The Effect of a Single Infusion of Zymosan-Activated Plasma on the Pulmonary Microcirculation of Sheep

Structure–Function Relationships

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Activation of the complement cascade is one of the mechanisms through which endotoxin may cause acute lung damage. The structural and functional changes following infusion of complement-activated plasma are described. In five anesthetized open-chest sheep, the authors monitored pulmonary and systemic artery pressures for 1 hour before and for 4 hours following the start of zymosan-activated plasma (ZAP) infusion (2 ml/min over a 20-minute period). Cardiac output, blood gases, and the number of circulating white cells were also measured. In addition, we took lung biopsy tissue at baseline, 7.5, 15, 30, 60, 120, 180, and 240 minutes following the start of infusion. Lung lymph flow and protein concentration were also monitored in 2 sheep. Following ZAP infusion there was an early phase of leukopenia and marked pulmonary hypertension, followed by a phase characterized by a modest increase in the flow of protein-rich lung lymph. By light microscopy pulmonary sequestration of granulocytes was evident just 7.5 minutes following the start of ZAP infusion. Peripheral lung granulocytes increased threefold above control values by 7.5 minutes, increasing to sevenfold by 30 minutes. Electron-microscopic studies showed that some of the granulocytes were disrupted, and specific and azurophilic granules were seen in the lumen. By 15 minutes endothelial damage was apparent, and intravascular monocytes were surrounded by a proteinaceous coat. Edema accumulation and an infiltration of inflammatory cells in the lungs' connective tissue regions increased to 2 hours. From 2 hours, lung injury was less marked, and the number of peripheral lung granulocytes, fewer. Sequestration of granulocytes occurred with the onset of pulmonary hypertension and leukopenia, and was most marked when lung injury was most severe. Transient endothelial damage and edema preceded the physiologic changes interpreted as an increase in pulmonary vascular permeability. Although pulmonary sequestration of granulocytes was at least as great as that with endotoxemia, unlike endotoxemia, ZAP caused only transient endothelial injury and modest changes in vascular permeability. (Am J Pathol 1984, 114:32–45)

A SINGLE INFUSION of *Escherichia coli* endotoxin into sheep causes an early and striking accumulation of both granulocytes and lymphocytes in the lungs' microcirculation. Later, persistent endothelial cell damage and disruption occurs.[1] Using anesthetized, open-chest sheep, we have shown a temporal relationship between the structural and functional changes during endotoxemia. Margination of leukocytes occurs during an initial phase of marked pulmonary hypertension,[2] and an increase in pulmonary resistance[3] and endothelial cell damage occurs, when there is an increase in "pulmonary vascular permeability."[2] One of the suggested mechanisms through which endotoxin causes these functional and structural changes is by activation of the alternative complement pathway.[4] Further, activation of the complement pathway has been suggested as a mechanism in the lung injury associated with dialysis[5] and in many experimental forms of the adult respiratory distress syndrome.[6,7]

The purpose of the present study was 1) to relate the time course of structural changes following a single infusion of complement-activated plasma to the physiologic changes and 2) to compare these changes with those we have described in sheep during

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endotoxemia. We took lung biopsy tissue sequentially over the 4-hour period following the start of the infusion of zymosan-activated plasma (ZAP) from open-chest anesthetized sheep, while monitoring pulmonary and systemic artery pressure, cardiac output, arterial blood white cell number, and blood gases.

Materials and Methods

Seven yearling sheep—5 experimental and 2 control—weighing between 36 and 40 kg were given sodium thiopental (Abbott Laboratories, Chicago, Ill) and anesthetized with a mixture of halothane (Fluothane, Ayerst Laboratories, Inc., New York, NY), 80% oxygen, nitrous oxide, and air. A 7 French Swan-Ganz thermodilution catheter was placed in the pulmonary artery, and a systemic arterial line into the aorta by way of the neck vessels. Pulmonary and systemic artery pressures were monitored with the use of strain gauges (Micron Instruments, Los Angeles, Calif) and an electronic recorder (Hewlett-Packard Model 7754B). Arterial blood was sampled for measurement of white cell number (Coulter Counter, Model ZBl, Coulter Electronics, Hialeah, Fla), blood gases, and pH (Instrumentation Blood Gas Analyzer, Model 510, Instrument Laboratories Inc., Lexington, Mass). In addition, differential counts of Wright's stained arterial blood were made. Cardiac output was measured by thermodilution with the use of a cardiac output computer (Model 9529A, Edwards Laboratories, Santa Ana, Calif). After catheter insertion, the sheep were placed on their backs, and the lungs were exposed by splitting of the sternum.

Biopsy Procedure

The lungs were inflated with air to a constant pressure of 35 cm water and a curved clamp placed around the piece of peripheral lung to undergo biopsy. The piece of lung enclosed by the clamp measured approximately 3 × 2 × 1 cm. A second clamp was placed behind the first, and the lung between the two clamps was cut. The biopsy tissue was placed immediately into 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2) for fixation. The biopsy site was sutured.

Zymosan-Activated Plasma

Autologous ZAP was produced on the day of experimentation. Heparinized (10 U/ml) sheep plasma was incubated at 37 C for 45 minutes with 10 mg/ml of zymosan (Sigma Chemicals Co., St. Louis, Mo). The zymosan was removed after incubation by centrifugation (2000*g* for 15 minutes) followed by passing the plasma through a Millipore filter (0.45-μ pore size)(Millipore Corp., Bedford, Mass).

Experimental Protocol

Five of the prepared sheep received a single infusion of 40 ml of autologous ZAP at a rate of 2 ml/min over a 20-minute period. The remaining 2 control sheep received a single infusion of 40 ml of autologous plasma over the same time period. Each sheep was monitored over a baseline period of 1 hour. At the end of this period baseline lung biopsy tissue was taken. Hemodynamics were monitored continuously over the ensuing 4 hours of the study, and biopsies were performed on the lungs 7.5, 15, 30, 60, 120, 180, and 240 minutes following the start of infusion. Lung biopsy tissue was taken from five different lobes of the lung, and the order in which the lobes underwent biopsy was varied between animals. When a lobe underwent biopsy twice, the second piece of tissue was taken as far as possible from the first.

In order to check that the structural changes were widespread throughout the lung, at the end of each experiment the lungs and trachea were removed intact and fixed by airway inflation with 10% formol-saline from a pressure of 35 cm of water. After 7 days of fixation at constant pressure, at least two blocks were taken from each lobe, one from the posterior region of the lobe and one from the anterior, for routine light-microscopic examination.

In order to confirm that our experimental procedure produced the changes in lung lymph that have been previously described[8,9] in 3 of the above sheep, we also monitored lung lymph flow and protein concentration. In 2 of the animals that received ZAP and 1 that received plasma alone, we placed neck lines, and we cannulated the caudal mediastinal lymph node[10] 1 week before the experiment was carried out. In these animals, lung lymph flow was assessed by measurement of the amount accumulated in a heparinized tube over a 15-minute period. In addition, lymph samples were pooled every half hour, aortic blood was drawn every 30 minutes and after centrifugation, protein concentration was determined by a modified biuret method[11] with an automated system (Autoanalyzer, Technicon Instruments, Tarrytown, NY).

Structural Studies

After an initial 5-minute fixation period in 2.5% glutaraldehyde, the clamp was removed from the biopsy tissue, and a slice was taken from the center. This slice was cut into cubes (2 cu mm) and fixed in glutaraldehyde for a further 90 minutes. After this

time, the blocks were rinsed three times in cacodylate buffer, left overnight at 4 C in the final rinse, postfixed in 1% osmium tetroxide, dehydrated, and embedded in Epon–araldite. One-micron sections were cut from each animal at each time period, stained with toluidine blue, and examined by light microscopy. Pale gold sections were cut, with the use of an LKB Ultrotome V and a diamond knife, from the block containing the most alveolar tissue. The sections were stained with 1% uranyl acetate in methanol followed by lead citrate and examined in a Philips EM-300 electron microscope at 60 kv.

After removal of the tissue slice for electron microscopy, the remainder of the tissue was placed in 10% formol–saline overnight, processed, and embedded in wax for routine light-microscopic examination. Five-micron sections were cut and stained with hematoxylin and eosin.

Quantitation of Peripheral Lung Granulocytes

Using an eyepiece reticule and a X 40 objective, we counted the number of granulocytes in peripheral lung tissue in at least 10 microscopic fields from each animal at each time studied. The number of alveoli in these fields was also counted. Each field contained only alveolar tissue and gave an area of 0.05 sq mm. The number of granulocytes per 100 alveoli was calculated and expressed as a percentage of baseline in each animal.

Statistics

Because there was no difference between the saline-treated controls from our endotoxin study[1] and the plasma-treated controls of the present study, these control results were combined. All variables between sheep receiving ZAP and controls were subjected to statistical analysis by the use of a split plot in time design—BMDP2V of the BMDP computer program series (analysis of variance and covariance with repeated measures).[12]

Results

Physiologic Studies

Pulmonary artery pressure increased early following the start of ZAP infusion, reaching a peak by just 7.5 minutes, but by 60 minutes had returned to the control values (Figure 1). The number of circulating arterial blood white cells also fell 7.5 minutes following the start of ZAP infusion, remained at this level at 15 minutes, and by 30 minutes was within the normal range. Differential counts of arterial blood showed that the reduction in peripheral blood white cells involved exclusively the granulocytes (percentage of granulocytes in control sheep: mean, 53 ± 7 (SE); ZAP-treated sheep 7.5 and 15 minutes following the start of infusion: mean, 9 ± 4 and 15 ± 6, respectively). From 7.5 minutes occasional band granulocytes were seen, the peak being at 30 minutes, when 3% (± 1.5) were band forms. Cardiac output showed no significant change throughout the period of study. Systemic artery pressure was reduced at each time studied, significantly so when compared with time-matched controls at 30 and 60 minutes and 2 hours ($P < 0.05$). Arterial Po_2 varied from animal to animal. In 3 animals it was reduced by approximately 50% throughout the study, and in the remaining 2 it was within the control range. Pco_2 was within the normal limits at all times studied.

The 2 sheep to which we gave ZAP and monitored pulmonary lymph flow and lymph/plasma protein concentration showed a response similar to that already described[8,9]; there was a minimal increase in lung lymph flow and protein concentration. At most, lymph flow showed an 80% increase during the 30 minutes following the start of ZAP infusion, although flow did remain elevated throughout the entire period of study (Figure 2). The lymph/plasma protein concentration in 1 sheep showed a gradual increase throughout the period of study (Figure 2); whereas in the other sheep the increase, although present, was more modest. We interpret these changes as a minimal increase in pulmonary vascular permeability.[13]

Infusion of autologous plasma into the 2 control sheep, including the one in which we followed lymph flow and protein concentration, caused no significant change in any of the variables monitored throughout the period of study. For this reason the plasma control data has been combined with our previous saline control data[1] (Figures 1, 2, and 4).

Structural Studies—Light Microscopy

The most striking change following the start of ZAP infusion, seen by light microscopy, was the marked accumulation and margination of granulocytes in the alveolar capillaries (Figure 3) and small arteries and veins. Quantitation of the number of granulocytes in peripheral lung tissue showed a threefold increase just 7.5 minutes following the start of infusion, increasing to sevenfold by 30 minutes (Figure 4). From 2 hours, the number of accumulated granulocytes was less than at the earlier times, but still three to four times baseline, and showed little variation throughout the remainder of study. These results are shown normalized to baseline in Figure 4.

From 30 minutes following the start of ZAP infu-

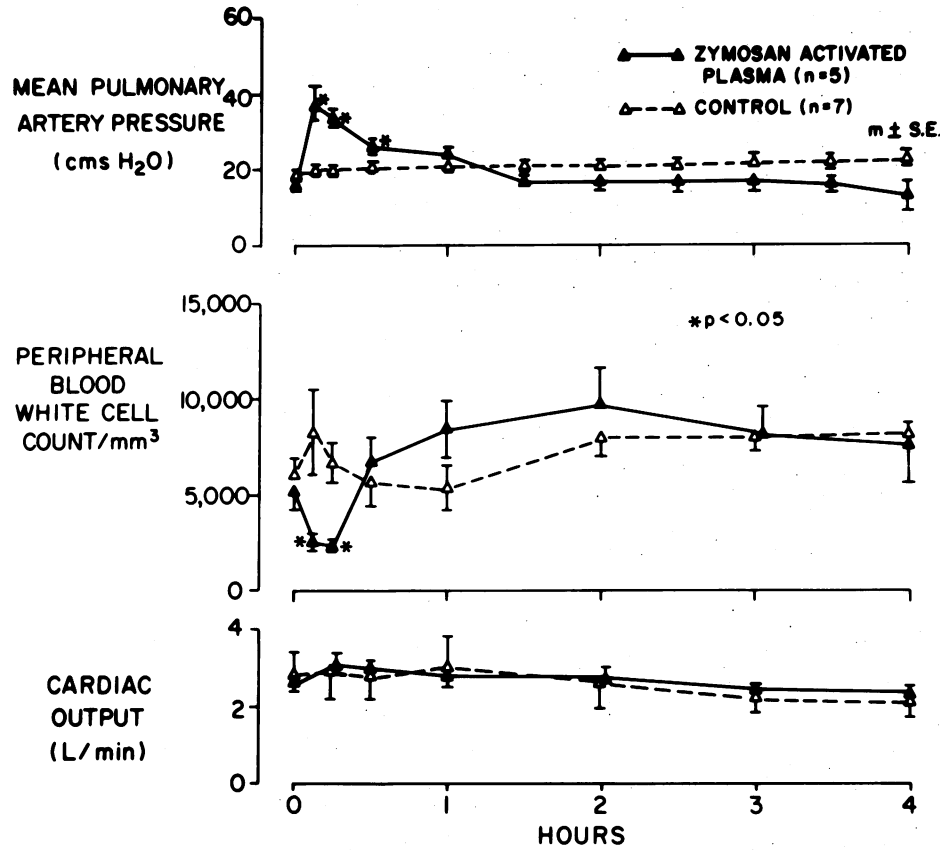


Figure 1—Effect of a single infusion of ZAP over a 4-hour period on pulmonary artery pressure, arterial blood white cell number, and cardiac output in anesthetized sheep. Analysis of variance for both intervention and time significant at the <0.001 level for pulmonary artery pressure and arterial white blood cell number: $*P < 0.05$—ZAP-treated animals compared with time-matched controls.

sion, the alveolar walls were thicker than normal, and pale pink staining edema fluid was seen in the perivascular spaces, subpleural connective tissue, and interlobular septa. Additionally, from 30 minutes, accompanying the appearance of edema in these regions, there was an infiltrate of mononuclear cells and granulocytes. Isolated groups of alveoli also contained pale-staining fluid. By 60 and 120 minutes, the cellular infiltrate and edema were more marked and the edema fluid was more eosinophilic than at 30 min-

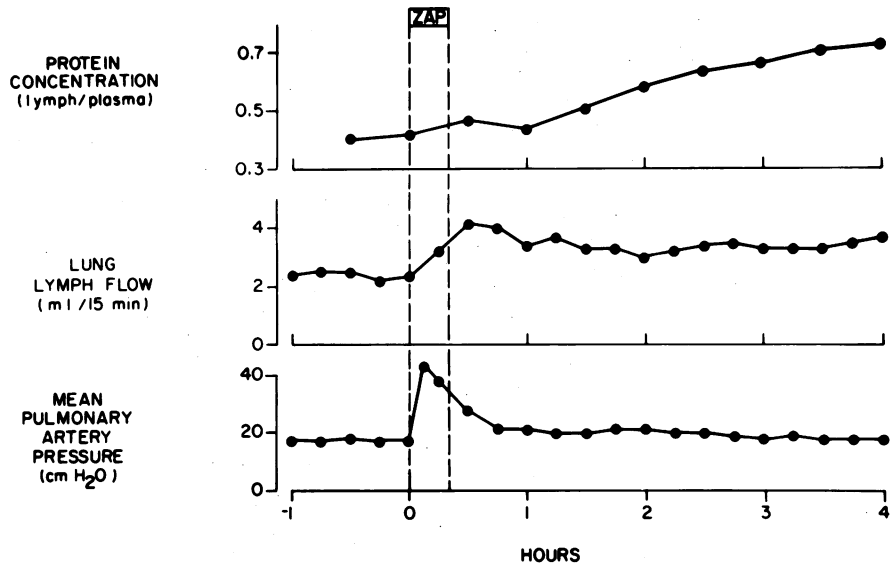


Figure 2—Effect of a single infusion of ZAP over a 4-hour period on lung lymph/plasma protein concentration ratio, lung lymph flow, and pulmonary artery pressure in an anesthetized sheep.

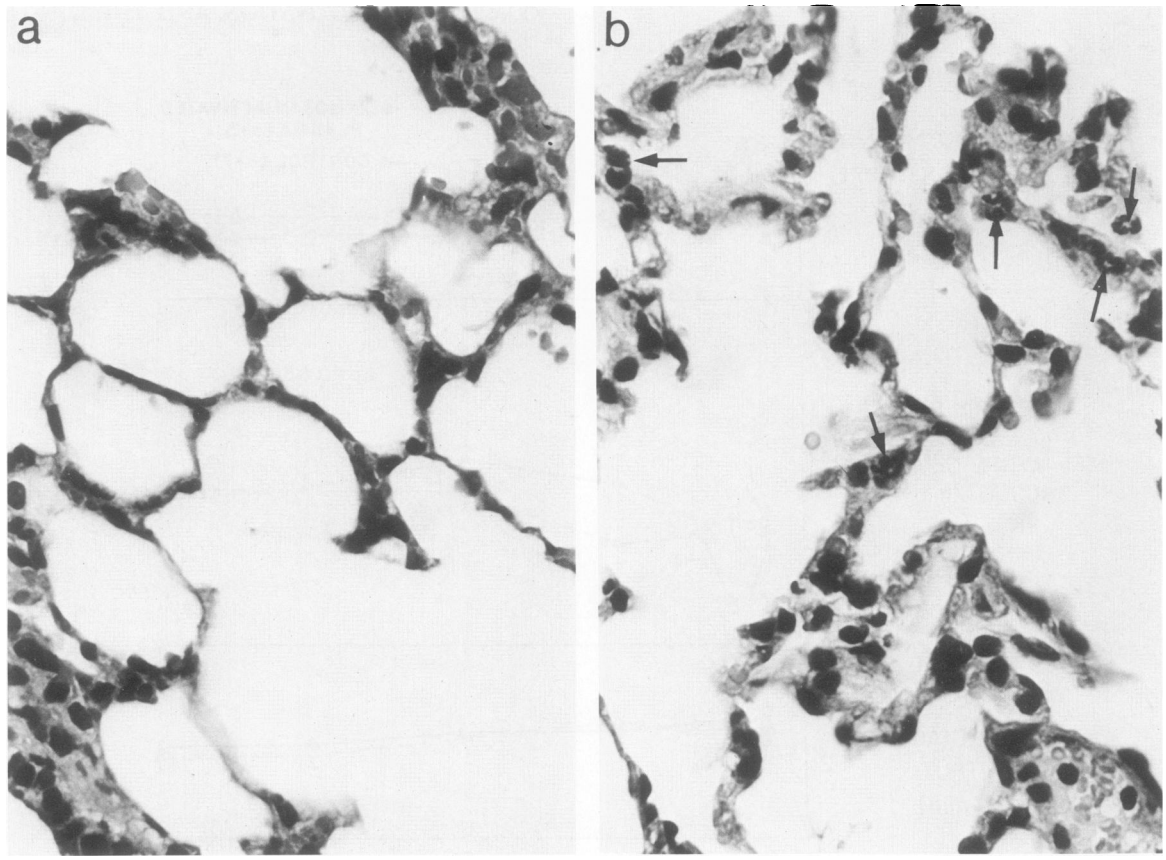


Figure 3 — Light micrographs of peripheral lung tissue obtained at biopsy. **a** — From a control sheep 60 minutes after start of plasma infusion. **b** — From a sheep 60 minutes after the start of ZAP infusion. The number of granulocytes (↑) in the microcirculation is increased in **b**. (H&E, ×580)

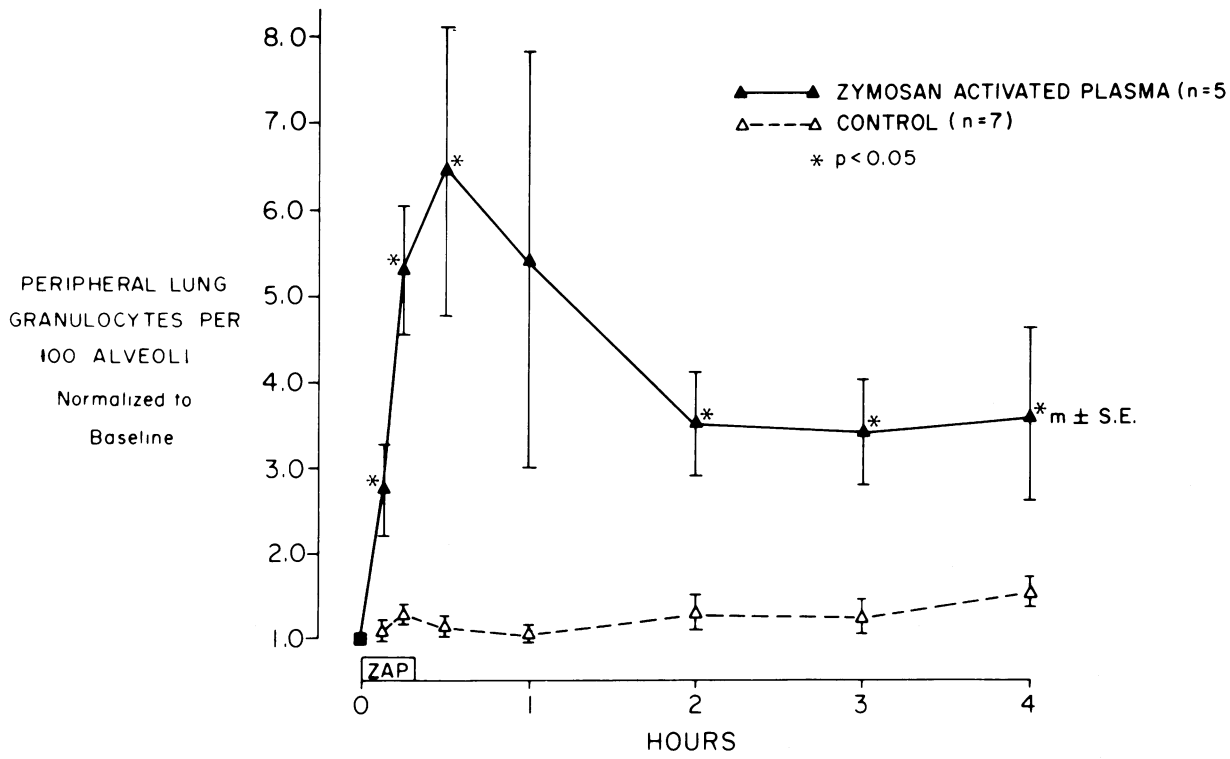


Figure 4 — Number of peripheral lung granulocytes per 100 alveoli over a 4-hour period following the start of ZAP infusion to anesthetized sheep. The results are normalized to baseline in each sheep. Analysis of variance for both intervention and time significant at the <0.001 level: $*P < 0.05$ — ZAP-treated animals compared with time-matched controls.

utes. Dilated lymphatics were also noted at 60 and 120 minutes. From 2 hours after the start of ZAP infusion, these morphologic changes were not as marked as at the earlier times. At each time examined, intraalveolar granulocytes and mononuclear cells were rare.

Electron Microscopy

Just 7.5 minutes following the start of ZAP infusion, there was a marked accumulation of granulocytes in the alveolar capillaries and small pulmonary vessels. Occasionally, part of a granulocyte cell membrane was damaged or missing, and specific and azurophilic granules were seen either emptying into or lying free within the vessel lumen (Figure 5).

At 15 minutes following the start of infusion, margination of granulocytes was more striking than at 7.5 minutes. The majority of granulocytes were still normal in appearance, but occasional ones appeared damaged (Figure 6), and some were necrotic. Occasional lymphocytes and monocytes were also seen in the vessel lumen; a proteinaceous coat was often identified around the monocytes. Also from 15 minutes, in some regions there was evidence of endothelial cell damage and alveolar wall edema. Endothelial damage was seen as an increase both in electron density and in number of pinocytic vesicles, and the edema, as electron-lucent areas in the thick portions of the blood–gas barrier (Figure 7) or between the endothelial cell and its basement membrane.

At 30 minutes, the changes were as described for 15 minutes, but the endothelial cell damage was more widespread, and occasional electron-dense pericyte processes were found in association with the damaged endothelium (Figure 7). In addition, in 1 animal, a granulocyte was found between an endothelial cell and its basement membrane, a position suggestive of migration from the capillary lumen to the alveolar interstitium (Figure 8). This was the only such leukocyte found at capillary level throughout the entire study.

At 60 minutes, the changes were similar to those at 30 minutes, but all monocytes were now "coated" (Figure 9) and often contained autophagic vacuoles. In addition, in 3 of the 5 animals (infused with ZAP) we found several leukocytes (neutrophils, monocytes, lymphocytes, and eosinophils) "migrating" through

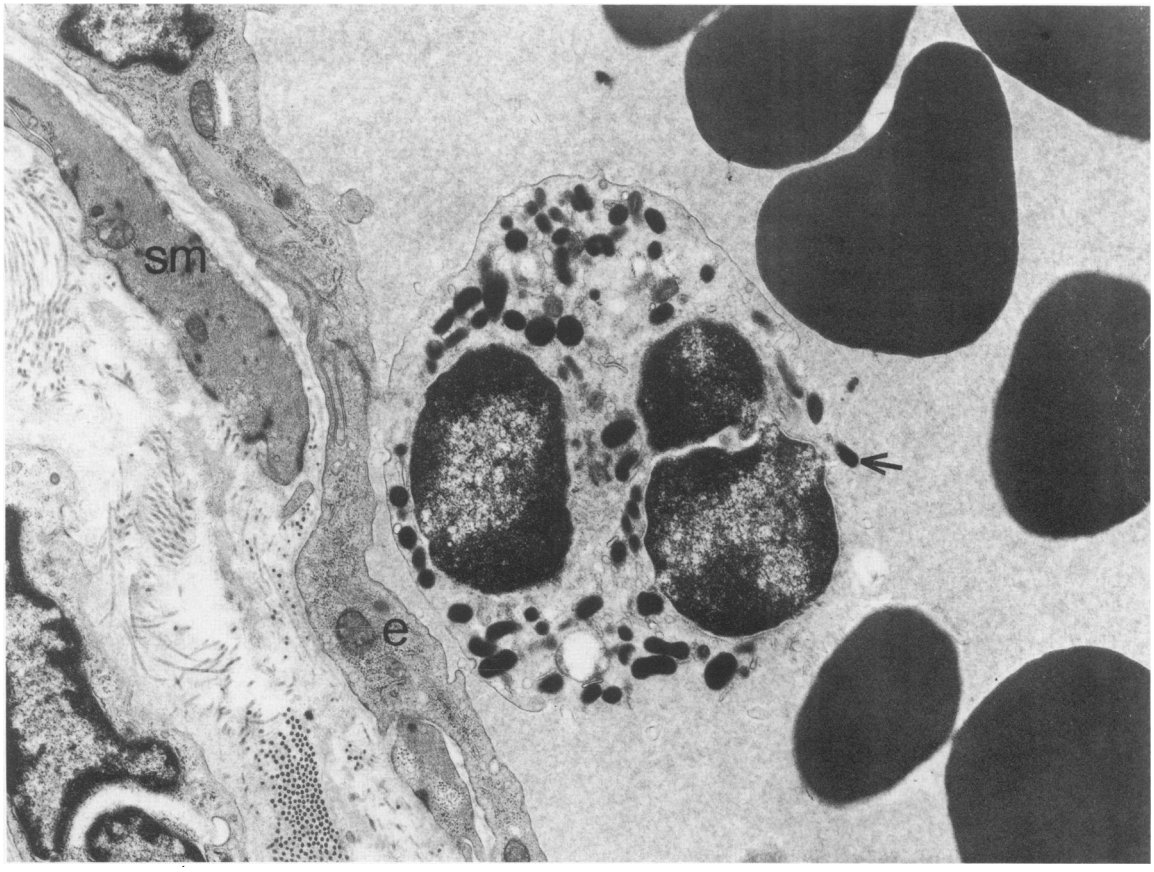


Figure 5—Electron micrograph, 7.5 minutes after the start of ZAP infusion, showing a damaged granulocyte. Part of the cell membrane is missing, and some of its granules (↑) are emptying into the vessel lumen. *e*, endothelial cell, *sm*, smooth muscle cell (×11,600)

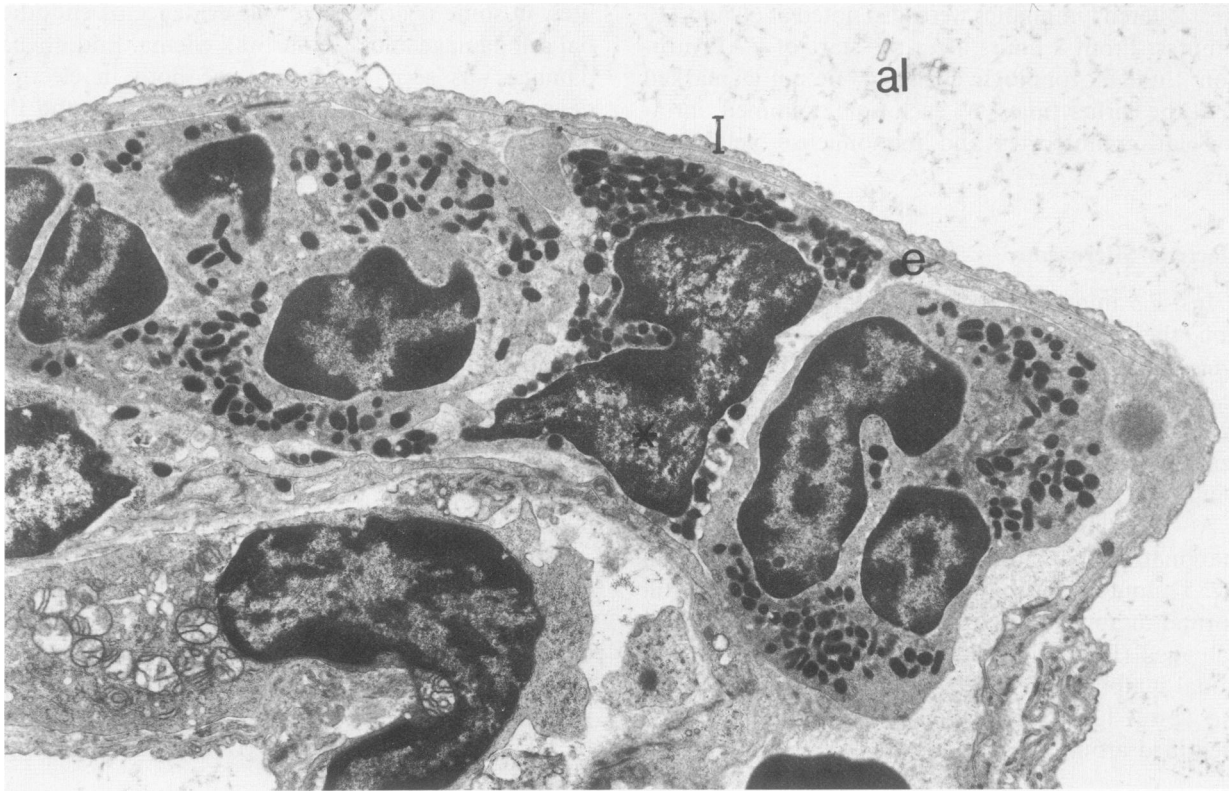


Figure 6 – Electron micrograph, 15 minutes after the start of ZAP infusion, showing margination of an accumulation of granulocytes in a lung capillary. Three of the granulocytes are normal in appearance, but one (*) is more electron-dense than normal, a condition suggesting its degeneration. *al*, alveolus; *e*, endothelial cell; *I*, Type I pneumonocyte. (×7500) (With a photographic reduction of 5%)

the walls of the small muscular vessels (Figure 10). Migration of leukocytes at this level was also seen occasionally in our control animals.

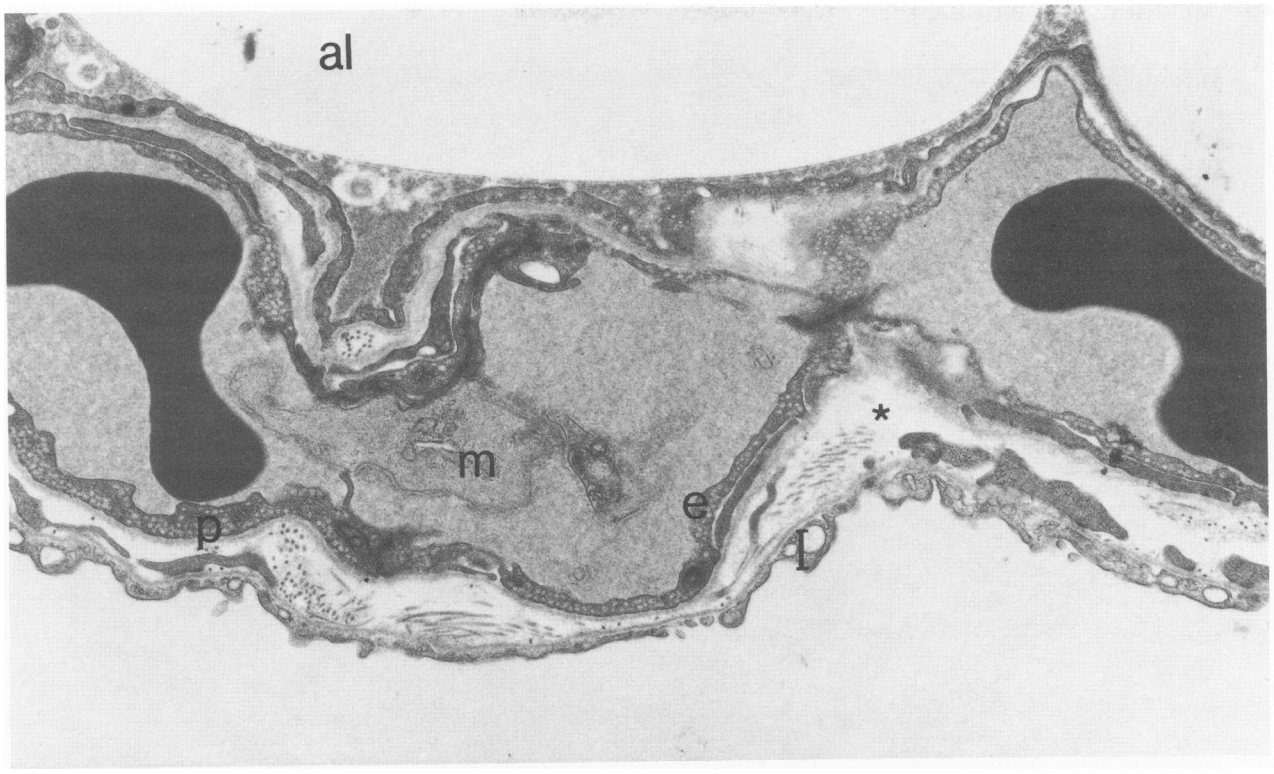
From 2 hours, the endothelial cells had, in general, returned to a normal appearance, although margination of granulocytes and interstitial edema were still prominent features. At this time, the edema appeared more electron-dense and floccular than at 15, 30, and 60 minutes (Figure 11). Also, from 2 hours, rare electron-dense Type I pneumonocytes were encountered; and in 1 animal, at 4 hours, we found a region where the Type I pneumonocytes had sloughed, leaving a bare area of basement membrane.

In all animals, at each time examined, the Type II pneumonocytes and mast cells were similar to those seen in our control animals. The number of alveolar macrophages was never strikingly increased, although from 30 minutes, the macrophages were hypertrophied. At each time studied, the intercellular junctions between the endothelial cells were tight. In general, the platelets were normal in appearance, distribution, and number throughout the study. Only in 1 animal, 3 hours following the start of ZAP infusion, did we find platelet accumulations in occasional capillaries.

Discussion

The present study has followed the pathophysiologic response of the lung, over a 4-hour period, to a single infusion of complement-activated plasma in catheterized, open-chest sheep. Use of serial lung biopsy tissue allowed correlation of morphologic with physiologic changes in the same animals.

Figure 7 – Electron micrograph, 30 minutes after the start of ZAP infusion, showing electron-lucent areas, which probably represent regions of edema (*) in the thick region of the blood-gas barrier. The endothelial cells (*e*) are more electron-dense than normal and contain increased numbers of pinocytic vesicles (*p*). The associated pericyte processes are also more electron-dense than normal. Part of a cell, probably a monocyte (*m*), surrounded by a proteinaceous coat, is seen in the capillary lumen. Edema fluid is also seen in the alveolus (*al*). *I*, Type I pneumonocyte. (×12,300) **Figure 8** – Electron micrograph, 30 minutes after the start of ZAP infusion, showing a granulocyte (*g*) lying beneath a "damaged" endothelial cell (*e*) and superficial to the capillary basement membrane (*bm*) and to pericyte processes (*pe*). *al*, alveolus. (×7700)



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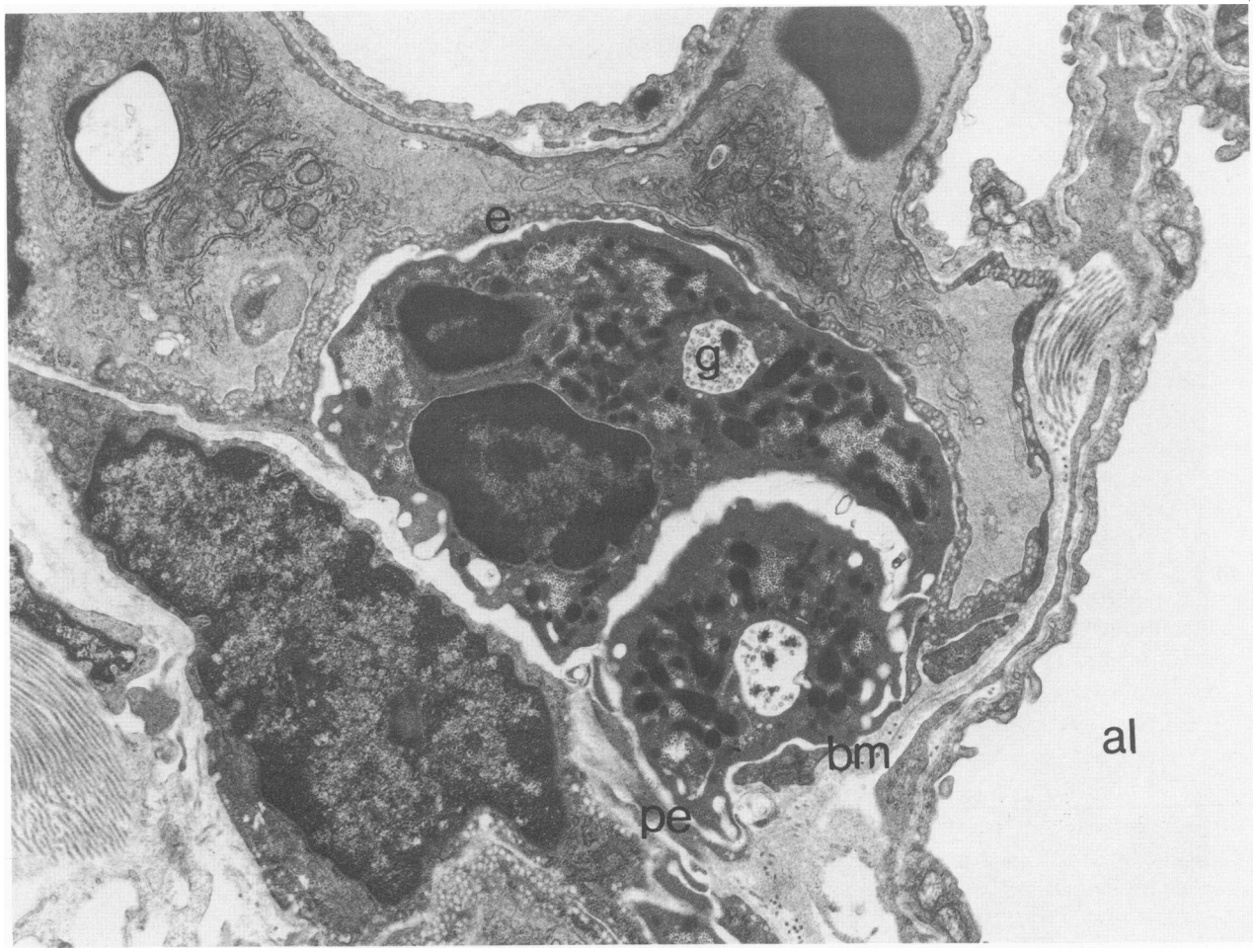

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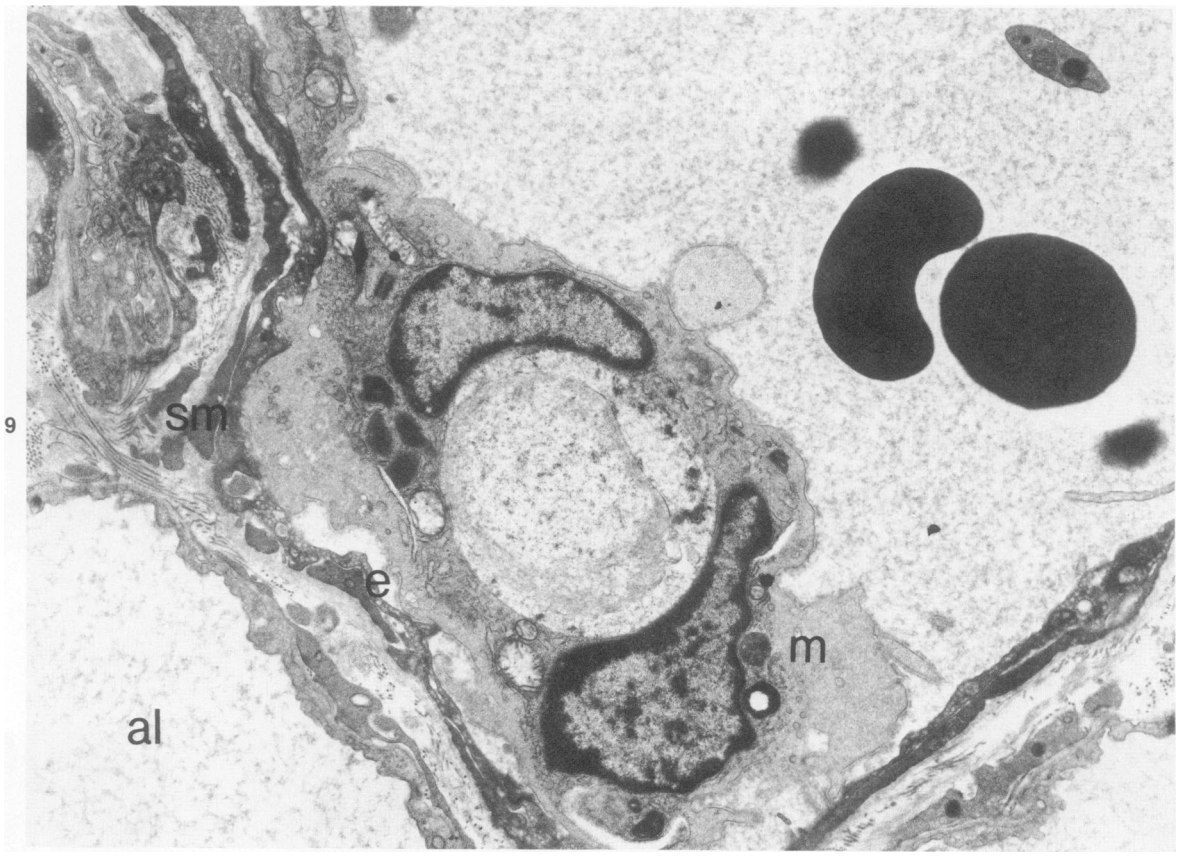

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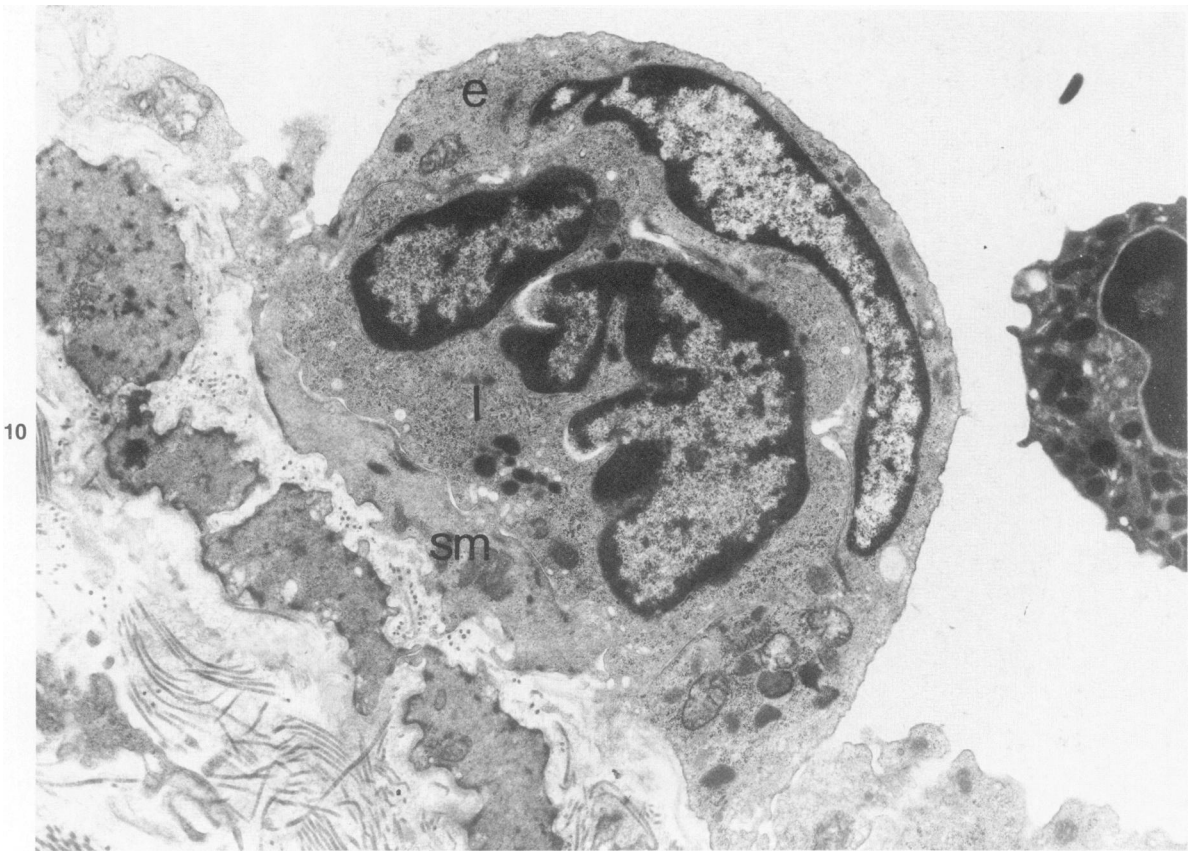

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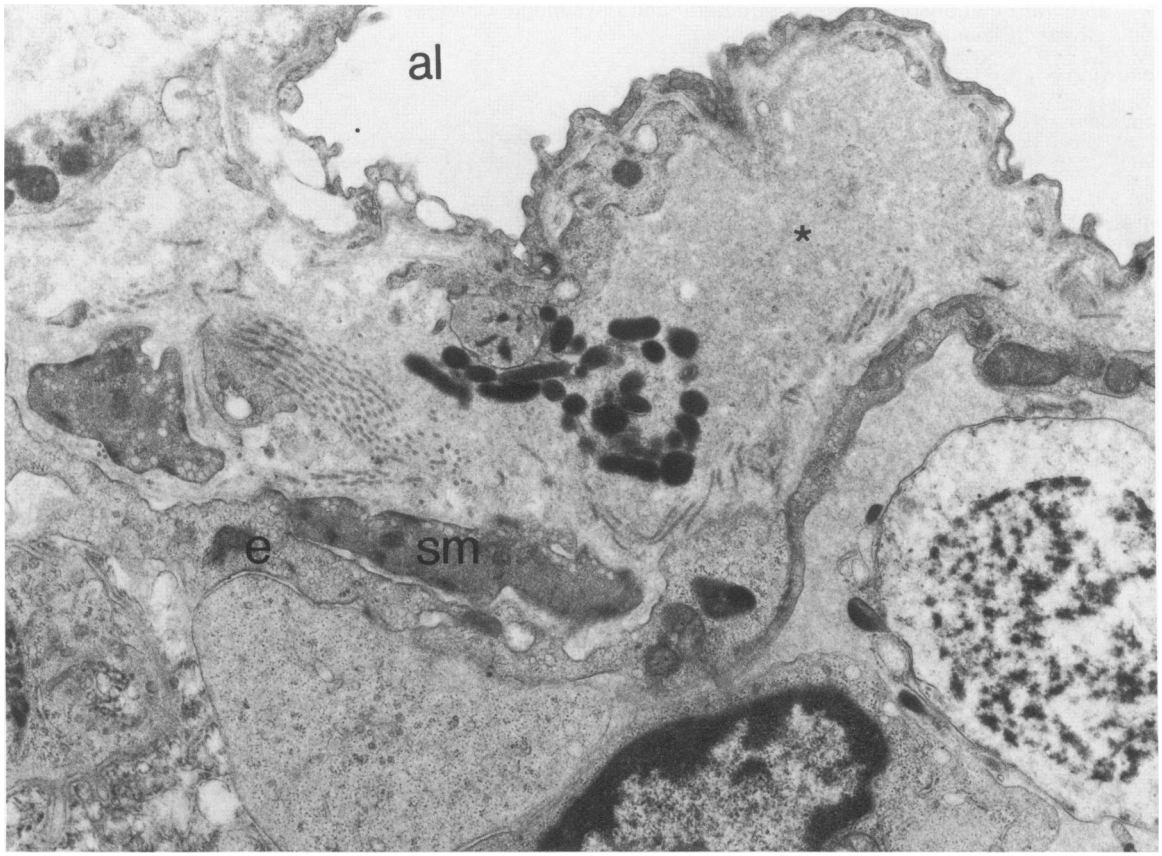

Figure 11 — Electron micrograph of part of an alveolar wall, 2 hours after the start of ZAP infusion. The edema in the interstitium (*) is more electron-dense than at earlier times (see Figure 7). The edematous area contains specific and azurophilic granules presumably from a damaged granulocyte that has migrated from a vessel lumen. The endothelial cells (*e*) and smooth muscle cells (*sm*) of the partially muscular vessel appear normal (compare with Figures 7, 8, and 9). *al*, alveolus (×13,800)

Physiologic Changes

Infusion of complement-activated plasma into sheep causes an early and transient period of leukopenia and marked pulmonary hypertension[8,9,14]; the latter is accompanied by a modest increase in the flow of protein-poor lung lymph. Later, pulmonary artery pressure returns to normal, but lymph flow remains elevated, and its protein content is above baseline. These latter changes we interpret as a minimal increase in pulmonary vascular permeability. Similar changes have been reported previously in acutely prepared sheep following infusion of ZAP[8] and by us in unanesthetized chronically catheterized sheep.[9]

Structural Changes

Examination of the biopsy tissue by both light and electron microscopy reveals that infusion of complement-activated plasma into sheep causes transient injury to the lungs' microvasculature. The changes are summarized in Table 1. As early as 7.5 minutes following the start of infusion, a marked sequestration of granulocytes in the pulmonary microcirculation occurred; some of these sequestered cells showed evidence of damage and disruption. This was followed at 15 minutes by endothelial cell damage, minimal edema accumulation in the alveolar walls, and the appearance of a proteinaceous coat around intravascu-

Figure 9 — Electron micrograph, 60 minutes after the start of ZAP infusion, showing a monocyte (*m*) in the lumen of a small muscular vessel. The monocyte is coated by a proteinaceous layer. Both the endothelial (*e*) and associated smooth muscle cells (*sm*) in the wall of the vessel are more electron-dense than normal. *al*, alveolus. (×11,500) **Figure 10** — Electron micrograph, 60 minutes after the start of ZAP infusion, showing part of a leukocyte (*1*), probably a monocyte, in the wall of a muscular vessel. The leukocyte lies under the endothelial cell (*e*) and superficial to a smooth muscle cell process (*sm*). (×9500)

Table 1—Temporal Relationship Between Structural and Physiologic Changes Following Infusion of Zymosan-Activated Plasma to Anesthetized Sheep

Time following start of ZAP infusion	Structural changes	Functional changes
7.5 minutes	Margination, accumulation, and degranulation of granulocytes	Increased pulmonary artery pressure; leukopenia
15 minutes	Some granulocyte death; focal regions of alveolar wall edema and endothelial cell damage, protein halo to intravascular monocytes	Increased lung lymph flow
30 minutes	Perivascular edema and isolated regions of intraalveolar edema	Pulmonary artery pressure falling toward baseline
60 minutes	Leukocyte migration in small vessels; dilated lymphatics, cellular infiltrate around small arteries, veins, and airways	Normal pulmonary artery pressure
120 minutes on	Peripheral lung granulocyte number of decreasing; endothelial cells of normal appearance; edema concentration subsiding	Increased lymph/plasma protein

lar monocytes. By 30 minutes the edema was more extensive, involving the connective tissue sheaths of the lung as well as occasional alveoli. Edema accumulation in the connective tissue regions was accompanied by an infiltration of monocytes and granulocytes. Migration of leukocytes through the walls of the small muscular vessels was also noted. From 2 hours lung injury was subsiding, the edema and endothelial cell damage was less marked, and the number of sequestered granulocytes was decreasing. The alveolar epithelium was spared any major and diffuse damage.

Fifteen minutes following the start of ZAP infusion, a floccular coating was seen to completely surround the intravascular monocytes. All of the other blood cells and elements were devoid of this coat. Further examination of pictures from our endotoxin study reveals that this coating is present also on the blood monocytes of sheep following endotoxemia (eg, Figures 6, 9, and 10 of Meyrick and Brigham[1]). A proteinaceous coat has been described around certain bacteria when mixed with plasma as seen by both light and electron microscopy; and, in such cases, the coat was thought to have some of the characteristics of fibrin—coat formation was inhibited by heparin.[15] As far as we are aware, this coating around the monocyte has not previously been described. Its appearance indicates that it is protein in nature and could perhaps represent enhanced lysozyme secretion by the monocyte,[16,17] an immunoglobulin, an antigen/antibody reaction, or one of the components of the complement cascade. In the present study, the coated monocytes were not associated with fibrin deposits, although following endotoxin fibrin was occasionally seen to lie in close proximity to the coated monocytes. Monocytes have long been implicated in the damage induced by endotoxin[4] and perhaps also in complement-induced damage. That the coating is restricted only to this cell type perhaps indicates that this cell is important in both reactions.

Administration of complement-activated plasma has been shown previously to produce acute lung damage when given either intravenously or into the airways.[7,18,19] When instilled into the airways of rabbits, neutrophils accumulate in the alveolar spaces; but when given intravenously, this phenomenon does not occur.[18] Intravenous administration of plasma that has been activated by cobra venom factor, to rats, leads to changes similar to those described for the present study, including sequestration of neutrophils in the capillaries and endothelial cell damage; the alveolar epithelium remains intact.[7] These workers also alluded to the transient nature of the response. By 4 hours they were unable to detect any remaining neutrophil aggregates in the capillaries or any endothelial cell damage. On the other hand, as seen by light microscopy, intravenous infusion of ZAP to rabbits has been reported to produce no significant lung damage.[6] Damage is found, however, when the infusion is preceded by surgical trauma or anoxia.[19,20] Thus, the mode of complement activation may be significant in the production of acute lung injury, and/or its action may be species-dependent. The dose of complement-activated plasma delivered to an animal is also important.[9]

Relationship of Structural to Functional Change

In Table 1, we show the temporal relationship between the structural and functional changes following an infusion of complement-activated plasma. Even though sequestration of granulocytes in the lungs' microcirculation occurs concurrently with systemic leukopenia and pulmonary hypertension, the pulmonary hypertension is probably not caused by vascular obstruction with leukocytes. Evidence of endo-

thelial cell damage is first detected when lung lymph flow is increasing. As the protein concentration of lung lymph increases, edema accumulates within the lung, and perivascular cellular infiltrates are seen. Later, when pulmonary vascular permeability is increased, damage to the lung is resolving. These results suggest that even when damage to the pulmonary endothelial cells is transient, the injury may still result in a small but measurable increase in pulmonary vascular permeability. Thus, damage to the endothelial cell is a forerunner or marker of a later increase in pulmonary vascular permeability.

Mechanisms of Complement-Induced Lung Injury

Granulocytes have been implicated in the response of both lung[5,21] and skin[22] to complement-induced injury. An intradermal injection of ZAP to neutropenic rabbits causes a striking reduction in the increased permeability and red cell extravasation seen normally following ZAP injection.[23] Similarly, in rats made leukopenic, lung injury and increased permeability following an intravenous infusion of cobra-venom-activated plasma is markedly attenuated.[7] These workers also found a significant degree of protection by intravenous injection of superoxide dismutase and catalase, suggesting a particular role for toxic oxygen products of granulocytes in the lung damage, particularly to the endothelial cells. Similarly, in sheep given ZAP, pretreatment with superoxide dismutase has been shown to attenuate the increase in lung vascular permeability; the initial pulmonary hypertension, however, does not seem to be affected.[24,25]

The present study shows release of specific and azurophilic granules from the granulocytes into the vessel lumen. Similar fragmentation and degranulation is seen also during endotoxemia in sheep[1] and monkeys[26] and in rabbits during the development of the generalized Shwartzman reaction.[27] It may be that the enzymes released from these granules are responsible for the endothelial cell damage. Lysosome release from granulocytes has previously been suggested to contribute to endothelial cell damage.[23]

Attempts have been made to identify the particular component of the complement cascade responsible for inducing lung damage. Henson and colleagues have isolated C5a and C5a des Arg from complement and given the fragments intratracheally to rabbits. They found that the accumulation of intralveolar neutrophils was more pronounced following C5a des Arg than following C5a or ZAP or serum administration.[20,28] That C5a des Arg is a powerful agent for inducing leukocyte infiltration and increased permeability has also been shown in skin.[23] *In vitro* studies have shown that both C5a and C5a des Arg induce the pulmonary macrophage to produce a chemotactic factor.[28] Whether the intravascular monocyte also produces a chemotactic factor under these circumstances is not known, but the present study does suggest a special role for this cell type in complement-induced lung injury. A recent study has shown that the alveolar macrophage, as well as neutrophil, of the rat can produce toxic oxygen products.[29]

Comparison of ZAP-Induced Changes With Those of Endotoxin

Activation of the alternative complement pathway has been implicated in the structural and functional response of the lung to endotoxin.[4,21] The present study and a similar one in sheep following a single infusion of *E coli* endotoxin[1] allow comparison of the structural and physiologic changes induced by the two agents. Overall, the physiologic response following the two interventions is qualitatively similar; there is an initial phase of pulmonary hypertension followed by an increase in lung vascular permeability. With complement activation, however, the pulmonary hypertension and leukopenia occur and resolve faster than with endotoxin, and the increase in pulmonary vascular permeability is minimal when compared with the reaction to endotoxin.

At first glance the structural changes following both interventions also follow a similar pattern. Both include accumulation and margination of granulocytes in the lungs' microcirculation, degranulation and migration of leukocytes, and endothelial cell damage. However, there are marked differences. For example, the sequestration of leukocytes following ZAP infusion is faster and initially more severe than that following endotoxin. Whether this finding is a direct effect of the immediate availability of complement-activated plasma to the granulocyte and microcirculation is not apparent. Also, during endotoxemia the sequestration of leukocytes includes both granulocytes and lymphocytes in approximately equal numbers[1]; whereas with ZAP the majority of sequestered leukocytes (approximately 80%) are granulocytes. Thus, lymphocytes do not seem to play a major role in complement-activated lung injury, whereas following endotoxin infusion their sequestration has been linked to the initial pulmonary hypertension.[30] Following endotoxin, the endothelial cell damage increased in severity over the 4-hour period of the study; whereas with ZAP the damage is transient, lasting for only a 2 or 3-hour period (see above). Fol-

lowing endotoxin, leukocyte migration occurs mainly at capillary level; whereas following complement activation, at least in sheep, it is mainly in the small muscular vessels. Why endotoxin and complement-activated plasma should lead to differences in the region where leukocyte migration primarily occurs is not clear. Following complement-induced damage, endothelial injury is minimal and similar at all levels of the microcirculation examined in the present study. It is possible that the small muscular vessels are the site of leukocyte migration, leading to the cellular infiltrate seen in the perivascular sheaths.

In summary, the present study has followed the structural and functional changes following an infusion of complement-activated plasma. Just 7.5 minutes following the start of infusion there was a striking accumulation of granulocytes in the microcirculation that coincided with both a systemic leukopenia, due exclusively to a reduction in number of granulocytes, and a marked increase in pulmonary artery pressure. Earlier studies, in sheep, have shown that the initial pulmonary hypertension during endotoxemia is not dependent on granulocytes, but the second phase of the response, the increase in pulmonary vascular permeability, is markedly attenuated in granulocyte-depleted sheep.[31] Because both the structural and functional changes following infusion of either complement-activated plasma or endotoxin are similar, it is likely that following complement activation granulocytes play a major role in the increased vascular permeability. Studies in the granulocyte-depleted rat given cobra venom complement-activated plasma confirm this idea.[7] The initial pulmonary hypertension following both endotoxin and complement-activated plasma infusion is more likely to be linked to the increased production of thromboxane A_2.[32,33] The source of the thromboxane in the lung is as yet unidentified. Endothelial cell damage following complement-activated plasma, like that of endotoxin, precedes the increase in pulmonary vascular permeability and suggests an association between the two changes. Endothelial cell damage should perhaps even be considered an indicator of this change.

Of special note is the finding that infusion of ZAP in the present study caused at least as much pulmonary sequestration of granulocytes as did infusion of gram-negative endotoxin, but the magnitude and duration of endothelial injury with ZAP was much less. We interpret this to mean that granulocyte activation resulting in marked pulmonary leukostasis *per se* is not sufficient to cause prolonged, severe microvascular injury. Identification of additional factors brought into play by endotoxemia will require further the study.

It may be difficult to generalize results of experiments describing complement-dependent phenomena among species because of differences in complement systems,[34] but comparisons between different interventions in the same species are especially important in this area.

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