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Review article

Pulmonary intravascular macrophages: a review of immune properties and functions

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Summary — Pulmonary intravascular macrophages (PIMs) are mononuclear cells found apposed to the lung capillary endothelium in a number of mammalian species. Although first described in the 1970s, it was not until the 1980s that they were more completely described. In several species of veterinary interest (bovine, porcine, ovine, and feline), PIMs are very important in blood clearance, which is not the case in mice and rats. Only recently have the immunological activities of PIMs been verified. In this review, we present an overview of PIM research with particular emphasis on the immune functions of this highly reactive macrophage population.

macrophage / lung / immunity / intravascular

Résumé — Les macrophages intravasculaires pulmonaires : revue des propriétés et fonctions immunitaires. Les macrophages intravasculaires pulmonaires (MIP) sont des cellules mononucléées apposées à l'endothélium des capillaires pulmonaires chez divers mammifères. Alors que leur découverte date des années 1970, leur description n'a été complétée que dans les années 1980. Chez plusieurs espèces animales domestiques (bovins, porcins, ovins et félins), les MIP ont un rôle important dans la clairance du sang, ce qui n'est pas le cas chez les souris et rats. Leurs propriétés immunologiques ont été établies seulement au cours de ces dernières années. Dans cette revue de la littérature, nous présentons un aperçu de la recherche sur les MIP en insistant tout particulièrement sur la fonction immunitaire de cette population de macrophages très réactifs.

macrophage / poumon / immunité / intravasculaire

INTRODUCTION

Pulmonary intravascular macrophages (PIMs) are members of the reticuloendothelial system found apposed to the pulmonary capillary endothelium in a number of mammalian species. The structural and

functional properties of PIMs were first reviewed by Winkler (1988), and the cell biology and possible pathogenic role of this unique macrophage population were reviewed by Warner and Brain (1990). Recently, a number of immunological functions have been attributed to PIMs. In this

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review, we provide an overview of PIM research, with particular emphasis on the newly elucidated immunological roles of PIMs.

HISTORY

Although it was recognized as early as 1918 that blood-borne particles localized in the lungs of certain species of animals (Winkler, 1988), pulmonary intravascular macrophages (PIMs) were not identified until the early 1970s. Identification of PIMs was difficult because of their close association with the capillary endothelium and because of the low resolving power of light microscopes at the time (Winkler, 1988). As a result, the uptake of particles by the lungs in the species of animals with PIMs was attributed to a lack of activity of hepato-splenic clearance (Niehaus *et al*, 1979), and not to a normal lung function.

Schneeberger-Keeley and Burger (1970) identified phagocytic mononuclear cells adhered to the pulmonary endothelium of cats. They hypothesized that these cells were hepatic Kupffer cells, which had migrated to the lungs in response to open chest ventilation. The existence of a resident population of PIMs was not considered at the time.

Shortly thereafter, Rybicka *et al* (1974) described PIMs in 3- to 4- month-old calves. Macrophages adhering to the pulmonary endothelium were observed in animals treated with various anesthetics, as well as in control animals. Because these calves had numerous PIMs and few pulmonary alveolar macrophages (PAMs), the authors hypothesized that these cells were the precursors of PAMs. It was not until the mid-1980s that bovine PIMs were identified as resident lung macrophages

that played a major role in blood clearance (Warner and Brain, 1984a, b).

Porcine PIMs were identified as a result of experiments designed to study animal models of bacterial sepsis; researchers first noted that infused bacteria were localized in mononuclear phagocytes of pig lungs (Crocker *et al*, 1981a, b; Dehring *et al*, 1983). These results prompted Wismar (1981) to hypothesize that PIMs were a new type of cell and, as such, merited additional study. In that same year, Crocker *et al* (1981b) reported that the pulmonary clearance of blood-borne bacteria by porcine PIMs was an important nonrespiratory function of the lungs. Three years later, Winkler and Cheville (1984) described porcine PIMs in a publication on the morphology of the terminal airways in the neonatal porcine lung.

Although sheep, goats and pigs were often used in studies of respiratory sepsis, several years passed before PIMs were identified in their pulmonary vasculature. Atwal and Saldanha (1985) identified the erythrophagocytic role of PIMs in goat lungs. A year later, Warner *et al* (1986) and Wheeldon and Hansen-Flaschen (1986) reported that the cells responsible for particulate and bacterial clearance in sheep lungs were PIMs.

Although responsible for blood clearance in ruminants, pigs, and cats, PIMs may play a lesser and somewhat different role in the other species in which they occur. They have been identified in man (Dehring and Wismar, 1989), as well as baboon (Francica *et al*, 1988), rat (Warner *et al*, 1989; Chang *et al*, 1990), dog (Niehaus, 1989), and rabbit lungs (Schultz *et al*, 1988; Carrasco *et al*, 1991). It is believed that under normal circumstances in the 5 latter species, PIMs are not numerous, or active in blood-clearance, unless a failure occurs in hepato-splenic clearance.

PIMs were first isolated from lungs by Staub *et al* (1987). They obtained large numbers of PIMs by perfusing the isolated lungs of sheep and goats with Ca/Mg-free Hank's buffer containing EGTA and EDTA. These PIMs had a number of properties that identified them as true macrophages. Morton and Bertram (1988) described a technique to isolate PIMs utilizing a collagenase solution to release PIMs from the vasculature, and subsequently Fowler *et al* (1991) described another technique using collagenase, but leaving the lungs *in situ* during the perfusion.

More recently, researchers have begun to associate the stimulation of PIMs with pulmonary vascular reactivity (Miyamoto *et al*, 1986a, b, 1987; Warner *et al*, 1987). Bertram *et al* (1987, 1988a, b, c, 1989) demonstrated that isolated PIMs were indeed capable of metabolizing arachidonic acid to its vasoactive metabolites by both the cyclooxygenase and lipoxygenase pathways. In addition, Miyamoto *et al* (1988b) theorized that PIMs, rather than endothelial cells, neutrophils, or perivascular interstitial cells, may be the source of circulating arachidonic acid metabolites. Because of the above properties, Winkler (1988) and Warner and Brain (1990) suggested that PIMs may play as large a role in the mechanisms of pulmonary disease as they do in pulmonary surveillance and blood clearance.

In 2 separate studies, Bertram (1985, 1986) suggested that PIMs are involved in *Haemophilus* (*Actinobacillus*) *pleuropneumoniae*-infected pigs. Intravascular macrophages were present in the lungs of infected pigs and contained cellular debris and fibrin as early as 6 hours post-infection, possibly indicating that the early effects of this pathogen involve macrophage and platelet activation (Bertram, 1985). In a subsequent study, Bertram (1986) reported that the PIM population changed from

immature to mature within 24 h after pigs had inhaled virulent *A pleuropneumoniae*.

Whiteley *et al* (1991) reported that PIMs may have an important role in the early intravascular inflammatory events in calves infected with *Pasteurella haemolytica*. They also noted that PIMs localized *Pasteurella* LPS within their cytoplasm (Whiteley *et al*, 1990), possibly indicating that endotoxin is the primary stimulus for inducing pulmonary inflammation through its interaction with pulmonary effector cells such as PIMs.

The most recently elucidated properties of PIMs relate to their susceptibility (or lack thereof) to viral infection. Sierra *et al* (1990) found that porcine PIMs were highly susceptible to infection by African swine fever virus and were the cells of the lung most responsible for the replication of this virus. However, Chitko-McKown *et al* (1990) reported that porcine PIMs were less susceptible than PAMs to infection by pseudorabies virus. Carrasco *et al* (1991) studied the role of PIMs in rabbit haemorrhagic disease, a viral disease characterized by vascular phenomena and pulmonary edema. Although PIMs were found in both infected and control animals, the PIMs in the experimental animals were more rounded in appearance and showed fewer junctions with endothelial cells. These differences were attributed to the cytopathic effect suffered by these cells.

In the past 20 years, the information available on PIMs has grown from the theory that these cells were migrating hepatic Kupffer cells to the realization that they comprise a resident population of lung macrophages. Because of the difficulty involved isolating these cells, most studies have been performed *in situ*; despite this, we presently know the morphological as well as some of the functional characteristics of PIMs.

ORIGIN AND DEVELOPMENT

Resident macrophages are generally assumed to begin as bone marrow cells, become circulating blood monocytes, and finally differentiate into mature macrophages at a suitable location. Winkler and Cheville (1985a) first presented evidence for the monocytic origin of the porcine PIM. They reported that blood monocytes colonize the porcine lung perinatally, replicate within the capillaries postnatally, and attach to the endothelium by intercellular junctions during differentiation. These data were supported by subsequent work, which described a doubling in the relative volume density of PIMs in piglets from birth to 7 days of age (Winkler and Cheville, 1987). These authors reported that undifferentiated mononuclear cells were enclosed in the capillaries of newborn pigs; however, by 7 days of age, numerous macrophages were observed in the capillaries. These macrophages were commonly associated with the endothelium, and few undifferentiated monocytes were identified. Additionally, Wismar *et al* (1984) reported transitional stages between circulating, small, mononuclear cells and large, sessile PIMs in pigs, with mitosis of the small mononuclear cells occurring within the capillaries.

Factors other than age may play a role in the differentiation and/or replication of PIMs in the porcine vasculature. A study of PIMs in pigs infected with *A pleuropneumoniae* suggested that the PIM population could change from immature to mature macrophages or immature epithelioid cells within 24 h (Bertram, 1986). These findings indicate that the microenvironment of the lungs may indeed be responsible for the subsequent colonization and differentiation of PIMs in the vasculature.

Little information is available on the development of PIMs in ruminants. Possibly,

this cell population is more differentiated at birth in calves than in pigs. Warner *et al* (1986) suggested that PIMs arise from bone marrow cells, which then attach firmly to the endothelium or migrate into the pulmonary interstitium and subsequently into the capillary lumen. However, a subsequent report by the same researchers states that no evidence was found to support the migration of PIMs to or from the lung (Warner and Brain, 1986). Therefore, ruminant PIMs, in fact, may comprise a self-replicating population (Warner *et al*, 1986).

MORPHOLOGY

Pulmonary intravascular macrophages are members of the mononuclear phagocyte system, found apposed to the underlying endothelium in the pulmonary capillaries in a number of mammalian species including ruminants, pigs, cats, rats, dogs, rabbits, baboons, and man (Schneeberger-Keeley and Burger, 1970; Winkler and Cheville, 1984, 1985a, b, 1987; Bertram, 1985, 1986; Warner *et al*, 1986; Wheeldon and Hansen-Flaschen, 1986; Dehring *et al*, 1987; Francica, 1988; Miyamoto *et al*, 1988b; Dehring and Wismar, 1989; Niehaus, 1989; Carrasco *et al*, 1991). *In vivo*, these cells are large, generally from 20–40 μm in diameter, irregular in shape, and have variably sized nuclei and abundant cytoplasm (Schneeberger-Keeley and Burger, 1970; Wismar *et al*, 1984; Atwal and Saldanha, 1985; Bertram, 1986; Warner and Brain, 1986, 1990; Wheeldon and Hansen-Flaschen, 1986; Dehring *et al*, 1987; Winkler and Cheville, 1987; Winkler, 1988; Fowler *et al*, 1991). *In vitro* comparisons of cells adhered to plastic indicated that PIMs are about half the diameter of PAMs, *ie* 8.2 μm vs 16.1 μm , respectively (Morton and Bertram, 1988).

Nuclear features include margination of the chromatin, concentration of chromatin in the centers of the nuclei, and interruption of marginated chromatin by nuclear pores (Atwal *et al*, 1989). In 3-day-old pigs, PIMs have been observed with mitotic figures and fat droplets in the nucleoplasm (Winkler and Cheville, 1985a, 1987).

The cytoplasm of PIMs contains various organelles. These include phagosomes, lysosomes, siderosomes, coated pits, rough endoplasmic reticulum, ribosomes, golgi membranes, numerous lamellar structures resembling micropinocytosis vermiformis (also described as worm-like bodies), and mitochondria (Schneeberger-Keeley and Burger, 1970; Rybicka *et al*, 1974; Crocker *et al*, 1981a; Dehring *et al*, 1983; Winkler and Cheville, 1984, 1985a, 1987; Atwal and Saldanha, 1985; Bertram, 1986; Warner and Brain, 1986; Warner *et al*, 1986; Wheeldon and Hansen-Flaschen, 1986; Albertine *et al*, 1987; Dehring *et al*, 1987; Warner *et al*, 1987; Miyamoto *et al*, 1988b; Atwal *et al*, 1989). In the calf, the mitochondria are restricted to the side of the PIM adjacent to the endothelium (Atwal *et al*, 1989).

In addition to the cytoplasmic organelles listed above, PIMs also contain an extensive cytocavitary network (Bertram, 1985). This network consists of tubular structures with electron dense central lamellae (Winkler and Cheville, 1985a, 1987), which have also been designated as microtubules and filaments (Atwal and Saldanha, 1985; Atwal *et al*, 1989). These structures give rise to the overall appearance of PIMs, which is that of mature macrophages possessing numerous pseudopodia and filopodia (Rybicka *et al*, 1974; Winkler and Cheville, 1984, 1985a; Atwal and Saldanha, 1985; Bertram, 1985; Wheeldon and Hansen-Flaschen, 1986; Morton and Bertram, 1988).

The PIM cell coat has been described as a fuzzy, electron-opaque, glycoprotein coat or glycocalix (Rybicka *et al*, 1974; Winkler and Cheville, 1985a, 1987; Atwal and Saldanha, 1985), and it is usually only partially preserved upon fixation (Wheeldon and Hansen-Flaschen, 1986). By staining with tannic acid as a component of paraformaldehyde-glutaraldehyde fixation, Atwal *et al* (1989) determined that in goats and calves, the PIM cell coat is actually a lipoprotein coat and is composed of regularly sized spherical globules ranging in size from 40–120 nm.

The amount of capillary volume occupied, as well as the amount of free surface area of PIMs exposed to blood, appears to be species-dependent. In cats, PIMs occupy 3.65% of the total capillary volume (Schneeberger-Keeley and Burger, 1970). However, ovine PIMs have been reported to occupy 15.3% of the intravascular volume (Warner *et al*, 1986), with 15.9 m² of free surface available for blood contact with these macrophages (Warner *et al*, 1986). However, another report estimated the capillary volume occupied and the surface area of the capillary covered by ovine PIMs to be 2.5% and 4.8%, respectively (Wheeldon *et al*, 1986). Apparently, the area occupied by PIMs may be dependent upon several factors, such as the age of the animal studied. In 30-day-old and newborn pigs, intravascular macrophages occupied 25 and 6% of capillary volume, respectively, and 16 and 2% of capillary surface was covered, respectively (Winkler and Cheville, 1987).

Pulmonary intravascular macrophages preferentially adhere to the thick side of the air-blood barrier (Winkler and Cheville, 1987) with peg-like processes (Wismar *et al*, 1984; Dehring *et al*, 1987). These processes have been described as intercellu-

lar junctions, or intracellular adhesion plaques (Morton and Bertram, 1988), containing electron-dense material, with 12–20 nm membrane interspace (Winkler and Cheville, 1985a, 1987; Warner *et al*, 1986; Morton and Bertram, 1988). Similar types of cell junctions are found between hepatic Kupffer cells and the endothelium (Bertram *et al*, 1988a; Morton and Bertram, 1988), and the latter authors hypothesized that both these structures serve the common purpose of anchoring phagocytes to endothelial surfaces.

Morton and Bertram (1988) demonstrated that isolated porcine PIMs have a mean diameter of 8.2 μ m and a ruffled membrane, adhere to endothelial surfaces of pulmonary artery and aorta, form junction-like adhesion plaques 15–20 nm wide containing electron-dense material, and contain organelles similar to those described above. However, Fowler *et al* (1991) reported a range in diameter of porcine PIMs of 10.4–16.5 μ m. Differences in diameter are no doubt due to the isolation procedures used, and to the different external stimulation available to the cells *in vitro* as compared to *in vivo*.

FUNCTION

Ultrastructural studies have revealed that under normal as well as some disease conditions PIMs actively phagocytose erythrocytes and platelets, as well as cell debris and fibrin (Rybicka *et al*, 1974; Winkler and Cheville, 1984, 1985a; Bertram, 1985, 1986; Dehring *et al*, 1987; Staub *et al*, 1987; Miyamoto *et al*, 1988b; Dehring and Wismar, 1989). Because of their pulmonary location, PIMs are exposed to 100% of the blood circulating in the body (Crocker *et al*, 1981b), and are therefore in a near-perfect location to perform the task of blood clearance in the species in which they are numerous. In or-

der to further study this possibility, infused particles were used in a number of experiments to determine blood clearance capabilities of PIMs. Tracer particles used in these experiments have included colloidal carbon, magnetic iron oxide, gelatinized ^{131}I -RE test lipid, polystyrene microspheres, monastral blue, colloidal gold, liposomes, and bacteria (Schneeberger-Keeley and Burger, 1970; Rybicka *et al*, 1974; Niehaus *et al*, 1979; Crocker *et al*, 1984a; Wismar, 1981; Dehring *et al*, 1983; Warner and Brain, 1984a, 1984b, 1986; Wismar *et al*, 1984; Atwal and Saldanha, 1985; Warner *et al*, 1986, 1987; Wheeldon *et al*, 1986; Wheeldon and Hansen-Flaschen, 1986; Albertine *et al*, 1987; Staub *et al*, 1987; Miyamoto *et al*, 1986a, 1986b, 1987, 1988a, 1988b).

While studying the effects of various concentrations of infused *Pseudomonas aeruginosa* in pigs, Crocker *et al* (1981b) observed that substantial lung clearance of blood-borne bacteria occurred in all experimental groups. These researchers found that as the lung clearance of bacteria decreased, the blood concentrations of bacteria progressively increased. Because most identifiable bacteria were located in mononuclear cells within small pulmonary vessels, they suggested that these predominant pulmonary capillary mononuclear cells played a key role in lung clearance.

The results described above, and others like them, necessitated clarifying which factors of bacteremia-induced pulmonary failure were dependent upon the type of animal tested. Therefore, a study was designed by Crocker *et al* (1981a) to compare the effects of bacterial infusion and the role of the lungs in localizing blood-borne bacteria in both dogs and pigs. In this study, *P. aeruginosa* was infused into the internal jugular vein of the animals. Physiological parameters were measured,

as were bacterial clearance and tissue retention. The data indicated that bacterial infusion caused severe hypoxemia and increased pulmonary shunting in the pigs within 1 hour. Although both pigs and dogs showed increased minute ventilation, no change in shunt fraction was demonstrated in dogs. Results also indicated that in pigs, 56 to 63% of the circulating bacteria were cleared with each pass through the lungs during the first hour of infusion, but then clearance decreased to a low value of 35%. In contrast, clearance values in dogs were constant at about 20%, which was significantly less than in pigs during the first 2 hours of infusion. Furthermore, pigs retained the majority of the cleared bacteria in the lungs, whereas the greatest tissue retention in dogs occurred in the spleen and liver. Electron microscopic study of the pig lungs showed that bacteria were located within the phagosomes of PIMs. In contrast, any bacteria observed in dog lungs were within the phagosomes of granulocytes.

Dehring *et al* (1983) performed a second experiment to determine if the respiratory pathophysiology and bacterial clearance observed previously were unique to *P aeruginosa*. This additional study compared the effects of *P aeruginosa* and *E coli*, both Gram-negative bacteria, and *S aureus*, a Gram-positive bacterium, in pigs. Pigs infused with *S aureus* maintained a high rate of lung bacterial clearance (75%), accompanied by pulmonary hypertension and a nonsignificant decrease in PaO_2 . These animals presented relatively normal lungs at necropsy. However, pigs infused with the Gram-negative bacteria developed systemic hypotension, pulmonary hypertension, hypoxemia, and decreased pulmonary clearance; 45 to 65% for *P aeruginosa* and 35% decreasing to 15–20% for *E coli*. All 3 types of the cleared bacteria

were found in greater numbers in the lungs than in the liver or spleen. Subsequently, these authors proposed that: 1), the quantity of bacteria or particulate matter cleared by the lungs varies between species; 2), high lung clearance occurs in species susceptible to acute respiratory failure; and 3), pulmonary clearance is not sufficient to cause respiratory failure as demonstrated with *S aureus*.

To determine the role of PIMs in the removal of bacteria from the bloodstream of sheep, Warner *et al* (1987) infused sheep and rats with a single dose of *P aeruginosa*. Kinetics of clearance, organ uptake, and morphological changes in the lungs and liver were compared. In rats, 97.5% of the organisms were removed from the blood in the first 20 minutes post-infusion, and 99.3% were removed within 60 minutes. Results in sheep were similar; 97% of the organisms were removed within 60 minutes. Hepatic and splenic clearance accounted for 93.7% of the localized bacteria in rats; however, in sheep this value was only 6.1%. In contrast, pulmonary uptake accounted for 93.2% of the bacteria in sheep, but only 1.6% in rats. Furthermore, pathological changes were noted in sheep lungs, but not in rat lungs. In the rat, most pathological changes were apparent in the liver, where the most bacteria were cleared; however, the sheep liver was morphologically normal. These data prompted the authors to suggest that the uptake of bacteria by PIMs causes their activation, and may mediate the subsequent inflammatory changes seen in the lungs.

An additional study compared blood clearance in sheep and rats, by studying the organ localization of both radiolabeled gold colloid and magnetic iron oxide particles (Warner *et al*, 1986). Gold colloid was rapidly cleared during the first 5 minutes after injection. Hepatic clearance account-

ed for 88.8% of the recovered dose of gold in the rat, but only 34.8% in sheep. In contrast, pulmonary localization of gold colloid in the rat was 0.3% as compared to 59.6% in sheep. Blood clearance of iron oxide followed the same trends in both species. Hepatic clearance of iron oxide accounted for 86.9% in the rat but was undetectable in the sheep, whereas pulmonary clearance was 3.6% in the rat and 99.8% in the sheep. These results demonstrated that the cells responsible for the pulmonary localization of gold colloid and iron oxide particles in the sheep lung were PIMs. The greater pulmonary localization of iron oxide was attributed to differences in particle size, surface characteristics, and mass of the injected dose.

Blood clearance using radiolabeled gold colloid and magnetic iron oxide was also studied in calves and a goat; once again, rats were used for comparison (Warner and Brain, 1986). The organ distribution of gold colloid in calves and goats showed nearly equal uptake by the lungs and liver; however, rats had predominantly hepato-splenic uptake. The uptake of the iron oxide particles showed more striking differences, with nearly exclusive pulmonary uptake in calves that were euthanized 1 hour post-injection. Rats demonstrated the same pattern of uptake for iron oxide as for colloidal gold, *ie*, predominantly hepatic uptake, but also some splenic localization.

An additional study of PIM function involved the effects of liposomes in sheep. Miyamoto *et al* (1988b) found a close correlation between liposome retention and PIMs; a test dose of liposomes injected intravenously into sheep caused an increase in pulmonary arterial pressure, which could be blocked completely by indomethacin and 75% by a thromboxane synthase inhibitor. They concluded that

liposomes affect pulmonary arterial pressure by a mechanism involving the arachidonic acid cascade, principally thromboxane. A subsequent report suggested that both the composition and the surface charge of the liposome are important factors affecting this mechanism (Miyamoto *et al*, 1988a).

When procedures became available to isolate PIMs, several groups proved that this population of cells was indeed active in metabolizing arachidonic acid (Bertram *et al*, 1987, 1988a, b, c, 1989; Staub, 1988). Although several abstracts have been published describing this function of PIMs (Bertram *et al*, 1987, 1988a, c; Staub, 1988; Bertram *et al*, 1989), we know of only one comprehensive study (Bertram *et al*, 1988b). Bertram *et al* (1988b) compared the profiles of arachidonic acid metabolites produced by PAMs and PIMs. They reported that PIMs appeared to be more active metabolically than PAMs when stimulated by the calcium ionophore, A23187. Additionally, PAMs formed at least 5 identifiable metabolites from exogenous arachidonic acid as compared to PIMs which formed at least 8. In contrast to PAMs, PIMs contained a 12-lipoxygenase as well as a 5-lipoxygenase and cyclooxygenase pathway for metabolizing arachidonic acid. They hypothesized that, because of these metabolic capabilities, PIMs play a significant role in the acute events of pulmonary inflammation and microvascular pressure.

The conclusion from the above experiments is that, in the animal species localizing blood-borne particulates in the lungs, PIMs are the cells responsible for this clearance. In some cases, because of the functional capability of metabolizing arachidonic acid, PIMs may be involved in the subsequent pathology associated with this metabolism.

IMMUNOLOGIC ACTIVITY OF PULMONARY INTRAVASCULAR MACROPHAGES

Few full-length scientific papers have been published describing the immunologic activity of PIMs; however, the currently available literature contains a number of abstracts on PIM immune function. Below, we have discussed the most detailed studies describing specific immunologic activities of PIMs. Additionally, table I provides a summary of published PIM immunological properties and activities in several mammalian species.

Staub's group reported that isolated PIMs stained positively for nonspecific esterase, secreted lysozyme, had receptors for the Fc fragment of immunoglobulin, generated superoxide and H_2O_2 when stimulated with PMA, and secreted a tumor necrosis factor (TNF)-like substance that could be inactivated by anti-TNF- α antibodies (Staub *et al*, 1987; Staub, 1988). These data clearly suggest an active immunologic role for PIMs. However, until recently, aside from the functions described above, little was known about the immunological activity of PIMs.

Because pulmonary alveolar macrophages (PAMs) have been firmly established as an immunologically active cell population, we felt it was logical to use them as a benchmark for comparing the immunological functions of PIMs in both pigs and calves.

In our first study, we used a modification of the technique previously described by Morton and Bertram (1988) to isolate PIMs and PAM from 6 pigs averaging 17.9 kg in weight. We then compared several immune parameters in the 2 macrophage populations: bactericidal and phagocytic activity; antibody-dependent cellular cytotoxicity; non-MHC restricted cellular cyto-

toxicity (NMRC); tumoricidal activity; and the production of the cytokines IL-1 and TNF- α (Chitko-McKown *et al*, 1991a). We found that PIMs were as active as PAMs in most parameters measured, but were less effective than PAMs in the phagocytosis assay and at certain ratios of effector to target cells in the tumoricidal assay. In contrast, PIMs lysed 6 times as many virally infected target cells in the NMRC assay as did PAMs.

Because of our results in the pig study, we performed a second study comparing the immune activity of PIMs to PAMs in 7 bull calves averaging 42.7 kg in weight. We performed all of the assays described for the pig experiment with the exception of the tumoricidal assay and, in addition, performed an assay measuring the production of nitrite (NO_2^- ; Chitko-McKown *et al*, 1991b). Calf PIMs were equal to PAMs in bactericidal and phagocytic activity, but as in the pig study, PIMs were significantly more cytotoxic than PAMs. When stimulated with LPS, PIMs produced more IL-1 than did PAMs; however, non-stimulated PIMs and PAMs produced similar amounts of the cytokine. In contrast, LPS-stimulated PAMs produced more TNF- α and NO_2^- than LPS-stimulated PIMs. Non-stimulated macrophages from both populations produced similar amounts of these substances.

Data from both of these studies suggest that PIMs comprise an immunologically important cell population. Differences between the degrees of activity of PIMs and PAMs indicate that these macrophage populations may have different roles in lung surveillance.

CONCLUDING REMARKS

A significant amount of research has involved describing the morphology and

Table 1. Immune properties or activities of pulmonary intravascular macrophages in various animal species.

<i>Species</i>	<i>Property/Activity</i>	<i>References</i>
Bovine	Antibody-dependent cellular cytotoxicity	Chitko-McKown <i>et al</i> , 1991b
	Bactericidal activity	Chitko-McKown <i>et al</i> , 1991b
	IL-1 production	Chitko-McKown <i>et al</i> , 1991b
	LPS immunoreactivity	Whiteley <i>et al</i> , 1990
	Non-MHC restricted cytotoxicity	Chitko-McKown <i>et al</i> , 1991b
	NO ₂ ⁻ production	Chitko-McKown <i>et al</i> , 1991b
	Phagocytosis	Rybicka <i>et al</i> , 1974; Warner and Brain, 1984a, b, 1986; Atwal <i>et al</i> , 1989; Whiteley <i>et al</i> , 1990, 1991; Chitko-McKown <i>et al</i> , 1991b
	TNF- α production	Chitko-McKown <i>et al</i> , 1991b
Caprine	Esterase positive	Staub <i>et al</i> , 1987; Schultz <i>et al</i> , 1988
	Lysozyme positive	Staub <i>et al</i> , 1987
	Peroxide production	Staub <i>et al</i> , 1987
	Phagocytosis	Warner and Brain, 1984b; Atwal and Saldanha, 1985; Albertine <i>et al</i> , 1987; Miyamoto <i>et al</i> , 1987; Staub <i>et al</i> , 1987; Atwal <i>et al</i> , 1989
	Plastic adherence	Staub <i>et al</i> , 1987
	Fc receptor positive	Staub <i>et al</i> , 1987
	Superoxide production	Staub <i>et al</i> , 1987; Schultz <i>et al</i> , 1988
	Thromboxane production	Miyamoto <i>et al</i> , 1987
	TNF- α production	Schultz <i>et al</i> , 1988
	TNF-like activity	Staub <i>et al</i> , 1987
Feline	Phagocytosis	Schneeberger-Keeley and Burger, 1970
Human	Phagocytosis	Dehring and Wismar, 1989
Lapine	Esterase positive	Schultz <i>et al</i> , 1988
	Phagocytosis	Warner <i>et al</i> , 1989; Carrasco <i>et al</i> , 1991
	Superoxide production	Schultz <i>et al</i> , 1988
	Thromboxane production	Schultz <i>et al</i> , 1988
	TNF- α production	Schultz <i>et al</i> , 1988
Murine (<i>Rattus norvegicus</i>)	Phagocytosis	Warner <i>et al</i> , 1989; Chang <i>et al</i> , 1990
Ovine	Esterase positive	Warner <i>et al</i> , 1986; Wheeldon and Hansen-Flaschen, 1986; Staub <i>et al</i> , 1987
	Lysozyme positive	Staub <i>et al</i> , 1987
	Peroxide production	Staub <i>et al</i> , 1987
	Phagocytosis	Warner <i>et al</i> , 1986; Wheeldon <i>et al</i> , 1986; Wheeldon and Hansen-Flaschen, 1986; Albertine <i>et al</i> , 1987; Miyamoto <i>et al</i> , 1987; Staub <i>et al</i> , 1987; Warner <i>et al</i> , 1987; Miyamoto <i>et al</i> , 1988b

Table 1 (continued)

	Plastic adherent	Staub <i>et al</i> , 1987
	Fc receptor positive	Staub <i>et al</i> , 1987
	Superoxide production	Staub <i>et al</i> , 1987
	TNF-like activity	Staub <i>et al</i> , 1987
Porcine	Antibody-dependent cellular cytotoxicity	Chitko-McKown <i>et al</i> , 1991a
	Adherence to plastic	Bertram <i>et al</i> , 1987; Bertram <i>et al</i> , 1988b, 1988c; Morton and Bertram, 1988; Bertram <i>et al</i> , 1989; Fowler <i>et al</i> , 1991; Chitko-McKown <i>et al</i> , 1991a
	Bactericidal activity	Chitko-McKown <i>et al</i> , 1991a
	Esterase positive	Morton and Bertram, 1988; Fowler <i>et al</i> , 1990; Fowler <i>et al</i> , 1991
	TGF β gene expression	Fowler <i>et al</i> , 1990
	IL-1 production	Chitko-McKown <i>et al</i> , 1991a
	Arachidonic acid metabolism	Bertram <i>et al</i> , 1987; Miyamoto <i>et al</i> , 1987; Bertram <i>et al</i> , 1988b, 1988c; Bertram <i>et al</i> , 1989
	Non-MHC restricted cytotoxicity	Chitko-McKown <i>et al</i> , 1991a
	Phagocytosis	Crocker <i>et al</i> , 1981a, 1981b; Wismar, 1981; Dehring <i>et al</i> , 1983; Winkler and Cheville, 1984; Wismar, 1984; Bertram, 1985; Winkler and Cheville, 1985a; Bertram, 1986; Albertine <i>et al</i> , 1987; Dehring <i>et al</i> , 1987; Miyamoto <i>et al</i> , 1987; Bertram <i>et al</i> , 1989; Sierra <i>et al</i> , 1990; Chitko-McKown <i>et al</i> , 1991a; Folwer <i>et al</i> , 1991
	TNF- α production	Chitko-McKown <i>et al</i> , 1991a
	Tumoricidal activity	Chitko-McKown <i>et al</i> , 1991a

anatomical location of PIMs, leaving no doubt that they comprise an active lung macrophage population. What remains to be determined is the extent to which PIMs participate in pulmonary physiology. Now that several techniques to isolate PIMs are readily available, much more information should be forthcoming on their other roles in lung function, pathology, and immunity.

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