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THE USE OF MICROPEPTIDE MAPS AND MONOCLONAL ANTIBODIES FOR ANTIGENIC VARIATION ANALYSIS OF INFLUENZA A VIRUSES ISOLATED IN CHINA FROM 1972 TO 1983

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Summary. — ^{125}I-labelled haemagglutinin (HA) micropeptide mapping and haemagglutinin inhibition (HAI) tests with monoclonal antibodies were used in analysing antigenic variation of six strains of influenza A virus isolated from 1972 to 1983 in China. The results from micropeptide mapping generally coincided with those of sequence analysis. ^{125}I-labelled HA micropeptide mapping is a simple, reproducible and practical method for surveillance of influenza, especially in combination with HAI test using monoclonal antibodies.

Key words: influenza virus haemagglutinin; peptide mapping; monoclonal antibodies; antigenic variation

Introduction

Influenza has still remained a major disease of man. There are two types of variation of the surface antigens of influenza A viruses: antigenic drift and antigenic shift, which are main causes for epidemic and pandemic influenza, respectively (Webster *et al.*, 1982; Palese and Young, 1982). Haemagglutinin (HA) is one of the two main surface antigens of influenza A viruses. The analysis of tryptic peptides of HA has been central to the development of the theory of antigenic variation of influenza A viruses (Laver and Webster, 1973; Webster *et al.*, 1975). According to WHO, peptide map was useful in comparing the HA of antigenically different viruses (WHO Memorandum, 1981). For usual peptide map analysis, however, a large amount of samples was required. Even for micropeptide maps described by Moss *et al.* (1980) 0.5—2.0 nmol/l (100—400 μg) HA was required. It is not easy apply this technique in the routine laboratory for studying epidemiology of influenza.

In this paper we used ^{125}I-labelled HA micropeptide maps, for which only 10—20 μg virus was needed in combination with haemagglutinin inhibition tests using monoclonal antibodies, to analyse antigenic variation of HA from six influenza A viruses isolated in epidemics from 1972 to 1983 in China.

Materials and Methods

Viruses. Seven strains of human influenza A viruses used were A/Guangtong/72/243 (H3N2), A/Hubei/75/8 (H3N2), A/Guangtong /77/38 (H3N2), A/Beijing/79/2, A/Wuhan/82/135, A/Wuhan/83/15, and A/PR/8/34 (H1N1). One strain of influenza B virus was B/Hunan/71/2. The viruses were inoculated into allantoic cavities of 11-day-old hen eggs. Viruses were precipitated by 7.5% PEG 6000 and collected by centrifugation at 4000 rev/min in Hitachi rotor at 4 °C for 60 min. The pellets were suspended in 0.1 mol/l Tris-HCl buffer (pH 7.4), layered onto a 5—30% continuous sucrose gradient in 0.1 mol/l Tris-HCl (pH 7.4) and centrifuged in a swing-out MSE rotor at 40,000 rev/min for 60 min at 4 °C, as described by Skehel and Schild (1971). The purified viruses were resuspended in 0.1 mol/l Tris-HCl (pH 7.4) and contained 0.3—1.0 mg protein/ml.

SDS polyacrylamide gel electrophoresis (SDS-PAGE). SDS-PAGE was carried out according to Laemmli (1970). The viruses were mixed with 2% SDS in 0.02 mol/l Tris-HCl (pH 6.8), heated at 100 °C for two min, and 50 μl vol layered onto slab gels measuring 18×18×0.2 cm. A 3.5% stacking and a 16.5% running polyacrylamide system were used utilizing discontinuous buffers when electrophoresed for 16 hr at 100 V. The gels were stained with 0.5% Coomassie blue.

^{125}I-labelling of HA. ^{125}I-labelling of HA was modified from the method described by Elder *et al.* (1977). The gel slices containing the HA were cut into small slices. After washing and drying, the following solutions were added: 0.5 mol/l phosphate (pH 7.5) 20 μl, Na^{125}I (16.7 MBq/ /ml, Beijing Institute for Atomic Energy) 10 μl, Chloramine T (3 mg/ml) 15 μl. The mixture was incubated at 25 °C for 30 min and the reaction was stopped with 0.5 ml of sodium metasulphite (1 mg/ml). By 15 min later, the ^{125}I-labelled gel slices were transferred into 10% methanol for washing out the unlabelled ^{125}I until the count ratio of washing methanol to gel slices was less than 0.03. The gel slices containing ^{125}I-labelled HA were dried at 40—50 °C.

Tryptic digestion. The gel slices containing ^{125}I-labelled HA were digested by 0.1 mg/ml TPCK-trypsin (Sigma) in 0.05 mol/l $NaHCO_3$ (pH 8.0) at 37 °C for 10 hr. The supernatant was retained, lyophilized and dissolved in 10—20 μl distilled water.

Tryptic peptide maps. Tryptic digest was applied in 1 μl to acetocellulose coated plates (20×20×0.25 cm) and separated in two dimensions by electrophoresis and chromatography. Electrophoresis was performed in the first direction in pH 4.4 buffer (pyridine: acetic acid: water, 100 : 4 : 900) and ascending chromatography was performed in butanol : pyridine : acetic acid : water (150 : 1000 : 3 : 100) in the second direction. Plates were dried at 80 °C and exposed to X-ray film for 1—2 days.

Haemagglutination inhibition. HAI tests were done using post-infection chicken sera against A/Guangtong/72/243, A/Hubei/75/8, A/Beijing/79/2 and A/Wuhan/83/15 viruses and 3 panels of monoclonal antibodies against influenza A X-31 (a recombinant of A/Aichi/1/68), influenza A/Texas/1/77 and A/HK/1/82 kindly provided by Dr. J. J. Skehel, WHO Influenza Centre, London.

Results

The results of haemagglutination inhibition of these viruses as tested with chicken antisera are shown in Table 1. The decreasing reactivity of late isolates to earlier antisera and of earlier isolates to late antisera is clear, but no more precise identification of the isolate was possible from these results.

The patterns of tryptic peptide maps of H3 viruses are all completely different from the H1 virus (A/PR/8/34) and from type B virus (B/Hunan/ /71/02). There were some similarities and some clear discernible differences among the six isolates of H3 viruses (Figs 1—6). The peptide map of A/ /Guangtong/72/243 HA differs from that of A/Hubei/75/8 HA at 4 positions and the peptide map of A/Guangtong/77/38 differs from that of A/Hubei/ /75/8 at three positions. There are three different peptide spots between A/Guangtong/77/38 and A/Beijing/79/2. Since the appearance of A/Beijing/

Table 1. Results of haemagglutination-inhibition tests using chicken antisera

Virus	Antiserum			
	A/Guangtong/72/243	A/Hubei/75/8	A/Beijing/79/2	A/Wuhan/83/15
A/Guangtong/72/243	**60**	20	20	20
A/Hubei/75/8	80	**160**	20	10
A/Guangtong/77/38	160	160	320	160
A/Beijing/79/2	80	80	**320**	160
A/Wuhan/82/135	10	20	160	80
A/Wuhan/83/15	10	10	160	**320**

/79/2 only 1—2 changes can be found between any two isolates. The peptide maps of A/Wuhan/82/135 and A/Wuhan/83/15 HA appear the same except the latter has another new spot (Figs 5—6). The changes of peptide maps of different HA are summarized in Table 2. It can be shown from the changes of the peptide spot number and of the amino acid sequence that their trend was generally similar.

The results of HAI using monoclonal antibodies are shown in Table 3. A/Guangtong/72/243 and A/Hubei/75/8 were antigenically very similar. Both could react with most X-31 monoclonal antibodies but not with all monoclonals to A/HK/1/82. A/Guangtong/72/243 did not react with all monoclonals to A/Texas/1/77, but A/Hubei/75/8 reacted with some of them. A/Guangtong/77/38 and A/Beijing/79/2 viruses reacted similarly, namely with most of A/Texas/1/77 and with some of A/HK/1/82 monoclonals, but not with the most of X-31 monoclonals. Influenza A/Wuhan/82/135 and A/Wuhan/83/15 apparently have some similar reaction patterns. Influenza A/Wuhan/83/15 reacted with all A/HK/1/82 monoclonals and A/Wuhan/82/135 with all anti-A/HK/1/82 monoclonals but HC 10.

Table 2. Comparison of changes of peptide map spot and amino acid sequence

Change of peptide map spot		Changes of amino acid sequence			
Epidemic variant in China	Number of spots changed	Similar representive variant	Number of amino acids changed	%	References
A/Guangtong/72/243	—	A/England/42/72	—	—	Laver, W. G. and Air, G. M. (1981)
A/Hubei/75/8	4*	A/Victoria/1/75	11	3.7	ibid.
A/Guangtong/77/38	3	A/Texas/1/77	8	1.9	ibid.
A/Beijing/79/2	3	A/Bangkok/1/79	5	1.5	Skehel, J. J. *et al.* (1983)
A/Wuhan/82/135	2	A/Hong Kong/1/82	6	1.8	ibid.
A/Wuhan/83/15	1	A/Netherland/246/82	2	0.6	ibid.

* Compared with earlier epidemic variant. "—" indicates not shown.

Table 3. Results of haemagglutination-inhibition tests with monoclonal antibodies

Virus	Monoclonal antibody to														
	X−31						A/Texas/1/77				A/HongKong/1/82				
	HC19	HC21	HC63	HC67	HC68	HC100	HC3	HC59	HC87	HC194	HC3	HC6	HC10	HC12	HC29
A/Guangtong/72/243	1 600	—	6 400	6 400	6 400	12 800	—	—	—	—	—	—	—	—	—
A/Hubei/75/8	800	—	6 400	3 200	3 200	12 800	800	25 600	—	25 600	—	—	—	—	—
A/Guangtong/77/38	—	—	—	—	—	—	400	25 600	800	25 600	25 600	—	—	25 600	25 600
A/Beijing/79/2	—	100	—	—	—	—	6 400	400	—	25 600	25 600	1 600	200	225 600	25 600
A/Wuhan/82/135	400	200	6 400	—	—	—	—	25 600	100	25 600	400	400	—	800	25 600
A/Wuhan/83/15	—	—	—	—	—	—	—	—	—	25 600	6 400	1 600	800	25 600	25 600

"—" indicates HAI titre was less than 100.

Discussion

The results presented here showed that ^{125}I-labelled HA micropeptide mapping method in combination with HAI using monoclonal antibodies is very useful for comparing the antigenic variation of influenza A viruses. Since the appearance of Hong Kong influenza in China two large outbreaks occurred in 1968—1969 and 1970, respectively. Three years later the variant represented by A/Guangtong/72/243 (similar to A/England/ /42/72) resulted in an outbreak in 1972. In 1975—1976 the variant represented by A/Hubei/75/8 (similar to A/Victoria/1/75) resulted in another outbreak. The HA micropeptide maps of these two isolates clearly showed four differences, although they reacted with the X-31 monoclonal antibody in a same way. After further variation of H3N2 viruses, two outbreaks occurred in 1977 and 1979 respectively, mainly in young people and children. The representative variants recovered from these outbreaks were A/Guangtong/77/38 and A/Beijing/79/2 (similar to A/Texas/1/77 and A/Bangkok/1/79, respectively). The HA micropeptide maps of these two viruses were quite different from that of A/Guangtong/72/243 and A/Hubei/75/8 and from each other. HAI tests using monoclonal antibodies showed similar results. Since 1979 antigenic drift proceeded steadily but undramatically for several years. Both A/Wuhan/82/135 and A/Wuhan/83/15 have 2—3 differences at micropeptide maps from earlier A/Beijing/79/2. The micropeptide map of A/Wuhan/82/135 only has one more spot than that of A/Wuhan/83/15. The results obtained with monoclonal antibodies directed against A/HK/1/82 also support this conclusion.

Sequence analysis of antigenically distinct isolates is valuable to understand the mechanism of antigenic change in influenza viruses and of influenza epidemiology (Skehel *et al.*, 1983). It was shown that the results from micropeptide maps can coincide with sequence analysis (Table 2). The micropeptide mapping is much easier to do than sequencing although it is not so precise as sequencing. Usual peptide mapping can be used in analysing structural changes of influenza viruses (Laver and Webster, 1973; Webster *et al.*, 1975; Laver and Air, 1981), but it is difficult to be applied in the routine laboratory for epidemiological analysis because the sample amount for usual peptide mapping is too large and especially some epidemic strains grow poorly. The micropeptide mapping presented here is high reproducible, inexpensive and the sample amount required is very small. It is easy to be introduced into routine laboratory work. We used this method to analyse antigenic changes of some strains isolated from pigs and obtained interesting results (in preparation).

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Explanation to Figures (Plates IX—X):

Figs 1—8. Micropeptide maps of ^{125}I-labelled haemagglutinin from: *1—6* influenza A (H3N2) viruses; *7* — influenza A virus (H1N1); *8* — influenza B virus strain.
Abscissae: direction of electrophoresis; ordinates: direction of chromatography.