Immunogenicity of a Synthetic Peptide Corresponding to a Portion of the Heavy Chain of H3N2 Influenza Virus Haemagglutinin

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SUMMARY

Rabbits were immunized with a synthetic octadecapeptide corresponding to the sequence ser-91 to leu-108 of the haemagglutinin heavy chain of H3N2 influenza A viruses. They developed antibodies reactive in solid-phase radioimmunoassay (SPRIA) with the peptide and with haemagglutinins of various H3N2 viruses but not of heterotypic H1N1 and H2N2 viruses. The antibodies were also non-reactive in the haemagglutination-inhibition or neutralization test. Influenza H3N2 virus replicated in the lungs of mice immunized with the peptide to the same extent as in the control mice. Of 27 human sera possessing anti-H3N2 activity or seven sera from rabbits immunized with either virions or haemagglutinins of various influenza A viruses, none was reactive with the peptide in SPRIA.
Table 1. Reactivity of sera from peptide-immunized rabbits with synthetic peptides and homotypic haemagglutinin by SPRIA

<table>
<thead>
<tr>
<th>Rabbit no.</th>
<th>Immunogen*</th>
<th>Test antigen†</th>
<th>Reactivity index‡ after indicated number of doses</th>
</tr>
</thead>
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<tr>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>1</td>
<td>OP-1 (HPLC)</td>
<td>OP-1 + BSA</td>
<td>8.2</td>
</tr>
<tr>
<td></td>
<td>+ TT-CDI</td>
<td>H</td>
<td>0.6</td>
</tr>
<tr>
<td>2</td>
<td>OP-1 (HPLC)</td>
<td>OP-1 + BSA</td>
<td>5.4</td>
</tr>
<tr>
<td></td>
<td>+ TT-GA</td>
<td>H</td>
<td>0.9</td>
</tr>
<tr>
<td>3</td>
<td>OP-2</td>
<td>OP-2 + BSA</td>
<td>2.1</td>
</tr>
<tr>
<td></td>
<td>+ TT-GA</td>
<td>H</td>
<td>0.9</td>
</tr>
<tr>
<td>4</td>
<td>OP-2</td>
<td>OP-2 + BSA</td>
<td>5.4</td>
</tr>
<tr>
<td></td>
<td>+ TT-GA</td>
<td>H</td>
<td>1.4</td>
</tr>
<tr>
<td>5</td>
<td>OP-2 (HPLC)</td>
<td>OP-2 + BSA</td>
<td>12.8</td>
</tr>
<tr>
<td></td>
<td>+ TT-CDI</td>
<td>H</td>
<td>1.2</td>
</tr>
<tr>
<td>6</td>
<td>OP-2 (HPLC)</td>
<td>OP-2 + BSA</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>+ TT-GA</td>
<td>H</td>
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<tr>
<td>7</td>
<td>OP-2 (HPLC)</td>
<td>OP-2 + BSA</td>
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<tr>
<td></td>
<td>+ TT-CDI</td>
<td>H</td>
<td>0.7</td>
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<tr>
<td>8</td>
<td>OP-2 (HPLC)</td>
<td>OP-2 + BSA</td>
<td>14.0</td>
</tr>
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<td></td>
<td>+ TT-CDI</td>
<td>H</td>
<td>2.4</td>
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<tr>
<td>9</td>
<td>TT</td>
<td>OP-2 + BSA</td>
<td>1.0</td>
</tr>
<tr>
<td>(control)</td>
<td>TT</td>
<td>H</td>
<td>1.1</td>
</tr>
</tbody>
</table>

* (HPLC), purified by HPLC; TT, conjugated to tetanus toxoid; CDI, coupled using carbodiimide, GA, coupled using glutaraldehyde.
† BSA, conjugated to bovine serum albumin; H, haemagglutinin derived from A/Philippines or A/Victoria (H3N2) influenza virus. Concentration of immunoglobulins was approximately 0.3 mg/ml.
‡ Ratio of c.p.m. of test serum to c.p.m. of preimmune serum, values equal to or exceeding 2.0 are underlined.

antigens: (i) 2 µg per well of HPLC-purified peptide coupled to BSA; (ii) 2 µg per well of haemagglutinins of homotypic H3N2 A/Philippines/85 and A/Victoria/75 and heterotypic [A/swine/31 (H1N1), A/PR8/34 (H1N1), A/Ostrava/80 (H1N1)] viruses, prepared from purified virions by bromelain cleavage (Brand & Skehel, 1972), and (iii) 0.5 µg per well of virions purified according to Laver (1964). Various dilutions (starting at 1 : 10) of the Ig preparations were tested. The reaction was considered positive if the ratio of c.p.m. of test serum to c.p.m. of preimmune serum was 2.0 or more.

The immunization procedure and the development of antibodies reactive with the synthetic peptides (OP-1 or OP-2) and H of H3N2 virus are shown in Table 1. It can be seen that antibodies reactive with either OP or with both OP and H developed in all rabbits. Two of the three rabbits which did not develop anti-H reactivity had been immunized with a preparation not purified by HPLC; this suggests a lower immunogenicity of this preparation. No marked differences in the immunogenic activity of OP-1 and OP-2 were encountered and no marked influence on the immune response was produced by the coupling procedure used. The anti-peptide antibodies were nearly always detected earlier and attained higher levels than those reactive with H. This might be associated with the higher number of antibody-binding sites in the wells coated with the peptide than those coated with H. Alternatively the differential reactivity may have been due to a heterogeneity of the OP preparation which might consist of molecules possessing the conformation of the native protein as well as molecules with other conformations and antigenicities; thus antibodies capable of reacting with antigens present in OP preparations but not in H might have developed.

The reactivity in SPRIA of selected sera of OP-immunized rabbits with purified H and complete virions of homotypic and heterotypic influenza viruses are shown in Fig. 1 and 2. The antibody was reactive with H preparations from H3N2 viruses but not with those derived from H1N1 and H2N2 viruses (Fig. 1). These results, demonstrating specificity of the reaction of OP-induced antibodies, were in sharp contrast to the cross-reactivity observed when whole virions
Fig. 1. Reactivity in SPRIA of Ig fractions from sera of rabbit no. 1 (Table 1), with haemagglutinins isolated from homotypic and heterotypic influenza A viruses (a) A/Philippines (H3N2), (b) A Victoria (H3N2), (c) A/swine (H1N1), (d) A/PR8 (H1N1), (e) A/Ostrava (H1N1) and (f) A/Singapore (H2N2). O, pre-immune serum; ▲, serum after 5th dose; ●, serum after 6th dose.

Fig. 2. Reactivity in SPRIA of immunoglobulin fractions from sera of rabbit no. 1 (Table 1), with purified virions of homotypic and heterotypic influenza A viruses (a) A/Philippines (H3N2), (b) A/PR8 (H1N1) and (c) A/Chile (H1N1). Symbols as in Fig. 1.

were used as antigens (Fig. 2). Sera from the other OP-immunized rabbits reacted in the same way. The nature of the heterotypic reactions with whole virion antigens, also observed by another group of investigators (Arnon & Shapira, 1984) is not understood at this time.

In further tests the Ig (diluted 1:2, 1:4 and 1:8) from rabbit sera proved negative in haemagglutination inhibition tests (Tučková et al., 1968) and in a sensitive modification of the neutralization test in chick embryos using 1 and 10 EID₅₀ of H3N2 Victoria/75 virus. Using OP as antigen we also examined a group of 27 human sera and seven rabbit immune sera in SPRIA. Human sera were collected from healthy subjects vaccinated with a subunit influenza vaccine (Subinvira, Imuna, Czechoslovakia) containing H3N2 and H1N1 viruses or unvaccinated, as well as from subjects experiencing a recent H3N2 virus infection. All of them contained homotypic haemagglutination inhibiting antibody ranging in titre from 1:20 to 1:5120. Sera originated from rabbits immunized with various complete H3N2 and H1N1 viruses or purified-preparations from the same viruses. The homotypic HI antibody titres of these sera varied from 1:256 to 1:2560. Neither human nor rabbit sera gave a positive reaction in SPRIA with OP.

Finally, the capability of OP to confer protection in mice was examined. Mice were immunized intraperitoneally with 50 μg of the OP-1-TT conjugate in complete Freund's adjuvant. Two additional doses (each comprising 25 μg of the conjugate) in incomplete Freund's adjuvant were injected subcutaneously at 3-week intervals. One week after the third dose pooled sera were examined for antibody; only mice immunized with OP-1-TT were reactive with OP and H of H3N2 virus. At the same time the remaining animals were challenged intranasally with
10^6 EID of H3N2 Aichi/68 virus which replicated well in mouse lungs but did not kill the animals. Three days later their lungs were removed and individually tested for infectivity in embryonated eggs. Replication of the virus in immunized mice was comparable to that in the control mice (data not shown). These data are inconsistent with the recent observations by Shapira et al. (1985) which suggested a protective activity of the same synthetic peptide in mice.

In accordance with Müller et al. (1982) the present data indicate that a synthetic octadecapeptide corresponding to the sequence ser-91 to leu-108 of the HA-1 subunit of H3N2 is capable of inducing antibodies reactive with immunizing peptide and homotypic H preparations. However, both the serological data and experiments in mice suggest that the immune reactions are not of any significance for protection against infection. We also did not detect antibodies reactive with OP in SPRIA in any sera from humans who had experienced natural infection and/or immunization with split H3N2 vaccine or in sera of animals immunized with H preparations or complete virions of different influenza A viruses. This indicates that the region of HA-1 corresponding to OP is not immunogenic under conditions of natural infection or immunization with the more complex virus materials.

REFERENCES


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