Pulmonary intravascular macrophages: their contribution to the mononuclear phagocyte system in 13 species

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Brain, Joseph D., Ramon M. Molina, Malcolm M. DeCamp, and Angeline E. Warner. Pulmonary intravascular macrophages: their contribution to the mononuclear phagocyte system in 13 species. Am. J. Physiol. 276 (Lung Cell. Mol. Physiol. 20): L146–L154, 1999.—The organ uptake of intravenously injected particles was examined in 13 species. All animals were injected intravenously with $^{198}$Au colloid and magnetic iron oxide particles. Vascular clearance kinetics of $^{198}$Au colloid ranged from 17 to 60% in sheep, calves, pigs, and cats but was <1.1% in monkeys, hyraxes, rabbits, guinea pigs, rats, mice, and chickens. For iron oxide particles, pulmonary uptake ranged from 80 to 99% in sheep, calves, pigs, goats, and cats and 15 to 18% in hamsters, hyraxes, and monkeys and was <10% in rabbits, chicken, mouse, rats, and guinea pigs. In all species, the bulk of the remainder of particle uptake was in the liver. Pulmonary intravascular macrophages are the cellular site of lung uptake in calves, cats, pigs, goats, and sheep, whereas monocytes and neutrophils predominate in other species. Kupffer cells were the site of uptake in the liver. Our data show marked species differences in the fate of circulating particles; ruminants, pigs, and cats have extensive pulmonary localization due to phagocytosis by pulmonary intravascular macrophages.

Pulmonary intravascular macrophages (PIMs) are large capillaries are responsible for particle uptake. These pulmonary intravascular macrophages (PIMs) are large (20- to 80-μm-diameter), mature macrophages that are bound to the pulmonary capillary endothelium. PIMs have characteristic morphological features of differentiated macrophages, such as irregular shape, an indented nucleus, lysosomal granules, pseudopods, phagosomes and phagolysosomes, tubular micropinocytosis vermiciforms structures, and a fuzzy glycocalyx (24, 31). Atwal et al. (1) described in detail the electron-dense surface coat of PIMs that appears to be primarily lipoprotein in nature.

PIMs also form membrane-adhesive complexes with underlying endothelial cells. These adhesions have an intercellular separation of 12–15 nm and electron-dense material present both in the intercellular space and subjacent to the plasma membrane of both cells (24, 30). These adhesion complexes most closely resemble adherent or intermediate junctions of epithelia, which have a 20-nm interspace and some associated cytoplasmic filaments. These adhesive complexes are believed to be important in anchoring macrophages to the microvasculature. Their presence is a useful criterion for distinguishing PIMs from adherent monocytes. PIMs attach preferentially to the thick portion of the air-blood barrier (29, 31), thus perhaps minimizing any potential interference with gas exchange at the air-blood barrier. This may be important because ~15 m$^2$ of the sheep lung capillary endothelial surface is covered with PIMs (24).

There is functional evidence consistent with the existence of PIMs from particle uptake studies (9, 17) carried out more than 70 years ago. More recently, other investigators (2, 16, 31) have demonstrated the existence of PIMs. However, inevitably, different investigators have used a variety of tracer particles and protocols to study removal of particles from the circulation and to demonstrate the existence of PIMs. In this paper, we describe a systematic comparative survey of 13 species with respect to the clearance kinetics and organ distribution of intravenously injected $^{198}$Au colloid and magnetic iron oxide particles. Radioactive $^{198}$Au colloid was used to determine clearance kinetics and organ uptake. Iron oxide particles were also used to identify the cell types responsible for organ uptake of particles because these particles are visible by light and electron microscopy.

MATERIALS AND METHODS

Animals. The characteristics of the animals used in this study are summarized in Table 1. Four healthy adult animals...
of each of the 13 species (two for goats) were obtained from various sources, kept in conventional housing, and given commercial food and water ad libitum. The calves and sheep were studied as part of previously published reports (24, 25). All animals were confirmed free from pneumonia or other significant pulmonary pathology at postmortem examination.

Radiolabeled 198Au colloid: clearance and organ uptake. Four animals of each species except goat and hamster were injected intravenously with 0.01 mg/kg of 198Au colloid (0.001 mg/kg for sheep and calves due to their large size; Amersham, Arlington Heights, IL). The average particle diameter was 20 nm. The colloid concentration was 0.01 mg198Au colloid/ml. It was given through the jugular (sheep, calves, hyrax, guinea pigs, and pigs), tail (rats and mice), wing (chicken), ear (rabbits), or cephalic (cats and macaque monkeys) vein. Duplicate aliquots of blood were taken before and at 1, 3, 5, 10, 15, 20, 25, 30, 40, 50, and 60 min after injection of 198Au. The volume of blood collected was adjusted depending on the estimated total blood volume of each species (from <1% of blood volume in calves to 10% in mice). The rates of vascular clearance were determined by measuring radioactivity in sequential blood samples. The animals were humanely killed, and the heart, left lungs (right lungs were used for morphological examination), liver, spleen, kidney, and samples of bone marrow and skeletal muscle were removed. Whole organs were weighed, and the radioactivity in up to 10 randomly selected samples of each tissue was determined in a Packard model 2001 gamma counter. Radioactivity, expressed as counts per minute per gram of tissue, was determined for each organ or tissue, and the percentage of the total recovered dose localized in each organ was computed. The organ and tissue weights not measured in all species were estimated (as a percentage of total body weight) as follows: bone marrow, 3%; skeletal muscle, 45%; and peripheral blood, 7% (8, 11, 12). The total lung weight was calculated using the estimate that the left lung is 67% of the right lung based on measurements made in normal animals of each species.

Magnetic iron oxide organ uptake. Thirty minutes after 198Au colloid administration, all animals were injected intravenously with a 1 mg/ml suspension of magnetic iron oxide particles [γ-hematite (γ-Fe₂O₃)] in sterile pyrogen-free saline at a uniform dose of 5 mg/kg. The particles were 0.5-μm agglomerates of 0.05- to 0.1-μm subunits generated by combustion of iron pentacarbonyl vapors (22). The suspension was sonicated immediately before injection. Two additional species (goat and hamster) were injected with only the iron oxide particle suspension. The tissue samples collected for 198Au analysis were also examined for iron oxide particle content by magnetometry. Samples were subjected to a brief pulse (10 ms) of a homogeneous 0.1-T (1 T = 1000 gauss) external magnetic field. When the external field was removed, the particles within tissue samples remain magnetized and aligned, generating a measurable remanent field (expressed as μT/g tissue) that is proportional to the amount of magnetic iron oxide present. This remanent magnetic field strength was measured with a fluxgate magnetometer (Forster Instruments, Corapolis, PA). The total recovered dose of iron oxide particles and percentages of recovered doses within each organ were also calculated as for 198Au.

Table 1. Animal characteristics

<table>
<thead>
<tr>
<th>Common Name</th>
<th>Scientific Name</th>
<th>Order</th>
<th>Sex</th>
<th>n</th>
<th>Body Weight, g</th>
<th>Lung Weight, g</th>
<th>Lung Wt/Body Wt, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sheep</td>
<td>Ovis aries</td>
<td>Artiodactyla</td>
<td>F</td>
<td>4</td>
<td>40.200 ± 1.650</td>
<td>586 ± 13.4</td>
<td>1.46 ± 0.05</td>
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<tr>
<td>Calf</td>
<td>Bos taurus</td>
<td>Artiodactyla</td>
<td>M</td>
<td>4</td>
<td>52.800 ± 6.470</td>
<td>813 ± 75.5</td>
<td>1.56 ± 0.06</td>
</tr>
<tr>
<td>Pig</td>
<td>Sus domesticus</td>
<td>Artiodactyla</td>
<td>M</td>
<td>4</td>
<td>8.800 ± 85.4</td>
<td>116 ± 14.2</td>
<td>1.31 ± 0.16</td>
</tr>
<tr>
<td>Goat</td>
<td>Capra domestica</td>
<td>Artiodactyla</td>
<td>M</td>
<td>2</td>
<td>31.000 ± 2.000</td>
<td>422 ± 33</td>
<td>1.37 ± 0.00</td>
</tr>
<tr>
<td>Cat</td>
<td>Felis domestica</td>
<td>Carnivora</td>
<td>M</td>
<td>4</td>
<td>2.300 ± 204</td>
<td>31.3 ± 4.63</td>
<td>0.35 ± 0.13</td>
</tr>
<tr>
<td>Monkey</td>
<td>Macaca fascicularis</td>
<td>Anthropoidea</td>
<td>M</td>
<td>4</td>
<td>3.240 ± 436</td>
<td>15.5 ± 2.31</td>
<td>0.48 ± 0.02</td>
</tr>
<tr>
<td>Hyrax</td>
<td>Procavia capensis</td>
<td>Hyracoidea</td>
<td>M, F</td>
<td>4</td>
<td>2.260 ± 245</td>
<td>11.5 ± 1.79</td>
<td>0.50 ± 0.03</td>
</tr>
<tr>
<td>Hamster</td>
<td>Mesocricetus auratus</td>
<td>Rodentia</td>
<td>M</td>
<td>4</td>
<td>116 ± 4.99</td>
<td>0.7 ± 0.03</td>
<td>0.61 ± 0.03</td>
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<tr>
<td>Rabbit</td>
<td>Oryctolagus cuniculus</td>
<td>Lagomorpha</td>
<td>M</td>
<td>4</td>
<td>2.040 ± 43.5</td>
<td>12.2 ± 2.58</td>
<td>0.61 ± 0.14</td>
</tr>
<tr>
<td>Guinea pig</td>
<td>Cavia porcellus</td>
<td>Rodentia</td>
<td>M</td>
<td>4</td>
<td>459 ± 104</td>
<td>2.62 ± 0.37</td>
<td>0.60 ± 0.30</td>
</tr>
<tr>
<td>Rat</td>
<td>Rattus norvegicus</td>
<td>Rodentia</td>
<td>M</td>
<td>4</td>
<td>352 ± 6.5</td>
<td>1.84 ± 0.13</td>
<td>0.52 ± 0.04</td>
</tr>
<tr>
<td>Mouse</td>
<td>Mus musculus</td>
<td>Rodentia</td>
<td>M</td>
<td>4</td>
<td>314 ± 0.84</td>
<td>0.24 ± 0.01</td>
<td>0.77 ± 0.07</td>
</tr>
<tr>
<td>Chicken</td>
<td>Gallus gallus</td>
<td>Galliformes</td>
<td>M</td>
<td>4</td>
<td>935 ± 78.9</td>
<td>6.32 ± 0.58</td>
<td>0.68 ± 0.06</td>
</tr>
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</table>

Data are means ± SE. n = no. of animals. Lung wt/body wt, lung weight-to-body weight ratio; monkey, cynomologous macaque monkey; rat, Sprague-Dawley rats; mouse, BALB/c mice.
RESULTS

Clearance kinetics of intravenously injected radioactive $^{198}$Au colloid. The clearance kinetics of intravenously injected $^{198}$Au colloid particles from the blood in the 11 species studied were similar (Fig. 1A). The fact that the individual curves are indistinguishable demonstrates the similarity in rates of vascular clearance of $^{198}$Au colloid. In every species studied, the blood was cleared of gold particles very rapidly during the first 5 min. The mean clearance half-times did not differ significantly and ranged from 1.04 (calves) to 2.14 (hyraxes) min (Fig. 1B). After 10 min, >90% of the $^{198}$Au had been cleared in all species. The small remaining fraction of $^{198}$Au was cleared more slowly over the next hour. At 60 min, essentially 100% of the injected $^{198}$Au had disappeared from the circulating blood.

Organ distribution of recovered $^{198}$Au colloid. The distribution of recovered $^{198}$Au from the lungs and liver of 11 species 1 h postinjection is shown in Fig. 2A. In all species, there was detectable pulmonary uptake of $^{198}$Au colloid particles. Ninety percent or more of the recovered radioactivity was found in the lungs of the monkey, hyrax, rabbit, guinea pig, rat, mouse, and chicken, and minimal gold uptake was detected in the lungs. On the other hand, moderate to substantial pulmonary uptake was observed in the cat, pig, calf, and sheep (17–60%). In all species studied, the lungs and the liver together accounted for 89.1 (rat) to 96.6% (chicken) of the total radioactivity recovered from the carcass. Nearly all of the injected dose of $^{198}$Au colloid was recovered from each animal. The percentage of injected dose recovered from the entire carcass ranged from 94 to 100%.

Organ distribution of recovered magnetic iron oxide. The distribution of recovered magnetic iron oxide in the lungs and livers of the 13 species studied is shown in Fig. 2B. In the sheep and calf, the lungs virtually removed 100% of the recovered iron oxide. The pig had 95.6% lung uptake, whereas the goat and cat showed 91.9 and 84.3% lung uptake, respectively. The monkey had a moderate pulmonary uptake of 17.8%. Common laboratory species such as the rat and mouse had considerably less lung uptake (<5%) and exhibited predominant removal by the liver. Hepatic uptake also dominated in the hamster, rabbit, and guinea pig. As was true for $^{198}$Au colloid, the liver and lungs together accounted for at least 83% (mouse) of uptake, whereas the other organs made a quantitatively less important contribution. The patterns of iron oxide distribution in various organs or tissues examined in the cat, monkey, and guinea pig are shown in Fig. 2, C–E. As shown, the spleen, bone marrow, and skeletal muscle were usually the other sites of detectable activity. Similar data were obtained from the other species.

Cells responsible for lung uptake. Iron oxide particles were rarely detectable in a light-microscopic section of lungs from animal species with <5% pulmonary uptake, e.g., rat (Fig. 3A). In contrast, those species with predominantly lung uptake, e.g., sheep, had abundant particles visible in lung sections (Fig. 3B).

In all the species examined, pulmonary localization of iron oxide particles was due to phagocytosis by PIMs or by circulating and adherent leukocytes such as monocytes and neutrophils. No particles were observed free in the plasma or as aggregates blocking vessels. The cells responsible for lung uptake of iron oxide particles in the sheep, calf, pig, goat, and cat were exclusively PIMs. A typical PIM observed in a cat lung after iron oxide phagocytosis is shown in Fig. 4A. The cell contains numerous ingested iron oxide particles as well as additional phagocytic vacuoles. It has an irregu-
lar outline and, in some regions, is closely applied to the underlying capillary endothelium. It has the characteristics of a fully mature mononuclear phagocyte and thus can be easily distinguished from circulating monocytes (Fig. 4B). Characteristic adhesion complexes were seen between PIMs and subjacent endothelial cells in the sheep, calf, goat, pig, and cat (Fig. 4A, inset).

In other species where pulmonary uptake was less dominant, circulating monocytes (Fig. 4B) and neutrophils (Fig. 4C) accounted for the lung retention. The
The presence of PIMs in the lungs of sheep, calves, goats, pigs, and cats is not triggered by injection of the tracer particles because sheep (24) and pigs (31) showed PIMs with characteristic ultrastructure even without prior injection of any tracer particle. A characteristic PIM from a normal un.injected calf is shown in Fig. 4E.

Cells responsible for pulmonary uptake. Particles found in the lungs were taken up by Kupffer cells. In species where hepatic uptake dominated, sections containing iron oxide particles within Kupffer cells were commonly observed (Fig. 5A). A typical Kupffer cell from a guinea pig with phagocytosed iron oxide particles is shown in Fig. 6. The amount of localized iron oxide particles in the liver of animal species with PIMs was so low that sections showing any particles were rarely seen (Fig. 5B).

DISCUSSION

Clearance of particles from the blood. The half-times for clearance of a nonsaturating dose of very small (20-nm) $^{198}$Au colloid particles from the circulation were not different among the species tested (sheep, calf, pig, cat, monkey, hyrax, rabbit, guinea pig, rat, mouse, and chicken). Thus the presence or absence of a dominant pulmonary compartment in the MPS does not alter the clearance rates of injected $^{198}$Au colloid, only the organ distribution of cleared particles.

Attempts to quantitate vascular clearance of iron oxide particles were abandoned because the clearance was too fast to accurately measure. In the sheep and calves, it occurred during the first pass through the lungs, and no particles were detected in blood samples at 1 min postinjection. The more rapid clearance and the greater likelihood of first-pass clearance of iron oxide particles compared with $^{198}$Au colloid is likely due to their larger particle size, which increases the likelihood of particle-phagocyte receptor interaction as particles pass through the pulmonary circulation. A second possible reason is the larger number of gold particles due to their much smaller size. The relatively longer circulation time (i.e., less complete first-pass clearance) of smaller and more plentiful $^{198}$Au colloid particles may also explain the greater liver uptake of $^{198}$Au colloid than of iron oxide in calves, pigs, and cats despite the presence of PIMs (Fig. 2A). In these species, iron oxide particles never reach the liver when given intravenously because of pulmonary uptake.

Pulmonary versus hepatic uptake. The quantitative measurement of organ uptake of intravenously injected particles was used as a functional assay for the presence of phagocytic cells lining endothelial cells in various organs. More than 80% of the iron oxide particles rapidly removed from the blood were found within the lungs of sheep, calves, pigs, goats, and cats. It has been previously shown (5) that if iron oxide particles are presented first to the liver in the sheep, they are efficiently removed by Kupffer cells. Thus the lack of iron oxide in the liver of these species reflects the effectiveness of PIMs, not a dysfunction of hepatic macrophages. The hepatic uptake of radioactive $^{198}$Au colloid in species with PIMs likely reflects the fact that many of these smaller particles escape initial clearance.
during pulmonary circulation. For both particles, the lungs and liver accounted for the bulk of their removal from the blood in all species. On the basis of other studies in our laboratory, the distribution patterns of the same dose of intravenously injected iron oxide were similar regardless of prior $^{198}$Au colloid administration. This was true in rats (without PIMs) (27) and sheep (with PIMs) (unpublished data). The differences in pulmonary uptake of both $^{198}$Au colloid and iron oxide particles among animal species with PIMs may be due to variation in their PIM volume densities or in the phagocytic activities of PIMs. A comparative morphometric estimation of PIM numbers or volumes or rates of phagocytosis by isolated PIMs from different species is necessary to elucidate this observation.

Function of PIMs. It can be assumed that natural endogenous targets similar in size and number to $^{198}$Au colloid and iron oxide would be removed from the blood
PIMs have a role in the clearance of circulating bacteria, an important host defense function of the MPS. This function is usually ascribed to hepatic macrophages. Intravascular infusion of bacteria into pigs has been used as a model of septicemia, and investigators measured pulmonary clearance of bacteria by quantitative culture of blood samples drawn simultaneously from the aorta and pulmonary artery. Efficient clearance of Escherichia coli, Pseudomonas aeruginosa, and Staphylococcus aureus during pulmonary circulation was demonstrated (6). In sheep, Warner et al. (28) showed >90% uptake of recovered intravenously injected P. aeruginosa in the lungs. A subsequent study (26) documented similar pulmonary uptake of injected radiolabeled endotoxin in sheep.

Thus the results seen here would be anticipated in a general way for a wide variety of targets given experimentally by intravenous injection or entering naturally via lymphatics or venous drainage. However, PIM-containing species can have effective hepatic clearance. Thus, when bacteria enter through the gut and are first presented to the liver through the portal circulation, liver uptake will be far greater (5). Interestingly, endotoxin is still retained to a greater extent in the lungs in the sheep even when given through the portal vein.

by PIMs in a pattern resembling that seen here. During an inflammatory response, porcine PIMs were shown to engulf neutrophils and fibrin after Haemophilus pleuropneumoniae-induced pneumonia (3). Several investigators have noted erythrophagocytosis by PIMs in, for example, normal goat (2), sheep (28), and cat (21). These findings suggest that PIMs may play a role in normal removal of effete erythrocytes or fibrin and cell debris during inflammation.

Fig. 5. Overview of liver after injection of iron oxide particles. A: section of rat liver showing numerous dense iron oxide particles (arrows; glycol methacrylate; magnification, ×7,440). B: in sheep, no iron oxide particles were observed (glycol methacrylate; magnification, ×7,200).

Fig. 6. Hepatic macrophage responsible for liver uptake. Guinea pig liver shows a large Kupffer cell (K) with crystalline electron-dense iron oxide particles (arrows) within phagosomes. S, sinusoid; H, hepatocyte. Bar, 1 µm.
Perhaps this is due to its slower clearance and smaller macromolecular aggregate size.

Is pulmonary uptake beneficial to the host? There are circumstances when PIMs may benefit the host. Their capacity to remove cell debris, bacteria, immune complexes, and endotoxin from the circulation may protect critical downstream organs such as the brain or heart. However, pathogen phagocytosis by PIMs can also produce an inflammatory response in the microvasculature and elicit acute lung injury. Once activated, PIMs may influence events in the pulmonary microvasculature through release of inflammatory mediators.

Acute respiratory distress syndrome, a clinical entity seen frequently in humans after major trauma, burns, or sepsis (14), is frequently associated with gram-negative sepsis or endotoxia. Many animal species, such as rats and rabbits, are quite resistant to endotoxin-induced acute lung injury, but three species that have been commonly used as animal models for this process are sheep, goats, and pigs (10, 18, 20). Their increased sensitivity to endotoxin is likely due to the presence of abundant PIMs.

Dehring and Wismar (7) reported large mononuclear cells with phagocytic vacuoles in clinical human lung biopsy specimens, but their number was not estimated. A morphometric study of human lung (32) did not show presence of abundant PIMs.

process are sheep, goats, and pigs (10, 18, 20). Their
produce an inflammatory response in the microvascula-
text of particulate material (manganese dioxide) administered intravenously to the dog, rabbit, guinea pig, rat, chicken, and turtle. J. Exp. Med. 33: 231–238, 1921.


References


