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*J Clin Invest.* 1980;66(2):375-382. <https://doi.org/10.1172/JCI109866>.

### Research Article

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# Role of Complement Activation in a Model of Adult Respiratory Distress Syndrome

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**ABSTRACT** The adult respiratory distress syndrome is characterized by arterial hypoxemia as a result of increased alveolar capillary permeability to serum proteins in the setting of normal capillary hydrostatic pressures. Because bacterial sepsis is prominent among the various diverse conditions associated with altered alveolar capillary permeability, we studied the effect of bacteremia with attendant complement activation on the sequestration of microorganisms and the leakage of albumin in the lungs of guinea pigs. Pneumococci were injected intravenously into guinea pigs and their localization was studied. Unlike normal guinea pigs, complement-depleted guinea pigs did not localize injected bacteria to the lungs. Preopsonization of organisms did not correct this defect in pulmonary localization of bacteria in complement-depleted animals, suggesting that a fluid-phase component of complement activation was required. Genetically C5-deficient mice showed no pulmonary localization of bacteria. C5-sufficient mice demonstrated the usual pulmonary localization, thus further suggesting that the activation of C5 might be important in this localization. The infusion of activated C5 increased alveolar capillary permeability to serum proteins as assayed by the amount of radioactive albumin sequestered in the lung. Neutropenic animals did not develop altered capillary permeability after challenge with activated C5. Thus, complement activation through C5, in the presence of neutrophils, induces alterations in pulmonary alveolar capillary permeability and causes localization of bacteria to the pulmonary parenchyma. Complement activation in other disease states could potentially result in similar clinical manifestations.

## INTRODUCTION

The proteins of the complement system play a major biological role as mediators of inflammation and opsonization. Many studies have suggested a role for complement in the development of shock in experimental animals (1). The vasoactive products of complement activation were identified and termed anaphylatoxins, since, on injection, they produce a syndrome similar to that seen in anaphylactic shock. The anaphylatoxins have been characterized as low molecular weight polypeptides released during activation of the third, fourth, and fifth components of complement (C3, C4, and C5) (2, 3). These peptides, termed C3a, C4a and C5a, are characterized by the ability to enhance vascular permeability after subcutaneous injection, to release histamine from mast cells, and to cause smooth muscle contraction. In addition, C5a is a potent chemoattractant for polymorphonuclear leukocytes (4). It is significant that complement activation in humans with gram-negative sepsis is associated with the development of shock and subsequent mortality (5).

Because a frequent companion of septic shock is respiratory insufficiency, we have studied the role of complement activation in the pathogenesis of this syndrome. The altered pulmonary alveolar capillary permeability associated with sepsis is commonly referred to as the adult respiratory distress syndrome (ARDS)<sup>1</sup> (6). Although sepsis is a frequent cause of ARDS, many other clinical entities are also associated with this disorder. Complement activation is a potential common denominator in the pathogenesis of ARDS associated with many of these disease states. For this reason we have evaluated the role of com-

Presented in part at the Annual Meeting of the American Federation for Clinical Research, San Francisco, Calif. 28 April-1 May 1978.

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Received for publication 31 December 1979 and in revised form 11 April 1980.

<sup>1</sup> *Abbreviations used in this paper:* ARDS, adult respiratory distress syndrome; C4D-GP, guinea pig deficient in C4; C5D-mice, mice deficient in C5; CVF, cobra venom factor; NIH-GP, NIH outbred strain of guinea pigs; PBS, phosphate-buffered saline; Zx, zymosan-bound convertase.

plement activation in altering alveolar capillary permeability in an experimental model.

## METHODS

**Animals.** Guinea pigs weighing 300–400 g were obtained from the National Institutes of Health (NIH) Small Animal Farms. In addition to the NIH outbred strain (NIH-GP), some experiments employed a strain of guinea pigs deficient in C4 (C4D-GP). These animals possess an intact alternative pathway of complement activation, but have a complete block in classical pathway activity. Animals were killed by CO<sub>2</sub> inhalation. Guinea pigs were made neutropenic by the intraperitoneal injection of 100 mg/kg of cytoxan on day 5 and 50 mg/kg of cytoxan on day 1 before study. Total polymorphonuclear leukocyte counts were 600 cells/mm<sup>3</sup> or less on the day of study (normal range, 2,000–5,000/mm<sup>3</sup>). Mice weighing 20–30 g were obtained from Jackson Laboratories, Bar Harbor, Maine. The two inbred strains employed were B10/OSN and B10/NSN. The former animals have a deficiency of C5 (C5D-mice).

**Bacteria.** Type 7 pneumococci (American Type Culture Collection, Rockville, Md.) were stored on blood agar plates with monthly passage in white mice or guinea pigs to maintain virulence. Organisms were grown 10–12 h in trypticase soy broth, washed, and resuspended in phosphate-buffered saline (PBS) at the appropriate concentration before use. Pneumococci were radiolabeled by the addition of 50  $\mu$ Ci of <sup>59</sup>Fe (Amersham Corp., Arlington Heights, Ill.) to the TSB. The pH was returned to 7.4 with 1 N NaOH. The organisms were heat-killed at 56°C for 30 min and washed until no significant radioactivity was recovered in the supernate. For clearance studies, live organisms were injected intravenously via the hind paw and blood colony counts monitored as previously.<sup>2</sup> For organ localization studies, animals were infused intravenously via the hind paw with heat-killed, radiolabeled bacteria containing ~10<sup>6</sup> cpm. After sacrifice, whole organs were removed and radioactivity determined in a Beckman gamma counter (Beckman Instruments Inc., Fullerton, Calif.). Background counts were consistently <200 cpm. Organisms were preopsonized by incubation in normal pooled guinea pig serum diluted 1:4 with veronal-buffered saline with added gelatin, calcium, and magnesium ion (7) for 1 h, washed twice in PBS, and then resuspended in the same.

**Complement components and titrations.** Hemolytic complement titrations of CH<sub>50</sub>, C<sub>3</sub>, and C<sub>5</sub> were performed in standard fashion (7). Cobra venom factor (CVF) was obtained from Cordis Laboratories Inc. (Miami, Fla.) and, after reconstitution, was injected at a dose of 200 U/kg i.v. via the hind paw in guinea pigs (CVF-GP) and intraperitoneally in mice (CVF-mice). CVF-treated animals were used 16 h after CVF administration. Guinea pigs so treated had a regular fall in C3 titer to <1% normal at this time.

**Purification of C5.** The purification of C5 from human plasma is given below and is derived in part by a modification of the procedure of Tack and Prahl (8). Approximately 10 U of human plasma shown to be free of hepatitis B antigen was obtained from the NIH Blood Bank and clarified by centrifugation at 4°C. The supernate was treated with appropriate inhibitors as described and the 5–12% polyethylene glycol fraction was obtained. After the removal of plasminogen from the C3/C5 cut by cyanogen bromide

coupled lysine-Sepharose 4B affinity chromatography, the C3/C5 containing effluent was sequentially purified by diethylaminoethyl cellulose (DE-52, Whatman Chemicals, Div: W&R Balston, Maidstone, Kent, England) chromatography, gel filtration on Sepharose 6B (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.) and hydroxylapatite chromatography on Bio-Gel HT (Bio-Rad Laboratories, Richmond, Calif.).

Human C5 was obtained from a hydroxylapatite chromatographic step by elution with 2 M salt before to the elution of bound C3. The pooled C5 was concentrated to 2.2 mg/ml and when tested by immunodiffusion analysis was negative for C3, immunoglobulin (Ig)M, factor B, and  $\beta$ 1H, but contained some IgA and a significant level of IgG. At 21,000 U C5/ml input, no hemolytic C3, C6, C8, or C9 could be detected. C7 contamination was less than one part in 60,000. To remove most of the contaminating IgG, the C5 pool was passed over a protein A Sepharose C1-4B column (Pharmacia Fine Chemicals Inc.). All C5 hemolytic activity was recovered in this step. Although immunochemical analysis of this preparation at 3.8 mg/ml showed a weak precipitin line against anti-IgG and anti-IgA, sodium dodecyl sulfate-acrylamide disc gel electrophoresis of 15.0  $\mu$ g of a reduced sample could demonstrate only the  $\alpha$ -(115,000 daltons) and  $\beta$ -(80,000 daltons) chains of C5. Coomassie Blue-stained bands for the heavy and light chains of IgG and IgA were not detectable indicating <1% contamination of the C5 by these proteins. A nonreduced sample exhibited only one stained band at about 185,000 daltons. C5a was prepared from the highly purified C5 by incubation with a zymosan-bound convertase (Zx). Boiled, washed zymosan obtained from ICN Nutritional Biochemicals (Cleveland, Ohio) was incubated with fresh normal guinea pig sera at a concentration of 4 mg/ml at 37°C for 30 min. Zx was removed by centrifugation and resuspended in 60% dextrose buffer with addition of Ca<sup>++</sup> and Mg<sup>++</sup> to 1 mM concentration at pH 7.4. Three 1.5-ml reaction mixtures were then prepared. (a) Control, Zx alone with Ca<sup>++</sup> and Mg<sup>++</sup>; (b) activated C5, Zx with Ca<sup>++</sup> and Mg<sup>++</sup> plus 50  $\mu$ l of purified C5 at a concentration of  $2 \times 10^6$  hemolytic U/ml; and (c) Ca<sup>++</sup> and Mg<sup>++</sup> plus 50  $\mu$ l of purified C5 in 60% dextrose and buffer. In the final reaction mixtures, Zx was present at a concentration of 2 mg/ml. Each solution was incubated at 37°C for 30 min. Greater than 90% of C5 was activated in solution (2) by this procedure as determined by fall in hemolytic C5 titer. 5 min after infusion of each of the above preparations in normal or neutropenic animals previously infused with radiolabeled albumin, the guinea pigs were killed and lungs removed and counted *in toto* to determine the amount of <sup>125</sup>I-albumin therein.

**Radiolabeling of guinea pig albumin.** Radiolabeling of purified guinea pig albumin (Sigma Chemical Co., St. Louis, Mo.) was accomplished with the Bolton-Hunter reagent (iodinated *p*-hydroxyphenylpropionic acid, *N*-hydroxysuccinimide ester; New England Nuclear, Boston, Mass.) using the technique of Dr. John Inman, NIH.<sup>3</sup> Guinea pig albumin at a concentration of 0.5 mg/ml in PBS, pH 7.4 was used. 250  $\mu$ Ci of Bolton-Hunter reagent were introduced into the bottom of a clean glass tube and evaporated under gently flowing nitrogen gas. Then, 0.5 ml of bicine buffer [*N,N*-bis(2-hydroxyethyl)glycine], pH 8.3, was added and stirred with a fine glass rod for 3 min to resolubilize the dried iodinated ester; 0.5 ml of guinea pig albumin was then added to the chilled mixture and stirred for 3 min. The reaction mixture was incubated on ice for 3 h and dialyzed against 500 ml of the sodium chloride buffer at 4°C for 48 h with three changes of dialysis fluid. 25  $\mu$ g of <sup>125</sup>I-labeled albumin was administered intravenously 30 min before study. To complete the

<sup>2</sup> Hosea, S. W., E. J. Brown, and M. M. Frank. The critical role of complement in experimental pneumococcal sepsis. Submitted for publication.

<sup>3</sup> Personal communication.

TABLE I  
Organ Distribution of Radiolabeled *Pneumococci* (50,000 cpm Injected) after Intravenous Administration in Guinea Pigs

	NIH-GP (6)	CVF-GP (4)	CVF-GP + pre- opsonization (4)
	% cpm		
Spleen	7.9±2.3	18.5±2.9	14.4±3.4
Liver	70.3±1.3	69.8±1.6	82.5±3.4
Lung	20.6±2.9	6.1±1.2	2.6±0.5
Kidney	1.5±0.2	1.0±0.3	0.8±0.3

All values are mean±SEM. The numbers in parentheses represent the number of animals in each group. There are significantly more counts per minute in the lungs of normal NIH-GP ( $P < 0.001$ ) compared with CVF-GP±preopsonization. In addition, CVF-GP have significantly more bacteria-associated radioactivity in the spleen than do NIH-GP.

studies reported herein, a second preparation of  $^{125}\text{I}$ -albumin was used for the Forssman shock experiments. The specific activity of this preparation in counts per minute per microgram albumin was one-third that of the original preparation. The amount of  $^{125}\text{I}$ -albumin organ-associated radioactivity was determined by counting the whole organ in a Beckman gamma counter.

**Statistics.** Statistical analysis was accomplished using the Student's *t* test.

## RESULTS

The intravenous infusion of  $5 \times 10^8$  heat-killed radiolabeled type 7 pneumococci was followed by their rapid clearance from the blood stream. Soon after

injection the majority of counts 78.2% localized to the reticuloendothelial system (liver and spleen) as shown in Table I. However, 20.6% of the radioactivity was found in the lung in both NIH-GP and C4D-GP. In animals depleted of C3-C9 and the alternative pathway components by the injection of CVF, only 6.1% of radioactivity was found in the lung. A proportionate increase in sequestration of organisms by the spleen was also noted in CVF-GP. Preopsonization of organisms by incubation in normal guinea pig serum, washing and then injecting into CVF-GP resulted in improved blood stream clearance<sup>2</sup> without an increase in the pulmonary localization of radioactivity. There was a significant difference ( $P < 0.001$ ) for lung-associated radioactivity in NIH-GP compared to CVF-GP with or without preopsonization of organisms. In addition, opsonization before infusion significantly decreased the role of the spleen in intravascular clearance in favor of the liver in CVF-GP. The lack of pulmonary localization in CVF-GP injected with preopsonized bacteria suggested that a fluid-phase component of complement activation was responsible for this phenomena.

The time-course of pulmonary sequestration of radiolabeled bacteria and of complement activation was examined (Table II). 10 min after injection, bacteria were found in the pulmonary parenchyma concomitant with a fall in circulating leukocytes and a modest decrease in C3 and C5 titers. Despite continued clearance of bacteria from the blood stream,<sup>2</sup> radiolabeled organisms ceased to accumulate in the lungs. By 6 h, the pulmonary-associated radioactivity had returned to base

TABLE II  
Time-Course of Pulmonary Sequestration of Radiolabeled Bacteria in Relation to Total Polymorphonuclear Leukocyte Count and Hemolytic C3 and C5 Titers

	Preinjection	Hours after injection		
		0.17	1.0	6.0
Pulmonary sequestration of bacteria, % cpm recovered	—	21	13	5
Polymorphonuclear leukocytes per mm <sup>3</sup>	3,017	1,152	787	2,403
C3 titer, hemolytic units	7,600	5,400	5,100	6,300
C5 titer, hemolytic units	9,400	6,200	8,600	9,200

The rapid accumulation of organisms in the lung after intravenous injection is associated with a fall in circulating polymorphonuclear leukocytes and a decrease in hemolytic C3 and C5 titers. Each value for pulmonary sequestration represents the average of two animals. The white count and hemolytic titers are sequential values in the same animals infused with the same preparation of pneumococci. The coefficient of variation for each hemolytic titer is 20%. There is a significant decrease in C5 titer at 0.17 h (10 min) compared with preinjection values ( $P < 0.001$ ).

line concomitant with a return in C5 titers to normal and a rising C3 titer. These data raised the possibility that activation of either C3 or C5 was responsible for the pulmonary sequestration of bacteria.

To more directly evaluate the role of C5 in the pulmonary localization of bacteria, C5D-mice (B10/OSN) were employed. In Table III, one can see that there is a significant difference ( $P < 0.001$ ) in the radioactivity associated with the pulmonary parenchyma in normal (17.3%) compared to C5D-mice (4.6%). In addition, the administration of CVF significantly decreased the pulmonary localization of radioactivity in normal mice, just as in the CVF-GP.

To further explore the relationship between the pulmonary localization of bacteria and the pathogenesis of the ARDS, an assay for altered pulmonary alveolar capillary permeability was developed. 25  $\mu$ g of  $^{125}$ I-labeled guinea pig albumin was infused intravenously. 10 min after various experimental manipulations, animals were killed. The lungs were removed, and the amount of radioactivity determined as a measure of alveolar capillary permeability to albumin. To test whether this was a valid assay in cases of altered alveolar capillary permeability, a model of Forssman shock was used (9). Both NIH-GP and C4D-GP received 0.8 ml i.v. of anti-Forssman antibody. NIH-GP died within 10 min. C4D-GP tolerated the infusion without incident but were also killed at 10 min. As shown in Table IV, the lungs of the NIH-GP had a significantly greater amount of radioactivity associated with the lung than the C4D-GP ( $P < 0.005$ ). In addition, the amount of  $^{125}$ I-albumin in the lungs of C4D-GP was comparable to that of NIH-GP given the same preparation of radiolabeled albumin without anti-Forssman antibody (not shown). Since the pathology of Forssman shock is a hemorrhagic pulmonary edema, the amount of  $^{125}$ I-albumin in the lung was taken to be a sensitive assay of the integrity of the pulmonary alveolar capillary bed.

TABLE III  
Organ Distribution of Radiolabeled Pneumococci after Intravenous Administration in Mice

	Normal (4)	C5D-Mice (4)	Normal + CVF (2)	C5D-Mice + CVF (2)
% cpm recovered				
Spleen	10.5 $\pm$ 1.8	14.6 $\pm$ 2.0	17.6 $\pm$ 1.7	13.3 $\pm$ 0.8
Liver	62.4 $\pm$ 1.1	70.8 $\pm$ 0.8	71.0 $\pm$ 0.8	71.1 $\pm$ 2.7
Lung	17.3 $\pm$ 1.3	4.6 $\pm$ 0.3	5.2 $\pm$ 0.6	2.4 $\pm$ 3.3
Kidney	7.0 $\pm$ 1.0	7.2 $\pm$ 0.6	5.7 $\pm$ 0.9	6.7 $\pm$ 0.3

All values are mean $\pm$ SEM. The numbers in parentheses represent the number of animals in each group. There are significantly more counts in the lungs of normal mice ( $P < 0.001$ ) compared to C5D, normal + CVF, or C5D + CVF.

TABLE IV  
Organ Localization of  $^{125}$ I-albumin in Forssman Shock in NIH-GP and C4D-GP

	NIH-GP (4)	C4D-GP (3)	Significance
Lung	18,171 $\pm$ 1,340	9,013 $\pm$ 1,491	<0.005
Spleen	567 $\pm$ 76	653 $\pm$ 76	NS
Kidney	3,640 $\pm$ 611	3,463 $\pm$ 168	NS
Liver	30,500 $\pm$ 2,720	29,766 $\pm$ 536	NS
Heart	1,908 $\pm$ 273	2,408 $\pm$ 712	NS

The numbers of animals in each group is shown in parentheses. There is a significant increase in  $^{125}$ I-albumin in the NIH-GP who died a respiratory death secondary to pulmonary edema.

In Fig. 1 the pulmonary localization of  $^{125}$ I-albumin is measured in normal and bacteremic animals. After infusion of  $^{125}$ I-albumin, control animals received 0.5 ml of PBS and bacteremic animals received 0.5 ml of  $1 \times 10^9$  type 7 pneumococci i.v. There was a significant increase in pulmonary  $^{125}$ I-albumin in bacteremic compared to control animals ( $P < 0.001$ ). Bacteremic animals tolerated the infusions without obvious evidence of respiratory difficulties. By 60 min after injection of bacteria, the pulmonary-associated  $^{125}$ I-albumin in bacteremic animals was similar to controls.

The specific role of activated C5 in altering alveolar capillary permeability was then evaluated. A highly purified preparation of human C5 was used. Control animals received either purified, nonactivated C5, or the supernate from incubation of Zx in 60% dextrose buffer. A significant increase in pulmonary  $^{125}$ I-albumin was seen only in those animals infused with activated

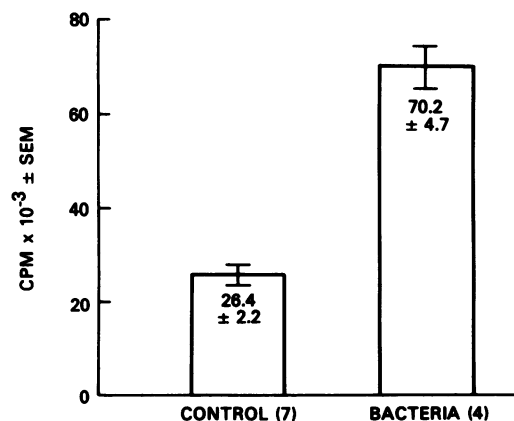


FIGURE 1 Pulmonary localization of  $^{125}$ I-albumin in normal and bacteremic guinea pigs. The number in parentheses represents the number of animals in each group. After the infusion of  $1 \times 10^9$  type 7 pneumococci, there is significantly increased pulmonary associated albumin in the bacteremic group ( $P < 0.001$ ).

C5 (Fig. 2). Neutropenic animals infused with activated C5 had pulmonary radioactivity similar to controls. This strongly suggests that circulating neutrophils were required to achieve altered pulmonary capillary permeability. A large amount of activated C5,  $4 \times 10^4$  hemolytic U per animal, was required to demonstrate increased alveolar capillary permeability. Lesser amounts were ineffective, suggesting rapid inactivation in vivo. The amount of activated C5 infused corresponds to the total amount of C5 activated after infusion of  $1 \times 10^8$  type 7 pneumococci, or  $4.8 \times 10^4$  hemolytic U of C5. The latter number was calculated by multiplying the difference in C5 titers at 0 and 10 min (Table II) by the approximate plasma volume of a 400-g NIH-GP (15 ml). Lesser amounts of activated C5 were insufficient to alter alveolar capillary permeability.

To gain some insight into the significance of the pulmonary sequestration of organisms in the clearance of bacteria from the blood stream, a clearance study was done. Animals made neutropenic with cytoxan exhibited a rate of blood stream clearance of bacteria identical to normal animals for the first 6 h of the study, as shown in Fig. 3. However, neutropenic animals infused with radiolabeled pneumococci had fewer pulmonary-associated bacteria (12.4 vs. 32.9%) than normal NIH-GP ( $P < 0.005$ ), a difference noted only in the early minutes of clearance.

## DISCUSSION

The pulmonary sequestration of microorganisms after intravenous injection is a theoretically unexpected, yet

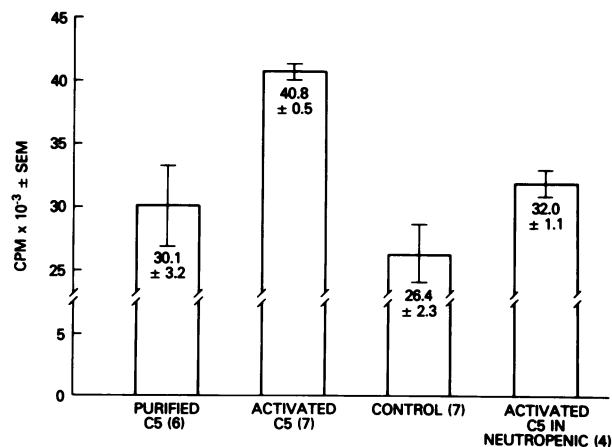


FIGURE 2 The pulmonary localization of  $^{125}\text{I}$ -albumin in animals infused with activated C5, purified, unactivated C5, and 60% dextrose supernate from Zx. Activated C5 was also infused into a group of animals made neutropenic with cytoxan. There is a significant increase in pulmonary  $^{125}\text{I}$ -albumin in those animals infused with activated C5 compared to controls ( $P < 0.005$  for purified C5 and  $P < 0.001$  for control). Only the neutropenic animals infused with activated C5 did not show a significant difference from controls.

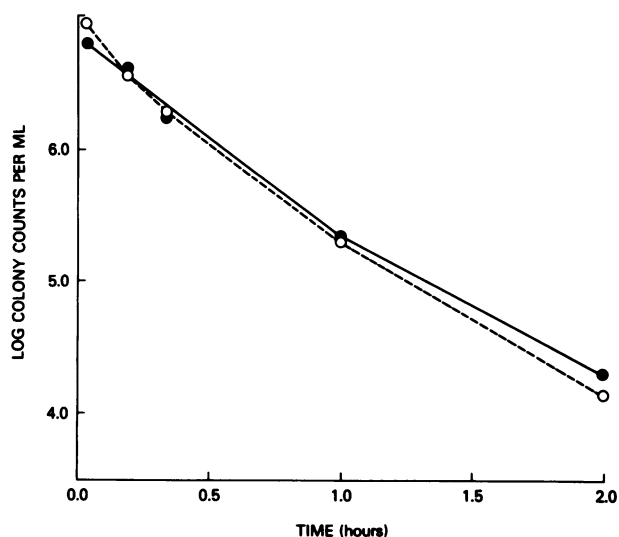


FIGURE 3 The clearance of  $5 \times 10^8$  type 7 pneumococci after intravenous injection in four NIH-GP (—) and four neutropenic guinea pigs (---). Colony counts are plotted semi-logarithmically on the ordinate vs. time on the abscissa. There is no significant difference in early bloodstream clearance in normal vs. neutropenic animals.

well-described phenomena (10, 11). Histologically, bacteria are found to be ingested by polymorphonuclear leukocytes within the alveolar capillaries. This study confirms that  $>20\%$  of radiolabeled bacteria may be recovered in the lung early after intravenous administration. The important role of complement in effecting the localization of organisms to the lung is seen in experiments using the CVF-GP. These animals are deficient in the proteins of the alternative pathway and the proteins C3 through C9 due to depletion by cobra venom (12). CVF-GP demonstrated no sequestration of organisms in the lung. To evaluate the role of membrane-bound complement components, preopsonization of microorganisms was accomplished before injection. However, preopsonized organisms did not localize to the lungs of CVF-GP. This data suggested that a fluid-phase component of complement activation was necessary for this phenomena. The cleavage fragments C3a and C5a are known to be important vasoactive peptides (2) and, thus, were considered to be prime candidates as mediators of this pulmonary sequestration of bacteria. More recently, C4a has also been shown to have vasoactive properties (3). That pneumococci localize to the lung in C4D-GP suggests that C4a plays no role in this phenomenon.

The localization of bacteria to the pulmonary parenchyma occurs soon after injection. This occurs simultaneously with a marked fall in circulating leukocyte counts and in hemolytic C3 and C5 titers. Wood and his associates (13) showed that polymorphonuclear leukocytes adhere to capillary endothelium and

phagocytize bacteria minutes after intravenous injection. It is noteworthy that, in these experiments, pulmonary localization of bacteria is a transient phenomena. This may be due, in part, to the powerful inactivators of the anaphylatoxins that are present in the circulation. Only the rapid, massive activation of complement, soon after administration of organisms, is sufficient to produce the observed phenomena. Ulevitch and co-workers, (14) demonstrated that complement-mediated hypotension required significant complement activation (30–40% of C3 activated) over a short period of time (14). Maintenance of normal body hemostasis would require the rapid inactivation of such potent vasoactive substances.

To assess the relative importance of C3 and C5 in the pulmonary clearance of bacteria, a strain of C5D-mice was employed. The complete lack of specific pulmonary clearance in the C5-deficient animals suggested that C5 was critical. A major difference in biologic activities of C3a and C5a is that C5a formed by activation of serum induces a chemotactic response in polymorphonuclear leukocytes (4). Moreover, the chemotactic factors derived from activated C5 also induce neutropenia when injected into rabbits (15). In addition, C5a will induce aggregation of granulocytes *in vitro* (16) and *in vivo* (17). Thus, C5a is a reasonable candidate for the fluid-phase factor of complement activation which is responsible for the localization of bacteria to the lungs and the neutropenia which occurs after intravenous injection of bacteria.

The effect that C5a has on granulocytes would suggest that these cells are also an important component of pulmonary sequestration of organisms. Neutropenic animals had significantly less organisms present in the pulmonary parenchyma than did normal animals. Although macrophages are present in the lung, their intra-alveolar position makes them ill-suited for mediating intravascular clearance. Thus, neutrophils also appear to be necessary for the lung clearance of bacteria. That this pulmonary clearance of microorganisms is not necessary for efficient intravascular clearance is shown by the identical rates of clearance in normal and neutropenic animals. The importance of neutrophils in host defense stems from their ability to mobilize to areas of infection not accessible by the fixed macrophages of the reticuloendothelial system. The *in vivo* significance of margination of neutrophils in the pulmonary capillaries was further investigated.

Because increased pulmonary alveolar capillary permeability is often associated with bacterial sepsis, an *in vivo* assay of altered capillary permeability was devised. The hypothesis that increased alveolar capillary permeability would be manifest by increased

serum proteins in the lung was tested in Forssman shock. Mortality in Forssman shock is due to a complement-mediated acute pulmonary edema (9). The amount of iodinated albumin in the lungs of animals dying of Forssman shock was significantly greater than that of controls given a similar injection. The amount of radioactive albumin associated with the pulmonary parenchyma was taken as a measure alveolar capillary permeability to plasma proteins.

Infusion of pneumococci also induced a dramatic increase in alveolar capillary permeability. This effect lasted only a matter of minutes since the amount of radioactive albumin sequestered in the lung returned to base line by 1 h after infusion. This perhaps reflects the rapid *in vivo* inactivation of the anaphylatoxins by inhibitors such as carboxypeptidase B (2). In addition, an increase in fluid in the pulmonary interstitium is rapidly mobilized to the pulmonary lymphatics (18), which is an important protective mechanism in preserving efficient gas exchange in the lungs. It is noteworthy, in this regard, that hyperventilation is often an early sign of bacterial sepsis (19). These data raise the possibility that the pathophysiology of this clinical sign may be in part explained by bacteremia inducing complement activation with granulocyte accumulation in the lung and subsequent respiratory insufficiency due to altered alveolar capillary permeability. Direct damage to the lung by endotoxin is a potential contributing factor (20), but other data suggest a primary role to complement activation secondary to endotoxin (21). Although gram-negative sepsis is more commonly associated with ARDS, high grade pneumococcal bacteremia may also be associated with pulmonary edema (22). The activation of complement is common to both of these clinical conditions (5, 23).

The contribution of C5a to alterations in alveolar capillary permeability in this animal model was clearly demonstrated by infusion of a highly purified, activated C5 preparation into animals. Neither nonactivated C5 or the supernate from the Zx induced leakage of albumin. Moreover, the activated C5, which caused increased pulmonary alveolar capillary permeability in the normal animal, produced none in the neutropenic guinea pigs. The increased permeability required both activated C5 and the presence of circulating neutrophils. These findings are in accord with those of Craddock and co-workers (24), who have evaluated the pathophysiology of neutropenia and pulmonary insufficiency that occurs in hemodialysis patients (24). They have suggested that complement activation induces alterations in the granulocyte membrane, which result in margination and clumping of granulocytes in the pulmonary microvasculature. These aberrations are the consequence of the effect of

C5a on polymorphonuclear leukocytes. The endothelial damage that ensues is presumed from in vitro experiments to be secondary to unidentified oxygen products released by adherent, aggregated granulocytes (25). The findings that high-dose corticosteroids can inhibit the membrane altering effects of C5a in vitro (18, 26) provides some rationale for the use of the same in shock and the ARDS.

The localization of intravenously injected bacteria to the pulmonary parenchyma requires both the activation of C5 and the presence of circulating polymorphonuclear leukocytes. Bacteria are trapped in the lung presumably as a consequence of the polymorphonuclear leukocytes being trapped there. However, alterations in alveolar capillary permeability do not require the presence of microorganisms, but rather any substance or particle that can cause complement activation. The ARDS is associated with a wide variety of disease states (6). The activation of complement is common to many of these disorders. Although C5a is usually rapidly cleared from the circulation, high levels of circulating C5a have been detected in traumatized patients at risk for ARDS and in bacteremic patients. The presence of C5a in the latter was significantly associated with the development of ARDS (27). Thus, complement activation in the setting of hemodialysis, bacterial sepsis, trauma, and pancreatitis (28) results in the formation of C5a. It is reasonable to suggest that the effect of C5a on the granulocyte to induce margination and leukoembolization leads to altered alveolar capillary permeability and contributes to ARDS. Complement activation is one common denominator in the pathogenesis of ARDS in these various disease states.

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