Histol Histopath (1992) 7: 421-425

Histology and Histopathology

Kupffer cells and PIMs in acute experimental African Swine Fever

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Summary. An ultrastructural study of Kupffer cells and pulmonary intravascular macrophages (PIMs) of healthy and African Swine Fever (ASF)-infected pigs was carried out. A vascular perfusion method was performed in order to obtain an optimal intravascular morphology and tissue fixation. The infection developed acute ASF lesions in both organs. Both Kupffer cells and PIMs were studied at different stages of infection. The differences observed in both macrophagic cells from uninfected and infected tissues are shown and discussed.

Key words: African Swine Fever, Kupffer cells, Pulmonary intravascular macrophages

Introduction

The origin of Kupffer cells is still controversial. Like other macrophages, there is good evidence that Kupffer cells arise from bone marrow monocytes (Lloyd and Triger, 1975). However, when there is a local demand in the mature liver, there is good evidence of local proliferation as well as extrahepatic recruitment (Bouwens et al., 1984, 1986).

Kupffer cells are clearly in a strategic position to clear the portal blood stream of bacteria and endotoxins, of viruses and immune complexes, and of thromboplastins and fibrin complexes that might appear in disseminated intravascular coagulation (DIC) (Wardle, 1987).

Pulmonary intravascular macrophages (PIMs) are mature mononuclear phagocytic cells that inhabit the pulmonary capillaries of certain mammalian species (Winkler, 1988).

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Probably, they originate from undifferentiated monocytes, which perinatally colonize lung capillaries. Postnatally, proliferating monocytes are junctionally attached to capillary endothelium, and differentiate into phagocytic intravascular macrophages (Winkler and Cheville, 1985, 1987; Winkler, 1988).

It has been established that intravascular macrophages are the principal sites of pulmonary retention of substantial numbers of blood cells, blood borne bacteria and particulates in pigs, calves, sheeps, goats and cats (Rybicka et al., 1974; Crocker et al., 1980, 1981; Bertram, 1986; Warner and Brain, 1986; Warner et al., 1986).

Clasically, the uptake of blood-borne particles has been shown to be carried out in the spleen, liver and lung. However, the lung containing PIMs seems to be central in this role (Crocker et al., 1980, 1981; Niehaus et al., 1980; Warner and Brain, 1986; Warner et al., 1986, 1987).

The 75 - 80 per cent of *P. aurinigosa* is retained in the lung when it is inoculated intravenously into pigs (Dehring and Wismar, 1989). Equally, the pig's lung retains more blood-borne bacteria than spleen and liver in infections with *Staphilococcus spp.* (Dehring et al., 1983).

African Swine Fever (ASF) is a haemorrhagic disease of domestic pigs caused by an intracytoplasmic deoxyvirus at present classified as a member of the Iridoviridae (Almeida et al., 1967; Enjuanes et al., 1976). The virus replicates primarily in cells of the mononuclear phagocyte system (MPS) (Maurer et al., 1958).

The central pathology is the development of thrombocytopenia, haemorrhage and circulatory disturbance which results in oedema, extravasation of fluid, shock and death (Anderson, 1986; Anderson et al., 1987).

In the present work the morphological changes observed in both cells, Kupffer cells and PIMs, at different stages of ASF infection will be shown.

Materials and methods

For this study, nine Large White X Landrace male pigs were used, each whith a live weight of approximately 20 kg at the beginning of the experiment and free from parasitic and infectious disease. They were divided into three groups, each consisting of three animals; two animals from each received intramuscular inoculations of 5 x 10^5 50 per cent haemadsorbing doses (HAD50) of the high virulent E70 isolate of the ASF virus (supplied by Instituto Nacional de Investigaciones Agrarias of Madrid); the third animal served as a control. Animals were sacrificed at 3, 5 and 7 days post inoculation (dpi) by vascular perfusion following induction of deep anaesthesia with azaperone and thiopental.

Organs were fixed by perfusion in 2.5 per cent glutaraldehyde in 0.1 M phosphate buffer; samples of liver and lung parenchyma were embedded in araldite.

For ultrastructure, 60 nm sections embedded in araldite were stained with uranyl acetate and lead citrate.

The number of cells found in the liver and lung tissue of all 9 animals was counted by the method previously described (Sierra et al., 1990).

Results

Liver

In the control animals the Kupffer cells showed a large size but did not show phagosomes (Fig. 1). The number of these cells was 30 cells in 40 fields of 90 µm x 90 µm (Tables 1, 2).

At 3 dpi, the Kupffer cells increased in number (Tables 1, 2) and cell hypertrophia was evident. The Kupffer cells showed many phagosomes, erytrophagocytosis and in some of them haemad-sorption associated to cells presenting viral replication sites.

Furthermore, Kupffer cell necrosis was also seen in many of the infected cells. Moreover, some mitosis were found in this group (Table 2).

A decrease in the number of Kupffer cells was present at 5 dpi (Tables 1, 2), 50 per cent of which had morphological changes of necrosis. Although virus replication was constant, the mitosis rate decreased (Table 2).

In the counted area, no mitosis were found at 7 dpi, and the number of necrotic Kupffer cells increased with a decrease in the total number of these cells (Fig. 2) (Table 2).

At 5 and 7 dpi many hepatocytes showed virus replication sites as well as necrotic signs.

Lung

Using electron microscopy in the control animals, the presence of PIMs was observed, as those cells showing intercellular junctions with endothelial cells, an elongated nucleus, a tongue-like cytoplasm with poorly-developed organelles and small cytoplasmic projections (Fig. 3). The mean number of these cells observed in 40 fields of 90 x 90 µm was 32 (Tables 1, 3).

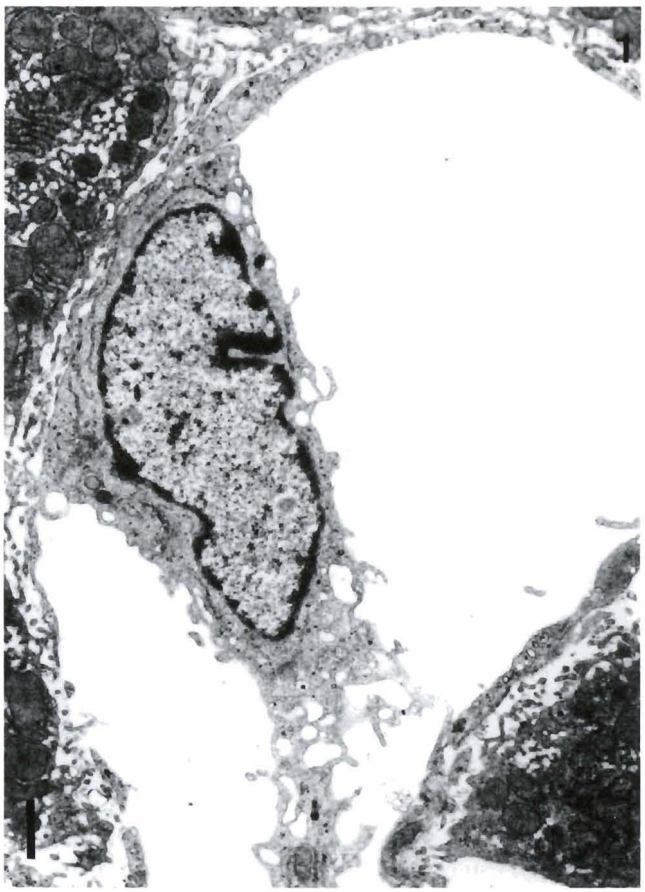
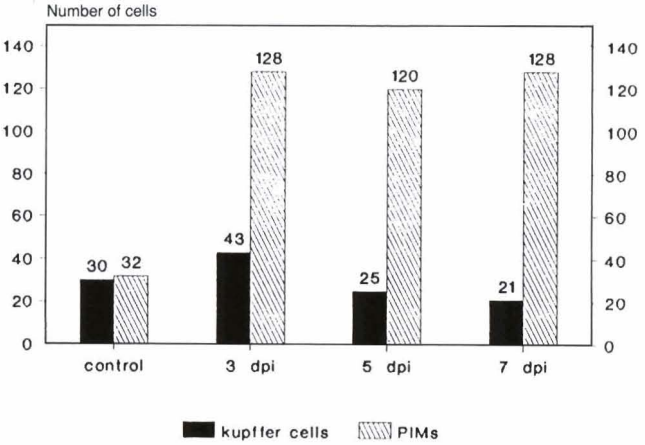


Fig. 1. Kupffer cell in a control animal. Bar. 1 µm

At 3 dpi the number of PIMs was increased, with 128 cells in 40 fields (Tables 1, 3). These cells were enlarged and rounded, with scarce, short intercellular

Table 1. Number of Kupffer cells and PIMs in 40 fields of 90 x 90 µm. Number of cells.



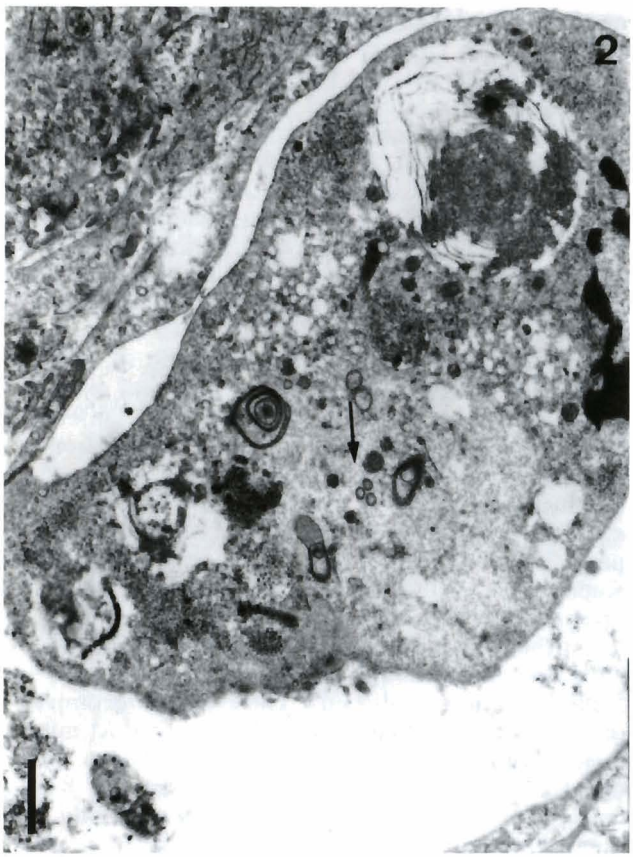


Fig. 2. Kupffer cell showing virus replication (arrow) and phagosomes. ASFV-E70, 7 dpi. Bar 1 µm

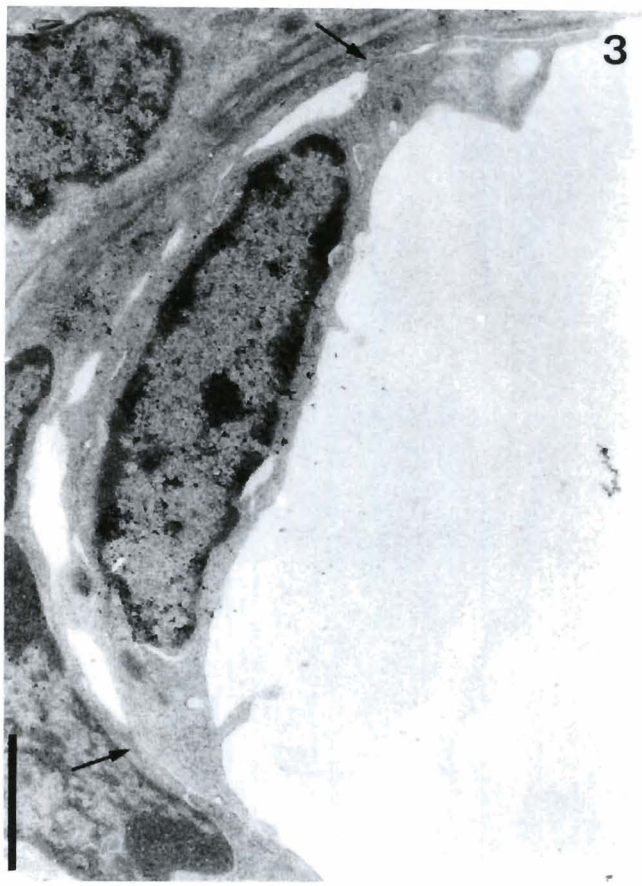


Fig. 3. A PIM in a control animal showing intercellular junctions (arrows) and poorly developed organelles. Bar 1 µm

junctions binding to endothelial cells and, sometimes, with large secondary lysosomes formed by spherical or oval membranous structures. Four of these cells showed marked peripheral margination of chromatin and a rounded organoid-free area in the cytoplasm containing viral particles at different stages of development; half of these PIMs with viral replication sites had necrotic changes (Table 3).

At 5 dpi there were 120 PIMs (Tables 1, 3), that showed similar characteristics to those found at 3 dpi. Lysosomes containing structures similar to those previously described, and there was also phagocytosis of granulocytes and erythrocytes. The cells with viral

Table 2. Changes observed in Kupffer cells, in 40 fields of 90 x 90 µm.

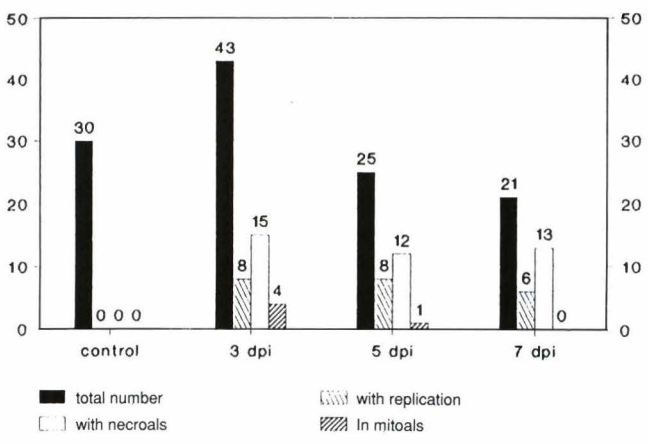
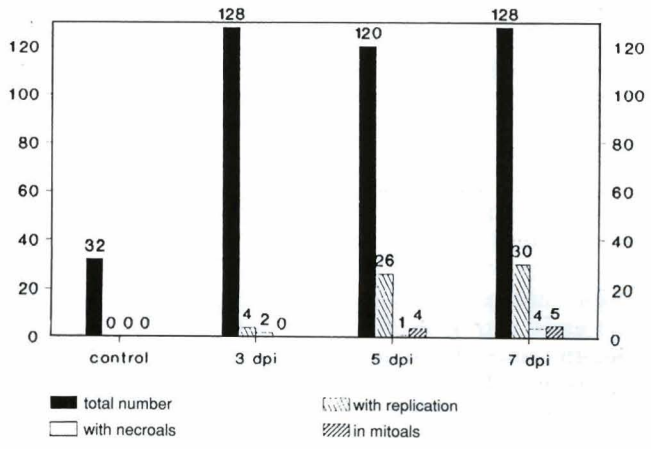


Table 3. Changes observed in PIMs in 40 fields of 90 x 90 µm



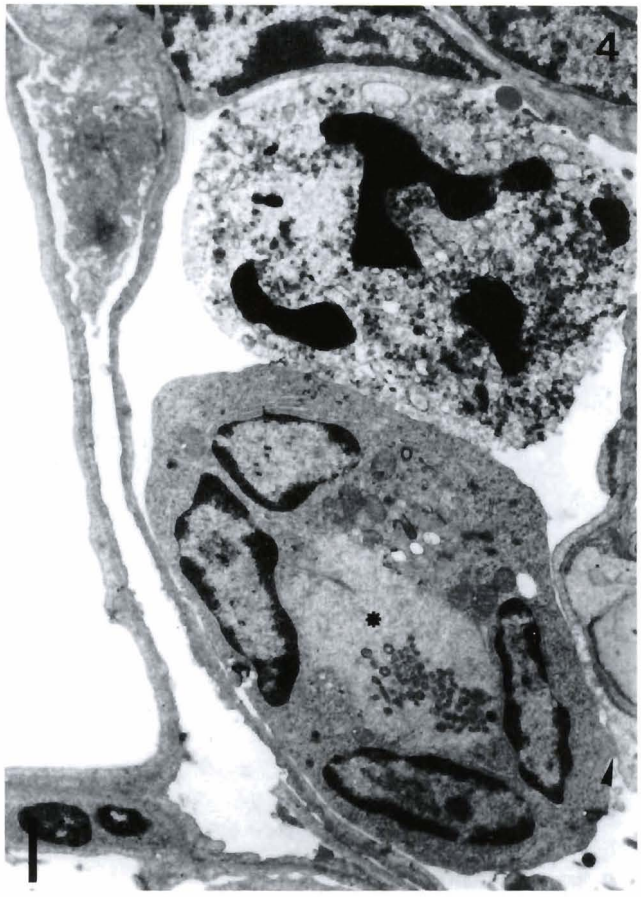


Fig. 4. A rounded PIM with short intercellular junctions (arrowhead) and a viral replication site (*). Close to a necrotic cell. ASFV-E70, 5 dpi. Bar 1 μm

replication sites were more abundant (Fig. 4), 26 cells in 40 fields, and some PIMs of this group (4 cells) showed mitotic figures (Table 3).

At 7 dpi 128 PIMs were present (Tables 1, 3), with similar characteristics to the 5 dpi PIM. Cells with viral replication sites were more numerous (30 cells), and those with mitotic figures and necrotic changes were also more numerous (5 and 4 cells respecitvely) (Table 3).

At 7 dpi, the progressive increase of cell debris in the lung capillaries or included in intracytoplasmic phagosomes of PIMs was also very significative.

Discussion

Both Kupffer cells and PIMs are the main representatives of the MPS in the liver and lung respectively (Winkler, 1989). They play several important roles in the pathophysiology of different systemic and local diseases (Warner et al., 1987). In healthy animals the quantity of these cells is similar (Winkler, 1989), as we have also observed in the present work, showing morphological properties of «inactivated macrophages» (David and Reinke, 1987).

ASF virus replicates primarily in MPS cells resulting in cell necrosis (Coggins, 1974). Necrotic cell debris and viremia produced at early stages of infection is likely to be a significant stimulus for macrophages maturation (Bertram, 1986). After this first stimulation, the morphological changes observed in both Kupffer cells and PIMs are defined as «activation» (David and Reinke, 1987). However, a significative increase in PIMs was evident. They have a rapid rate of maturation when compared to other blood derived macrophages (Niehaus, 1989; Winkler, 1989).

Although both cells are susceptible to infection (Sierra et al., 1987, 1990), the rate of viral replication at early stages of infection was higher in Kupffer cells, being very low in PIMs. The lack of replication at 3 dpi and the significative increase of replication after 5 dpi in PIMs, with a low rate of cell necrosis, could explain a different behaviour of this macrophage population with respect to the virus, when compared to Kupffer cells or other macrophages located in lymphoid organs, where they are destroyed earlier (Pan, 1987).

Furthermore, intracytoplasmic immunocomplexes have been found in PIMs but not in Kupffer cells after 5 dpi (data not published). Those immunocomplexes may induce a continuing stimulation for PIM mitosis, maturation and phagocytosis, as was observed at 5 and 7 dpi. However, the early and rapid decrease of Kupffer cells, probably due to a higher cell susceptibility to be destroyed by high virulent viruses, opened up the possibility for this virus to cause damage to the hepatocyte (David and Reinke, 1987). This hepatic cell necrosis associated to virus replication was previously observed (Sierra et al., 1987) and would participate in the high virus titres that are normally found in infected livers (Hess, 1971), causing severe hepatic impairments. Although a specific receptor, described in Vero cells and swine macrophages, seems to be necessary to cause a productive infection (Alcami et al., 1989, 1990), this has not been investigated in non-macrophagic cells, like hepatocytes.

In the lung, the increase in PIMs number and cell size which occurs in this disease as under other pathological conditions may induce venoconstriction and pulmonary hypertension (Winkler, 1989). Furthermore, the involvement of PIMs in lung injury seems to be evident because they secrete vasoactive and inflamatory agents that mediate many circulatory mechanisms (Bertram et al., 1989). Therefore, it is well-known that the principal lesions in acute ASF are haemorhage and circulatory disturbance, characterized by an acute alveolar oedema in the lung (Pan, 1987), where PIMs in response to phagocytosis would secrete arachidonic acid metabolites that would participate in vascular injury and pulmonary hypertension with oedema (Bertram et al., 1989).

The cytological changes in Kupffer cells and PIMs infected with ASF virus were similar, but the general behaviour of both cell populations in each organ was

not. So, the MPS cell in the liver is soon destroyed, allowing parenchymal damage and liver function impairment. However the MPS cell in the lung suffers a marked hyperplasia and hypertrophia, being infected later in the course of infection and destroyed at a minor rate, when compared to other macrophages. These cells also showed a marked hypertrophia with a great amount of intractyoplasmic phagocytic material in phagosomes (Sierra et al., 1990), which could be related to the capacity of these cells to produce and secrete active inflammatory mediators (Bertram et al., 1989), these probably being involved in acute ASF lung lesions.

References

Alcami A., Carrascosa A.L. and Viñuela E. (1989). Saturable binding sites mediate the entry of African Swine Fever into Vero cells. Virology 168, 393-398.

Alcami A., Carrascosa A.L. and Viñuela E. (1990). Interaction of African Swine Fever virus with macrophages. Virus Research. 17, 93-104.

Almeida J.C., Waterson A.P. and Plowright W. (1967). The morphological characteristics of African Swine Fever virus and its resemblance to Tipula iridescent virus. Arch. Ges. Virusforsch. 20, 392-396.

Anderson E.C. (1986). African Swine Fever: Current on its pathogenesis and immunology. Rev. Sci. Tech. Off. Int. Epiz. 5, 477-586.

Anderson E.C., Williams S.M., Fisher-Hoch S.P. and Wilkinson P.j. (1987). Arachidonic acid metabolites in the pathophysiology of thrombocytopenia and haemorrhage in acute African Swine Fever. Res. Vet. Sci. 42, 387-394.

Bertram T.A. (1986). Intravascular macrophages in lungs of pigs infected with Haemophilus pleuropneumoniae. Vet. Pathol. 23, 681-691.

Bertram T.A., Eling T.E. and Brody A.R. (1989). Arachidonic acid metabolism in isolated cells. In: The pulmonary intravascular macrophage. Norman C. Staub (ed.). Futura Publishing Company. New York. pp 101-121.

Bouwens L., Baekeland M. and Wisse E. (1984). Importance of local proliferation in the expanding Kupffer cell population of rat liver after zymosan stimulation and partial hepatectomy. Hepatology 2, 213-218.

Bouwens L., Knoop D.L. and Wisse E. (1986). Local proliferation and and extrahepatic recruitment of liver macrophages in partial body irradiated rats. J. Leukocyte Biol. 39, 687-697.

Coggins L. (1974). African Swine Fever virus. Pathogenesis. Prog. Med. Virol. 18, 48-63.

Crocker S.H., Eddy D.O., Wismar R.N., Obenauf R.N. and Lowery B.D. (1980). Bacteremia-induced pulmonary failure and substantial pulmonary clearance of blood-borne organisms in pigs but not in dogs. Surg. Forum. 31, 39-41.

Crocker S.H., Lowery B.D., Eddy D.O., Wismar B.L., Bueching W.J. and Obenauf R.n. (1981). Pulmonary clearance of blood-borne bacteria. Surg. Gynecol. Obstet. 153, 845-851.

David H. and Reinke P. (1987). The concepts of the «perisinusoidal functional unit» of the liver. Importance to pathological processes. Exp. Pathol. 32, 193-224.

Dehring D.J., Crocker S.H., Wismar B.L., Steinberg S.M., Lowery B.D. and Coultier C.T. (1983). Comparison of live bacteria infusions in a porcine model of acute respiratory failure. J. Surg. Res. 34, 151-158.

Dehring D.J. and Wismar B.L. (1989). Animal models of sepsis. In: The pulmonary intravascular macrophage. Normal C. Staub (ed). Futura Publishing Company, Inc. New York. pp 79-100.

Enjuanes L. Carrascosa A.L. and Viñuela E. (1976). isolation and properties of the DNA of African Swine Fever. (ASF) virus. J. Gen. Virol. 32, 479-492.

Hess W.R. (1971). African Swine Vever Virus. Virology Monographs 9, 1-33.

Lloyd R.S. and Triger D.R. (1975). Studies on hepatic uptake of antigen. Immunology 29, 253-263.

Maurer F.D., Griesemer R.A. and Jones T.C. (1958). The pathology of African Swine Fever , a comparison with hog cholera. Am. J. Vet. Res. 19, 517-539.

Niehaus G.D. (1989). Role in systemic host defense. In: The pulmonary intravascular macrophage. Norman C. Staub (ed). Futura Publishing Company. New York. pp 39-58.

Niehaus G.D., Schumacker P.R. and Saba T.M. (1980). Reticulo endothelial clearance of blood borne particulates in sheep: revelance to experimental lung microembolization and vascular injury. Ann. Surg. 191, 479-487.

Pan I.C. (1987). Spontaneously susceptible cells and cell culture methodologies for African Swine Fever virus. In: African Swine Fever. y. Becker (ed.). Martinus Nijhoff Publishing. Boston. pp 81-127.

Rybicka K., Daly B.D.T., Migliore J.J. and Norman J.C. (1974). Intravascular macrophages in normal calf lung. An electron microscopic study. Am. J. Anat. 139, 353-368.

Sierra M.A., Bernabé A., Mozos E., Méndez A. and Jover A. (1987). Ultrastructure of the liver in pigs with experimental African Swine Fever. Vet. Path. 24, 460-462.

Sierra M.A., Carrasco L., Gómez-Villamandos J.C., Martín de las Mulas J., Méndez A. and Jover A. (1990). Pulmonary intravascular macrophages in lungs of pigs inoculated with African Swine Fever virus of differing virulence. J. Comp. Path. 102, 323-334.

Wardle E.N. (1987). Kupffer cells and their function. Liver 7, 63-75.

Warner A.E., Barry B.E. and Brain J.D. (1986). Pulmonary intravascular macrophages in sheep. Morphology and function of a novel constituent of the mononuclear phagocyte system. Lab. Invest. 55, 276-288.

Warner A.E. and Brain J.D. (1986). Intravascular pulmonary macrophages: a novel cell removes particles from blood. Am. J. Physiol. 250, R728-R732.

Warner A.E., Molina R.M. and Brain J.D. (1987). Uptake of blood-borne bacteria by pulmonary intravascular macrophages and consequent inflammatory responses in sheep. Am. Rev. Respir. Dis. 136, 683-690.

Winkler G.C. (1988). Pulmonary intravascular macrophages in domestic animal species: review of structural and functional properties. Am. J. Anat. 181, 217-234.

Winkler G.C. (1989). Developmental biology in pigs. In: The pulmonary intravascular macrophage. Norman C. Staub (ed). Futura Publishing Company. New York. pp 1-22.

Winkler G.C. and Cheville N.F. (1985). Monocytic origen and postnatal mitosis of intravascular macrophages in the porcine lung. J. Leukoc. Biol. 38, 471-480.

Winkler G.C. and Cheville N.F. (1987). Postnatal colonization of porcine lung capillaries by intravascular macrophages: an ultrastructural morphometric analysis. Microvas. Res. 33, 224-232.

Accepted March 10, 1992