Acta virol. **26**: 113–118, 1982

CORRELATION BETWEEN REPRODUCTIVE AND INTERFERING ACTIVITIES OF EPIDEMIC AND THERMOSENSITIVE INFLUENZA VIRUS STRAINS

G. I. ALEKSANDROVA, *N. I. DEKHTYAREVA, T. E. MEDVEDEVA, **M. A. SHNEIDER

Research Institute of Experimental Medicine, U.S.S.R. Academy of Medical Sciences, 197022 Leningrad; *All-Union Research Institute of Influenza, U.S.S.R. Ministry of Public Health, Leningrad; and **All-Union Research Technological Institute of Antibiotics and Medically Important Enzymes, Leningrad, U.S.S.R.

Received April 17, 1981

Summary. — Differences in interfering activities were demonstrated between low-passage epidemic and thermosensitive cold-adapted strains of influenza viruses A and B in experiments on chick embryos inoculated on the chorioallantoic membranes and on white mice. At a high multiplicity of infection the thermosensitive variants showed a higher interfering activity as compared with the original viruses which had undergone a limited number of chick embryo passages.

Key words: *influenza virus*; *thermosensitivity*; *interference*

Introduction

We demonstrated that virulent and attenuated strains of influenza viruses A and B differ in their interfering activities, i. e. in their abilities to inhibit the reproduction of indicator virus, in a susceptible line of diploid human embryo lung fibroblast (DHL) cells. The interfering activity was the highest in cold-adapted thermosensitive strains, innocuous for men of various age groups including 1–6 years old children (Aleksandrova *et al.*, 1980).

The interference test was reproducible only in certain DHL cell lines susceptible to influenza virus. The results were negative in both insusceptible lines of DHL cells and chick embryo cell (CEC) cultures. The susceptibility to influenza virus of the latter varies widely (Petrunova *et al.*, 1977). In experiments on CEC the interfering abilities of influenza virus were independent of the number of chick embryo passages and in no relation with thermosensitivity and virulence for man of the virus strains (Dekhtyareva and Medvedeva, 1976; Dekhtyareva *et al.*, 1977). The investigations mentioned showed that the intesity of interference is related with the ability of the cell system to support the replication of influenza virus. To elucidate the mechanism of the differences in the interfering activities of low-passage and cold-adapted strains we compared their reproduction in a highly susceptible system — the chorioallantoic membranes (CAM) of developing chick embryos — at various multiplicities of infection. The higher interfering activities of cold-adapted strains were confirmed. To check the connection between interfering activity and the degree of adaptation of the test viruses to chick embryos, we studied the interfering abilities of cold-adapted and low-passage strains also in white mice, a heterologous system.

Materials and Methods

Viruses. The epidemic influenza virus strain A/Leningrad/538/2/74 (H3N2) and B/USSR/5/69 had undergone respectively 2 and 5 chick embryo passages; they are thermoresistant. The attenuated cold-adapted variants of these two strains had undergone respectively 41 and 60 passages in chick embryos at lowered (28-25 °C) temperature. These A/Leningrad/538/41/ /74 and B/USSR/60/69 variants are thermosensitive, i. e. unable to reproduce at increased temperature: the difference between titres at optimal (32 °C) and nonpermissive (40 °C for influenza virus A and 38° C for influenza virus B) was 6.75 log EID_{50}/0.2 ml (Aleksandrova *et al.*, 1980; Medvedeva *et al.*, 1980).

Inoculation of chick embryos on the CAM. Gas from the air sac was sucked off by a rubber bulb through a hole in the shell. In this way the shell membrane was separated from the CAM. Thereafter part (1.5 cm^2) of the shell membrane was removed through an additional hole made in the shell at the lateral surface of the embryo. Virus inoculum was then placed on the exposed area of the CAM.

Determination of the interfering activity of influenza virus on chick embryo CAM. Vaccinia virus in the form of standard dry smallpox vaccine was used as indicator virus. The activity of vaccinia virus was evaluated based on the formation in the inoculated areas of characteristic white pock lesions, easily identifiable macroscopically. The starting preparation had a titre of 7.25 - 7.5 log pock-forming units (PkFU) per 0.2 ml. The test influenza virus strains were inoculated in a dose of 7 log EID_{50}/0.2 ml on the chick embryo CAM. After incubation for 24 hr at 37 °C, serial tenfold dilutions of vaccinia virus were inoculated on the same areas of the CAM. A parallel group of chick embryos was not infected with influenza virus and served for titration of vaccinia virus infectivity (log PkFU/0.2 ml). The titres of indicator virus in experimental and control embryos were determined after incubation of the embryos for 48 hr at 37 °C. The interfering activity was expressed as the difference in titres of vaccinia virus (in log PkFU/0.2 ml) found in the experiment and control. The results (mean values) were evaluated statistically by the t-test.

Determination of the reproductive activities of influenza viruses in chick embryos. Groups of 3 embryos each were inoculated into the allantoic cavities with medium and high (2-3 and 7 log EID_{50}/0.2 ml) doses of the strains tested and incubated at 30 °C for 48 or 72 hr with influenza virus A or B, respectively. In pooled samples of the allantoic fluids we determined the haemagglutinin and infectivity titres. The latter were assayed in chick embryos incubated at 32 °C.

Determination of the interfering activities of influenza virus in white mice. Groups of 20 mice each were inoculated intranasally with 0.05 ml of the influenza virus strains tested (their titres reached 5-6 log EID_{50}/0.2 ml). After 24 hr the animals were challenged with a standard dose (100 MLD_{50}) of the pathogenic indicator virus A/Leningrad/32/49 (H0N1) and observed for 9 days. This period corresponded to that of the maximal lethality of the indicator virus. The control consisted of an equivalent mouse group given intranasally saline instead of the test influenza virus. The indices of interference were evaluated based on the inhibition of the lethality of the A/Leningrad/32/49 indicator virus. The intensity of interference was scored as low (+; up to 40% of the infected animals survived), moderate (++; survival rate 40–60%) and high (+++; survival rate > 60%).

Results

Reproductive activities of virulent and attenuated influenza virus strains in chick embryos

All the virus strains tested reproduced well in the allantoic cavities of chick embryos on inoculation with the low dose (2 log EID_{50}/0.2 ml) (Table 1).

Table 1. Infectious and HA titres of low-passage and cold-adapted influenza virus strains in relation to the size of inoculum

Virus	RCT40/38 marker log	Inoculum					
		2 log EID_{50}/0.2 ml			7 log EID_{50}/0.2 ml		
		I	II	III	I	II	III
Low-passage							
A/Leningrad/538/2/74	0.25	8.0	1.8	6.2	5.0	2.7	2.3
B/USSR/5/69	0.5	7.25	1.8	5.45	4.25	2.1	2.15
Cold-adapted							
A/Leningrad/538/41/74	6.75	7.25	2.1	5.15	8.75	3.0	5.75
B/USSR/60/69	6.75	6.75	1.5	2.25	8.5	2.7	5.8

I – log EID_{50}/0.2 ml.
II – log HA units.
III – EID_{50}/HA ratio.

The viruses reached infectious titres from 6.75 to 8 log EID_{50}/0.2 ml and haemagglutinin titres from 1.5 to 2.1 log haemagglutinating (HA) units.

Differences between low-passage strains and the thermosensitive variants passed for long periods at lowered temperature became manifested by differences in infectious virus titres after inoculation of chick embryos with the high virus dose. Under these conditions the HA activities of the low-passage strains changed only little, whereas their infectivity dropped rapidly. There was an enhanced production of noninfectious virus particles that retained their HA ability (von Magnus phenomenon).

The course of reproduction of the thermosensitive A/Leningrad/538/41/74 and B/USSR/60/69 variants after inoculation with the high virus dose was quite different, without the appearance of the von Magnus phenomenon. The infectious virus titres were even by 1–2 log units higher than after inoculation of the embryos with the low dose.

The ratios of the infectivities to the HA activities of the low-passage and the thermosensitive viruses changed correspondingly. This ratio was markedly lowered for low-passage viruses inoculated at the high dose, but remained unchanged for thermosensitive variants inoculated at either the high or low dose.

The thermosensitive viruses were thus characterized by a high reproductive activity in chick embryos which did not depend on the size of inoculum and by the maintained ability to produce infectious progeny on inoculation with a massive dose.

Interfering activities of virulent and attenuated influenza virus strains

Epidemic and thermosensitive strains of influenza virus significantly differed from each other by their abilities to inhibit vaccinia virus in chick embryos (Table 2). The virulent low-passage strains A/Leningrad/538/2/74 and B/USSR/5/69 were characterized by minimal indices of interference, lowering the titres of vaccinia virus by 2.25–3 log PkFU/0.2 ml. The at-

Table 2. Interfering activities of low-passage and cold-adapted influenza virus strains

Virus	Interference in					
	chick embryos				white mice	
	I	II	I-II	Diff.	III	Diff.
A/Leningrad/538						
/2/74 (low-pass.)	7.5	5.25	2.25 ± 0.3	4.5	25.0 ± 2.88	45.0
/41/74 (cold-adapted)	7.5	0.75	6.75 ± 0.08		70.0 ± 2.8	
B/USSR/						
5/69 (low-pass.)	7.25	4.25	3.0 ± 0.16	3.25	61.6 ± 1.66	28.4
60/69 (cold-adapted)	7.25	1.0	6.25 ± 0.08		90.0 ± 5.77	

I and II — Titre of vaccinia virus (log PkFU/0.2 ml) in the control and in the presence of influenza virus, respectively.
Diff. — difference between cold-adapted and low-passage virus.
III — Rate of protection against A/Leningrad/32/49 virus in %.

tenuated cold-adapted variants A/Leningrad/538/41/74 and B/USSR/60/69 lowered the titres of indicator virus by 6.25—6.75 PkFU/0.2 ml, i. e. they showed a higher interfering activity against the indicator virus.

In connection with the marked effect on the intensity of interference of the intensity of virus reproduction on the CAM it could be assumed that the differences between the cold-adapted and low-passage strains could have been due to the degree of adaptation to chick embryos. To test this assumption, we assayed the interfering activities of the same viruses in white mice, a heterologous system. Like in experiments on CAM, the marked interfering activity of cold-adapted strains as compared with the low-passage viruses was manifested again.

The A/Leningrad/528/74 and B/USSR/69 strains tested and their cold-adapted variants were avirulent for white mice, but reproduced in their lungs, lowering the pathogenicity of the virulent indicator virus.

As shown in Table 2, the original strain A/Leningrad/538/2/74 protected only 25% of the infected animals from lethal infection. Under the effect of the cold-adapted variant A/Leningrad/538/41/74 the protection rate increased to 70%, indicating that the interference of this virus with the indicator strain A/Leningrad/32/49 was more active. Experiments with variants of the B/USSR/69 virus yielded similar results. The interfering activity of the cold-adapted variant was higher as compared with the low-passage virus, but the absolute indices of interference of the latter were higher than those of the low-passage A/Leningrad/538/2/74 virus.

Discussion

In the present study on the reproduction of original and cold-adapted strains of influenza viruses A (H3N2) and B we found significant differences in the accumulation of infectious virus progeny after inoculation of high doses of virus.

With low-passage viruses, the ratio of infectivity to HA activity depended on the size of inoculum. With increasing doses the yield of infectious particles decreased, but the HA titres remained high. An increase in the inoculated dose to 6-7 log EID_{50}/0.2 ml led to an increased yield of infectious virus particles, the level of haemagglutinin having been preserved. Consequently, the ratio of infectivity to HA activity decreased on the account of an increased concentration of noninfectious haemagglutinins.

But this phenomenon, well known under the designation of the von Magnus phenomenon, did not occur in experiments on the cold-adapted variants reproduced in chick embryos inoculated with a massive dose of virus. Their reproduction was even enhanced as compared with a medium-sized inoculum which proved optimal for low-passage epidemic strains.

The thermosensitive cold-adapted viruses were thus characterized by their ability of active reproduction yielding complete virus after massive inocula which, with low-passage strains, yielded the so-called incomplete virus.

In the interference test we used maximal doses of the test influenza virus strains, because the differences between cold-adapted and low-passage influenza virus strains were most marked at a high multiplicity of infection (Medvedeva *et al.*, 1972; Dekhtyareva and Medvedeva, 1976). The intensity of interference induced by virulent and cold-adapted strains varied in respect to vaccinia virus. Virulent strains interfered with vaccinia virus in chick embryos less intensively, lowering its titres by not more than 3 log units. The interfering activity of the cold-adapted thermosensitive strains was considerably higher (6.25 – 6.75 log units).

The higher interfering activity of the cold-adapted strains was also manifested in experiments on white mice.

Our results indicate that the differences observed between low-passage and cold-adapted viruses are due to the properties and peculiarities of reproduction of thermosensitive viruses rather than to the degreee of their adaptation to chick embryos.

The differences in the interfering activities of the low-passage and cold-adapted viruses are in accordance with the previously observed higher interferon production in various susceptible systems: in chick embryos and human leukocyte cultures (Medvedeva *et al.*, 1972; Polezhayev *et al.*, 1974). The observed difference between thermosensitive and virulent strains could be used for their laboratory differentiation, as well as in practical interferon induction, taking into account the higher interferon-inducing and interfering activities of thermosensitive strains of influenza viruses A and B.

References

Aleksandrova, G. I., Dekhtyareva, N. I., and Medvedeva, T. E. (1980): Correlation between thermosensitivity and interfering activity of influenza virus (in Russian). *Rom. J. med. Virol.* **31**, 161 – 167.

Dekhtyareva, N. I., and Medvedeva, T. E. (1976): Genetic markers of virulent and attenuated strains of influenza virus, passed at optimal and lowered temperature (in Russian). *Problemy Grippa i ORZ* **15**, 70 – 77.

Dekhtyareva, N. I., Medvedeva, T. E., Polyakov, Yu. M., Golubev, D. B., and Aleksandrova, G. I. (1977): Differentiating markers of virulent and attenuated (cold-adapted) strains of influenza virus A (in Russian). *Vop. Virus.* **22**, 173 – 178.

Medvedeva, T. E., Aleksandrova, G. I., and Smorodintsev, A. A. (1972): Interferon production in developing chick embryos infected with psychro- and thermophilic strains of influenza virus type A2 (in Russian). *Problemy Grippa i ORZ*, **1**, 143–150.

Medvedeva, T. E., Dekhtyareva, N. I., and Aleksandrova, G. I. (1980): Differences in the interfering activities of influenza viruses with respect to virulence for men (in Russian), pp. 141–147. In *Faktory nespetsificheskoi Rezistentnosti pri virusnykh Infektsiakh*, R. A. Poliak and T. Ya. Lusıanina (eds.), Leningrad.

Petrunova, S., Mikeeva, A. V., and Ghendon, Yu. Z. (1977): Study of the reproduction of human influenza viruses in chick embryo fibroblast cell culture. Communication III. Formation of viral ribonocleoproteids (in Russian). *Zh. Epidem.* (*Sofia*) **14**, 15—18.

Polezhaev, F. I., G. S. Makariev, and Aleksandrova, G. I. (1974): Interferon-inducing ability in human leukocyte cultures of influenza viruses of different virulence. *Acta virol.* **18**, 362.