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Heterogeneity Among Influenza H3N2 Isolates Recovered During an Outbreak

Brief Report

By

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With 2 Figures

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Summary

Isolates of the H3N2 subtype recovered during an outbreak have been analyzed at the genomic and protein level. No reassortant genomes were detected. By T1-oligonucleotide fingerprinting of RNA segments coding for hemagglutinin (HA), nucleoprotein (NP), and neuraminidase (NA) two genotypes were found.

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Antigenic variability is a characteristic property of influenza and many other RNA viruses. From epidemiological point of view two patterns of influenza evolution have been obersved. One in which a virus spreads from one area to another, and the other, more frequently observed, in which local epidemics caused by similar viruses develop at the same time in widely separated regions (1). The first pattern is associated to sudden changes in the structure of external antigens (antigenic shift) probably due to the existence of a virus gene pool in a variety of animals. The second is related to continuous and more subtle changes in antigenicity (antigenic drift). Since long these changes have been demonstrated for the external antigens hemagglutinin (HA) and neuraminidase (NA) and more recently for internal virion proteins, nucleoprotein (NP) and matrix protein (M1) (2). These changes originate in a general genetic variability of influenza virus RNA that leads to a continuous accumulation of point mutations in all viral genes

(3, 4, and 5). Genetic variability can be amplified by reassortment among co-circulating strains (6), specially since 1977, when H1N1 subtype virus was reintroduced in man and co-circulated with viruses of the H3N2 subtype.

Following our initial studies on the genetic variation of influenza virus field isolates (4), we have analyzed the virus obtained during one outbreak in the 1980—1981 season, regarding antigenic characterization of circulating strains, the presence or absence of reassortant viruses among the isolates and their antigenic and genetic heterogeneity.

During the 1980—1981 season, only mild influenza activity was detected in Madrid province; eighteen isolates were obtained from one outbreak in the period December 1980 to February 1981. All virus strains isolated were characterized as of H3N2 subtype by hemagglutination inhibition tests (7). The absence of circulation of B type or H1N1 subtype viruses in this season was confirmed by a serological survey carried out with sera covering all age groups collected at the beginning and the end of the period (data not shown). The serological characterization of the isolates indicates that they are similar to A/Bangkok/79 (KB) but with a clear cross-reaction to A/Texas/77 (Tex), a common feature presented by many isolates of that year (8). They are also antigenically heterogeneous (Table 1), as has been shown for other outbreaks of type A or B influenza viruses (10, 11, 12, 13).

Table 1. *Serological characterization of influenza virus isolates*

Strains	Isolation date	Reference Ferret Antisera			
		Vict[a]	Tex	BK	BK-2
Reference virus					
A/Victoria/3/75		*320*[c]	160	80	80
A/Texas/1/77		160	*1280*	640	640
A/Bangkok/1/79		160	320	*2560*	640
A/Bangkok/2/79		40	320	640	*5120*
Field isolates					
A/Madrid/9492/80[b]	2/12/80	40	640	640	160
A/Madrid/9688/80	11/12/80	40	320	640	320
A/Majadahonda/9689/80	11/12/80	40	320	640	320
A/Majadahonda/9744/80	15/12/80	40	320	640	320
A/Majadahonda/716/81	26/01/81	160	320	1280	320
A/Majadahonda/1088/81	9/02/81	40	640	1280	640

[a] Abbreviations for strains: Vict, A/Victoria/3/75; Tex, A/Texas/1/77; BK, A/Bangkok/1/79; BK-2, A/Bangkok/2/79

[b] Field isolates will be named only by its isolation number: 9492, 9688, 9689, 9744, 716 and 1088

[c] HI titers are the inverse of the sera dilution able to inhibit four hemagglutinating units of the analyzed virus

To investigate the presence of H3N2 subtype viruses with a reassortant genotype, similar to those described by YOUNG and PALESE (6), viral RNA was analyzed by polyacrylamide-urea gel electrophoresis (14), after *in vivo* labelling with ^{32}P-phosphate (4). The mobility of every RNA segment of

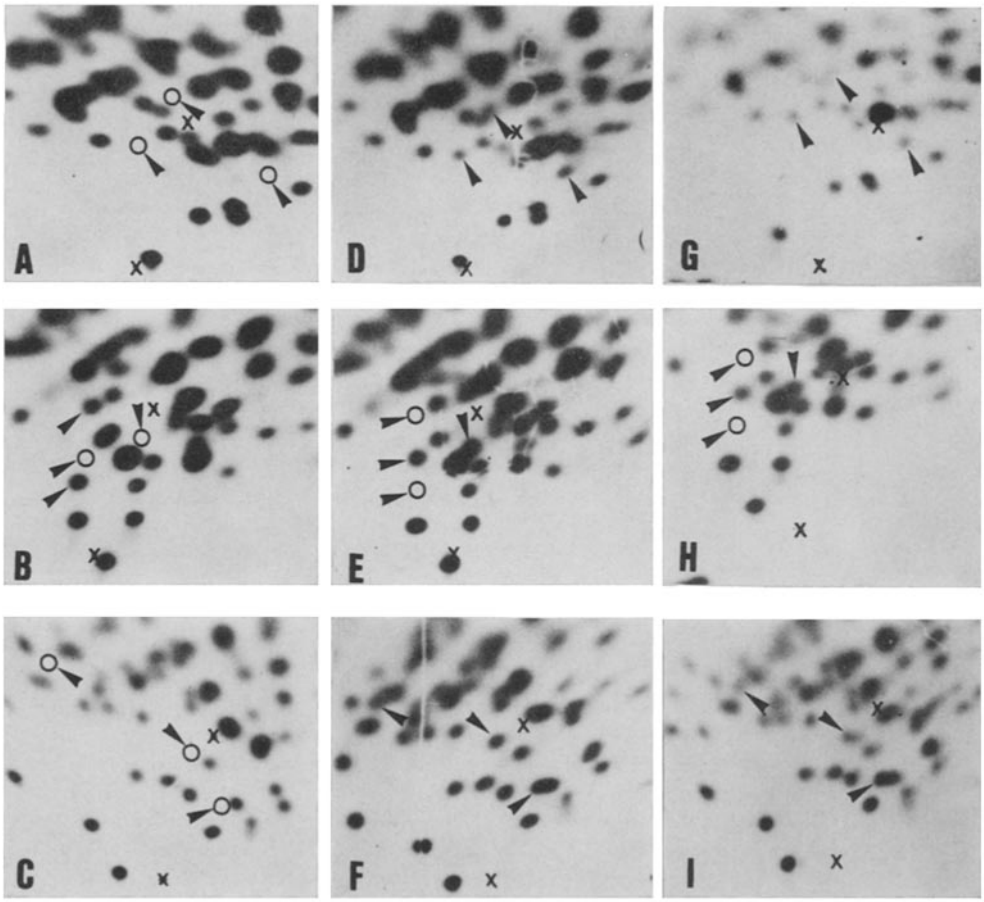

Fig. 1. T1-oligonucleotide fingerprinting of HA, NP and NA RNA segments from isolates and BK reference strains. vRNA labelled *in vivo* with ^{32}P-phosphate was hybridized to the corresponding cDNA clones (4); the eluted material was processed for oligonucleotide fingerprinting (15); Xs shows the position of Xylene-cyanol and bromophenol-blue dye markers; arrow-heads show the unique oligonucleotides, and open circles the missing oligonucleotides. *A* HA of 9744 virus; *B* NP of 9744 virus; *C* NA of 9492 virus; *D* HA of 9688 virus; *E* NP of 9688; *F* NA of 9688; *G* HA of BK; *H* NP of BK; *I* NA of BK. The HA map of 9744 viruses (Fig. A) is indistinguishable of those of 9492, 9689, 716 and 1088; the NP map of 9744 (Fig. B) is indistinguishable of 9492, 9689, 716 and 1088; the NA map of 9492 (Fig. C) is indistinguishable of 9689, 9744, 716 and 1088. The 9688 virus shows maps identical to the BK reference strain in the three genes HA (Fig. D and G), NP (Fig. E and H) and NA (Fig. F and I). The 9688 viruses shows 3—4 differences with other field isolates in the HA, NP and NA compare Fig. A and D, B and E and C and F

virus isolates was indistinguishable of those of reference strain BK (data not shown). The absence of reassortant viruses among isolates was confirmed by analysis of *in vivo* ^{35}S-Met labelled viral proteins by SDS-polyacrylamide gel electrophoresis according to Laemmli (9) (data not shown).

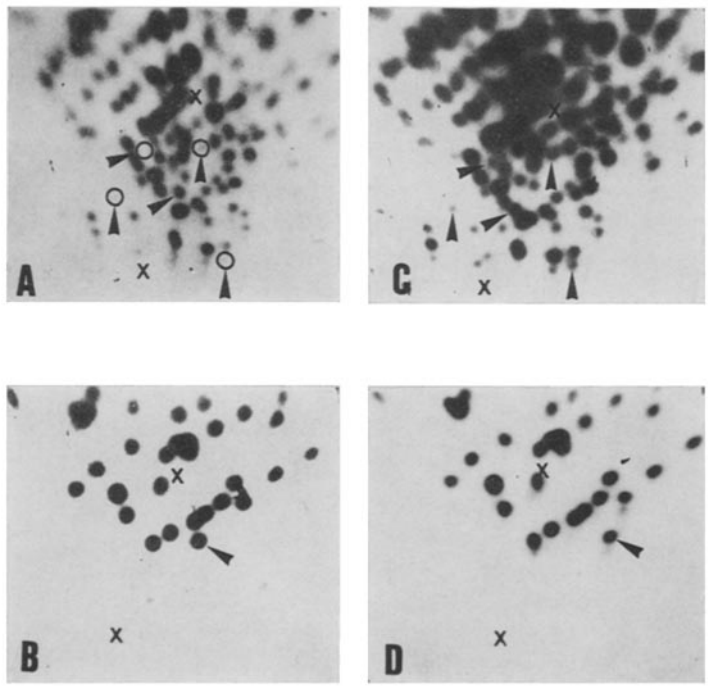

Fig. 2. Comparison of Polymerases and non structural protein RNA fragments of BK and 9688 viruses. ^{32}P-vRNA was separated by polyacrylamide-urea gel electrophoresis, and Px and NS RNA fragments were eluted from the gels (4) and processed for oligonucleotide fingerprinting (15). Symbols are as in Fig. 1. *A* Px of BK virus; *B* NS of BK; *C* Px of 9688 and *D* NS of 9688. The Px maps of BK and 9688 differs in 5 oligonucleotides (Fig. A and C), and the NS maps of BK and 9688 shows one oligonucleotide difference (Fig. B and D)

The genetic heterogeneity of virus isolates was further analyzed by T1-oligonucleotide fingerprinting (15) of individual genomic RNA segments. Isolate 9688 shows oligonucleotide maps for HA, NP and NA RNA segments indistinguishable from those of reference strain BK. The corresponding maps of isolates 9492, 9689, 9744, 716 and 1088 show 3—4 differences with BK strain (Fig. 1). Thus, two genotypes were detected among cocirculating viruses, with distinguishable HA, NP and NA RNA segments. Since 20—30 per cent of the sequences are screened in the oligonucleotide maps of individual genes, the variations detected would represent mutations at 0.6—0.8 per cent of the RNA residues, assuming that each map change is caused by a point mutation. Isolate 9688, however differed from BK strain

in several mutation in the non structural protein (NS) and the polymerases (Px) RNAs (Fig. 2). The number of oligonucleotide variations suggests differences in 0.3—0.7 per cent of the RNA residues.

Genetic heterogeneity among cocirculating influenza viruses has been previously detected by RNA, polypeptide migration analysis (16) and T1 oligonucleotide fingerprinting of viral RNA (1, 4, 18, 19). Antigenic heterogeneity among viruses recovered within s ngle influenza epidemics has also been detected by monoclonal antibody analysis (12, 13, 20). In the present report, variations in the internal and external protein-coding genes of viruses isolated during and epidemic outbreak have been detected by T1 fingerprinting of individual gene segments. This is in agreement with the suggestion of a general RNA variability in this virus (3, 4, 5). The origin of the different genotypes found is not known but its detection may be relevant to the design of idoneous vaccines. Furthermore, the inclusion of a continuous and systematic analysis of the complete genome of viruses that are circulating could be adequate in epidemiological surveillance systems, in search for a possible relation between biochemical changes and different biological properties of viruses.

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