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STUDIES OF BIOLOGICAL PROPERTIES OF THE RECOMBINANTS BETWEEN HUMAN INFLUENZA AND FOWL PLAGUE VIRUSES AS RELATED TO GENOME COMPOSITION

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Summary. — Some biological properties of recombinants obtained by crossing of fowl plague and human influenza viruses were studied. The capacity of the recombinants to reproduce in chick embryo fibroblast cultures was in reverse correlation to the number of genes coding for P proteins derived from the human influenza virus. The genome composition was of importance for the expression of ts-phenotype of the recombinants in different systems. Substitution of at least one gene in the fowl plague virus genome by a corresponding human influenza virus gene resulted in the decrease of virulence for 1-day-old chickens. The presence of three P genes from human influenza virus genome in the genome of the recombinant proved to be insufficient for the capability of the recombinant to reproduce in organ cultures of human origin.

Key words: *orthomyxoviruses*; *recombinants*; *genome composition*; *biological properties*; *pathogenicity*

Introduction

Thorough investigations of the genome composition and phenotypic expression of different influenza virus strain recombinants allowed, on the one hand, to determine which proteins are encoded by certain genes (Palese, 1977; Scholtissek, 1978), and, on the other hand, to reveal the correlation between the manifestation of biological properties and expression of certain genes (Scholtissek et al., 1977; Rott *et al.*, 1979; Ghendon *et al.*, 1979; Sugiura and Ueda, 1980; etc).

Recombinants between temperature-sensitive (ts) mutants of fowl plague virus (FPV) and human influenza viruses with a characterized genome composition were obtained in our laboratory. The present paper describes some biological properties of these recombinants which inherited the majority of genes from FPV while others from human influenza virus.

Materials and Methods

Viruses. The following virus strains were used: FPV Weybridge strain (H7N7); human influenza virus A/Krasnodar/101/59(H2N2); recombinants between ts-mutants of FPV and human influenza virus A/Krasnodar obtained by the method described previously (Ghenkina and Ghendon, 1979).

Analysis of the recombinant genome was performed using a technique described earlier (Ghendon *et al.*, 1979). Briefly, chick embryo fibroblast (CEF) cultures were infected with the recombinants under study and parent viruses (100 EID_{50}/cell) and incubated in the presence of cycloheximide (100 μg/ml) at 36 °C for 60 min. ^3H-uridine was then added and incubation continued for 4 hr. ^3H-labelled complementary RNA (cRNA) was extracted from the infected cells and hybridized with an excess of unlabelled virion RNA (vRNA), isolated from purified virions of parent strains. The material was further treated with nuclease S1, precipitated with ethanol and analysed by electrophoresis in a 4% polyacrylamide gel.

One-cycle reproduction of viruses in CEF cultures. CEF cultures were infected with the viruses at multiplicity of infection 1—3 PFU/cell. After the viruses at multiplicity of infection 1—3 PFU /cell. After 30 min adsorption at room temperature the cells were washed twice and supplemented with warm medium 199. Incubation lasted for 10—12 hr at 36 °C following which the cells were frozen and thawed. Plaques were prepared in CEF cultures under agar overlay.

Studies of ts phenotype were carried out by titration of viruses in CE and in CEF cultures at temperatures of 36 °C and 42 °C, respectively.

Pathogenicity of viruses for 1-day-old chickens. One-day-old chickens, "Russian white" strain, were used. Chickens were inoculated intramuscularly with 10-fold virus dilutions, the virus dose ranging from 0.001 to 10 PFU. The number of deaths was registered for 3 days. Virulence of viruses was estimated by a LD_{50}/PFU ratio which was calculated for each day of observation.

Studies of virus reproduction in organ cultures of human tissues. Nasal polyps of adults were used for preparing organ cultures. The material obtained in an otolaryngological clinic by a polypotomy procedure was washed in medium with antibiotics, purified from necrotic tissues, cut into 1-3 mm fragments and placed into flasks containing medium 199 (4 fragments per each flask). After 24 hr of incubation at 36 °C the explants were subjected to microscopy. Only those explants were used which revealed ciliary motion and made the medium acid. After removal of the medium, the fragments were infected with either virus at 10^4 EID_{50} per flask. After 1 hr incubation at 36 °C, the explants were washed twice and supplemented with MEM. The dynamics of virus reproduction in organ cultures was studied for 3 days, samples of virus-containing fluid were taken for titration in CE.

Results

Reproduction in CEF cultures

As seen from Table 1, substitution of FPV genes coding for the P proteins by some genes of human influenza virus strain A/Krasnodar results in the decrease of the capacity to reproduce in CEF. Substitution of either genes 1, 1 and 2 or 1 and 3 caused a 10-to 70-fold decrease of infectious virus yields in our system, while substitution of all three P genes of FPV by the corresponding genes of human influenza viruses (recombinants 51, 54) result, in a 1,000 to 70,000-fold decrease of the reproductive capacity of viruses. At the same time, decrease in the plaque sizes was observed, although no direct correlation between these two parameters was revealed.

Expression of a ts-phenotype in different systems

Table 1 summarizes the data of studies on a ts-phenotype of the recombinants in two systems — in CEF and CE. Titration in CEF cultures showed that a difference in titres of the recombinants at 36 °C and 42 °C was 0.2—1.5 log PFU irrespective of the genome composition. At the same time different results were obtained by titrations in CE. Recombinants, in which gene 1 had been substituted (recombinants 8, 14), differed by 0.5—2.0 log EID_{50} at these two temperatures, while substitution of two or three segments resulted in dras-

Table 1. Biological properties of the recombinants obtained by crossing of FPV and A/Krasnodar strain of human influenza virus

Recombinant	RNA segment derived from A/Krasnodar strain	Virus yields by one-cycle growth in CEF, PFU/cell	Plaque size, (mm)	ts-phenotype CEF log PFU/ml	ts-phenotype CE log EID_{50}/0.1 ml
8	1	2	1 —1.5	6.5/5.0**	7.0/5.0**
14	1	5	2.0—2.5	7.2/6.5	7.5/7.0
46	1,2	2	1 —1.5	7.0/6.2	7.5/2.5
48	1,2	1	1 —1.5	7.0/6.7	8.0/3.0
51	1,2.3	0.04	2.0—2.5	8.0/7.8	8.0/3.0
52	1,3	6	2.0—2.5	7.0/6.7	8.0/2.5
53	1,3	3	2.0—2.5	7.8/6.7	8.5/2.5
54	1,2,3	0.001	1 —1.5	7.0/6.5	8.5/1.5
FPV (control)		70	2.5—3.5	8.6/8.6	9.5/9.0
A/Krasnodar (control)		< 0.0001	—	—	8.5/2.5

* CEF cultures were infected with each virus at a multiplicity of 1—3 PFU/cell. After 10—12 hr incubation at 36 °C the cells were frozen and thawed; virus titre was determined in CEF under agar overlay.

** virus titres: nominator = at 36 °C, denominator = at 42 °C.

tic changes in the ts-phenotype: the difference in titres of these recombinants at 36 °C and 42 °C was 5-7 log EID_{50}.

Pathogenicity for chickens

Table 2 shows the results of studies on pathogenicity of the viruses for 1-day-old chickens. Virulence was reduced in all recombinants as compared to the parent FPV strain, however, it was expressed in different ways: in the recombinant 48, reduction of virulence was observed throughout the observation period (3 days), in others it consisted of changes in the dynamics of

Table 2. Virulence for one-day-old chickens of the recombinants obtained by crossing of FPV and A/Krasnodar strain of human influenza virus

Recombinant	RNA segment derived from A/Krasnodar strain	Observation day		
		1	2	3
8	1	< 0.3 *	10	30
14	1	0.5	0.5	12
46	1,2	0.1	1.4	14
48	1,2	0.1	0.1	0.1
52	1,3	0.1	10	30
54	1,2,3	0.1	0.3	10
FPV (control)		25	25	25

One-day-old chickens were infected intramuscularly with 10-fold dilutions of each virus (ranging from 0.001 to 10 PFU);* data expressed in LD_{50}/PFU.

chicken deaths. So, if FPV causes a quick death of chickens as early as 1 day after infection, then the recombinants 8, 12, 14, 46, 53 and 54 turned out to be significantly less virulent on the first observation day. However, sort of these recombinants had gradually "cought up" the FPV, as on observation day 3 the LD_{50}/PFU ratio became approximately the same as that of FPV. Thus, despite of the fact that substitution of one or two FPV gene by the corresponding genes of human influenza virus can decrease the virulence of recombinants for chickens, substitution of even three RNA segments--coding for the P proteins in FPV (recombinant 54) — by corresponding genes of human influenza virus apathogenic for chickens does not result in a complete loss of virulence; a recombinant with such a genome composition is still able to cause death of chickens, although later than FPV.

Reproduction in nasal cavity polyp organ cultures

At a dose of infection used, all recombinants failed to reproduce in nasal cavity polyp organ cultures of adults alike to FPV and unlike to human influenza virus.

Discussion

As shown previously in our laboratory (Mikheeva *et al.*, 1977) the A/Krasnodar human influenza virus strain does not form infectious progeny in CEF cultures, aparently because haemagglutinin cleavage is impaired in this system. In the present investigations wer have obtained data indicating that the presence in the genome of the recombinants under study of one or several P genes inherited from human influenza virus, drastically reduces infectious yields in CEF cultures despite of the fact that the haemagglutinin had been coming from FPV. In this connection one can assume that failure of A/Krasnodar virus to reproduce in this system is not only due to impairment in haemagglutinin cleavage, but also due to certain impairments in functioning of the P proteins encoded by the corresponding genes of human influenza virus.

Data were published that substitution of one RNA segment coding for P3 protein in the FPV genome resulted in loss of virus capacity to form plaques in a certain cell system (Almond, 1977). In our investigations substitution of three genes coding for the P proteins did not affect this feature (except a reduction in plaque sizes); but in a one-cycle experiment formation of infectious progeny of the recombinants had been drastically reduced. One can assume that functions of the influenza virus P proteins are host-cell dependent.

Different expressions of the ts-phenotype of the recombinants reproducing in CEF cultures and in CE at high temperature found in our experiments resemble to the situation described by Israel (1980). The author obtained a ts-mutant of Dobson strain FPV having a ts-mutation in the gene coding for P1 protein; this mutant failed to form plaques at high temperature in one system, but formed plaques well under same conditions in another. Scholtissek and Murphy (1978) described FPV ts-mutants having lesions in genes P1 and P3; unlike wild-type virus, they lost their capacity to form plaques

in MDCK cells even at permissive temperature. All these phenomena are, probably, associated with the influence of some cellular factors on the function of the transcriptase-polymerase complex which includes the P group proteins; the influence of these factors may depend on the temperature of incubation. When discussing the possible influence of cellular factors on biological functions of the recombinant influenza virus strains, one should bear in mind that an impairment in reproduction, or in manifestation of biological properties of the recombinants in a certain system may not be directly due to a defect in the function of some gene or a protein encoded by it, but due to a change resulting from recombination of optimal gene constellation.

There is only one report that substitution of one gene in the FPV genome by the corresponding RNA segment of human influenza virus may result in changes in virulence of the virus for chickens (Scholtissek *et al.*, 1977). Similar results were obtained in our experiment with other FPV strains and human influenza viruses. Thorough investigations on the dynamics of deaths of chickens revealed that recombinants with identical gene composition (recombinant 46 and 48) had shown different degrees of virulence for chickens. Apparently, additional mutations may emerge in the process of obtaining and passaging of the recombinants which are not detected by the method of genome analysis used in our experiments, but which may be of great importance for reproduction of viruses in the organism of a susceptible animal.

In our experiments, changes in virulence of the majority of recombinants for chickens caused that the dynamics of their death became slower (Table 2). Recently McCahon et al. (1981) have shown that reduction in virulence for suckling mice of a number of ts-mutants of foot-and-mouth disease is associated with their slower reproduction. One can not rule out the possibility that a similar situation may take place in our experiments.

Recent investigations by Ogawa and Ueda (1981) on virulence for chickens and CE of recombinants obtained by crossing of virulent and avirulent avian influenza virus strains have shown that the recombinants which inherited HA, NA and M genes from the virulent parent, completely retained their virulence for chickens. The authors concluded that the main gene responsible for virulence for chickens is the gene coding for the haemagglutinin, however, involvement of the NA and M genes is necessary for a complete expression of virulence. In our experiments the recombinants which had inherited the HA, NA and M genes from the virulent strain, and one or several genes P from the avirulent strain, showed a reduced virulence for chickens. These data testify the fact that proteins encoded by P gene may be also involved in mediating the virulence of avian influenza strains for chickens.

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