PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 161, 519-521 (1979)

Variant-Specific Antihemagglutinin Serum Response to Type A Influenza Natural Infection and Inactivated Vaccines in Adults (40587)[1]

J. A. KASEL, H. R. SIX, R. B. COUCH, S. B. GREENBERG, AND T. R. CATE

Influenza Research Center, Department of Microbiology and Immunology, Baylor College of Medicine, Houston, Texas 77030

The hemagglutinin (HA) subunit of type A influenza viruses possesses cross-reactive and strain-specific determinants that give rise to antibody populations having the capacity to neutralize infectious virus under *in vitro* conditions (1-5). In a murine model, antibodies to variant-specific determinants afforded a higher level of resistance to disease than comparable concentrations of antibody to cross-reactive antigens (6).

In previous reports, we have described the serologic responsiveness of individuals to inactivated vaccines ((7, 8), Couch, unpublished data). Immunization with type A H3N2 vaccines resulted in the formation of antibody directed toward the cross-reactive determinant(s); less than 50% of persons developed a response to the specific antigenic site on the vaccine virus. In view of the lack of similar data evaluating the antibody response to natural infection, we undertook to determine whether an A/England/72 (A/Eng/72) (H3N2) infection was more effective than immunization with inactivated vaccines in evoking a variant-specific in addition to a cross-reactive antibody response.

Materials and methods. Specimens from the serum collection of the Influenza Research Center were used in this study. These were obtained from normal healthy individuals between the ages of 18 and 40 years who participated in an evaluation of commercially available inactivated vaccines with an A/Eng/72 component and persons who experienced a virologically documented natural infection with the same variant. In the vaccine trial, a single 0.5-ml dose of either whole virus (Eli Lilly and Co.) or subvirion vaccine (Wyeth Laboratories, Inc.) that contained 700 and 300 chicken cell agglutination units of strains antigenically representative of A/Eng/42/72 and B/Hong Kong/5/72, respectively, was administered by the intramuscular route. Serum specimens were collected prior to and 30 to 40 days after infection and immunization. In order to be able to detect variant-specific antibody responses after adsorption, only postexposure sera having a neutralizing antibody titer of 32 or greater to A/Eng/72 virus were evaluated.

Sera were adsorbed with a formalin-inactivated zonal centrifuge purified A/Hong Kong/68 (A/HK/68) (H3N2) whole virus preparation by a previously described method (5). In brief, 1 vol of adsorbing virus (100,000 hemagglutination units) was added to 1 vol of undiluted serum and incubated for 60 min at 25°C. Following incubation, the virus-serum mixture was centrifuged for 90 min at 68,000*g* in the cold. Any excess virus in the supernatant portion was removed by the addition of 0.5 vol of a 50 chick red blood cell suspension. The latter mixture was held at 4° C for 90 min with agitation every 15 min. After removal of erythrocytes by low-speed centrifugation, the fluid phase was assayed for residual hemagglutination activity. Occasional sera needed additional adsorption with A/HK/68 to complete the removal of antibody to this virus or with red blood cells to remove residual A/HK/68 virus. The absence of neutralizing activity of adsorbed sera to A/HK/68 and A/Eng/72 viruses was considered to be an antigenic response to the shared determinant(s) only. Antibody that remained after adsorption with A/HK/68 virus was interpreted as being a response to the variant-specific antigen(s).

Serum antihemagglutinin antibody titers (expressed as the reciprocal of serum dilutions) were determined by the hemadsorption-inhibition neutralization test (9). Anti-

[1] This study was supported by Contract No 1 A1 42528 from the National Institute of Allergy and Infectious Diseases.

0037-9727/79/090519-03$01.00/0

genic hybrids possessing the hemagglutinin of A/Eng/72 or A/HK/68 and the neuraminidase of A/Equine 1/Prague/56 (Heq1Neq1) were used as test viruses.

Results. Prior to infection and vaccination, serum neutralizing antibody to A/HK/68 and A/Eng/72 was demonstrable in approximately one-half to one-third of the study population, respectively. The geometric mean antibody titer for A/HK/68 was 11 and that for A/Eng/72 was 6.3. The distribution of preexisting antibody levels to both variants was essentially the same for the vaccine and the infection groups.

The analysis of sera after the antigenic stimulus with A/Eng/72 is presented in Table I. Antibody titers were determined before and after adsorption with A/HK/68 whole virus using antigenic hybrid viruses to detect responses only to the desired HA. The assessment of unadsorbed sera showed that all individuals had developed at least a fourfold or greater rise in neutralizing antibody to both homologous and heterologous viruses. Response to the A/HK/68 were highest in the vaccine groups, but the mean titers to the A/Eng/72 virus were not different for any of the groups.

For each group, adsorption of sera to remove all measurable antibody to A/HK/68 reduced the mean titer to A/Eng/72 virus. However, among 36 individuals who experienced infection, 26 (72%) had residual A/Eng/72 specific antibody in their adsorbed postexposure sera. For the 10 others, adsorption of sera with A/HK/68 virus removed antibody activity to both test viruses. Administration of whole virion and subvirion vaccines resulted in the occurrence of variant-specific in addition to cross-reactive antibodies in 8 (40%) and 11 (42%) of individuals, respectively. While these frequencies of response were similar, they were significantly less than that observed after natural infection (in each case, $P < 0.05$). The frequency of detection of A/Eng/72 specific antibody in the postexposure sera did not vary according to the height of the antibody titer to either test virus before adsorption (in each case, $P > 0.10$, Wilcoxon).

To evaluate the data further, we examined the frequency of variant-specific responses according to the absence and/or presence of antibody to the A/HK/68 and A/Eng/72 variants before exposure to A/Eng/72 (Table II). Results of the two vaccine groups were combined since the patterns of response were quite similar. After infection, the distribution of sera exhibiting variant-specific antibody was similar in each of the preexposure serologic classifications. The same pattern of distribution was observed among vaccinated persons.

Discussion. In the present investigation, adsorption studies revealed that serum neutralizing antibody induced by infection or vaccination was mainly directed toward determinants shared by A/Eng/72 and A/HK/68 hemagglutinin subunits. Perhaps this should be expected, since the molecule carries multiple altered and identical cross-reactive determinants, and the total response would reflect the combined antibody specificites ((10), Six and Kasel, unpublished data).

The finding that antibody specific to the A/Eng/72 HA was more frequently demonstrable after infection indicates that this mode of antigenic stimulation provided the broadest antibody response. While the reason(s) for this were not identified in this study, it is possible that infection resulted in a relatively larger antigenic mass than that contained in the vaccine. Alternatively, the manner of presentation of antigen may have been the determining factor.

The relative contribution of cross-reactive

TABLE I. INFLUENZA VIRUS ANTIBODY TITERS IN POSTEXPOSURE SERA BEFORE AND AFTER ADSORPTION WITH A/HONG KONG/68 (H3N2) VIRUS

	Geometric mean serum neutralizing antibody titers					
		Before adsorption			After adsorption	
Type of exposure	Number of persons	A/Eng/72	A/HK/68	Number of persons	A/Eng/72	A/HK/68
Natural infection	36	284	207	26	14	<8
Whole virion vaccine	20	256	609	8	19	<8
Subvirion vaccine	26	285	392	11	16	<8

TABLE II. OCCURRENCE OF A/ENGLAND/72 (H3N2) VARIANT SPECIFIC RESPONSES ACCORDING TO LEVEL OF PREEXISTING ANTIBODY

Preexisting antibody titer to the indicated virus		Incidence of adsorbed sera that exhibited neutralizing activity to A/Eng/72			
		Natural infection		Vaccination	
A/Eng/72	A/HK/68	Number	Percentage	Number	Percentage
<8	<8	12/17[a]	71	7/19	37
<8	≥8	5/6	83	6/15	40
≥8	≥8	9/13	69	6/12	50

[a] Number of variant-specific responses/number in antibody category.

and variant-specific antibody specificities in host resistance against disease has not been fully defined. Present information from human studies indicates that a neutralizing antibody titer of 32 to cross-reactive determinants correlates with a reduction in infection and illness (Couch, unpublished data). Similar data for comparable or lower levels of variant-specific antibody are not available.

Summary. Antigenic stimulation by infection was more effective than immunization with inactivated vaccine in evoking a variant-specific serum antibody response. In both groups, occurrences of such a response could not be associated with the height of postexposure antibody titer or absence and/or presence of preexisting antibody to homologous and heterologous viruses.

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Received February 22, 1979. P.S.E.B.M. 1979, Vol. 161.