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EFFECT OF NEURAMINIDASE ANTIBODY ON HONG KONG INFLUENZA

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Summary The relation between antineuraminidase antibody (A.N.) and natural influenza infection in 1968 was investigated in the community of Tecumseh, Michigan. The outbreak was caused by Hong Kong influenza virus, which contained a new hæmagglutinin antigen (H_3), while the neuraminidase antigen (N_2) was more closely related to that of Asian viruses circulating since 1957. In the study group of two hundred and seventy-four randomly selected adults (aged 20–45), titres of N_2 neuraminidase antibody were detected in a hundred and fifteen (42%) serum samples collected before the outbreak. Influenza infection during the course of the outbreak was identified serologically. The frequency of infection decreased significantly at increasing levels of pre-existing A.N. antibody. In those subjects who were not protected from infection, A.N. antibody significantly suppressed the clinical expression of infection. It is concluded that antibody against the neuraminidase of the influenza virus prevented or modified infection in a situation in which hæmagglutinin antibody had no effect.

Introduction

IMMUNE resistance to influenza is related to circulating antibody as determined by the hæmagglutination-inhibition (H.I.) test.[1] Antibody so measured is now known to be directed mainly against the hæmagglutinin [2]—one of two surface glycoprotein antigens of the influenza virion. Antibody can also be elicited by neuraminidase, the second surface antigen,[3] but tests for its measurement have only become available in recent years. Thus, the role of antineuraminidase (A.N.) antibody in infections of man has not been established. Possibly A.N. may modify the clinical expression of influenza infection [4] or, alternatively, may even completely prevent infection.[5]

All studies on the effect of A.N. antibody have been conducted in the laboratory or in volunteers artificially challenged with laboratory-grown virus.[4–9] The role of A.N. antibody in natural infection is unknown. The major problem in such an investigation is that between major shifts of antigen, influenza strains all possess similar hæmagglutinin and neuraminidase antigens and the effect of each antigen cannot be separately identified. However, in 1968, the Hong Kong variant of type-A influenza appeared with a new hæmagglutinin subtype (H_3) but with a neuraminidase (N_2) of similar subtype to that of the previous Asian strains (H_2N_2).[10,11] Therefore, individuals in 1968 could possess pre-existing A.N. antibodies to the Hong Kong strain in the absence of hæmagglutinin antibodies. During this period a prospective study of respiratory infections was under way in the community of Tecumseh, Michigan, and the present report describes the effect of pre-existing neuraminidase antibodies on illness and infection during the 1968 outbreak of Hong Kong influenza.

Methods

Population

The study of respiratory illness in Tecumseh has been described in detail elsewhere.[12] Families in which the parents were under 45 years of age were randomly selected. Surveillance of these families was started on a staggered basis, and thereafter families were contacted at weekly intervals for 1 year to determine the occurrence of acute illness. Specimens were collected for viral isolation from persons reporting respiratory symptoms within 2 days of onset; standard methods for viral propagation and identification were used.[13] Blood-samples were obtained at recruitment, and again 6 and 12 months thereafter. Before the 1968–69 Hong Kong influenza outbreak, specific vaccine had been administered to school-age children as part of a community trial.[14] Adults had not been inoculated and the vaccine was not generally available at the time. Therefore this present report gives data from the two hundred and seventy-four adults whose serum samples spanned the outbreak.

Antibody Determinations

The standard hæmagglutination-inhibition test (H.I.) was performed in microtitre plates using chicken erythrocytes and four units of antigen; the virus used was A/Aichi/2/68 (H_3N_2), which contained the H_3 hæmagglutinin of Hong Kong influenza and the N_2 neuraminidase present in Asian and Hong Kong strains. A.N. antibodies were determined by a micro-modification of the neuraminidase-dependent hæmagglutinin-inhibition test (N.-H.I.) using 1/60 goat anti-human IgG serum to enhance the pattern of inhibition.[15] The antigen used (4 units) was the recombinant virus X 15-HK, which contained an equine influenza hæmagglutinin (H $equi_1$) and the neuraminidase of Hong Kong influenza (N_2). Since man does not have an antibody against this equine hæmagglutinin,[3] the antibody measured in the N.-H.I. test is directed exclusively against the N_2 neuraminidase of recent A strains. For both H.I. and N.-H.I. tests, sera were treated with *Vibrio choleræ* receptor-

destroying enzyme, heated at 56°C for a half-hour and titrated from an initial dilution of 1/8. A change in antibody titre was judged to be significant when antibody appeared in the post-epidemic serum at 1/16 after being absent in the pre-epidemic serum, or if there was a fourfold rise in antibody titre. For the N.-H.I. test only, the presence of a definite cell button in the first cup, which did not form a " tear-drop " when plates were tilted, was scored as a " trace " of A.N. antibody (reciprocal titre 8 [trace]). This method used in scoring end-points was confirmed by testing 48 of the sera for antibodies by a chemical neuramidase-inhibition (N.I.) technique (with fetuin substrate), similar to that adopted by the Influenza Centre for the Americas.[16] Sera with a titre of <8 in the N.-H.I. test had a titre of <5 in the chemical test, whereas sera that had been scored at 8 (trace) in the N.-H.I. test were found to have a mean titre of 5 in the chemical test.

Results

Protection Against Influenza Infection

The Hong Kong variant of influenza was first isolated from a specimen collected in the Tecumseh area on Nov. 17, 1968. The virus continued to be isolated through the week of Jan. 19, 1969, for a period of 10 weeks. Illness-rates were increased for a 6-week period extending from the week of Nov. 24 to the week of Dec. 29. Blood-samples were obtained routinely from all persons in the study, so that specimens had been collected before the appearance of the Hong Kong influenza variant, and others had been collected from the same individuals after the outbreak. Pairs of serum-samples spanning the outbreak had been collected from two hundred and seventy-four adults, age 20–45 years. The first serum of the pair was initially tested by N.-H.I. to determine the frequency of A.N. antibody before the appearance of Hong Kong influenza. In a hundred and fifty-nine sera (58%) no antibody was detected at 1/8 dilution. Fifty-six sera (20·4%) had a trace of A.N. antibody at that titre. Antibody at 1/8 titre was exhibited by 35 (12·8%), and antibody at 1/16 or greater was found in 24 (8·8%) of the sera.

This pre-existing antibody to Hong Kong influenza neuraminidase was a consequence of infection or vaccination with earlier Asian influenza virus strains, whose neuraminidases were of the same antigenic subtype (N_2) as the neuraminidases of Hong Kong influenza.[10,11] In contrast, the hæmagglutinins of the Asian influenza viruses were of a subtype (H_2) distinct from that of the Hong Kong influenza hæmagglutinin (H_3).[10,11] Thus, the specific effects of pre-epidemic A.N. antibodies on infection with Hong Kong influenza could be determined under circumstances where no effect could be attributed to the possession of antibodies to hæmagglutinin.[17]

TABLE I—RELATION OF PRE-OUTBREAK A.N. ANTIBODY TO INFECTIONS DURING 1968–69 HONG KONG INFLUENZA OUTBREAK

A.N. titre pre-outbreak (reciprocal)	No. with titre	Total infected†	
		No.	%
<8	159	62	39·0
8 (trace)*	56	16	28·6
8	35	9	25·7
≥16	24	5	20·8
Total	274	92	33·6

* Trace of antibody at 1/8 by N.-H.I.
† Infections determined by rise in H.I. antibody titre.

TABLE II—RELATION OF PRE-OUTBREAK A.N. ANTIBODY TO RESPIRATORY ILLNESS, WITH AND WITHOUT EVIDENCE OF INFLUENZA INFECTION, DURING 10-WEEK INFLUENZA OUTBREAK

A.N. titre pre-outbreak (reciprocal)	Persons with one or more illnesses			
	Not infected*		Infected†	
	No.	%	No.	%
<8	43/97†	44·3	47/62	75·8
8 (trace)	14/40	35·0	11/16	68·8
8	15/26	57·7	6/9	66·7
≥16	12/19	63·2	3/5	60·0
Total	84/182	46·2	67/92	72·8

* Hong Kong influenza infection determined by rise in H.I. antibody titre.
† Number with illness per number in group.

To detect these infections, pre and post epidemic sera from the two hundred and seventy-four individuals were tested by N.-H.I. and the standard H.I. method. In no case was there a significant increase in A.N. antibody without a concomitant increase in H.I. titre. However, 38% of persons with rise in H.I. titre did not have a simultaneous increase in A.N. antibody titre. The total number of infections determined serologically was detected by H.I. (table I) and was classified according to the A.N. antibody titre present in the pre-epidemic serum. Individuals who had pre-epidemic neuraminidase antibodies in their sera had a lower rate of influenza infection than those without pre-epidemic A.N. antibody. The proportion of individuals infected fell steadily as pre-epidemic A.N. antibody levels rose, and this regression was statistically significant ($\chi^2=5{\cdot}09$, $P<0{\cdot}05$). Therefore, the pre-existing neuraminidase antibody appeared capable of reducing the frequency of infection with Hong Kong influenza in the 1968–69 epidemic.

Effect of Neuraminidase Antibodies on Illness

Pre-existing A.N. antibody may modify clinical expression of influenza infection resulting from artificial challenge in volunteers.[4] Influenza virus had been isolated during a 10-week period in Tecumseh. During this time, weekly records of acute respiratory illness were obtained by means of telephone calls or house visits. Several other respiratory agents circulated in the community at that time, and illnesses of non-influenzal ætiology must have occurred [13]; the frequency of these illnesses should have been the same in individuals with comparable influenza A.N. antibodies. Thus, any differences in illness-rates would be related to influenza.

For individuals with no detectable pre-outbreak A.N. antibody, respiratory illness was much more common among those infected with influenza than among those who were not ($\chi^2=15{\cdot}23$, $P<0{\cdot}005$) (table II); this finding indicates that influenza infections were most commonly expressed as clinical illness in those with no pre-existing A.N. antibody. There was a difference in illness frequency among persons with a trace of A.N. antibody at 1/8 ($\chi^2=5{\cdot}39$, $P<0{\cdot}05$), but in persons with A.N. antibody levels of 1/8 or greater the difference had disappeared ($\chi^2=0{\cdot}10$, $P>0{\cdot}05$). No other differences in the illness-rates shown in table II were statistically significant. A similar reduction in illness frequency as A.N. anti-

body titre increased was found when only respiratory illnesses with fever were considered, or when the 6-week period of peak influenza prevalence was examined. These findings, therefore, indicate that influenza infections were less commonly expressed as clinical illness when A.N. antibody was present than when it was absent.

Discussion

In laboratory studies A.N. did not neutralise influenza virus directly, as did hæmagglutinin antibody, but it dampened infection by limiting viral spread from affected to adjacent cells.[6,7] There is an association between A.N. antibody and protection against influenza in vivo. Chickens vaccinated with neuraminidase antigens were protected from lethal challenge with homologous influenza viruses, but not as effectively as when hæmagglutinin was used as antigen.[9] Previously, mice had similarly been protected by active immunisation, and also by passive transfer of A.N. antibody.[8] The latter observation demonstrated that the antibody itself, and not cell-mediated immunity, was responsible.

In man, determination of the protective role of A.N. antibody has been complicated by the problem of differentiating between effects of hæmagglutinin and A.N. antibodies in persons who may have naturally acquired both. Discriminate analysis of the variables in a study conducted in persons with hæmagglutinin and A.N. antibodies against the antigens of the challenge strain disclosed that the A.N. antibody was of greatest importance in protection.[5] Participants in another study were preselected for absence of H_3 antibodies.[4] Upon challenge of the twenty-one volunteers by a laboratory-propagated Hong Kong (H_3N_2) virus, no protection from infection could be demonstrated. However, various degrees of illness severity were identified, which were significantly related to the amount of pre-challenge A.N. antibody.

In the present study, the possible effect of hæmagglutinin was eliminated because the initial sera were obtained before virus with the new H_3 hæmagglutinin had appeared in the Tecumseh area. The effect of A.N. antibodies was studied for the first time with a natural virus challenge; the population was large, randomly selected, and was observed to determine the consequences of the outbreak. The decrease in infection-rates observed with increasing levels of pre-existing A.N. antibody is convincing evidence that A.N. antibody can protect against epidemic influenza. Suppression of clinical expression of the infection agreed with this finding. In those cases in which the antibody does not prevent infection it may make it inapparent.

The present data do not permit any estimate of the relative protective effect of A.N. or hæmagglutinin antibodies in man, since the study was designed to examine the independent effect of A.N. However, there are several indications that neuraminidase is a relatively poor immunogen. Outbreaks of Asian influenza had been reported in Tecumseh during the 10 years before the appearance of Hong Kong influenza in 1968. Yet only 42% of the adults tested had detectable titres of A.N. antibodies, mostly at low levels. In the Hong Kong outbreak, rises in A.N. antibody titre were not as common as, and never occurred independently of, rises in H.I. antibody titre. Repeated exposures to neuraminidase may be necessary before detectable levels of A.N. antibody are achieved by infection or vaccination.[18–21] The apparently poor immunogenicity of neuraminidase may result from its being only a minor component of the naturally isolated influenza viruses.[22–24] Through genetic manipulation, the amount of neuraminidase in the virus may be increased.[25] Preparation of such virus strains and their incorporation into vaccines should usefully improve A.N. antibody response.

Our present results indicate the advisability of eliciting A.N. and hæmagglutinin antibodies by vaccination to ensure maximum protection against influenza. In addition to the potential additive or synergistic effect in protection, there is another advantage which may be realised, based on the genetic independence of the two surface antigens. Major shifts in hæmagglutinin without accompanying major changes in neuraminidase occurred not only in 1968 but possibly also in 1947 and 1933.[11] Development of vaccines with sufficient potency to maintain high levels of A.N. antibody in the population would improve prospects for moderating epidemic influenza if a similar antigenic event should occur in the future.

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