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ANTIBODY RESPONSE TO INFLUENZA VACCINES CONTAINING THE ASIAN STRAIN[1]

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The appearance of a new subgroup of type A influenza viruses has given further impetus to the study of protection by influenza vaccines. Accumulated experience since the wide scale outbreaks of Asian (A2) influenza, beginning in 1957, has shown that widespread morbidity (1) may occur and that under some circumstances mortality is not inappreciable (2, 3).

At any given time and location, however, outbreaks of respiratory disease are often due to more than one agent, and estimates of the protective capacity of a vaccine may be minimized by the concurrent presence of diseases of comparable symptomatology but different etiology. Specific etiologic diagnosis thus becomes of importance, and when serodiagnostic methods are employed, the antibody response engendered by influenzal vaccines becomes an important consideration. Complement fixation tests utilizing "soluble" antigen afford a promising tool in the serologic assessment of influenzal disease (4), although difficulties are not completely avoided (5).

Hemagglutination-inhibiting antibody response is usually considered to reflect a more strain-specific reactivity and may also afford a useful index of prior experience with influenza viruses of similar antigenic composition (6, 7). During early experience with Asian influenza infections, as well as with the response to vaccines, variable, often minimal, titer responses were encountered (8, 9).

MATERIALS AND METHODS

The study vaccines. Two aqueous influenza vaccines, an adjuvant preparation and a placebo, were studied in terms of antibody response. All of the vaccines were commercially prepared and were intended for evaluation of protective effects. The first vaccine (aqueous vaccine I) was a monotypic aqueous preparation containing only the A2/Japan/305/57 strain. Ostensibly, the concentration was 400 CCA units/1-cc dose (given subcutaneously) although retesting disclosed that the concentration may have been as little as 250 CCA units/cc (8). The second preparation was also an aqueous vaccine, containing 200 CCA units/cc, and was given in a 2-cc dose, subcutaneously.

The third vaccine was of the adjuvant type. It contained 150 CCA units of the A2/Japan/305/57 strain and 50 CCA units each of the Swine/31, A/PR/8/34, and the A1/AA/1/56 strains as the type A components, together with 50 CCA units of the B/GL/1/54 strain as the B component. These amounts of influenza virus were contained in the 0.25-cc dose, given intramuscularly. The placebo, consisting of formalinized saline, was given in a 1-cc dose, subcutaneously.

The study group. The three active preparations and the placebo were given to a company of military trainees (young adult males) on April 22, 1958. Just prior to administration of the preparations a prevaccination blood sample was obtained from each man. Postvaccination blood samples were obtained at 7, 10, 14, 21, 35 and 56 days. The period of assessment was chosen to minimize the effects of naturally occurring influenza due to Asian viruses. A major outbreak had occurred at this installation during the summer and early fall of 1957 (8), and another sharp outbreak had occurred during the middle of the spring of 1958. In contrast, influenzal disease was at a low ebb during the period under consideration, although the disease could not be assumed to be completely absent. Men entering the study were at the beginning of their period of

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basic training, newly entering both the army and this installation, but could well have had prior experience with Asian influenza as civilians.

Two hundred and nineteen men entered the study. They were randomly divided into four approximately equal groups. A few of the men in each group were lost to the study or their serum specimens were unsatisfactory. Such cases are excluded from the tabulations presented in this report.

Serologic tests. Determinations of the complement-fixing antibody response to a soluble antigen were made by methods previously described (10). The A1/Berkeley/5/48 strain was used to make the antigen. Hemagglutination-inhibition tests were carried out by a generally accepted method (10). The A1/Albany/1/55 virus was used to make a representative A1 (A-prime) hemagglutinating antigen and the A2/Japan/305/57 strain was chosen as the A2 (Asian) strain. Potassium periodate was used to remove nonspecific inhibitors.

Presentation of data. In this report antibody titers are presented as the reciprocals of the initial serum dilution. Geometric means were calculated for the titer distributions and where the term "mean" is used or implied, the geometric mean is to be understood. Since the initial serum dilution employed was 8, an assumption is required where titers were <8; *i.e.*, for the purpose of calculating geometric means, titers of <8 were considered to have a value of 4. Geometric mean titers are presented in terms of two significant digits.

RESULTS

The "S" antibody response to the vaccines. Figure 1 presents the antibody response to aqueous vaccine I, aqueous vaccine II, the adjuvant vaccine and the placebo in terms of the geometric mean. Before vaccination the mean titer was 21 in the 49 men who received vaccine I. Seven days after vaccination the mean titer had more than doubled to 44, and at 10 days a mean titer of 58 was attained. At 14, 21 and 35 days after vaccination mean titers were 55, 59 and 50 respectively. At 56 days the mean titer was 40.

The mean titer before vaccination in the 55 men who received vaccine II was also 21, and at 7 days there was an increase to 31. Ten days after vaccination the maximal mean titer of 35 was attained. This same level was also found at 14

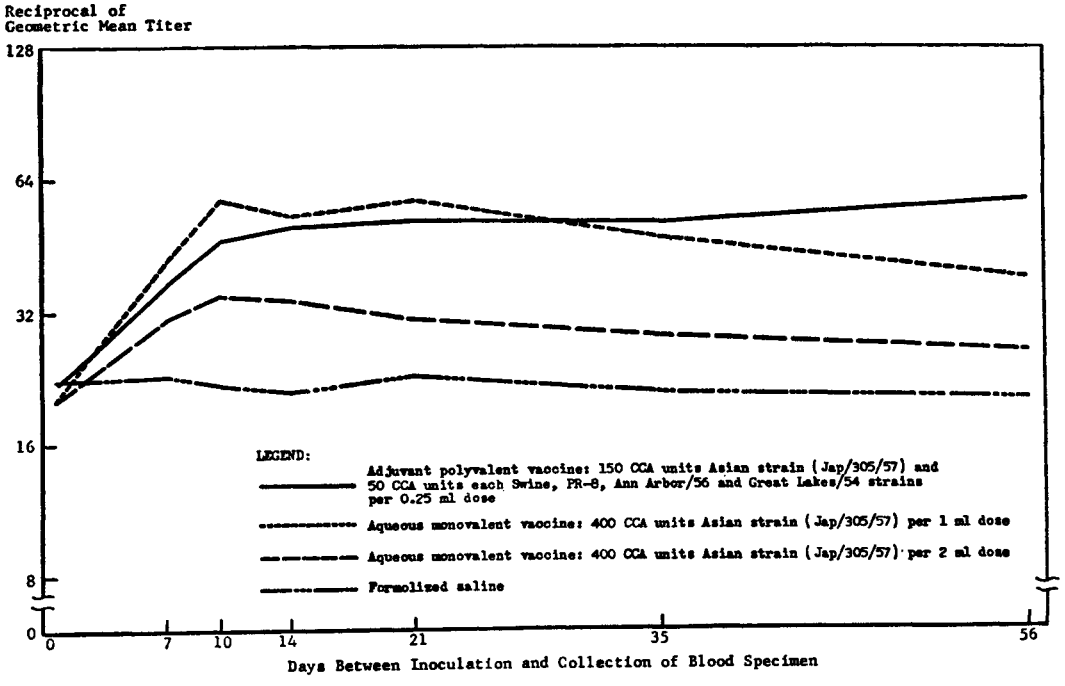

Figure 1. Complement-fixing antibody response to soluble antigen of influenza A virus (A/Berkeley/5/48).

days, and at 21 days the mean titer was 31. At 35 and 56 days, respectively, mean titers of 29 and 27 were found.

The mean titer before vaccination in men who received the adjuvant vaccine was 23. Seven days after vaccination the mean titer was 38, at 10 days it was 49, and at 14 days it was 52. Unlike the aqueous vaccines I and II, there was no evidence of regression in mean antibody levels during the period of observation. At 21 days the mean titer was 54, at 35 days it was 53, and at 56 days after vaccination the mean titer was 59.

Fifty-eight men received the placebo. During the 56-day period of observation there was essentially no evidence of any change in titer. Before vaccination the mean titer was 24, at 56 days it was 22, and at no time did variations in mean titer exceed these limits.

Hemagglutination-inhibiting antibody response. With respect to aqueous vaccine I the virtual lack of hemagglutinating antibody response has already been described (8). No field trial of vaccine II was obtained, and hemagglutination-inhibiting antibody response was not measured.

Determinations of the hemagglutination-inhibiting antibody response were made with the adjuvant vaccine and compared to the titers of men who had received the placebo. Determinations of antibody response were made on a randomly selected sample of 25 men from the group that had received the adjuvant vaccine and an equal number of men who had received the placebo, utilizing both antigens. Because of the importance of the differences in response to the A-prime antigen and to the Asian antigen, the data on the response to the vaccine is presented in three Tables (I, II and III). A description of titers in individuals who received the placebo may be abbreviated by stating that no response was detected with either antigen.

Table I presents the individual titers and geometric mean titers before and after vaccination as measured with the A1/Albany/1/55 antigen. Most of the men had prevaccination titers in the low ranges with the resultant geometric mean titer of 10. At 7 days after vaccination the mean titer was 14; at 10 days after vaccination the mean titer was 26; at 14 and 21 days the mean titer was 29; at 35 days the mean titer was 27; and at 56 days a further increase to 34 was found. When individual titers

TABLE I

Hemagglutination-inhibiting antibody response in 25 men to adjuvant vaccine as measured with an antigen made with the A1/Albany/1/55 strain

Reciprocal of Antibody Titer	Days from Inoculation to Collection of Specimen						
	0	7	10	14	21	35	56
<8	9	7	1	1	1		
8	6	6	3	3	2	4	4
16	5	4	8	5	7	8	5
32	2	2	7	10	9	6	8
64	3	5	3	3	3	5	2
128		1	2	2	2	1	5
256			1	1	1	1	1
Geometric mean titer	10.3	13.9	26.4	28.6	28.6	27.1	33.8

are considered, it will be noted that the patterns tend to follow a distribution which is fairly regular.

When the hemagglutination-inhibiting antibody response in the same men was measured utilizing an antigen made with the A2/Japan/305/57 strain, a very different pattern emerged. In 9 of the 25 men there was little or no titer response, and these men had a distinctively different pattern of response from that seen in 16 men whose response was of much greater magnitude. The pattern of antibody response in the "slight response" group is presented in Table II. Herein it will be seen that before vaccination the geometric mean titer was 4.0 and that it remained at this level for 14 days after the vaccine had been given. At 21 days a dubious increase to 4.3 was found; at 35 days the mean level was 7.4; and at 56 days the mean level was 19. Even the increases in mean titer at 35 and 56 days are unduly weighted by an 8-fold response (occurring in a single individual) between 21 and 35 days, and a further 4-fold response (in the same man) occurring between 35 and 56 days.

Table III presents the titer response to the Asian antigen in the 16 men who showed a marked response. Prevaccination titers in these men were distinctly higher than in the "slight response" group, the mean titer being 14. Increases in titer were more prompt and of much greater magnitude. At 7 days the mean titer was 450; at 10 days the mean titer was 1448; and at 14 days the peak level of 1649 was attained. At 21 days a dubious decrease in mean titer to 1579 was found; at 35 days essentially the same

TABLE II

Hemagglutination-inhibiting antibody response in nine men showing slight response to adjuvant vaccine as measured with an antigen made with the A2/Japan/305/57 strain

Reciprocal of Antibody Titer	Days from Inoculation to Collection of Specimen						
	0	7	10	14	21	35	56
<8	9	9	9	9	8	5	2
8					1	2	
16						1	4
32							2
64						1	
128							
256							1
Geometric mean titer	4.0	4.0	4.0	4.0	4.3	7.4	18.7

TABLE III

Hemagglutination-inhibiting antibody response in 16 men showing marked response to adjuvant vaccine as measured with an antigen made with the A2/Japan/305/57 strain

Reciprocal of Antibody Titer	Days from Inoculation to Collection of Specimen						
	0	7	10	14	21	35	56
<8	2						
8	3						
16	8						
32	2						
64	1	1					
128		2	1				
256		6	3	2	2	1	1
512		2	1	3	3	4	4
1024		3	4	4	3	4	6
2048		1	2	1	3	3	3
4096			2	2	2	2	1
8192				3	2		
16384		1	3	1	1	2	1
Geometric mean titer	14.0	449.6	1448.2	1649.2	1579.2	1512.3	1166.1

level, *e.g.*, 1512, was maintained. At 56 days after vaccination the mean titer was still 1166. Further confirmation of the magnitude of the difference may be detected by examining the individual titer values presented in Table III. Here it will be seen that all of the individuals had prevaccination levels of 64 or less. Seven days after vaccination all but one of the 16 men had titers greater than 64. At 10 days and thereafter all of the men had titers greater than 64.

DISCUSSION

The complement-fixing antibody response to a soluble antigen affords points of interest in relation to the usefulness of this method in detecting influenzal infections in a vaccinated population. With respect to the two aqueous preparations, it may be noted that the peak of the response was attained in about 10 days. Thereafter, titer levels remained essentially stationary, perhaps declining slightly toward the end of the 56-day period of assessment. These data suggest that titer increases in men who had received one of these vaccines could be attributed to influenza if they occurred more than 10 days after vaccination.

There were distinct differences in the complement-fixing antibody response to the two preparations although both were given in ostensibly the same dosage. Soluble antibody to vaccine I increased nearly 3-fold at 10 days whereas mean increases of 1.5-fold were elicited by vaccine II. Although the latter preparation was given in a 2-cc volume, it is questionable that this explains the difference. For diagnostic purposes it is clear that preparations more closely resembling vaccine II would be preferable since serodiagnostic distinctions between "titer rise due to vaccine" and "titer rise due to disease" could be made with greater ease. Furthermore, it is to be hoped that further attention to this aspect might result in vaccines with an even lesser S reactivity. If this were practical in vaccine production, field trials would be facilitated.

When the soluble antibody response to the adjuvant vaccine is considered, it is seen that the extended period of reactivity presents a diagnostic difficulty. Furthermore, descriptions of titer responses in terms of geometric mean antibody level may be misleading since 4-fold titer increases were seen up to but not after 10 days. The further slight increases in geometric mean titer were due to a few 2-fold increases in titer. A summative effect might lead to error but, nonetheless, with a 4-fold titer increase concurrent with appropriate illness, the diagnostic interpretation would suggest infection with type A influenza viruses.

With respect to the magnitude of titer increase after administration of an adjuvant vaccine, it appears that with this preparation the increases are of the same order of magnitude as with the more reactive aqueous preparation. The only real evidence of an "adjuvant effect" is the lack of receding mean titers in the later sampling periods. These considerations lend credence to the hope that an adjuvant vaccine

may be manufactured that will have minimal reactivity in terms of stimulation of soluble antibody.

Differences in the pattern of hemagglutination-inhibiting antibody response to the adjuvant vaccine were notable when an Asian antigen was used as the method of measure and contrasted sharply to the response assayed with an A-prime strain. With the latter antigen antibody titers followed a distribution suggesting a gradient of previous experience. Postvaccination antibody response was also distributed in the manner usually found after an adjuvant vaccine is given. The degree of postvaccination antibody response was not high; possibly this is related to the fact that the vaccine contained only 50 CCA units of an A-prime strain.

The very differing pattern of hemagglutination-inhibiting antibody response when an Asian virus was used suggests considerations of both theoretic and practical import. The adjuvant vaccine (containing 150 CCA units of the A2/Japan/305/57 virus) elicited a very prompt and very early antibody response in the men with the marked response. Within 7 days titers had increased many fold, rising from a mean level of 14 to a mean level of 450. By 14 days a further increase to 1649 had occurred. In contrast, the group with a slight response exhibited no change during the first 14 days after vaccination, and only later were titer rises of comparatively meager magnitude detected. Even these might represent natural infection.

It may be further noted that the prevaccination antibody titers of the strongly reactive men were 3-fold higher than the prevaccination titers of the men exhibiting a slight response. Herein is an apparent exception to the frequent observation that high prevaccination antibody titers inhibit postvaccination response. One may suspect that this tendency is being overcome in this case by the effect of prior experience in a manner analogous to that reported in another study (7). In other terms, the enhancement of response suggests the effect of prior experience.

If the difference is attributed to the effect of prior experience, some implications of potentially practical importance also arise. The rationale for the use of adjuvant influenza vaccines lies in their demonstrated capacity to elicit both high and sustained levels of hemmaglutinating antibody. These data suggest that in some instances prior experience may be important in the adjuvant effect. The previous history of the men who participated in this study is unknown in terms of exposure to influenza viruses, but it is not inappropriate to note that they entered this study 9 or 10 months after Asian influenza became widespread in the United States. The evidence presented herein suggests that about one-third of these men escaped infection during this period. This evidence is not inconsistent with other reports (11). In turn, if hemagglutinating antibody response is considered reflective of protection to be anticipated, it appears that 9 of the 25 men would experience much less protective effect from this vaccine and that the protective effect engendered would also be delayed. These considerations may be of real importance in recommendations for vaccination in a young adult group. They tend to affirm the desirability of repeated exposure, in the antigenic sense, to the viruses of current importance, just as repeated exposures are desirable in the case of children (12).

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SUMMARY

The S antibody response to vaccination with influenza vaccines containing an Asian strain was evaluated in young adult males over a period of 56 days. Two of the vaccines were aqueous monotypic preparations, and the third was of the polyvalent adjuvant type. With the aqueous vaccines the highest levels of S antibody were attained in about 10 days. A somewhat different response was seen with the adjuvant vaccine. Four-fold titer increases occurred in the first 10 days after vaccination but there was also evidence of further, although slight, titer increases thereafter. Differences were noted between the two aqueous vaccines, one engendering about half of the soluble antibody elicited by the other. These patterns of S antibody response suggest that useful distinctions may be made in the serodiagnosis of influenza in vaccinated populations.

Studies of the hemagglutination-inhibiting antibody response to the adjuvant vaccine disclosed that 9 of 25 men had a minimal and equivocal response as measured by an A2/Japan/305/57

antigen. In 16 of the 25 men, the response occurred more promptly and was of much greater magnitude. Such a distinction was not apparent when the A1/Albany/1/55 virus was utilized to make an otherwise comparable antigen. These differences suggest that prior experience may affect the antibody response to adjuvant vaccines.

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