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On the Origin of the H1N1 (A/USSR/90/77) Influenza Virus

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SUMMARY

The influenza virus H1N1 (the A/USSR/90/77 strain) that reappeared in 1977 after the H1N1 influenza viruses had disappeared from the human population, is compared with the A/FM/1/47 and the A/FW/1/50 influenza viruses by the method of oligonucleotide mapping of individual segments of the viral RNAs. Seven genes of the A/USSR/90/77 virus appear to be very similar to the corresponding genes of the A/FW/1/50 virus, whereas the gene coding for the M protein displays considerable homology to the corresponding gene of the A/FM/1/47 virus. The data demonstrate that the A/USSR/90/77 strain is a recombinant virus.

The return of the H1N1 virus in 1977 after a 20 year absence from circulation of the H1N1 viruses among the human population, raises the question of its origin. The H1N1 (A/USSR/90/77) virus appears to be antigenically closely related to the A/FW/1/50 virus that was circulating in the 1950s and less related to viruses that were circulating in the 1940s or in the mid-1950s (Kendal *et al.*, 1978; Zhdanov *et al.*, 1978). The origin of the 1977 H1N1 virus has been explained by its preservation in human or animal reservoirs or re-introduction of the virus into the human population from storage in an ice-box (Nakajima *et al.*, 1978; Scholtissek *et al.*, 1978).

To find an answer to this question, we compared the structures of genes of the A/USSR/90/77 virus to those of the A/FW/1/50 and the A/FM/1/47 viruses which belong to the same serotype (H1N1) by the method of oligonucleotide mapping. The three influenza virus strains were designated as USSR (A/USSR/90/77), FM (A/Fort Monmouth/1/47) and FW (A/Fort Warren/1/50) (Chanock *et al.*, 1971).

Viruses were grown in fertile hens' eggs, purified by centrifugation in a sucrose density gradient and RNA was extracted with SDS–phenol (Palese & Schulman, 1976). The virus RNA segments were separated by electrophoresis in 1·6% agarose (Locker, 1979) or 2·8% polyacrylamide gels (Palese & Schulman, 1976) containing urea. The genes coding for P1 and P3 proteins could not be well separated and therefore they were analysed together. The RNA segments eluted from the gel were treated with RNase T1, the resulting oligonucleotides were labelled with ^{32}P and subjected to two-dimensional electrophoresis in polyacrylamide gel (see legend to Fig. 1). This system gives an optimal resolution for oligonucleotides over ten bases long and thus allows a considerable part of sequences of each gene to be analysed.

Fig. 1 presents fingerprints of M (*a*) and NP (*b*) genes of the three influenza strains studied and diagrams to reflect the distribution of the analysed oligonucleotides. The fingerprints of NP genes of the USSR and FW viruses are very similar but differ considerably from that of FM virus. In contrast, the M genes of the USSR and FM viruses are quite similar, whereas they differ from the FW virus gene. The fingerprints of the other six genes are not presented in this paper.

Analysis of fingerprints of all eight genes is given in Table 1. Every gene is analysed pairwise: USSR with FW and USSR with FM viruses. The table shows that the oligonucleotides analysed varied from 28 to 66% of the length of the corresponding genes.

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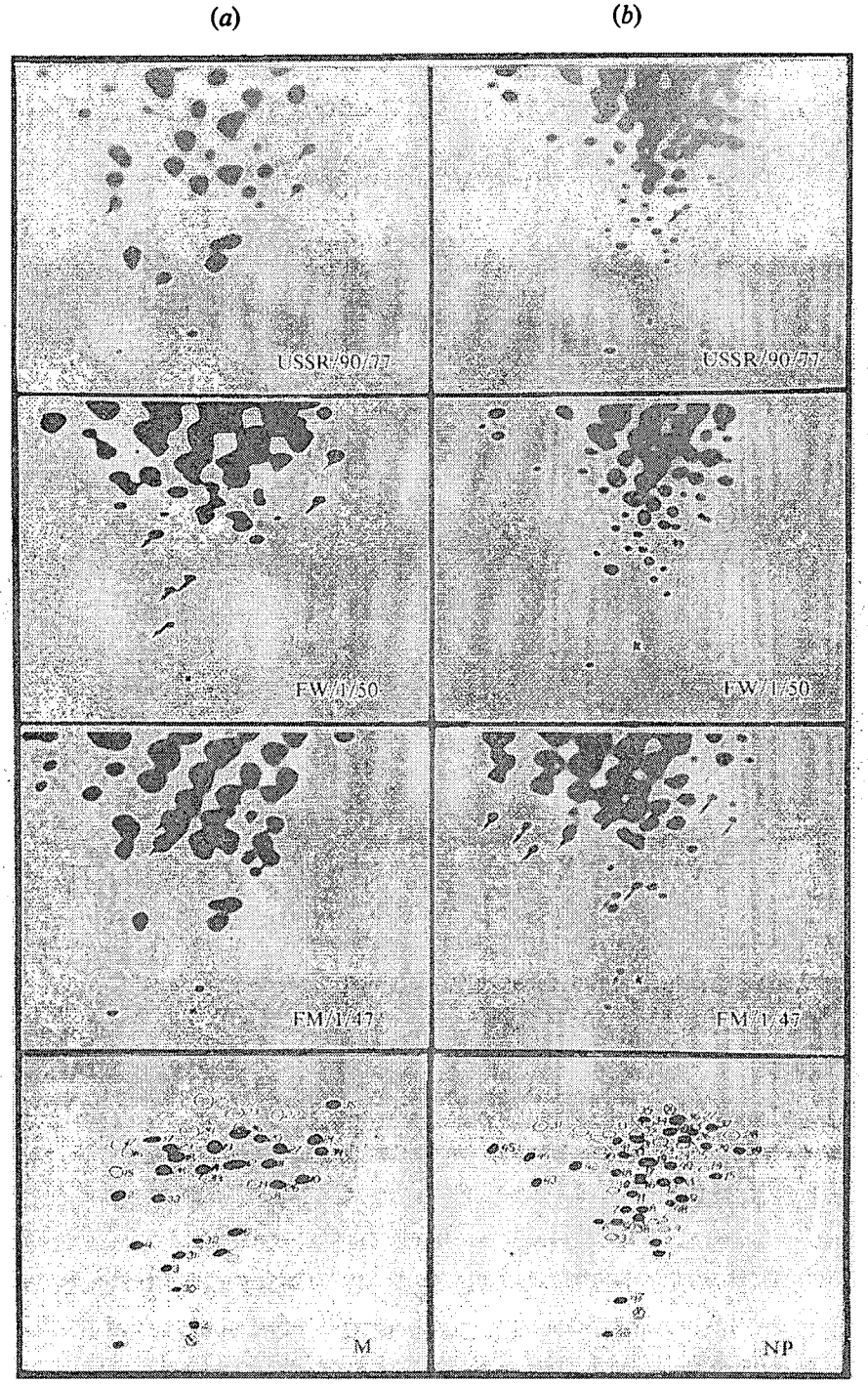

Fig. 1. Fingerprints of M (*a*) and NP (*b*) genes of USSR, FM and FW influenza viruses. A 1 μg amount of RNA was hydrolysed with T1 RNase in the presence of *Escherichia coli* alkaline phosphatase and resulting oligonucleotides were treated with polynucleotide kinase in the presence of γ-^{32}P-labelled ATP by the method of Efstratiadis *et al.* (1977). Two-dimensional electrophoresis in polyacrylamide gel was carried out by the method described by Pedersen & Haseltine (1980). Electrophoresis in the first dimension (left to right) was at pH 3·5 in 10% gel containing 25 mM-citrate and 6 M-urea at 1700 V for 2 h; in the second dimension (bottom to top) in 22·8% gel in 50 mM-tris–borate pH 8·3, 1 mM-EDTA

Table 1. *Analysis of fingerprints of RNA segments of USSR, FW and FM influenza viruses*

RNA segment*	Pairs of viruses compared	Corresponding segment analysed (%)†	Distribution of oligonucleotides			Minimal number of base changes	
			Common	(USSR⁺)‡	(USSR⁻)§	No.‖	%¶
P1 + P3	USSR/FW	27·5	72	12	6	15	1·05
	USSR/FM		55	29	22	40	2·80
P2	USSR/FW	34·5	56	1	3	3	0·35
	USSR/FM		45	12	15	21	2·50
HA	USSR/FW	29·5	28	7	10	13	2·30
	USSR/FM		18	17	13	23	4·00
NA	USSR/FW	42·5	41	3	8	9	1·50
	USSR/FM		33	11	11	16	2·60
NP	USSR/FW	38·0	36	2	1	2	0·33
	USSR/FM		15	23	12	29	4·80
M	USSR/FW	48·0	10	15	8	19	4·80
	USSR/FM		21	4	4	6	1·50
NS	USSR/FW	66·0	24	5	3	6	1·40
	USSR/FM		21	8	9	13	3·00

* P1 + P3, Genes coding for the P1 and P3 proteins; P2, P2 protein; HA, haemagglutinin; NA, neuraminidase; NP, nucleoprotein; M, matrix protein; NS, non-structural protein.

† The mol. wt. of influenza virus RNA segments obtained by Desselberger & Palese (1978) were used. The length of the analysed oligonucleotides was measured by alkaline hydrolysis of the eluted oligonucleotides followed by electrophoresis in polyacrylamide gels.

‡ USSR⁺, Oligonucleotides are present in the USSR strain RNA segments but not in the corresponding RNA segments of FW or FM viruses.

§ USSR⁻, Oligonucleotides are present in FW or FM strain RNA segments but not in the corresponding RNA segments of the USSR virus.

‖ According to the calculations by Nakajima *et al.* (1978).

¶ Percentage of the minimal number of base changes in the analysed part of the segments. This value is the number of base changes per 100 bases of compared sequences and characterizes the divergency of the genes compared.

The relative amount of base substitutions in the USSR/FW pairs of genes was rather low for P1 + P3, P2 and NP genes (0·3 to 1·05%) and considerably higher for HA, NA and NS genes (1·4 to 2·3%). It was rather high for the corresponding genes of the USSR/FM pair (2·5 to 4·8%) with one exception: the M gene of the USSR virus was close to that of the FM virus (1·5%) but not to the FW virus (4·8%).

The results demonstrate that the USSR influenza virus displays a higher degree of similarity to the FW influenza virus in the structure of P1 + P3, P2 and NP genes and a lower degree in that of HA, NA and NS genes. In contrast, M genes display a higher degree of homology in the USSR and the FM viruses. It should be noted that this protein is the most stable in influenza viruses (Dimmock *et al.*, 1980), whereas others undergo variations resulting in the antigenic drift of the haemagglutinin and neuraminidase proteins (Webster & Laver, 1975). However, changes at the genomic level may not always result in change of amino acid sequence. Using this method Palese and co-workers analysed only 7·6% (Nakajima *et al.*, 1978) or 10·2% (Young *et al.*, 1979) of the total viral genome. In our work we have analysed from 28% up to 66% of the individual segment of the virus genome.

Based on our results, the A/USSR/90/77 virus should be considered not as a conserved form of the A/FW/1/50 virus stored in human or animal populations or elsewhere as was

at 1000 V for 7 h. The gels were autoradiographed. Homogeneous oligonucleotides are numbered; white spots in the diagrams represent the oligonucleotides common for all the three influenza strains. The arrows indicate the oligonucleotides unique for a given strain. The positions of the dye markers xylene cyanol FF and bromophenol blue are denoted (X). In the quantitative analysis only good, clear intensive spots which were reproduced from one experiment to another constantly were included.

suggested by Palese and co-workers (Nakajima *et al.*, 1978; Young *et al.*, 1979) but as a recombinant virus whose genes originate from at least two parental strains. When and where such recombination has occurred and how this recombinant virus has been introduced into the human population remains to be elucidated.

It has been shown recently that later H1N1 strains isolated during the winter of 1978/1979 significantly differ from that of 1977 and are recombinant viruses, having several genes (P1, P2, P3 and NP) derived from an H3N2 virus (Young & Palese, 1979). Our data further suggest that genetic variation in influenza strains of the same serotype is not restricted to mutational changes, but may involve recombination (reassortment).

[1]*Institute of Molecular Biology*
The U.S.S.R. Academy of Sciences
Vavilova St. 32, Moscow 117984

J. V. KOZLOV[1]
V. G. GORBULEV[1]
A. G. KURMANOVA[1]
A. A. BAYEV[1]

[2]*The D. I. Ivanovsky Institute of Virology*
The U.S.S.R. Academy of Medical Sciences
Gamaleya St. 16, Moscow 123098, U.S.S.R.

A. A. SHILOV[2]
V. M. ZHDANOV[2]*

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