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EXPERIMENTAL PATHOLOGICAL STUDIES ON MECHANISM OF ABORTION CAUSED BY EQUINE ARTERITIS VIRUS

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Equine viral arteritis (EVA) is an acute infectious disease caused by equine arteritis virus (EAV). EAV infected horses show various clinical signs such as pyrexia, edema of the legs, conjunctivitis, and diarrhea. Abortion occurs in pregnant mares infected with EAV, at a rate of approximately 50% during field epizootics. In the breeding areas, abortion is the most significant manifestation of EVA, however the pathogenesis is not well understood. The purpose of this study is to elucidate the mechanism of abortion in EVA through histopathological, immunohistochemical and electron microscopic examinations.

1. The morphogenesis of EVA in the maternal genital tract and in the fetus has never been fully investigated, therefore, electron microscopy and immunocytochemistry of a modified Bucyrus strain of EVA in BHK-21 cells were performed.

Findings showed bacillary tubules, an increase in rough endoplasmic reticulum (RER) and accumulated ribosomes in the cytoplasm at 8 hr postinfection (PI). Mature virions, 79 to 122 nm in diameter (101 nm on average), were observed in the cisternae of the RER at 12 hr PI, or later. They had isometrical cores, morphological subunits in the outer layer, and a covering envelope with projections. Budding occurred from the RER and the outer nuclear membrane, but not from the cell surface. Small-sized particles about 40 nm in diameter, seemingly in a process of maturation, were observed in the cytoplasmic vesicles. These morphological findings were similar to those found in the coronavirus.

The tubules have perviously been considered as nucleocapsids of EAV. However, structural linkage was detected between the tubule and the virus core. This means that the tubule is in fact the precursor of the core. Aberrant strands were occasionally demonstrated within the nucleus at 12 hr PI. Immunofluorescence and immunogold labeling revealed that the tubules in the cytoplasm and the aberrant strands in the nucleus were identical in antigenicity.

2. Immunohistochemical techniques can be useful in investigating the pathogenesis of EAV infection. Preservation of the morphological details of tissues or cells as well as the antigenicity is important. Formalin-fixed and paraffin-embedded sections are a suitable preservation format. Thus, treatment methods for the deparaffinized-sections were developed for the detection of EAV antigen using indirect fluorescent antibody (IFA) techniques.

Paraffin sections made from maternal uterus samples and fetal organs were collected from mares which aborted after experimental infection with EAV. After deparaffinization, sections were treated with TUF (target unmasking fluid) or pronase. IFA staining with an anti-EAV monoclonal antibody was carried out after treatment with TUF at 90°C for 10 min, or 0.1% pronase at room temperature for 1 min, in order to facilitate the detection of EAV antigens. The fluorescent antigens were detected in tissue containing a virus titer of $10^{3.5}$ pfu/g or greater.

Although direct fluorescent antibody techniques were performed on frozen sections, immunohistochemistry on paraffin sections has never been conducted. To detect the antigens of microorganisms on paraffin sections, enzymatic treatment of sections is frequently needed before immunohistochemical staining. In most cases, treatment with proteinase for several minutes or more is recommended. In the case of EAV, treatments with pronase at room temperature for a few minutes were enough to detect the antigens. Treatment with TUF is also recommended because of its stable ability to unmask antigenicity.

3. Experimental EAV infection of pregnant mares was carried out to elucidate the mechanism of abortion. Samples from the maternal reproductive organs, placentas and fetuses were investigated histopathologically, immunohistochemically, electron-microscopically, and virologically.

Five pregnant mares, at 6 to 8 months in gestation, were intranasally inoculated with the Bucyrus strain of EAV at a titer of $5 \times 10^{5.3}$ pfu/ml. Of the 5 mares, 4 aborted at 10 or 12 days PI and 1 died at 8 days PI. Aborted fetuses were dead and enveloped with fetal membrane. Microscopically, common lesions in the maternal reproductive organs showed myometritis consisting of a degeneration of the myocytes and an infiltration of the mononuclear cells. Epithelial cells of the endometrial gland showed sporadic degeneration. Lesions in the fetal tissue included an atrophy of the lymphoid follicles in the spleen and the lymph nodes with cellular necrosis. The placentas were edematous, with degenerated fibroblasts and with an infiltration of macrophages, monocytes and lymphocytes in the subvillous layers. Through the IFA method by use of the monoclonal antibody, EAV antigens were detected in the myometrium and the endometrial gland in the dams, in the subvillous layer of the placentas, and in the liver, spleen, kidney, lung, thymus and mesenteric lymph node of the fetuses. EAV antigens were detected in the smooth muscle cells of the myometrium, the epithelial cells of the endometrial gland and the infiltrated mononuclear cells in the maternal uterus; in the degenerated fibroblasts and the infiltrating mononuclear cells in the placenta; and in the infiltrating mononuclear cells and the tissue macrophages in the fetal tissues and organs mentioned above. Electron-microscopy detected short tubules in the smooth muscle cells of the myometrium, fibroblasts and macrophages in the placenta, and monocytes in the splenic capsules of the fetus. EAV was isolated from all samples of maternal, placental and fetal tissues. The

placentas had the greatest amount of viruses.

From these results, transplacental infection of EAV to the fetus was demonstrated. Intranasally inoculated EAV showed a tropism for the myometrium of the pregnant mares and replicated in the smooth muscle cells, infiltrating macrophages and lymphocytes, and epithelial cells of the endometrial gland. Free and cell-associated EAV apparently passed through the maternal-placental barrier, attached to the epithelium of the chorions, and infected the trophoblasts and macrophages in the subvillous layer of the placentas, traveling to the fetuses via the umbilical cord. The fetal infection was regarded as an important factor in the EAV abortion process.

In field studies, abortion due to EAV has occurred in both the acute and convalescent stage of the disease in pregnant mares. However, clinical signs have not always been obvious before abortion. This study suggests that fetal infection played an important role in the pregnant mares.

In conclusion, EAV abortion is apparently caused by myometritis in the acute phase among diseased mares. It might be the result of a lethal infection of the fetus in the recovering stages, accompanied with the latent infection of the mare. It is therefore considered that EAV infection of the fetus *in utero* occurs during the gestation period.

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