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**Research Article**

Intravascular activation of the complement system with cobra venom factor results in acute lung injury, which has been quantitated by increases in lung vascular permeability. Cobra venom factor preparations devoid of phospholipase A2 activity retain full lung-damaging capacity. The lung injury is associated with the preceding appearance of chemotactic activity in the serum coincident with the development of a profound neutropenia. The chemotactic activity is immunochemically related to human C5a. Morphologic studies have revealed discontinuities in the endothelial cell lining of lung alveolar capillaries, damage and/or destruction of endothelial cells in these areas, plugging of pulmonary capillaries with neutrophils that are in direct contact with vascular basement membrane, the presence of fibrin in alveolar spaces and in areas adjacent to damaged endothelial cells, and intraalveolar hemorrhage. Lung injury is dramatically attenuated in animals that have been previously neutrophil depleted. Teh intravenous injection of superoxide dismutase or catalase also provides significant protection from the pulmonary damage. Very little protection from the pulmonary damage. Very little protection is afforded by pretreatment of rats with antihistamine. These studies suggest that intravascular activation of the complement system leads to neutrophil aggregation and activation, intrapulmonary capillary sequestration of neutrophils, and vascular injury, which may be related to production of toxic oxygen metabolites by complement-activated neutrophils.



# Intravascular Activation of Complement and Acute Lung Injury

## DEPENDENCY ON NEUTROPHILS AND TOXIC OXYGEN METABOLITES

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ABSTRACT Intravascular activation of the complement system with cobra venom factor results in acute lung injury, which has been quantitated by increases in lung vascular permeability. Cobra venom factor preparations devoid of phospholipase $A_2$ activity retain full lung-damaging capacity. The lung injury is associated with the preceding appearance of chemotactic activity in the serum coincident with the development of a profound neutropenia. The chemotactic activity is immunochemically related to human C5a. Morphologic studies have revealed discontinuities in the endothelial cell lining of lung alveolar capillaries, damage and/or destruction of endothelial cells in these areas, plugging of pulmonary capillaries with neutrophils that are in direct contact with vascular basement membrane, the presence of fibrin in alveolar spaces and in areas adjacent to damaged endothelial cells, and intraalveolar hemorrhage. Lung injury is dramatically attenuated in animals that have been previously neutrophil depleted. The intravenous injection of superoxide dismutase or catalase also provides significant protection from the pulmonary damage. Very little protection is afforded by pretreatment of rats with antihistamine. These studies suggest that intravascular activation of the complement system leads to neutrophil aggregation and activation, intrapulmonary capillary sequestration of neutrophils, and vascular injury, which may be related to production of toxic oxygen metabolites by complement-activated neutrophils.

## INTRODUCTION

The observations of neutropenia developing in patients during hemodialysis, leukapheresis, and cardiopulmonary bypass procedures have led to the recognition that intravascular activation of the complement system is related to the phenomenon of neutropenia and intrapulmonary sequestration of neutrophils (1–3). The subsequent in vitro demonstration of the ability of chemotactic factors to cause aggregation and activation of leukocytes (4) and the fact that neutropenia can readily be induced by the in vivo activation of the complement system (5, 6) or by intravenous infusion of chemotactic factors (7) has opened up a broad area of study. It has been suggested that intravascular activation of complement leads to stimulation of neutrophils, sequestration within capillaries of these activated leukocytes, and subsequent damage of endothelial cells. The findings of Sachs et al. (8) have provided in vitro evidence that activation of leukocytes with complement-derived chemotactic products results in a cytotoxic effect on endothelial cells. The target cells are protected in the presence of superoxide dismutase and catalase.

Our earlier observations have indicated that complement activation, when produced within the alveolar compartment by the deposition of immune complexes, triggers a series of reactions resulting in considerable damage to the lung parenchyma (9). The pathogenesis of this damage is related to the influx of neutrophils into alveolar spaces, their activation (presumably by phagocytic uptake of immune complexes), and the subsequent production by leukocytes of $H_2O_2$ (or its derivatives) (10). In this model, acute lung damage can be averted in a dose-dependent manner by the presence of catalase.

In this paper we provide direct evidence that intravascular activation of the complement system will lead to acute lung injury and that this injury is apparently related to damage of endothelial cells caused by the generation from neutrophils of toxic oxygen products.

## METHODS

*Animals.* Young adult male Long Evans rats were used in these studies. The measurement of lung injury was determined by the increase in lung vascular permeability,

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which was estimated by the leakage of $^{125}$I-rat IgG into the extravascular compartment of the lung. At time zero, 0.5 ml phosphate-buffered (pH 7.4) saline (PBS) (with or without the test material) containing 1 μg $^{125}$I-rat IgG was injected intravenously. At the time of sacrifice, animals received an intraperitoneal injection of 30 mg ketamine hydrochloride (Parke Davis & Co., Detroit, MI), followed by exsanguination with aortic transsection. 1 ml blood was collected for assessment of radioactivity. The lung vasculature was then perfused with 5.0 ml saline to clear any radioactivity remaining within the vasculature. Lungs were assessed for content of radioactivity, which was expressed as a ratio to the amount of radioactivity present within 1.0 ml blood. This value represents the lung permeability value.

*Cobra venom factor (CVF)*[1]. This was isolated from crude lyophilized cobra (*Naja naja*) venom by ion-exchange chromatography and gel filtration (11). When used, an amount of 80 μg (containing 4 inhibitor units) was injected intravenously (12). This resulted in the complete abolition of $CH_{50}$ hemolytic activity (<5 $CH_{50}$ U/ml serum) within 2–3 min after intravenous infusions of CVF. In some experiments the CVF was initially treated with *p*-bromophenacyl bromide (BPB) to inactivate the contaminating phospholipase $A_2$ activity, followed by extensive dialysis, as described by Shaw et al. (13). For in vivo comparisons, acute lung injury after the intravenous infusion of CVF (before or after treatment with BPB) was compared with effects on lung of infused phospholipase $A_2$ that had been isolated from cobra venom (Sigma Chemical Co., St. Louis, MO.). Phospholipase $A_2$ activity was measured by the rate of hydrolysis of lecithin, according to procedures described in the Worthington Manual (Worthington Biochemical Corp., Freehold, NJ).

*Analysis of chemotactic activity.* This was determined according to the depth of penetration within micropore filters of rat peritoneal neutrophils suspended in Hanks' medium containing 0.1% bovine serum albumin. For these studies, 50 μl serum was diluted to 1.0 ml in Hanks'-Tris-buffered (pH 7.4) solution and applied to the lower compartment of a chemotaxis chamber. Using the leading front method, we measured the depth of penetration of migrating cells into micropore filters, according to the technique of Zigmond and Hirsch (14).

*Blood leukocyte counts.* We assessed these in the usual manner, using both total cell counts as determined in hemocytometers as well as cell differential counts as determined by methanol-fixed and stained (Wright's) blood smears.

*Inhibitors.* Various reagents were used to determine any protective effect against lung injury produced by the intravascular injection of CVF. Neutrophil depletion was accomplished by the intraperitoneal injection 18 h earlier of rabbit serum (1.0 ml/rat) containing antibody to rat neutrophils (9). The treatment of animals in this fashion resulted in a profound neutropenia, with <500 neutrophils/mm$^3$ blood. This antiserum did not reduce the platelet counts in blood. Companion control animals were treated with the same volume of normal rabbit serum, which did not result in a neutropenia. Antihistamine treatment included the intraperitoneal injection 30 min earlier of 25 mg diphenhydramine hydrochloride (Parke Davis & Co.), which over a 30-min period of observation ablated the vasopermeability response in rats after the intradermal injection of 20 μg histamine. When catalase (Sigma Chemical Co.) was used, 20 or 40 mg (10,000 U/mg) were injected intravenously together with 0.5 ml PBS containing the CVF. Superoxide dismutase (Diagnostic Data, Inc., Mountain View, CA) in an amount of 10 or 30 mg (4,000 U/mg) was similarly dissolved in PBS and injected intravenously. The characteristics of these last two reagents have been described in considerable detail in a recent communication (15).

## RESULTS

*Acute lung injury associated with intravascular complement activation.* In four negative control animals and eight positive control animals a volume of 0.5 ml PBS (pH 7.4) or a similar volume of PBS containing 80 μg (4 U of complement inactivator) CVF was injected intravenously via a penile vein. Together with these materials, 1 μg rat IgG (containing 1 μCi $^{125}$I) was also injected intravenously. Animals were killed 30 min later, and the lung-associated radioactivity was measured (after perfusion of the lung vasculature with saline) and calculated as a ratio to the amount of radioactivity present in 1.0 ml blood obtained at the time of sacrifice. As the data in Table I indicate, there was an increase in the lung vascular permeability values over the 30-min interval ranging from a 2- to a 3.5-fold increase when compared with the values from animals receiving an intravenous injection of PBS. In the latter group no increase in lung permeability occurred during the same interval of time. Morphologically, the major changes in animals undergoing complement activation in vivo were related to an intrapulmonary, intracapillary sequestration of neutrophils along interstitial capillary channels (Fig. 1A). In contrast, the lungs of PBS-infused rats failed to show any evidence of intravascular aggregates of neutrophils (Fig. 1B). To obtain quantitative estimates of differences in numbers of neutrophils in vessels of lungs, sections similar to those shown in Fig. 1 were obtained from eight control rats and eight rats

TABLE I
*Induction of Acute Lung Injury by Intravascular Complement Activation*

| Treatment | Lung injury | |
|---|---|---|
| | Individual values* | Mean±SEM |
| PBS | 0.17, 0.20, 0.23, 0.22 | 0.21±0.01 |
| CVF | 0.45, 0.51, 0.62, 0.44<br>0.66, 0.48, 0.68, 0.54 | 0.55±0.03 |

* Relative permeability value, calculated by the ratio of radioactivity remaining in PBS-perfused lungs to the amount of radioactivity present in 1.0 ml blood.

[1] *Abbreviations used in this paper:* BPB, *p*-bromophenacyl bromide; CVF, cobra venom factor; PBS, phosphate-buffered saline.

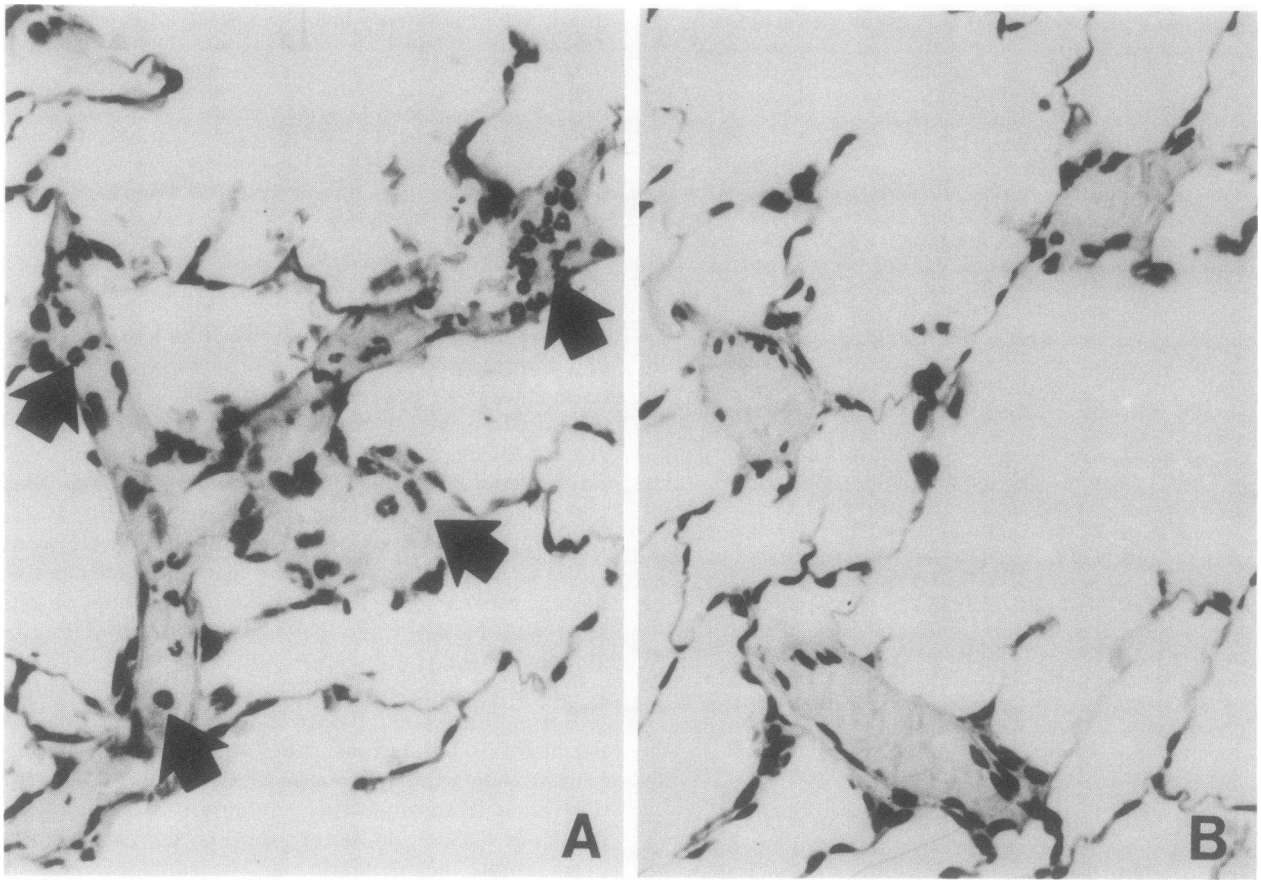


FIGURE 1 (A) Lung from a rat that 30 min earlier received an intravenous injection of 80 μg CVF. The interstitial capillaries are dilated and contain large numbers of neutrophils. (B) Lung from rat injected intravenously with PBS. No intravascular accumulates of neutrophils are present (Hematoxylin and eosin, ×130).

injected with CVF. Three lung sections per animal were examined. The numbers of neutrophils in the control rats were 0.8±0.25 (SEM), whereas in the complement-activated rats these were 29.1±2.0 cells per field. These differences reinforce the morphological observations described above. In animals injected with CVF, there was also focal intraalveolar hemorrhage, which was never seen in the animals infused with PBS. By transmission electron microscopy, the outstanding features noted in animals 30 min after the intravenous infusion of CVF were the plugging of pulmonary capillaries with neutrophils and the presence of erythrocytes and fibrin (identified by the characteristic periodicity of its fibrils) deposits within alveolar spaces and in perivascular locations (Fig. 2A). Alveolar lining epithelial cells appeared intact. Under higher resolution, structural defects in capillary walls could be detected in areas in which vessel lumina were plugged with neutrophils. In such areas the endothelial cell lining was discontinuous, which resulted in direct contact of neutrophils with vascular basement membrane. In these areas fibrin deposits were often found on the extraluminal side of the capillary walls (Fig. 2B).

In other tissue sections, clear evidence of endothelial cell damage and/or destruction was apparent. Complete loss of endothelial cell lining of capillaries occurred in some areas with the presence of disintegrated endothelial cells (Fig. 3). In these areas it also appeared that the vascular basement membrane was discontinuous. By 4 h we were unable to detect evidence either of leukoaggregates within the pulmonary vasculature or of endothelial cell injury.

*Inability of phospholipase $A_2$ to induce lung injury.* In view of an earlier report describing lung injury produced by the airway instillation of CVF that contained phospholipase $A_2$ activity (13), experiments were designed to determine whether the acute lung injury produced by the intravenous infusion of CVF could be related to the presence of contaminating phospholipase activity. As expected, the infusion of 4

CVF (containing 10.4 U phospholipase $A_2$) produced a mean lung permeability value of 0.62±0.02 as compared with the value obtained in animals receiving an infusion of PBS (0.16±0.03) (Table II). In the former group the serum $CH_{50}$ values were unmeasurable (<5 U/ml) at the time of sacrifice (30 min). The prior heating (80°C, 2 min) of the CVF preparation abolished the complement inactivator activity and caused a slight increase in the phospholipase activity (13.1 U) in the preparation. Animals treated with this material failed to develop evidence of complement reduction, and there was no evidence of lung injury (lung permeability value of 0.12±0.02). CVF pretreated with BPB was devoid of measurable phospholipase $A_2$ activity. The infusion of this material resulted in abolition of detectable serum complement activity; this preparation was fully active in producing lung injury (lung permeability value of 0.66±0.05). The infusion of 12 U purified phospholipase $A_2$ did not reduce the serum level of complement and failed to produce evidence of lung injury (lung permeability value of 0.16±0.03). These data indicate that acute lung injury produced by the infusion of CVF is not related to the presence of contaminating phospholipase $A_2$ activity.

*Time-course of lung injury, neutropenia, and in vivo generation of chemotactic activity.* The time-course of lung injury after the intravascular infusion of CVF was studied over a 4-h period. In all experiments described in Fig. 4, five animals were studied at each point. As demonstrated in Fig. 4 (upper frame), significant evidence of lung injury was first seen at 15 min, reaching a peak at the 30-min interval, followed by a progressive decline over the next 3.5 h. By the end of this period of time the lung permeability value had returned to the normal range, which suggests that the increase in lung permeability is a reversible phenomenon. In a companion series of animals, the intravenous infusion of PBS did not result in evidence of increased lung permeability (Fig. 4, upper frame).

Sera were obtained sequentially from animals over a 1-h period. Each animal served as its own control, with sequential bleedings after the infusion of 0.5 ml PBS, followed 2 d later by injection of CVF and serial bleedings. In animals sampled over a 1-h period after the infusion of PBS, no chemotactic activity was detected in the serum samples (Fig. 4, middle frame). In striking contrast, after the intravenous infusion of CVF, a sharp increase in the serum levels of chemotactic activity was found during the first 5 min, with a rapid disappearance of the serum-associated chemotactic activity by 15 min. When serum containing chemotactic activity generated after contact with CVF was examined immunochemically, antibody to human C5a (produced in our own laboratories) blocked the activity. For instance, 10 μl antibody blocked 50% of the chemotactic activity (assessed by the modified Boyden chamber technique) in 1.0 ml serum, whereas 25 μl antibody totally blocked the chemotactic activity. These data indicate that CVF in rat serum generates a chemotactic fragment that is immunochemically closely related to human C5a.

As would be predicted, a converse relationship was found between the presence of chemotactic activity appearing in the serum (Fig. 4, middle frame) and the concentration of neutrophils in the blood (Fig. 4, bottom frame). Indeed, few if any neutrophils could be detected in blood samples 5 min after the intravenous infusion of CVF. By 15 min, blood neutrophils had returned to normal levels, only to increase at the 20-min interval to a level that was ~25-fold above the initial level. After this period of time, there was a gradual reduction toward normal levels, which were

TABLE II
*Inability of Phospholipase $A_2$ to Produce Acute Lung Injury*

| Material infused intravenously* | Complement inactivator activity‡ | Phospholipase $A_2$ activity§ | Serum $CH_{50}$‖ | Lung injury (permeability)¶ |
|---|---|---|---|---|
| | *U* | | *mean±SEM* | |
| CVF | 4 | 10.4 | <5 | 0.62±0.02 |
| CVF, 80°C, 2 min | <1 | 13.1 | 141±4.5 | 0.12±0.02 |
| CVF, pretreated with BPB | 4 | <0.01 | <5 | 0.66±0.05 |
| Phospholipase $A_2$ | <1 | 12.0 | 159±7.6 | 0.15±0.03 |

* All materials were injected in a total volume of 0.5 ml. Animals were killed 30 min later. In each group, four animals were used.
‡ Defined according to reference 12.
§ Amount of titratable acidity released from lecithin, at pH 8.9, 25°C. 1 U enzyme liberates 1 μmol fatty acid/min.
‖ Whole, hemolytic complement activity in serum obtained at time of death.
¶ Leakage of $^{125}$I-rat IgG into lung parenchyma. See text for details.

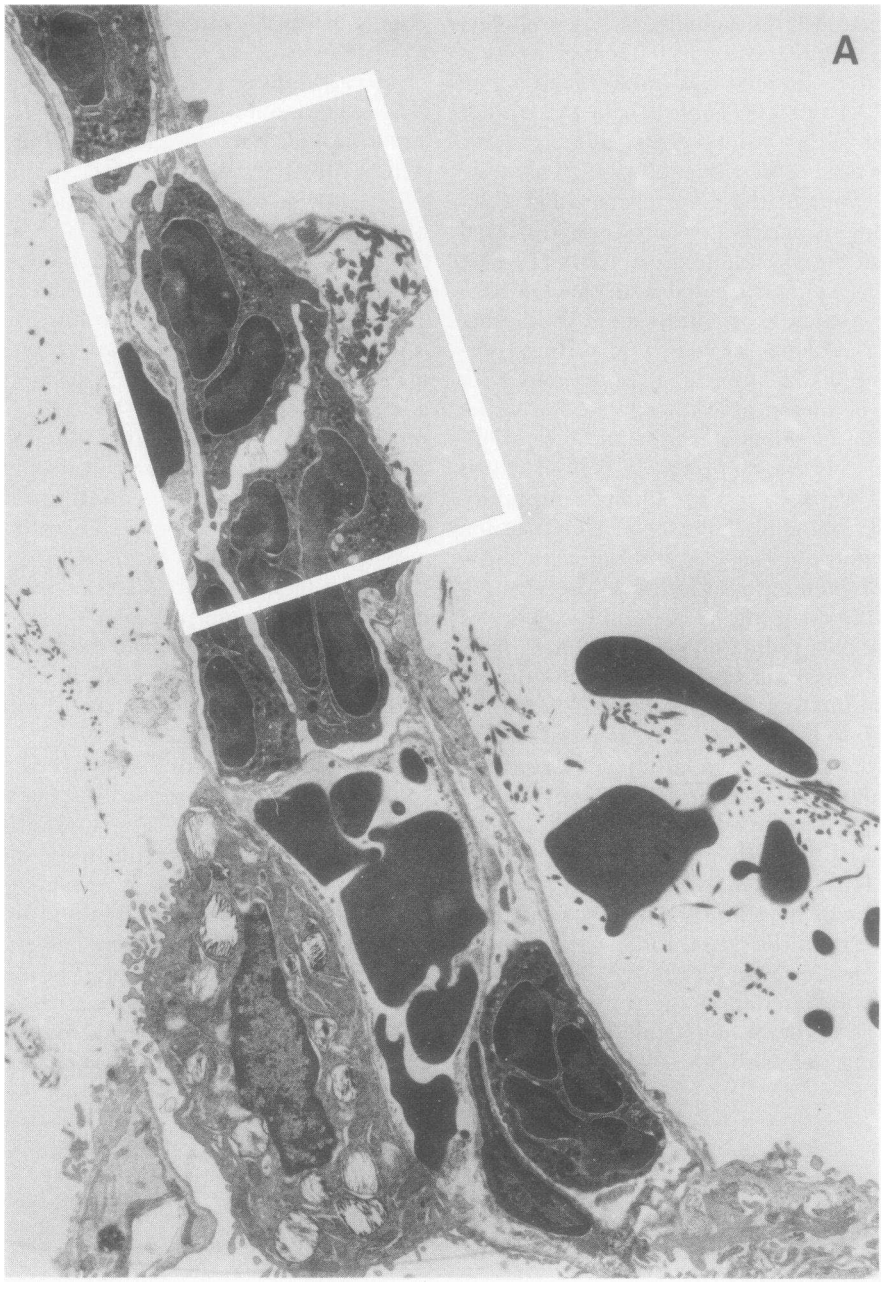


FIGURE 2 (A) Transmission electron micrograph of lung from rat 30 min after the intravenous injection of CVF. The septal capillaries are filled with neutrophils that are in intimate contact with vascular walls. Erythrocytes and fibrin deposits are present within alveolar spaces, but alveolar lining (type II) cells are well preserved, with typical microvilli projecting from their surfaces (×3,000). (B) The inset from (A) shows an area in which the endothelial cell lining is absent and neutrophils (as indicated by arrows) are in direct contact with vascular basement membrane. Fibrin deposits are present on the extraluminal surface of the capillary wall (×8,750).

reached by the 4th hour. Changes in numbers of blood mononuclear cells (monocytes and lymphocytes) after the intravenous infusion of CVF were far less impressive. No initial drop was found; within 20 min the number of blood mononuclear cells was approximately double the starting level, slowly returning towards

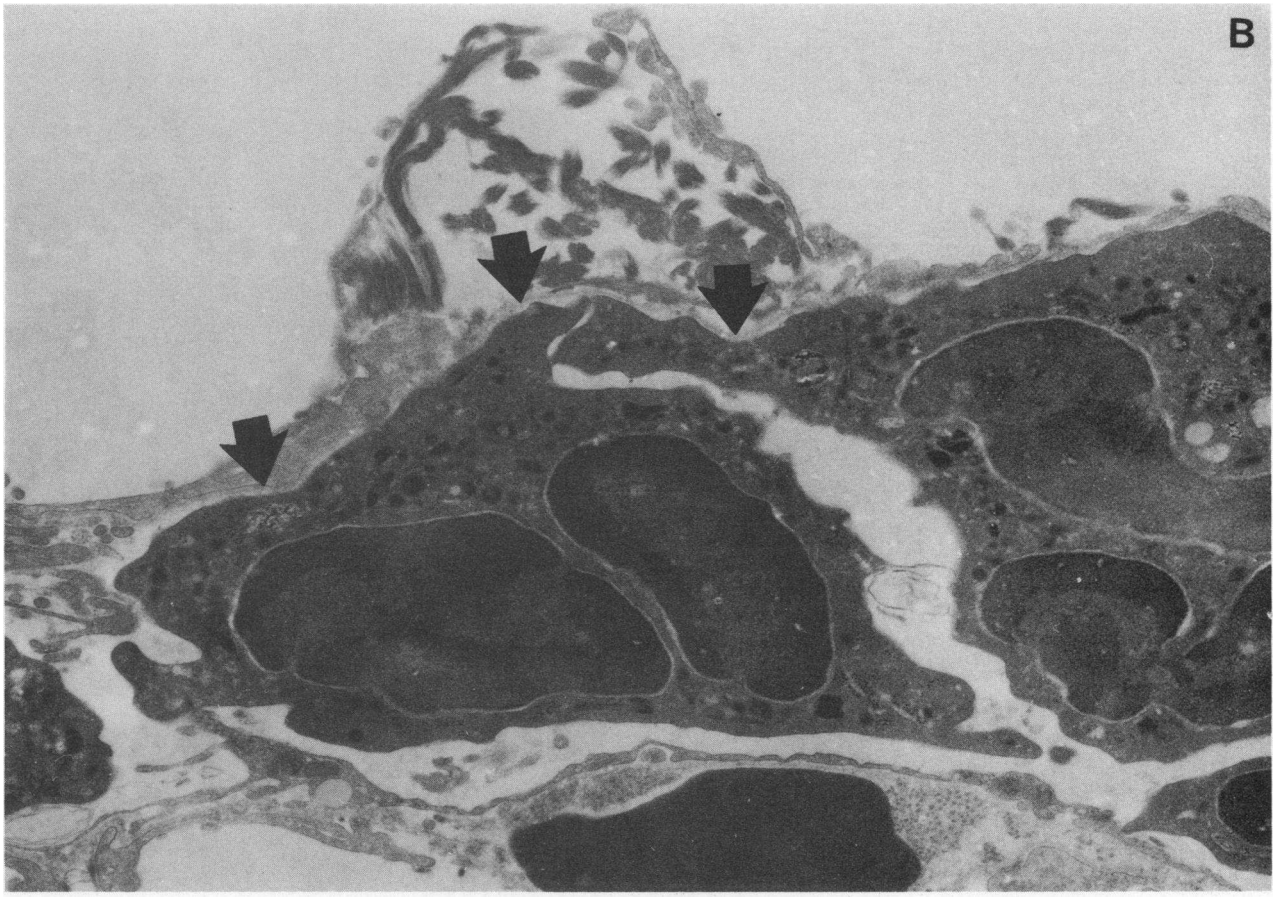


FIGURE 2 (*Continued*)

normal counts by the 2nd hour. The data in this study demonstrate the association between the intravascular generation of chemotactic activity, the development of neutropenia, and subsequently the appearance of lung injury.

*Inhibition of acute lung injury.* Several manipulations were undertaken to assess the mediators involved in lung injury occurring 30 min after the intravenous infusion of the CVF. This interval of time was selected since it represents the point of maximal lung injury (Fig. 4). The data for these experiments are shown in Table III. As expected, animals injected with 80 μg CVF showed a mean threefold increase in lung vascular permeability (0.66±0.05 as compared with the 0.22±0.02 value for the PBS-infused animals). Individual permeability values in the cobra venom factor-treated group increased over a range of 2.0- to 4.5-fold above the values for the PBS-injected group. The prior intraperitoneal treatment (30 min before infusion of the CVF) of rats with 25 mg diphenhydramine hydrochloride led to limited protection (16%) from the lung injury. In contrast, prior depletion of neutrophils (<500 neutrophils/$mm^3$ blood) by the use of specific antibody caused a marked reduction (68% protection) in the extent of lung injury after intravascular complement activation. The combined effects of neutrophil depletion and antihistamine treatment appeared to be additive, leading to 84% protection from acute lung damage. The simultaneous intravascular infusion of 20 or 40 mg catalase together with the CVF produced 44 and 66% protection, respectively, from acute lung injury. The treatment of rats with 10 or 30 mg superoxide dismutase resulted in 25 and 53% protection, respectively, from acute lung injury. In experiments designed to determine the effects of the simultaneous administration of 20 mg catalase and 10 mg superoxide dismutase, the infusion of both reagents into four animals provided 45% protection, whereas the protection afforded by the same dose of catalase alone or superoxide dismutase alone (infused into two

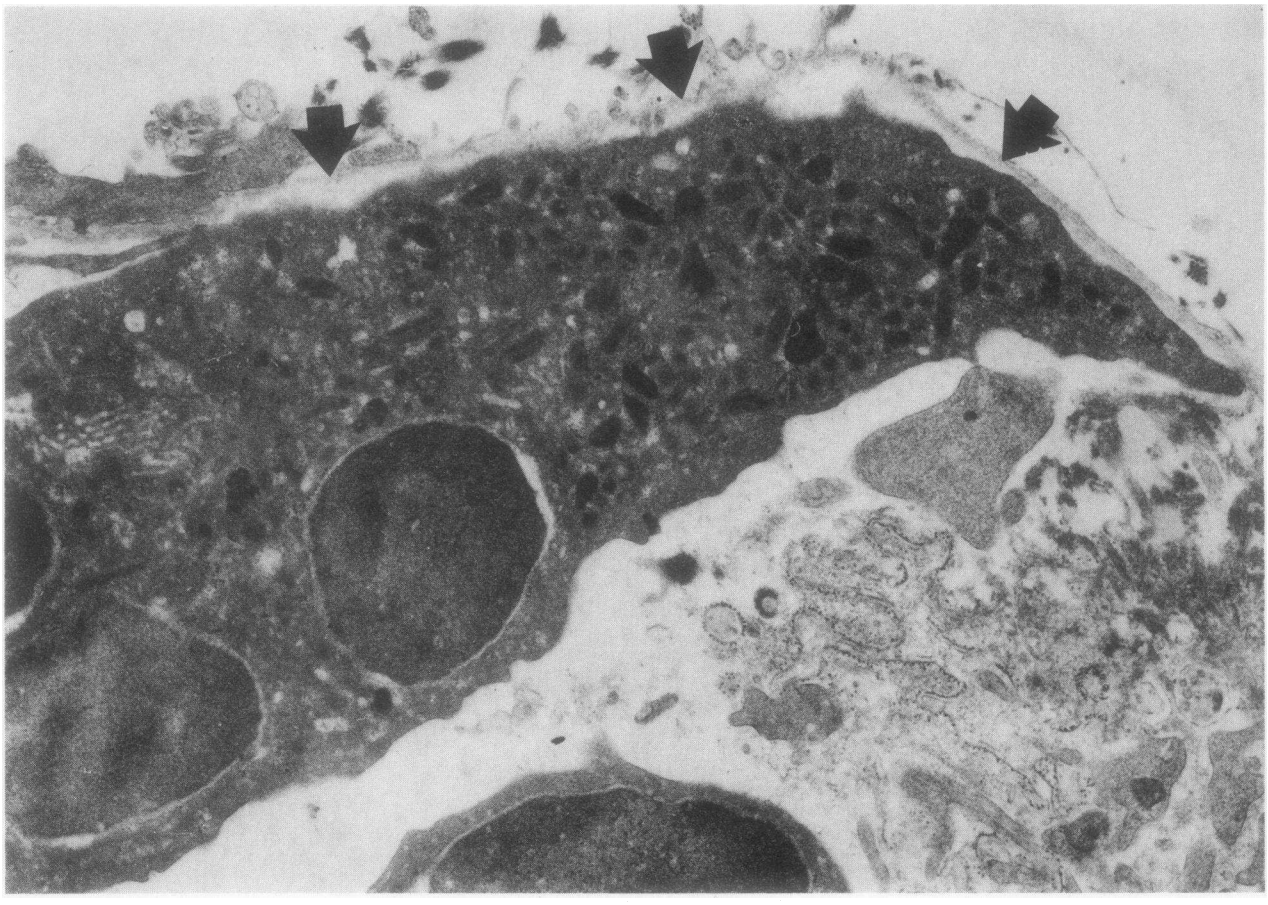

FIGURE 3 Transmission electron micrograph of lung from animal treated similarly to that described in Fig. 2. Neutrophils (as indicated by arrows) are in direct contact with pulmonary capillary walls, which, in some areas, no longer contain an endothelial cell covering. In these areas fibrin deposits are present on the extraluminal surface of the capillary wall. In one area the capillary wall is disrupted by the presence of cell debris, which intrudes into the vessel lumen (×15,550).

groups of four rats each) was 35 and 27%, respectively. Thus, it would appear that the combined administration produces a greater effect than either inhibitor infused alone. These data indicate the neutrophil dependence of lung injury associated with intravascular activation of the complement system, and further suggest the likelihood that oxygen-dependent products ($O_2^-$, $H_2O_2$ and/or derivatives of these materials) from neutrophils are responsible for the pathogenesis of the lung vascular injury.

## DISCUSSION

The data presented in this paper document that intravascular activation of the complement system can lead to acute lung injury, as defined by an increase in lung vascular permeability. In addition, the data provide direct morphological evidence of injury to endothelial cells in the lung capillaries. The limited duration of the increased lung permeability changes (Fig. 4) suggests that the endothelial cell injury is reversible. This is reminiscent of the recent report by Reidy and Schwartz (16), in which injury to rat aortic endothelial cells results in a rapid restoration of the endothelial cell lining in the absence of a proliferative response of the surviving endothelial cells. It is possible that endothelial cells adjacent to areas of injury migrate into denuded areas, hereby covering the defects. This is in contrast to the persistent defects in the endothelial cell covering when the area of injury and denudation involves a much wider zone of endothelium (16). The injury described in our studies is similar to the first example, where focal injury related to the presence of neutrophils has been observed.

Recently, Henson et al. (17) have presented evidence that intravascular activation of the complement system produces lung injury, provided the lung has first been subjected to an insult such as anoxia. On the other hand, others have provided evidence that the intravascular infusion of complement-activated serum or C5 activation products directly results in acute lung injury (18, 19). Although Shaw et al. (13) have concluded that airway instillation of CVF produces acute lung injury that is due to the action of contaminating phospholipase $A_2$ and is not related to the complement activating activity of the preparation, our studies show that the converse is the case. This is not particularly surprising, in that our experiments have featured intravascular (intravenous) infusion, whereas the experiments of Shaw et al. (13) involved airway instillation of the CVF, which in the latter case would permit the phospholipase to interact directly with lung cells.

Further support of the role of complement-activation products in pulmonary dysfunction states is found in the recent report by Chenoweth et al. (20) demonstrating the presence of C3- and C5-derived anaphylatoxins in the plasma of patients undergoing cardiopulmonary bypass. Similarly, C5a has been reported in the plasma of patients with acute respiratory distress syndrome (21). In these patients, evidence of neutrophil sequestration in the pulmonary vascular bed has also been obtained. In addition to this type of evidence has been the finding in patients with acute respiratory distress syndrome of the presence in bronchoalveolar fluids of inactive $\alpha_1$-antitrypsin and the presence of free elastase activity (22). These data have been interpreted to suggest that oxygen-derived free radicals, perhaps produced by complement-activated leukocytes, have brought about oxidation of the critical methionyl residue in $\alpha_1$-antitrypsin, leading to functional inactivation of the antiprotease and the subsequent loss of the protease shield within the lung (23).

Experimentally, the concentration of chemotactic stimulus required to cause activation and release of ~50% of total lysosomal enzyme in human neutrophils is ~100 times less than the concentration of chemotactic stimulus producing a similar response in rat neutrophils.[2] Accordingly, if lung injury occurs in humans owing to intravascular activation of complement, much less activation of the complement system may be required than was implied by the experiments in rats.

In our experimental model, it should be emphasized that lung injury occurs only after the initial, very early finding in serum of chemotactic activity, which is coupled with the simultaneous onset of profound neutropenia. The neutropenia can be readily explained by intravascular leukoagglutination and sequestration of aggregated cells within the interstitial pulmonary capillaries after contact with C5a. It seems likely that the most important effect of the intravascular injection of CVF is the production of chemotactic (C5-related) peptides that directly stimulate blood neutrophils, which would result in their activation and production of oxygen metabolites including $O_2^-$ and $H_2O_2$. The ability of superoxide dismutase and catalase to protect the lung vasculature from injury provides strong evi-

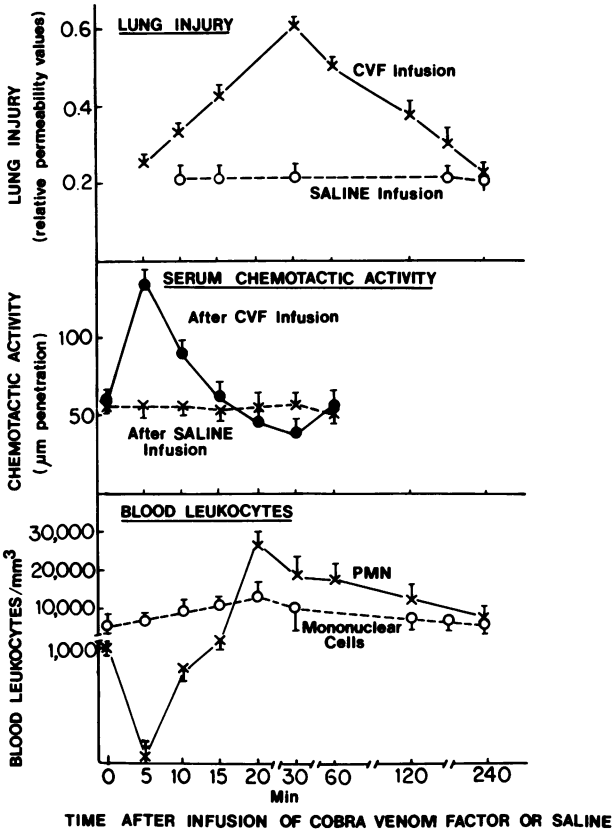


FIGURE 4 Composite of changes in the blood and lungs of rats after the intravenous infusion of CVF or PBS. For each point in the figure the standard error of the mean is shown. Upper frame, changes in lung vascular permeability. Middle frame, chemotactic activity in the serum of rats after the intravenous infusions of CVF or PBS. Lower frame, changes in blood counts of neutrophils and mononuclear cells (monocytes and lymphocytes) after the intravenous infusion of CVF. Mean values are shown. Although not shown in the frame, values for standard errors of the means did not vary from the mean values by > 10%. After the intravenous infusion of PBS, no significant changes in numbers of either blood neutrophils or mononuclear cells were found. PMN, polymorphonuclear leukocytes; Saline, PBS.

[2] Marasco, W., J. C. Fantone, and P. A. Ward. Unpublished data.

TABLE III

*Protection from Lung Injury Produced by Intravascular Complement Activation*

| Treatment* | No. animals | Lung injury (permeability)‡ Individual values | Mean±SEM | Protection§ |
|---|---|---|---|---|
| | | | | % |
| PBS | 4 | 0.20, 0.19, 0.23, 0.28 | 0.22±0.02 | |
| CVF | 10 | 0.69, 0.60, 0.91, 0.46, 0.86, 0.67, 0.46, 0.61, 0.63, 0.79 | 0.66±0.05 | |
| CVF + antihistamine | 5 | 0.49, 0.37, 0.54, 0.53, 0.59 | 0.59±0.09 | 16 ($P < 0.05$)‖ |
| CVF + neutrophil depletion | 9 | 0.42, 0.41, 0.41, 0.23, 0.47, 0.21, 0.35, 0.54, 0.34 | 0.36±0.03 | 68 ($P < 0.001$) |
| CVF + neutrophil depletion + antihistamine | 5 | 0.18, 0.33, 0.24, 0.34, 0.34 | 0.29±0.03 | 84 ($P < 0.001$) |
| CVF + catalase | | | | |
| 20 mg | 3 | 0.40, 0.58, 0.42 | 0.47±0.0 | 44 ($P > 0.05$) |
| 40 mg | 5 | 0.30, 0.41, 0.32, 0.45, 0.35 | 0.37±0.03 | 66 ($P < 0.01$) |
| CVF + superoxide dismutase | | | | |
| 10 mg | 4 | 0.57, 0.68, 0.50, 0.45 | 0.55±0.04 | 25 ($P > 0.05$) |
| 30 mg | 6 | 0.31, 0.42, 0.50, 0.41, 0.46 | 0.43±0.03 | 53 ($P < 0.01$) |

* See text for details. All materials were infused intravenously at time 0, except for neutrophil depletion, which was accomplished by the intraperitoneal injection of specific antibody 18 h earlier. Antihistamine treatment commenced 30 min before the intravascular infusion of CVF.

‡ Amount of lung-associated radioactivity as compared with that present in blood obtained at the time of death. Calculations are described in text.

§ Reduction in lung permeability calculated by the ratio of the lung injury value (corrected, by subtraction, for the PBS values) in the experimental group to the corrected value (0.44) in the CVF group, ×100.

‖ Student's *t* test values, all compared with the CVF treatment alone.

dence that production of $O_2^-$ and $H_2O_2$ (and/or their conversion products) from leukocytes is the most proximate event related to the vascular injury. Leukocytes in direct contact with pulmonary vascular walls would therefore be likely to generate these potentially toxic products, which could lead to damage of endothelial cells and/or the underlying basement membrane, resulting in an increase in vascular permeability. The findings described in this paper may have broad significance in view of the increasing body of clinical evidence that relates complement activation to the acute respiratory distress syndrome and other examples of acute pulmonary dysfunction.

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## REFERENCES

1. Craddock, P. R., J. Fehr, A. P. Dalmasso, K. L. Brigham, and H. S. Jacob. 1977. Hemodialysis leukopenia. Pulmonary vascular leukostasis resulting from complement activation by dialyzer cellophane membranes. *J. Clin. Invest.* **59:** 879–888.
2. Haslam, P. L., P. J. Townsend, and M. A. Branthwaite. 1980. Complement activation during cardiopulmonary bypass. *Anaesthesia.* **25:** 22–26.
3. Nusbacher, J., S. I. Rosenfeld, J. L. MacPherson, P. A. Thiem, and J. P. Leddy. 1978. Nylon fiber leukapheresis-associated complement component changes and granulocytopenia. *Blood.* **51:** 359–365.
4. O'Flaherty, J. T., D. L. Kreutzer, and P. A. Ward. 1977. Neutrophil aggregation and swelling induced by chemotactic agents. *J. Immunol.* **119:** 232–239.
5. O'Flaherty, J. T., P. R. Craddock, and H. S. Jacob. 1978. Effect of intravascular complement activation on granulocyte adhesiveness and distribution. *Blood.* **51:** 731–739.
6. Craddock, P. R., D. E. Hammerschmidt, C. F. Moldow, O. Yamada, and H. S. Jacob. 1979. Granulocyte aggregation as a manifestation of membrane interactions with complement: possible role in leukocyte margination,

micro-vascular occlusion, and endothelial damage. *Semin. Hematol.* **16:** 140–147.
7. O'Flaherty, J. T., H. Showell, and P. A. Ward. 1977. Neutropenia induced by systemic infusion of chemotactic factors. *J. Immunol.* **118:** 1586–1589.
8. Sachs, T., C. F. Moldow, P. R. Craddock, T. K. Bowers, and H. S. Jacob. 1978. Oxygen radical mediated endothelial cell damage by complement-stimulated granulocytes. An in vitro model of immune vascular damage. *J. Clin. Invest.* **61:** 1161–1167.
9. Johnson, K. J., and P. A. Ward. 1974. Acute immunologic pulmonary alveolitis. *J. Clin. Invest.* **54:** 349–357.
10. Johnson, K. J., and P. A. Ward. 1981. Role of oxygen metabolites in immune complex injury of lung. *J. Immunol.* **126:** 2365–2369.
11. Pepys, M. B. 1976. Role of complement in the induction of immunological responses. *Transplant. Rev.* **32:** 93–120.
12. Ballow, M., and C. G. Cochrane. 1969. Two anticomplementary factors in cobra venom: hemolysis of guinea pig erythrocytes by one of them. *J. Immunol.* **103:** 944–952.
13. Shaw, J. O., M. F. Roberts, R. J. Ulevitch, P. Henson, and E. A. Dennis. 1978. Phospholipase $A_2$ contamination of cobra venom factor preparations. Biologic role in complement-dependent in vivo reactions and inactivation with p-bromophenacyl bromide. *Am. J. Pathol.* **91:** 517–530.
14. Zigmond, S. H., and J. G. Hirsch. 1973. Leukocyte locomotion and chemotaxis. New methods for evaluation and demonstration of cell-derived chemotactic factor. *J. Exp. Med.* **137:** 387–410.
15. Johnson, K. J., J. C. Fantone, III, J. Kaplan, and P. A. Ward. 1981. In vivo damage of rat lungs by oxygen metabolites. *J. Clin. Invest.* **67:** 983–993.
16. Reidy, M. A., and S. M. Schwartz. 1981. Endothelial regeneration. III. Time course of intimal changes after small defined injury to rat aortic endothelium. *Lab. Invest.* **44:** 301–308.
17. Henson, P. M., G. L. Larsen, R. O. Webster, B. C. Mitchell, A. J. Goins, and J. E. Henson. Pulmonary microvascular alterations and injury induced by complement fragments: synergistic effect of complement activation, neutrophil sequestration and prostaglandins. *Ann. N. Y. Acad. Sci.* In press.
18. Hohn, D. C., A. J. Meyers, S. T. Gherini, A. Beckman, R. E. Morkison, and A. M. Chung. 1980. Production of acute pulmonary injury by leukocytes and activated complement. *Surgery (St. Louis).* **88:** 48–58.
19. Hosea, S., E. Brown, C. Hammer, and M. Frank. 1980. Role of complement activation in a model of adult respiratory distress syndrome. *J. Clin. Invest.* **66:** 375–382.
20. Chenoweth, E. D., S. W. Cooper, T. E. Hugli, R. W. Stewart, E. H. Blackstone, and J. W. Kirklin. 1981. Complement activation during cardiopulmonary bypass. Evidence for generation of C3a and C5a anaphylatoxins. *N. Engl. J. Med.* **304:** 497–503.
21. Hammerschmidt, D. E., L. J. Weaver, L. D. Hudson, P. R. Craddock, and H. S. Jacob. 1980. Association of complement activation and elevated plasma-C5a with adult respiratory distress syndrome: pathophysiological relevance and possible prognostic value. *Lancet.* **I:** 947–949.
22. Lee, C. T., A. M. Fein, M. Lippmann, H. Holtzman, P. Kimbel, and G. Weinbaum. 1981. Elastolytic activity in pulmonary lavage fluid from patients with adult respiratory-distress syndrome. *N. Engl. J. Med.* **304:** 192–196.
23. Carp, H., and A. Janoff. 1979. In vitro suppression of serum elastase-inhibitory capacity by reactive oxygen species generated by phagocytosing polymorphonuclear leukocytes. *J. Clin. Invest.* **63:** 793–797.