[Frontiers in Bioscience, 2, d232-241, June 1, 1997]

THE COMPARATIVE BIOLOGY OF PULMONARY INTRAVASCULAR MACROPHAGES

Kim E. Longworth, Ph.D.

Department of Surgical and Radiological Sciences, School of Veterinary Medicine, University of California, Davis

TABLE OF CONTENTS

ABSTRACT

Pulmonary intravascular macrophages are an important part of the mononuclear phagocyte system in some species of mammals, mainly sheep and other ruminants, pigs, and horses. These cells phagocytize foreign particles, cell debris and pathogens that pass through the pulmonary circulation. Species with intravascular macrophages localize intravenously injected tracer particles and bacteria predominantly in the lung rather than the liver, and exhibit pulmonary hypertension when these cells are activated. Both *in vivo* and *in vitro* studies show that pulmonary intravascular macrophages have distinct secretory and immune capabilities. Consequently, the pulmonary intravascular macrophages play an important role in pulmonary inflammation in species that have them.

2. INTRODUCTION

Pulmonary intravascular macrophages are a distinct population of cells that permanently reside within the pulmonary capillaries of some species of mammals. Research on the physiological significance of these cells has only developed in the last 10 years. However, it is clear from this research that, in species that have them, the macrophages play an important role in the animal's response to some invading pathogens, endotoxin or foreign particles (1-7).

Since 1988, reviews (1, 2, 3, 4, 5) a monograph (6) and a book chapter (7) have focussed on various aspects of pulmonary intravascular macrophage biology. As the first publication of this topic to be presented in an electronic forum, this review will emphasize some of the key features of the anatomy and physiology of these cells that were touched upon in published reviews.

Received 4/11/97 Accepted 5/21/97
To whom correspondence should be addressed to: Kim Longworth, VM: Surgical and Radiological Sciences, University of California, Davis, CA 95616. Tel: (916)-754-9391 Fax: (916)-752-6042 E-mail: kelongworth@ucdavis.edu

The mononuclear phagocyte system in mammals is comprised of phagocytic cells throughout the body responsible for removing particles from the circulation (8, 9). The liver has historically been regarded as the functional center of that system because of the Kupffer cells (stellate macrophages) lining its sinusoids and because of the high fraction of systemic blood flow (about 20%) that it receives. The spleen and bone marrow, with their associated macrophage populations, are secondary sites for particle clearance, mainly because their blood flows are low..

This hepatic orientation of regional phagocytosis is based on studies in typical laboratory mammals, and humans (9, 10). Early studies showed that intravenously injected foreign particles are mostly retained by the liver and spleen in rats, rabbits and dogs (11, 12). However, although these studies suggested that the lung could retain a substantial fraction of blood-borne particles in some species, this observation was largely ignored for many decades (1). More recently, the macrophages responsible for lung retention of particles were considered to be displaced Kupffer cells by Schneeberger-Keeley and Burger (13), but later were formally identified as a resident population of macrophages by Rybicka *et al.* (14). Later studies confirmed the pulmonary orientation of phagocytosis in sheep, pigs, cats and calves (15, 16, 17, 18, 19).

The mononuclear phagocyte system of the lung is comprised of cells in 3 compartments; airway and alveolar macrophages, interstitial macrophages and, in some species, intravascular macrophages (20) (Fig 1). All of these macrophages may derive from sequestered monocytes in the pulmonary capillaries that migrate to the respective compartments, or may multiply by mitosis in situ (9, 21). These cells act as a portion of the lungs' defense mechanism against airborne pathogens or foreign particles that infiltrate the lung airspaces. In all mammals, blood-borne pathogens or particles that infiltrate the pulmonary tissues may be attacked by interstitial macrophages. In those species with

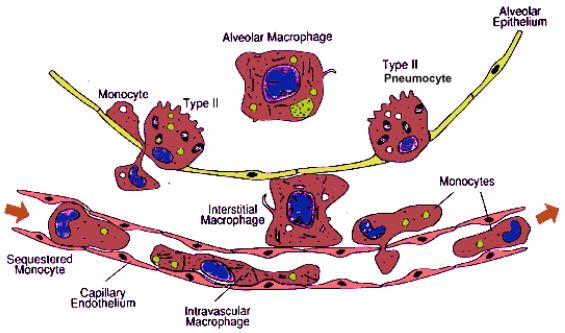

Figure 1. Mononuclear phagocytes in the lung are a dynamic system. Large numbers of monocytes pass through the pulmonary circulation and may become transiently sequestered in pulmonary capillaries. Some of these sequestered cells migrate into the interstitium or alveolar spaces, and differentiate into mature macrophages. In some species of mammals, monocytes permanently adhere to the endothelium and differentiate into mature pulmonary intravascular macrophages. Reprinted with permission from Reference 4.

intravascular macrophages, all particles circulating in the blood can potentially be phagocytized by intravascular macrophages before entering the interstitium.

3. MORPHOLOGY AND DISTRIBUTION

Rybicka *et al.* first identified pulmonary intravascular macrophages as a distinct population of cells in calves (14). The mononuclear cells they observed in pulmonary capillaries of normal calf lungs had all the characteristics of mature macrophages rather than circulating monocytes. Later, Winkler and Cheville (22) and Warner and co-workers (16) showed that the cells form membrane adhesion complexes with the underlying endothelial cells, supporting the concept that they are resident rather than merely circulating cells. Workers attempting to isolate the cells by washing calcium chelators through blood-free lungs have found it difficult to break down the adhesion complexes without using digestive enzymes (23, 24, 25): such tenacious attachment to the underlying endothelium suggests that these cells are resident macrophages rather than mobile or circulating cells. In addition, putative intravascular macrophages, isolated from pig lungs, form adhesion complexes with endothelial cells (23).

As are other mature macrophages, pulmonary intravascular macrophages are larger (20-80 µm) than monocytes and have larger and more extensively developed organelles (nucleus, Golgi apparatus, mitochondria, rough endoplasmic reticulum) (Figures 2 and 3). They have numerous pseudopods that allow a large portion of the cell surface to be in contact with the endothelium. Their plasma membranes, coated with a glycocalyx, have invaginations that form micropinocytosis vermiformis; these features indicate active receptor-mediated endocytosis (2). Atwal and co-workers have extensively studied the morphological function of the surface coat (26, 27, 28). Even in normal lungs, large phagosomes contain cellular debris, indicating that clearance of effete cells from the circulation is a normal function of the cells (16, 18, 22, 29, 30).

In certain species, abundant macrophages have been identified histologically in pulmonary capillaries. In 1-month-old pigs, intravascular macrophages occupy 25% of the capillary lumen and are estimated to number 14 x 10^3 cells per mm^3 of lung parenchyma (30); in adult sheep, this fraction is 15% and the number is 8 x 10^3 cells per mm^3 of lung parenchyma (16). By contrast, in other species the presence of even a small population of pulmonary intravascular macrophages is disputed (4). Although Warner et al. (2) found no electron microscopic evidence of any pulmonary intravascular macrophages in rats, Niehaus (31) suggests that they do exist in rats in small numbers. In one study in humans, investigators examined biopsies of lung tissues obtained during surgery (32). Because the samples were biopsies, the blood was not removed from capillaries, a technique which improves identification of resident intravascular macrophages. The authors reported finding occasional large mononuclear cells that appeared similar to intravascular macrophages in other species. They concluded that the population of intravascular macrophages must be relatively smaller in human lungs.

4. PHYSIOLOGY

Because of this uncertainty relating to the anatomical definition of a resident phagocyte in pulmonary capillaries of fixed tissue, it is useful to use functional criteria to determine whether pulmonary intravascular macrophages are a biologically significant cell component in the normal mammalian lung. One functional criterion is whether the lung is capable of retaining a substantial fraction of blood-borne particles.

Comparative studies have examined the uptake of intravenously injected non-biological tracer particles in mammals (Figure 4) (11, 19, 33-39). The result of combining the data from these studies shows a pattern of particle retention in the lung that is related to mammalian taxonomic orders. All seven species studied in two mammalian orders, Artiodactyla and Perissodactyla, show high lung retention of particles (generally >40%). All three species in another order, Rodentia, show low (<10%) lung retention of particles. On the other hand, of the two carnivores in which more than one particle has been tested, the cat shows variable but generally high retention and the dog shows low retention.

Studies using bacteria or endotoxin in paired species comparisons support this lung retention pattern. Pigs and sheep have a high lung retention of Pseudomonas bacteria while rats and dogs have a low retention (40, 41). Regardless of the significance of the distribution among orders, it is clear that in certain species the anatomically observed pulmonary intravascular macrophages are numerous enough to effect lung localization of foreign particles or cell debris in the blood. Thus, these species could be described as having a functional pulmonary intravascular macrophage population.

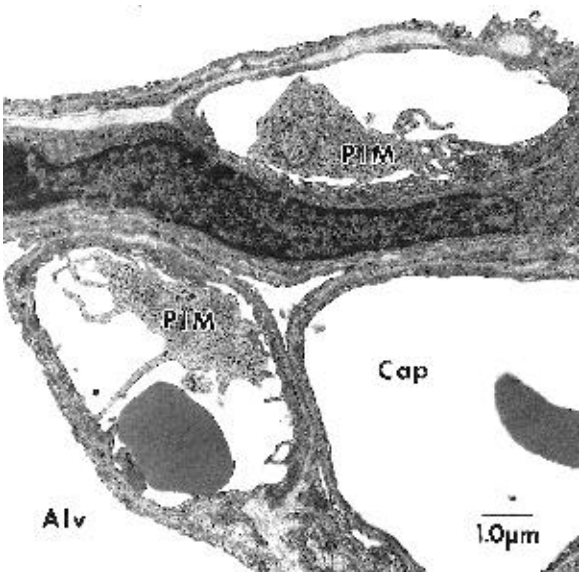

Figure 2. Electron micrograph of capillaries (Cap) surrounded by alveolar air spaces (Alv) in a lamb lung fixed by vascular perfusion. Pulmonary intravascular macrophages (PIM) are in intimate contact with the capillary endothelium by pseudopods. Reprinted with permission from Reference 48.

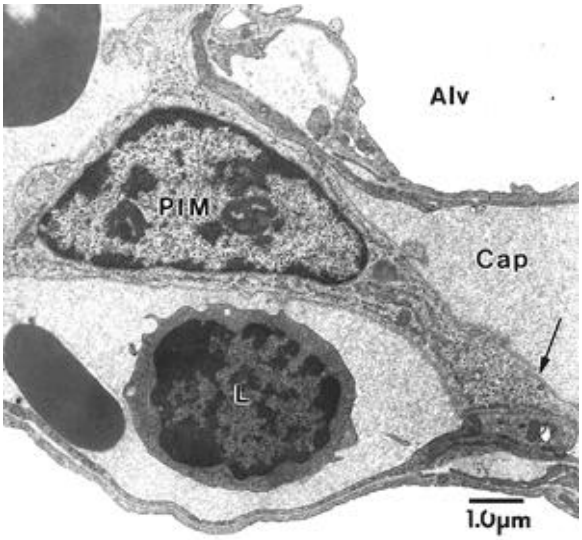

Figure 3. Electron micrograph of capillaries (Cap) surrounded by alveolar air spaces (Alv) in a lamb lung fixed by tracheal insufflation to preserve the vascular contents. The pulmonary intravascular macrophage (PIM) has a characteristic fuzzy coat (glycocalyx; shown by arrow) that is not apparent in lungs fixed by vascular perfusion. L, lymphocyte. Reprinted with permission from Reference 48.

However, this description of experimental observations of the fate of intravenously injected particles may not reflect the degree to which, in the natural condition, pulmonary intravascular macrophages play a role in phagocytosis of particles from the circulation. In fact, most particles injected into the portal circulation in sheep are mainly taken up in the liver by Kupffer cells (42).

A relevant criterion that could be used to assign pulmonary intravascular macrophages a primary role in the mononuclear phagocyte system is whether the cells elicit a physiological response over and above phagocytosis when they encounter particles. The ability of the cells to effect pathophysiological changes would have a bearing on their significance in the whole body immune defense system.

Two sets of data relate to this concept, one is the pulmonary circulatory hemodynamic response when particles are phagocytized by intravascular macrophages, and the other is the cells' secretory and immune capabilities.

4.1. Effect on pulmonary hemodynamics

Intravenous injection of particles induces pulmonary hypertension in species with pulmonary intravascular macrophages (Figure 5). In sheep, calves, pigs, llamas, goats, reindeer and horses, the tracer particles Monastral blue or liposomes induce a transient (3-5 min) increase in pulmonary arterial pressure of 16-43 mm Hg, depending on the dose and species (19, 35-39, 43). The pulmonary hemodynamic response to tracer particles also occurs in sheep when particles are injected intraarterially (33, 43). The particles are not retained in the systemic circulation, and return to the pulmonary circulation via the pulmonary artery. Species without the macrophages (dogs, ferrets, rabbits and monkeys) have almost no hemodynamic response to the same particles (39). The only exception to this observation is the cat. The cat has many macrophages that phagocytize injected particles; however, intravenous injection of particles into cats does not induce pulmonary hypertension.

The pattern in species-specific response is similar when foreign blood is injected. In goats, when rabbit or rat blood is injected intravenously they induce an increase in pulmonary arterial pressure of 24 and 18 mmHg, respectively (44). No increase in pressure greater than 7 mmHg occurs in rabbits injected with goat or rat blood, or rats injected with goat or rabbit blood. In addition, sheep pulmonary intravascular macrophages phagocytize platelets bound to injected anti-sheep platelet serum; this event is associated with pulmonary hypertension (45). No pulmonary hemodynamic response occurs in rats after anti-sheep platelet serum is injected.

The transient pulmonary hypertension after intravenous injection of particles is due to pulmonary venous and arterial constriction (35) . Studies in goats, ponies and sheep have demonstrated either no change or a decrease in cardiac output concurrent with pulmonary hypertension (33, 38, 46, 47). The vasoconstriction is caused by large quantities of thromboxane A_2 (a potent vasoconstrictor metabolite of cell membrane arachidonate) released into the pulmonary circulation (measured as the pulmonary arterial-systemic arterial difference in the stable metabolite thromboxane B_2) (19, 45, 46, 47) .

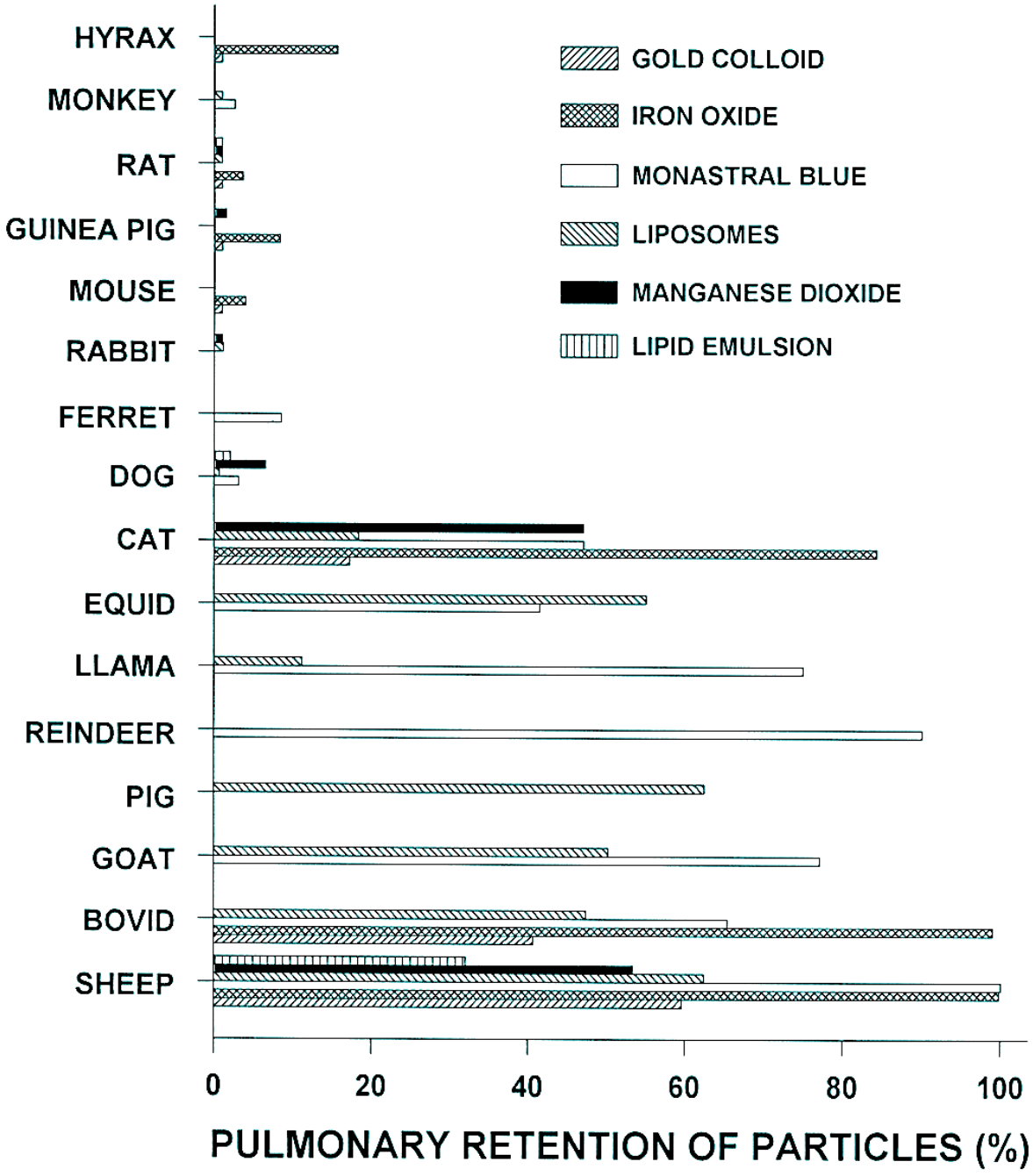

Figure 4. Summary of data from comparative studies showing pulmonary retention of tracer particles injected into the circulation of 16 species of mammals in 6 taxonomic orders. Orders represented (from bottom to top) are Artiodactyla (sheep, bovid [cattle, calf], goat, pig, reindeer and llama); Perissodactyla (equid [pony, horse]); Carnivora (cat, dog, ferret); Lagomorpha (rabbit); Rodentia (mouse, guinea pig, rat); Primate (monkey) and; Hyracoidea (hyrax). Particles used for injection are gold colloid, iron oxide, Monastral blue, liposomes, manganese dioxide and lipid emulsion. Data from 11, 19, 33-39.

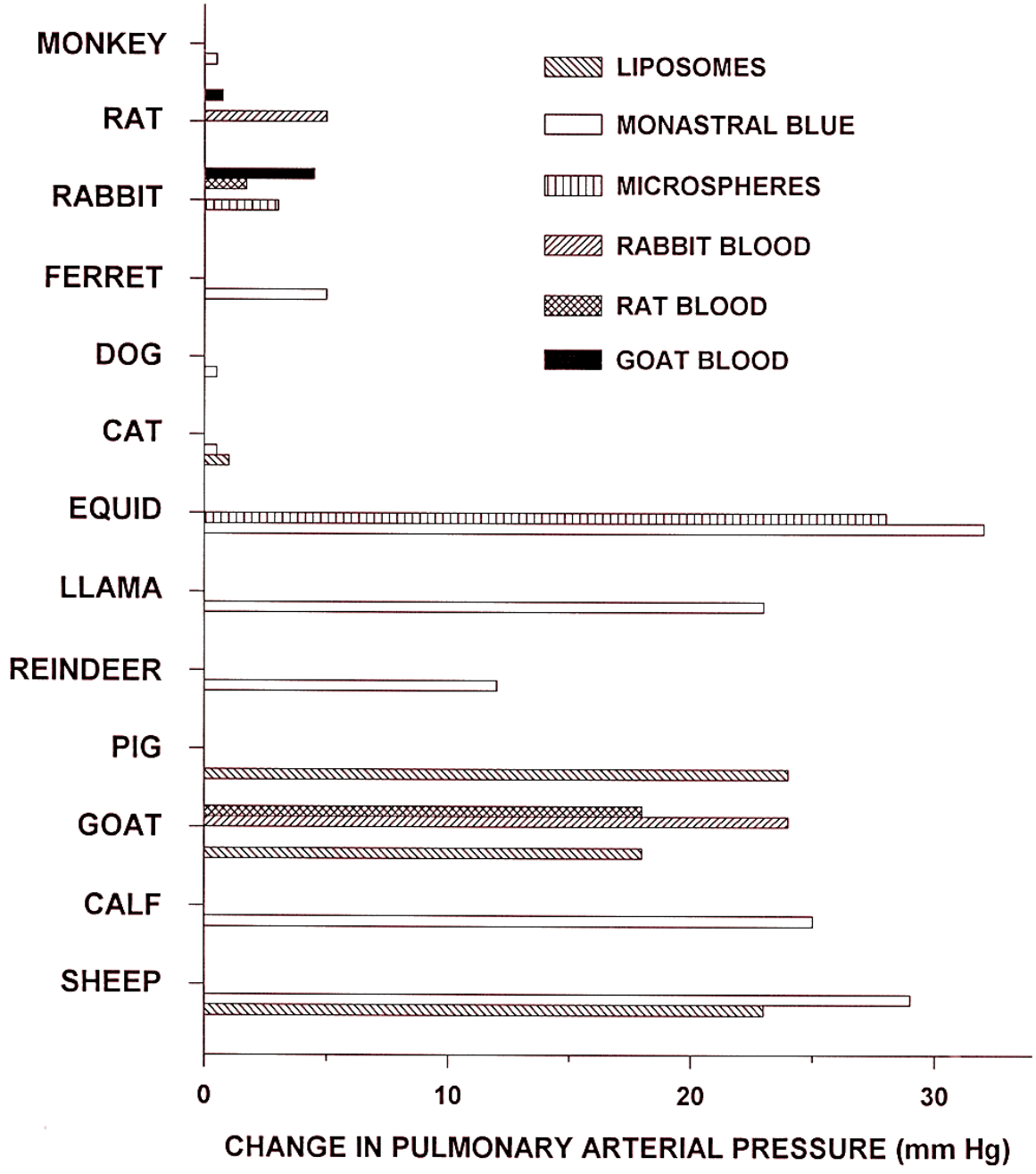

Figure 5. Summary of data from comparative studies showing change in pulmonary arterial pressure after injection of tracer particles into the circulation of 13 species of mammals in 5 taxonomic orders. Orders represented (from bottom to top) are Artiodactyla (sheep, calf, goat, pig, reindeer and llama); Perissodactyla (equid [pony, horse]); Carnivora (cat, dog, ferret); Lagomorpha (rabbit); Rodentia (rat); and Primate (monkey). Particles used for injection are liposomes, Monastral blue, microspheres, rabbit blood, rat blood and goat blood. Data from 19, 33, 35-39, 43.

In lambs, the pulmonary vascular reactivity has been linked to the presence of the macrophages (46). In newborn (1-2 day old) lambs, few macrophages are present in the pulmonary capillaries (48), and the animals show no hemodynamic response to intravenous Monastral blue or liposomes. Particles are mostly retained by the liver. By two weeks, a substantial population of macrophages appears in the capillaries, the animals respond to particles with an increase in pulmonary vascular driving pressure (left atrial-pulmonary arterial pressure difference), and particles are mostly retained by the lung. The increase in driving pressure is due to an increase in the pulmonary production of thromboxane (46).

The species differences in the pulmonary hemodynamic response to tracer particles are similar to differences observed when bacteria are injected intravenously. In response to the same relative dose of Pseudomonas aeruginosa, anesthetized pigs had a 220% increase in pulmonary arterial pressure and a 300% increase in pulmonary vascular resistance within one hour, compared to no change in pressure and only a 30% increase in resistance in anesthetized dogs (40).

The presence of pulmonary intravascular macrophages causes the pulmonary circulation to be sensitive to relatively small doses of intravenously injected endotoxin. Experimental doses of endotoxin used in species that have the cells are three orders of magnitude smaller than those used in species without the cells. For example, 0.03 to 1.3 micrograms per kilogram intravenous injection of endotoxin are used to induce pulmonary hypertension in sheep and horses, and changes in lymph flow and pulmonary inflammation in sheep (49, 50, 51, 52). In contrast, experimental endotoxin doses that are used to induce systemic hemodynamic changes in rats, rabbits, dogs and non-human primates are in the range of 0.1 to 5 milligrams per kilogram of endotoxin (53-56); large doses of 0.15 mg/kg in the dog (54) and 5 mg/kg in the monkey and rabbit (53) are needed to elicit any detectable change in pulmonary arterial pressure. The maximum dose used in experimental endotoxemia in humans (4 ng/kg) does not produce any increase in pulmonary arterial pressure (57).

There is, therefore, a clear link between the presence of pulmonary intravascular macrophages and the pulmonary hemodynamic response to blood-borne particles. Among the species studied, whenever the cells are present, pulmonary hypertension occurs when foreign particles are injected into the peripheral circulation. The reactive macrophages can alter pulmonary hemodynamics and potentially alter pulmonary fluid balance.

4.2. Secretory and immune functions

Some studies have examined various secretory properties and immune activities of *in vivo* and isolated pulmonary intravascular macrophages (3). A few studies have focused on comparisons between these cells and alveolar macrophages. Both pulmonary intravascular and alveolar macrophages isolated from pigs and incubated with arachidonic acid produced lipoxygenase and cyclooxygenase metabolites (Prostaglandin F_2, hydroxyheptadecatrienoic acid (HHT), and 5-, 12- and 15-hydroxyeicosotetraenoic (HETE)). However, intravascular macrophages also produced thromboxane B_2 prostaglandin D_2 and prostaglandin E_2 (58, 59). When exposed to various particles and soluble stimuli (calcium ionophore A23187, asbestos, iron spheres, zymosan, lipopolysaccharide) both types of cells produced leukotriene B_4. However, there were differences in the amounts of metabolites released by the two cell populations. The authors concluded that intravascular macrophages are generally metabolically more active than alveolar macrophages (3).

A comparison of immunological functions between isolated intravascular and alveolar macrophages from pigs showed similar bactericidal and antibody-dependent cellular cytotoxic activities (60). Although intravascular macrophages were less effective at phagocytosis and non-MHC restricted cellular cytotoxicity, they had more tumoricidal activity than alveolar macrophages. Both cell populations produced similar concentrations of interleukins 1 and 2 and tumor necrosis factor alpha (60). In studies with endotoxin-stimulated intravascular and alveolar macrophages, the former produced more T-cell proliferative cytokines and the latter more tumor necrosis factor alpha and nitric oxide (61).

In vivo studies suggest that there are differences in the secretory capabilities of intravascular macrophages and Kupffer cells (4). The pulmonary circulations of species with intravascular macrophages release thromboxane into the circulation in response to injected particles (19, 44, 46, 47) . In contrast, in species without intravascular macrophages, although injected particles are taken up by the liver, there is no apparent release of thromboxane into the systemic circulation.

These studies show that pulmonary intravascular macrophages are as metabolically active as other macrophage populations, and that they have a full range of secretory and immune capabilities, apparently different from alveolar macrophages and Kupffer cells. Taken together, the data indicate that the macrophages are important in mediating pulmonary pathophysiological changes.

5. ROLE IN DISEASE

A few studies have specifically focused on the role of pulmonary intravascular macrophages in pulmonary disease. When *Pseudomonas aeruginosa* bacteria are intravenously injected into sheep or pigs, the pulmonary capillaries become congested with red cells, neutrophils, platelets, some lymphocytes and fibrin clumps, and intravascular macrophages (40-41, 62). Lung interstitium shows varying degrees of edema, from widening around larger vessels to intraalveolar edema with atelectasis. One hour after injection, bacteria in the lung parenchyma are contained within phagosomes of intravascular macrophages (41); by 24 hrs they are also found in neutrophils (62). These morphologic changes accompany physiological changes, such as pulmonary hypertension and delayed systemic hypotension, an increase in lung lymph flow (initially due to hydrostatic pressure and later to increased

vascular permeability), hypoxemia, fever and leukopenia (40, 62).

In contrast, rats and dogs injected with *Pseudomonas* show almost no pulmonary morphologic changes and no features of pulmonary failure (40-41). In the rat, most bacteria are retained in the liver by Kupffer cells, and it is the hepatic sinusoids that are congested with neutrophils and platelets (40). In dogs, the hemodynamic effect of bacterial infusion is limited to systemic hypotension and decreased cardiac output (41).

Other studies on different pathologic organisms demonstrate the involvement of intravascular macrophages in lung infections. Bertram showed that after intratracheal inoculation with *Haemophilus pleuropneumoniae,* the relative volume of intravascular macrophages in pig lungs increased in areas of inflammation and necrosis (63). The cells matured within 24 h of inoculation, increasing in cytoplasmic volume (due to an increase in the number of organelles), and increasing intracellular adhesion to underlying capillary endothelial cells. Bertram concluded that the intravascular macrophages clear cellular and acellular debris from the blood in pneumonitis.

A similar role has been proposed for pulmonary intravascular macrophages in the pulmonary response to intratracheal inoculation of *Pastuerella haemolytica* in calves (64), in experimentally induced African Swine Fever (a virally-induced hemorrhagic disease) in pigs (65, 66), and in the response to spontaneously occurring *Actinomyces pyogenes* lesions in cattle (67).

6. PERSPECTIVE

Experimental studies suggest that pulmonary intravascular macrophages have a dominant role in the mononuclear phagocyte system of mammals in the orders Artiodactyla and Perissodactyla. These cells are involved in clearance of particles and debris from the circulation and the immune response against blood-borne and airborne pathogens. It is less clear what their importance is to animals in the natural environment. Although various hypotheses have been proposed to account for the presence of these macrophages, it is uncertain why such a substantial and distinctive population has evolved in some species and not others (4).

Much of the current research focuses on whether pulmonary intravascular macrophages are induced in humans in certain pathological conditions. Humans normally have few intravascular macrophages; foreign particles in the circulation are localized in the liver, phagocytized by Kupffer cells. However, gram-negative septicemia or endotoxemia in humans often leads to acute lung injury (Adult Respiratory Distress Syndrome, or ARDS), and it not understood how pulmonary inflammation develops from systemically introduced pathogens. Sheep and pigs are both used as experimental models for ARDS in humans; possibly, humans develop intravascular macrophage-type cells in the lung after endotoxemia or liver injury, making their pulmonary circulations behave more like those in species with resident intravascular macrophages. To determine if this is possible, workers are attempting to induce a pulmonary intravascular macrophage population in species without the cells by chronic endotoxin infusion (4, 5, 7, 68).

7. REFERENCES

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

2. Warner AE & Brain JD: The cell biology and pathogenic role of pulmonary intravascular macrophages. *Am. J. Physiol.* 258, L1-L12 (1990)

3. Chitko-McKown CG & Blecha F: Pulmonary intravascular macrophages, a review of immune properties and functions. *Ann. Rech. Vet.* 23, 201-214 (1992)

4. Staub NC: Pulmonary intravascular macrophages. Annu. Rev. Physiol. 1994; 56, 47-67 (1994)

5. Warner AE: Pulmonary intravascular macrophages. Role in acute lung injury. *Clinics Chest Med.* 17, 125-35 (1996)

6. Staub NC: Ed. The pulmonary intravascular macrophage. Mount Kisco, NY, Futura (1989)

7. Brain JD, Molina RM, & Warner AE: Pulmonary intravascular macrophages. In: Lipscomb MF & Russell SW, eds. Lung Macrophages and Dendritic Cells in Health and Disease. New York, Marcel Dekker, Inc., 131-149 (1997)

8. Van Furth R: Origin and kinetics of monocytes and macrophages. *Semin. Haematol.* 7, 125-141 (1970)

9. Lasser A: The mononuclear phagocyte system. *Hum. Pathol.* 14, 108-126 (1983)

10. Buchanan JW & Wagner HN: Regional phagocytosis in man. In: Reichard SM & Filkins JP, eds. The reticuloendothelial system: a comprehensive treatise. New York: Plenum Press. 247-270 (1985)

11. Lund CC, Shaw LA, & Drinker CK: Quantitative distribution of particulate material (Manganese dioxide) administered intravenously to the dog, rabbit, guinea pig, rat, chicken and turtle. *J. Exp. Med.* 33, 231-238 (1921)

12. Wislocki GB. On the fate of carbon particles injected into the circulation with especial reference to the lungs. *Am. J. Anat.* 32, 423-445 (1924)

13. Schneeberger-Keeley EE & Burger EJ. Intravascular macrophages in cat lungs after open chest ventilation. *Lab. Invest.* 22, 361-369 (1970)

14. Rybicka K, Daly BDT, Migliore JJ, & Norman JC. Ultrastructure of pulmonary alveoli of the calf. *Am. J. Vet. Res.* 35, 213-222 (1974)

15. Crocker SH, Lowery BD, Eddy DO, Wismar BL, & Beusching WJ. Pulmonary clearance of blood-borne bacteria. *Surg. Gynecol. Obstet.* 153, 845-851 (1981)

16. Warner AE, Barry BE, & Brain JD. Pulmonary intravascular macrophages in sheep: morphology and function of a novel constituent of the mononuclear phagocyte system. *Lab. Invest.* 55, 276-288 (1986)

17. Warner AE & Brain JD. Intravascular pulmonary macrophages: a novel cell removes particles from blood. Am. J. Physiol. 250 (Regulatory Integrative Comp. Physiol. 19), R728-R732 (1986)

18. Wheeldon EB & Hansen-Flaschen JH. Intravascular macrophages in the sheep lung. *J. Leuk. Biol.* 40, 657-661 (1986)

19. Miyamoto K. Comparative hemodynamics and lymph dynamic reaction to particles. In: Staub, NC, eds. The pulmonary intravascular macrophage. Mount Kisco, NY: Futura, 59-78 (1989)

20. Brain JD. Lung macrophages: How many kinds are there? What do they do? *Am. Rev. Respir. Dis.* 137, 507-509 (1988)

21. Shellito J, Esparza C, & Armstrong C. Maintenance of the normal rat alveoler macrophage cell population. the roles of monocyte influx and alveolar macrophage proliferation *in situ*. *Am. Rev. Respir. Dis.* 135, 78-82 (1987)

22. Winkler GC & Cheville NF. The neonatal porcine lung: ultrastructural morphology and postnatal development of the terminal airways and alveolar region. *Anat. Rec.* 210, 303-313 (1984)

23. Morton D & Bertram TA: Isolation and preliminary *in vitro* characterization of the porcine pulmonary intravascular macrophage. *J. Leuk. Biol.* 43, 403-410 (1988)

24. Fowler AA, Carey PD, Walsh CJ, Sessler CN, Mumaw VR, Bechard DE, Leeper-Woodford SK, Fisher BJ, Blocher CR, Byrne TK, & Sugerman HJ: *In situ* pulmonary vascular perfusion for improved recovery of pulmonary intravascular macrophages. *Microvasc. Res.* 41, 328-344 (1991)

25. Rogers RA, Tasat DR, Warner AE, & Brain JD: Quantitative recovery of pulmonary intravascular macrophages from sheep lungs. *J. Leuk. Biol.* 56, 692-701 (1994)

26. Atwal OS, Minhas KJ, Ferenczy BG, Jassal DS, Milton D, & Mahadevappa VG: Morphology of pulmonary intravascular macrophages in ruminants: ultrastructural and cytochemical behavior of dense surface coat. *Am. J. Anat.* 186, 285-299 (1989)

27. Singh B, Atwal OS, & Minhas KJ: Ultracytochemical study of multiple dose effect of monastral blue uptake by equine pulmonary intravascular macrophages (PIMs). *J. Submicrosc. Cytol. Pathol.* 26, 235-243 (1994)

28. Singh B, Atwal OS, & Minhas KJ: Surface coat of sheep pulmonary intravascular macrophages is reconstituted following brefeldin A-mediated endocytosis. *J. Submicrosc. Cytol. Pathol.* 27, 235-249 (1995)

29. Atwal O & Saldanha KA: Erythrophagocytosis in alveolar capillaries of goat lung: ultrastructural properties of blood monocytes. *Acta Anat.* 124, 245-254 (1985)

30. Winkler GC & Cheville NF: Postnatal colonization of porcine lung capillaries by intravascular macrophages: an ultrastructural, morphometric analysis. *Microvasc. Res.* 33, 224-232 (1987)

31. Niehaus GD: Role in systemic host defense. In:The pulmonary intravascular macrophage. Ed: Staub NC. Mount Kisco, NY: Futura. 39-58 (1989)

32. Dehring DJ & Wismar BL: Intravascular macrophages in pulmonary capillaries of humans. *Am. Rev. Respir. Dis.* 139, 1027-1029 (1989)

33. Niehaus GD, Saba TM, Edmonds RH, & Dillon BC: Leukocyte involvement in pulmonary localization of blood-borne microparticulates: relationship to altered lung fluid balance. *Circ. Shock* 12, 95-105 (1984)

34. Brain JD, Warner AE, Molina RM, & DeCamp MM: Pulmonary intravascular macrophages are an important part of the mononuclear phagocyte system in ruminants and cats. *Am. Rev. Respir. Dis.* 137, 147A (1988)

35. Miyamoto K, Schultz E, Heath T, Mitchell M, Albertine K, & Staub NC: Pulmonary intravascular macrophages and hemodynamic effects of liposomes in sheep. *J. Appl. Physiol.* 64, 1143-1152 (1988)

36. Longworth KE, Steffey EP, Woliner M, Tyler WS, & Staub NC: Comparative physiology of pulmonary intravascular macrophages. *FASEB J.*; 6, A1242 (1992)

37. Staub NC, Nicolaysen A, Nicolaysen G, Bjertnaes L, Olafsen K, & Folkow L: Pulmonary intravascular macrophages in reindeer. *FASEB J.* 6, A1242 (1992)

38. Longworth KE, Jarvis KA, Tyler WS, Steffey EP, & Staub NC: Pulmonary intravascular macrophages in horses and ponies. *Am. J. Vet. Res.* 55, 382-388 (1994)

39. Longworth KE, McClure DE, Nicolaysen A., Jarvis KA, Smith BL, & Staub NC: Update on the comparative physiology of pulmonary intravascular macrophages. *Physiologist* 37, A92 (1994)

40. Crocker SH, Eddy DO, Obenauf RN, Wismar BL, & Lowery BD: Bacteremia: host-specific lung clearance and pulmonary failure. *J. Trauma* 21, 215-220 (1981)

41. Warner AE, Molina RM, & Brain JD: Uptake of bloodborne bacteria by pulmonary intravascular macrophages and consequent inflammatory responses in sheep. *Am. Rev. Respir. Dis.* 136, 683-690 (1987)

42. DeCamp MM, Warner AE, Molina RM, & Brain JD: Hepatic versus pulmonary uptake of particles injected into the portal circulation of sheep. *Am. Rev. Respir. Dis.* 146, 224-231 (1992)

43. Albertine KH & Staub NC: Vascular tracers alter hemodynamics and airway pressure in anesthetized sheep. *Microvasc. Res.* 32, 279-288 (1986)

44. Enzan K, Wang Y, Schultz E, Stavros F, Mitchell MD, & Staub NC: Pulmonary hemodynamic reaction to foreign blood in goats and rabbits. *J. Appl. Physiol.* 71, 2231-2237 (1991)

45. Nakano T, Miyamoto K, Nishimura A, Aida A, Aoi K, & Kawakami Y: Role of pulmonary intravascular macrophages in anti-platelet serum-induced pulmonary hypertension in sheep. *Resp. Physiol.* 98, 83-99 (1994)

46. Longworth KE, Westgate AM, Grady MK, Westcott JY, & Staub NC: Development of pulmonary intravascular macrophage function in newborn lambs. *J. Appl. Physiol.* 73, 2608-2615 (1992)

47. Wang Y, Enzan K, Schultz E, Mitchell MD, Stavros F, & Staub NC: Pulmonary hypertensive response to rabbit blood components in goats: role of thromboxane. *Am. Rev. Respir. Dis.* 147, 927-933 (1993)

48. Longworth KE, Albertine KH, & Staub NC: Ultrastructural quantification of pulmonary intravascular macrophages in newborn and 2-week-old lambs. *Anat. Rec.* 246, 238-44 (1996)

49. Brigham KL, Bowers RE, & Haynes J: Increased sheep lung vascular permeability caused by Escherichia coli endotoxin. *Circ. Res.* 45, 292-297 (1979)

50. Warner AE, DeCamp MM, Molina RM, & Brain JD: Pulmonary removal of circulating endotoxin results in acute lung injury in sheep. *Lab. Invest.* 59, 219-230 (1988)

51. Clark ES, Gantley B, & Moore JN: Effects of slow infusion of a low dose of endotoxin on systemic haemodynamics in conscious horses. *Equine Vet. J.* 23, 18-21 (1991)

52. Longworth KE, Smith BL, Staub NC, Steffey EP, & Serikov VB: Use of detergent to prevent initial responses to endotoxin in horses. *Am. J. Vet. Res.* 57, 1063-1066 (1996)

53. Kuida H, Gilbert RP, Hinshaw LB, Brunson JG, & Visscher MB: Species differences in effect of gram-negative endotoxin on the circulation. *Am. J. Physiol.* 200, 1197-1202 (1961)

54. Hales CA, Sonne L, Peterson M, Kong D, Miller M, & Watkins WD: Role of thromboxane and prostacyclin in pulmonary vasomotor changes after endotoxin in dogs. *J. Clin. Invest.* 68, 497-505 (1981)

55. Lindsey DC, Emerson TE Jr, Thompson TE, John AE, Duerr ML, Valdez CM, Kuo HS, Bouffard RB, Irwin RG, Canivel D, & Fournel MA: Characterization of an endotoxemic baboon model of metabolic and organ dysfuction. *Circ. Shock* 34, 298-310 (1991)

56. McClure D & Staub NC: Non-human primate responses to endotoxin infusions. *FASEB J.* 9, A887 (1995)

57. Suffredini AF, Fromm RE, Parker MM, Brenner M, Kovacs JA, Wesley RA, & Parrillo JE: The cardiovascular response of normal humans to the administration of endotoxin. *New Engl. J. Med.* 321, 280-287 (1989)

58. Bertram TA, Overby LH, Danilowicz R, Eling TE, & Brody AR: Pulmonary intravascular macrophages metabolize arachidonic acid *in vitro*: comparison with alveolar macrophages. *Am. Rev. Respir. Dis.* 138, 936-944 (1988)

59. Bertram TA, Overby LH, Brody AR, & Eling TE: Comparison of arachidonic acid metabolism by pulmonary intravascular and alveolar macrophages exposed to particulate and soluble stimuli. *Lab. Invest.* 61, 457-466 (1989)

60. Chitko-McKown CG, Chapes SK, Brown RE, Phillips RM, McKown RD, & Blecha F: Porcine alveolar and pulmonary intravascular macrophages: comparison of immune functions. *J. Leuk. Biol.* ; 50, 364-372 (1991)

61. Chitko-McKown CG, Reddy DN, Chapes SK, McKown RD, & Blecha F: Immunological characterization of pulmonary intravascular macrophages. *Reg. Immunol.* 4, 236-44 (1992)

62. Dehring DJ, Fader RC, Traber LD, & Traber DL: Cardiopulmonary *changes occuring with pulmonary intravascular clearance of live bacteria in sheep.* Circ. Shock 29, 245-256 (1989)

63. Bertram TA: Intravascular macrophages in lungs of pigs infected with Haemophilus pleuropneumoniae. *Vet. Pathol.* 23, 681-691 (1986)

64. Whiteley LO, Maheswaren SK, Weiss DJ, & Ames TR: Alterations in pulmonary morphology and peripheral coagulation profiles caused by intratracheal inoculation of live and ultraviolet light-killed Pasteurella haemolytica A1 in calves. *Vet. Pathol.* 28, 275-285 (1991)

65. Sierra MA, Carrasco L, Gomez-Villamandos JC, Martin de las Mulas J, & Mendez AJA: Pulmonary intravascular macrophages in lungs of pigs inoculated with African swine fever virus of differing virulence. *J. Comp. Pathol.* 102, 323-34 (1990)

66. Carrasco L, Gomez-Villamandos JC, Mozos E, Méndez A, & Jover A: Kupffer cells and PIMs in acute experimental African swine fever. *Histol. Histopath.* 7, 421-425 (1992)

67. Leifsson PS, Basse A, Jensen HE, Bloch B, & Aalbaek B: Pulmonary intravascular macrophages in the pathogenesis of bovine pulmonary lesions caused by Actinomyces pyogenes. *J. Comp. Pathol.* 112, 197-206 (1995)

68. Tsubouchi T, English D, & Doerschuk CM: Monocyte accumulation in the lung after chronic endotoxemia in rabbits. *Am. Rev. Respir. Dis.* 143, A329 (1991)