CHANGES IN THE MANIFESTATIONS OF EXPERIMENTAL INFLUENZA WHEN COMBINED WITH PARAINFLUENZA

G. V. Shastina and N. A. Koneva

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When albino mice were infected with influenza virus and with a combination of influenza and parainfluenza viruses, the disease followed a more severe clinical course and involvement of the lung tissue was more widespread in animals with the combined infection.

Acute respiratory virus infections are very frequent diseases both in children and in adults. The study of postmortem material at the Leningrad Pediatric Medical Institute, in agreement with data in the literature [2, 5, 6], has shown that the severest forms of these diseases are observed if a combination of different virus infections is present: influenza and parainfluenza, influenza and adenovirus infection, and so on.

The object of the present investigation was to study inflammatory changes in the lungs arising in albino mice after infection with type A2 influenza virus or combined infection with two viruses: A2 influenza virus and type 3 parainfluenza virus. Particular attention was paid to the severity of the lesion and the concentration of virus excreted.

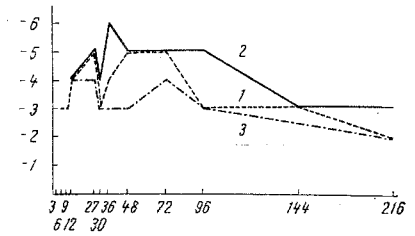

Fig. 1. Dynamics of reproduction of A2 influenza virus, strain 21/65, in lungs of albino mice infected with influenza virus and receiving normal culture fluid 24 h later (1), infected with influenza virus and 24 h later with type 3 parainfluenza virus (2), and infected with influenza virus only (3). Ordinate, titer of influenza virus (in log EID/0.2 ml); abscissa, time after administration of influenza virus (in h).

EXPERIMENTAL METHOD

Experiments were carried out on 120 albino mice divided into three groups. The animals (50) of group 1 were infected with A2 influenza virus; the mice (40) in group 2 were infected with A2 influenza virus followed 24 h later by type 3 parainfluenza virus. The animals of group 3 (30 mice) were infected with the same strain of influenza virus, but 24 h later they received the culture fluid from an uninfected HEp-2 cell line.

The A2 influenza virus, strain 21/65, used in the experiments was maintained by passage through developing chick embryos (hemagglutination titer 1:256-1:512, infectious titer 10^6-10^7 EID_{50}/0.2 ml). Type 3 parainfluenza virus, strain Na-1, was subcultured in cells of the transplantable line HEp-2 (infectious titer on the 4th day after infection of the cells was 10^5 CPD_{50}/0.2 ml).

The animals were infected under light ether anesthesia by instillation of 0.05 ml of virus-containing or culture fluid into the nose. To study the dynamics of the lung lesions and accumulation of the viruses in the lungs of the infected mice, the animals were sacrificed 3, 6, 9, 12, 27, 30, 36, 48 and 72 h and 4, 6, and 9 days after

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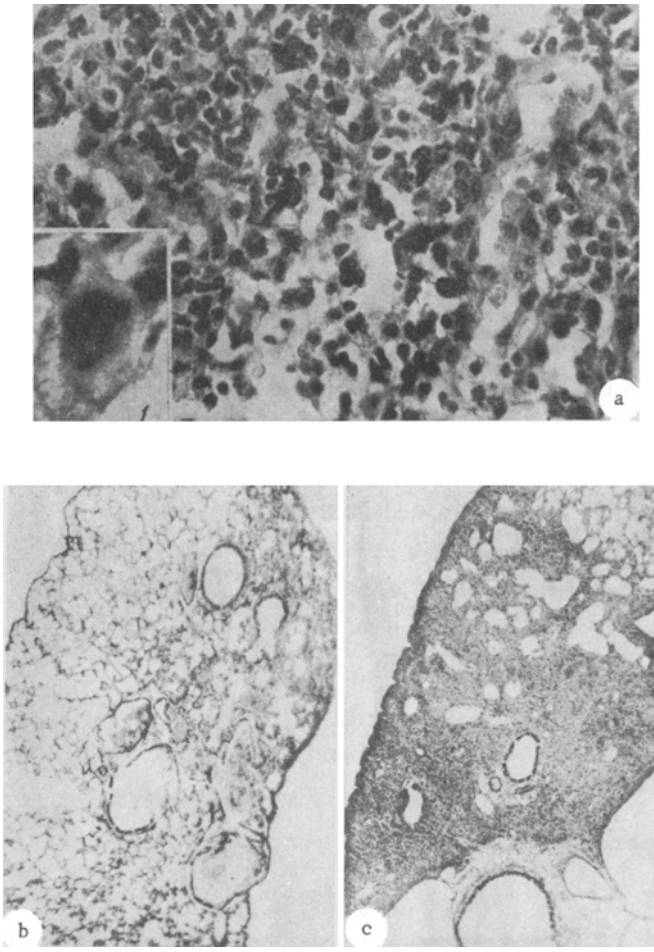

Fig. 2. Histological changes in lung parenchyma of albino mice infected with A2 influenza virus, strain 21/65: a) focus of pneumonia with exudate containing leukocytes and macrophages 30 h after infection (azure-eosin, 300×); 1) detail of section, giant mononuclear cell arising from alveolar epithelial cell (azure-eosin, 1 300×); b) 48 h after infection with influenza virus: multiple small, frequently confluent, pneumonic foci can be seen (azure-eosin, 35×); c) 48 h after infection with influenza virus and 24 h after administration of type 3 parainfluenza virus; almost the whole lobe of the lung is filled with confluent pneumonic foci (azure-eosin, 35×).

the beginning of infection with influenza virus. At each time, four animals were usually investigated in all three series of experiments.

After perfusion with physiological saline through the heart the lungs of two mice were ground in a mortar with quartz sand and a 10% suspension was prepared in blood-digest maintenance medium. Tenfold dilutions of this suspension in a volume of 0.2 ml were used to infect a 48-h culture of HEp-2 cells or 10-12-day developing chick embryos. Influenza virus was indicated by the hemagglutination test with chick erythrocytes, and parainfluenza virus by its cytopathogenic action on the HEp-2 cell line. The lungs of the other two mice were investigated histologically. They were fixed in 10% formalin solution and embedded in celloidin-paraffin. Sections were stained with hematoxylin-eosin, azure-eosin, and by the Gram-Weigert method.

EXPERIMENTAL RESULTS

A study of the fate of the viruses administered to the mice showed that type A2 influenza virus, strain 21/65, actively propagated in the animal's lungs (Fig. 1). Preliminary infection of the mice with influenza

virus did not interfere with propagation of the parainfluenza virus. In addition, if the animals were infected with two viruses, at certain times a higher concentration of A2 influenza virus was found in the lungs than in animals infected with influenza virus alone and in the lungs of the control mice which received normal culture fluid instead of parainfluenza virus. Combined infection of the mice with two viruses increased the severity of the clinical course of the disease and the mortality among the animals.

None of the mice in group 1 died. Microscopic examination 3-6 h after infection showed circulatory disturbances, focal emphysema, and atelectasis of the lungs. The changes were most marked in the paravertebral regions. In the bronchi degenerative changes were found in the epithelial cells: the cytoplasm of many of them was heterogeneous, with small basophilic inclusions surrounded by a border of translucency frequently present in it. At the same time, large, round, oxyphilic inclusions also were seen. The changes in the cells of the bronchial epithelium are in agreement with those described elsewhere [4, 8]. The nuclei of most cells were pale and shadow-like, while in other cells the nuclei were pycnotic and hyperchromic.

In the respiratory regions some cells of the alveolar epithelium were cubical or circular in shape, with a large hyperchromic nucleus and homogeneous cytoplasm. Some of them were detached and were lying in the lumen of the alveoli. These alveoli also contained red cells, one or two leukocytes, and a few protein masses. No microorganisms were present. The cells of the bronchial epithelium 9-12 h after infection were separated, their cytoplasm was homogeneous, and their nuclei pycnotic. Cytoplasmic oxyphilic inclusions, surrounded by an oxyphilic border, were found in many cells. The inflammatory foci in the lungs were larger and occupied two or three alveoli, in which the leukocytes were broken down and were pinkish-purple in color.

Marked disturbances of the circulation and large hemorrhages were observed in the respiratory areas areas of the lungs 30-60 h after administration of the virus. The pneumonic foci were located mainly around the bronchi. The alveoli contained an exudate in which very large mononuclear cells were mixed with the scattered macrophages and leukocytes (Fig. 2a). Cells of the same type may also be observed in cases of death caused by A2 influenza [7]. Some leukocytes had disintegrated. No bacterial microflora could be found in the foci of inflammation.

The most widespread involvement of the lung tissue was observed on the second to fourth day after infection (Fig. 2b). At this time the respiratory areas were severely underaerated through atelectasis and the presence of exudate in the alveoli. The exudate consisted of protein masses, large cells with a foamy or homogeneous cytoplasm, and a few leukocytes. In some places the interalveolar septa were saturated with protein, thickened, and oxyphilic.

After the sixth day the inflammatory foci in the lung decreased in size. The remains of the exudate were absorbed by macrophages and the lung tissue restored.

In the 40 mice of group 2, infected with A2 influenza virus, strain 21/65, and 24 h later with type 3 parainfluenza virus, seven of the mice died on the fifth-sixth day.

The character of the morphological changes in the lungs of the mice of this group differed somewhat from that described above. To begin with, the area of lung tissue involved was much greater (Fig. 2c) and it reached a maximum on the 2nd day after the first infection, and 24 h after administration of the parainfluenza virus. Lung function was impaired: well-marked atelectasis and emphysema with perivascular edema and hemorrhages were present. The alveoli contained a serous exudate with leukocytes and a few red cells and macrophages. Meanwhile, proliferation of the bronchial epithelium was observed, so that it began to appear stratified, a characteristic feature of parainfluenza in general [3, 4]. A small quantity of exudate appeared in the lumen of the bronchi, mainly along their walls.

The mice of group 3, which received culture fluid 24 h after infection with influenza virus, can be regarded as the control animals for the mice of group 2. The morphological changes in their lungs were indistinguishable from those observed in the animals of group 1.

The results of these experiments show that after combined infection with A2 influenza virus and type 3 parainfluenza virus the disease follows a more severe course and is more likely to cause death of the animals. This is due to the more rapid propagation of the influenza virus in the lungs in the case of combined infection and to superposition of the morphological changes produced by parainfluenza virus on those due to the influenza virus.

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