Reticuloendothelial Clearance of Blood-borne Particulates

Relevance to Experimental Lung Microembolization and Vascular Injury

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The rapid increase in sheep lung vascular permeability observed during *Pseudomonas aeruginosa* bacteremia may be due to embolization of the pulmonary microvasculature by blood-borne particulates. Since alterations in lung microvascular permeability during mild septicemia in sheep may reflect inefficient RES phagocytic clearance of bacteria as well as products of bacterial induced intravascular coagulation, the opsonic and phagocytic aspects of RES function in sheep (30–50 kg) were compared to other species. RES function was evaluated by both the clearance and relative organ uptake of gelatinized I^{131} RE test lipid emulsion and gelatinized colloidal carbon. Immunoreactive opsonic α_2SB glycoprotein levels were determined by electroimmunoassay. The phagocytic index for RES clearance of the gelatinized (500 mg/kg) test lipid in sheep was 0.019 ± 0.002 corresponding to a half-time of 16.65 ± 1.74 minutes. With colloidal carbon (64 mg/kg), the phagocytic index in sheep was 0.080 ± 0.026, corresponding to a half-time of 6.16 ± 1.99 minutes. The per cent of injected lipid emulsion (%ID) in major RE organs, on a total organ basis (TO), was: liver = 15.69 ± 1.65%; spleen = 2.09 ± 0.78%. Localization in the lung = 31.39 ± 6.2%. The per cent of carbon localized in major RE organs (%ID/TO) was: liver = 21.37 ± 1.9%; spleen = 1.95 ± 0.55%. Localization in the lung = 32.70 ± 4.55%. In contrast, clearance and organ distribution of the blood-borne test microparticles in rats and dogs at the same relative challenging dose revealed a much more intense and rapid liver and spleen RES uptake with minimal lung localization (1–2%). Immunoreactive opsonic protein concentrations varied greatly with species and directly correlated with efficiency of RES function. Levels observed were: dog = 1285 ± 135 μg/ml; mouse = 1077 ± 67 μg/ml; rat = 400 ± 31 μg/ml; human = 297 ± 10 μg/ml; and sheep = 184 ± 13 μg/ml. After intravenous particulate challenge, circulating immunoreactive opsonic protein in the sheep was depleted ($p < 0.05$) rapidly with partial recovery at 24 hours and mild rebound hyperopsonemia at 48 hours. This pattern is in contrast to the rapid restoration seen in dog and rat within three to six hours postchallenge. Thus, in sheep, the extensive pulmonary localization of blood-borne microparticles appears related to inefficient RES clearance function mediated by a relative deficiency of circulating opsonic protein (plasma fibronectin).

NUMEROUS CLINICAL STUDIES have demonstrated a progressive deterioration in pulmonary vascular integrity leading to pulmonary insufficiency, especially when sepsis is superimposed on burn and trauma.[4,33] Recent data suggest that the pulmonary alterations may be due to lung vascular microembolization and injury indirectly related to failure of reticuloendothelial system (RES) clearance of blood-borne microaggregates during sepsis after trauma.[30,33] RE phagocytic cells avidly remove blood-borne foreign or denatured particulates from the circulation with the liver Kupffer cells representing the largest clearance compartment. Depression of RES function has been correlated with decreased resistance to experimental shock and sepsis[14] and an increase in pulmonary localization of blood-borne particulates.[27–29] Such particulate localization in the lung may lead to pulmonary capillary occlusion or generate focal sites of altered hemodynamics or permeability which could contribute to edema with altered organ function.[4]

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Kupffer cell phagocytosis of nonbacterial particulate such as collagenous debris and fibrinogen/fibrin complexes is mediated by opsonic α_2 surface binding (SB) glycoprotein.[5-7] Opsonic glycoprotein is identical to plasma fibronectin or cold-insoluble globulin (CIg).[5] Plasma fibronectin exhibits a high affinity for collagen and gelatin,[6,12] as well as fibrinogen–fibrin complexes which are cleared by the RES.[34] Humoral control of Kupffer cell phagocytic function as modulated by plasma opsonin is further supported by the observation that opsonic deficiency and RES phagocytic depression following surgical trauma can be reversed by infusion of purified opsonic α_2SB glycoprotein.[29] Indeed, reversal of opsonic deficiency in septic surgical, trauma and burn patients with pulmonary insufficiency leads to a marked improvement of host defense and pulmonary function.[30,33]

Endotoxemia and sepsis can elevate the concentration of circulating particulates by inducing disseminated intravascular coagulation.[2,38] RE clearance and hepatic uptake are depressed following disseminated intravascular coagulation (DIC)[15] and the hemodynamic pressures in the lungs are elevated.[20] Dogs with thrombin-induced DIC manifest gas exchange abnormalities and exhibit pulmonary edema[20,32] if DIC develops during opsonic deficiency and RES phagocytic blockade,[32] but not if DIC is induced in the presence of normal RE function.

Pulmonary fluid flux is governed by the interaction of hydrostatic and oncotic pressures between lung capillaries and the surrounding interstitial space. A net flow into the extravascular space occurs when either capillary hydrostatic pressure or interstitial space oncotic pressure increases. Edema would be expected when the net transvascular fluid movement into the interstitial space exceeds the pulmonary lymphatic drainage capacity.[35] Development of the sheep lung lymph preparation[36] has provided an experimental animal model in which the hydrostatic and oncotic aspects of edema could be evaluated. This valuable animal model has been utilized to investigate pulmonary vascular integrity following hemorrhage,[22] burn,[11] sepsis[8] and endotoxin infusion.[26]

An observation of direct relevance to the present study is that sheep receiving intravenous infusion of 10^5–10^{10} *Pseudomonas aeruginosa* exhibit an acute elevation in pulmonary vascular permeability even in the absence of sustained increases in pulmonary arterial and wedge pressures.[8] The basis for such extensive alteration in pulmonary function in sheep with minimal bacterial challenge is unknown, but inefficient reticuloendothelial function might be a factor, since the RES provides the major acute defense against systemic bacteremia.[25] In addition, bacteremia stimulates intravascular coagulation[2,38] and the byproducts of disseminated intravascular coagulation, which can alter lung function, are cleared from circulation by the reticuloendothelial system.[34] Inhibition in hepatosplenic phagocytic function results in depressed clearance of fibrinogen: fibrin complexes.[34] Also, decreased Kupffer cell uptake of bacteria results in elevation of pulmonary localization of the micro-organisms.[17]

These observations collectively suggest that the functional status of the reticuloendothelial system may modulate the degree of pulmonary localization and associated injury due to blood-borne particulates, especially during sepsis. We postulate that the sheep RES may be incapable of efficiently clearing bacteria and or particulates during sepsis. This could contribute to pulmonary microvascular embolization and altered pulmonary vascular integrity. To evaluate this concept, the present investigation defined the humoral and cellular parameters of systemic reticuloendothelial function in the sheep in comparison to the reticuloendothelial system in laboratory animals (*i.e.*, rat, dog) which are known to manifest apparent resistance to organ dysfunction during experimental bacteremia. A comparative study of immunoreactive opsonic α_2SB glycoprotein (plasma fibronectin) levels in mouse, rat, dog, sheep and man was performed in order to place perspective on this humoral parameter.

Methods

Serum and Plasma Collection

Adult sheep (20–55 kg) used in this study were provided food and water *ad libitum*. In sheep, blood samples were collected via catheters positioned in either a carotid artery or a jugular vein. For comparative species studies, male Sprague-Dawley rats (250–300 g), male C57BL/6 mice (18–24 g), and male mongrel dogs (11–16 kg) were evaluated in the nonfasted state. Human blood was obtained by vena puncture from seven healthy volunteers (18–38 yr) with appropriate informed consent. Blood from all species was allowed to clot for 30–60 minutes at room temperature. Blood was centrifuged at 6,000 *g* for 10 minutes and serum was collected for analysis by electroimmunoassay.

Immunoreactive Opsonic Protein

Serum immunoreactive opsonic α_2SB glycoprotein was determined by electroimmunoassay as described by Blumenstock et al.[5,7] Monospecific antiserum to the isolated sheep, human, rat, mouse and dog opsonin was developed in rabbits as previously described.[5-7] The antigen was isolated by affinity chromatography using a gelatin-sepharose affinity column[6,12,31] supple-

mented with mercaptoethanol. Antiserum to the respective opsonic protein was added to a 1% agarose in barbital buffer solution and the warmed mixture was spread on a 5 × 10 inch glass plate to a thickness of 1.8 mm. Serum, diluted to 10% with distilled water, was placed in wells (10 μl) punched in the agar and subjected to electrophoresis with a LKB multiphore system at 82 volts for 21–22 hours at 15–17 C. The resulting immunoelectrophoretic rockets were stained as previously described[7] and immunoreactive opsonin concentrations determined from a standard curve by double reciprocal plot of opsonic protein concentration (μg) vs rocket height (mm) using standardized serum. Immunoreactive opsonic α_2SB glycoprotein (plasma fibronectin) levels are expressed as μg/ml serum. For all species, plasma concentrations are slightly higher than in serum, but this difference is minimized if blood is allowed to clot at room temperature as opposed to in the cold.

Reticuloendothelial Phagocytic Index

Reticuloendothelial function was evaluated by the colloid clearance technique[3,27] using two separate test colloids. Gelatinized I^{131} RE-test lipid emulsion was prepared at an anhydrous base concentration of 20% in a 0.3% gelatin-supplemented sterile 5% dextrose and water solution. The gelatinized RE-test lipid emulsion (500 mg/kg) was injected intravenously and arterial blood samples were collected at two minute intervals (2–14 minutes) with the animal sacrificed at 15 minutes postinjection for determinations of particle distribution. The concentration of test colloid in each blood aliquot was determined using a 300 sample Auto-Gamma-Scintillation System with a 2″ NaI Crystal (Searle, Des Plaines, IL). The second particulate used to evaluate reticuloendothelial function was colloidal carbon (C11/1431a Pelikan, Gunther-Wagner, Hanover, Germany).[3] The gelatinized colloidal carbon was injected intravenously (64 mg/kg) and 5.0 ml aliquots of arterial blood were collected at two minute intervals up to 14 minutes after injection and analyzed for carbon concentration. The 0.5 ml aliquot of blood was hemolyzed by addition of 4.0 ml of 0.1 M sodium carbonate and carbon concentration was determined by optical density at 650 using a Perkin Elmer 55 spectrophotometer as previously described.[3] The spectrophotometer was zeroed against a blank containing 0.5 ml of blood collected before carbon injection. The half-time for particle clearance from blood was determined from semilogarithmic plots of colloid concentration against time. The phagocytic index (K) was calculated as K = 0.301/half-time in minutes, where 0.301 is the log base 10 of 2. The liver, lung and spleen distribution of both the carbon and the lipid colloid was evaluated at 15 minutes postinjection. The organs were excised, rinsed with cold saline, blotted dry and weighed.[27] To determine colloid content, four representative samples of each organ were either analyzed isotopically to determine lipid localization or spectrophotometrically, to quantitate carbon localization. Sample preparation for spectrophotometric analysis involved boiling each gram of tissue for 90 minutes in 10 ml of 30% Potassium Hydroxide. Variable pigmentation was reduced with five drops of H_2O_2 and then each gram of hydrolyzed tissue was diluted to 100 ml with distilled water. Carbon content was determined by comparing sample absorbance at 570 nm (Perkin Elmer 55 Spectrophotometer) against a standard curve. Localization of either test colloid was expressed as the percentage of the administered test dose per total organ (%ID/TO) and per gram of tissue (%ID/g).

Experimental Depletion of Circulating Opsonic Protein

A heparinized, polyethylene catheter (PE 160) was percutaneously positioned in a jugular vein and sutured in place. Opsonic protein depletion was induced by injecting the gelatin-coated nonradiolabelled RE-test lipid emulsion (500 mg/kg) through the catheter. This approach was utilized due to its effectiveness in inducing opsonic deficiency in the rat[7] and dog.[9] Gelatin is denatured collagen and the opsonic protein (plasma fibronectin) has a high affinity for denatured collagen. Serum samples were collected through the catheter at 0.5, 1, 2, 3, 4, 5, 24 and 48 hours after colloid injection for determination of alterations in circulating immunoreactive opsonic protein in order to test if acute depletion would develop comparable to other species.[7,9]

Experimental Induction of Disseminated Intravascular Coagulation

Five adult sheep (25–55 kg) were anesthetized with sodium pentobarbital (15 mg/kg; Abbott Laboratories, Chicago, IL), paralyzed with pancuronium bromide (35 μg/kg; Organon Inc., West Orange, NJ), intubated and mechanically ventilated (Harvard Apparatus, Millis, MA) at a tidal volume of 12 ml/kg. Throughout the remainder of the study the anesthetics were given as required to maintain a light plain of anesthesia. The animal was placed in an anterior recumbancy and a sampling catheter surgically inserted into the right carotid artery. Control serum and heparinized plasma samples were collected before 5×10^9 *P. aeruginosa* was injected intravenously with a parallel intraperitoneal injection of 5×10^{10} bacteria. Serum and heparinized plasma samples were collected at hourly intervals for the next four hours and analyzed for clottable immuno-

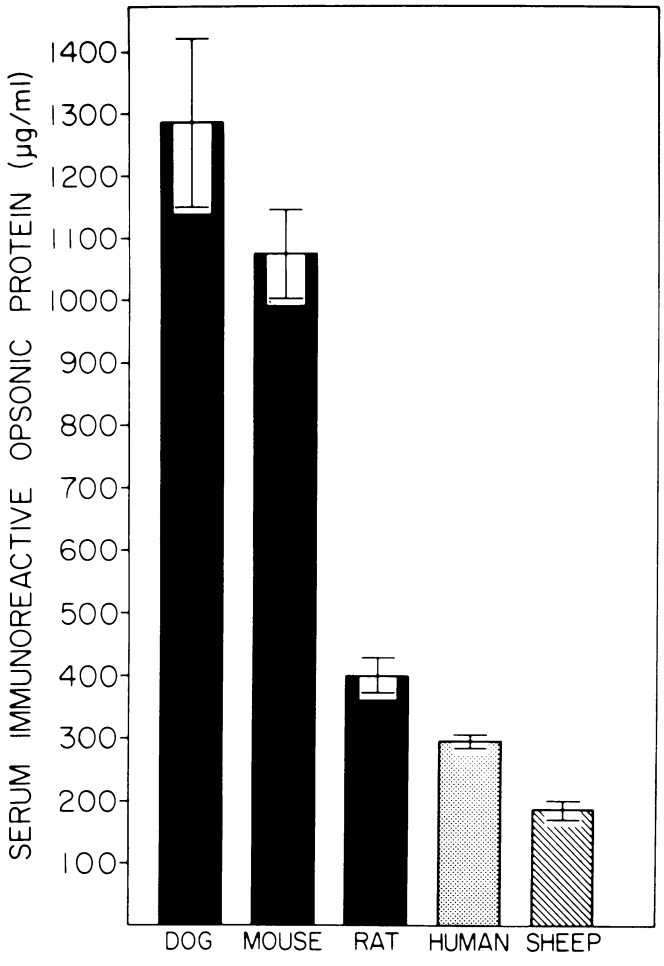

FIG. 1. Comparative immunoreactive opsonic protein levels (μg/ml) in the sheep, human, rat, mouse and dog. The measured serum opsonin concentrations were determined by electroimmunoassay against monospecific antiserum developed against antigen isolated from each species.

reactive fibrinogen and nonclottable immunoreactive fibrin degradation products. A decrease in plasma fibrinogen and an increase in serum fibrin degradation products is indicative of intravascular coagulation.[16]

Immunodiffusion Assay of Fibrinogen and Fibrin Degradation Products

Agarose (Sigma) was dissolved in phosphate buffered saline (PBS), pH 7.2, to a concentration of 2% by careful heating to 100 C while stirring. The 2% agarose was then transferred to a 63 C waterbath, and warmed monospecific antiserum diluted in PBS was mixed with the agarose to yield a final antiserum dilution of 1:64 in 1% agarose (dependent on the titer of the antiserum). The volume of agarose–antiserum solution is then poured onto a clear, warm, level glass plate. Sixteen milliliters of agarose–antiserum solution, poured onto a 8.3 × 10.2 cm glass plate, yields a gel thickness of about 2.0 mm. After the agarose had hardened, 20 wells (3 mm in diameter) were cut in the agarose plate 2 cm apart. Ten microliters of the sample or of purified fibrinogen (standard) were added to each well. The samples were allowed to diffuse for 24 hours at room temperature in a humidified chamber. The plates were washed overnight at 37 C in 0.15 M saline with 2.5–3 L of saline per plate. Prior to staining, the agarose plate was pressed and dried by covering the agarose plate with a wetted filter paper and several centimeters of a tissue-cloth absorbant material. Plates were pressed under a pressure of at least 5 g/cm^2 for 15 minutes. The paper absorbant and filter paper were peeled off the compressed agarose slide and the slides were dried using a warm air hair-dryer. Slides were stained for three minutes in 0.5% Coomassie blue R-250 consisting of 5 g Coomassie Brilliant Blue R-250 in a solution of 450 ml of 95% ethanol and 100 ml of glacial acetic acid. Slides were destained by washing 3–4 times in a solution of 95% ethanol:acetic acid:water (5:2:9) for four minutes per wash, and allowed to dry. The ring diameter was measured with a Finescale comparator and quantitated as milligrams per milliliter by comparison to a standard curve. The standard curve was constructed by analysis of purified fibrinogen of known protein content and was unique for each plate. A plot of log concentration versus ring diameter was linear.[16]

Statistical Analysis of Data

Data were analyzed using the unpaired Student's t-test, with the confidence level placed at 95%. All data were expressed as the mean ± SE of the mean.

Results

Immunoreactive serum opsonic protein levels in the sheep (184.6 ± 13.0 μg/ml serum) were compared (Fig. 1) to opsonin concentration in dog (1285 ± 135.0 μg/ml), mouse (1076.9 ± 66.99 μg/ml), rat (400 ± 31.0 μg/ml) and human (297 ± 10.1 μg/ml), in order to determine if this humoral parameter correlated with the functional RES clearance capacity (Fig. 2). Species with high concentrations of immunoreactive opsonic protein, such as the dog, demonstrate rapid clearance and intense liver Kupffer cell uptake as compared to the slow RE phagocytic function and low opsonin concentrations in the sheep. Indeed, the level in the sheep is even less than that observed in humans who also have a high susceptibility to organ dysfunction, *i.e.*, pulmonary insufficiency during sepsis especially after trauma.

RES phagocytic function in sheep, rat, mouse and dog is compared in Figure 2. Respective gelatinized lipid emulsion (500 mg/kg) clearance half-times of 3.7,

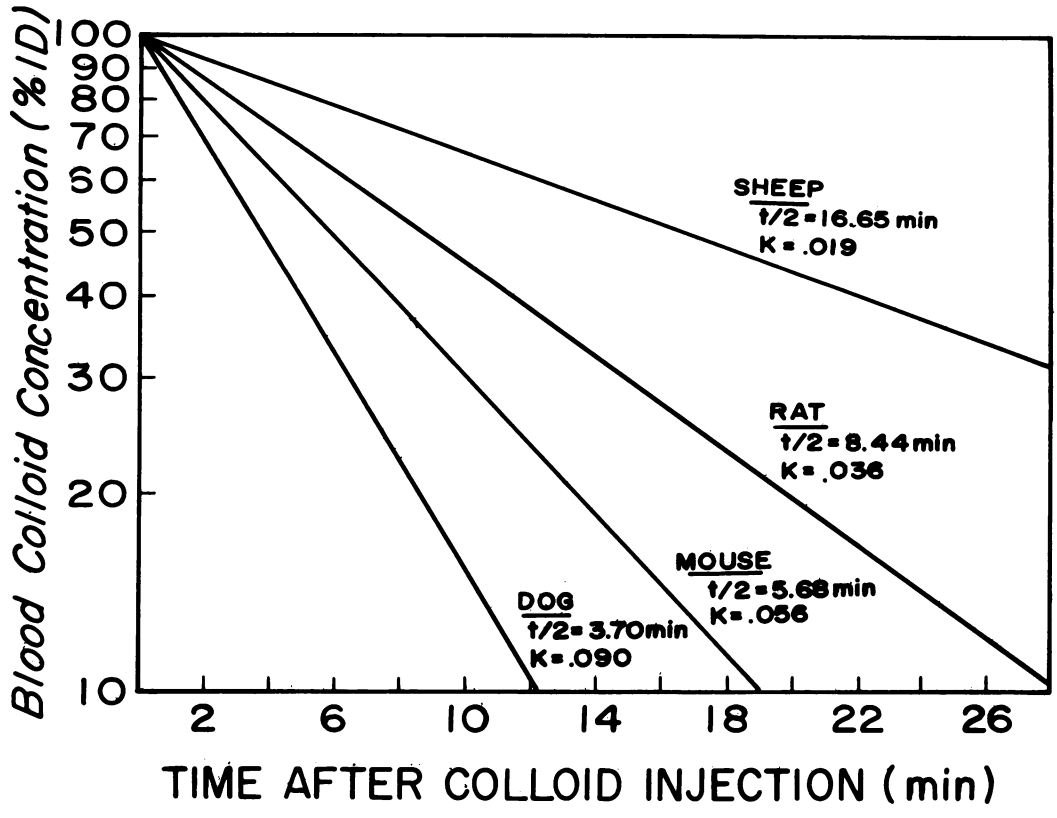

FIG. 2. Comparison of systemic RES phagocytic clearance capacity in the sheep, rat, mouse and dog. Vascular clearance capacity was determined isotopically after intravenous injection of gelatinized I^{131}-labelled RE-test lipid emulsion (50 mg/100 g). Values represent the mean determination.

5.7 and 8.4 minutes were observed in the dog, mouse and rat. The sheep clearance half-time for the gelatinized RE test lipid emulsion was 16.65 ± 1.74 minutes, with a corresponding phagocytic index (K) of 0.019 ± 0.002 (n = 5 sheep).

The relative localization of intravenously injected, gelatinized RE test lipid emulsion (500 mg/kg) in liver, spleen and lungs of the dog, rat and sheep is presented in Figure 3. Hepatic uptake of the blood-borne test microparticles is intense in the dog and rat with the lung in these two species demonstrating localization of the particulate equivalent to only 1–2% of the test dose. In marked contrast, the sheep exhibits much less hepatic RES clearance of the test colloid with a corresponding pronounced elevation ($p < 0.01$) in pulmonary localization amounting to 31.37% of the injected dose.

The comparative pattern of localization of gelatinized colloidal carbon and gelatinized I^{131} labelled RE test lipid by the liver, lung and spleen is graphically presented in Figures 4 and 5. This pattern was evaluated to determine the specificity of the response in terms of the types of colloid. Lung localization of either carbon or lipid exceeds liver and spleen uptake on both a per 100 g and per total organ basis in the sheep. The lung exhibits a similar and extensive localization of both lipid and carbon even though the size (1.0 μ vs 250Å), total dose (500 mg/kg vs 64 mg/kg) and characteristics (metabolizable vs inert) of these particles differ markedly.[3,9] Both particles are, however, dependent on opsonic protein (plasma fibronectin) for efficient RES phagocytosis.[7,27,37]

Since RE organ size is a factor in clearance capacity, the relative liver and spleen size in the varied species investigated was compared (Table 1). Liver and spleen size, expressed as per cent of total body weight in the dog, rat and mouse is more than 100% greater than in the sheep.

To further evaluate the humoral aspects of RES function, serum was collected from the jugular vein before and at specific intervals after intravenous infusion of gelatinized RE test lipid emulsion (Fig. 6). This was done to evaluate the effect of intravenous infusion of colloid on opsonic protein levels in the sheep. At each sampling time, there is a close intra-animal correlation in opsonin concentrations. Immunoreactive opsonin was rapidly depleted by 30 minutes postparticle infusion ($p < 0.05$), exhibited slow restoration during the next 4.5 hours and demonstrated recovery to prechallenge levels by 24 hours.

We have postulated that the reported increase in pulmonary microvascular permeability in the sheep following Pseudomonas bacteremia[8] may reflect inefficient RES phagocytic clearance of products of sepsis induced

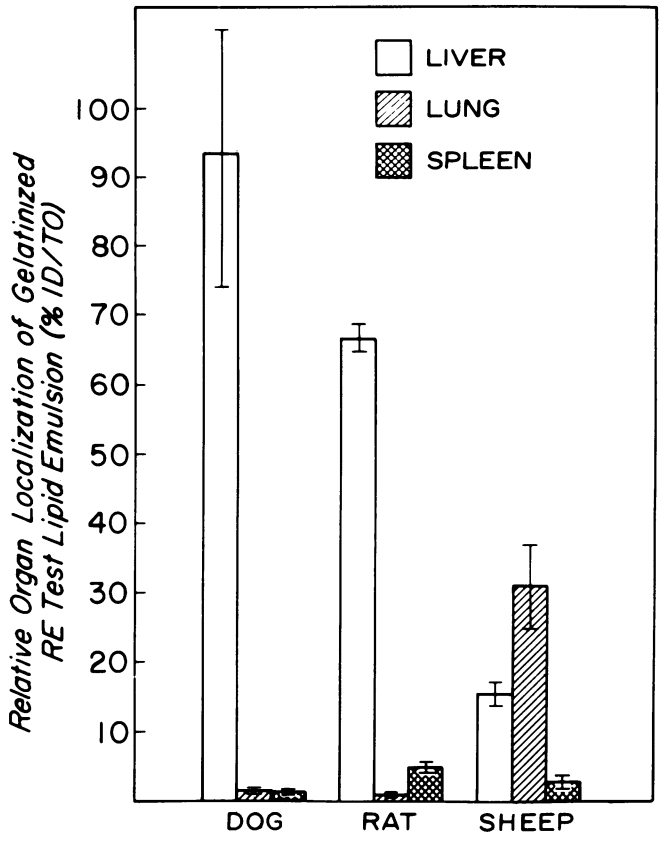

FIG. 3. Liver, spleen and lung distribution of intravenously injected gelatinized RE-test lipid in the sheep, rat and dog. Values represent mean ± SE of the mean are expressed as per cent of injected dose localized per total organ (%ID/TO). Tissue distributions were determined at 15 min postinjection.

intravascular coagulation. That postulate is based on the concept that bacteremia can cause disseminated intravascular coagulation. To test that concept a study was conducted to determine the effect of sepsis on the sheep intravascular coagulation mechanisms. The data in Table 2 support that concept by demonstrating a significant ($p < 0.05$ at four hours postbacterial challenge), progressive, sepsis induced decrease in plasma immunoreactive fibrinogen and an increase in serum immunoreactive fibrin degradation products.

Discussion

The reticuloendothelial system (RES) actively removes foreign and effete particulate matter from the vascular compartment.[27] Investigation of this RES systemic host-defense function has demonstrated a correlation between RE clearance capacity and altered resistance to various forms of experimental shock which include hemorrhage, endotoxemia, trauma and bowel ischemia.[1,14,28,37] Due to its significant vascular perfusion and extensive reticuloendothelial cell population (Kupffer cells), the liver provides a major fraction of the systemic phagocytic clearance capacity in mice, rats, dogs and man.[3,7,9,28] Kupffer cell phagocytic removal of nonbacterial blood-borne particulates such as collagenous debris and products of hemostasis correlates with immunoreactive[7,29] and bioassayable[27] opsonic α_2SB glycoprotein. This large molecular weight glycoprotein has a high affinity for and readily opsonizes foreign particulates[7,27] and its deficiency is the basis for RES blockade induced by colloid injection and undoubtedly is related to the altered resistance to shock manifested during RE blockade.

When compared to other species, the sheep exhibits a deficient liver and spleen phagocytic activity and an elevated lung localization of blood-borne RE test particles. This restricted RES clearance capacity in sheep is correlated with low immunoreactive opsonin levels (Fig. 1) and a relatively small liver and spleen (Table 1). On a per cent of total body weight basis, the liver and spleen of the dog, rat and mouse are more than twice as large as measured in the sheep (Table 1). Thus, large fractions of injected RE test particles may localize in the lung both because the insufficient opsonic α_2SB

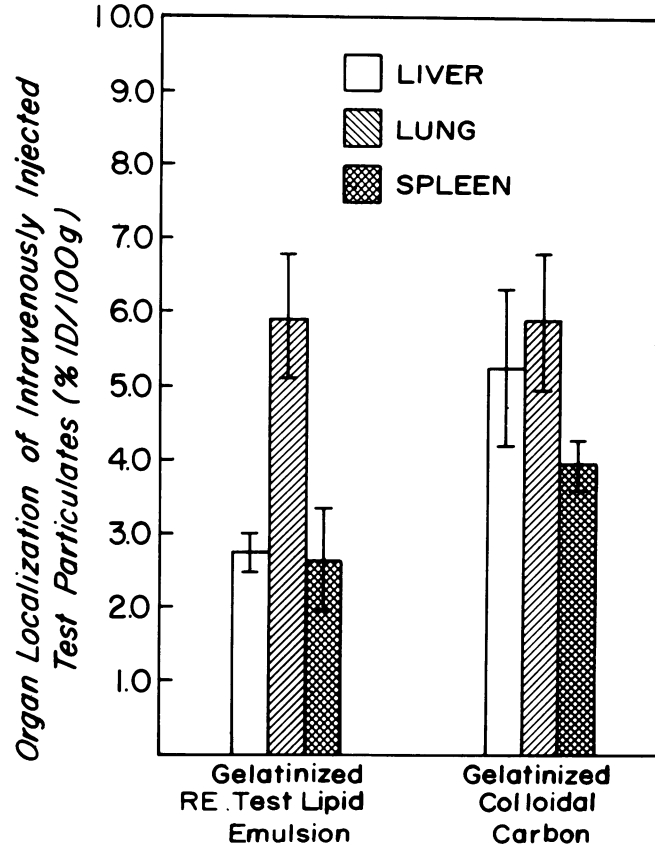

FIG. 4. Comparative liver, spleen and lung distribution of intravenously injected gelatinized colloidal carbon and gelatinized RE-test lipid emulsion, expressed as per cent injected dose per 100 g tissue (%ID/100 g). Each point represents the mean ± SE of the mean with five sheep per group.

glycoprotein concentrations appear to limit RES clearance capacity and because of the relatively small size and minimal phagocytic capacity of the liver and spleen.

The temporal patterns of opsonin levels after colloid injection indicate a 24 hour repletion period is required to return depleted opsonin concentrations to control levels. By comparison, the rat requires only six hours for total restoration of immunoreactive opsonin levels after a similar RE blockade induced by the gelatinized RE test lipid emulsion.[7] The sheep reticuloendothelial system, therefore, has a small liver and spleen; is associated with very low opsonic protein concentrations; and displays a pattern of delayed opsonic restoration after acute depletion by blood-borne particulates. It is possible that these features may make the sheep very susceptible to increased pulmonary microembolism during episodes of disseminated intravascular coagulation or after soft tissue trauma, especially during septicemia. The basis for this response may be the coexistence of severe RES depression during sepsis after trauma as well as elevated blood-borne particulates.[30,33]

Support for this hypothesis is the observation of opsonic deficiency (plasma fibronectin deficiency) in surgical, trauma and burn patients, especially during septicemia and its correlation with both peripheral vascular failure and pulmonary insufficiency.[30,33] In contrast, reversal of opsonic deficiency in such patients by intravenous infusion of cryoprecipitate which is rich in opsonic protein, results in improvement of gas exchange and augmented limb perfusion as well as reactive hyperemia.[30,33] This improved response may be due to augmented RES clearance of particulate material that might otherwise have embolized in the lung, thus leading to a better matching of ventilation and perfusion.

Brigham reports[8] that sheep receiving a 30 minute infusion of 10^5–10^{10} *P. aeruginosa* exhibit immediate but transient chills, fever, leukopenia and hypoxemia. This response is associated with increased pulmonary artery pressure and lymph flow and decreased lymph protein concentration.[8] At three to five hours postinfusion, the vascular pressures and lymph protein concentrations return to normal, while lymph flow increased to a new steady-state three to ten times higher than baseline. All variables return to baseline values in 24–72 hours.[8] The high sensitivity of the sheep to bacteria-induced alterations in pulmonary vascular permeability may be related to an inefficient RES clearance capacity in this animal. Such an inefficient RES clearance capacity may result in altered pulmonary vascular permeability via mechanisms as schematically described in Figure 7. In this concept, the inefficient RES function in the sheep may permit bacteria to remain in circulation for prolonged periods, thus allowing greater stimulation of disseminated intravascular coagulation, either directly or via endotoxin release.

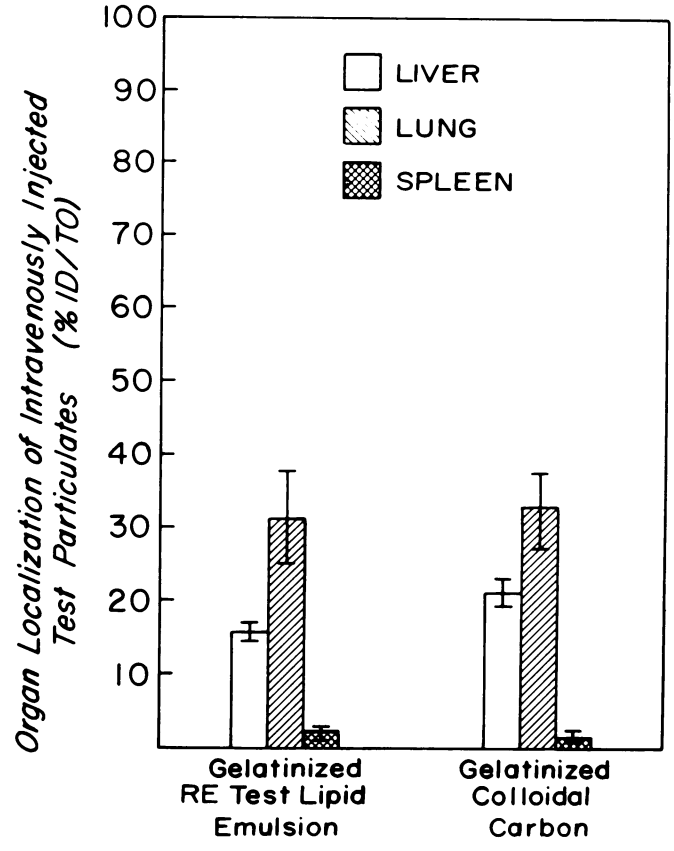

FIG. 5. Comparative liver, spleen and lung distribution of intravenously injected gelatinized colloidal carbon and gelatinized RE-test lipid emulsion, expressed as per cent injected dose per total organ (%ID/TO). Each point represents the mean ± SE of the mean with five sheep in each group.

This hypothesis is supported by our quantitation of serologic parameters which are reflective of intravascular coagulation[16] in sheep after experimental sepsis (Table 2). The depletion of plasma clottable fibrinogen coupled with the elevation of plasma degradation products after *P. aeruginosa* challenge demonstrates directly in the sheep that bacteremia can lead to intra-

TABLE 1. *Interspecies Comparison of Liver and Spleen Weight*

Species	Liver (%BW)	Spleen (%BW)	Liver and Spleen (%BW)
Mouse	5.92	0.32	6.24
Dog (9)	3.36	0.25	3.61
Rat	3.35	0.29	3.64
Human (20)	2.31	0.21	2.52
Sheep	1.42	0.19	1.61

Organ weights are expressed as per cent of total body weight (%BW). Listed values represent means. Standard error of the means for mouse, rat and sheep were less than 10% of the mean but data for dog and human were not available.

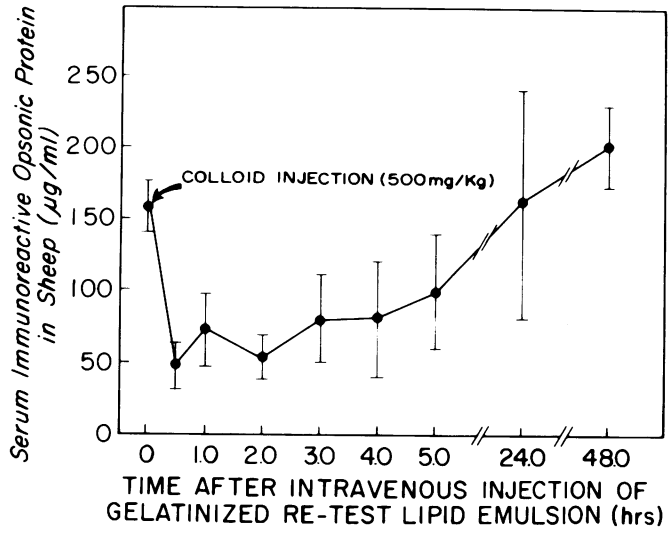

FIG. 6. Temporal pattern of serum opsonic α_2SB glycoprotein concentration during colloid induced RE blockade in sheep. RES blockade was induced by intravenous injection of gelatinized RE-test lipid emulsion (500 mg/kg). Each point represents the mean ± SE of the mean with three sheep studied.

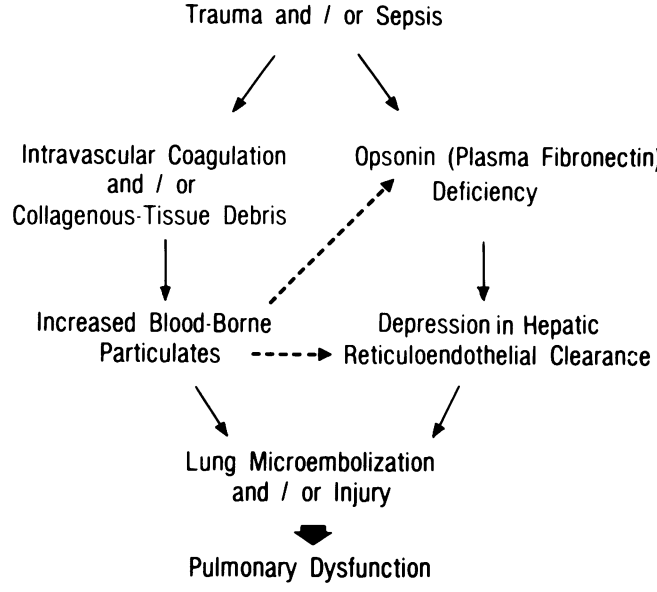

FIG. 7. Mechanisms by which trauma and/or sepsis could result in depletion of opsonic α_2SB glycoprotein, inhibition of reticuloendothelial host-defense function and, eventually, pulmonary insufficiency.

vascular coagulation and that this may be a factor in the cause of increased lung vascular permeability in the septic sheep as reported by Brigham.[8] Augmented intravascular coagulation coupled with exposure of basement membrane collagen to vascular fluid via endothelial injury, especially in the trauma patient with coexistent sepsis, may rapidly deplete opsonic protein and thus further limit reticuloendothelial removal of products of bacterial sepsis. Prolonged circulation of such particulates might exacerbate aggregation and formation of microaggregates which could embolize the pulmonary vasculature and alter vascular permeability.[10,18,19] Such a response would be exaggerated when trauma preceeds sepsis since opsonic deficiency as documented after injury in burn and trauma patients is quite severe and is associated with pulmonary distress.[30,33]

TABLE 2. *Plasma Fibrinogen Concentrations and Serum Fibrin Degradation Products in Sheep Following Pseudomonas Challenge**

Time (hr)	Plasma Immunoreactive† Clottable Fibrinogen mg/ml	Per Cent Control	Serum Immunoreactive† Fibrin Degradation Products mg/ml	Per Cent Control
0	4.39 ± 0.9	100	0.105 ± 0.04	100
1	4.09 ± 0.8	93	0.105 ± 0.04	100
2	3.62 ± 0.8	82	0.115 ± 0.05	110
3	3.37 ± 0.9	77	0.134 ± 0.05	128
4	2.53 ± 0.8	58	0.140 ± 0.05	133

* Sheep under pentobarbital anesthesia were challenged with *Pseudomonas aeruginosa* and received an intravenous dose of 5×10^9 bacteria in parallel with an intraperitoneal dose of 5×10^{10} bacteria.

† Fibrinogen and fibrin degradation products were measured by immunoassay prior to (0 Hr) and at hourly intervals after induction of sepsis. The decrease in fibrinogen and increase in fibrin degradation products reflect activation of intravascular coagulation. By four hours after bacterial challenge both fibrinogen and fibrin degradation products differ significantly ($p < 0.05$) from control. Each value represents the mean and standard error of the mean for five sheep.

Failure of the hepatic reticuloendothelial system in man to effectively function as a "prepulmonary filter" for noxious, blood-borne particulates may augment the development of pulmonary insufficiency during intravascular coagulation and/or sepsis following nonthoracic trauma.[28,30,33] In addition, the opsonic α_2SB glycoprotein (plasma fibronectin; cold-insoluble globulin) may be a regulator of the RES clearance capacity. Clinical evidence to document the interaction of plasma fibronectin and the coagulation system was recently reported by Mosher and Williams.[21] They demonstrated severe plasma fibronectin deficiency in patients with disseminated intravascular coagulation and correlated the level of fibronectin deficiency to the degree of organ failure. The relationship between DIC and organ failure was not addressed in the study, but has been investigated by others.[10,13,18,19] Embolization of fibrin, fibrin degradation products and platelets in the lung has been associated with the onset of significant pulmonary injury and gas exchange insufficiency.[19,20] Lung fibrin deposition secondary to thrombin infusion results in increased shunting and an increase in pulmonary extravascular lung water.[13,18,20] Recently, Costabella et al. have demonstrated an increase in pulmonary vascular permeability and consequent pulmonary edema after thrombin infusion with fibrinolytic blockage of the dog.[10] Since clearance techniques for

directly measuring RE function are invasive and blockade RE phagocytic activity,[7,27] it does not currently seem prudent to directly measure RE phagocytic colloid clearance function in patients who may already have marginal host defense capacity in order to further verify the correlation of immunoreactive opsonin to clearance activity. In this context, the present data indicate that the sheep provides an excellent model for investigation of the role of the RES in the etiology of pulmonary injury and organ failure during sepsis as it may relate to the clinical setting. Indeed, the similarity of the human and sheep reticuloendothelial system, from the standpoint of circulating levels of immunoreactive opsonic α_2SB glycoprotein, coupled with the capability to investigate pulmonary vascular permeability using the sheep lung lymph preparation, lends further support to the concept that this animal may provide an excellent model for investigation of clinically relevant problems with respect to pulmonary insufficiency during endotoxemia and bacteremia.

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