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ANTIGENIC ANALYSIS OF RECENT H1N1 INFLUENZA VIRUSES WITH MONOCLONAL ANTIBODIES

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Summary. - Antigenic analysis of recently isolated H1 influenza viruses was performed using haemagglutination inhibition (HI) assay with monoclonal antibodies to the haemagglutinin (HA) subunit. Tests using monoclonal antibodies against the HA of the A/England/333/80 (H1N1) and A/Yamagata/120/86 (H1N1) viruses revealed that the major antigenic drift occurred in 1985 or 1986 and A/Dunedin/6/83-like virus became a major strain after 1986.

Key words: influenza virus; monoclonal antibodies; antigenic drift

Introduction

Although the antigenic drift of influenza virus has been studied by using monoclonal antibodies (Gerhard *et al.*, 1981; Webster *et al.*, 1979; Yamada *et al.*, 1984), a detailed antigenic analysis of recent H1N1 influenza viruses has not yet been performed. Monoclonal antibodies to H1N1 influenza viruses were originally produced by Webster *et al.* (1979), and they have been used for a number of antigenic analyses. However, as a result of progressive antigenic drift, most of the monoclonal antibodies produced by Webster's group do not react with the isolates obtained after 1983. Since April 1986, influenza A (H1N1) viruses that are readily antigenically distinguishable from isolates before 1985 have been detected in the Far East (WHO 1986 *a, b*).

To obtain information on the antigenic drift of recent H1N1 influenza viruses, we produced monoclonal antibodies to the haemagglutinins (HA) of A/England/333/80 and A/Yamagata/120/86, which are representative isolates for 1980 and 1986, respectively.

Materials and Methods

Viruses. Thirty-three strains of influenza virus A (H1N1) were used. The viruses were grown in

11-day-old chicken embryos and purified by adsorption to and elution from chicken erythrocytes followed by differenntial centrifugation and sedimentation through a sucrose gradient (10–40 % sucrose, 0.5 mol/l NaCl) (Laver, 1969).

Serological test. Haemagglutination inhibition (HI) tests were performed as described previously (Webster *et al.*, 1979).

Monoclonal antibodies and chicken antisera. Hybrid cell lines producing antibodies to the HA of A/England/333/80 (H1N1) and A/Yamagata/120/86 (H1N1) viruses were prepared as described previously (Yamada *et al.*, 1984). For fusion, myeloma cells (X63-Ag 8-6.5.3) were used as parental cells.

Chicken antisera against the influenza viruses were raised against purified influenza viruses (10,000 HAU) injected either intravenously and intramuscularly into chickens; serum was collected 10 days post inoculation.

Competitive binding assay. The competitive binding assay was performed as modified ELISA (Kimura *et al.*, 1983). The purified influenza A/England/333/80 virus at limiting concentration was used as antigen. Serial dilutions of each competing antibody obtained from culture fluid were added to the wells of microplates, to which the A/England/333/80 virus was adsorbed. Culture medium alone was used as control. After 2 hr incubation at room temperature, the wells were washed and horseradish peroxidase (HRPO) - labelled monoclonal antibody (OD_{490} of 1.0) was added. The amount of competitive binding was estimated from the absorbance at 490 nm in the presence or absence of unlabelled competing antibodies. The percentage competition was determined by the formula $|100\,(A-n)|/(A-B)$, where A is the OD in the absence of any competing antibody, B is OD in the presence of homologous antibody (10^4 ELISA units), and n is OD in the presence of a competitive antibody (10^4 ELISA units).

Table 1. Antigenic analysis of H1N1 virus by chicken antiserum

Virus	Chicken antiserum to:			
	A/USSR/92/77	A/Bangkok/10/83	A/Dunedin/6/83	A/Yamagata/120/86
A/FM/1/47	1024	1024	128	64
A/Omachi/1/53	256	256	16	<16
A/USSR/92/77	**512**	256	64	16
A/Kumamoto/37/79	128	128	32	<16
A/Bangkok/10/83	256	**256**	128	32
A/Dunedin/6/83	256	128	**256**	32
A/Kyoto/10/84	256	256	128	32
A/Yokohama/4/86	64	128	256	2048
A/Sendai/10/86	64	64	64	256
A/Kanagawa/12/86	64	64	64	256
A/Kanagawa/13/86	64	128	128	2048
A/Yamagata/115/86	64	128	128	2048
A/Yamagata/120/86	64	64	128	**1024**
A/Yamagata/123/86	64	128	128	1024
A/Yamagata/32/87	128	128	128	256

Values indicating the HI titre with the homologous virus in bold type.

Results

Antigenic analysis of recent H1 viruses

Antigenic analysis of 25 strains (17 strains isolated before 1983 and 8 strains isolated after 1986) by HI test was carried out using chicken antisera (Table 1). Antigenic differences were not striking among the isolates in 1986 and 1987, but a large antigenic difference was detectable between viruses isolated in 1986/1987 on one hand and the viruses isolated earlier on the other hand.

Analysis of antigenic determinants of the HA of A/England/333/80

Monoclonal antibodies prepared against A/England/333/80 and A/Yamagata/120/86 reacted strongly with the homologous viruses in HI assays (Table 2), but did not react with other influenza A subtypes or influenza B viruses (data not shown). Thirteen monoclonal antibodies to England/333/80 and five to A/Yamagata/120/86 HA were obtained and used in this study.

To analyse the epitopes on the HA protein of A/England/333/80 virus, a competitive binding assay was performed with four types of HRPO-labelled monoclonal antibodies (clones 5, 15, 30 and 139). Thirteen monoclonal antibodies were tested for their competitive binding to each HRPO-labelled antibody, and could be divided into five groups by the results of the competitive binding assay (Fig. 1).

Table 2. Isotype and HI activity of the monoclonal antibodies

	Monoclonal antibodies to: A/England/333/80				Monoclonal antibodies to: A/Yamagata/120/86		
No.	isotype	HI	ELISA (log10)	No.	isotype	HI	ELISA (log10)
5	IgG2	1280	5.0	14	IgG1	160	7.0
30	IgG2	5120	5.0	19	IgG3	160	7.0
75	IgG2	2048	5.0	24	IgG3	2560	6.0
20	IgG2	2560	5.0	29	IgG1	1280	5.0
90	IgG2	1280	5.0	30	IgG3	1280	5.0
117	IgG2	20480	7.0				
139	IgG2	20480	5.0				
15	IgG2	20480	5.0				
96	IgG2	20480	6.0				
21	IgG2	20480	6.0				
39	IgG2	20480	6.0				
41	IgG2	20480	6.0				
151	IgG2	20480	6.0				

All group I monoclonal antibodies inhibited the binding of an HRPO-labelled monoclonal antibody 30 (HRPO-30), but none of the antibodies from the other groups did so. HRPO-139 was blocked completely by homologous antibody and partially by group II antibodies (by 70 to 80 %). In contrast, group I antibodies did not block HRPO-139. HRPO-15 was blocked completely by the homologous antibody. Partial competition by group II and group III antibodies (by 60 to 50 %) was higher than by group I and V antibodies (by 10 to 30 %). HRPO-5 was blocked completely by antibodies of groups III, IV and V, but not by those of groups I and II. Thus, the thirteen monoclonal antibodies against A/England/333/80 could be divided into five groups, with the antibodies belonging to each group recognizing different epitopes.

Antigenic analysis of recently isolated H1N1 viruses

An antigenic analysis of recently isolated H1N1 influenza viruses was performed using corresponding monoclonal antibodies. The H1N1 viruses isolated from different regions of the world before 1984 were similar in their reactivities to the England strain, execept for Dunedin/6/83 and Yamagata/233/84. These two strains failed to react with any of the monoclonal antibodies raised against the England strain (Table 3).

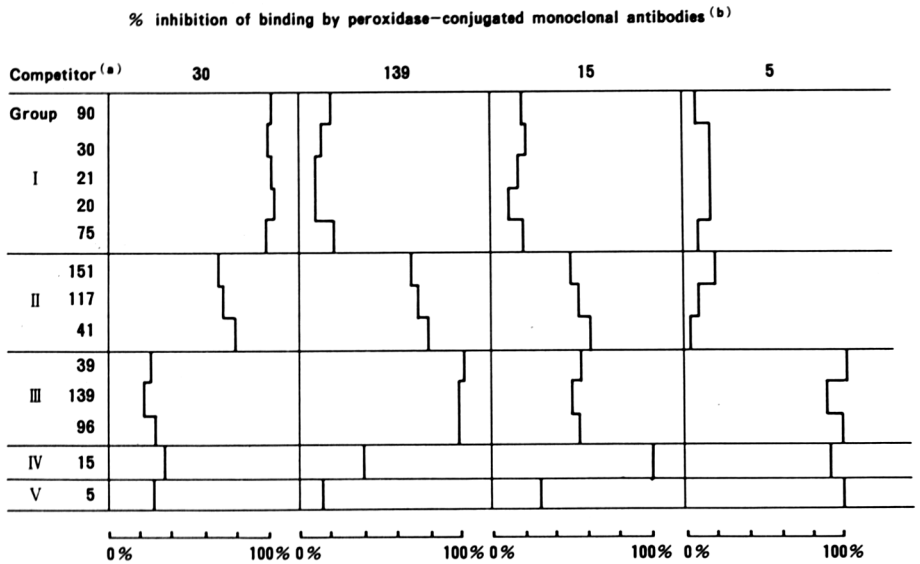

Fig. 1

Results of the competitive binding assays

(a) Competitors were used at the limiting concentration of 10^4 ELISA units

(b) % inhibition

Table 3. Antigenic drift of H1N1 influenza viruses

Virus	Reaction patterns in HI tests with the following monoclonal antibodies													
	A/England/333/80(HA)									A/Yamagata/120/86/(HA)				
	5	30	75	20	90	117	139	15	96	14	19	24	29	30
FM/1/47		+				+								
Omachi/1/53						+								
USSR/92/77	+	+	+	+	+	+						+	+	+
Kumamoto/37/79		+	+	+		+								
Bangkok/10/83	+	+	+	+	+	+	+	+	+					
Dunedin/6/83												+	+	+
Kyoto/10/84	+	+	+	+	+	+	+	+	+					
Y/210/84		+	+	+										
Y/217/84		+	+	+										
Y/233/84														
Y/234/84		+	+	+								+	+	+
Y/237/84		+	+	+										
Y/239/84		+	+	+						+	+	+	+	+
Y/242/84		+	+	+										
Y/246/84		+	+	+								+	+	+
Yokohama/4/86												+	+	+
Sendai/10/86												+	+	+
Kanagawa/12/86												+	+	+
Y/115/86												+	+	+
Y/120/86										+	+	+	+	+
Y/123/86												+	+	+
Y/163/86												+	+	+
Y/190/86												+	+	+
Y/192/86												+	+	+
Y/260/86												+	+	+
Y/32/87												+	+	+
Y/36/87												+	+	+
Y/40/87												+	+	+
Y/52/87												+	+	+
Y/54/87												+	+	+
Y/62/87														
Y/63/87												+		
Y/65/87														

+; HI titre less than 10-fold different from that for the homologous virus
-; greater than 10-fold decrease in HI titre from that for the homologous virus
Y; Yamagata

The H1N1 viruses isolated in 1984 in Yamagata Prefecture were all similar in their reactivity with the monoclonal antibodies, except for A/Yamagata/233/84. The strains isolated during or after 1986 showed more pronounced antigenic differences from those isolated before 1984; none of the later isolates reacted with monoclonal antibodies to A/England/333/80. This change in reactivity was also evident when using chicken antisera raised against A/Yamagata/120/86 (Table 1). Some of the isolates from before 1984 (A/USSR/92/77, A/Dunedin/6/83, A/Yamagata/234/84, A/Yamagata/239/84, and A/Yamagata/246/84) reacted with monoclonal antibodies to A/Yamagata/120/86, but most of the isolates did not react with these antibodies.

These findings indicate that H1 influenza viruses isolated after 1986 were antigenically distinct from those isolated before 1984. It is interesting that some of the earlier viruses (such as A/Dunedin/6/83) showed the same reactivity pattern as those isolated after 1986/1987.

Monoclonal antibodies to the HA of A/England/333/80 and A/Yamagata/120/86 showed a spectrum of reactivity patterns with H1N1 viruses isolated in Yamagata City between 1984 and 1987. In 1984, 4 strains (Y/210/84, Y/217/84, Y/237/84, and Y/242/84) showed the same reactivity patterns, while another 3 strains (Y/234/84, Y/239/84, and Y/246/84) showed a separate pattern. The only virus which did not fit either of the two patterns was Y/233/84, did not react with any of the monoclonal antibodies.

Discussion

An antigenic analysis of H1N1 viruses isolated between 1980 and 1986 was performed using monoclonal antibodies to the HA molecules of the viruses and HI assay. These studies revealed that minor antigenic variation occurred throughout this period, presumably due to the accumulation of point mutations, and that a major antigenic drift occured between 1984 and 1986. This result may explain the fact that patients in the epidemics before 1984 were mainly children, while after 1986 mainly adults were infected by the H1N1 virus.

It is not known why this major antigenic drift occurred, but it seems to be of importance that A/Dunedin/6/83 showed the same reactivity pattern as the major strains in 1986/87. This suggests that an A/Dunedin/6/83-like virus became a major strain by the gradual progress of antigenic drift. It is also interesting that A/Yamagata/233/84, isolated from children in Yamagata City in January 1984, showed a reactivity pattern similar to 3 strains isolated in 1987 (Yamagata/62/87, Yamagata/63/87, and Yamagata/65/87).

The main isolates in April and May 1986 showed reactivity patterns similar to the sporadic isolates in July (Yamagata/260/86), and they showed similar patterns to the main isolates in January and February 1987.

This finding suggests that influenza virus survives through the spring in sensitive humans and then causes a new epidemic by the next season.

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