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Pathology and Pathogenesis of African Swine Fever
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Introduction
African swine fever (ASF) is a viral haemorrhagic disease that causes an acute haemorrhagic fever with mortality rates up to 100 % in domestic pigs it, although some low-virulence isolates, with chronic and unapparent clinical forms have been reported. ASF virus (ASFv) causes inapparent persistent infections in its natural hosts, warthogs (Phacochoerus aethiopicus), bushpigs (Potamochoerus porcus), and soft ticks (Ornithodoros moubata), which inhabit warthog burrows1.

ASFv is a large, icosahedral, double-stranded DNA virus which replicates in the cytoplasm of infected cells and has been classified as the only member of a virus family, the Asfarviridae2.

The virus replicates in monocytes-macrophages (m-MÆ) mainly. Macrophage infection by ASFv is of foremost relevance for pathogenesis, taking into consideration that MÆ play important roles in both innate and acquired immune responses. Relevant in the role of MÆ as orchestrators of immune and inflammatory responses, they synthesize cytokines that have an impact on the development of these responses. However, the role of these cells in ASF is unclear, with contradictory results.

Disseminated intravascular coagulation develops during the late phase of acute course and the initial phase in subacute course of the disease, and this may lead to the characteristic hemorrhagic syndrome. Widespread apoptotic cell death occurs in both T and B lymphocytes of lymphoid tissues, with severe lymphopenia.

Changes in monocytes-macrophages
The main target cells of ASFv are m-mÆ3-8, with virus replication in epithelial and mesenchimal cells after. Viral replication is ultrastructurally characterized by cytoplasmic replication sites containing elongated membranous structures and hexagonal viral particles of 175-195 nm diameter, some of them with an electron-dense nucleoid. Cytopathogenic effect is characterized by rounding of nuclei, peripheral margination of chromatin and development of cytoplasmic vacuoles4-8. Additional effect of viral replication in m-MÆ is haemadsorption reaction in vivo, which is the cause of virus particles adhered to erythrocytes8.

The morphological changes no related with viral replication m-MÆ are the proliferation of primary lysosomes, increased phagocytosis and micropinocytic vesicles and cell enlargement which are characteristics of activation coinciding with the presence of ASFv in different organs. During the middle phase of the disease there is an infiltration of MÆ in different organs10. These cells are activated and became infected by the virus. In vitro studies have demonstrated that viral ASFv replication inhibit the releasing of cytokines by MÆ11 and that host cells antiviral gene expression pattern is modified12. However, other in vitro studies have showed secretion of proinflammatory cytokines by infected MÆ13,14. and early transcriptional activation of proinflammatory cytokines genes15. Experimental inoculation in pigs with virulent ASFv have showed that there is a significant increase in the number of MÆ expressing IL-1β, IL-6 and TNF-α, results that have been confirmed by different techniques15-20. These results supported the hypothesis, borned of morphological results, that main key in the pathogenesis of ASF is the release of cytokines by the MÆ. Others findings indicative of MÆ activation in ASF is the significant increase in PGE21 and an increase in swine leukocyte antigen expression in animals inoculated with virulent isolates16,20.

These differences between experimental animal studies and in vitro studies may have two causes: 1) the complex network of chemical mediators that develops in animals and which cannot be reproduced in cell cultures; and 2) the fact that production of these cytokines is not restricted to infected cells, but mostly involves cells showing signs of activation but not infection.

The changes induced by activation in MÆ of infected animals do not hinder virus replication, which in turn do not hinder phagocytosis. These findings concur with in vitro studies which show that the virus infects and replicates in MÆ without inhibiting their phagocytic activity, although a previous paper has reported an impairment of antibody mediated phagocytosis in these cells21. Thus, the decline of phagocytosis by MÆ in pigs infected with in ASFv would not appear to be due to virus replication but to the excess of material phagocytosed by these cells and their becoming hyperactive.

Haemorrhages
Mebus2 reports that the most consistent and indicative lesion in acute ASF, along with splenic lesions22, is haemorrhagic node enlargement, particularly evident in gastro-hepatic and renal lymph nodes. As well as, renal haemorrhages are a consistent result of the inoculation of pigs with virulent or moderately virulent strains of ASFv.
Lymph-node haemorrhages in swine inoculated with virulent ASFv strains started at 3 dpi\textsuperscript{23}, and renal haemorrhages\textsuperscript{10} are observed from 5dpi. At that time no replication of the virus in the endothelial cells of the interstitial capillaries is observed. However, coinciding with the presence of haemorrhages, there is endothelial damage consisting of phagocytic activation of the endothelial cells with proliferation of lysosomes and phagocytized cell debris, increased capillary fenestration and even necrosis and loss of endothelial cells; the latter lesions resulted in exposure of the capillary basement membrane, to which activated platelets are attached\textsuperscript{10}. This may be one of the causes of the DIC characteristic of ASF\textsuperscript{24,25}. The endothelial lesions described constitute the morphological manifestation of the biochemical values indicative of endothelial dysfunction in acute ASF, such as high levels of factor VIII\textsuperscript{28}, a reduction in prostacyclin\textsuperscript{1} and the increase in tissue plasminogen activator\textsuperscript{27}. This increase is indicative of excessive activation of the fibrinolytic system and this, together with the vasodilatory role of PGE\textsubscript{2} could prompt a significant aggravation of the hemorrhages caused by the endothelial lesions.

The absence of a resident macrophage population in lymph-nodes and renal interstitial capillaries may influence the phagocytic activity of endothelial cells in these vessels. There is an absence of phagocytic activity in endothelial cells of the liver\textsuperscript{4}, spleen\textsuperscript{22} and lungs\textsuperscript{26} in ASF, as these organs have resident macrophage populations which clear the vascular lumen of cellular debris.

In other hand, cytokines, released by m-MÆ, may play an important role in the establishment of DIC. Activated MÆ and/or MÆ containing virus replication sites are observed in the vicinity of damaged vessels, and it has been suggested that proinflammatory cytokines could be released by these cells during ASF.

The kidneys of pigs inoculated with the moderately virulent strains reveal an intense, widespread haemorrhage, but neither endothelial lesions nor intravascular coagulation are observed. Morphometrical and ultrastructural data suggest that these haemorrhages are associated with intense vasodilation; this would be expected to give rise to increased vascular permeability, which in turn would be responsible for the intense interstitial oedema and diapedesis observed\textsuperscript{10}. Diapedesis of erythrocytes would seem to be the cause of haemorrhage in the kidneys of pigs inoculated with moderately virulent strains, in which immunological processes may play a main role\textsuperscript{29}.

**Thrombocytopenia**

In acute forms the disorder is generally observed in the final phase, after haemorrhages are detected, and often goes undetected due to the sudden worsening and death of affected animals\textsuperscript{24,25}. However, there is an important damage of bone marrow and virus replicate in stromal and haematopoietic cells\textsuperscript{30}. In subacute ASF, a transitory thrombocytopenia occurs between the initial and middle phases of the disease, disorder that could be important in the pathogenesis of haemorrhages in this form of the disease\textsuperscript{26}. There is a marked parallel in time between ultrastructural changes in megakaryocytes, especially the appearance of denuded megakaryocytes, and the transitory thrombocytopenia observed in animals inoculated with moderately-virulent strains of ASFv. The megakaryocytes show three stages in the course of the disease: a compensatory stage, represented by cytoplasmic projections, hyper-maturity stage, represented by denuded megakaryocytes, and regenerative stage, represented by clusters of immature megakaryocytes. These changes could explain the early and transitory thrombocytopenia detected in subacute ASF\textsuperscript{30}.

The considerable increase in denuded megakaryocytes numbers in ASF may be due to an accelerated maturation of these cells resulting from the action of cytokines\textsuperscript{33} and/or peripheral platelet consumption, detected a few days after inoculation with a moderately-virulent ASFv isolate\textsuperscript{25,31}.

**Lymphopenia**

Apoptosis of lymphocytes has been reported as a cause of lymphopenia in a number of virus diseases, and there is an increase in the number of apoptotic lymphocytes in infiltrate lymphocytes\textsuperscript{5} and lymphoid structures\textsuperscript{34,35} in ASF, which may be the cause of the lymphopenia in this disease. Moreover, the inhibition of lymphocyte proliferation would aggravate lymphopenia, and it is probably aggravated in the final phase of the disease by the destruction of the lymphoid structures as a result of vascular lesions\textsuperscript{5,22,23}.

A number of different pathogenic mechanisms have been proposed for the programmed cell death of lymphocytes in viral diseases and may be directly or indirectly related to infection of the cell. Virus replication in lymphocytes as a possible pathogenic mechanisms of apoptosis of lymphocytes must be ruled out in ASF, since although the virus may infect the lymphocytes, it does not replicate in them\textsuperscript{36}. Moreover, the ASFv contains a gene that may prevent apoptosis in infected cells\textsuperscript{37} which could account for the epithelial hyperplasia associated with virus replication\textsuperscript{1}. Activated m-MÆ, and/or MÆ containing virus replication sites, may synthesize and release cytokines which would trigger the apoptosis of the lymphocytes. The fact that apoptosis is more widespread in diffuse lymphoid tissue, where activated MÆ or MÆ containing virus replication sites are more numerous, would tend to support this hypothesis. Activation of m-MÆ, with a significant increase in the
number of cells immunostained with anti-IL-1α, anti-IL-6 and anti-TNF-α in pigs affected of acute ASF, suggest that this cytokine may also be involved in apoptosis of lymphocytes in this disease.35,36

References


