Archives of Virology 66, 265—269 (1980)

Archives of Virology

Protection of Chickens Against Challenge with Virulent Influenza A Viruses of Hav5 Subtype Conferred by Prior Infection with Influenza A Viruses of Hsw1 Subtype

Brief Report

By

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Accepted June 12, 1980

Summary

Prior infection of six-week-old chickens with influenza A viruses of Hsw1 haemagglutinin subtype and irrelevant neuraminidase subtypes reduced the deaths and sickness in groups of those birds challenged with A/tern/S. Africa/61 (Hav5Nav2/3) and A/chicken/Scotland/59 (Hav5N1).

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On the recommendations of the WHO Expert Committee (13) influenza viruses are classified into types A, B or C on the basis of the ribonucleoprotein antigen and influenza A viruses into subtypes on the basis of the haemagglutinin (H) or neuraminidase (N) antigens. However, relationships have been revealed amongst H subtypes originally thought to be distinct by serological tests (6, 8, 10) and, in the case of the relationship between Heq1 and Hav1 subtypes, by protection studies in chickens (9). More recently immuno-double-diffusion tests with antisera against isolated antigens have suggested several interrelationships between subtypes and a reorganization of the classification system for influenza A viruses has been recommended (11, 14, 15). Although Schild *et al.* (11) do not report any relationship between Hav5 (proposed H5) and Hsw1 (proposed H1), low-level cross relationships between these subtypes have been reported (2, 12). In the present study we have examined the ability of viruses of Hsw1 subtype to protect chickens against virulent Hav5 viruses with irrelevant N subtypes.

The viruses and their sources have been described (1, 2) with the exception of A/duck/Alberta/35/76 (Hsw1N1) (7) and A/duck/Hong Kong/196/77 (Hsw1N2) which were received from Dr. K. F. Shortridge, Hong Kong University, Hong Kong. In protection studies six-week-old chickens were infected by intramuscular inoculations of about 10^8 EID_{50} of primary virus and reinfected by the same route with a similar dose three weeks later. Two weeks after the second dose birds were bled and challenged with 0.1 ml of diluted infectious allantoic fluid contain-

0304-8608/80/0066/0265/$ 01.00

ing about 10^6 EID_{50} of challenge virus by intramuscular injection. Birds were examined twice daily for signs of disease. Those alive but too sick to eat or drink were killed and recorded as dead at the next observation. Experiments were restricted to those in which the primary infecting virus and the challenge virus had dissimilar N subtypes as antibodies to this antigen also afford protection (4, 9). Uninfected fully susceptible controls were also challenged and A/equine/Prague/1/56 (Heq1Neq1) and A/turkey/England/63 (Hav1Nav2/3) were used for primary infection and challenge as controls for susceptibility and protection. A/turkey/England/N28/73 (Hav5N2), which is of low virulence for chickens, was used to demonstrate protection by an Hav5 virus.

The serological responses seen after primary infection and challenge and the signs of disease and deaths are shown in Table 1. All susceptible birds challenged with A/tern/S.Africa/61 became sick and died with a mean death time (MDT) of 5.1 days. Primary infection with dk/H.K./196, ty/Eng/250 and dk/Alb/35 conferred considerable resistance to challenge with tn/S.A./61, only 2/10 birds dying from each group infected with dk/H.K./196 and ty/Eng/250 and 1/10 with dk/Alb/35. While 5/10, 3/10 and 3/10 respectively showed signs of disease. Protection by A/swine/Cambridge/39 (Hsw1N1) was not so marked, 9/10 birds showing signs of disease but only 6/10 dying. Prior infection with the virus of Hav5 subtype, ty/Eng/N28, induced complete protection to challenge with tn/S.A./61. One bird primary infected with eq/Prague survived challenge with tn/S.A./61, although the low post challenge haemagglutination inhibition (HI) titre to tn/S.A./61 in this bird may indicate that infection was never established. The other nine eq/Prague infected birds all became sick and died in a noticeably shorter time than susceptible controls. With the exception of ty/Eng/N28 infected birds, none had shown prechallenge HI titres to tn/S.A./61. All birds surviving challenge were positive by HI tests to tn/S.A./61 and all individual birds in the ty/Eng/250, dk/H.K./196 and dk/Alb/76 groups showed increased HI titres to the primary infecting virus after challenge. Although earlier work indicated that ck/Scot/59 was as virulent as tn/S.A./61 (5) in the present study only 8/10 susceptible controls were sick and 7/10 died after challenge with ck/Scot/59. All three surviving birds showed high HI titres to ck/Scot/59 indicating that they had been infected. Prior infection with dk/H.K./196 produced considerable protection to challenge with ck/Scot/59. One bird was found dead on day 4 after challenge but this was the only bird to show any signs of disease. Birds infected with ty/Eng/N28 were fully protected against challenge with ck/Scot/59. As a further control, selected viruses were used as primary infecting viruses prior to challenge with turkey/Eng/63 (Hav1Nav2/3). Birds were not protected against this virus by sw/Camb/39 and, as seen with eq/Prague/56 and tn/S.A./61, deaths and onset of sickness occurred noticeably sooner than with challenged susceptible birds. One bird primary infected with dk/H.K./196 survived challenge with ty/Eng/63, the other nine becoming sick and dying at about the same time as in suceptible controls. The surviving bird showed a high HI titre to ty/Eng/63. Eq/Prague/56 conferred a high level of protection to challenge with ty/Eng/63, only one bird showing signs of disease and dying. Calculation of pathogenicity indices (Table 1) for the challenged birds gave a good indication of the virulence of the challenge viruses and the degree of protection conferred by the primary viruses.

Table 1. *Effect of prior infection of chickens on challenge with virulent influenza A viruses*

Primary infecting virus	Prechallenge HI titres[a]				Postchallenge HI titres in surviving birds				Sick		Dead		Pathogenicity[b] index
	Primary virus		Challenge virus		Primary virus		Challenge virus		No.	MTO[d] (days)	No.	MDT[e] (days)	
	MLT[c]	Range	MLT	Range	MLT	Range	MLT	Range					
Challenge with A/tern/S. Africa/61 (Hav5Nav2)													
None	—	—	<1	—	—	—	—	—	10/10	3.8	10/10	5.1	1.51
A/ty/Eng/250/79 (Hsw1N1)	5.3	3—6	<1	—	7.1	5—10	5.0	4—6	5/10	5.4	2/10	(at 5 and 15 days)	0.40
A/dk/Hong Kong/D196/77 (Hsw1N2)	4.6	3—6	<1	—	6.4	4—13	4.7	1—10	3/10	4.7	2/10	(at 4 and 14 days)	0.32
A/sw/Cambridge/39 (Hsw1N1)	8.4	7—10	<1	—	7.0	6—8	5.2	5—6	9/10	3.9	6/10	4.3	1.17
A/dk/Alberta/35/76 (Hsw1N1)	5.3	4—7	<1	—	5.5	5—7	5.8	1—7	3/10	4.0	1/10	(on day 6)	0.19
A/ty/England/N28/73 (Hav5N2)	5.0	3—6	2.8	1—4	5.7	5—8	3.2	2—6	0/10	—	0/10	—	0.00
A/eq/Prague/1/56 (Heq1Neq1)	6.2	5—8	<1	—	7	—	3	—	9/10	2.7	9/10	3.0	1.64
Challenge with A/chicken/Scotland/59 (Hav5N1)													
None	—	—	<1	—	—	—	8.0	8	8/10	2.5	7/10	3.6	1.34
A/dk/Hong Kong/D196/77 (Hsw1N2)	5.3	4—8	<1	—	6.4	3—9	5.9	4—9	1/10	4	1/10	4	0.16
A/ty/England/N28/73 (Hav5N2)	5.1	3—6	3.8	2—5	7.0	6—10	5.4	4—8	0/10	—	0/10	—	0.00
Challenge with A/turkey/England/63 (Hav1Nav3)													
None	—	—	<1	—	—	—	—	—	10/10	3.0	10/10	3.7	1.67
A/sw/Cambridge/39 (Hsw1N1)	8.5	7—9	<1	—	—	—	—	—	10/10	2.2	10/10	2.7	1.81
A/dk/Hong Kong/D196/77 (Hsw1N2)	5.0	2—6	<1	—	5	—	11	—	9/10	3.1	9/10	3.7	1.40
A/eq/Prague/1/56 (Heq1Neq1)	5.6	4—8	1.6[f]	<1—4	8.8	6—11	6.4	3—9	1/10	3	1/10	4	0.17

[a] Haemagglutination inhibition (HI) titres are expressed as $\log_2$ of the reciprocal of the highest dilution of serum to inhibit 4 HA units of virus

[b] Pathogenicity indices were calculated by scoring 2 for each dead bird, one for each sick bird and 0 for each normal bird each day over the 15 day observation period after challenge and calculating the average score per bird per day

[c] MLT — mean $\log_2$ titre

[d] MTO — mean time of onset of sickness

[e] MDT — Mean death time

[f] Mean of the six birds showing a titre ≥ 1

These results indicate that Hsw1 and Hav5 subtypes are related. Serological tests have suggested that a very low-level cross relationships may exist between viruses of these groups (2, 12) and the lesser protection afforded by the Hsw1 viruses compared to the avirulent Hav5 virus, A/ty/Eng/N28/73 is an indication that the degree of antigenic relatedness may be quite low. Although none of the Hsw1 viruses gave 100 per cent protection against challenge with the Hav5 viruses, some levels of protection were at least as high as those reported by BUTTERFIELD and CAMPBELL (3) in protection studies with the avirulent A/ty/Oregon/71 (Hav1Nav2) and challenge with virulent Hav1 viruses.

In the past the use of protection studies to ascertain the relatedness of influenza A virus antigens has not always produced meaningful results. This has been mainly due to a lack of understanding of shared surface antigens in the vaccine and challenge viruses. However, in some cases, serological relationships seen *in vivo* have been later confirmed by laboratory techniques (9, 14). In the present study only viruses with irrelevant neuraminidases have been compared, controls have been used to exclude the effect of other virus antigens and the homologous system should remove the possible effect of host-derived antigens. It appears that there is a real indication of antigenic relatedness of the H antigens of the Hsw1 and Hav5 viruses tested.

The Hav5 and Hsw1 subtypes have been placed in separate groups, H5 and H1 respectively, on the evidence available to a WHO Expert Committee considering the revision of influenza A nomenclature (15). While it must be stressed that criteria used for the system of nomenclature do not necessarily exclude immunological relationships which may be measured in other systems, evidence obtained in laboratory studies, including RNA-RNA hybridization studies with H antigen genes of viruses of the H5 and H1 groups, indicates that these subtypes are not related. The results obtained in the present study therefore represent an anomaly within current concepts of influenza A immunology. Nevertheless, *in vivo* observations such as these may be important in the full understanding of protection and susceptibility of animals, including man, to influenza virus infections.

Acknowledgments

We thank Miss R. Hull for her excellent assistance.

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Received April 11, 1980