Bull. Org. mond. Santé
Bull. Wld Hlth Org. } 1969, **41**, 647-650

The Role of Antineuraminidase Antibody in Immunity to Influenza Virus Infection *

JEROME L. SCHULMAN [1]

Experimental studies in mice have provided evidence that antibody specific for viral neuraminidase markedly inhibits influenza virus replication in the lungs of animals challenged with virus containing homologous neuraminidase. In contrast to antihaemagglutinin antibody, antineuraminidase antibody does not increase resistance to the initiation of infection, but does reduce the capacity for subsequent transmission of infection.

The 1968 Hong Kong virus was found to possess a haemagglutinin antigen markedly different from previous A2 strains but its neuraminidase is indistinguishable from the enzyme antigens of 1967-68 A2 viruses, and protection of mice immunized with 1957 and 1967 A2 viruse and challenged with Hong Kong virus was entirely attributable to antineuraminidase antibody.

The desirability of systematically examining the protective effects of antineuraminidase antibody in man and of revising current methods for selection and standardization of influenza vaccines are discussed.

Thirty-six years of research with influenza viruses have provided abundant evidence relating immunity against influenza to the presence of antibody specific to viral haemagglutinin in the sera and/or the respiratory secretions of previously infected or immunized subjects. Antibody produced in response to dissociated haemagglutinin can neutralize virus *in ovo* (Davenport et al., 1964) and can completely inhibit plaque formation in tissue culture (Jahiel & Kilbourne, 1966); and human subjects immunized with dissociated haemagglutinin protein are protected against influenza (Hennessy & Davenport, 1966).

However, influenza virus possesses a second virus-coded protein antigen in its surface envelope—neuraminidase—and antibody specific for viral neuraminidase has been demonstrated in man and in laboratory animals following infection or parenteral immunization with inactivated virus (Kilbourne, Christenson & Sande, 1968; Fedson, 1969; Kilbourne et al., 1968; Schulman, Khakpour & Kilbourne, 1968).

In contrast to the effects of antihaemagglutinin antibody, antibody to neuraminidase (except in very high concentration) does not reduce infectivity titres of pre-inoculation mixtures of virus and antibody, but virus replication in *in ovo* and tissue culture systems is inhibited by the continuous presence of antineuraminidase antibody (Kilbourne et al., 1968; Webster & Laver, 1967; Webster, Laver & Kilbourne, 1968).

Experiments in mice have demonstrated marked reductions of pulmonary virus titres and prevention of lung lesions in animals immunized with the isolated neuraminidase antigen of the challenge virus. In other experiments which utilized recombinant viruses in which haemagglutinin and neuraminidase antigens were derived from viruses of 2 different subtypes, specific immunization against the enzyme component of the challenge virus provided protection comparable to that achieved by immunization against the haemagglutinin antigen (Schulman, Khakpour & Kilbourne, 1968).

These observations raise the question whether immunity to epidemic influenza in man is exclusively

* This investigation was conducted in part under the auspices of the Commission on Influenza, Armed Forces Epidemiological Board, and was supported in part by the US Army Medical Research and Development Command, Department of the Army, under Research Contract No. DA-49-193-MD-2795; in part by US Public Health Service Research Grant AI-09304 from the National Institutes of Allergy and Infectious Diseases; and in part by the Health Research Council of the City of New York under Contract No. U-1023.

[1] Department of Microbiology, Mount Sinai School of Medicine of The City University of New York, New York, N.Y. 10029, USA.

related to antibody specific for viral haemagglutinin, or if it results in part from antibody to the viral enzyme as well. A second, related question is whether the epidemiology of influenza is influenced by antigenic changes in neuraminidase.

Detailed analysis of representative strains of influenza A2 virus by plaque assay in human conjunctival cells and by tests of cross-reactivity in enzyme-inhibition and haemagglutination-inhibition reactions provided evidence that antigenic variation of the 2 virus-coded envelope proteins occurs independently in nature, resulting in new viruses which differ from older strains more with respect to one antigen than the other (Schulman & Kilbourne, 1969). In particular, the 1968 Hong Kong virus was found to have a haemagglutinin antigen which is markedly different from that of earlier A2 strains, but its neuraminidase was demonstrated to be antigenically related to the enzymes of 1957–64 A2 strains and identical to the enzyme antigens of 1967–68 strains.

The effects of these antigenic relationships on immunity against Hong Kong virus challenge were investigated in mice, utilizing hybrid viruses in which haemagglutinin and enzyme antigens were derived from parents of 2 different subtypes. Mice were immunized with A2/Japan/305/57 virus, A2/England/67 virus and with hybrid viruses derived by recombination of each of the A2 strains with A0/NWS virus. Four such recombinant viruses were studied: a hybrid virus with haemagglutinin protein derived from A0/NWS and a neuraminidase antigen derived from A2/Japan/305/57 virus (A0 E [J. 305]); a similar recombinant possessing A0 haemagglutinin and the enzyme antigen of A2/England/67 (A0 E [Eng.]); a hybrid virus containing A2/Japan/305/57 virus haemagglutinin and the enzyme of A0/NWS virus (A2/Jap. 305/e), and a recombinant possessing the haemagglutinin of A2/England/67 virus and A0/NWS virus enzyme (A2/Eng./e).

As shown in Table 1, following challenge with mouse-adapted Hong Kong virus, lower pulmonary virus titres and less extensive lung lesions were observed in mice immunized with the parent A2 viruses. However, equivalent reductions of virus titres and lung lesions were achieved by immunization with the hybrid viruses possessing A0/NWS virus haemagglutinin and enzyme antigens derived from the A2 virus parents (A0 E [J.305], and A0 E [Eng.], and no protection was observed in animals immunized with the hybrid viruses possessing the haemagglutinin antigens but not the neuraminidase antigens of A2/Japan/305 and A2/England viruses (A2/Jap. 305/e and A2/Eng./e). In addition, mice immunized with the same wild-type A2 viruses were not protected against challenge with a mouse-adapted recombinant virus possessing the haemagglutinin of Hong Kong virus and neuraminidase derived from A0/NWS virus (Hong Kong/e). Thus, as stated earlier in this Conference, the antigenic relatedness of Hong Kong virus to previous A2 strains is much more evident with respect to its enzyme antigen than to its haemagglutinin antigen and only antineuraminidase antibody induced by prior immunization with earlier A2 viruses protected mice against Hong Kong virus challenge. It is interesting to speculate whether the epidemiology of Hong Kong influenza in human populations was similarly influenced by antineuraminidase antibody resulting from prior infection or immunization with previous strains of influenza A2 virus. The A1 subtype introduced in 1947 was antigenically dissimilar to A0 strains with respect to its haemagglutinin but was cross-reactive with later A0 strains with respect to its neuraminidase antigen (Paniker, 1968). Although widespread epidemics occurred in association with the appearance of the A1 subtype, pandemic influenza did not occur on a scale comparable to the events of 1957–58 with the introduction of the A2 viruses which were antigenically novel with respect to both surface proteins.

TABLE 1

PROTECTIVE EFFECTS OF IMMUNIZATION WITH INFLUENZA A2 VIRUSES AND WITH RECOMBINANT VIRUSES DERIVED FROM A2 AND A0 VIRUS PARENTS ON HONG KONG VIRUS CHALLENGE

Immunizing virus [a]	Pulmonary virus titres at 48 hours [b]	Lung lesions at day 7 (%)
A2/Japan/305/57	6.3	25
A0 E (J. 305) [c]	6.1	16
A2/Jap. 305/e [d]	7.0	50
A2/England/67	5.9	15
A0 E (Eng.) [e]	5.9	4
A2/Eng./e [f]	7.0	47
Saline	7.1	55

[a] Subcutaneously in Freund's adjuvant 6 weeks before challenge.

[b] EID_{50}, log_{10}.

[c] Haemagglutinin of A0/NWS; enzyme of A2/Jap. 305.

[d] Haemagglutinin of A2/Jap. 305; enzyme of A0/NWS.

[e] Haemagglutinin of A0/NWS; enzyme of A2/England.

[f] Haemagglutinin of A2/England; enzyme of A0/NWS.

Immunization experiments in mice provided evidence indicating the immunological significance of antigenic differences as well as similarities of the neuraminidase of Hong Kong virus and the enzyme antigens of earlier A2 strains. Mice were immunized subcutaneously with purified neuraminidase derived from a 1957 strain of A2 virus or with a hybrid virus containing the enzyme of the 1957 A2 strain and haemagglutinin derived from A0/NWS virus. As may be seen in Table 2, following challenge with either A2/Japan/305/57 virus or A2/Aichi/68 virus, pulmonary virus titres were lower in the groups of animals immunized with the 1957 A2 enzyme or with a hybrid virus containing that enzyme than in control animals. However, the degree of protection as expressed by the amount of reduction of virus titres was greater when challenge was with the A2/Japan/305/57 virus, which contains an enzyme homotypic to the immunizing neuraminidase antigen. The cross-reactivity of the neuraminidase antigens of Hong Kong virus and 1957 A2 virus demonstrable in enzyme-inhibition and plaque-assay systems was reflected in challenge experiments in immunized mice but the antigenic differences in the enzyme antigens of these 2 viruses were also demonstrated.

TABLE 2

EFFECTS OF IMMUNIZATION WITH A 1957 A2 NEURAMINIDASE ON PULMONARY VIRUS TITRES IN MICE CHALLENGED WITH A2/Japan/305/57 AND A2/Aichi/68 VIRUSES

Immunization [a]	Challenge infection [b]	
	A2/Japan/305/57	A2/Aichi/68
X-7 (A0 E[1957])	5.8	6.1
Purified A2 enzyme (1957)	4.1	5.5
Saline	7.6	6.7

[a] Subcutaneously, 4 weeks before challenge; enzyme, inactivated virus or saline mixed with Freund's adjuvant.

[b] Pulmonary virus titres 48 hours after challenge; EID_{50}, log_{10}.

In addition to its effects on virus replication in mouse lungs, antineuraminidase antibody may influence the capacity of infected mice to transmit infection. Mice were immunized passively by intraperitoneal inoculation of rabbit antiserum prepared against isolated influenza A2 virus enzyme or with antiserum to X-9 virus (A2 e)—a recombinant possessing the haemagglutinin antigen of A2/RI/5/57 virus (Kilbourne et al., 1967), and neuraminidase derived from A0/NWS virus. As may be seen in Table 3, following challenge with influenza A2/Japan/305/57 virus, pulmonary virus titres and lung lesions were reduced in both groups. However, infector mice immunized with antiserum to the haemagglutinin antigen of the challenge virus transmitted infection to uninfected contacts as readily as control animals. In contrast, transmission of infection by animals immunized passively with antineuraminidase antibody was markedly reduced. The decreased transmission of infection by animals with antineuraminidase antibody is not merely a reflection of reduced virus titres. Comparable reductions of virus titres in animals with antihaemagglutinin antibody did not reduce the capacity to transmit infection (Schulman, 1967). Although these observations are preliminary, they suggest that antineuraminidase antibody may influence transmission by a specific inhibitory effect on the shedding of virus from respiratory epithelial cells, reducing the availability of virus for expulsion into the environment. Such an effect is in accord with other biological effects of antineuraminidase antibody in inhibiting the release, but not the attachment of virus to cell surfaces (Brown & Laver, 1968; Kilbourne et al., 1968; Seto & Rott, 1966; Webster & Laver, 1967; Webster, Laver & Kilbourne, 1968).

TABLE 3

EFFECTS OF INTRAPERITONEAL IMMUNIZATION OF MICE WITH RABBIT ANTISERA TO PURIFIED A2 NEURAMINIDASE AND TO A2 HAEMAGGLUTININ ON TRANSMISSION OF INFLUENZA A2/Japan/305/57 VIRUS INFECTION

Immunization [a]	Pulmonary virus titres at 48 hours [b]	Lung lesions at day 7 (%)	Contacts infected (%)
Anti-enzyme serum	5.1	0	10
Anti-X-9 (A2 e) serum	6.1	9	50
Normal rabbit serum	7.2	67	60

[a] Intraperitoneally 24 hours prior to challenge.

[b] EID_{50}, log_{10}.

Conversely, other experiments have demonstrated that mice immunized passively with purified enzyme do not have increased resistance to the initiation of infection transmitted by other mice, but contact animals with antihaemagglutinin antibody are much less susceptible to the initiation of infection (Schulman, 1967, 1970).

Therefore, antibody to either surface antigen of

influenza virus profoundly influences the course of influenza infection in mice, leading to reduced virus titres and less extensive lung lesions. Resistance to the *initiation* of infection is correlated with anti-haemagglutinin antibody, whereas reduced *transmission* of infection by immune animals is more closely related to the presence of antineuraminidase antibody.

These observations strongly suggest, by their possible implications for immunity in man, that the protective effects and duration of antineuraminidase antibody should be investigated systematically in human populations. If similar results are obtained, consideration should be given to the adequacy of current criteria for choosing strains of virus for vaccines and for standardizing the potency of such vaccines. Vaccine-induced immunity might prove to be even more effective if, as is the practice with haemagglutinin, the neuraminidase content of proposed vaccines is optimal with regard to both antigenicity and its antigenic similarity to the enzymes of recent isolates. In instances in which significant antigenic drift of haemagglutinin occurs too late to modify vaccine strains, residual homology of the neuraminidase antigens of vaccine virus and the new antigenic variant might be particularly important in determining vaccine efficacy.

Furthermore, the independent antigenic variation of haemagglutinin and neuraminidase proteins in nature makes it necessary to revaluate the system employed to classify human influenza A viruses according to antigenic subtypes. The present system of designation, although operationally useful, may be misleadingly simplistic, depending as it does largely on antigenic relatedness assessed by cross-reactivity in tests of haemagglutination-inhibition and haemadsorption-inhibition. It is evident that a more comprehensive taxonomy would include antigenic analysis of both surface proteins.

Finally, by way of speculation, the specific effects of antineuraminidase antibody on the capacity to transmit influenza virus infection might provide a useful epidemiological tool. Theoretical advantages might result from an immunization programme in which only selected susceptible members of the population would be inoculated with vaccines containing optimal concentrations of both surface antigens of influenza virus. At the same time, mass immunization with purified neuraminidase might induce herd immunity by reducing the spread of infection by immunized individuals. If this were possible, broad population immunity might be achieved without accelerating the emergence of new viruses with antigenically novel haemagglutinin antigens.

ACKNOWLEDGEMENT

The able technical assistance of F. Kaye Leitzinger and Tatiana Tershakovec is gratefully acknowledged.

REFERENCES

Brown, J. & Laver, W. G. (1968) *J. gen. Virol.*, **2**, 291-295

Coleman, M. T., Dowdle, W. R., Pereira, H. G., Schild, G. L. & Chang, W. K. ((1968) *Lancet*, **2**, 1384-1386

Davenport, F. M., Hennessy, A. V., Drescher, J. & Webster, R. G. (1964) *Analytical serological and clinical experiences with the hemagglutinin subunits of influenza A virus.* In: Wolstenholme, G. E. W. & Knight, J., ed., *Cellular biology of myxovirus infections*, Boston, Little Brown, pp. 272-287

Fedson, D. S. (1969) *Ann. intern. Med.* **71**, 386-389

Hennessy, A. V. & Davenport, F. M. (1966) *J. Immunol.*, **97**, 235-238

Jahiel, R. I. & Kilbourne, E. D. (1966) *J. Bact.*, **92**, 1521-1534

Kilbourne, E. D., Christenson, W. N. & Sande, M. (1968) *J. Virol.*, **2**, 761-762

Kilbourne, E. D., Laver, W. G., Schulman, J. L. & Webster, R. G. (1968) *J. Virol.*, **2**, 281-288

Kilbourne, E. D., Lief, F. S., Schulman, J. L., Jahiel, R. I. & Laver, W. G. (1967) *Perspect. Virol.*, **5**, 87-106

Paniker, C. K. J. (1968) *J. gen. Virol.*, **2**, 385-394

Schulman, J. L. (1967) *J. exp. Med.*, **125**, 467-478

Schulman, J. L. (1970) *Progr. med. Virol.* (in press)

Schulman, J. L., Khakpour, M. & Kilbourne, E. D. (1968) *J. Virol.*, **2**, 778-786

Schulman, J. L. & Kilbourne, E. D. (1969) *Proc. nat. Acad. Sci. (Wash.)*, **63**, 326-333

Seto, J. T. & Rott, R. (1966) *Virology*, **30**, 731-737

Webster, R. G. & Laver, W. G. (1967) *J. Immunol.*, **99**, 49-55

Webster, R. G., Laver, W. G. & Kilbourne, E. D. (1968) *J. gen. Virol*, **3**, 315-326