capacity at all. Thus antihaemagglutinin was likely to determine the end-points of these antisera.

For the indirect neutralization described above we suggest the following. Neuraminidase represents non-critical sites for the initiation of the infection. After interaction of antiglobulin with antineuraminidase attached to the virus, antiglobulin molecules might interfere
with neighbouring critical sites (haemagglutinin), thus leading to the neutralization of the virus. An alternative explanation for the indirect neutralization would be the stabilization of virus-antineuraminidase complexes by the antiglobulin, but this possibility seems to be less probable since it was shown that antineuraminidase antibodies were of the same avidity as antihaemagglutinin antibodies (Webster, Laver & Kilbourne, 1968). No significant neutralization was achieved by antiglobulin added to the virus previously treated with antihaemagglutinin. One explanation of this observation is the absence of infectious virus-antihaemagglutinin complexes indicating that entire surface haemagglutinin might represent critical sites. Using antiglobulin, cross-neutralization could be shown with adenoviruses which did not cross-react in direct neutralization test (Kjellén & Pereira, 1968). In the indirect neutralization test no cross-reactions between Ao/Bel and A2/Singapore viruses could be observed confirming the distinct antigenic properties of the two viruses. This test might be found useful in the antigenic analysis of influenza strains for vaccine production.

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REFERENCES


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