JOURNAL OF VIROLOGY, July 1968, p. 761-762

Vol. 2, No. 7
Printed in U.S.A.

Antibody Response in Man to Influenza Virus Neuraminidase Following Influenza

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Received for publication 25 April 1968

Genetic recombination of influenza viruses of different subtype may result in segregation of hemagglutinin and neuraminidase in antigenically hybrid viruses [E. D. Kilbourne et al., *in* M. Pollard (ed.), *Perspectives in Virology*, vol. 5, p. 87, Academic Press, Inc., N.Y., 1967; and W. G. Laver and E. D. Kilbourne, Virology **30**:493, 1966]. The selective use of such antigenic hybrids in serological reactions permits the independent measurement of antibodies to the envelope proteins of the virion, i.e., hemagglutinin, and neuraminidase (E. D. Kilbourne et al., J. Virol. **2**:281, 1968). In conventional hemagglutination-inhibition (HI) reactions, antibody to the hemagglutinin is titrated and is considered a measure of response to immunization or infection. Recently, by the use of recombinant hybrids and isolated viral neuraminidase, it has been shown that experimental infection of mice produces large amounts of antibody to the neuraminidase of the infecting influenza virus (J. L. Schulman et al., J. Virol., *in press*). In the present paper, we present evidence that similar increase in antineuraminidase occurs in man during and after natural infection.

Serum was obtained from six adult patients during the acute phase of illness and 14 to 23 days later. In the fatal case 1012, the bleeding interval was only 3 days. HI tests were conducted as described previously after serum inhibitors were inactivated by heat and periodate (S. C. Wong and E. D. Kilbourne, J. Exptl. Med. **113**: 95, 1961). Neuraminidase (enzyme)-inhibition tests were conducted as described previously (E. D. Kilbourne et al., J. Virol. **2**:281, 1968), employing a virus [X-7(F1)] with A_0 hemagglutinin and A_2 (E) enzyme. Six viruses were used in HI tests: A_2/Cornell/1001/67, A_2/RI/5/57 ("+" and "−" variants, P. W. Choppin and I. Tamm, J. Exptl. Med. **112**:895, 1960), A/equine/1/56, and two recombinants, X-9 and X-15 (see footnote to Table 1). Recombinant X-15 is uniquely susceptible to in vitro inhibition with antibody to A_2 neuraminidase (anti-E), so that its inhibition in HI tests is a specific measure of A_2 neuraminidase antibody (E. D. Kilbourne, Science **160**:74, 1968). Because the recombinants used to measure neuraminidase antibody [X-15 and X-7(F1)] had derived their enzyme component from A_2/RI/

TABLE 1. *Titration of influenza A_2 hemagglutination-inhibiting and neuraminidase-inhibiting antibodies in acute and convalescent phase sera of patients with influenza*[a]

Case	Antihemagglutinin (A_2) titers[b]				Antienzyme (E) titers	
	$\frac{A_2^-E^-}{A_2/\text{Cornell}/67}$	$\frac{A_2^-E^+}{A_2/RI/5^-/57}$	$\frac{A_2^-E^-}{A_2/RI/5^-/57}$	$\frac{A_2^-e}{X-9}$	$\frac{EqE^+}{X-15}$	Enzyme inhibition
998	8[c]	16	8	32	32	16[d]
	64	64	64	64	32	24
1000	16	64	8	16	16	64
	256	256	128	256	256	>256
1008	<8	<8	8	<4	16	<4
	256	64	128	128	128	16
1009	32	256	<8	4	<4	<4
	256	256	64	64	64	16
1012	64	1,024	32	16	64	64
	2,048	8,192	1,024	512	1,024	>256
1015	32	256	128	32	64	64
	128	512	256	128	64	64

[a] $A_2^+E^+$: inhibitor sensitive, antibody-avid "+" or I virus (A_2/Cornell/67 is homologous to the epidemic). $A_2^-E^-$: inhibitor insensitive, antibody-nonavid "−" or i variant of A_2/RI/5/57. A_2^-e: recombinant containing A_2^- hemagglutinin and neuraminidase heterologous to E^+ and E^-. EqE^+: recombinant containing A/equine/1 hemagglutinin and neuraminidase from A_2/RI/5^+/57 (E^+) virus. A_2, Eq, and A_0 represent hemagglutinin (major) antigen. E, eq, and e represent neuraminidase (minor) antigen. Note: reading vertically, the first figure represents the acute-phase and the second the convalescent-phase serum titer.

[b] Hemagglutination-inhibition titers.

[c] Reciprocal of serum dilution at titration end point.

[d] Reciprocal of serum dilution causing 50% inhibition of enzyme activity of E-containing recombinant X-7(F1) (A_0E).

5+/57, HI antibody was titrated with this virus as well as with A_2/Cornell/1001/67—the epidemic virus and similarly "+" or antibody avid. Recombinant X-9, which contains the hemagglutinin but not the neuraminidase of the 1957 virus, was used in comparison with A_2/RI/5−/57—similarly antibody nonavid.

The results (Table 1) demonstrate, first, that the 1957 strains and their derivative, X-9, were almost equal to the epidemic strain in detecting antibody increase in HI tests. Secondly, fourfold or greater increases in antibody to A_2 neuraminidase were shown in four out of six cases, measured either by direct enzyme inhibition or by HI titration with X-15. (Antibody to A/equine/1/56 was not detected in HI tests in any serum.)

Although humoral antibody to influenza virus hemagglutinin has been correlated with infection with and resistance to influenza virus, the role of antibody to viral neuraminidase in recovery from and resistance to influenza in man is unknown. Our studies demonstrate that natural infection stimulates production of antineuraminidase. It will be important to establish whether or not immunization with standard inactivated influenza virus vaccines can also do so, particularly because antineuraminidase contributes substantially to immunity of mice to challenge with influenza viruses (J. L. Schulman et al., *in preparation*).

This investigation was conducted under the auspices of the Commission on Influenza, Armed Forces Epidemiological Board, and was supported by the U.S. Army Medical Research and Development Command, Department of the Army, under research contract no. DA-49-193-MD-2795; by Public Health Service research grant AI-01595 from the National Institute of Allergy and Infectious Diseases; and by the Health Research Council of the City of New York under contract no. U-1023.