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Antigenic and Genetic Variation in the Hemagglutinins of H1N1 and H3N2 Human Influenza A Viruses in the Shanghai Area From 2005 to 2008

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Continued rapid evolution of the influenza A virus is responsible for annual epidemics and occasional pandemics in the Shanghai area. In the present study, the representative strains of A/H1N1 and A/H3N2 influenza viruses isolated in the Shanghai area from 2005 to 2008 were antigenically and genetically characterized. The antigenic cartography method was carried out to visualize the hemagglutination-inhibition data. Antigenic differences were detected between circulating A/H1N1 strains isolated from 2005 to 2006 and the epidemic A/H1N1 strains isolated in 2008, which were found to be associated with the amino acid substitution K140E in HA1. The present vaccine strain A/Brisbane/59/2007 is considered to be capable of providing sufficient immunity against most of the circulating A/H1N1 viruses isolated in 2008 from the Shanghai population. The study showed that there were significant antigenic differences between the epidemic A/H3N2 strains isolated in 2007 and 2008, suggesting that antigenic drift had occurred in the A/H3N2 strains isolated in 2008. The P194L mutation was thought to be responsible for the antigenic evolution of influenza A/H3N2 viruses isolated from Shanghai in 2008. Evidence of antigenic drift suggests that the influenza A/H3N2 vaccine component needs to be updated. ***J. Med. Virol. 83:1113–1120, 2011.***

KEY WORDS: influenza virus; antigenic cartography; hemagglutinin; Shanghai

INTRODUCTION

Influenza is a respiratory infection caused by the influenza virus and is responsible for approximately half a million deaths worldwide annually [van den Dool et al., 2008]. Influenza viruses are divided into three types, designated A, B, and C. Influenza virus type A is the most virulent and causes the most severe disease. The most common prevailing influenza A subtypes that infect humans are H1N1 and H3N2. The influenza A genome consists of eight segments of RNA with negative-sense polarity, which encode 11 proteins (PB1, PB2, PA, NP, HA, NA, M1, NS1, NS2, M2, and PB1-F2) [Zhirnov et al., 2007]. The hemagglutinin (HA) protein is the major viral surface antigen which induces protective antibody responses. The HA protein consists of two polypeptides, HA1 and HA2. The HA1 polypeptide mutates more frequently than the HA2 polypeptide and plays a crucial role in natural selection [Chi et al., 2005]. The sequence evolution of HA1 results in antigenic drift as antigenic properties change with time [Blackburne et al., 2008]. Antigenic drift strains escape from host defense mechanisms of acquired immunity and cause annual epidemics. Thus, HA is the focus of influenza virus

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surveillance, and is also the primary component of influenza vaccines that needs to be updated annually.

In the present study, recent changes in the antigenic and genetic characteristics of circulating influenza viruses isolated from clinical specimens collected around the Shanghai area from 2005 to 2008 were examined. Antigenic differences between strains were measured by hemagglutination inhibition (HI) assays. Antigenic cartography methods were used for visualization and quantitative analysis of antigenic differences among strains [Smith et al., 2004; Liao et al., 2009]. The results were compared with genetic changes, which were shown by genetic maps to reveal important amino acid variations in the HA1 sequences.

MATERIALS AND METHODS

Viruses

Nasopharyngeal swab specimens were collected from individuals suffering from influenza and influenza-like illness in influenza sentry hospitals located in Shanghai and surrounding areas from 2005 to 2008. Isolation of influenza viruses was carried out on MDCK cell cultures. All isolates were typed and subtyped by reverse transcription-polymerase chain reaction (RT-PCR). Viral RNA was extracted from the culture supernatants using Trizol LS reagent (Invitrogen, Carlsbad, CA). Reverse transcription and amplification of the HA genes was conducted using AMV reserve transcriptase (Takara Biotechnology (Dalian) Co., Ltd., Dalian, China) and Ex Taq Polymerase (Takara), respectively. The sequence of primers used were 5′-AGC AAA AGC AGG GGA AAA TA-3′ (H1F), 5′-AAC AAG GGT GTT TTT CCT CA-3′ (H1R), 5′-AGC AAA AGC AGG GGA TAA TTC-3′ (H3F), and 5′-CTC AAA TGC AAA TGT TGC ACC-3′ (H3R). Purified PCR products were sequenced by the Shanghai Sunny Biotechnology Company (Shanghai, China).

Hemagglutination Inhibition Assays

The HI tests were performed according to the procedures described [Kendal et al., 1982] using post-infection hamster antisera. The strains used in the HI assays were representative strains of H1N1 and H3N2 viruses isolated from the Shanghai area, as shown in Tables I and II. The following vaccine strains provided by the Shanghai Institute of Biological Products were included: A/New Caledonia/20/1999 (H1N1), A/Brisbane/59/2007-like (H1N1), A/Sydney/5/1997 (H3N2), A/Fujian/411/2002 (H3N2), and A/Brisbane/10/2007-like (H3N2). All antisera were serially diluted two-fold starting at 1:8.

Antigenic Cartography

A web server, Analytical Tool for Influenza Virus Surveillance (ATIVS) [Liao et al., 2009], based upon antigenic cartography methods [Smith et al., 2004] was used for analyzing HI data. Two-dimensional (2D) antigenic maps were generated to reflect the antigenic relationships among strains. One unit of antigenic distance on the map corresponds to a two-fold dilution of antisera in the HI assay. The outer circle of the antigen point is used to express the error (uncertainty of its location).

Genetic Analysis

Sequences were assembled using MEGA software version 4.0 [Tamura et al., 2007], and multiple sequence alignments were conducted with the ClustalW application. The numbers of amino acid substitutions between pairs of strains used in the antigenic analysis were calculated by the alignment of HA1 sequences. The principal coordinate analysis [Higgin, 1992] was used to visualize the amino acid distance matrix and produce 2D genetic maps, which facilitate a side-by-side comparison with the antigenic maps. The principal coordinate analysis was performed using R statistical software version 2.9.2 [Venables et al., 2009].

RESULTS

Fourteen representative strains of influenza A/H1N1 viruses that were collected from 2005 to 2008 and eleven representative strains of influenza A/H3N2 viruses from 2007 to 2008 were used in this study. There were no A/H1N1 strains isolated during 2007.

A/H1N1 Influenza Virus

The antigenicity of fourteen representative strains of H1N1 viruses was characterized by HI assays. Cross-reactive HI titers are presented in Table I, which consists of 14 circulating strains and two vaccine strains (A/New Caledonia/20/1999 and A/Brisbane/59/2007). Subsequently, the HI data were used to construct a 2D antigenic map, as shown in Figure 1. In general, the antigen points could be divided into two categories along the vertical center line although there was some cross-protection between them. Five isolates circulating during 2008 gathered together on the left side with A/Brisbane/59/2007, which is the influenza A (H1N1) component recommended for the 2008/2009 and 2009/2010 influenza vaccine. Four of the five isolates were in positions within a radius of two antigenic units (4-fold in HI) of A/Brisbane/59/2007, denoting similar antigenicity between the circulating strains and A/Brisbane/59/2007. Strain A/Shanghai/MH79/2008 was considered an antigenic variant to vaccine virus A/Brisbane/59/2007. Nine circulating strains isolated in the period 2005–2006 clustered together on the right side with the A/New Caledonia/20/1999 vaccine component used from 2000/2001 to 2006/2007. As presented in Figure 1, the nine isolates from 2005 to 2006 were antigenically similar to the vaccine virus A/New Caledonia/20/1999.

TABLE I. Antigenic Analyses of Representative Influenza A/H1N1 Isolates by Hemagglutination-Inhibition (HI) Tests

		HI titer															
		Post-infection hamster antisera															
Virus strain	Isolation date	Ne/20/99	S174/05	J14/05	J16/05	J11/06	CDC12/06	CDC22/06	CDC32/06	CDC37/06	CDC40/06	Br/59/07	MH79/08	MH87/08	MH111/08	MH135/08	MH208/08
A/New Caledonia/20/1999	09-06-99	1,024	512	512	1,024	1,024	512	512	512	128	512	1,024	512	1,024	128	256	512
A/Shanghai/S174/2005	19-04-05	256	256	256	512	512	64	128	64	128	256	512	512	512	128	128	256
A/Shanghai/J14/2005	03-04-05	256	128	256	512	256	64	128	64	128	256	256	256	512	32	32	256
A/Shanghai/J16/2005	02-04-05	256	256	256	512	512	64	256	128	128	256	512	128	256	128	128	256
A/Shanghai/J11/2006	10-01-06	128	256	256	512	256	64	256	128	256	256	512	128	256	128	64	64
A/Shanghai/CDC12/2006	25-07-06	256	256	256	512	512	128	1,024	256	128	256	256	512	256	128	512	512
A/Shanghai/CDC22/2006	25-07-06	256	256	128	512	512	128	1,024	128	256	512	256	16	256	128	256	512
A/Shanghai/CDC32/2006	03-08-06	128	256	256	512	512	128	1,024	128	128	128	128	512	128	64	256	256
A/Shanghai/CDC37/2006	09-08-06	256	128	256	256	512	256	512	256	256	512	512	256	256	64	256	128
A/Shanghai/CDC40/2006	23-08-06	256	64	64	128	128	64	256	128	128	256	128	128	256	64	128	128
A/Brisbane/59/2007	01-07-07	128	512	256	1,024	512	64	64	128	64	64	1,024	1,024	1,024	512	256	512
A/Shanghai/MH79/2008	06-03-08	32	32	32	128	128	32	256	32	16	64	256	256	256	64	128	64
A/Shanghai/MH87/2008	10-03-08	64	128	256	512	256	32	128	64	64	64	1,024	1,024	1,024	128	128	256
A/Shanghai/MH111/2008	23-03-08	32	128	256	64	128	128	256	128	128	128	512	256	256	128	128	1,024
A/Shanghai/MH135/2008	01-07-08	128	32	32	256	128	64	512	64	32	128	1,024	512	512	64	1,024	1,024
A/Shanghai/MH208/2008	05-08-08	16	32	64	256	128	64	256	64	32	64	512	512	256	64	256	1,024

TABLE II. Antigenic Analyses of Representative Influenza A/H3N2 Isolates by HI Tests

		HI titer													
		Post-infection hamster antisera													
Virus strain	Isolation date	Sy/5/97	Fu/411/02	CDC188/07	CDC193/07	CDC199/07	NB55/07	Br/10/07	PT7/08	MH41/08	MH43/08	MH59/08	MH61/08	BT18/08	MH229/08
A/Sydney/5/1997	23-06-97	1,024	32	16	8	<8	128	<8	<8	256	8	32	32	128	16
A/Fujian/411/2002	11-08-02	256	512	8	8	16	64	8	8	8	16	8	16	64	32
A/Shanghai/CDC188/2007	Feb-07	<8	<8	512	256	512	1,024	1,024	256	1,024	128	1,024	256	512	1,024
A/Shanghai/CDC193/2007	Mar-07	<8	8	256	256	512	1,024	1,024	256	1,024	128	1,024	256	512	1,024
A/Shanghai/CDC199/2007	Feb-07	<8	8	512	128	512	1,024	1,024	256	1,024	64	1,024	256	512	1,024
A/Ningbo/NB55/2007	14-01-07	<8	32	256	64	128	512	1,024	256	512	512	1,024	512	512	256
A/Brisbane/10/2007	06-02-07	<8	8	1,024	256	512	1,024	1,024	256	1,024	128	1,024	512	1,024	1,024
A/Shanghai/PT7/2008	11-02-08	<8	64	64	16	64	128	128	64	512	256	1,024	128	256	256
A/Shanghai/MH41/2008	27-02-08	<8	64	128	32	128	128	256	128	512	256	512	128	256	256
A/Shanghai/MH43/2008	27-02-08	<8	32	128	32	128	64	256	64	128	128	1,024	256	256	128
A/Shanghai/MH59/2008	03-03-08	<8	32	64	8	64	128	32	64	512	512	1,024	128	128	32
A/Shanghai/MH61/2008	05-03-08	<8	64	128	32	128	512	512	128	512	512	1,024	128	128	128
A/Wuxi/BT18/2008	Mar-08	<8	16	64	8	64	128	128	64	128	64	512	64	128	64
A/Shanghai/MH229/2008	11-08-08	<8	64	128	32	128	128	512	128	256	256	1,024	256	512	256

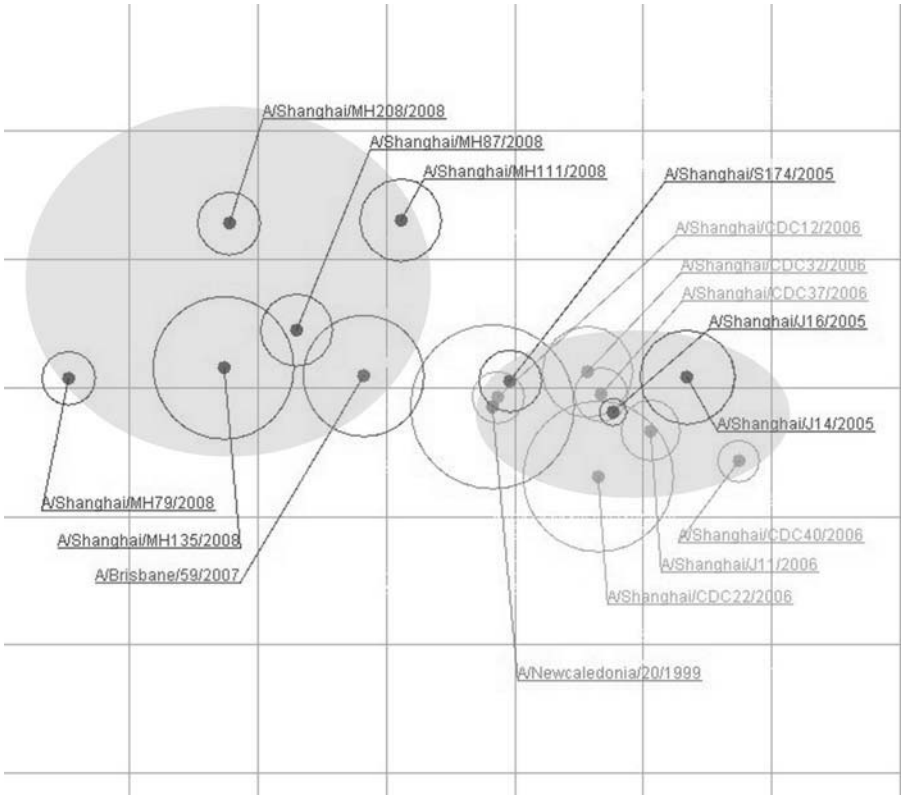

Fig. 1. Antigenic map of influenza A/H1N1 viruses isolated around the Shanghai area from 2005 to 2008. The total error is 158.54 and the average error is 0.62.

The constructed 2D genetic map is shown in Figure 2. The circulating strains during 2005 were closely genetically related to A/New Caledonia/20/1999 but to a lesser extent with the epidemic strains isolated in 2006. Three isolates circulating in the spring of 2008 (MH79, MH87, MH111) gathered together with A/Brisbane/59/2007; however, they were distant from the two epidemic strains isolated in the summer of 2008 (MH135, MH208).

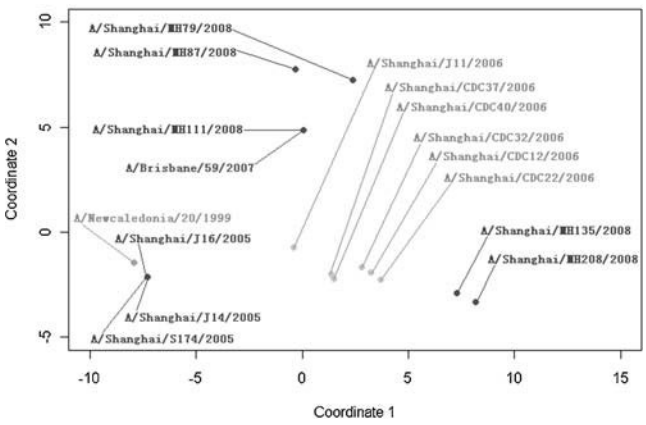

Fig. 2. Genetic map of influenza A/H1N1 viruses isolated in the Shanghai area from 2005 to 2008. The percentage of variance explained by the first two coordinates was 72%.

The amino acid changes in the HA1 subunit of H1N1 viruses are shown in Table III. Compared with vaccine strain A/New Caledonia/20/1999 and circulating strains during 2005 and 2006, the HA genes of A/Brisbane/59/2007 and epidemic strains isolated in 2008 contained the same K140E mutation. All isolates from 2006 contained the following amino acid changes: T82K, Y94H, R145K, R208K, and T266N. The two isolates circulating in the summer of 2008 exhibited eight common amino acid changes when compared with the three epidemic strains isolated in the spring of 2008; these were: N35D, S36N, I47K, E68G, K82R, R145K, T193K, and K273E.

A/H3N2 Influenza Virus

Antigenic characterization using HI tests was performed on 11 representative strains of H3N2 viruses and three vaccine strains (A/Sydney/5/1997, A/Fujian/411/2002, and A/Brisbane/10/2007; Table II). The antigenic map is presented in Figure 3. The circulating strains from 2007 and 2008 had undergone significant antigenic changes in the HA protein compared with vaccine strains A/Sydney/5/1997 and A/Fujian/411/2002. There were clear-cut antigenic differences between epidemic strains isolated in 2007 and 2008, except that strain A/Ningbo/NB55/2007 was antigenically close to the isolates circulating during 2008.

TABLE III. Amino acid Changes in HA1 of Influenza A/H1N1 Vaccine Strains and Representative H1N1 Strains Collected From 2005 to 2008

Virus strain	Amino acid position in HA1[a]																														
											Sa[b]		Ca		Sa	Ca	Ca	Sb	Sb	Sb	Sb	Sb	Sb	Sb		Ca									
	35	36	47	56	63	66	68	82	94	103	125	128	140	145	161	165	167	184	186	188	189	192	193	194	195	204	208	214	251	252	266	273	283	314	315
A/Brisbane/59/2007	N	S	I	S	G	E	E	K	H	E	N	V	**E**	R	S	A	N	I	D	K	A	H	T	E	N	H	K	A	R	Y	N	K	G	V	T
A/New Caledonia/20/1999	D	.[c]	.	.	.	.	.	T	Y	.	.	.	**K**	.	.	V	.	.	N	R	.	.	.	.	.	.	R	.	W	.	T	E	.	.	.
A/Shanghai/J14/2005	D	.	.	.	.	.	.	T	Y	.	.	.	**K**	.	.	.	.	.	.	R	.	.	.	.	.	.	R	.	.	F	T	E	.	A	.
A/Shanghai/J16/2005	D	.	.	.	.	.	.	T	Y	.	.	.	**K**	.	.	.	.	.	.	R	.	.	.	.	.	.	R	.	.	F	T	E	.	A	.
A/Shanghai/S174/2005	D	.	.	.	.	.	.	T	Y	.	.	.	**K**	.	.	.	.	.	.	R	.	.	.	.	.	.	R	.	.	F	T	E	.	A	.
A/Shanghai/CDC12/2006	D	N	.	.	.	.	.	.	.	.	K	.	**K**	K	.	.	.	.	.	M	T	.	.	.	.	.	.	.	.	.	.	E	.	.	.
A/Shanghai/CDC22/2006	D	N	.	.	.	.	.	.	.	.	.	.	**K**	K	.	.	.	.	.	M	T	.	.	.	.	N	.	.	.	.	.	E	R	.	.
A/Shanghai/CDC32/2006	D	N	.	.	.	.	.	.	.	.	.	.	**K**	K	.	.	.	.	.	M	T	.	.	.	.	.	.	.	.	.	.	E	.	.	.
A/Shanghai/CDC37/2006	D	.	.	.	.	.	.	.	.	.	.	I	**K**	K	.	.	.	.	.	R	T	Y	.	D	.	.	.	.	.	.	.	E	.	.	.
A/Shanghai/CDC40/2006	D	.	.	N	.	.	.	.	.	.	.	I	**K**	K	.	.	.	.	V	R	T	Y	.	D	.	.	.	.	.	.	.	E	.	.	.
A/Shanghai/J11/2006	D	.	.	.	.	.	.	.	.	.	.	.	**K**	K	.	.	.	.	.	R	.	.	.	.	I	.	.	.	.	.	.	E	.	.	.
A/Shanghai/MH79/2008	.	.	.	.	R	K	.	.	.	.	.	.	**.**	.	N	.	.	.	.	N	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.
A/Shanghai/MH87/2008	.	.	.	.	.	.	.	.	.	G	.	.	**.**	.	.	.	.	.	N	.	.	.	.	.	.	.	.	V	.	.	.	.	.	.	.
A/Shanghai/MH111/2008	.	.	.	.	.	.	.	.	.	.	.	.	**.**	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
A/Shanghai/MH135/2008	D	N	K	.	.	.	G	R	.	.	.	.	**.**	K	.	.	.	.	.	M	T	.	K	.	.	.	.	.	.	.	.	E	.	.	P
A/Shanghai/MH208/2008	D	N	K	.	.	.	G	R	.	.	.	.	**.**	K	.	.	S	T	.	M	T	.	K	.	.	.	.	.	.	.	.	E	.	.	.

[a]Results are reported as amino acid differences between the HA1 sequences of the isolates and the HA1 sequence of the A/Brisbane/59/2007 vaccine strain.
[b]Antigenic sites are shown [Igarashi et al., 2010].
[c]No change.
[d]Amino acid 140 is indicated in bold.

Strain A/Brisbane/10/2007 exhibited high antigenic correlation with the epidemic strains isolated in 2007, and a relatively low degree of antigenic cross reactivity with isolates circulating in 2008.

The genetic map was generated based on an amino acid distance matrix (Fig. 4). Strains A/Brisbane/10/2007 and A/Ningbo/NB55/2007 were grouped with the epidemic strains isolated in 2008, while they were relatively distant from the other three isolates circulating during 2007. The strains A/Shanghai/MH59/2008 and A/Shanghai/PT7/2008 demonstrated remarkable amino acid variations compared with the other isolates from 2008.

Details concerning amino acid changes in the HA1 domain are shown in Table IV. The HA genes of A/Ningbo/NB55/2007 and circulating strains isolated in 2008 contained the mutation P194L. There were only two amino acid substitutions occurring between A/Shanghai/MH41 (or MH43)/2008 and A/Brisbane/10/2007. The three isolates circulating during 2007 (CDC188, CDC193, CDC199) had the same four mutations (E50G, I140K, R142G, and N144D), and two of the three isolates had an additional amino acid substitution (V112I).

DISCUSSION

The World Health Organization (WHO) annually recommends an influenza vaccine composition for the prevention and control of seasonal influenza. This vaccine composition is based on antigenic and genetic characteristics of circulating viruses, therefore the occurrence of new antigenic changes is of considerable importance in formulating vaccine compositions. In this study, the representative strains of A/H1N1 and A/H3N2 influenza viruses in the Shanghai area from 2005 to 2008 were antigenically and genetically characterized.

Antigenic differences have been detected between circulating A/H1N1 strains isolated in the period 2005–2006, and epidemic A/H1N1 strains isolated in 2008. Despite extensive differences in HA1 sequences, there was a close correlation between the difference in antigenicity of epidemic strains isolated in 2008 compared with circulating strains isolated from 2005 to 2006 and the presence of the amino acid change, K140E (lysine to glutamic acid), in HA1. It was therefore suggested that the single amino acid substitution, K140E, possibly has a strong antigenic effect. This observation is consistent with previous results [WHO Influenza Centre, 2007; Rossi, 2008]. Amino acid 140 is located in a known antigenic site [Igarashi et al., 2010] that straddles the subunit interface of the trimer. It is thought that K140E can be responsible for the antigenic evolution of influenza A/H1N1 viruses isolated around Shanghai in 2008.

The amino acid sequence analyses show that multiple amino acid substitutions occurred between epidemic A/H1N1 strains isolated in 2005 and 2006. These include T82K, Y94H, R145K, R208K, and

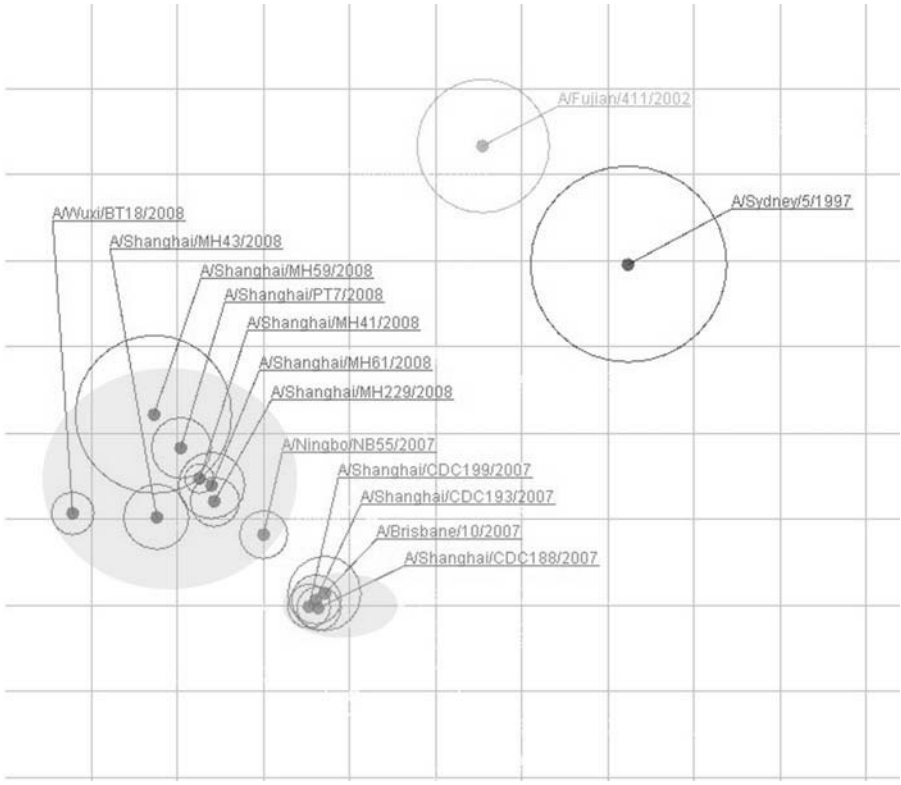

Fig. 3. Antigenic map of influenza A/H3N2 viruses isolated in the Shanghai area from 2007 to 2008. The total error is 111.01 and the average error is 0.57.

T266N; however, these substitutions were not located in antigenic sites. The circulating strains isolated in 2006 were antigenically close to the vaccine strain A/New Caledonia/20/1999 and isolates from 2005. Antigenic analyses demonstrated that the majority of H1N1 viruses isolated in 2008 were antigenically matched to the vaccine strain A/Brisbane/59/2007, except for A/Shanghai/MH79/2008, which is a recent antigenic variant of A/Brisbane/59/2007. Strain A/Brisbane/59/2007 is considered to be capable of providing sufficient immunity against most of the circulating A/H1N1 viruses isolated in 2008 amongst the Shanghai population.

Previous studies have shown that there were significant antigenic differences between epidemic A/H3N2 strains isolated in 2007 and 2008, suggesting that antigenic drift had occurred in the A/H3N2 strains isolated in 2008. The amino acid changes

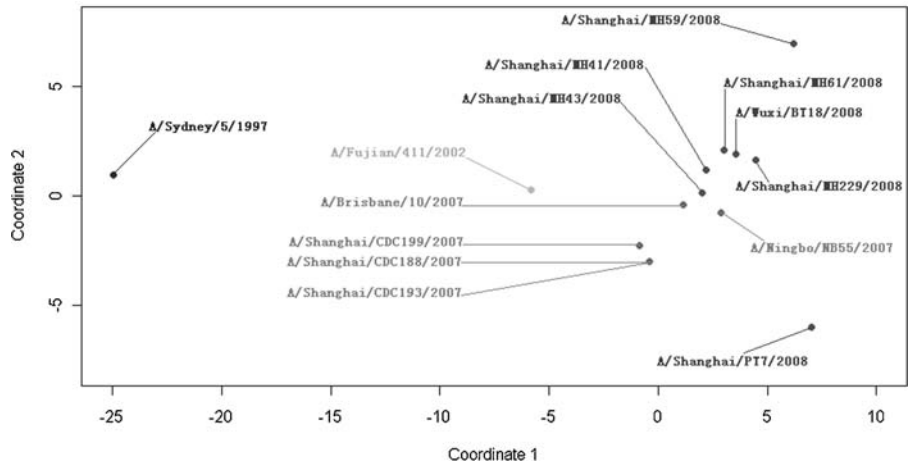

Fig. 4. Genetic map of influenza A/H3N2 viruses isolated in the Shanghai area from 2007 to 2008. The percentage of variance explained by the first two coordinates was 79%.

TABLE IV. Amino acid Changes in HA1 of Influenza A/H3N2 Vaccine Strains and Representative H3N2 Strains Collected From 2007 to 2008

Amino acid position in HA1[a]

Virus strain	3	12	25	50	57	75	83	109	110	112	124	121	137	140	142	144	145	155	156	157
				C[b]	E	E	E	E			A	A	A	A	A	A	A	B	B	B
A/Brisbane/10/2007	L	T	I	E	Q	Q	K	R	S	V	S	T	S	I	R	N	N	T	H	L
A/Fujian/411/2002	.[c]	.	.	G	.	.	.	.	.	.	.	.	.	K	.	.	K	.	.	.
A/Sydney/5/1997	I	.	L	R	R	H	E	.	.	.	.	A	Y	K	S	I	K	H	Q	.
A/Shanghai/CDC188/2007	.	.	.	G	.	.	.	.	.	I	.	.	.	K	G	D	.	.	.	.
A/Shanghai/CDC193/2007	.	.	.	G	.	.	.	.	.	I	.	.	.	K	G	D	.	.	.	.
A/Shanghai/CDC199/2007	.	.	.	G	.	.	.	.	.	.	.	.	.	K	G	D	.	.	.	.
A/Ningbo/NB55/2007	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
A/Wuxi/BT18/2008	F	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
A/Shanghai/MH41/2008	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
A/Shanghai/MH43/2008	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
A/Shanghai/MH59/2008	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
A/Shanghai/MH61/2008	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
A/Shanghai/MH229/2008	.	K	.	.	.	.	.	.	.	.	N	.	.	.	.	.	.	.	.	.
A/Shanghai/PT7/2008	.	.	.	.	H	.	.	M	L	.	.	.	.	.	.	.	.	.	.	S

Virus strain	158	159	171	172	173	182	186	189	192	193	194	202	214	221	222	225	226	227	229	233	234	257	261	269	275	278	280
	B	B	D	D	D	D	B	B	B	B	B		D				D	D	D				E		C	C	C
A/Brisbane/10/2007	K	F	N	E	K	V	G	N	I	F	**P**	I	I	P	R	N	I	P	R	Y	W	Y	R	R	G	N	E
A/Fujian/411/2002	.	Y	.	.	.	.	.	S	.	S	**L**	.	.	.	.	D	V	S	.	.	.	.	.	.	.	.	.
A/Sydney/5/1997	.	Y	.	D	.	.	S	S	T	S	**I**	V	.	.	W	G	.	S	.	H	.	.	.	.	.	.	.
A/Shanghai/CDC188/2007	.	.	.	.	.	.	.	.	.	.	**.**	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
A/Shanghai/CDC193/2007	.	.	.	.	.	.	.	.	.	.	**.**	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
A/Shanghai/CDC199/2007	.	.	.	.	.	.	.	.	.	.	**.**	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
A/Ningbo/NB55/2007	.	.	.	.	.	.	.	.	.	.	**L**	.	.	H	.	.	.	.	.	.	.	.	Q	.	.	.	.
A/Wuxi/BT18/2008	R	.	.	.	N	.	.	.	.	.	**L**	.	.	.	.	.	.	.	.	.	.	.	.	.	S	.	.
A/Shanghai/MH41/2008	.	.	.	.	Q	.	.	.	.	.	**L**	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
A/Shanghai/MH43/2008	.	.	K	.	.	.	.	.	.	.	**L**	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
A/Shanghai/MH59/2008	.	.	.	.	Q	.	.	.	.	.	**L**	.	F	.	.	.	.	.	I	F	L	N	.	K	.	.	.
A/Shanghai/MH61/2008	.	.	.	.	Q	.	.	.	.	.	**L**	.	.	.	.	.	.	.	.	.	.	.	.	K	.	.	.
A/Shanghai/MH229/2008	R	.	.	.	E	.	.	.	.	.	**L**	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
A/Shanghai/PT7/2008	.	.	.	.	.	I	.	.	.	.	**L**	.	.	.	.	.	.	.	.	.	.	.	Q	.	.	I	D

[a]Results are reported as amino acid differences between the HA1 sequences of the isolates and the HA1 sequence of the A/Brisbane/10/2007 vaccine strain.
[b]Antigenic sites are shown [Ndifon et al., 2009].
[c]No change.
[d]Amino acid 194 is indicated in bold.

revealed a close correlation between the antigenicity of circulating strains isolated in 2008, including A/Ningbo/NB55/2007, and the presence of the substitution P194L (proline to leucine), which indicates that this particular single amino acid induces antigenic drift. Amino acid 194 resides in the receptor binding site of the HA protein, and is also located within proposed antigenic site B on the H3 HA molecule [Ndifon et al., 2009]. It was identified as being under positive selection by Bush et al. [1999]. Therefore, P194L was thought to be responsible for the antigenic evolution of influenza A/H3N2 viruses isolated around the Shanghai area in 2008.

The three A/H3N2 isolates circulating during 2007 (CDC188, CDC193, CDC199) exhibited four amino acid differences (E50G, I140K, R142G, and N144D) in two antigenic sites of the HA1 molecule compared with A/Brisbane/10/2007; however, they were still closely related, on an antigenic basis, to the vaccine strain A/Brisbane/10/2007. Nevertheless, the antigenicity of circulating strains isolated in 2008 had already drifted from the present vaccine strain A/Brisbane/10/2007, suggesting that the influenza A/H3N2 vaccine component needs to be updated.

Sequencing allows for the rapid and sensitive detection of minor genetic changes, but some genetic mutations may exert a disproportionately antigenic effect [Smith et al., 2004; McHardy and Adams, 2009], as our results demonstrated. Antigenicity is the primary criterion for influenza vaccine strain selection [Redlberger et al., 2007]. Careful surveillance of antigenic and genetic changes in the hemagglutinin proteins can facilitate the rapid identification of newly emerging strains with epidemiological significance and the choice of appropriate influenza vaccine composition.

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