Acta virol. **27** : 434—438, 1983

HIGH REPRODUCTION CAPACITY OF RECOMBINANTS BETWEEN H3N2 HUMAN INFLUENZA AND FOWL PLAGUE VIRUSES IS DUE TO THE GENE CODING FOR M PROTEINS

A. KLIMOV, Y. GHENDON, *H. ZÁVADOVÁ, *J. BROUČEK, **T. MEDVEDEVA

Moscow Research Institute of Viral Preparations, 109088 Moscow, U.S.S.R.; *Institute of Sera and Vaccines, Prague, Czechoslovakia; and **Institute of Experimental Medicine, Leningrad, U.S.S.R.

Received July 22, 1982; revised January 18, 1983

Summary. — Recombinants between H3N2 human influenza viruses (A/Victoria/3/75 and A/Bangkok/1/79, low-yielding parents in chick embryos) and fowl plague virus (FPV, a high-yielding parent in chick embryos) have been obtained. The high reproductive capacity of recombinants in chick embryos has been shown to be due to the gene coding for M proteins.

Key words: *influenza viruses*; *recombinants*; *high-yielding*; *M-protein*

Several authors (Palese *et al.*, 1976; Brand *et al.*, 1977; Schulman and Palese, 1978; Oxford *et al.*, 1978; Ghendon, 1979; Baez *et al.*, 1980) made attempts to elucidate the genes responsible for high reproduction capacity of influenza virus recombinants in chick embryos (CE). It was shown that this capacity was not associated with genes coding for haemagglutinin or neuraminidase (Palese *et al.*, 1976; Schulman and Palese, 1978; Ghendon *et al.*, 1979). At the same time several data (Schulman and Palese, 1978; Baez *et al.*, 1980) indicated that the gene coding for M protein could play an important role in the acquisition of high reproductive capacity in CE by recombinants, though one cannot rule out the possibility that other genes might also take part in the transfer of the above property (Brand *et al.*, 1977; Oxford *et al.*, 1978; Schulman and Palese, 1978, Baez *et al.*, 1980). It should be noted, however, that among the recombinants investigated by these authors there was no one which would have inherited the M gene only from a high-yielding parent, while all other genes from a low-yielding parent.

The present report shows that we succeeded in obtaining such recombinants between the low-yielding human influenza virus strains (A/Victoria/3/75 and A/Bangkok/1/79) and the high-yielding fowl plague virus (FPV) which turned out to be high-yielding in CE.

The A/Victoria × FPV recombinants were obtained by dual infection of chick embryos with UV-inactivated FPV (Weybridge strain, H7N7) and A/Victoria/3/75 (H3N2) influenza virus. The A/Bangkok × FPV recombinants

Table 1. Haemagglutinating and infectious activity of parent influenza virus strains and recombinants on reproduction in chick embryos

Viruses*	HAU/ml	log EID_{50}/0.1 ml
R (H3N2)	2560	10.0
R2 (H3N2)	2560	10.5
R8/9/6 (H3N2)	1024—2048	9.5—10.0
R8/9/8 (H3N2)	1024—2048	9.5—10.0
R7/1/2 (H3N2)	2048—4096	9.2—10.0
R7/1/3 (H3N2)	2048—4096	9.5—10.5
R7/1/4 (H3N2)	2048—4096	9.0—10.0
R7/1/6 (H3N2)	1024—2048	9.5—10.5
R7/9/4 (H3N2)	2048—4096	8.5— 9.5
R7/9/7 (H3N2)	2048—4096	9.2—10.5
FPV (H7N7)	2048—4096	10.0
A/Victoria/3/75 (H3N2)	160	6.5
A/Bangkok/1/79 (H3N2)	128— 256	6.5— 7.0

Chick embryos were infected with 10^3 EID_{50} of the viruses and incubated at 36 °C for 48 hr following which haemagglutinating and infectious activities were determined in the allantoic fluids.

* R1 and R2 — recombinants obtained by crossing of FPV and A/Victoria/3/75; R8/9/6, R8/9/8, R7/1/2, R7/1/3, R7/1/4, R7/1/6, R7/9/4, R7/9/7 — recombinants obtained by crossing of FPV and A/Bangkok/1/79.

were obtained by dual infection of CE with A/Bangkok/1/79 (H3N2) virus inactivated by heating for 96 hr at 37 °C and FPV. Infected CE were incubated for 48 hr at 35 °C followed by two passages using limited dilutions of virus-containing materials in CE at 35 °C in the presence of anti-FPV serum. The serotype of the recombinants obtained was determined in haemagglutination-inhibition and neuraminidase-inhibition tests (Wong and Kilbourne, 1961; Aymard-Henry *et al.*, 1973).

From the viruses thus obtained recombinants of H3N2 serotype that gave high yields of virus in CE were selected (Table 1). It should be stressed that all recombinants listed in Table 1 failed to multiply in CE at an enhanced temperature (42 °C) like a parent A/Victoria/3/75 and A/Bangkok/1/79 viruses (PFV titres were not decreased at 42 °C as compared to its titres at 36 °C) (not shown).

Analysis of the recombinant genome was carried out according to Hay *et al.* (1977) as described earlier (Ghendon *et al.*, 1979); ^{3}H-uridine labelled complementary RNA (cRNA) isolated from chick embryo fibroblast (CEF) culture infected with recombinants and incubated in the presence of cycloheximide was hybridized with an excess of unlabelled virion RNA (vRNA) of parent strains: then the hybrid molecules of double-stranded RNAs (dsRNA) formed in this way treated with nuclease S1 and analysed by electrophoresis in 4% polyacrylamide gel.

Fig. 1 shows the results of the genome composition analysis of the R2

recombinant obtained by recombination of A/Victoria/3/75 virus and FPV. One can see that the electrophoretic mobility of single fragment corresponding to FPV gene 7 does not change following treatment with nuclease S1 of double-stranded molecules resulting from hybridization of cRNA of the recombinant with vRNA of FPV. Vice versa, hybridization of cRNA of R2 with vRNA of A/Victoria/3/75 virus followed by treatment with nuclease S1 leads to disappearance of the band corresponding to gene 7, while all other bands remain. The electrophoretic mobilities of these bands are similar to those of corresponding fragments of dsRNA of the A/Victoria/3/75 virus. Thus, the recombinant R2 inherited only gene 7 coding for M proteins from FPV while all other genes having been derived from the low-yielding A/Victoria/3/75 virus. Similar results were obtained for R1 recombinant (not shown).

Alternatively, the results of the genome composition analysis of three recombinants (R7/1/2, R8/9/6, R8/9/8) between A/Bangkok/1/79 virus and FPV, presented in Fig. 2, show that these recombinants have inherited a single gene from FPV i.e. the gene 7. The analysis has shown that other A/Bangkok × FPV recombinants — R7/1/3, R7/1/4, R7/1/6, R7/9/4 and R7/9/7 — also inherited from FPV the gene 7 only (not shown). Thus, the data have shown that the capacity of recombinants to multiply well in CE was associated with the gene coding for M proteins only.

It has been shown earlier (Brand *et al.*, 1977; Schulman and Palese, 1978; Baez *et al.*, 1980) that all high-yielding recombinants obtained as a result of crossing of low-yielding influenza virus strains with the high-yielding A/PR8 virus had derived gene 7 (M) from the latter, though the genomes of these recombinants contained also some other genes from the low-yielding parent.

Apparently, on crossing of human influenza viruses, transfer of some other genes along with the M gene from a high-yielding parent can somewhat increase the recombinant yields in CE. This point of view is in agreement with the data obtained by Baez *et al.* (1980); they revealed that nearly all the most high-yielding recombinants inherited the greatest number of genes from A/PR8. Similarly WRL 94 and WRL 105 recombinants that inherited three genes from the high-yielding parent A/Okuda/57 (H2N2) (Hay *et al.*, 1977) gave higher yields (Brand *et al.*, 1977) than WRL 100 recombinant that derived only two genes from that parent. However, the observed transfer of a gene other than M from a high-yielding parent into the genome of a high-yielding recombinant along with the gene coding for M proteins (Schulman and Palese, 1978) could be a secondary phenomenon (for example, NP protein can join better, or even exclusively, with "its" M protein in the process of assembly).

Nevertheless, one can not rule out the possibility that in some cases (i.e. crossing of definite parents) the transfer of some gene other than the M gene can alone somewhat increase the yield of a recombinant in CE. Apparently such situation was observed by Brand *et al.* (1977) in the studies of a recombinant between A/Okuda/57 and A/New Jersey/8/76 (Hsw1 N1) influenza viruses. That recombinant appeared to be more high-yielding in CE than

its low-yielding parent A/New Jersey, though M gene was derived from the latter. It should be noted, however, that reproduction of that recombinant in CE was more poor than that of the high-yielding parent A/Okuda/57.

Oxford *et al.* (7978) studied six recombinants between A/PR8 and A/England/69 viruses and suggested that transfer of P2 and NP genes from the high-yielding parent resulted in the increase of a recombinant virus yields in CE. However, the method applied by the authors failed to determine from which of the parents the gene coding for M proteins was inherited.

Our results obtained by crossing of low-productive human influenza viruses H3N2 and high-productive FPV (H7N7) strain, which have shown that one gene coding for M proteins could transfer to a recombinant a high reproductive capacity in CE, suggest that M gene plays a major role in the transfer of a high reproductive capacity of influenza virus at least in crossing of human H3N2 viruses and FPV. Simultaneous transfer of other genes (revealed, for example, in crossing of genetically related human influenza viruses; Brand *et al.*, 1977; Schulman and Palese, 1978; Baez *et al.*, 1980) can produce the most optimal conditions for protein complex functioning, resulting in maximal increase of virus yields in CE, but is not, apparently, a primary factor responsible for a high reproductive capacity.

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Explanation of Figures (Plates XLV–XLVI):

Fig. 1. Analysis of R2 recombinant between A/Victoria/3/75 virus and FPV.
Electrophoretic mobility of dsRNAs obtained by hybridization of ^{3}H-cRNA of the tested viruses with vRNA of the parent strains.

CEF cells infected with viruses (100 EID_{50}/cell) were incubated in the presence of cycloheximide (100 μg/ml) and ^3H-uridine (100 μCi/ml) for 4 hr at 36 °C, then RNAs were isolated (cRNA) and hybridized with an excess of unlabelled vRNA of parental strains. The hybrid molecules thus obtained were treated with nuclease S1 (1000 U/ml) and analysed by electrophoresis in 4% polyacrylamide gel (75 V, 20 hr).
V = A/Victoria/3/75; F = FPV; R_2 = R2 recombinant.

Fig. 2. Analysis of recombinants between A/Bangkok/1/79 virus and FPV.
See legend under Fig. 1.
B = A/Bangkok/1/79; F = FPV; $R_{7.1.2.}$ = R7/1/2 recombinant; $R_{8.9.6.}$ = R8/9/6 recombinant. $R_{8.9.8.}$ = R8/9/8 recombinant. 1, 2, 3, 4, 5, 6, 7, 8 — corresponding dsRNAs.