INFECTION AND IMMUNITY, June 1979, p. 804-807
0019-9567/79/06-0804/04$02.00/0

Vol. 24, No. 3

Antigenic Determinants in Influenza Virus Hemagglutinin

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Received for publication 23 March 1979

Three antigenic determinants were revealed in H3 hemagglutinin of influenza A viruses isolated from 1968 to 1975. One of them was common for all viruses, and two others specified differences between the viruses possessing H3 hemagglutinin.

The antigenic drift which is characteristic of influenza A viruses circulating in human population has been revealed also in H3N2 viruses, whose prototype strain is A/Hong Kong 1/68 (3, 5, 8, 12, 13). The hemagglutination inhibition test (HIT) markedly reveals the decrease of relatedness among H3N2 influenza virus strains isolated from 1968 until now. This method, revealing antigenic changes in the adsorption site of the hemagglutinin (HA) molecule, does not give information concerning the existence of antigenic determinants in other parts of the HA molecule, however. For this purpose additional methods must be used, such as cross-adsorption, radioimmunoassay, complement fixation, and others, which were used in the study of several strains of H3N2 viruses (6, 10-13, 16, 17).

This paper presents the results of a comparative study on antigenic determinants of HA of H3N2 influenza viruses isolated from 1968 to 1975.

MATERIALS AND METHODS

Viruses. The following influenza virus strains were used: A/Hong Kong 1/68, A/Hong Kong 76/69, A/USSR 392/70, A/England 42/72, A/Port Chalmers 1/73, A/USSR 053/74, A/USSR 0249/75, A/Victoria 3/75, A/Tokyo 1/75 and A/MRC/11, a recombinant of A/PR8/34 and A/Port Chalmers 1/73. The viruses were propagated in the allantoic cavity of chicken embryos concentrated by adsorption and elution on Formalin-treated human erythrocytes (the zero group) and purified by differential centrifugation (9, 11). HA titers of concentrated viruses were 1:10,000 to 1:80,000.

HIT. HIT was performed in the volume of 0.5 ml with 5% human erythrocytes (the zero group). Neuraminidase activity of virus eluates was destroyed by heating at 58°C for 30 min and was determined before and after heating by the method of Warren (2, 15).

Immune sera. Immune sera were obtained by immunization of male rabbits with concentrated virus preparations intravenously and intraperitoneally within 4 weeks. Such sera were arbitrarily designated as immune sera. After 6 weeks the rabbits were reimmunized within 2 weeks and then were bled to obtain hyperimmune serum (9).

For obtaining immunoglobulins free from nonspecific inhibitors, the serum was mixed with concentrated influenza virus deprived of neuraminidase activity (see HIT). The formed precipitate was sedimented by high-speed centrifugation, the supernatant containing nonspecific inhibitors and antibodies to neuraminidase was removed, and the precipitate was covered with saline. The suspension was heated at 68 to 70°C for 30 min, and anti-hemagglutinins were released from the precipitate. Immunoglobulins obtained by this method were free from antibodies to neuraminidase that was checked in neuraminidase activity neutralization test (2, 4, 7, 10).

For the immunological analysis of antigenic determinants in hemagglutinins of the viruses studied, cross-adsorption of antibodies with the viruses adsorbed on Formalin-treated chicken erythrocytes was performed with the subsequent test of the sera in HIT (6, 10, 11). By employing this principle, monospecific antibodies to certain antigenic determinants were obtained.

Radioimmunoassay. Highly purified HA of A/MRC/11 virus and monospecific serum to antigenic H3.1 determinants were used for radioimmunoassay. The latter was prepared by the following method. Immune serum to A/Hong Kong 76/69 virus was mixed with a virus that has common H3.1 determinant and was deprived of neuraminidase activity with the former virus and two other determinants different from that (see Results), and the precipitate was sedimented by high-speed centrifugation. Under these conditions, only H3.1 antibodies were bound to the virus, whereas heterogenous antibodies remained in the supernatant. H3.1 antibodies were then released by heating as described earlier (preparation of inhibitor-free antisera).

In the test system for radioimmunoassay, A/MRC/11 virus purified in a linear sucrose gradient and monospecific to H3.1 determinant serum was used. The second serum was donkey immune serum to rabbit immunoglobulins.

The working dose of ^{125}I-labeled antigen has 5,000 cpm/10 μl, and the serum dilution was 1:15,000. Other details of the method are described elsewhere (1).

RESULTS

Results of the study of antigenic properties of HA of A/Hong Kong 76/69, A/USSR 392/70,

A/England 42/72, A/Port Chalmers 1/73, and A/USSR 053/74 viruses by the method of cross-adsorption of immune sera to these viruses are presented in Table 1.

The table shows that HAs of each virus contain three antigenic determinants. One of them is common for all five viruses and is designated as H3.1. There are two more antigenic determinants in A/Hong Kong 76/69 virus (or the corresponding antibodies in the virus-specific serum). One of them is common for A/Hong Kong 76/69 and A/Hong Kong 1/68 viruses (both viruses appear to be identical in their antigenic structure) and is designated as H3.2. The other is common not only for these two viruses, but also for A/USSR/392/70 virus and is designated as H3.3.

The table also shows that A/USSR 392/70 virus contains not only H3.1 and H3.3 determinants, but also antigenic determinant common with A/England 42/72 virus designated as H3.4. The latter virus contains H3.1, H3.4, and additional antigenic determinant common with A/Port Chalmers 1/73 virus designated as H3.5. The latter virus contains H3.1, H3.5, and additional antigenic determinant designated as H3.6. Finally, A/USSR 053/74 virus appears to be identical with A/Port Chalmers 1/73 virus.

Results of the comparative study of immune and hyperimmune sera to viruses isolated in 1975 (A/USSR 0249/75, A/Victoria 3/75, and A/Tokyo 1/75) are presented in Table 2.

The table shows that the immune sera did not react with A/Hong Kong 76/69 virus. After adsorption with A/England 42/72 virus, the sera reacted only with viruses isolated in 1975. One could conclude from these data that viruses isolated in 1975 have no relatedness with A/Hong Kong 76/69 virus and possess only two antigenic determinants in their HAs. However, such conclusion is premature.

A series of experiments was conducted to determine more specifically the antigenic determinants in HAs of the viruses studied. For this purpose hyperimmune sera were prepared to all viruses studied (see Materials and Methods), and experiments were repeatedly carried out with all viruses analogous to those indicated in Table 1. These experiments showed that hyperimmune inhibitor-free sera to all viruses isolated in 1975 contain antibodies to A/Hong Kong 76/69 virus (Table 2). After adsorption with A/Hong Kong 76/69 virus, the sera lose antibodies to that virus, but preserve antibodies to viruses isolated in 1972 to 1974 and 1975. After adsorption with A/England 42/72 or A/USSR 053/74 viruses, the sera do not react with these viruses and A/Hong Kong 76/69 virus, but react with viruses isolated in 1975.

It may be concluded from these experiments that viruses isolated in 1975 contain antigenic determinant H3.1 common for all of the Hong

TABLE 1. *Analysis of antigenic composition of HA of influenza A viruses isolated in 1968 to 1974*[a]

Inhibitor-free serum to virus	Adsorbant	HIT with 4 HU of viruses					
		Hong Kong 1/68	Hong Kong 76/69	USSR 392/70	England 42/72	Port Chalmers 1/73	USSR 053/74
A/Hong Kong 76/69	None	2,560	2,560	640	160	320	640
	England 42/72	640	640	320	0	0	0
	USSR 392/70	320	320	0	0	0	0
	Hong Kong 76/69	0	0	0	0	0	0
	Hong Kong 1/68	0	0	0	0	0	0
A/USSR 392/70	None	2,560	2,560	2,560	1,280	160	640
	USSR 053/74	640	640	640	80	0	0
	Hong Kong 76/69	0	0	320	80	0	0
	USSR 392/70	0	0	0	0	0	0
A/England 42/72	None	1,280	1,280	1,280	2,560	2,560	2,560
	Hong Kong 76/69	0	0	320	1,280	1,280	1,280
	USSR 392/70	0	0	0	640	80	160
	England 42/72	0	0	0	0	0	0
A/Port Chalmers 1/73	None	40	40	40	1,280	1,280	1,280
	Hong Kong 76/69	0	0	0	1,280	1,280	1,280
	England 42/72	0	0	0	0	160	320
	Port Chalmers 1/73	0	0	0	0	0	0
	USSR 053/74	0	0	0	0	0	0

[a] Figures show reciprocal dilutions of serum which completely inhibit HA.

TABLE 2. *Analysis of antigenic composition of HA of influenza A viruses isolated in 1975*[a]

Inhibitor-free serum to virus	Adsorbant	HIT with 4 HU of viruses					
		Hong Kong 76/69	England 42/72	Port Chalmers 1/73	USSR 0249/75	Victoria 3/75	Tokyo 1/75
A/USSR 0249/75 immune	None	0	640	160	320	320	640
	England 42/72	0	0	0	160	160	160
A/USSR 0249/75 hyperimmune	None	160	640	640	2,560	1,280	1,280
	Hong Kong 76/69	0	320	320	1,280	1,280	320
	England 42/72	0	0	0	640	1,280	1,280
	USSR 053/74	0	0	0	1,280	1,280	1,280
	USSR 0249/75	0	0	0	0	0	0
	Victoria 3/75	0	0	0	0	0	0
	Tokyo 1/75	0	0	0	0	0	0
A/Victoria 3/75 immune	None	0	40	80	80	320	640
	England 42/72	0	0	0	40	160	160
A/Victoria 3/75 hyperimmune	None	40	320	1,280	640	1,280	1,280
	Hong Kong 76/69	0	160	320	640	1,280	1,280
	England 42/72	0	0	0	80	320	320
	USSR 053/74	0	0	0	80	320	160
	Victoria 3/75	0	0	0	0	0	0
	Tokyo 1/75	0	0	0	0	0	0
A/Tokyo 1/75 immune	None	0	80	160	320	640	640
	England 42/72	0	0	0	160	320	320
A/Tokyo 1/75 hyperimmune	None	20	80	320	640	640	1,280
	Hong Kong 76/69	0	40	160	640	640	640
	England 42/72	0	0	0	320	640	640
	Port Chalmers 1/73	0	0	0	320	640	640
	USSR 053/74	0	0	0	320	640	640
	USSR 0249/75	0	0	0	0	0	0
	Victoria 3/75	0	0	0	0	0	0
	Tokyo 1/75	0	0	0	0	0	0

[a] For explanation, see Table 1.

Kong (H3N2) family of viruses, H3.5 common with virus isolated in 1972 to 1974, and a new antigenic determinant designated as H3.7 (3, 5, 6, 11).

The results of the experiments are summarized in Table 3. Antigenic determinants of H3 HA are designated with additional figures added to H3, e.g., H3.1, H3.2, H3.3, etc., thus showing that all determinants belong to the H3 family of HAs.

The table shows that the antigenic determinant H3.1 is common for HAs of all H3N2 viruses (the Hong Kong family) isolated from 1968 to 1975, but there are two additional antigenic determinants which are changing from year to year so that viruses isolated within subsequent years have one of two of them in common.

Experiments were conducted for quantitation of H3.1 antigen in viruses of H3N2 family by radioimmunoassay. The results are given in Table 4.

TABLE 3. *Antigenic determinants of H3 HA in influenza viruses circulated in 1968 to 1975*

Virus	Yr isolated	Antigenic determinant of HA		
A/Hong Kong 1/68	1968	H3.1	H3.2	H3.3
A/Hong Kong 76/69	1969	H3.1	H3.2	H3.3
A/USSR 392/70	1970	H3.1	H3.3	H3.4
A/England 42/72	1972	H3.1	H3.4	H3.5
A/Port Chalmers 1/73	1973	H3.1	H3.5	H3.6
A/USSR 053/74	1974	H3.1	H3.5	H3.6
A/USSR 0249/75	1975	H3.1	H3.5	H3.7
A/Victoria 3/75	1975	H3.1	H3.5	H3.7
A/Tokyo 1/75	1975	H3.1	H3.5	H3.7

The table shows that the content of H3.1 antigen common for H3N2 family of influenza

TABLE 4. *Quantitation of H3.1 determinant in HA of H3N2 viruses*

Virus	Titer (HU)	Content of H3.1 antigen (ng/ml)[a]
A/Port Chalmers 1/73	8	458
A/MRC/11	8	200
A/Victoria 3/75	16	47
A/Tokyo 1/75	8	52

[a] Figures of H3.1 antigenic quantity show to which quantity of HA test antigen in radioimmunoassay it is equivalent.

viruses decreases as far as antigenic drift proceeds.

DISCUSSION

This paper aimed to analyze the antigenic drift of HA of influenza H3N2 viruses isolated from 1968 to 1975. For this purpose inhibitor-free sera to viruses of this family were prepared, and antigenic determinants were revealed by cross-adsorption followed by HIT.

The results of this study clearly showed that all viruses studied, besides main antigenic determinants that specify their belonging to H3 viruses (this antigenic determinant was designated as H3.1), also possess two additional determinants designated by the symbols H3.2 to H3.7. These determinants may be common for viruses isolated at the same period or within the next years and differ in viruses whose isolation is separated by several years. Thus, the antigenic drift may be explained by formation of new antigenic determinants together with preservation of a basic antigen determinant that specify HA-shifting species.

Additional experiments with radioimmunoassay also show that the content of this basic antigen diminishes while the antigenic drift proceeds.

Our observations are in agreement with the data of Laver et al. (8) who found two antigenic determinants in HA of A/Hong Kong 1/68 virus and three in A/England 42/72 and with the data of Takachy and Barb (14) who revealed new antigenic determinants in late H3N2 viruses together with common antigens for early and late viruses.

All this may serve a basis for explanation why influenza virus strains that appear during the antigenic drift possess the ability to overcome immunity to previously circulating viruses of the same (e.g., H3N2) family, causing epidemic and pandemic spread.

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