—Preliminary Note—

Genomic analyses of influenza A (H3N2) viruses from Japan and Thailand, in the period of 1979 to 1981

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A/Bangkok (BKK)/1/79-like virus has been a representative epidemic or prevailing virus of Hong Kong (H3N2) subtype of type A influenza virus in the world¹⁻¹⁷⁾. In the rainy season (August) of 1980, two influenza virus strains, A/BKK/137 and 138/80 were isolated in Thailand¹²⁾, which were antigenically similar to A/Kyoto/C-1/81 that was isolated in February, and A/Kyoto/C-1/81-like virus became the major epidemic virus in Japan in the winter of 1981 to 1982¹⁵⁻¹⁸⁾. A/BKK/211/81 was isolated in January, which was antigenically similar to A/Niigata/102/81 that was isolated in March in 1981 and A/Niigata/102/81-like virus did not make an epidemic virus but only a few strains of the variant was isolated in Japan in winter of 1981 to 1982^{12, 15-18)}, though it was employed as a virus for manufacturing the inactivated influenza vaccine in Japan for the period.

Genetic changes in A/BKK/1/79, A/Niigata/102/81, A/Kyoto/C-1/81, and A/BKK/211/81 was followed by comparing the oligonucleotide pattern of the entire virus genome. Ribonuclease T1-resistant oligonucleotide mapping of RNA was done as described previously¹⁹⁾. Viruses were grown in chorioallantoic cavity of 10-day old chick embryo, and purified with one cycle of ultra-centrifugations on 30~60% sucrose cushion and in 30~60% sucrose density gradient at 24,000 rpm for 90 minutes with Beckman-Spinco SW 28 model. Viral RNAs were solubilized with SDS in the presence of proteinase and extracted with phenol at 56°C. Total viral RNAs were digested with ribonuclease T1. The resulting oligonucleotides were 5'-end labeled with [γ -³²P]-adenosine triphosphate, using a T4 polynucleotide kinase, and separated by two-dimensional gel electrophoresis and autoradiographic study were performed as described previously^{21, 22)}.

As described in **Fig. 1** and **Table 1**, RNA oligonucleotide mapping studies showed that there were 15 spot differences between the map of A/BKK/1/79 and either of those of A/Niigata/102/81 and A/Kyoto/C-1/81, which may be somewhat greater than expected from genomic evolution of a period of two years²⁰⁾. There were only four spot differences between maps of the two Japanese isolates. Oligonucleotide mapping study of A/BKK/

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Fig. 1 Oligonucleotide maps of the RNAs of A/BKK/1/79 (A), A/Niigata/102/81 (B), A/Kyoto/C-1/81 (C), and A/BKK/211/81 (D)

Spots on the diagram of A/BKK/1/79 were numbered 1 to 53 in the order of the molecular size. In the diagrams B, C, and D, closed spots point out oligonucleotides present only in the second of the two viruses, while open circles nominated alphabetically indicate oligonucleotides present only in the first of two: B, A/BKK/1/79 vs. A/Niigata/102/81 (15 differences); C, A/BKK/1/79 vs. A/Kyoto/C-1/81 (15 differences); D, A/BKK/1/79 vs. A/BKK/211/81 (22 differences).

Table 1 RNA oligonucleotide finger prints of H3N2 viruses

Virus	Spot										Numbers of spots different from		
	1	2	3	4	5	21	23	36	37	38	Bangkok/79	Niigata/81	Kyoto/81
A/Bangkok/1/79	+	+	+	+	+	+	+	+	+	+	0		
A/Niigata/102/81	-	+	+	+	-	-	+	+	+	+	15	0	
A/Kyoto/C-1/81	-	+	+	+	-	-	+	+	+	+	15	4	0
A/Bangkok/211/81	-	+	+	-	-	-	-	-	-	-	22	19	17

Virus	Spot																			
	a	b	c	d	e	f	g	h	i	j	k	l	m	n	o	p	q	r	s	t
A/Bangkok/1/79	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A/Niigata/102/81	+	-	+	+	-	+	+	-	-	-	+	+	-	-	-	+	+	+	+	+
A/Kyoto/C-1/81	+	-	+	+	-	+	+	-	-	-	+	+	-	-	+	+	+	+	+	-
A/Bangkok/211/81	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	-	-	-	-	-

Spots 6~20, 22, 24~35, and 39~53 were detected in all virus strains tested.

211/81 showed that there were 22 spot differences compared to the map of A/BKK/1/79 and 17 to 19 spot differences from those of the two Japanese viruses isolated in the same year.

From these results it is postulated that A/BKK/211/81 might represent a recombinant deriving several genes from a H1N1 epidemic strains which prevailed in Thailand^{12,14} and H3N2 influenza which were isolated in Japan (Niigata/81 and Kyoto/81) and in Thailand (BKK/102/81) in the early winter of 1981, clearly originated from different sources. Recombinant H1N1 viruses have been widely distributed in the world²³. Analysis with the HAI reactions by using mouse monoclonal antibodies showed close antigenic similarities between A/Niigata/102/81 and A/BKK/211/81, and apparent antigenic differences between A/Niigata/102/81 and A/Kyoto/C-1/81¹⁴⁻¹⁶. However, the HAI reactions with postinfection ferret sera did not always recognize the antigenic difference between A/Niigata/102/81 and A/Kyoto/C-1/81²³. Although monoclonal antibodies are capable of making further detailed distinctions between closely related viruses, only a limited number of epitopes on the hemagglutinin (HA) could be practically tested with monoclonal antibodies. Postinfection ferret serum may not always include antibodies to all the antigenic determinants of the infecting virus HA. The evidence for individual differences in the antibody response has been provided in natural infections with influenza virus²⁴. Assignment of oligonucleotide spots of entire viral RNA to gene segments and determination of epitopes recognized by the mouse monoclonal antibodies will further clarify the origin of influenza virus variants studied in this report.

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