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ANALYSIS OF TS MUTATIONS OF COLD-ADAPTED INFLUENZA A/LENINGRAD/134/57 VIRUS VARIANTS

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Summary. — By recombination of ts mutants of fowl plague virus belonging to different complementation groups with two cold-adapted variants of human influenza virus, the number and gene localization of ts mutations occurring in these variants was determined. In the course of passaging of human influenza virus at lowered temperature, the number of genes with ts mutations increased.

Key words: Orthomyxovirus; human influenza virus; fowl plague virus; ts mutants; recombination

Introduction

The existence of well known ts mutants in which genes with mutation damage have been identified makes it possible to evaluate the number and localization of ts mutations in the genome of cold-adapted influenza virus strains by the recombination test. The methodical approaches used in such investigations as elaborated by Ghenkina and Ghendon (1979) are based on the use of a collection of ts mutants of fowl plague virus (FPV) in which the genes with mutation damage and proteins induced by them have been identified. The method is based on the recovery of the ts phenotype in ts mutants of FPV due to an exchange of a mutant gene for a non-mutant gene on recombination with the test strain of influenza virus. Recombination is carried out under conditions under which either of the crossed partners is unable to produce infectious virus (Ghenkina and Ghendon, 1979). Based on the detection of ts mutants of FPV which are unable to undergo recombination with the test strain of influenza virus it is possible to determine which genes of the test virus carry these mutations. The method was used by Ghendon *et al.* (1981) to localize ts mutations in genes of two cold-adapted strains of human influenza virus. We are reporting the results concerning the number and gene localization of mutation damages in two cold-adapted variants of influenza virus A/Leningrad/134/57 (H2N2).

The first variant, A/Leningrad/134/17/57, had undergone 21 passages in chick embryos at the optimal temperature of 33 °C and 17 additional passages at 25 °C. This variant is characterized

Table 1. Recombinants of FPV ts mutants with cold-adapted variants of human influenza virus

ts mutant	Mutant gene	Mutant protein	PFU/ml in CEC cultures infected with							
			FPV mutants and human influenza virus						FPV mutants alone	
			A/Len./134/17/57		A/Len./134/47/57		A/Krasn./101/59			
			36 °C	42 or 40 °C	36 °C	42 or 40 °C	36 °C	42 or 40 °C	36 °C	42 or 40 °C
29	1	P3	8×10^7	2×10^3	3×10^7	$<10^2$	1×10^7	3×10^6	2×10^7	$<10^2$
131	2	P1	9×10^7	2×10^7	2×10^5	$<10^2$	4×10^5	1×10^4	1×10^5	$<10^2$
166	3	P2	1×10^7	8×10^5	5×10^6	1×10^5	3×10^6	1×10^5	3×10^6	$<10^2$
US1	5	NP	1×10^7	$<10^3$	1×10^6	$<10^2$	2×10^6	3×10^5	1×10^6	$<10^2$
303/1	7	M	3×10^7	$<10^3$	6×10^5	$<10^2$	6×10^5	6×10^3	5×10^5	$<10^2$
MN3	8	NS	7×10^7	3×10^6	1×10^6	$<10^2$	8×10^5	3×10^4	2×10^6	$<10^2$

by a marked ts phenotype (the difference in infectious virus titres in chick embryos at 32 and 40 °C reached 7.5 log EID_{50}/0.2 ml), is areactogenic in children (Alexandrova and Smorodintsev, 1965) and proved to be a good donor of attenuation in obtaining recombinant vaccine strains of live influenza vaccine for adults (Aleksandrova *et al.*, 1979). The second variant, A/Leningrad/134/47/57, represents the A/Leningrad/134/17/57 virus after an additional 30 passages at 25 °C. The two variants, A/Leningrad/17/57 and A/Leningrad/47/57, multiply well in chick embryos at 33 °C and do not differ from one another in the ts phenotype: their reproduction in chick embryos at nonpermissive temperature was reduced by 7.0—7.5 log EID_{50}. In recombination experiments we used ts mutants of FPV belonging to 6 complementation groups. Several of these ts mutants (29, 131, 166 and 303/1) were obtained by Markushin and Ghendon (1973); they carry ts mutations in genes 1, 2, 3 and 7, respectively (Ghendon and Markushin, 1980). Two other ts mutations (US1 and MN3), kindly supplied by Dr. B. W. J. Mahy (Cambridge University, U. K.), carry mutations in genes 5 and 8, respectively (Almond *et al.*, 1979).

Recombination experiments were carried out by the method described by Ghenkina and Ghendon (1979). Chick embryo cell (CEC) cultures were infected with the test variants of human influenza virus at a multiplicity of 1—2 EID_{50} per cell. After 30 min adsorption at 20 °C, the influenza virus inoculum was decanted and the cultures were challenged with ts mutants of FPV in doses from 10^1 to 10^6 plaque forming units (PFU) per culture. After 30 min adsorption at 20 °C, the cells were washed and overlaid with agar overlay. The plaques were counted after incubation for 3 days at 36, 40 and 42 °C (the nonpermissive temperature for ts mutants US1, MN3 and 303/1 is 40 °C, that for ts mutant 29, 131 and 166 is 42 °C). As control, FPV ts mutants were titrated at 36, 40 and 42 °C in the absence of human influenza virus. A control recombination test of the ts mutants with human influenza virus A/Krasnodar/101/59 (H2N2) was also included. With this virus, FPV mutants belonging to different complementation groups, formed recombinants at a high frequency (Ghenkina and Ghendon, 1979).

The results summarized in Table 1 show that the influenza virus variant A/Leningrad/134/17/57 on crossing with FPV ts mutants 131, 166 and MN3 produced plaque-forming recombinants. It practically did not undergo genetic interactions with ts mutants 29, US1 and 303/1, carrying mutations in genes 1, 5 and 7 which are coding for P3, NP and M proteins, respectively.

The virus variant A/Leningrad/134/47/57 formed no recombinants with 5 of 6 FPV ts mutants tested, namely with mutants 29, 131, US1, 303/1 and MN3, carrying mutations in genes 1, 2, 5, 7 and 8, coding for P3, P1, NP, M and NS proteins, respectively. These results suggest that mutations had occurred in the genome of this variant of human influenza virus.

Long-term passaging of influenza virus A/Leningrad/134/57 in chick embryos at 25 °C thus led to an accumulation of ts mutants in its genome; after 47 passages mutations were detected in 5 of 6 genes coding for non-glycosylated virus proteins.

References

Aleksandrova, G. I., Garmashova, L. M., Golubev, D. B., Polyak, R. Ya., Medvedeva, T. E., Polezhaev, F. I., Polyakov, Yu. M., Rumovsky, V. I., and Smorodintsev, A. A. (1979): Attempt at selection of innocuous thermosensitive recombinants of influenza A virus. *Vop. Virus.* **24**, 342—346.

Alexandrova, G. I., and Smorodintsev, A. A. (1965): Obtaining of an additionally attenuated vaccinating cryophyl influenza strain. *Rev. Roum. Inframicrobiol.* **2**, 179—186.

Almond, J. W., McGeoch, D., and Barry, R. D. (1979): Temperature-sensitive mutants of fowl plague virus: isolation and genetic characterization. *Virology* **92**, 416—427.

Ghendon, Y. Z., and Markushin, S. G. (1980): Studies on mutation lesions and physiology of fowl plague virus ts mutants. *Philos. Trans. roy. Soc.* **288**B, 383—392.

Ghendon, Y. Z., Klimov, A. I., Alexandrova, G. I., and Polezhaev, F. I. (1981): Analysis of genome composition and reactogenicity of recombinants of cold-adapted and virulent virus strains. *J. gen. Virol.* **53**, 215—224.

Ghenkina, D. B., and Ghendon, Yu. Z. (1979): Recombination and complementation between orthomyxoviruses under conditions of abortive infection. *Acta virol.* **23**, 97—106.

Markushin, S. G., and Ghendon, Yu. Z. (1973): Genetic classification and biological properties of temperature-sensitive mutants of fowl plague virus. *Acta virol.* **17**, 369—376.