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STUDIES OF NATURAL POPULATION VARIABILITY OF PARAINFLUENZA VIRUSES DURING THEIR EPIDEMIC CIRCULATION

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Summary. - The population of circulating serotype 3 parainfluenza virus strains isolated in different years proved to be sufficiently polymorphic concerning its antigenic and biological features as well as their virulence for newborn hamsters. The highly virulent strain population appeared to have an antigenic pattern different from that of the prototype strain. The epidemic caused by it in groups of school and preschool childern was more intensive as compared to that induced by avirulent strains population.

Key words: parainfluenza viruses; virulence; natural variability; epidemic activity

Introduction

Parainfluenza viruses are widespread and comprise an important group of agents arousing respiratory diseases in man. Parainfluenza virus of serotype 3 possesses the highest epidemic activity and is frequently associated with diseases of lower respiratory tract in children (Storey *et al.*, 1984).

Recent reports indicate the pleomorphism of natural parainfluenza virus populations in terms of their antigenic and biological properties. The application of monoclonal antibodies allowed to define the antigenic properties of various strains of parainfluenza viruses serotype 1 (Kessler *et al.*, 1985). The isolates obtained in different years differed in antigenic structure of haemagglutinin (HN). The strains were differentiated according to 3 out of 4 epitopes. Menlemans *et al.*(1986) analysed a large scale respiratory disease outbreak in pigeons induced by paramyxovirus type 1 which spread to most European countries. The population analysis of viruses isolated in 1982-83 showed that they had different virulence for 6-day-old chick embryos, thermostability of HN and electrophoretic mobility of P-protein.

The changes of antigenic and biological properties among natural populations of human parainfluenza viruses serotype 3 have been insufficiently

studied as yet. The present work was aimed at evaluating the level of changeability of circulating strains of serotype 3 parainfluenza virus isolated in different years, and at comparing the biological characteristics of natural populations and the level of their epidemic activity in organized groups of school and preschool children.

Materials and Methods

Virus isolation. During the period of 1976–80 in the same time about 80–90 children aged from 3 months to 4 years were followed up. Paired serum samples of sick children were examined in serologic tests with antigens of influenza and other viruses of acute respiratory diseases. For virologic studies nasopharyngeal smears were obtained from patients with acute respiratory diseases and healthy children in contact. Primary human embryo kidney cells (HEK) were infected with 0.2 ml of test material and incubated at 36 ^{0}C for 2 weeks and the presence of virus was determined by routine haemadsorption test. Freshly isolated strains were identified by haemadsorption inhibition test.

Determination of viral antigenic pattern. The strains were cloned by end-point dilution procedure in Hep-2 cells. Immune sera were raised in rabbits by intravenous administration of various strains of serotype 3 parainfluenza virus according to a conventional schedule. The serum was heated at 56 ^{0}C for 30 min and precipitated with rivanol. Cross haemadsorption inhibition reaction in Hep-2 cells was made using two-step dilution of immune sera. The homologous and heterologous titre of the same serum was compared against 100 infectious doses of different virus strains (ID_{50}/ml). The prototype strain (PG_3-C-243/57) and isolates of different years were used. To determine the antigenic relationship between isolates of different years and prototype strains, the mixture of equal volumes of viruses and sera to homo- and heterologous strains following a 2-hour exposure was introduced to Hep-2 cell culture with subesquent incubation of the culture at 36 ^{0}C. The serum titre was defined as its maximal dilution inducing complete inhibition of virus reproduction (evaluated on days 5–7 of incubation).

Virion thermostability. Parainfluenza virus strains (in doses of 10 000 ID_{50}/ml) were preheated at 42 ^{0}C for 1–5 hr. Thereafter, the samples were inoculated by serial 10-fold dilutions into Hep-2 cells. Virion thermostability was determined by difference in titres of the heated and natural viruses. Viruses were defined as thermostable if the decrease of their titre was not higher than 2.0 log ID_{50}/ml, while thermolabile types showed a decrease in titres (following 3–5 hr heating) of not lower than by 4.0 log ID_{50}/ml.

Susceptibility to β-inhibitors in the sera from various animal species. To determine the serum inhibiting activity, various virus strains (100 ID_{50}/ml) were added to each of 2-fold dilutions of normal animals sera or to Eagle's maintenance medium free of serum (control). The mixtures of serum with virus in equal volumes were kept at room temperature for 2 hr with subsequent introduction to Hep-2 cell culture-containing tubes and incubation at 36 ^{0}C. Serum titres were defined as its maximal dilution causing complete inhibition of viral reproduction.

Newborn hamsters were inoculated by intranasal route with 0.02 ml suspensions of PG-3 virus (strain PG_3/Leningrad/643/03/77 and PG_3/Leningrad/1364/01/78) in the dose of 1000 ID_{50}/ml. Their lungs were examined on day 3 p. i. by histologic and electron microscopic studies. For histologic examination haematoxylin-eosin stained paraffin sections were used, in which giant cells in bronchiolar epithelium and the development of local inflammatory reaction were regarded as specific for parainfluenza lesions. For electron microscopy the specimens were fixed in 2.5% glutaraldehyde in the cacodylate buffer (pH 7.2) with subsequent 2% OsO_4 solution, and embedded into Epon. Lesion-containing blocks were formed following preliminary examination of 1 μm sections stained with methylen blue. Ultrathin sections contrasted with uranyl acetate and lead citrate were examined using a JEM-100S electron microscope.


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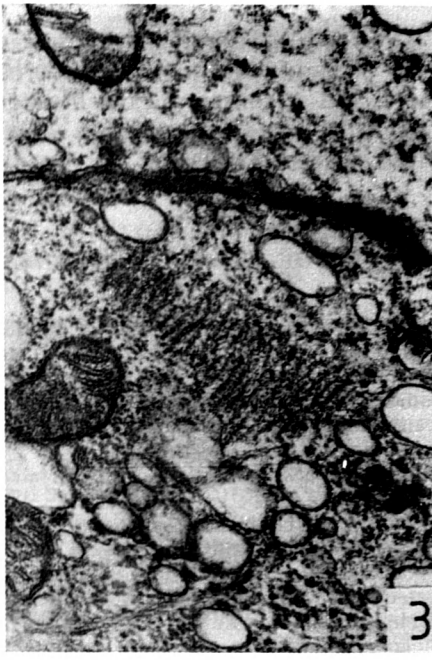
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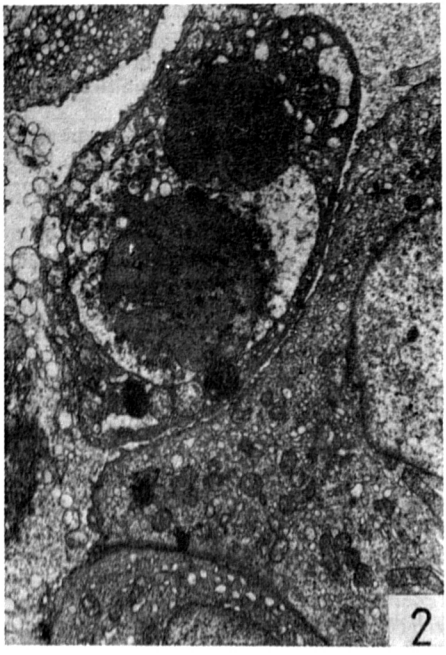
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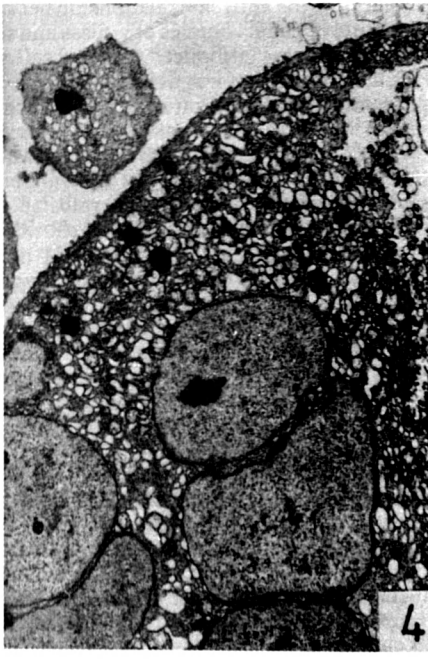
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Results

Analysis of natural viral populations in terms of their virulence for laboratory mice

Serotype 3 parainfluenza virus strains of varying dates of isolation proved to have different virulence for newborn hamsters. The 1977 isolates appeared to be more virulent than the 1978 strains. Thus, 1977 strains in constrast to the 1978 isolates induced intensive pneumonia and the most marked local inflammatory reaction in the lungs of newborn hamsters. The extent of this reaction was estimated considering the intensity of exudative and leukocytic infiltrate. On the contrary, the 1978 isolates induced leukocytic infiltration in the bronchi only. The strains differed substantially in the type of infection induced. The productive type of infection was typical for the 1977 isolates: electron microscopic examination revealed budding of virus particles on the external membrane of bronchial epithelium cells (Fig. 1). These cells were in contact with phagocytes (Fig. 2). On the contrary, in abortive type of infection typical of 1978 isolates no virion formation was detected on the cell membranes of bronchial epithelium. They formed polykaryocytes (Fig. 4) which cytoplasm regularly contained parallel filaments of parainfluenza virus ribonucleoprotein (Fig. 3). In these cases no leukocytic reaction was noted.

Analysis of natural viral populations in terms of their antigenic patterns

Typical for parainfluenza infection is the possibility of co-circulation of older known and novel natural antigenic variants. The majority of isolates of 1977 revealed weakly manifested antigenic relationship with the prototype virus as the prototype serum sample interacted with them up to its 1/8 – 1/6 titre. At the same time, 1978 virus isolates and the prototype strain had less manifested antigenic differences (Table 1). In addition to antigenically different variants,

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Fig. 1
Parainfluenza virions in the bronchial epithelium of newborn hamsters (day 3 after inoculation with PG_3/Leningrad/643/03/77 strain) × 90 000.

Fig. 2
Leukocyte accumulation near bronchial respiratory epithelium of newborn hamsters (day 3 after their inoculation with PG_3/Leningrad/643/03/77 strain) × 21 000.

Fig. 3
Accumulation of bands of ribonucleoproteins of parainfluenza virus in bronchial epithelium of newborn hamsters (day 3 after their inoculation with PG_3/Leningrad/1364/01/78) × 90 000.

Fig. 4
Symplasts of bronchial epithelium of newborn hamsters (day 3 after their inoculation with PG_3/Leningrad/1364/01/78 strain). Neither budding of virus particles on the cell membranes nor leukocytic reaction were noted. × 21 000.

Table 1. Antigenic characteristics of circulating strains of serotype 3 parainfluenza viruses

Virus	Serum to indicated strains									
	PG_3-C-243/57	643	645	1210	1364	1365	1419	1420	1424	1388
PG_3-C-243/57-prototype	128*	64	32/64	64	64/128	32/64	32	32	32	64
PG_3/Leningrad/643/03/77	8/16	128	64	32/64	8/16	4/8	nd	16	8	32
PG_3/Leningrad/645/03/77	8	64	128	64	8	4/8	nd	16	16	64
PG_3/Leningrad/1210/11/77	32/64	32/64	32/64	128	32	16/32	16	128	16	64
PG_3/Leningrad/1364/01/78	64	64	64	64	128	nd	16	128	16	64
PG_3/Leningrad/1365/01/78	64	64	64	64	nd	64	nd	nd	nd	nd
PG_3/Leningrad/1419/02/78	16	64	64/128	128	16	32	64	32	16	32
PG_3/Leningrad/1420/02/78	64	nd	nd	nd	32	32	64	128	nd	64
PG_3/Leningrad/1424/02/78	32	nd	nd	nd	64	32	64	128	32	nd
PG_3/Praha/1388/83	8/16	32	64	64	nd	64	nd	128	32	64

* Haemadsorption inhibition titres (dilution reciprocals)

strains similar to the prototype occurred among isolates of 1978 (Table 1). Consequently, we detected 2 antigenic variants in the natural population of serotype 3 parainfluenza viruses.

Polymorphism of natural populations in terms of virion thermostability levels

The strains of parainfluenza viruses isolated at different dates were also differentiated by their thermostability. Thus, PG_3/Leningrad/643/03/77 strain proved to be more thermostable as compared to PG_3/Leningrad/1364/01/78 strain since even after 5 hr heating of virus-containing fluid at 42 ^{0}C it still retained its infectivity. On the other hand, PG_3/Leningrad/1364/01/78 strain was completely inactivated after heating for 3 hr.

Polymorphism of natural viral populations in terms of their susceptibility to ß--inhibitors

Viral isolates of 1978 proved to be more stable to ß-inhibitors in rabbit sera than those from 1977 (Table 2). The comparison of biological properties of natural viral populations and their epidemic activity revealed that structural changes of circulating populations in terms of their biological properties correspond to changes in intensity of the epidemic process in organized groups of school and preschool children. In 1976–1980 the epidemic induced by parainfluenza virus serotype 3 was characterized by interchanging periods with relatively severe, moderate, or weak course of disease. The epidemic process was especially severe in 1976–1977. Thus at that time more outbreaks occurred (23); higher incidence (90.0) and infectivity (106.8) of parainfluenza were recorded as compared to similar periods of 1977–1980, when the data were 3–9; 21.6–81.6; and 21.6–96.0, respectively.

Consequently, in recent years a decrease in epidemic activity of parainfluenza virus circulating strains was noted.

Discussion

The analysis of the natural population of serotype 3 parainfluenza virus isolates of different years revealed its heterogeneity in terms of antigenic and biological properties. Different virulence for laboratory animals was also demonstrated. One can assume that the variability of circulating populations reflects changes in intensity of the epidemic process of parainfluenza infection. In our opinion, the epidemic process in the organized groups of school and preschool children was most marked in 1976–1977 and it was accounted for by the circulation of strain populations with the highest virulence, antigenic pattern appreciably different from the prototype, having moderate susceptibility to ß-inhibitors of rabbit sera and high thermostability.

There has been reported that the natural population of bakuloviruses may simultaneously consist of several genotypic and phenotypic variants (Brown *et al.*, 1985). Similar results were obtained in our studies.

Table 2. Susceptibility of different strains of parainfluenza viruses to inhibitors in sera of various animals species

Virus	Titres in neutralization test with sera:				
	horse	calf	guinea pig	rabbit	mouse
PG_3-C-243/57-prototype	0	16	32	16	2
PG_3/Leningrad/643/03/77	0	2	2	16	32
PG_3/Leningrad/645/03/77	0	2	4	16	32
PG_3/Leningrad/1210/11/77	0	4	2	16	32
PG_3/Leningrad/1364/01/78	0	4	2	2	32
PG_3/Leningrad/1365/01/78	0	4	2	2	32
PG_3/Leningrad/1419/02/78	0	4	2	2	32
PG_3/Leningrad/1424/02/78	0	4	2	2	32

Each antigenic variant apparently has a corresponding phenotypic profile. Nevertheless, we cannot rule out the presence of phenotypic variants in the composition of parainfluenza virus population since PG_3/Leningrad/1364/01/78 strain has its antigenic pattern similar to the prototype while its degree of susceptibility to ß-inhibitors of sera of various animals species was different from that of the prototype. It indicates appreciable polymorphism of circulating populations of serotype 3 parainfluenza virus.

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