COXSACKIEVIRUS B4-E2 STRAIN INFECTION OF GRAVID MICE

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Introduction

Genus *Enterovirus* belongs to the family *Picornaviridae* and consists of 12 species of which seven are known human pathogens (*Enterovirus A, B, C, D* a *Rhinovirus A, B, C*). Coxsackie B viruses (CVB) belong to the species *Enterovirus B*.

Enteroviruses are one of the most common human pathogens, ubiquitous in nature and found in all countries of the world. Infections by these viruses are common during pregnancy. A few case reports suggest that infection of the mother during pregnancy may lead to preterm births, or slow growth of the fetus, embryopathy and increase the risk of development of chronic diabetes type 1 in children aged 0-14 years born to these mothers.

Aim

To study the innate immune response and the influence of virus infection on the course of gravidity in dams infected with a coxsackievirus B4 strain.

Materials and methods

Virus: Coxsackievirus B4-E2
Mice: CD1 outbred mice
Infection: Gravid mice were orally inoculated with coxsackievirus B4-E2 at a dose of 0.2 x $10^{7.5}$ $TCID_{50}$/ml at different stages of gravidity:
a) in the 1st week on day 4 after the start of the gestation period,
b) in the 2nd week on day 10 after the start of the gestation period,
c) in the 3rd week on day 17 after the start of the gestation period.
Mock infected (with PBS parallel to the virus infection) mice served as controls. Infected dams were sacrificed at different days post infection (p.i.), depending on the time of infection. Four mice were sacrificed at day 3, 5 and 14 p.i.
Collected material: Heart, pancreas, serum
Quantitative measurement of genome copies: RNA isolation, Real-time RT-PCR
Histopathology: Sections of heart and pancreas tissue, haematoxylin and eosin staining
Cytokine assays: microarray Granulocyte-macrophage colony-stimulating factor (GM-CSF), monocyte chemoattractant protein 1 (MCP-1), interleukin-1α (IL-1α), IL-1β, IL-2, IL-5, IL-9, IL-10, IL-13, IL-17, interferon gamma (IFNγ), tumor necrosis factor alpha (TNFα), vascular endothelial growth factor (VEGF), regulated on activation normal T cell expressed and secreted (RANTES). Quantibody® cytokine antibody array 1 (RayBiotech).

Results and discussion

The highest copies of RNA were found in the heart of dams infected in the 2nd week of gravidity on day 5 p.i., whereas in the pancreas of dams infected in the 1st week on day 3 p.i. (Fig. 1). High cytokine levels were noted on day 3 p.i., levels of some increased further on day 5 p.i. (Fig. 2). Cytokines levels differed depending on the time of infection of the dams. Most of the cytokine levels were higher in dams infected in the 1st week of gravidity. We observed histopathological changes only in the pancreas of the dams infected in the 1st and 2nd week of gravidity either peripancreatic adipose tissue infiltration(Fig. 3 a) or pancreatitis(Fig. 3 b), whereas those infected in the 3rd week (Fig. 3c) and controls did not show any changes. Fig .d shows absence of changes in the hearts as observed in all the mice (infected and controls)

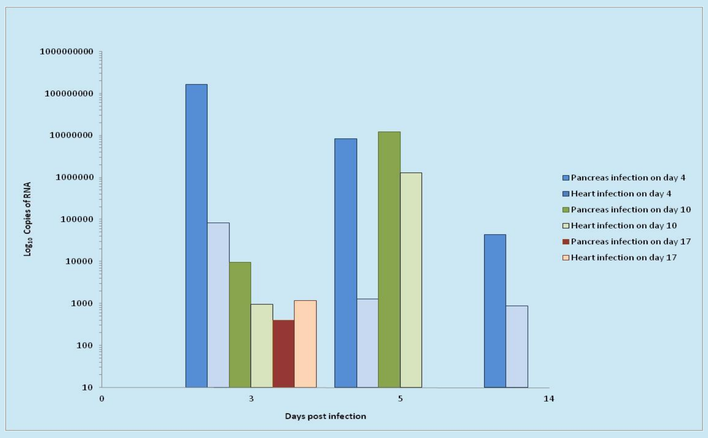

Fig. 1: Quantitation of viral load in heart and pancreas tissues of infected gravid mice.

Results and discussion

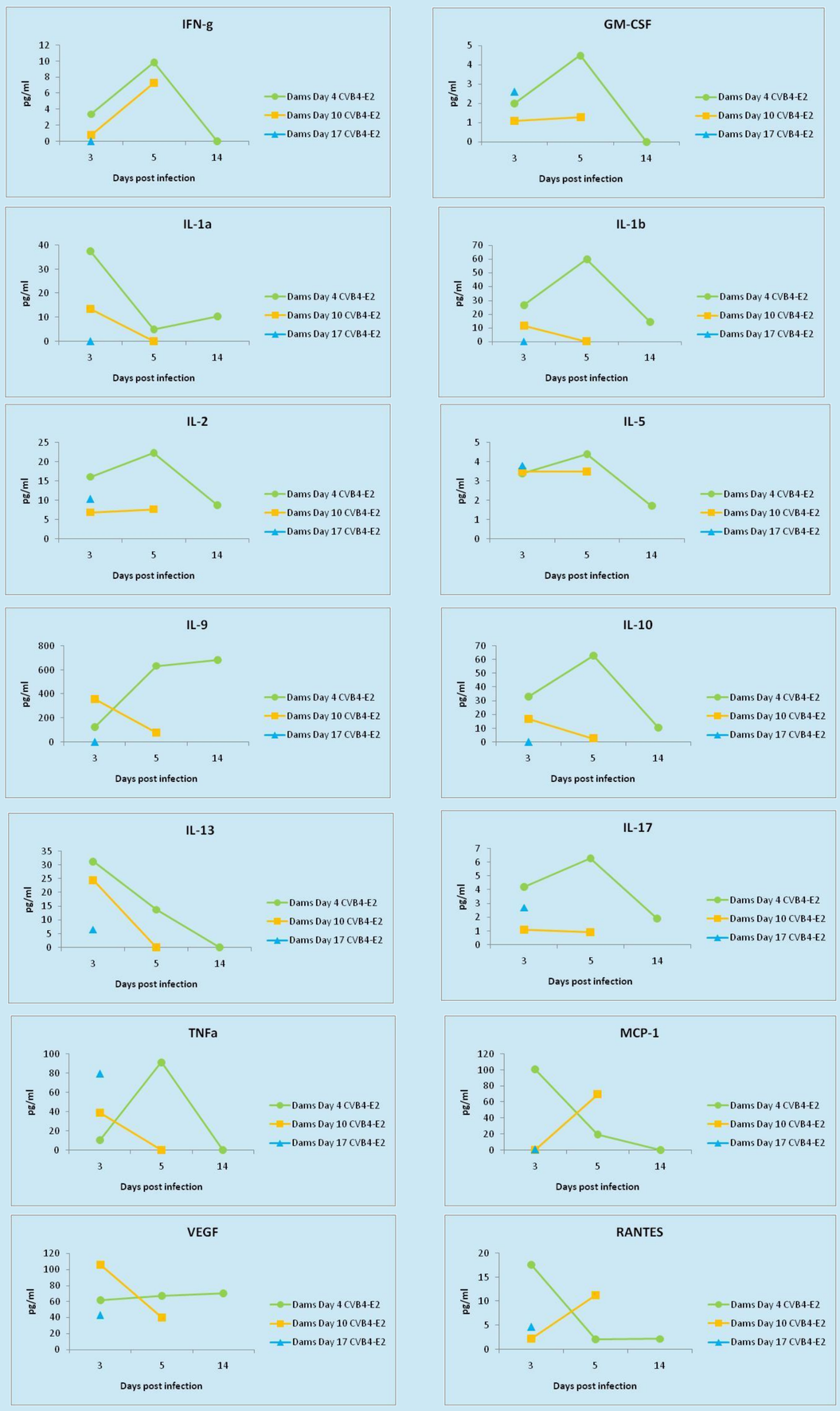

Fig. 2: Cytokine levels in sera of infected gravid mice.

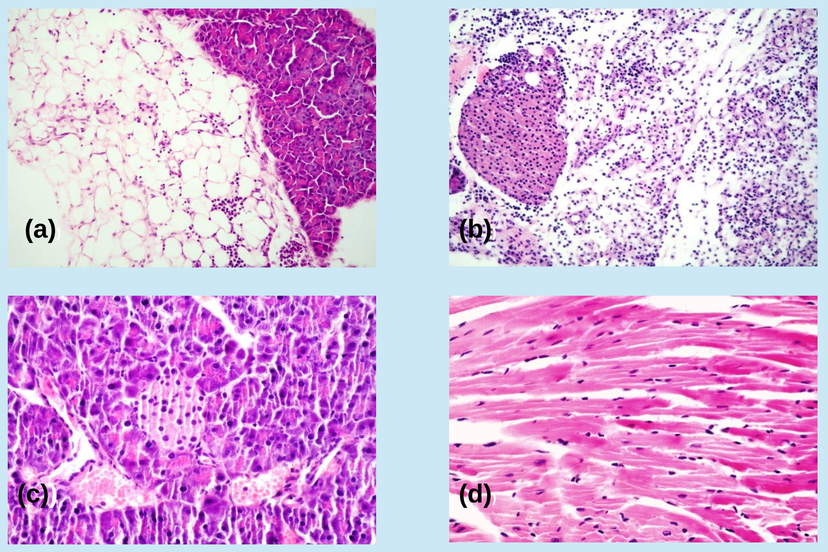

Fig. 3: Histopathological observations in the pancreas and heart tissues of infected gravid mice.

Conclusion

We conclude that the time of infection during gravidity rules the intensity of the infection, and also the innate and adaptive immune responses.

The study was approved by the Ethical Committee of the Slovak Medical University and the State Veterinary and Food Control Authority of the Slovak Republic
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