Antibody Response in Humans to Neuraminidase of A2/Aichi/68 and B/Mass/66 Influenza Viruses (35829)

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(Introduced by J. L. Melnick)

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Neuraminidase antibodies (1–6) may play an important part in immunity to influenza viruses in humans and in the epidemiology of influenza in general (7). Such antibodies have been detected in the sera of persons exposed to influenza infections or to vaccination by attenuated live and inactivated vaccines (8–11). The present study, concerned with the levels of hemagglutination-inhibiting (HI) and neuraminidase-inhibiting (NI) antibodies in sera of humans before and after vaccination, indicates that the strain-specific immunologic responses to hemagglutinin and neuraminidase are correlated, although these two envelope components of influenza viruses represent two distinct immunologic entities (12).

Materials and Methods. Sera. Serum specimens were obtained from persons before and 24 to 28 days after immunization with a single dose of different influenza virus vaccines. The preparations compared consisted of 400 chicken cell agglutinating (CCA) units (16) of A2/Aichi/68 and 300 CCA units of B/Mass/66 strains of influenza viruses. These were present either as inactivated intact virus or as subunits of virus disrupted by diethyl ether or tri-*n*-butyl phosphate (13).

Preparation of neuraminidase. The A2/Aichi/68 and B/Mass/66 influenza viruses were disrupted by tri-*n*-butyl phosphate (13) and subsequently incubated for 1 hr at 36° with trypsin (1 mg/ml) (14). Soybean trypsin inhibitor (1 mg/ml) was added and the preparations (1 ml) were centrifuged for 90 min in the Spinco rotor SW65 at 28,000 rpm. Supernatant solutions which contained less than 1% of hemagglutinin present before centrifugation, were used in the NI tests.

Neuraminidase inhibition (NI) tests. All sera were heated at 56° for 30 min. Serial twofold dilutions (0.2 ml) of the sera in 0.14 *M* NaCl, 0.01 *M* Tris(hydroxymethyl)aminomethane (TB) (pH 7.0) containing 0.001 *M* $CaCl_2$ (TBCa) were mixed with an equal volume of the neuraminidase solution (1 to 2.5 units/ml) (3). The mixtures were incubated for 30 min at 36° and at 4° overnight. A 0.1-ml portion of substrate solution (100 mg/ml of ovomucoid in TBCa) was added. The amount of neuraminic acid released after 35-min incubation at 36° was determined by the method of Aminoff (15), using thiobarbituric acid as the color reagent. The optical densities were read against a blank containing only the substrate. The amount of neuraminic acid present in the sera, or released during their incubation with neuraminidase in the absence of added substrate was negligible.

Hemagglutination inhibition (HI) *tests.* Assays for hemagglutinin and inhibition of hemagglutination by serum were performed as described elsewhere (16).

Separation of 7S and 19S antibodies. Aliquots (0.3 ml) of serum were submitted to rate zonal centrifugation at 65,000 rpm for 100 min in the Spinco rotor SW65. Four-milliliter gradients of 10 to 25% sucrose in TB were used. Fractions collected from the bottom of the centrifuge tubes were dialyzed

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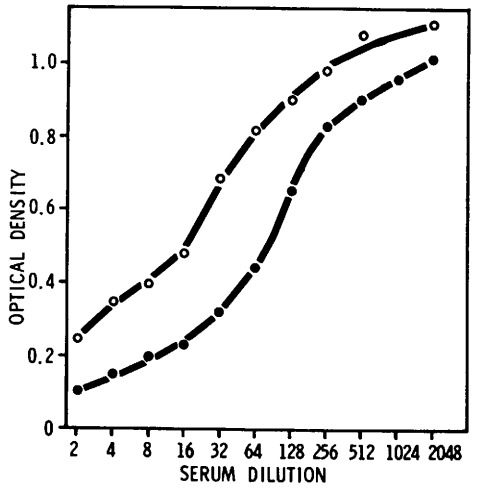

FIG. 1. Inhibition of A2/Aichi/68 influenza virus neuraminidase by antibodies from a pre- (○); and a postvaccination (●) serum.

against TB and assayed by the NI test.

Results. The inhibition of neuraminidase by pre- and postvaccination sera is illustrated in Fig. 1. Sigmoid curves were obtained when the amount of enzymatically released neuraminic acid was plotted against the serum dilution. The NI titer of the serum was expressed as the dilution at which a 50% inhibition of enzymatic activity occurred.

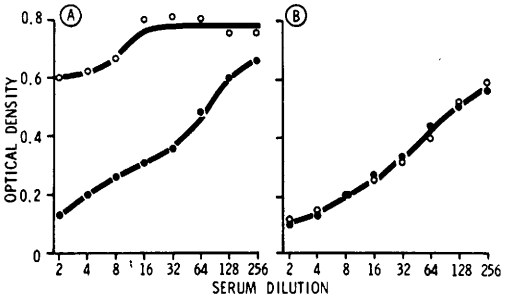

FIG. 2. (a) Evidence for the formation of NI antibodies against A2/Aichi/68 neuraminidase in serum of an individual with no detectable NI antibodies before vaccination. (b) Evidence for the unchanged NI-titers in the serum of a person given placebo instead of vaccine (see Fig. 1 for explanation of symbols).

This value was found on the abscissa on which the serum dilutions were plotted in a logarithmic scale. Figures 2a and b show NI curves for pre- and postvaccination sera from one person without initial measurable NI antibodies and from another given placebo instead of vaccine. They demonstrate the specificity of the reaction in response to vaccine and the minimal effect of serum without NI antibody. The NI titers against B/Mass/66 neuraminidase did not increase

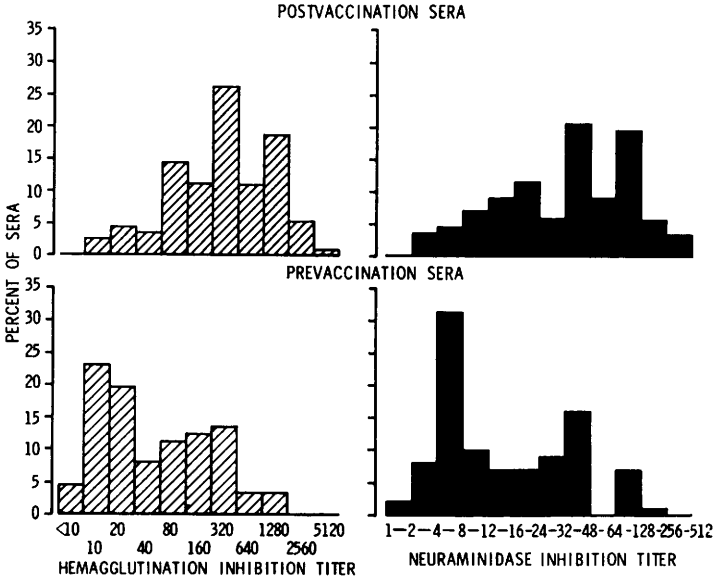

FIG. 3. HI (lined columns) and NI (black columns) antibody titers in sera of persons before and after immunization with A2/Aichi/68 influenza virus.

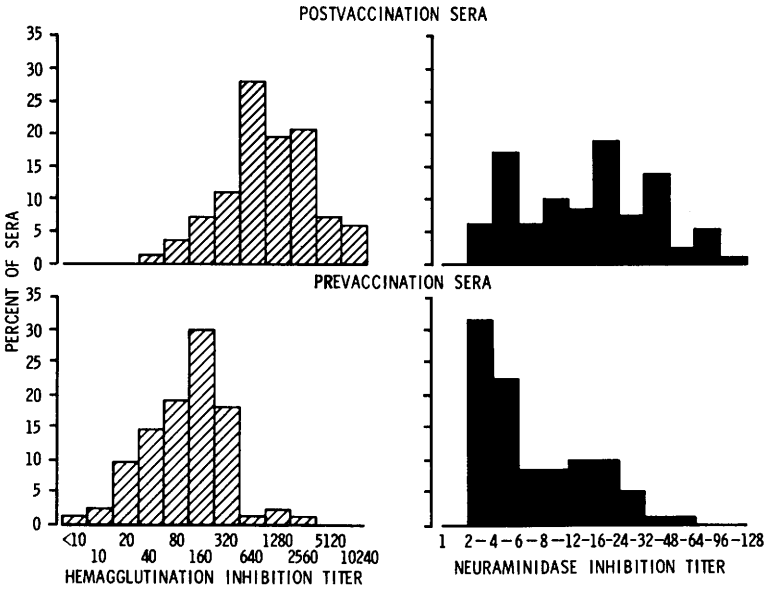

FIG. 4. HI (lined columns) and NI (black columns) antibody titers of sera of persons before and after immunization with B/Mass/66 influenza virus.

in sera of persons given a monovalent vaccine containing the A2/Aichi/68 virus. A difference of about 20% in NI titers could easily be detected; *i.e.*, a 1.2-fold rise in NI titer resulting from immunization could be considered as significant.

The vaccines did not differ significantly in their ability to induce rises of serum NI antibodies, although individual variations did occur. In the evaluations, all vaccine groups are considered together. To facilitate the comparison of HI and NI titers, the sera were grouped according to the range of the NI titers, as shown in the right part of Figs. 3 and 4. Figures 3 and 4 summarize the results obtained with 174 and 158 sera tested for HI and NI antibodies to A2/Aichi/68 and B/Mass/66 influenza viruses, respectively. It is evident that the percentage of sera with higher HI and NI antibody titers increased as the result of vaccination. However, the magnitude of the increases was greater for HI than for NI antibodies (Figs. 5 and 6). In some instances the NI titers did not increase, as was also the case for HI titers. The NI titers did not change in sera of persons given a placebo instead of vaccine.

To determine the physicochemical nature of NI antibodies formed as the result of vaccination, the postimmunization sera from three persons having no detectable NI antibodies before immunization were fractionated by rate zonal centrifugation. NI antibodies were detected in both the 7S and 19S fractions.

The results were examined to establish whether or not there was a correlation be-

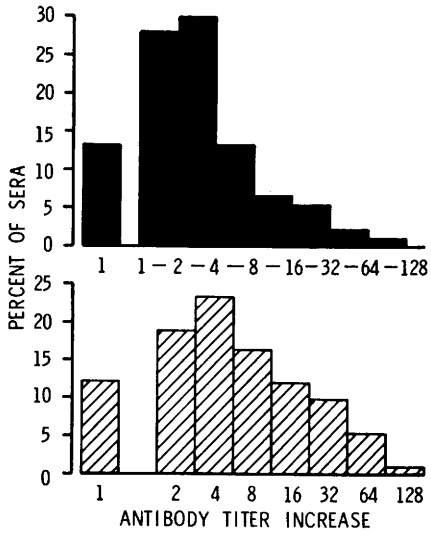

FIG. 5. Frequency of HI (lined columns) and NI (black columns) titer increases in sera of persons immunized with A2/Aichi/68 influenza virus.

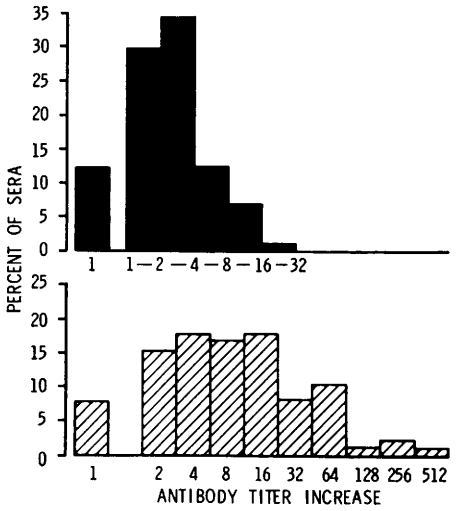

FIG. 6. Frequency of HI (lined columns) and NI (black columns) titer increases in sera of persons immunized with B/Mass/66 influenza virus.

tween the level of serum NI and HI antibodies. The sera were divided into groups with equal HI titers. The mean NI titers and the standard deviations of the mean were calculated for each group. Since no significant differences in NI titer between pre- and postvaccination serum specimens with equal HI titers were found, the results with all sera were combined and plotted in Figs. 7 and 8. Evidently, the antibody responses to hemagglutinin and neuraminidase of either of the two viruses seem to be correlated.

Discussion. The number of sera investigated exceeded by several times the total number of sera tested to date for NI antibodies according to published reports (8–11). The results show that upon testing pre- and postvaccination sera for antineuraminidase activity, using partly purified homologous neuraminidase, there is an antineuraminidase antibody response to vaccination. Control persons not given influenza vaccine did not show rises of NI antibody. The extent of the antibody response to the two distinct envelope antigens of influenza virus—neuraminidase and hemagglutinin—was correlated. This was true for both natural infections, as determined by examination of preimmunization sera, or following vaccination. Either inactivated whole virus or subunit vaccines prepared by two different procedures elicited both types of antibody. Because of the correlation between NI and HI antibody titers, the time-consuming determination of NI antibodies seems unnecessary. This finding may be gratifying to those accustomed to consider HI titers as the measure of antibody responses to influenza viruses.

A few vaccinees had no detectable prevaccination NI antibodies. Most of them, however, had already had previous experience with the A2/Aichi/68 and the B/Mass/66 viruses as indicated by the initial HI and NI serum antibodies. This previous experience could have contributed to the observed correlation between NI and HI titers. If antibody responses had been measured against a newly emerging influenza virus strain, having one but not the other envelope antigen immunologically related to older strains, it seems likely that the correlation between the HI and NI antibodies would not have been found, at least with the prevaccination sera.

Summary. Results of determinations of NI and HI antibodies in sera of persons before and after immunization with A2/Aichi/68 and B/Mass/66 influenza viruses are described. A correlation between the magnitude of HI and NI titers was established. Similar results were obtained with inactivated whole virus and virus subunit vaccines. The titer increases resulting from vaccination were, in

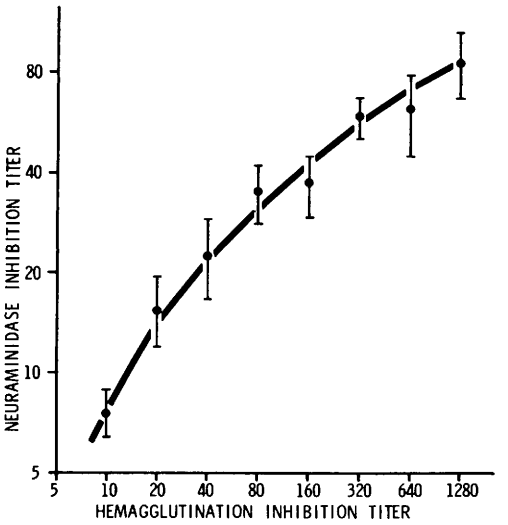

FIG. 7. Correlation between titers of NI and HI antibodies to A2/Aichi/68 influenza virus antigens.

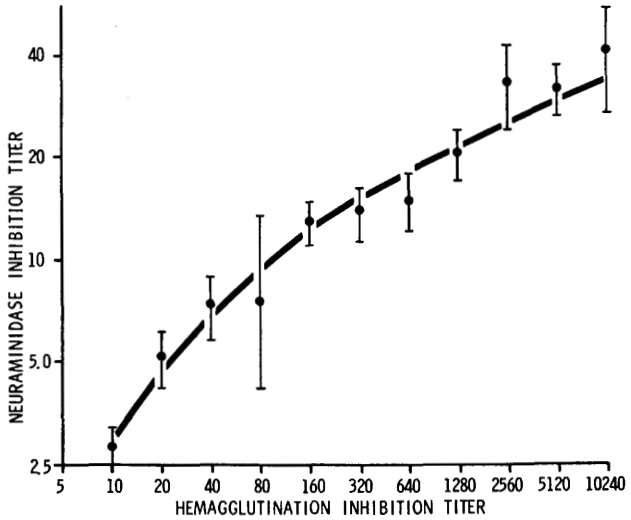

FIG. 8. Correlation between titers of NI and HI antibodies to B/Mass/66 influenza virus antigens.

general, higher for HI than for NI antibodies in accordance with the nonlinear correlation between the two titers.

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