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Effect of Influenza Anti-Neuraminidase Antibody on Virus Neutralization

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Antibody to influenza virus neuraminidase participates in tissue culture neutralization tests in which inhibition of hemadsorption is the end point. Presumably, by inhibiting virus release it functions additively with anti-hemagglutinin in reducing total virus replication.

Neutralization (in a conventional sense) of influenza virus is a function of antibody to the hemagglutinin antigen. It has been suggested that anti-neuraminidase antibody can produce apparent neutralization by interfering with viral release and by steric inhibition of virus adsorption (5, 6). Consequently, the use of conventional tissue culture neutralization tests for determinations of hemagglutinin antibody must take into account the presence of antibody to the enzyme antigen in a test specimen. The following study assesses the effect of anti-neuraminidase antibody in serum neutralization tests using wild and recombinant (hemagglutinin-specific) virus.

Serum specimens from adult male volunteers who participated in evaluation of an influenza virus vaccine monospecific for neuraminidase were used in this study (Couch et al., unpublished data). The experimental vaccine employed, X-32 (4), consisted of an inactivated, antigenically hybrid virus possessing the hemagglutinin of A/equine/Prague/1/56 virus and the neuraminidase of an H3N2 recombinant, X-31 (2). Thus, X-32 is Heq1N2. A type B vaccine containing B/Mass/66 virus was used as control. Sera were collected at the time of vaccination, 30 days after vaccination, and 28 days after virus challenge. The challenge inoculum used was prepared from a naturally occurring H3N2 virus.

Sera were assayed for antibody activity by the serum neutralization test against 32 50% tissue culture infectious doses ($TCID_{50}$) of H3N2 (Hong Kong) virus or the antigenic hybrid $H3N1_{(PR8)}$ and by the neuraminidase inhibition test using approximately three enzyme units of $HON2_{(HK)}$, as previously described (1, 6). In the neutralization procedure, virus-serum mixtures remained in the tissue culture system during the test period.

The data in Table 1 show that determinations for hemagglutinin antibody by neutralization tests may be influenced by the presence in the assay system of antibody to the neuraminidase antigen. This is clearly shown by postvaccination and post-virus-challenge serological results among individuals in the X-32 vaccine group. The relative frequencies and levels of antibody response were significantly greater in tests using H3N2 than in those employing H3N1, the antigenic hybrid containing an antigenically unrelated neuraminidase (in each case, $P < 0.005$). In addition, a similar contribution of anti-neuraminidase antibody to the level of response is suggested by the higher mean titer in sera after virus challenge in the type B vaccine group. Although not shown, postvaccination and post-virus-challenge serum specimens from three volunteers in the X-32 group were tested against H3N2 virus by neutralization tests in which serum-virus mixtures were removed from cell cultures after a 60-min incubation period. The results were similar to those seen in tests using H3N1 virus. None of the individuals exhibited antibody activity ($<1:2$) in their postvaccination serum specimens, and the mean antibody titer was twofold less after virus challenge. By contrast, in tests where H3N2 virus remained in the cell culture system during the test period, the mean postvaccination and post-virus-challenge antibody titers

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TABLE 1. *Apparent serum-neutralizing antibody responses after administration of neuraminidase (N2)-specific influenza A or control (influenza B virus) vaccines and challenge with H3N2 influenza virus*

Vaccine group[a]		Time period of serum collection	Geometric mean serum anti-neuraminidase antibody titer[b]	Serum-neutralizing antibody responses			
				Test virus	Incidence of significant rise		Geometric mean titer
Type	No. of men				No.	%	
A (X-32)	16	Postvaccination	83	H3N2	12	75	81
				H3N1	0	0	<4
		Post-virus-challenge	120	H3N2	16	100	89
				H3N1	7	44	10
B	10	Postvaccination	<4	H3N2	0	0	<4
				H3N1	0	0	<4
		Post-virus-challenge	24	H3N2	8	80	48
				H3N1	8	80	14

[a] Prevaccination serum of all participants lacked antibody (<1:4) to the hemagglutinin and neuraminidase of H3N2 virus.

[b] Titer expressed as the reciprocal of the serum dilutions and for geometric mean calculation a titer of less than 1:4 was classified as 1:2.

were 1:13 and 1:77, respectively. The results of neutralization tests were not attributable to any differences in susceptibility of the H3N2 and H3N1 viruses to H3 antibody. Reactivity of 6 $TCID_{50}$ of H3N1 and 16 $TCID_{50}$ of H3N2 virus to neutralization was measured with a monospecific anti-H3 goat antiserum. The reagent was provided by the Research Resources Branch, National Institute of Allergy and Infectious Diseases. In both cases, viral infectivity was inhibited by 1:12, 800 dilution of antiserum.

The findings presented show that antibody to the neuraminidase antigen is capable of contributing to apparent viral hemadsorption-inhibition neutralization in tests in which virus-serum mixtures are not removed from the assay system after an appropriate period for viral absorption. Presumably, by inhibiting virus release it functions additively with anti-hemagglutinin in reducing total virus replication. Thus, if such a method is used, it is essential to employ a test virus containing an antigenically unrelated neuraminidase, if true neutralization (mediated by anti-hemagglutinin) is to be measured.

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