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PULMONNARY INTRAVASCULAR MACROPHAGES (PIMs): WHAT DO WE KNOW ABOUT THEIR ROLE IN PRRSV INFECTION IN PIGS?

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Review Article

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Abstract

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PULMONARY INTRAVASCULAR MACROPHAGES (PIMs): WHAT DO WE KNOW ABOUT THEIR ROLE IN PRRSV INFECTION IN PIGS?

Porcine pulmonary intravascular macrophages (PIMs) have been recently known for their role of pulmonary surveillance. In the past few years, we have been investigating the role of PIMs in porcine reproductive and respiratory syndrome virus (PRRSV) infection. PRRSV antigen has been demonstrated in PIMs both *in vitro* and *in vivo*. PRRSV-infected PIMs reveal viral particles that tend to accumulate in the smoothed-wall vesicles and yield a high virus titer. The PRRSV infection induces either apoptosis or cell lysis. The bactericidal activity of *in vitro* PRRSV - infected PIMs is significantly decreased and the phagocytic activity is questionable as the pulmonary copper clearance, which measures the phagocytic activity of PIMs *in vivo*, is significantly decreased in PRRSV-infected pigs. This evidence supports the hypothesis that PRRSV - induced damage to PIMs results in increased susceptibility to bacteremic diseases. This result could explain the increase in the chronic bacterial respiratory diseases, septicemia, and mortality experienced in pigs on farms endemically- or epizootically-infected with highly virulent strains of PRRSV. The objective of this article is to summarize the current knowledge of the complex interaction between PRRSV and the PIMs.

Key words : Pulmonary intravascular macrophages, PRRS, pigs

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บทคัดย่อ

รุ่งโรจน์ ธนาวงษ์นุเวช

Pulmonary Intravascular Macrophages (PIMs) : บทบาทที่น่าสนใจของพิมส์ต่อโรค พี อาร์ อาร์ เอส ในสุกร

Pulmonary Intravascular Macrophages (PIMs) หรือพิมส์ เป็นที่รู้จักกันว่ามีบทบาทที่สำคัญในการเก็บกินสิ่งแปลกปลอมที่ผ่านมาทางกระแสเลือดภายในปอด ผู้วิจัยได้ทำการศึกษาบทบาทของพิมส์ในโรค พี อาร์ อาร์ เอส โดยพบแอนิเจนของเยื่อไวรัส พี อาร์ อาร์ เอส ในพิมส์ เมื่อทดลองทั้งในตัวสุกรและในห้องปฏิบัติการโดยจะพบปริมาณไวรัสไตเตอร์ค่อนข้างสูงในห้องปฏิบัติการ เมื่อใช้พิมส์เป็นเซลล์เพาะเลี้ยง และมักพบอนุภาคไวรัสสะสมอยู่ภายในถุง vesicles ภายในไซโตพลาส ส่วนความสามารถในการทำลายแบคทีเรียในห้องปฏิบัติการลดลงอย่างมีนัยสำคัญ เมื่อติดเชื้อไวรัส พี อาร์ อาร์ เอส ในขณะที่ความสามารถในการเก็บกินนั้นยังคลุมเคลือ อย่างไรก็ตามเมื่อทดลองวัดความสามารถในการเก็บกิน โดยใช้สารละลายทองแดงฉีดเข้าเส้นโลหิตสุกร พบว่าในสุกรที่ติดเชื้อไวรัสพิมส์มีความสามารถในการเก็บกินน้อยลงอย่างมีนัยสำคัญ ซึ่งผลการค้นพบช่วยสนับสนุนสมมุติฐานว่าเชื้อไวรัส พี อาร์ อาร์ เอส ก่อให้เกิดความเสียหายต่อพิมส์ โน้มนำให้สุกรป่วยเป็นโรคติดเชื้อแบคทีเรียในกระแสเลือดได้ง่ายขึ้น และยังสอดคล้องกับรายงาน ที่พบอัตราการป่วยและตายเพิ่มขึ้น จากการติดเชื้อร่วมกันของสุกรในฟาร์มที่เคยมีการระบาดของโรค พี อาร์ อาร์ เอส จุดประสงค์ของการเขียนบทความฉบับนี้เพื่อเสนอถึงบทบาทที่น่าสนใจของพิมส์ต่อโรค พี อาร์ อาร์ เอส ในสุกร

คำสำคัญ : Pulmonary intravascular macrophages, พี อาร์ อาร์ เอส, สุกร

1. Introduction

In the past, the information on pulmonary intravascular macrophages (PIMs) was based on the theory that these cells were migrating hepatic Kupffer cells. It was not until the mid-1980 that PIMs were identified as a resident population of lung macrophages (Warner and Brain,1984). PIMs are a member of the mononuclear phagocytic system (MPS) which also play an important role in lung defense mechanisms. Because of their intravascular location (Figure 1), PIMs perform the task of blood clearance and are the site of blood cell degradation in pig lungs. PIMs are tightly adherent to the endothelium, not readily displaced, and capable of specific actions beyond those attributable to activated monocytes. Within the order Artiodactyla (even-toed), PIMs have been reported in bovine (Rybicka et al., 1974; Warner and Brain,1984; Warner and Brain,1986; Leifsson et al., 1995), sheep (Wheeldon and Hansen-Flaschen, 1986; Warner et al., 1988; Longworth et al., 1992; Rogers et al., 1994), goats (Atwal and Minhas, 1992), pigs (Winkler and Cheville, 1985a; Winkler and Cheville, 1985b; Bertram, 1986; Morton and Bertram, 1988; Sierra, 1990), llama (Staub, 1989), deer (Carrasco et al., 1996), and reindeer (Staub et al., 1992). It was recently shown that PIMs also exist in the order Perissodactyla (odd-toed), such as horses (Staub, 1989; Atwal et al, 1992; Singh et al., 1994). However, there is no theory to explain the existence of PIMs in the cat (Schneeberger-Keeley and Burger, 1970). Re-

cent reports found that PIMs have been detected in rats with chronic biliary cirrhosis and in humans with malignancy and liver diseases (Chang and Ohara, 1996; Warner, 1996).

The first cases of porcine reproductive and respiratory syndrome (PRRS) were reported in the United States (Hill, 1990) and Canada (Harper, 1991) in 1987. In November of 1990, the first cases of PRRS in Europe were reported in Germany (Egbering, 1991). Subsequently, PRRS spread rapidly through North America, Europe, and Asia (Zimmerman et al., 1997b). Serologic evidence suggests that PRRS occurred in Canada as early as 1979 (Carmen, 1995), in the United States in 1985 (Yoon et al., 1992b), and in Thailand in 1989 (Damrongwatanapokin et al., 1996a). The PRRS virus (PRRSV), however, was first isolated in 1991 in the Netherlands (Wensvoort et al., 1991b), in 1992 in the US (Collins et al., 1992), and in 1996 in Thailand (Damrongwatanapokin et al., 1996a).

In regard to morphology, genome organization, replication strategy, and protein composition, PRRSV closely resembles lactate dehydrogenase-elevating virus (LDV), equine arteritis virus (EAV) and simian hemorrhagic fever virus, collectively termed Arteriviruses (Plagemann and Moenning, 1992; Meulenberg et al., 1993) in a new family, *Arteriviridae* (Cavanagh, 1997). Based on its virion and genome size and icosahedral nucleocapsid symmetry, PRRSV is similar to Togaviridae (Benfield et al., 1992). However, the genomic organization and translation strategy suggest a closer link to Coronaviruses and *Toroviruses* (Meulenberg et al., 1993) which are in the same newly estsblished order, Nidovirales (Cavanagh, 1997). Based on the sequence homology of the putative M and N genes of the Arterivirus group, PRRSV most closely resembles LDV, but the two do not cross-react serologically (Meng et al., 1995). Indeed no serological cross-reaction has been detected between PRRSV and any other virus (Goyal, 1993). Recent serological and biophysical data suggest that the U.S. and European PRRSV are the same virus, however, antigenic variation occurs, not only between the U.S. isolates and the European isolates, but also among different U.S. isolates (Wensvoort et al., 1992; Nelson et al., 1993). Lesser antigenic differences are also detectable between different European isolates (Drew et al., 1995). The multiplex PCR assay has been developed to distinguish between two genotypes of PRRSV directly from the supernatants of virus-infected cell cultures (Gilbert et al., 1997). Genetic comparisons indicate considerable differences between the U.S. and European isolates including deletions as well as point mutations allowed to divide PRRSV isolates into two distinct antigenic subgroups (U.S. and European) (Kwang et al., 1994; Murtaugh, 1995; Mardassi et al., 1994b; Mardassi et al., 1995; Meng et al., 1995). The Thai isolate, however, is more closely related to the American isolates than the European isolates (Damrongwatanapokin et al., 1996).

2. Does PRRSV infect PIMs?

The site of PRRSV replication has not been fully elucidated, although there appears to be a predilection for replication in PAMs or other tissue macrophages. PRRSV antigen has been detected in resident macrophages in various tissues such as lung, lymph node, tonsil, heart, thymus, spleen, Peyer's patches, liver, kidney, adrenal gland (Halbur et al., 1995a, 1995b, 1996a, 1996b), and brain (Molitor et al., 1996; Rossow et al., 1996; Thanawongnuwech et al., 1997a). Other cells in which viral antigen has been detected include pneumocytes, bronchiolar epithelium (Pol et al., 1991; Sur et al., 1996), endothelial cells in the heart, dendritic cells in the lymphoid tissues (Halbur et al., 1995a, 1996a), and muscle tissues (Magar et al., 1995). The lymphoid and respiratory systems have the most severe lesions and are likely the major sites of viral replication (Halbur et al., 1995b; Duan et al., 1997).

To date, pulmonary alveolar macrophages (PAMs) are the primary cell identified to support replication of PRRSV both in vitro and in vivo. Duan et al. (1997) found that the activation/maturation stage of macrophages have some effect on the susceptibility to PRRSV infection. Monocytes are inherently resistant to supporting virus progeny production. Monocytes, however, cultured in the presence of monocyte colony stimulating factor (M-CSF) express tissue macrophage markers and become monocyte-derived macrophages (MDMs) which are susceptible to PRRSV replication (Molitor et al., 1996). Increased PRRSV replication is also observed in the mature or activated PAMs based on surface marker expression and PAM functions. Like PAMs, PIMs are of the monocytic origin (Winkler, 1988). The marginated monocytes attaching to the endothelium via intercellular adhesion plaques (ICAPs) during differentiation to PIMs (Winkler and Cheville, 1985a) may modulate the susceptibility of PIMs to PRRSV infection as well. There is evidence that PRRSV antigen is present in PIMs by immunohistochemistry (IHC) (Halbur et al., 1996a; Rossow et al., 1996; Thanawongnuwech et al., 1998a). PIMs were successfully recovered by in situ pulmonary vascular perfusion with 0.025% collagenase in saline and were infected with PRRSV for electron microscopic examination (Thanawongnuwech et al., 1997b). PRRSV-infected PIMs in vitro revealed viral particles that tended to accumulate in the vesicles of the Golgi apparatus or endoplasmic reticulum and to be released from the vesicles via exocytosis by fusing of the vesicle wall to the cytoplasmic membrane (Thanawongnuwech et al., 1997b). PRRSV-infected PIMs contained aberrant ribosomes and tubules, numerous phagolysosomes, cytoplasmic vacuoles, and residual bodies filled with necrotic debris and damaged cellular components. Swollen mitochondria with cristolysis and flocculation of matrical proteins were also observed in the infected macrophages (Thanawongnuwech et al., 1998b).

3. Influence of pig age on PRRSV susceptibility

Functional and ultrastructural quantification differences between PIMs in newborn and older animals has been demonstrated (Winkler, 1988; Longworth et al., 1992; Longworth et al., 1996). Younger mice possess a higher proportion of LDV-permissive peritoneal macrophages than older mice and the persistent plasma LDV titers are also 10- to 100-fold higher than in older mice (Rowland et al., 1994). Similarly, young pigs are more susceptible to PRRSV infection and are more likely to have secondary bacterial infections (Rossow, 1998). We demonstrated that PIMs from 4-week-old pigs were more permissive and susceptible to PRRSV than PIMs from the 4-month-old pigs in term of PRRSV titers (Thanawongnuwech et al., 1998b). The levels of differentiation and activation of monocyte/macrophages have been reported to play an important role in determining their susceptibility to PRRSV (Molitor et al., 1996). Porcine peritoneal macrophages, another population of well-differentiated tissue macrophages, have been demonstrated to be resistant to PRRSV infection while activated monocytes are susceptible (Duan et al., 1997). Macrophage differentiation may reflect altered expressions of surface proteins, which may be viral receptors or transcription factors which are essential for virus replication. Macrophage activation may up- or down-regulate the expression of these factors which is a possible explanation for differences in macrophage susceptibility to PRRSV (Rutherfold et al., 1993). However, the reason for the change in PRRSV permissiveness of PIMs with age of pigs is unknown. One explanation for the decreased permissiveness and susceptibility to PRRSV in older pigs is a decrease in the proportion of macrophages that may express a surface protein which acts as the PRRSV receptor. This is why young pigs are

more susceptible to PRRSV infection.

4. Effects of PRRSV infection on phagocytosis and bactericidal activity

PRRSV was reported to stimulate PAMs to phagocytose and kill Streptococcus suis during the early infection *in vitro* (Pijoan et al., 1994). Similar to PRRSV-infected PAMs, PRRSV-infected PIMs are capable to internalize *Staphylococcus aureus* upto 24 hours post infection (Thanawongnuwech et al., 1997b). After 48 hours post PRRSV-infection, the phagocytic activity is apparently decreased (Thanawongnuwech, unpublished). This may be due to PRRSV inducing apoptosis or lysis of macrophages. Numerous phagolysosomes and residual bodies have been demonstrated in a number of the infected macrophages. These morphological changes may lead to advanced degeneration and lysis of the macrophages. In addition, PRRSV has been reported to induce apoptosis in PAMs (Suarez et al., 1996).

Following PRRSV infection, we found that the production of superoxide anion (SOA) and myeloperoxidase-H_2O_2-halide was significantly decreased (Thanawongnuwech et al., 1997b). This suggests that PRRSV-infected PIMs may be capable of internalization, but unable to kill bacteria. Likewise, peritoneal macrophages of mice persistently-infected with LDV produce less SOA than uninfected macrophages (Hayashi et al., 1993). PRRSV may induce a similar response in porcine macrophages. Human influenza virus which does not affect phagocytosis, however, triggers an oxidative burst in monocytes leading to reduce oxidative metabolism and depressed intracellular killing (Gardner and Lawton, 1982). This could explain why PRRSV infection significantly reduced the bactericidal ability of the PIMs at 48 hours post infection.

5. Effects of PRRSV infection on pulmonary clearance

Since, blood clearance of bacteria is one of the major roles of PIMs (Winkler, 1988; Staub, 1989), the loss of bactericidal function in PIMs may facilitate hematogenous bactericidal infection. An important feature of PRRSV infection is an increased incidence of concurrent bacterial disease (Zeman et al., 1993; Galina et al.,1994). Determination of pulmonary copper clearance in pigs is a relatively simple method to measure PIM function (Thanawongnuwech et al., 1998a). After intravenous administration of copper particles, the lungs of pigs consistently had the highest copper concentrations when compared to other organs. As in the previous studies (Smith et al., 1996; Thanawongnuwech et al., 1998a), the blue copper particles were easily observed in porcine PIMs with light microscopy. *In situ* pulmonary vascular perfusion with 0.025% collagenase recovered PIMs with numerous phagosomes containing copper as electron-dense material (Fig. 2). Similarly, iron particles given intravenously to sheep were found only in PIMs and not in alveolar and interstitial macrophages, endothelial cells, circulating monocytes, or neutrophils (Rogers et al., 1994). In horses, it has been demonstrated that PIMs are capable of internalizing copper particles without any manifestation of toxic insult when treated with multiple doses of copper particle suspension over a period of 96 hours (Singh et al., 1994). Copper particle internalization is thought to occur principally in the coated pits of the PIMs (Fig. 3).

The severity of PRRSV-induced damage to PIMs differs among strains. Pigs given a low virulence strain of PRRSV and uninfected control pigs had similar capacity to clear a single intravenous dose of copper particles, whereas the high virulent strain (VR-2385) significantly decreased copper clearance (Thanawongnuwech et al., 1998a). This suggests that the high virulence PRRSV-inoculated pigs had more extensive PRRSV-induced necrosis or apoptosis of PIMs. PRRSV has also recently been demonstrated to induce apoptosis

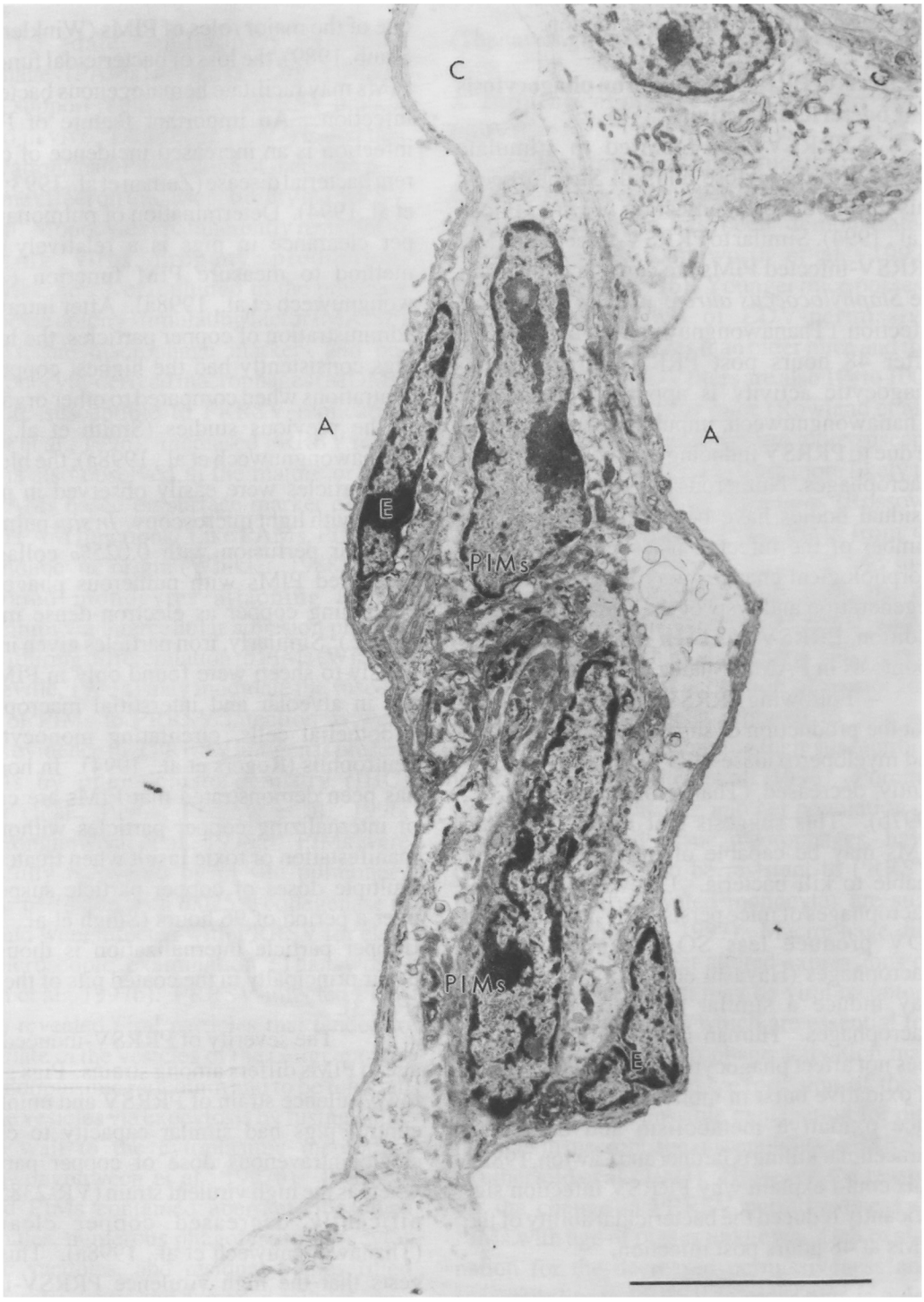

Figure 1. Lung; pig. Pulmonary intravascular macrophages (PIMs) A = Alveoli, C = Capillary lumen, E = Endothelial cell. Bar = 0.5 μm.

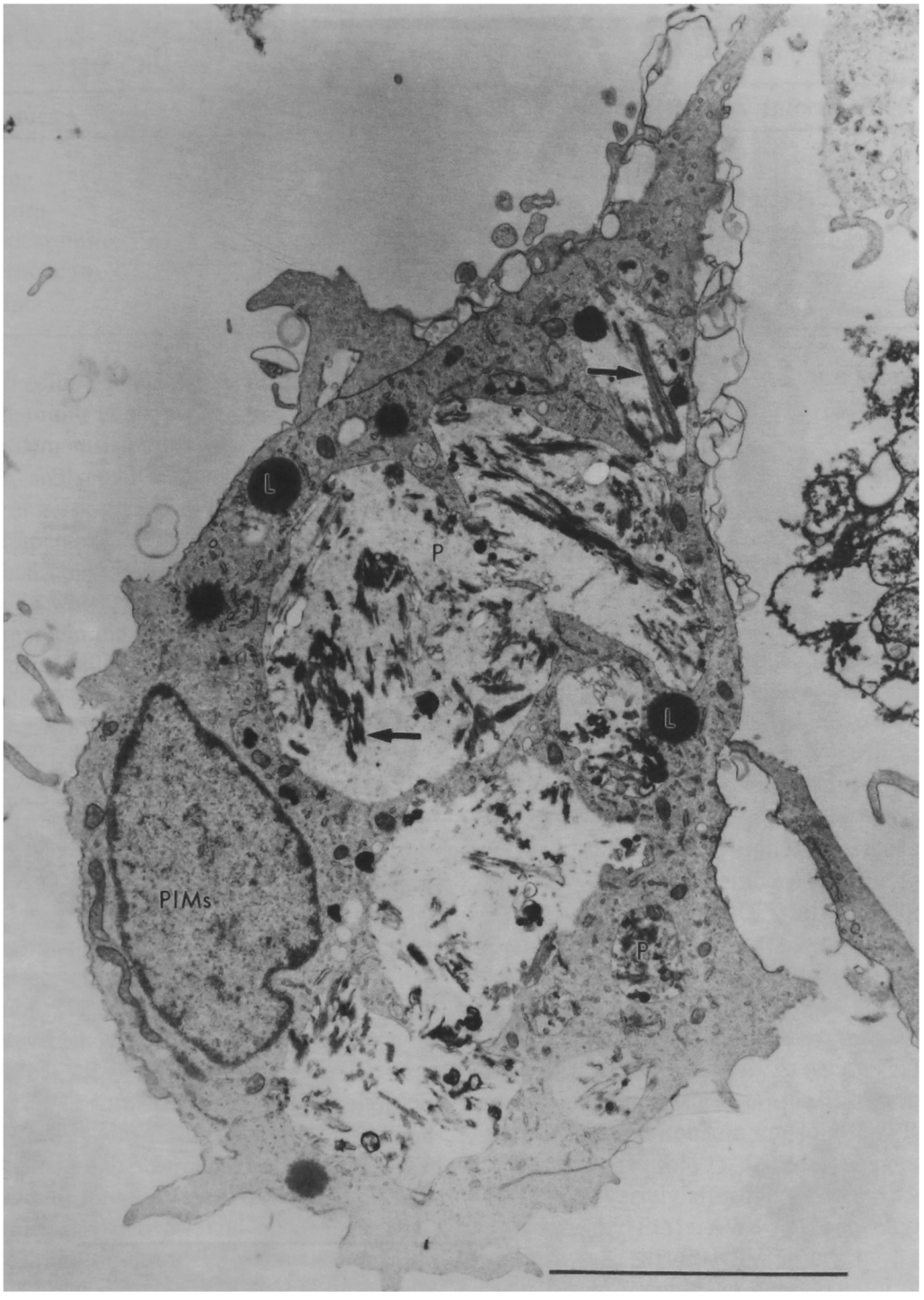

Figure 2. Primary cell culture; pig. Cultured PIMs recovered 7 days after copper infusion intravenously. Lysosomal lamellar of copper particles (arrow) found in phagolysosomes (P). L = Lysosomes. Bar = 0.5 μm.

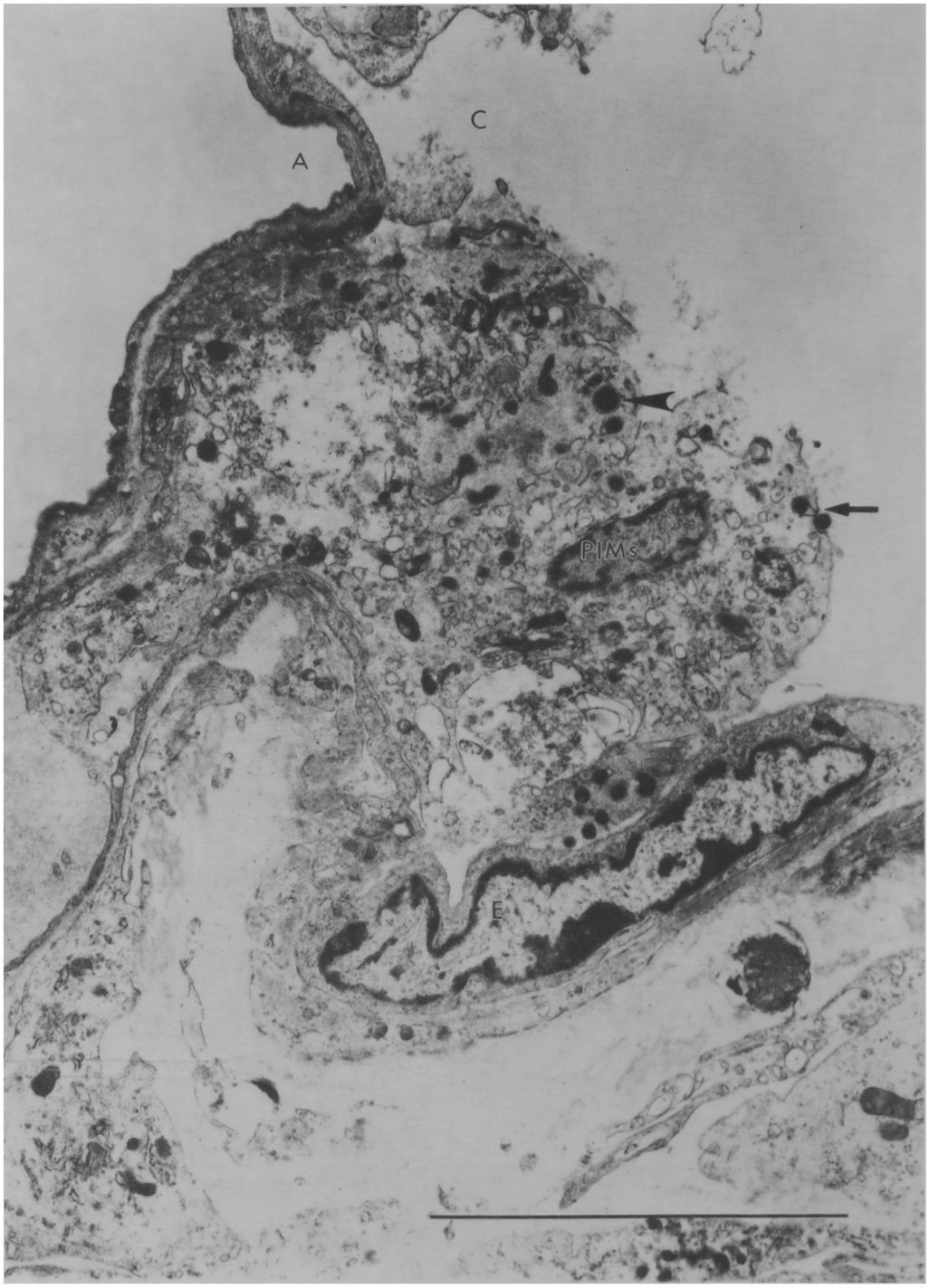

Figure 3. Lung; pig. PIMs internalized surfacecoat globules by receptor-mediated endocytosis (arrow). Globular complexes in endosomes (arrow head). Bar = 0.5 μm. see Fig. 1 for key

Table 1: *S. suis* serotype 2 recovered from tissues of pigs given *S. suis* intravenously (10^6 CFU/pig) when necropsied 2 days later.

Tissues	Control Pigs	PRRSV-infected Pigs
Lung	0/4	1/2
Pleura	3/4	1/2
Pericardium	0/4	2/2
Peritoneum	0/4	1/2
Joints	1/4	2/2

in cell culture and in tissues from infected pigs (Sirinarumitr et al., personal communication). It was demonstrated that large numbers of PAMs, PIMs, and septal mononuclear cells in lungs were either positive for PRRSV nucleic acids or were apoptotic. This suggests that PRRSV-induced apoptosis occurs in cells other than those in which PRRSV replicates. This may help explain the diffuse nature of PRRSV-induced interstitial pneumonia and the multifocal distribution of PRRSV antigen or nucleic acid in the lung. Differences in the ability to induce apoptosis may also explain difference in virulence of PRRSV isolates. In addition, in vivo studies using CDCD pigs found a marked difference in pathogenicity between PRRSV isolates (Halbur et al., 1995; Halbur et al., 1996). The low and high virulence strains of PRRSV had similar in vitro effects on PIMs in terms of virus titers, bactericidal activity, and SOA production (Thanawongnuwech et al., 1998b). However, the results of in vivo studies on the effect of PRRSV on PIMs did not parallel those observed in vitro. The experimental conditions in vivo may provide different microenvironments and macrophage activation factors important for replication of the viruses. In vitro cultures may activate and make most PIMs more susceptible to PRRSV infection than the whole population of PIMs in vivo in which a subpopulation of PRRSV-permissive PIMs may exist. In vitro bactericidal assays of PRRSV-infected PIMs did not correlate with known pathogenic differences of the selected PRRSV strains in vivo. Thus these in vitro assays may not be a good indicator of in vivo pathogenicity.

6. Interaction between PRRSV and *Streptococcus suis* serotype 2

Galina et al. (1994) were the first to demonstrate that PRRSV predisposes specific pathogen-free (SPF) pigs to infection and disease caused by *Streptococcus suis* serotype 2. Our preliminary results using 6 3-week-old pigs provided promising evidence for the interaction of the two pathogens (Thanawongnuwech et al., unpublished). Four uninfected-control pigs and 2 PRRSV-infected pigs were intravenously given 1 ml of 10^6 CFU of *S. suis* serotype 2. Blood was collected 5 minutes later and cultured for *S. suis*. The amount of *S. suis* recovered from blood of PRRSV-infected pigs was significantly higher than those from PRRSV-negative pigs (2500 vs. 430 CFU/ml, respectively). The co-infection pigs developed central nervous system signs and were necropsied 2 days after *S. suis* infusion. At necropsy, *S. suis* was frequently isolated from tonsil, lung, pleura, pericardium, peritoneum, and joints of PRRSV-infected pigs (Table 1). Our data suggests that PRRSV adversely affects pulmonary bacterial clearance by PIMs. We have developed an improved S. suis/PRRSV co-infection model which is in progress in our laboratory. This experiment was designed to further advance the understanding of the pathogenesis of PRRSV-induced increased susceptibility to bacterial infections.

7. Conclusion

Reduction in the numbers of PAMs has been demonstrated in pigs experimentally infected with PRRSV (Done and Paton, 1995). Associated with the altered lung cell dynamics in PRRSV-infected pigs is a decrease in the ability of PAMs to release SOA and to kill bacteria (Thanawongnuwech et al., 1997b). Similar to PAMs, the susceptibility of PIMs to PRRSV has been elucidated. We have provided further evidence that PIMs play an important role in pulmonary clearance of copper particles and *S. suis*. This evidence supports the hypothesis that PRRSV-induced damage to PIMs may result in increased susceptibility to bacteremic diseases. These results could explain the increase in the chronic bacterial respiratory diseases, septicemia, and death loss experienced in pigs on farms endemically- or epizootically-infected with high virulent strains of PRRSV. Consequently, a better understanding of the effects of PRRSV infection on PIMs will provide important information on the pathogenesis of PRRSV with respect to the porcine respiratory disease complex.

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