The importance of antineuraminidase antibodies in resistance to influenza A and immunologic memory for their synthesis

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SUMMARY

Eight hundred and seventy-seven sera from 360 adults aged 18–50 who were under permanent observation from October 1980 to March 1981 have been studied by haemagglutination-inhibition (HI) and erythrocyte elution-inhibition (EI) tests – a simplified method of antineuraminidase antibody titration. It was demonstrated in some subjects infected with influenza A H1N1 and H3N2 viruses that the antibody rise was to one of the surface antigens only – haemagglutinin or neuraminidase. These subjects made up 5·2–25·8% of all examinees. The protective effect of antibodies to neuraminidase was similar to that of anti-haemagglutinins. Interaction of both types of antibodies was observed in protection against the disease. Data have been obtained on the influence of antineuraminidase antibodies in decreasing the severity of natural infection with influenza A.

A study of heterologous immunologic responses to haemagglutinin and neuraminidase among persons immunized with live influenza A H1N1 and H3N2 vaccines and among children naturally infected with influenza A H3N2 demonstrated the presence of immunologic memory for antineuraminidase antibody synthesis. Thus, the suggestion of a common antigenic structure for neuraminidase N1 and N2 is made.

INTRODUCTION

Influenza virus has two surface antigens, a haemagglutinin and a neuraminidase. The importance of antihaemagglutinins in resistance to influenza has been sufficiently studied (reviewed by Potter & Oxford, 1979). Numerous studies reveal an inverse relationship between the level of haemagglutination-inhibiting (HI) antibodies and morbidity in natural and experimental conditions. Determination of HI titre is widely used for the evaluation of vaccine efficacy and seroepidemiologic studies. The problem of the participation of antibodies to the neuraminidase in immunity has been much less studied and data obtained by different authors has sometimes been contradictory. Thus, in experiments on animals, the presence of anti-neuraminidase antibodies led only to a decrease in the severity of disease but

did not effect the frequency of infection (Schulman, Ehakpour & Kilbourne, 1968; Rott, Becht & Orlich, 1974; Milovidova *et al.* 1974; Gorev, Titova & Korovina, 1977). Similar results were obtained in a study of the role of anti-neuraminidase antibodies on a small group of volunteers (Murphy, Kasel & Channock, 1972). On the other hand, during the 1967–9 pandemic of Hong Kong influenza persons who had antibodies to N2 neuraminidases were 2·0- to 2·6-fold less likely to suffer disease than persons without them (Monto & Kendal, 1973). Finally, during the A/Texas influenza outbreak in 1978 it was noted that resistance to the disease correlated well with the level of anti-neuraminidase antibodies and did not depend on the titre of HI antibodies (Nobuhisa *et al.* 1979).

The present paper gives data on the relation of serum HI and anti-neuraminidase antibody levels of the resistance of man to influenza caused by H1N1 and H3N2 viruses and data on immunologic memory for anti-neuraminidase antibody synthesis.

MATERIALS AND METHODS

Antibody determinations

Antibodies to haemagglutinin were determined by the HI method recommended by WHO (WHO,1959). Antibodies to neuraminidase were determined by erythrocyte elution inhibition (EI) (Appleyard & Oram, 1977) according to the modification of Topuria, Naikhin & Denisov (1980).

In the HI test the following influenza A variants resistant to serum thermostabile non-specific inhibitors were used: A/Swine/Iowa/15/30 (H1N1, according to the old nomenclature – HswN1), A/PR/8/34(H1N1, according to the old nomenclature – H0N1), A/Khabarovsk/74/77 (H1N1), A/Singapore/1/57 (H2N2), A/Hong Kong/1/68 (H3N2), A/Port Chalmers/1/73 (H3N2), A/Victoria/3/75 (H3N2), A/Texas/1/77 (H3N2) were used.

In EI tests the following recombinant strains were used: P7 (N1 of A/Khabarovsk/74/77, H7 (Heq1) of Aeq/Prague/1/56), X-7 (N2 of A/Singapore/1/57, H1 (Hsw) of Asw/33), X-15 (N2 of A/Hong Kong/1/68, H7 (Heq1) of Aeq/Prague/1/56), X-32 (N2) of A/Victoria/3/75), H1 of (A/PR/8/34), P-9 (N2 of A/Texas/1/77, H7 (Heq1) of Aeq/Prague/1/56). Recombinant strains P-7, P-9, X-32 were obtained from N. E. Gorev, Genetic Laboratory, All-Union Research Institute of Influenza, Ministry of Health, USSR; X-42, X-15 and X-7 were obtained from E. D. Kilbourne, Mount Sinai School of Medicine of the City University of New York.

Eight hundred and seventy-seven sera obtained from 360 students of age 18–26 who lived in hostels and were under observation from October 1980 to March 1981 were studied by both HI and EI. This period was characterized by an elevated circulation of influenza A H1N1 and H3N2 among the population. The original sera were obtained in October–November 1980 and the second sera in February–March 1981. In addition, paired sera collected at the onset of the disease and in the convalescent period have been studied in all persons with acute respiratory diseases (ARD) during the period of observation. In addition, 38 paired sera from children aged 3–6 years who had had ARD and 285 paired sera from subjects aged 18–23 years who were immunized with live H1N1 and H3N2 vaccines have been studied. Prior to testing sera were heated at 56 °C for 30 min.

RESULTS

Relationship between HI and EI antibody titres and frequency of infection

Fig. 1 presents the distribution of HI and EI antibody titres to influenza A viruses in sera collected from 360 persons at the beginning of the study period, i.e. in October–November 1980. The geometrical mean titre (gmt) to the H1 haemagglutinin was 1·5 times the gmt of antibody to the N1 neuraminidase and the gmt of antibody to the H3 haemagglutinin was 1·9 times the gmt of antibody to the N2 neuraminidase. Persons with low titres ($\leqslant 10$) of HI and EI antibody predominated in this group. An excess of HI antibody compared to EI antibody was noted in 23·9% (H3 *vs.* N2) and 25·0% (H1 *vs.* N1) of those examined. By contrast, EI antibody titres were higher than HI titres in 6·8% (N2 *vs.* H3) and 19·7% (N1 *vs.* H1) of cases.

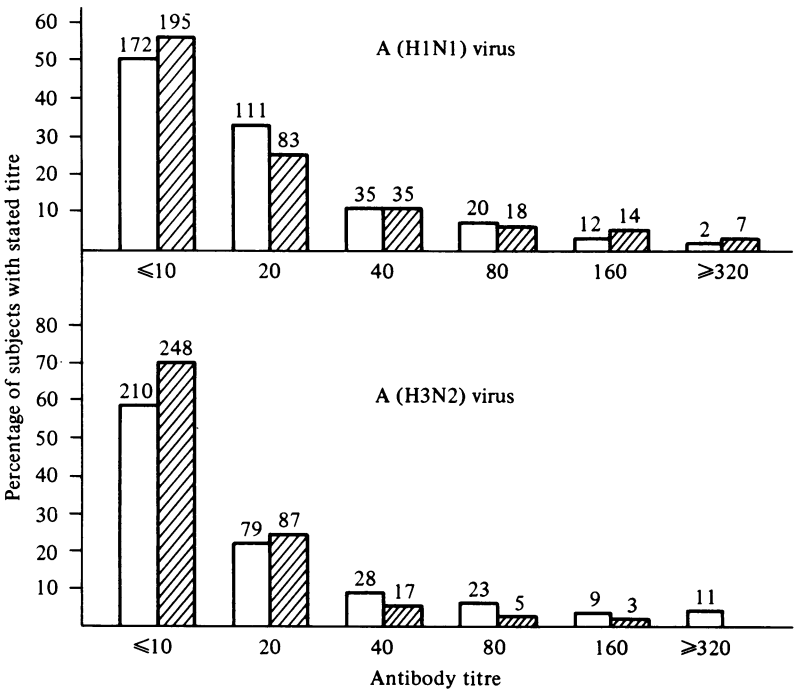

Fig. 1. Titre distribution of HI (□) and EI (▨) antibodies in sera obtained from people at the beginning of the observation period. Figures above columns indicate number of subjects.

With the H1N1 virus 36 subjects (10%) showed a four-fold or greater rise by HI but not by EI. With the H3N2 virus the figure was 34 (9·4%) (Table 1). EI showed diagnostic rises to N1 neuraminidase in 49 persons (13·6%) and to N2 neuraminidase in 59 persons (16·4%) which was not confirmed by HI using H1 and H3 antigens respectively.

Fig. 2 analyses the subjects infected with influenza A in relation to the initial level of EI and HI antibodies. All the groups demonstrate the inverse relationship between infection and presence or absence of either EI or HI antibodies. Thus, persons with an initial anti-neuraminidase antibody titre $\geqslant 40$ (groups B) were

Table 1. *Immunologic responses to haemagglutinin and neuraminidase of influenza A virus in naturally infected and immunized people*

Subjects	No. of paired sera studied	Antigens	No. (%) of ⩾ 4-fold rises: By HI but not EI	No. (%) of ⩾ 4-fold rises: By EI but not HI
Persons aged 18–26 years who were under observation from October 1980 to January 1981	360	A/Khabarovsk/ 74/77 (H1N1)	36 (10·0)	49 (13·6)
		A/Texas/1/77 (H3N2)	34 (9·4)	59 (16·4)
Persons aged 18–23 years immunized with live A/Khabarovsk/74/77 (H1N1) vaccine	133	A/Khabarovsk/ 74/77 (H1N1)	31 (23·2)	9 (6·9)
Persons aged 18–23 years immunized with live A/Texas/ 1/77 (H3N2) vaccine	172	A/Texas/1/77 (H3N2)	35 (20·3)	25 (14·6)

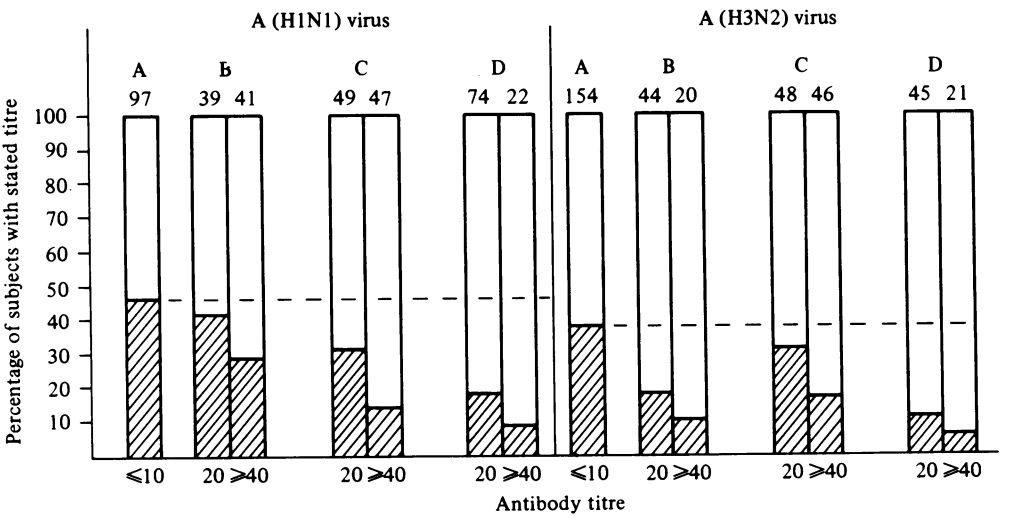

Fig. 2. Interrelationship of influenza A infection and the initial titre of HI and EI antibodies. ▨ indicated percentage who gave four-fold or greater rise in titre and □ those who did not. (A) Persons with antibody tires of ⩽ 10 to both haemagglutinin and neuraminidase; (B) persons with antibody titres to neuraminidase of 20 and 40 and to haemagglutinin of ⩽ 10; (C) persons with antibody titres to haemagglutinin of 20 and ⩾ 40 and to neuraminidase of ⩽ 10; and (D) persons with antibody titres to haemagglutinin and neuraminidase of 20 and ⩾ 40. Figures above columns indicate number of subjects.

1·6–3·7 times less frequently infected with A H1N1 and H3N2 than persons with antibody titres of $\leqslant 10$ (groups A). The equivalent indices of protection for HI antibody to H1 and H3 were 3·2 and 2·9 (groups C). Persons who had initial titres of 20 or $\geqslant 40$ of both EI and HI antibody (groups D) had the maximum protection and were 5·2- and 6·3-fold less frequently infected with A H1N1 and A H3N2 than groups A. The difference of infection indices of groups B, C, D, compared to groups A, is statistically significant ($P < 0{\cdot}05$).

Table 2. *Number and percentage of subjects with HI and EI antibody responses to different influenza A viruses.*

	Immunized with			Natural infection
	A/Vic/3/75 (Feb. 1977)	A/Tex/1/77 (Oct. 1979)	A/Khabar/74/77 (Oct. 1978 to Feb. 1979)	H3N2 epidemic (1979–80)
Age (years)	18–20	18–21	18–23	3–6
No. of subjects	83	127	75	38
Antigens (HI test)				
Hsw(A/Iowa/15/30)	0	0	0	0
H0(A/PR/8/34)	0	0	0	0
H1(A/Khabar/74/77)	1(1,2)*	35(27,6)	44(58,7)	14(36,8)
H2(A/Sing/1/57)	19(23,0)	79(62,2)	19(25,3)	0
H3(A/Hong Kong/1/68)	34(41,0)	80(62,9)	16(21,3)	0
H3(A/Port Chal/1/73)	25(30,0)	81(63,8)	15(20,0)	15(39,5)
H3(A/Vic/3/75)	35(42,0)	84(66,6)	14(18,7)	23(76,3)
H3(A/Tex/1/79)	—	86(67,8)	13(17,3)	29(76,3)
B/Singapore/222/79	0	0	0	0
Antigens (EI test)				
N1(A/Khab/74/77)	1(8,6)	34(26,8)	24(32,0)	15(39,5)
N2(A/Sing/1/57)	22(26,5)	40(31,4)	14(18,7)	0
N2(A/Hong Kong/1/68)	20(24,0)	45(35,4)	13(17,3)	0
N2(A/Port Chal/1/73)	22(26,5)	49(38,6)	13(17,3)	11(28,9)
N2(A/Vic/3/75)	35(42,0)	50(39,4)	14(18,7)	21(55,2)
N2(A/Tex/1/77)	—	54(42,5)	14(18,7)	24(63,2)

* Figures represent numbers (percentage) with a four-fold or greater rise in HI or EI antibody titre.

When analysing the distribution of clinically pronounced and asymptomatic cases of influenza A it was noted that, in persons with high initial EI antibody titres ($\geqslant 40$), clinically pronounced disease was 3·9-fold less frequent than asymptomatic infection. Such a difference in relation to HI antibody was found to be insignificant.

Immunologic memory in the system of anti-neuraminidase antibody synthesis

Table 2 gives the frequency of four-fold or greater rises in antibody to haemagglutinin and neuraminidase of different influenza A virus strains in immunized subjects and those with natural infection. Persons aged 18–20 years who were immunized with live A/Victoria/3/75 (H3N2) vaccine in early 1977, i.e. before the H1N1 recirculation, responded with an anamnestic HI and EI antibody rise to the different drift variants of H3, H2 and N2 antigens. They did not show sero-

conversion to surface antigens of viruses which circulated before their birth. After the influenza A (H1N1) epidemic in 1977–8 persons of the same age gave additional heterologous antibody rises to H1 and N1 in response to immunization with live vaccine prepared from influenza A (H3N2) – A/Texas/1/77 virus.

Persons 18–23 years of age immunized with live A/Khabarovsk/74/77 (H1N1) vaccine in October 1978 to February 1979, i.e. after the influenza A (H1N1) epidemic of 1977–8, manifested heterologous immunologic responses to different drift variants of both H3 and N2 antigens.

Similar results were obtained on examination of children at the age of 3–6 years who had had A/Texas/1/77 during the epidemic 1977–80. Heterologous immunologic responses were found only to surface antigens to which this age group has been exposed.

DISCUSSION

Kilbourne (1975) called the influenza pandemic of 1968–9 a great experiment of nature in detecting the participation of anti-neuraminidase antibody in the immunity to influenza. However, a more detailed study showed that nature did not perform this experiment quite correctly. The data of Monto & Kendal (1973) showed that only 12 % of the population on the eve of the epidemic had high titre antibodies to neuraminidase N2 and that 72 % had none.

Before the epidemics due to A/Victoria/35/72 (H3N2) in 1972–3, A/Port Chalmers/1/73 (H3N2) in 1974–5, A/Victoria/3/75 (H3N2) in 1975–6, A/Texas/1/77 (H3N2) in 1979–80 and A/Khabarovsk/1/77 (H1N1) the herd immunity to the neuraminidase of the above mentioned agents was found to be also at a low level (Naikhin *et al.* 1981). These data show that the epidemic spread of influenza A antigenic variants in the above mentioned cases occurred on a background of low anti-neuraminidase immunity of the population. Therefore, it cannot be concluded that the participation of anti-N antibodies in influenza resistance in the period of replacement of haemagglutinin antigenic structure was insignificant.

The data presented in this paper are based on a continuous observation in the community and indicate an importance of antineuraminidase antibodies in influenza resistance similar to that of antihaemagglutinins.

The results presented here comparing the HI antibody levels in healthy adults, the frequency of antibody formation in persons infected with influenza A and also the published results on the study of vaccine immunogenicity in respect to both influenza A surface antigens (Naikhin, Topura & Denisov, 1980) show an independence of antibody formation to haemagglutinin and neuraminidases in infection or immunization. Consequently, in persons who have no H1 antibody, protection against influenza A infection can be determined only by anti-neuraminidase antibodies. Our data shows that such persons constitute 5·2–25 % of those examined. Hence, to obtain more detailed characteristics of the immunologic status of the population and the characteristics of live and inactivated vaccine immunogenicity it is necessary to determine antibodies to both surface antigens.

Data indicating a minimal frequency of infection in a group of people who had high titres of both HI and EI antibodies show that the two antibodies cooperate

in protection against influenza. In addition, our results show more frequent asymptomatic infections, as compared to the clinically pronounced ones, in a group of persons with high initial EI antibody titres. In our opinion, this confirms experimental data on the influence of these antibodies in decreasing the severity of infection with influenza A.

Francis, Davenport & Hennessy (1953) have described the concept of original antigenic sin for HI antibody synthesis. Data presented in the second part of this study give grounds for believing that immunologic memory is formed in regard to antibody production to influenza A neuraminidase components as well. This agrees with the opinion of other investigators (Aymard, 1976, Molibog *et al.* 1979). Thus, in young adults immunized with A/Victoria/3/75 (H3N2) before the 1977 epidemic an antibody rise was observed to all the preceding neuraminidase N2 variants. However, the rise to the N1 neuraminidase was observed only in the case of immunization with H3N2 viruses after the influenza A H1N1 epidemic.

Heterologous immunologic responses were found to all antigenic neuraminidase N2 variants after immunization with A H1N1 vaccine. These facts indicate the presence of common antigenic structures in both known neuraminidases of influenza A.

In conclusion it should be noted that rises of heterotypic antibodies to different haemagglutinin and neuraminidase antigenic variants are of specific nature. Thus, non-specific HI activity did not effect the results of the HI test since this test used antigens resistant to thermostable inhibitors; the thermolabile inhibitors were removed by heating the sera under study. The EI results could not be influenced by the antigenic activity of other proteins of influenza A viruses since the mechanism of EI is based on the inhibition of the neuraminidase activity by specific antibodies. Such activity is absent in other proteins. Finally, the most convincing evidence of the specific antibody nature of heterotypic immunologic responses to haemagglutinin and neuraminidase is the fact that in influenza patients and immunized persons such responses were specified by the exposure to the given variants in the past, i.e. by the preservation of immunologic memory.

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