VIROLOGY

Experimental Modeling of Viral Diseases of the Locomotor System

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Dissemination of infectious inflammation was studied in experimental influenza and acute and chronic herpesvirus infections. The possibility of articular involvement into the infectious process was evaluated. Pathomorphological signs of changes in the articular tissue confirmed the effects of these viruses on the locomotor pathology. Results of virus infection simulation in experimental animals suggest this model for studies of the pathogenesis of diseases of viral etiology (including those with articular involvement) in humans.

Key Words: *experiment; respiratory viruses; herpesviruses; locomotor diseases*

Recent reports put forward a hypothesis that reactive arthritis is more incident after virus infection than after bacterial one [1,6]. It is also hypothesized that influenza viruses and some adenovirus types play the key role in the formation of reactive arthritis [4]. Intoxication lesions, reactive, inflammatory mono- and polyarthritis, and other infectious allergic processes predominate in the clinical picture of articular changes [5,8]. Some authors tried to detect histopathological changes in antigen-induced arthritis in animal experiments in order to evaluate the similarity of these lesions to those in arthritis in humans [7,10].

We simulated influenza and herpesvirus infections in order to evaluate dissemination of infectious inflammation and the possibility of articular involvement into the infectious process.

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MATERIALS AND METHODS

Laboratory animals were infected by influenza A/PR/8/34 (H1N1) reference strain. The infective titer was 7-8 lg EID_{50} in 0.2 ml. Human herpes simplex virus (HSV; strain L2) reference strain was obtained from the State Collection of Viruses, D. I. Ivanovsky Institute of Virology. The infective titer of this strain was 4.5 lg TCD_{50}/ml. Experimental studies were carried out on outbred albino mice (n=150). All experiments were carried out in accordance with regulations for studies on experimental animals (GLP) and with the Order of the Ministry of Health of the Russian Federation No. 267 of June 19, 2003. Influenza infection was induced in two groups of mice (10-12 g), 30 animals in each: group Ia included experimental animals intranasally infected with A/PR/8/34 (H1N1) virus (0.05 ml virus-containing fluid) under light ether narcosis and mice of control group Ib intranasally receiving saline (0.05 ml).

Herpetic infection was simulated in 3 groups of mice (20-22 g), 30 animals per groups: group IIa in-

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cluded experimental mice intraperitoneally infected with 0.2 ml HSV for inducing acute herpetic infection; mice of experimental group IIb were intraperitoneally infected with 0.02 ml HSV in order to induce chronic herpetic infection; and mice of control group IIc were intraperitoneally injected with 0.2 ml 0.9% saline.

Histological studies of the lungs, liver, kidney, and joints (knee joint and temporomandibular joint, TMJ) were carried out. The preparations were stained with hematoxylin and eosin and after van Gieson and Weigert. Virus antigens were detected in impression smears of cells from the trachea and bronchi, oral mucosa, urinary precipitate, inner surface of the knee joint and TMJ cavities by the immunofluorescence test (IFT) [3]. Dynamic study was carried out 2, 5, 7, 10, 15, 25, 30, and 40 days after infection.

RESULTS

By day 7 after influenza virus infection, involvement of the respiratory organs with acute respiratory failure and distant rale were observed in experimental mice. The process involved small bronchi, bronchioles, and lung parenchyma; focal influenza pneumonia developed with serous hemorrhagic or necrotic inflammation in the trachea and bronchi. During the terminal stage (days 10-12 postinoculation) changes in the respiratory organs were paralleled by pronounced symptoms of general intoxication (inertia, refusal from food, chill, photophobia, locomotor dyscoordination, convulsions). Influenza encephalitis was observed in 66.6% cases. Detection of influenza virus antigen in brain cells on day 7 after infection indicated the involvement of the nervous system.

Study (by IFT) of impression smears of the knee joints and TMJ on day 2 showed specific fluorescence of cells lining the synovial membrane.

Pathohistological changes developed in the following order. On day 2 after infection with influenza virus, accumulations of macrophageal and histiocytic cells were detected in the lung parenchyma. Foci of atelectasis and emphysema with pronounced circulatory disorders (plethora, stenosis, edema, focal hemorrhages) were detected. On day 7, small-focal bronchopneumonia developed, which progressed on day 10 into large-focal condition with signs of edema of the lungs and alveolar septa. Edematous fluid was detected in the knee joint; swelling and focal desquamation of the synovial membrane cells and devastation of the cartilaginous lacunae were observed (Fig. 1). Examination of the TMJ also showed moderate epiphyseal edema. Focal devastation of the cartilage lacunae was seen.

Hence, by day 10 of observation the inflammatory process in the lungs was paralleled by signs of increased vascular and tissue permeability of the articular synovial membrane, which attested to exudative phase of inflammation.

Simulation of acute herpesvirus infection led to the development of conjunctivitis in all animals 2 days after the virus injection; suppurative conjunctivitis developed in 3.3% cases. By day 7 postinfection, involvement of the CNS was observed in 23.3% animals, it was characterized by disorders of motor coordination and pareses of different severity. Herpetic involvement of the CNS and pneumonia led to 60% mortality in this group by day 7.

Immunofluorescent analysis of impression smears of the oral mucosa and urinary precipitate cells detected HSV antigen starting from day 2 until death. Specific fluorescence of cells was detected in lung tissue. This explained the development of pneumonia and determined the mortality level among infected animals. On day 7 of observation, the HSV antigen was detected in the synovial cells lining the knee joint and TMJ cavity in 50% cases.

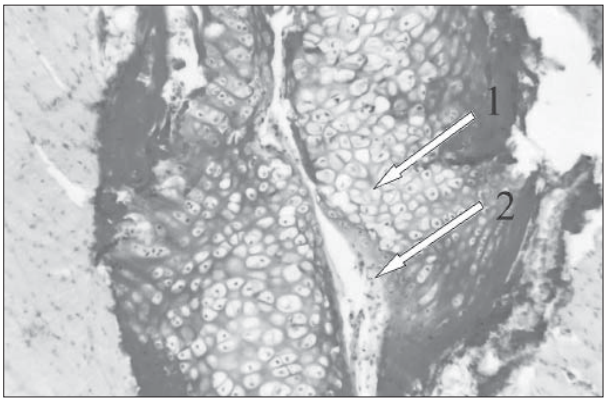

Fig. 1. Mouse knee joint after infection with influenza virus. *1*) devastation of cartilaginous lacunae of the epiphyseal body; *2*) desquamation of synovial membrane cells into articular cavity. Staining by methods of van Gieson and Weigert, ×100.

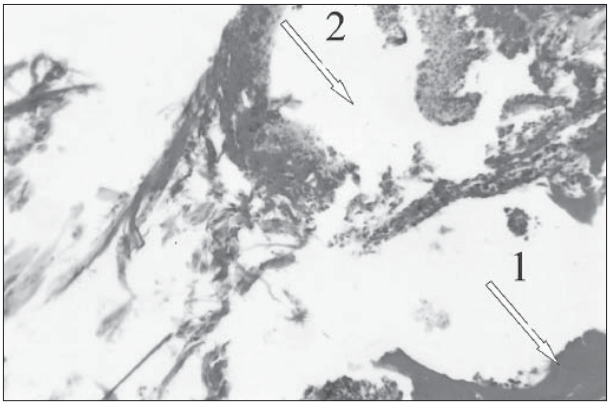

Fig. 2. Mouse TMJ (chronic experiment, terminal stage of HSV infection). *1*) hyperplasia of epiphyseal membrane structures; lymphoid infiltration; *2*) focal destruction of cartilage matrix with devastation of lacunae. Staining by methods of van Gieson and Weigert, ×100.

Histological study on day 2 after infection showed detachment, pronounced edema, hyperemia, leukocytic infiltration of the tracheobronchial epithelial cells. On day 5, atelectasis and emphysema with pronounced circulatory disorders were detected in the lungs. On day 7, small-focal bronchopneumonia with hemorrhagic foci developed, which progressed on day 10, transforming into large-focal bronchopneumonia involving 64.5% lung area with inflammatory macrophageal and histiocytic infiltrate. On day 15, hyperemia and stasis of small vessels and capillaries were observed in the liver, paralleled by diffuse vacuolar degeneration of hepatocytes with focal necrobiosis and necrosis. Significant histiocytic and macrophageal infiltration was detected in necrobiosis foci. On day 15 small and large lymphoid infiltration foci in the interstitium with compression of the tubules were seen in the kidneys, paralleled by degenerative changes in the tubular epithelium and glomerular and stromal capillary plethora. Focal desquamation and necrosis of the structures were seen in the knee joint synovial membrane, as well as sharply pronounced plethora of the microvessels. Moderate interstitial edema was seen in the articular epiphyseal plates, solitary desquamation of chondrogenic cells was detected in the articular cavity.

Hence, inflammatory process developed in the lungs by the end of the observation period in this group of animals, which progressed and involved 74.2% of the lungs. Structural changes were caused by increased vascular and tissue permeability typical of the exudative phase of inflammation. Marginal vacuolation and necrobiosis of hepatocytes were seen in the liver, degenerative changes of the tubular epithelium in the kidneys, and development of the pathological process (inflammation) in the articular tissue.

Simulation of chronic herpetic infection (>10 days) in group IIb showed that acute period was followed by epithelialization of ulcers in the oral mucosa and cessation of mucous suppurative discharge from the eyes. Rale in the lungs and photophobia disappeared on days 15-25.

After the clinical symptoms disappeared, HSV antigen was detected only in liver cells (in 56.6% cases). The antigen was detected in 33.3% cases in the synovial cells lining the cavities of the knee joints and TMJ throughout the entire period of observation.

Histological study on day 15 showed hyperemia, pyknosis, and desquamation of the vascular epithelium in the lungs. The alveoles were swollen like in emphysema. The bronchi at sites of infiltration were deformed and compressed. Necrotic cells with signs of degradation were seen in the bronchial lumen. Acute circulatory disorders (intensive hyperemia and hemorrhages, pronounced edema of the peribronchial and perivascular spaces) developed after day 15. The inflammatory process involved about 73.3% lung area. Elements of granulations, consisting of loose connective tissue layered by fatty tissue, were detected in the knee joints and TMJ. Foci of angiomatosis with predominating capillaries were detected. Vast areas of synovial membrane cell proliferation were seen. The infiltration was lymphoplasmocytic. Stable focal destruction of chondrocytes and partial devastation of the cartilaginous lacunae with the formation of sequester-like sites and foci of mucoid swelling of the cartilaginous matrix structures were seen in the epiphyseal plates of the cartilage (Fig. 2).

No clinical signs of infection or changes in the viscera and joints were detected in the control animals throughout the entire period of observation. Immunofluorescent analysis showed nothing but even basal fluorescence of cells.

Hence, simulation of viral infections on experimental animals showed that this model can be used for more profound study of the pathogenesis of viral diseases, including those with involvement of the joints in humans.

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