Delayed-Type Hypersensitivity and the Pathogenesis of Viral Hemorrhagic Fever (African Swine Fever)

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A potent inhibition of leukocyte migration, as high as 98%, was observed in superacute and acute forms and exacerbation of the chronic form of African swine fever. In an asymptomatic infection the inhibition is no more than 30%. Inhibition of the migration of porcine leukocytes infected in a cell culture was 73 to 90% after infection with four virulent strains and 15 to 36% after infection with two attenuated strains. The data on in vitro and in vivo leukocyte sensitization in African swine fever are indicative of a correlation between the severity of the pathogenetic picture of the disease and the degree of delayed-type hypersensitivity.

Key Words: hemorrhagic fevers; African swine fever; pathogenesis; delayed-type hypersensitivity; leukocyte migration inhibition test

Disorders of hemostasis leading to the development of the syndrome of disseminated intravascular coagulation with disorders of the microcirculation in organs and uncontrollable bleedings are the main triggering mechanisms in the pathogenesis of various viral hemorrhagic fevers [1]. In the course of development of a viral infection nonspecific pathological processes may be complicated due to the realization of specific immune mechanisms, including those related to the delayed-type hypersensitivity (DTH) phenomena. Induction of DTH mediators as mediators of inflammation may lead to the formation of granulomas, edema, and exudates. In the event of a sufficiently strong reaction, not only tissues and small vessels are injured, but larger vessels as well, resulting in hemorrhages [3-6]. Elucidation of the significance of nonspecific mechanisms and specific immunological reactions in the pathogenesis of hemorrhagic fevers is necessary for choosing ways and means of purposefully regulating their manifestation with a view to achieving a therapeutic effect.

The aim of this study was to analyze the relationship between the degree of DTH and the severity of a viral hemorrhagic fever, African swine fever (ASF).

MATERIALS AND METHODS

Experiments were carried out with Large White piglets weighing 25 to 30 kg. ASF virulent strains KIR, MOZ, L-57, and F-32 and the avirulent FK-135 and LK-111 were used. The dose for intramuscular inoculation was $10^6-10^7$ HAUs/ml. The development of DTH in infected animals was assessed in the leukocyte migration inhibition test (LMIT) at various times after inoculation. For this purpose leukocytes were isolated from heparin-treated blood [7] and after a 5-min centrifugation at 400 g glass capillaries were filled with the resultant cell sediment. The capillaries were cut into fragments 8 mm long and stuck with a silicone...
lubricant to the bottom of round glass chambers 20 mm in diameter. The chambers were filled with minimal Eagle’s medium with 30% inactivated porcine serum, covered with slides, and incubated for 18–24 h at 37°C. Areas of migration projected with a photographic enlarger on photographic paper were outlined, cut out, and weighed. The index of leukocyte migration inhibition (I) in % was calculated by the formula:

$$I = (1 - \frac{P_{\text{exp}}}{P_{\text{con}}}) \times 100,$$

where $P_{\text{exp}}$ is the mean weight of the projection area of leukocyte migration of infected animals and $P_{\text{con}}$ is the mean weight of the projection area of leukocyte migration in intact animals. During the assessment of cell migration inhibition under the effects of various ASF strains, LMIT was performed with a culture of porcine leukocytes from intact animals, this culture being infected during inoculation at a multiplicity of $2 \times 10^{-3}$–$2 \times 10^{-6}$ HAU$_{50}$/cell and cultured in a monolayer for 3–4 days at 37°C.

**RESULTS**

Piglets infected with virulent strains KIR, L-57, and F-32 developed an acute form of ASF with a lethal outcome. Starting from day 3 postinoculation, that is, from the time of appearance of the clinical signs of the disease, and till the death of the animals an intensive inhibition of migration was observed (Fig. 1). LMIT values during the disease were 61 to 98%. In piglets immunized with the FK-135 strain and surviving after subsequent inoculation with the virulent strain F-32 of a homologous serotype [2] an injection of the virulent strain KIR of a heterologous serotype induced a superacute disease. The animals died on days 3–5 after inoculation when they developed a severe clinical picture of the disease, with LMIT values from 84 to 94% (88±2% on average, $n=4$) on days 3-4 after infection with the KIR strain.

None of the 16 animals inoculated with the attenuated FK-135 strain showed a noticeable inhibition of leukocyte migration or clinical signs of the disease from day 3 to day 32 postinoculation. LMIT values were within the normal range or no more than 30%. In fact, in some animals migration increased to 30%. After the animals were injected with the attenuated strain LK-111, characterized by a higher reactogenicity in comparison with strain FK-135, LMIT values as high as 20–50% were more frequently observed, and only sometimes was a 20–30% stimulation of leukocyte migration recorded.

In some cases, when piglets preinoculated with homologous attenuated strains were infected with virulent strains, the development of a chronic ASF form resulted. Leukocyte migration inhibition in such animals was observed only during exacerbation of the disease. For example, pathomorphological examination of a piglet inoculated with attenuated strain LK-111 and infected for control purposes with strain L-57 revealed on day 22 after challenge with the virulent strain marked changes in the organs, the LMIT value being as high as 78%. Similar data were obtained with the material from two piglets inoculated with strain FK-135 and infected for control with strain F-32. On day 23 after control challenge, blood leukocyte migration

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![Fig. 1. Inhibition of blood leukocyte migration in animals with acute ASF. 1) piglets infected with strain L-57; 2) those infected with strain F-32; 3) those infected with strain KIR.](image1)

![Fig. 2. Mean LMIT values after inoculation with ASF virus strains of different virulence in a culture of porcine blood leukocytes.](image2)