Pulmonary intravascular macrophages and lung health: What are we missing?

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Schneberger D, Aharonson-Raz K, Singh B. Pulmonary intravascular macrophages and lung health: What are we missing? Am J Physiol Lung Cell Mol Physiol 302: L498–L503, 2012. First published January 6, 2012; doi:10.1152/ajplung.00322.2011.—Pulmonary intravascular macrophages (PIMs) are constitutively found in species such as cattle, horse, pig, sheep, goat, cats, and whales and can be induced in species such as rats, which normally lack them. It is believed that human lung lacks PIMs, but there are previous suggestions of their induction in patients suffering from liver dysfunction. Recent data show induction of PIMs in bile-duct ligated rats and humans suffering from hepatopulmonary syndrome. Because constitutive and induced PIMs are pro-inflammatory in response to endotoxins and bacteria, there is a need to study their biology in inflammatory lung diseases such as sepsis, asthma, chronic obstructive pulmonary diseases, or hepatopulmonary syndrome. We provide a review of PIM biology to make an argument for increased emphasis and better focus on the study of human PIMs to better understand their potential role in the pathophysiology and mechanisms of pulmonary diseases.

constitutive pulmonary intravascular macrophages; induced pulmonary intravascular macrophages; human lung

ONE OF THE STATEMENTS FROM a workshop on pulmonary immunobiology and inflammation sponsored by the National Heart, Lung, and Blood Institute in 1999 was, “In humans, the relative roles of endothelium, pulmonary intravascular macrophages, neutrophils, or other pulmonary cells in the clearance and processing of microorganisms and antigens from the systemic venous blood are poorly defined” (15). Since then, there has been significant advancement in understanding the roles of many of the indicated cells. However, the progress has been limited for the pulmonary intravascular macrophages (PIMs). We provide a focused review of the biology of PIMs to underscore a need to study their role in human lung disease.

The role of vascular inflammation in regulating the organ and systemic inflammatory responses is well established. In this context, the contributions of intravascular mononuclear phagocytes such as hepatic Kupffer cells in microbial inflammation are well understood. In contrast, the PIMs are much lesser known and appreciated for their potential influence on lung physiology. PIMs range from 20 to 80 μm in diameter and typically are firmly attached to the capillary endothelium on the thicker side of the alveolar septum, presumably to minimize the impact on gas exchange, in species such as cattle, horse, sheep, goat, and pigs (56, 64). PIMs’ identity as macrophages in species such as cattle and horse was confirmed in situ through phenotyping with anti-macrophage antibodies (36, 49). Although sheep lung is credited with the highest concentration of PIMs, which cover 20% of the capillary endothelium (56), the horse PIMs are the largest in size. The plasma membrane of PIMs exhibits a uniquely enhanced glycocalyx through decoration with lipid or lipoprotein globules of 50–200 nm in size (Fig. 1) (3, 43). To our knowledge, this is the only such arranged glycocalyx among eukaryotic cells. The globular surface coat of PIMs, probably anchored via a glycosyl-phosphatidyl inositol anchor, complexes with blood-borne materials such as tracer particles and endotoxins and mediates their endocytosis (3, 43, 46).

PIM colonization of the lung occurs either early after birth in some species (constitutive PIMs) (28, 65), later upon stimulation with lipopolysaccharide (LPS) and bacteria, or in conditions such as cirrhosis (induced PIMs) (11, 13, 45). Of the animals tested, the orders Artiodactyla (pigs, sheep, goats, llamas, reindeer, water buffalo) and Perissodactyla (horse; Fig. 2A), cats, and the cetacean suborder odontoceti (toothed whales) have constitutive PIM populations (3, 22, 36, 40, 54). Other animals such as rodentia (rats, mice, guinea pigs, hamster) and lagomorpha (rabbits) have induced PIMs (7). Among the primates, macaques lack PIMs, whereas baboons demonstrate significant particle retention in the lungs in marginated mononuclear cells (57). Induction of PIMs in animals normally devoid of PIMs suggested many years ago is now well established. Earlier studies showed increased phagocytic activity in the lungs of rats injected with increasing dosages of endotoxin (1–10 μg) (23). Later on, morphological evidence of induction of PIMs was obtained in rats injected with E. coli intraperitoneally or following ligation of bile duct (Fig. 2B) (11, 18, 45). The septal macrophages localized with immuno-histochemistry were confirmed with electron microscopy to be mostly PIMs. There is one ultrastructural report showing a few PIMs in humans (17). There is, however, indirect old evidence suggesting presence of induced PIMs in humans with liver disease/abnormalities leading to increased pulmonary clearance of 99mTc-sulfur colloid (24–26, 41). The use of sheep, which have constitutive PIMs, as a model to study lung inflammation in humans that appear to lack constitutive PIMs...
raises a question as to the relevance of the data for humans (8). Similar questions may be raised regarding the use of pigs as a model to study lung transplantation in humans (9). The mechanisms of induction of PIMs in normal or inflamed lungs are yet to be investigated and understood in a meaningful manner.

PIMS AS PHAGOCYTIC CELLS

One of the earliest notable features of PIMs was their robust phagocytic capacity (2). Phagocytosis of tracer particles such as Monastral blue in sheep induces pulmonary arterial resistance due to PIMs’ production of vasoconstrictors such as thromboxane (1, 32). Use of other tracer particles such as radiolabeled colloids, magnetic iron oxide, and fluorescent latex beads shows similar strong localization in the lung and in the PIMs (58, 59, 61). Clearance of single or multiple injections of particles by PIMs was highly efficient, with all particulates being removed within 1 min of the administration and most probably during the first pass of the particles through the lung vasculature (47, 54). PIMs phagocytose blood-borne particles at a faster rate compared with the uptake of airway-instilled particles by alveolar macrophages. This may reflect a higher probability of particle-cell interaction by PIMs (34). The phagocytic capacity may show interspecies variation since another study showed alveolar macrophage to be more phagocytic but less cytolytic than PIMs in pigs (14). PIMs were shown to be equally phagocytic and cytolytic as alveolar macrophages for bacteria but more cytolytic for virally infected cells (14). Nevertheless, the data show robust phagocytic capacity of the PIMs.

An examination of 13 different species showed similar kinetics of clearance of intravenously injected particles in sheep, pigs, calves, and cats with an elevated uptake in the lung compared with monkeys, rabbits, rats, mice, guinea pigs, and chickens (7). In contrast, this second group of animals showed particle uptake mostly by Kupffer cells in the liver (31). In normal pig and sheep, particulates and bacteria injected via the portal vein to simulate gut entrance of infections were selectively cleared by Kupffer cells in the liver (16). Oddly, the same was not true of LPS since LPS injected in the portal vein preferentially accumulated in PIMs (16). Therefore, endotoxins originating from the intestine may interface with constitutive or induced PIMs in animals, even in those with intact and functional Kupffer cells, and lead to pulmonary inflammation.

A role for PIMs has been shown in the removal of erythrocytes, fibrin, cellular debris, and immune cells (2, 62, 63). The β3 integrin subunit (CD61) is expressed at a much higher rate in PIMs than alveolar or interstitial macrophages (21). This subunit forms part of a dimer that is a receptor for fibrinogen, vitronectin, and extracellular matrix proteins that PIMs are believed to help clear from circulation. Induced PIMs in the rat lung take up significant quantities of viral particles (51) as do constitutive PIMs in the pig and sheep (20, 48). Several studies have shown PIMs to be targets of infection with parasite *Cytauxzoon felis* in cats (19), African swine fever (42) and hog cholera in pigs (10), and ovine lentivirus in sheep (48). The studies allude to the role of PIMs as a potential reservoir of viral and other infectious agents in the lungs, thus contributing to microbial burden in the lung.

PIMS INFLUENCE LUNG PHYSIOLOGY

One of the reasons for the gap in our knowledge of the biology of PIMs is our inability to isolate them for in vitro...
studies. PIMs are intimately attached to the capillaries’ endothelium and hence can be removed only with drastic enzyme treatments of lungs and through use of magnetic fields following phagocytosis of intravenously injected iron oxide (37). Although this procedure yields viable iron oxide-containing PIMs, the phagocytosis of iron oxide may make them unsuitable for further in vitro experiments. Our growing understanding of the pathophysiological role of PIMs is through in vivo studies of selective depletion or inactivation of PIMs and comparing the outcomes between animals with normal PIMs and those in which PIMs are depleted. PIM depletion is achieved via intravenous treatment with gadolinium chloride and clodronate liposomes. Gadolinium chloride is a heavy metal lanthanide. Because gadolinium chloride induces apoptosis only after its uptake by cells, it is rather selective for action against phagocytic cells, and apoptotic cells generally do not induce significant tissue reaction (33). Furthermore, following an injection of gadolinium chloride in the jugular vein, most of the effects are restricted to PIMs because of its fast clearance by PIMs during the first pass through the pulmonary vasculature. Using gadolinium chloride and clodronate liposomes, we and others have provided multiple lines of evidence to establish pro-inflammatory roles of PIMs in lung inflammation induced by bacteria, viruses, and endotoxin in many species such as cattle, horse, and sheep (29, 35, 44, 49, 53). It is worth noting that PIM depletion with gadolinium chloride reduced rejection of transplanted lungs in pigs along with reduced levels of thromboxane in pulmonary circulation to indicate PIMs as a major source of arachidonic acid metabolism (9). The depletion of induced PIMs in bile duct-ligated rats that are used as a model for hepato-pulmonary syndrome showed significant therapeutic effects such as reduced endotoxin-induced lung inflammation and mortality (18, 55). Collectively, these data show constitutive and induced PIMs’ critical role in shaping the outcomes of lung inflammation.

To understand the contributions of PIMs to endotoxin-induced lung inflammation, we have explored the expression of Toll-like receptors (TLR) on constitutive PIMs. TLRs sense and engage various microbial molecules (4). We have reported the expression of TLR4 and TLR9 on PIMs of horse, cattle, pig, and water buffalo (38–40, 50, 60). Interestingly, before the discovery of TLR4, we used the conventional and immunegold electron microscopy to show that intravenously infused LPS enters the cytoplasm and nucleus of PIMs within 10 min of administration, suggesting an efficient receptor-mediated uptake (43). Later on, we substantiated this with dual immunegold electron microscopy evidence showing colocalization of LPS with TLR4 in PIMs of horses to provide direct evidence of their interaction (50). PIM depletion significantly reduced the amount of TLR4 and TLR9 mRNA in the lungs, demonstrating the major contribution of PIMs in relation to these critical TLRs in the lung. Furthermore, treatment of horses with LPS led to an increase in the lung expression of TLR2 and TLR9 (38, 50).

The downstream effect of TLR signaling is cell activation and production of mediators such as TNF-α and IL-1β. We provided earlier electron microscopic evidence of increased secretory activity in the PIMs of LPS-treated sheep (43). We and others have localized TNF-α in constitutive and induced PIMs and showed a reduction in the expression of TNF-α in the lung following depletion of PIMs (10, 18, 35). We noticed aggregation of IL8-rich platelets around PIMs in calves intra-tracheally challenged with Mannheimia hemolytica, and the platelet aggregation was inhibited in PIM-depleted animals (49). These observations may be important considering the recently described role of the early recruited platelets in neutrophil recruitment and inflammation (30). Recently, we found that depletion of constitutive PIMs ameliorates clinical signs of recurrent airway obstruction (RAO) in horses, a disease similar to human asthma (Aharonson-Raz K, Lohmann KL, Townsend HG, Marquez F, Singh B, unpublished results). Reduced lung inflammation associated with PIM depletion may be due to considerable reduction of lung localization of microbes and endotoxins along with reduced lung expression of TLR4 and TLR9. The activation of PIMs following intravenously administered endotoxins or bacteria may be due to signal transduction from the alveolar space into the capillary lumen through a second messenger (27). These data show that PIMs may influence lung inflammation in response to both vascular and airway challenges.

Results similar to those with depletion of constitutive PIMs have been observed following depletion of induced PIMs. As previously mentioned, the most striking outcome of depletion of induced PIMs was inhibition of mortality following LPS treatment of bile duct-ligated rats (18). Pulmonary localization of intravenously administered adenovirus is increased in a rat model of hepato-pulmonary syndrome, and the virus was localized in the recruited PIMs and caused lung inflammation (51, 52). Recently, the Archer laboratory used a rat model of hepato-pulmonary syndrome to show the reversibility of a number of physiological responses on PIM depletion with gadolinium chloride. PIM depletion reduced capillary dilation and muscularization as well as angiogenesis in the lungs of rats with hepato-pulmonary syndrome (55). There was also an inhibition of ERK1 activation and suggestion of reduced VEGF and PDGF signaling in the PIM-depleted animals. The recruitment of PIMs may be related to the altered phenotype of pulmonary microvascular endothelium, including induction of chemokines and expression of adhesion molecules. Collectively, these data demonstrate proinflammatory roles of constitutive and induced PIMs in the host species.

**IMPLICATIONS OF PIMs FOR HUMAN LUNG DISEASES**

The conclusive work showing normal occurrence of PIMs in species such as cattle, horse, sheep, goat, and pigs, and the very little evidence of their occurrence in humans may have led to waning of interest in studying PIM biology in the 1990s. Other than one short report by Dehring and Wismar (17), there has not been any systematic study of the extent of PIMs in human lung. Even if the yet to be conducted studies show absence of PIMs in humans, there is indirect evidence of induced PIMs in humans suffering from liver dysfunction (23, 24). Interestingly, increasing uptake of intravenously injected radiolabels in the lungs had some correlation with reduced survival of the patients suffering from neoplasia or other systemic nonpulmonary diseases (24). These intriguing data allude to the recruitment and influence of PIMs on the lung physiology of human patients. Paucity of data on PIMs in humans seems to be due to the fact that we have not looked for PIMs in normal and diseased human lungs, and we may have attributed the contributions of PIMs to other cells such as interstitial or alveolar macrophages. A recent study on the role of human interstitial
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One of the long-standing questions relates to the reasons for the occurrence of PIMs only in certain species. Since PIMs can be induced under physiological stress in animals that do not have constitutive PIMs, one could speculate that the microbial molecules in the barn environment elicit constitutive PIMs in animals such as sheep, pig, and cattle. But such an explanation may not apply to occurrence of PIMs in cats and whales. Another question relates to the role of constitutive PIMs in normal physiology. Earlier evidence showed phagocytosis of red blood cells by the PIMs in lungs of normal goat (2).

The lack of a relevant model to study the biology of the PIM in humans is one of the major impediments. Recent use of bile duct-ligated animals to induce PIMs has provided a useful model to study the role of induced PIMs and the impact of liver dysfunction on lung physiology via the recruitment of PIMs. It is possible that induced PIMs, compared with constitutive PIMs, may form a less tight adhesion complex with the vascular endothelium in bile duct-ligated or other animal models. Therefore, it may be easier to isolate induced PIMs for in vitro studies.

PIMs may be induced to cope with systemic physiological stress such as sepsis in animals through expansion of the existing mononuclear phagocytic system. It is plausible that repetitive insults over the life of a human may lead to induced PIMs and corresponding increase in sensitivity for lung disease. Therefore, we need to search for PIMs in autopsied or biopsied lungs from patients who may have died due to various chronic pulmonary or nonpulmonary diseases. Another ap-
proach would be to probe lung tissues from various animal models of human lung injury for induced PIMs before examining the autopsied lungs. The prioritization of models could be based on those systemic or organ-specific diseases, such as pancreatitis, that do result in significant pulmonary complications.

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8. Chang SW, Ohara N. K.A.-R., and B.S. drafted the manuscript; D.S., K.A.-R., and B.S. edited and revised the manuscript; D.S., K.A.-R., and B.S. approved the final version of the manuscript.

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No conflicts of interest, financial or otherwise are declared by the author(s).

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