EXPERIMENTAL INFLUENZA CAUSED BY AN INCOMPLETE FORM OF THE VIRUS

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An incomplete form of influenza virus obtained by Nayak's method was administered intranasally to CBA and C57BL mice. The pathogenic agent was isolated from the lung tissues of the infected mice for a period of 45 days, but from the other internal organs only during the first few hours after infection. Morphological investigation of the lungs of animals infected with incomplete virus showed predominance of the proliferative component against the background of inflammatory changes. Circumscribed lymphoid structures consisting of monomorphic cells with hyperchromic nuclei were discovered in the lung tissue 3 months after infection. Later, marked proliferation of the alveolar and bronchiolar epithelium was observed, with marked cellular anaplasia in foci of papillomatous growth. Among lesions in other internal organs, glomangiomas of the mesentery were found in 18.7% of the CBA mice.

KEY WORDS: incomplete influenza virus; influenzal pneumonia; proliferation of epithelium.

Persistent and chronic forms of virus infection are among the most urgent problems in modern virology. Defective forms of the agent are of great importance in the mechanism of their formation [1-3]. The properties of these defective forms have been studied from genetic, structural, functional, and other aspects. Meanwhile, in order to understand the mechanisms of virus persistence it is important to study the pathogenesis of influenza caused by defective virions.

In this investigation some of the features of the course of experimental influenza were studied in mice of various lines after infection with incomplete virus.

EXPERIMENTAL METHOD

Experiments were carried out on 650 C57Bl and CBA mice which were divided into three groups. The animals of group 1 were given incomplete influenza virus obtained after centrifugation in a sucrose gradient [4]. The titer of virus was 10^7 EID_{50} and the infecting dose 10^3 EID_{50} in 0.05 ml, which was injected intranasally. The mice of group 2 were infected with complete (original) influenza virus of the A/Hong Kong/1.68 H_3N_2 strain in the same dose. The mice of group 3, which received inactivated influenza virus (60°C for 1 h) served as the control. By random sampling 5 mice from each group were killed after 1-5, 7, 15, and 30 days, and thereafter monthly, for virological and morphological investigations. Extracts (10%) were prepared from the lungs, liver, kidneys, and spleen, treated with ultrasound on the UZDN-IV 42 apparatus, and centrifuged; the resulting supernatant was used to infect chick embryos. In parallel tests pieces of the same organs were fixed in 10% neutral formalin, taken through alcohols of increasing strength, and embedded in paraffin wax. Sections 4-5 μ thick were stained with hematoxylin–eosin.

EXPERIMENTAL RESULTS

The results of determination of influenza virus in the lung, liver, kidney, and spleen tissues are given in Table 1. A virus was isolated for a longer time (up to 45 days from the time of infection) from the lungs of the animals of group 1 than from those of group 2. No virus could be isolated from mice infected with the inactivated virus.

Determination of hemagglutinins in extracts of the lungs, liver, kidneys, and spleen likewise revealed their prolonged circulation in the animals infected with incomplete influenza virus. Isolation of the virus was

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TABLE 1. Determination of Influenza Virus in Organs of C57BL Mice at Various Times after Infection

Organs	Group of animals	Titer of virus, log EID_{50} days after infection								
		1st	2nd	3rd	5th	7th	15th	30th	45th	60th
Lungs	1	1,0	2,0	3,2	4,5	4,0	1,0	1,0	1,0	—
	2	2,0	4,5	7,5	6,0	3,2	—	—	—	—
	3	—	—	—	—	—	—	—	—	—
Liver	1	—	0,2	0,6	—	2,5	1,0	—	—	—
	2	0,2	1,4	1,5	—	—	—	—	—	—
	3	—	—	—	—	—	—	—	—	—
Kidneys	1	—	1,5	—	—	1,0	—	—	—	—
	2	—	—	0,3	1,5	—	—	—	—	—
	3	—	—	—	—	—	—	—	—	—
Spleen	1	—	0,6	1,2	1,2	1,2	2,5	—	—	—
	2	—	0,3	0,8	—	—	—	—	—	—
	3	—	—	—	—	—	—	—	—	—

Legend. —) No virus detected.

confirmed by immunofluorescent tests, whereby specific fluorescence of virus antigen could be detected in the tissue of the internal organs.

During the study of viremia in the animals of groups 1 and 2 the earlier (after 6 h) appearance of infectious virus in the blood serum of the mice infected with incomplete virus was noted. In animals receiving the original strain positive results were obtained 24 h after infection. Maximal titers in the blood serum corresponded to the maximum of reproduction of the virus in the lungs. During the period from 48 to 72 h the titer of virus in the erythrocyte fraction was higher in the mice of group 1; complete disappearance of the virus from the erythrocytes took place in this group only after their treatment with ultrasound.

Morphological investigations of the organs of the animals also revealed differences in the mice of the difference experimental groups. After administration of the original strain an acute influenzal infection developed, in the form of proliferative–desquamative pneumonia with a marked hemorrhagic component, and with signs of peri- and panbronchitis. By the 15th to 20th day the foci of pneumonia were smaller, and by the 30th day most of the mice had recovered. In some animals the pneumonia had no tendency toward resolution and followed a chronic course.

In mice infected with incomplete virus, after the first day diffuse proliferation of septal cells was observed in the lungs against the background of marked edema and congestion of the alveolar septa. Degenerative changes were seen in the epithelium of the bronchi, and infiltration of the peribronchial cellular tissue and the submucosa with lymphocytes was only slight in degree of undetectable compared with that observed after injection of the complete form of the virus. Despite the fact that the infecting dose was the same, mortality in group 1 was significantly lower (27.4%) than in group 2 (72.1%). Starting with the third week, proliferation of the cells of the alveolar septa began to be found in the lungs of the animals of group 1 (C57BL mice), and by the third month circumscribed groups of lymphocytes appeared, numbering 10 to 12 in a section of the lung. On microscopic examination they consisted of collections of monomorphic dark cells with hyperchromic nuclei. The appearance of lymphoid formations in the lung tissue of the animals infected with incomplete influenza virus was evidently the result of the prolonged infection, accompanied by marked cell proliferation [5].

In the later stages (after 3 months) marked proliferation of the alveolar and bronchial epithelium was observed, in which papillomatous growths partly or completely closed the lumen of the alveoli and bronchi. The degree of proliferation was much greater than that usually observed in the late stages of the infection. Atypical cells were a marked feature.

Among lesions of other internal organs, neoplasms were observed in the mesentery, most frequently in CBA mice (in 18.7% of cases after infection with incomplete virus and 7.9% after infection with the original strain). In their structure these neoplasms were glomangiomas and they were characterized by a loose arrangement of cells with large nuclei, surrounded by angiomatous areas.

The results of this investigation suggest that a definite number of incomplete forms is present in a population of the mature virus. As well as a less severe acute infection, these incomplete forms give rise to early anaplasia of the bronchial and alveolar epithelium, and in the late stages to glomangiomas of the mesentery.

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SOME FEATURES OF GROWTH OF AN ORGAN CULTURE OF THE LIVER FROM MICE INFECTED WITH COXSACKIE A13 VIRUS

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Features of growth and proliferation of organ cultures of the liver from noninbred albino mice infected with a single dose of Coxsackie A13 virus were investigated. A marked zone of growth mainly of epithelial cells was found early in explants of the liver of the experimental group of mice, whereas growth of cells around the liver explants of the control mice either was absent or was very weak. Moreover, many lymphocytes uniformly distributed in the zone of growth of the liver cells were found in preparations of the liver of the experimental mice. In some explants the picture of adhesion of lymphocytes to the hepatocytes of the culture was seen, and in places where lymphocytes accumulated death of the liver cells and marked thinning of the cellular layer were observed on the 21st and 28th days of growth of the culture.

KEY WORDS: Coxsackie A13 virus; organ culture; proliferation; liver.

In recent years investigators have paid considerable attention to the ability of some viruses to stimulate cell division [4, 5, 8]. This phenomenon, known as the cytoproliferative activity of viruses, plays an important role in the pathogenesis of certain acute, chronic, and slow virus infections [2].

In this investigation an attempt was made to study the morphology of organ cultures of the liver of mice infected with Coxsackie A13 virus. Previous investigations showed increased mitotic activity of the liver cells of mice infected in the neonatal period with Coxsackie A13 virus [1].

EXPERIMENTAL METHOD

Experiments were carried out on noninbred female mice weighing 20-22 g. The mice were infected with virus-containing culture fluid, undiluted, containing $10^{-5.35}$ TCD_{50}/ml. Virus was injected intramuscularly into the animals as a single dose of 1 ml on the 7th day of pregnancy. The day of discovery of a vaginal plug was taken as the 1st day of pregnancy [9]. The mother rats were autopsied on the 13th, 15th, and 36th days after infection. Virus was isolated from the liver tissue of the experimental mice by the usual method.

Organ culture of the liver was carried out by Grobstein's method in the modification of Luria and P'yanchenko [3] in Conway dishes at the boundary between two media. Pieces of mouse liver were cultured on millipore filters (RUFS brand, Czechoslovakia) with a pore diameter of 1-2 μ. The liver explants were cultured in medium No. 199 with the addition of bovine serum, 40% glucose solution, 5% ascorbic acid solution, penicillin, and streptomycin. The gas mixture used for culture consisted of 60% O_2, 5% CO_2, and 35% atmospheric air.

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