

The Role of Granulocytes in the Pulmonary Response to Group B Streptococcal Toxin in Young Lambs

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ABSTRACT. Marked leukopenia and sequestration of granulocytes in the lung are consistently seen in severe early onset group B streptococcal (GBS) disease in human infants. To investigate the role of granulocytes as potential mediators in the pulmonary pathophysiology of this disease, the effects of intravenously administered GBS type III toxin were studied in young lambs before and after granulocyte depletion with hydroxyurea. Granulocyte depletion markedly reduced the 4-fold increase in total lung resistance and the decrease in dynamic compliance observed after GBS toxin. Granulocyte depletion significantly attenuated the pulmonary hypertension, hypoxemia and increased minute ventilation present during the first phase of the response (0.5–1 h after GBS toxin). It did not significantly alter the increase in body temperature, the marked increase in lung lymph thromboxane B₂ concentrations during the first phase or the increase in lung lymph flow and protein clearance during the second phase of the response (3.5–5 h after GBS toxin). The results indicate that granulocytes are involved as mediators of the changes in lung mechanics seen after GBS toxin infusion in young lambs. Granulocytes contribute to the pulmonary hypertension and decrease in arterial oxygenation, but other mediators appear to be responsible for the injury of the vascular endothelium. (*Pediatr Res* 21: 159–165, 1987)

Abbreviations

C_{dyn}, dynamic compliance
GBS, group B streptococcus
L/P, lymph/plasma protein ratio
P_{pa}, pulmonary artery pressure
R_L, resistance to air flow across the lungs
TXB₂, thromboxane B₂
TGV, thoracic gas volume
V_T, tidal volume
V_{min}, minute ventilation
P_{pl}, pleural pressure
V̇, flow
P_{ao}, airway opening pressure
P_{tp}, transpulmonary pressure
SG₁, specific conductance
f, frequency

Infection with GBS in the early neonatal period is associated with a high mortality in spite of appropriate antibiotic therapy (1). Severe disease presents with respiratory failure, leukopenia, and cardiovascular collapse. Isolation of a toxin from type III GBS cultures obtained from an infant who died with GBS pneumonia, has provided a tool for studies of the pathophysiology involved in this disease (2–6). Infusion of this toxin into sheep causes a 2-phase reaction with initial pulmonary hypertension associated with tachypnea, chills, and fever, followed by a second phase characterized by increased flow of lung lymph, rich in protein, indicating an increased vascular permeability. A similar reaction as well as alterations of lung mechanics has been described after *Escherichia coli* endotoxin infusion in sheep (7–9). As in GBS pneumonia in newborn infants, granulocytopenia with pulmonary sequestration of granulocytes occurs during the GBS toxin reaction in sheep (4).

Pulmonary vasoconstriction during the first phase of the GBS toxin reaction is assumed to be mediated by arachidonic acid metabolites, such as thromboxane A₂, which cannot be measured in body fluids due to its short half life. Plasma levels of its stable metabolite, TXB₂, increase during intravenous infusion with live GBS bacteria in piglets (10), and a marked increase in TXB₂ lung lymph concentrations is seen during the first phase of the GBS toxin reaction in sheep (5). The first phase pulmonary hypertensive response to GBS toxin infusion in sheep can be blocked by cyclooxygenase inhibition (5). The vascular permeability changes are, however, not blocked by cyclooxygenase inhibition.

The degree of granulocytopenia during the GBS toxin response correlates positively with the increase in vascular permeability, as indicated by an increased lung lymph protein clearance (4). Leukopenia and increased lymph protein clearance following GBS toxin can be blocked by pretreatment with methylprednisolone (6). Granulocytes have, therefore, been implicated as mediators of the vascular damage (4). There are *in vivo* and *in vitro* studies showing that granulocytes can release thromboxane after *E. coli* endotoxin challenge (11), so they might also play a role in mediating toxin-induced pulmonary vasoconstriction.

The purpose of this study was to test the hypothesis that the increased vascular permeability to protein, pulmonary hypertension, and alterations in lung mechanics seen after GBS toxin administration in sheep are mediated by granulocytes sequestered in the lung. Young lambs were studied before and after granulocyte depletion. Hydroxyurea was used to induce granulocyte depletion because it acts predominantly on the granulocytes, and it is well tolerated by the animals.

MATERIALS AND METHODS

Preparation. Twelve lambs were instrumented 1 to 10 days after birth with placement of catheters in a femoral artery and a

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tarsal vein. A 5 Fr. Swan Ganz catheter was introduced through a jugular vein sheath on the day of the study. A right thoracotomy was done to obtain a chronic lung lymph fistula. The efferent duct of the caudal mediastinal node was cannulated with a silastic catheter. To avoid contamination from abdominal and systemic lymphatics, the caudal portion of the lymph node and diaphragmatic lymph vessels were ligated. In the animals whose lung functions were studied, two pleural balloons (silastic envelopes) were placed in the right pleural space. A tracheostomy was performed the day prior to the first study. In the postoperative period, all lambs received 50,000 U/kg/day of procain penicillin and 40 mg/kg/day of dihydrostreptomycin intramuscularly for 3 days. The lambs were allowed to recover an average of 8 days (range 2–14 days) after surgery before they were studied the first time. Lambs with lymph preparations were chronically heparinized to decrease the risk of clots in the lymph fistula. All lambs received 2000 U heparin intravenously on the morning of the studies. Five lambs were used for lymph studies, five lambs for studies of lung mechanics and two lambs for both.

Study protocol. All lambs were studied at least two times. First, they were studied in a control state with normal white cell count and the second time they were studied after depletion with 100 mg/kg/day of hydroxyurea (Hydrea, E. R. Squibb & Sons Inc., Princeton, NJ), until the granulocyte count was below 200 cells/mm³. The hydroxyurea was dissolved in 10 ml normal saline, filtered through a millipore filter, and given intravenously. While in the granulocyte-depleted state, the lambs had an increased susceptibility to infections. Therefore, antibiotics in the form of penicillin 50,000 U/kg/day and dihydrostreptomycin 40 mg/kg/day or gentamycin 5 mg/kg/day were restarted before the control study and were given during granulocyte depletion until repletion had occurred. The lambs were only studied in the depleted state if they were afebrile and appeared healthy, were eating well, gaining weight, and were free of obvious infections. Outcome of the lambs following the study in the depleted state varied. Five lambs were studied after spontaneous recovery of the granulocyte count 6–12 days (mean 10 days) later. Four lambs were killed 0–48 days (mean 21 days) later because of slow repletion of the granulocyte count, catheter failure, or scheduling problems. Three lambs were killed 4–7 days (mean 5 days) later because of illness.

The average age of the lambs during the first study was 15 days (range 4–22 days), 23 days (range 19–41 days) during the second study and 31 days (range 26–37 days) during the third study. Their mean weights were 7.6 kg (range 3.6–11.2 kg) on the day of the first study, 8.7 kg (range 4.4–13 kg) on the day of the second study, and 9.3 kg (range 7.3–14.5 kg) on the day of the third study.

After stable baseline measurements during 1.5 to 2 h, 100 µg/kg of GBS toxin mixed in 30 ml of normal saline was infused intravenously during 12 min. The response was monitored for at least 3 h in all lambs and for 5 h in most lambs.

Measurements. The lambs were studied unanesthetized standing in a loose sling. Respiratory rate and rectal temperature (YSI tele-thermometer, Yellow Springs Instrument Co., Yellow Springs, OH) were recorded every 15 min. Heart rate and vascular pressures (pressure transducers: Statham P23Gb Gould Statham Instruments, Inc., Hato Rey, Puerto Rico; electronic recorder: model 8800S Hewlett-Packard Co., Palo Alto, CA) were measured continuously and recorded every 15 min. The transducers were kept at the level of the heart of the lambs. Pulmonary capillary wedge pressures were measured through a Swan Ganz catheter, after that the balloon had been inflated with 0.8 ml CO₂. Lung lymph was collected into a graduated tube coated with heparin, EDTA, and indomethacin. Lymph flow was recorded every 15 min, and lymph was pooled every 30 min for analyses. Lymph protein concentration was determined on every collection. Lymph concentrations of TXB₂ and 6-keto-PGF_{1α} (a prostacyclin metabolite) were determined during baseline, and every hour for 5 h after toxin infusion. Plasma total protein concentration was determined every hour. Two white

cell counts were done during baseline and at 60, 90, 120, 180, 240, and 300 min after toxin infusion. Arterial blood gases and pH were analyzed during baseline, at the peak of pulmonary hypertension in phase I, and every 60 min throughout the experiment. A respiratory quotient of 0.8 was assumed when alveolar arterial differences were calculated. Lymph protein clearance was calculated by multiplying lymph/plasma protein ratio by lymph flow.

Lung mechanics. During the lung mechanics measurements, the lamb was standing in a whole body pressure-compensated integrated-flow plethysmograph. The volume of the box was 165 liters. The cuffed, rigid tracheostomy tube was connected via a flexible non-collapsible tubing extending through the wall to a valve. The valve could automatically occlude the airway for TGV determinations. The solenoid-activated shutter in this valve occluded the airway within the first few milliseconds of inspiration and held it closed a preset time of 1 to 5 s. The shutter was triggered by zero crossing of the plethysmograph pressure from negative to positive. There was a constant bias air flow through the valve to reduce the dead space of the tubing. V_T was measured by pressure compensating the integrated pressure signal from the plethysmograph. V_{min} was calculated as an average V_T times respiratory f or by integrating V_T over 1 min with a Hewlett-Packard respiratory integrator. V was obtained by electrically differentiating the volume signal. P_{pl} was measured in a pleural balloon placed in the pleural space. P_{ao} was measured through a catheter with multiple side holes located 0.5 to 1 cm past the distal end of the tracheostomy tube. P_{ip} was measured as the difference between P_{pl} and P_{ao}. Validyne differential transducers (model MP-45, Validyne Engineering Corp., Northridge, CA) and Hewlett Packard amplifiers (Hewlett-Packard Medical Products, Andover, MA) were used. The signals from the transducers with connecting catheters and pleural balloons were tuned to eliminate phasic distortion up to 8 Hz.

Before each measurement of lung mechanics, the lungs of the lamb were inflated to 20 cm H₂O of P_{ao}. Simultaneous V/V and V/P_{ip} curves were displayed on a Tektronix dual-beam storage oscilloscope and photographed for further calculations of lung mechanics. C_{dyn} was calculated as V_T/P_{ip} at points of zero flow. Total R_L was calculated as P_{ip}/V at midtidal volume by using the method of von Neergaard and Wirz (12). Specific dynamic lung compliance was calculated as C_{dyn}/TGV and SG_L as 1/R_L/TGV. TGV was measured according to DuBois *et al.* (13) using the P_{ao} and plethysmographic volume changes obtained during airway occlusion at end expiration. Measurements were performed every 5 to 15 min.

Laboratory analysis. White blood cells were counted using a model 2BI Coulter counter (Coulter Electronics, Hialeah, FL), specifically adjusted for counting sheep. A blood smear was stained with Wright's stain to determine the number of granulocytes and lymphocytes/100 white cells. The same procedures were used for determination of lymph white cells. Protein content of blood and lymph was analyzed by a modified biuret method on an automated analysis system (Auto Analyzer Technicon Instruments Corp., Tarrytown, NY). TXB₂ and 6-keto-prostaglandin F_{1α} concentrations in lung lymph were measured with a radioimmunoassay as previously described in detail (6). Arterial blood gas determinations were made with a blood gas analyzer (Corning model 158, Corning Medical and Scientific, Medfield, MA).

Statistics. Each lamb was studied in control and depleted states. The results are presented as mean ± SEM. To test statistical significance of differences over time in each of the two states, control and granulocyte depleted, and to test the significance of differences between states we used two-way analysis of variance. The null hypothesis was rejected for $p < 0.05$. Duncan's multiple range test was then used for significance of differences within states at the $p < 0.05$ level. Lambs who did not have complete data for all time periods in both states, *e.g.* due to catheter failure or sample loss, were not used for statistical analysis of differences.

RESULTS

Effect of granulocyte depletion on baseline variables. Hydroxyurea markedly reduced total white blood cell counts (Fig. 1). After hydroxyurea, the granulocyte count had a range of 0–175 cells/mm³. The blood lymphocyte count was also reduced but not to the same extent as the granulocyte count. The cell count in lymph, which predominantly consisted of lymphocytes, was significantly reduced from 23171 ± 9454 to 4951 ± 541 cells/mm³ after hydroxyurea. C_{dyn} was significantly higher after hydroxyurea treatment (11.7 ± 1.7 versus 15.1 ± 2.3 ml/cm H₂O). Other baseline variables were not significantly altered by hydroxyurea.

Response to GBS toxin. Results of leucocyte, hemodynamic, and lymph measurements are presented as group means ± SEM of single measurements every 15–60 min for 5 h after GBS toxin in Figures 1 and 2. Group means ± SEM of peak values during phase I (0.5–1 h after GBS toxin) and averaged values during phase II, the stable period, 3.5–5 h after GBS toxin were also calculated, as was done in our previous reports of the GBS toxin response (2–6).

White Cells. Total white blood cell and granulocyte counts decreased abruptly at 1 h after GBS toxin in the control studies and increased progressively thereafter as shown in Figure 1. The lymphocyte count also decreased at 1 h but remained decreased. Figure 1 (bottom) illustrates the corresponding cell counts during the depleted studies. The lymphocyte count also decreased significantly in lung lymph during the control studies from 23171 ± 9454 to 7909 ± 3994 cells/mm³ during phase II. The corre-

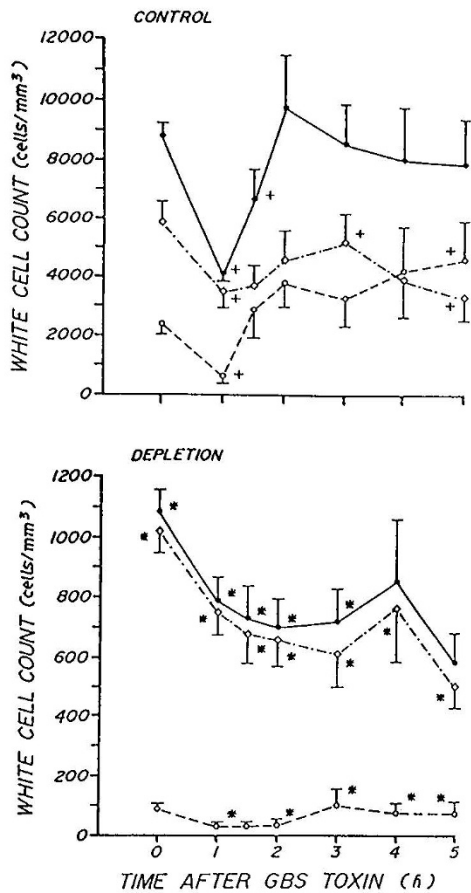


Fig. 1. Effect of GBS toxin on total white blood cell (●—●) granulocyte (○—○) and lymphocyte (◇—◇) counts in young lambs studied before (control) and after granulocyte depletion. Values are mean ± SE; n = 11 until 3 h and 7–8 thereafter. † Significantly different compared to baseline. * Significantly different compared to control state; p < 0.05.

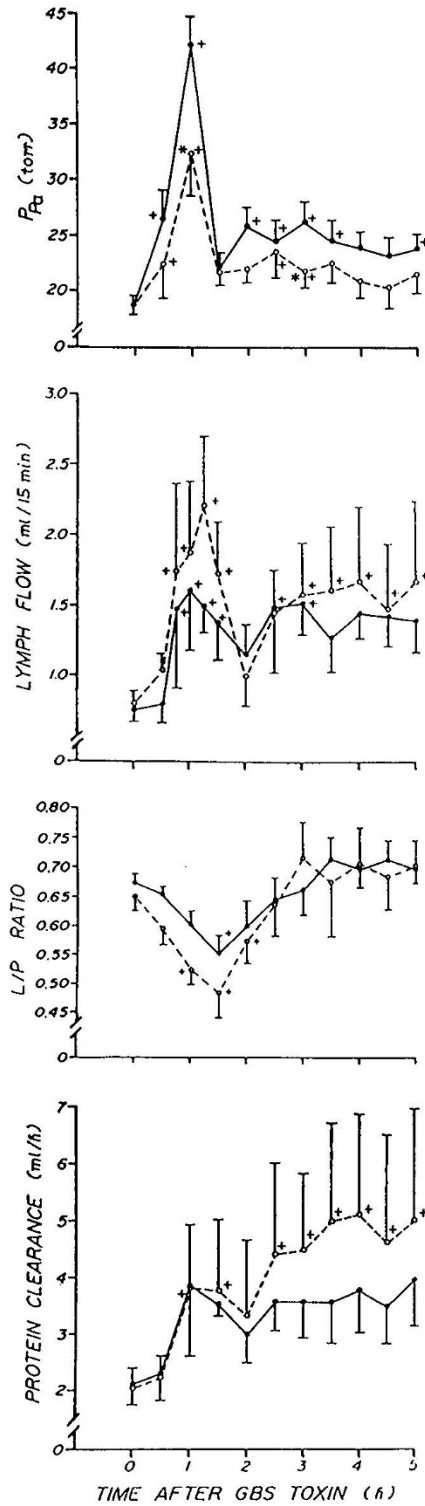


Fig. 2. P_{pa}, lung lymph flow, L/P ratio, and lung lymph protein clearance changes after GBS toxin in young lambs studied before (●—●) and after granulocyte depletion (○—○). Values are mean ± SE; n = 11 until 3 h and 7–9 thereafter for P_{pa}, n = 7 for lymph flow and n = 6 for L/P ratio and protein clearance. † Significantly different compared to baseline, * significantly different compared to control state, p < 0.05.

sponding change from 4951 ± 541 to 1765 ± 794 cells/mm³ during the depletion studies was not statistically significant.

Blood Pressure and Heart Rate. Systemic blood pressure increased significantly in phase I in both states (81 ± 3 to 88 ± 4 and 83 ± 3 to 90 ± 3 torr, respectively). No significant changes

were observed in heart rate during the GBS toxin reaction. A biphasic increase in pulmonary arterial pressure was noted after GBS toxin (Fig. 2). The first peak of the pressure response occurred at 45–60 min after GBS toxin infusion. The second phase of the P_{pa} increase was highest 2–3 h after toxin infusion, and it lasted longer. P_{pa} did not reach baseline values at the end of the control study. After granulocyte depletion, P_{pa} after GBS toxin infusion followed the same pattern but was significantly lower than in the control state at 1 and 3 h. Pulmonary wedge pressures increased significantly during phase I in both the control and depleted state (3.3 ± 0.8 to 8.6 ± 1.4 and 5.3 ± 1.1 to 8.4 ± 1.9 torr, respectively).

Lung Fluid Balance. A biphasic increase in lung lymph flow occurred in the control state, which was not significantly different in the depleted state (Fig. 2). L/P ratios decreased in phase I and increased thereafter (Fig. 2). There was not a significant or consistent effect of granulocyte depletion on lung lymph protein clearance (Fig. 2). Compared to the control state, the increase in protein clearance during phase II of the GBS toxin response was greater in the depleted state in three lambs, smaller in two, and unchanged in one lamb.

Prostaglandins. Lung lymph TXB_2 concentrations increased significantly in phase I in both control and depleted states. Control and depletion measurements were not significantly different. Figure 3 illustrates that the increase in TXB_2 lung lymph concentrations was discrete at 1 h after GBS toxin. No significant changes were seen in 6-keto-prostaglandin $F_{1\alpha}$ lymph concentrations after GBS toxin.

Temperature. Body temperature increased significantly and peaked at 1 h and 15 min after GBS toxin in both control and depleted states (39.7 ± 0.1 to 40.9 ± 0.1 and 39.8 ± 0.1 to $40.7 \pm 0.3^\circ C$, respectively).

Lung Mechanics. A biphasic response was observed with peak values at 0.5–1 and 2–3 h. Results of lung mechanics measurements are presented as percent of baseline in Figure 4. In the control study, total lung resistance was increased at 0.5–1 and 2–3 h after GBS toxin infusion. After granulocyte depletion, no significant changes in R_L compared to baseline values were observed after toxin. Compared to the control study, the reaction in the depleted state was significantly attenuated. The same differences were observed in specific conductance. Compared to baseline, there was a decrease in C_{dyn} 0.5–1 and 2–3 h after toxin infusion in the control study. These changes were not found in the granulocyte-depleted state, which had significantly higher C_{dyn} at these times compared to the control study. The same differences were observed in specific dynamic compliance. The

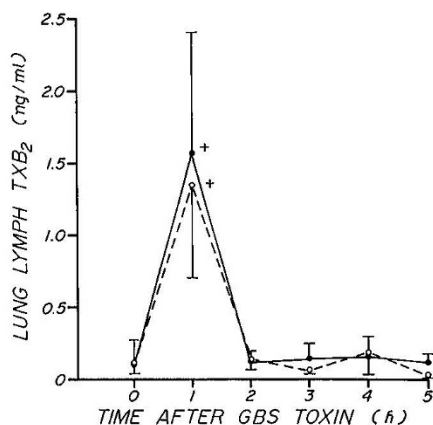


Fig. 3. Effect of GBS toxin on lung lymph concentrations of TXB_2 in young lambs studied before (●—●) and after granulocyte depletion (○—○). Values are mean \pm SE; $n = 5$ until 1 h and 2–5 thereafter. † Significantly different compared to baseline: $p < 0.05$.

changes in TGV were less obvious although there was a trend toward a biphasic decrease in TGV coinciding with the changes in lung mechanics.

Ventilation and Gas Exchange. Compared to baseline, there was a slight but insignificant increase in tidal volume and a significant increase in respiratory rate at 0.5–1 h after toxin infusion in the control study (47 ± 4 to 55 ± 6 breaths per min). The significant increase in \dot{V}_{min} during phase I in the control state (2507 ± 309 to 3473 ± 484 ml/min) was significantly reduced (2366 ± 204 to 2788 ± 389 ml/min) after depletion. Arterial PO_2 was lower compared to baseline measurements at 0.5–1 and 2–3 h after toxin in the control study (89 ± 3 , 68 ± 3 , and 81 ± 3 torr, respectively). This decrease in PO_2 was attenuated to 77 ± 3 torr at 0.5–1 h after toxin in the depleted state. Arterial PCO_2 and pH did not change significantly in either state. Alveolar-arterial PO_2 differences ($\Delta AaPO_2$) were increased at 1 and 2–5 h after GBS toxin as illustrated in Figure 5. At 1 h after GBS toxin, $\Delta AaPO_2$ was significantly lower in the depleted compared to the control state.

Repletion. Five lambs were studied three times, before granulocyte depletion, after depletion, and finally after spontaneous granulocyte repletion. Due to the long interval between the studies several catheters were no longer functioning, and complete studies could not be performed. All lambs, however, had measurements of P_{pa} and white blood cell counts. There was not a significant difference between the GBS toxin-induced maximum increase in P_{pa} during the first control studies and the studies in the repleted state (127 ± 31 and $112 \pm 39\%$, respectively). The decrease in granulocyte count after GBS toxin was not significantly different when the first control studies was compared to the studies in the repleted state (55 ± 20 and $66 \pm 13\%$, respectively).

DISCUSSION

In this study, we found that granulocyte depletion with hydroxyurea markedly attenuated the GBS toxin-induced changes in lung mechanics in young lambs. Reductions of the pulmonary hypertension and decrease in oxygenation were also seen. We found, however, no significant effect on lymph protein clearance, an index of vascular permeability to protein or lung lymph prostaglandin metabolites.

In the control state, before hydroxyurea-induced granulocyte depletion, a similar response to GBS toxin was seen in the 1- to 2-wk-old lamb as has earlier been described in the adult sheep (3). The GBS toxin-induced biphasic changes in lung mechanics were characterized by a significant increase in R_L and a decrease in C_{dyn} . A slight decrease in TGV with a biphasic tendency paralleling the changes in lung mechanics was also noticed.

Inhibition of the arachidonic acid metabolism by indomethacin blocks the pulmonary hypertension after both *E. coli* endotoxin (14) and GBS toxin (5). Meclofenamate (8) attenuates the *E. coli* endotoxin-induced early changes in lung mechanics and pulmonary pressure. This has led to the assumption that prostaglandins, such as thromboxane A_2 , are mediators of toxin-induced pulmonary hypertension, and that they also may be involved in the changes of lung mechanics during the first phase of the response.

Granulocyte depletion delayed and markedly attenuated the GBS toxin-induced biphasic increase in R_L and decrease in C_{dyn} . The changes in specific conductance and specific dynamic compliance were also attenuated in the depleted state. The reduction of the GBS toxin-induced changes in lung mechanics, TGV, and oxygenation are similar to what have been found in leukopenic sheep after *E. coli* endotoxin infusion (7).

Pulmonary hypertension was significantly reduced in the first phase of the GBS toxin response in the depleted lambs, and the average P_{pa} was consistently lower throughout the response. In contrast, granulocyte depletion with hydroxyurea did not signif-

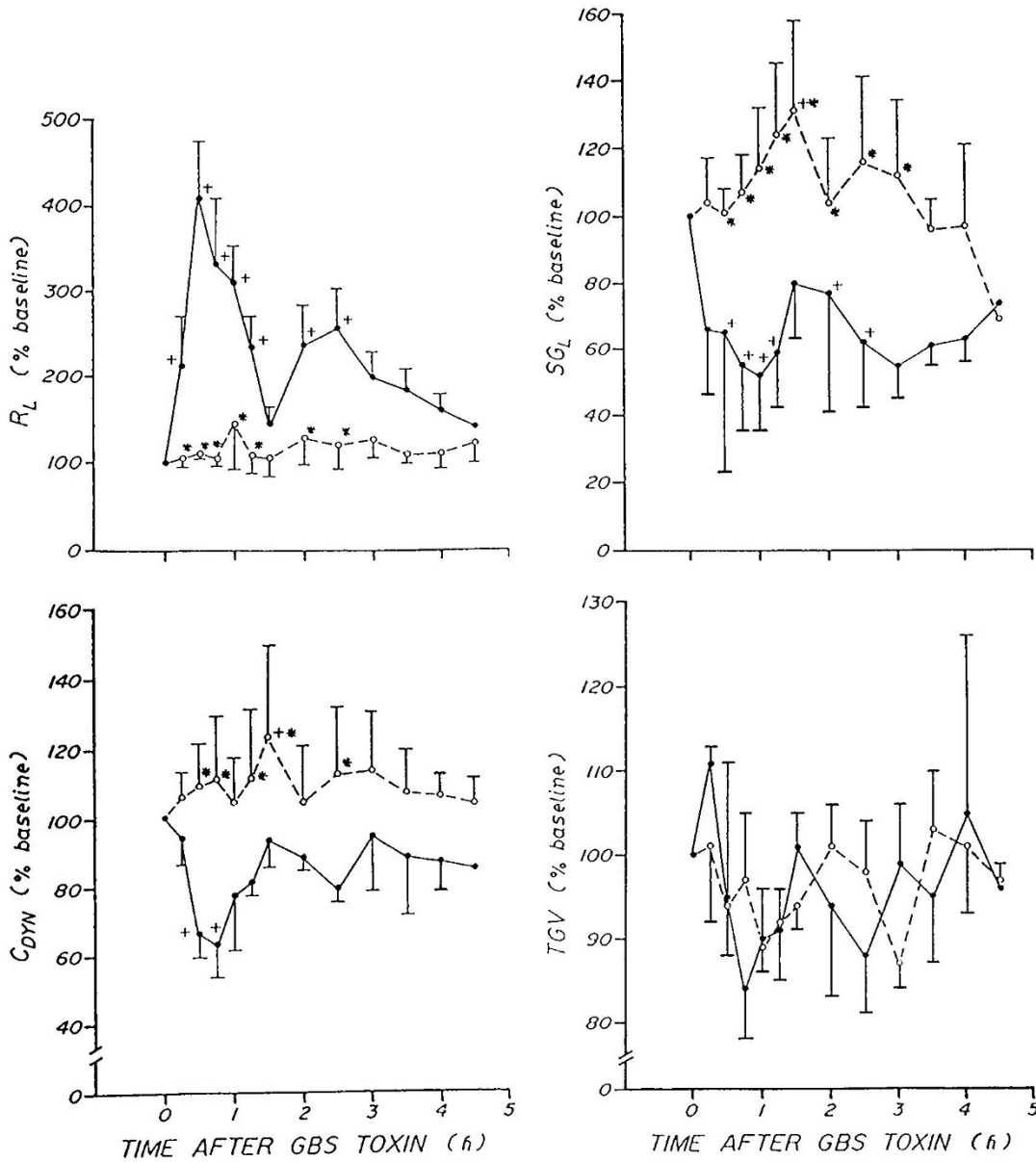


Fig. 4. R_L , SG_L , C_{dyn} , and TGV changes in young lambs before (●—●) and after granulocyte depletion (○—○). Values are mean \pm SE; $n = 7$ until 3 h and 2-6 thereafter. † Significantly different compared to baseline. * Significantly different compared to control state: $p < 0.05$.

icantly affect the *E. coli* endotoxin-induced pulmonary hypertension (7, 9) although the endotoxin-induced pulmonary hypertension was attenuated after granulocyte depletion with nitrogen mustard (15). *In vivo* and *in vitro* experiments have shown that granulocytes are capable of synthesis of vasoactive substances after endotoxin stimulation (8, 11). Granulocyte depletion should, therefore, reduce the source for these vasoactive metabolites, thereby explaining the reduction in pulmonary hypertension found in our study. Participation of other cells in the lung, such as the endothelial cells in the synthesis of vasoactive arachidonic acid metabolites is, however, not excluded (16).

The marked increase in lung lymph levels of TXB_2 was not attenuated by granulocyte depletion. These results and those of Hinson *et al.* (7), who studied the *E. coli* endotoxin reaction, therefore, indicate that granulocytes might not be the main source of TXB_2 concentrations in lung lymph following toxin administration. Since the changes in lung mechanics were markedly attenuated by granulocyte depletion but not the marked increase in lung lymph levels of TXB_2 , it is not likely that

thromboxane- A_2 is the only mediator of the changes in lung mechanics. It is, however, possible that lung lymph levels of TXB_2 do not reflect local thromboxane concentrations in the airways.

In this study, there was no consistent effect of granulocyte depletion on the GBS toxin-induced increase in lung lymph protein clearance. We expected to find a reduced protein clearance response to GBS toxin, since we previously have demonstrated a significant correlation between the decrease in circulating granulocyte counts and the increase in protein clearance and have found that granulocytes sequester in the lungs after GBS toxin (4). The pulmonary response to GBS toxin could theoretically be explained by release of active substances from activated granulocytes, *e.g.* arachidonate products (17), oxygen free radicals (18, 19), and proteolytic enzymes (20). Participation of granulocytes has been described in other forms of pulmonary injury, *e.g.* adult respiratory distress syndrome (21), and experimentally in sheep from *E. coli* endotoxin (7-9, 15), microembolization (22), and oxygen toxicity (23). Pulmonary sequestra-

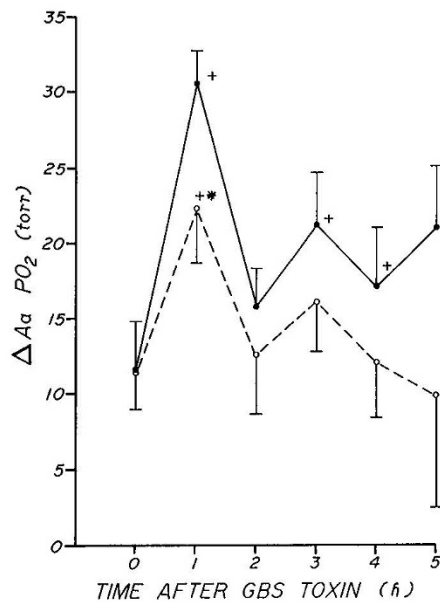


Fig. 5. Alveolar-arterial PO_2 differences ($\Delta AaPO_2$) in young lambs given GBS toxin before (●—●) and after granulocyte depletion (○--○). Values are mean \pm SE; $n = 12$ until 3 h and 5-9 thereafter. † Significantly different compared to baseline. * Significantly different compared to control state: $p < 0.05$.

tion of granulocytes does, however, not in itself necessarily induce severe vascular injury as illustrated in some clinical situations, e.g. in patients undergoing hemodialysis (24), cardiopulmonary bypass (25), or experimentally in animals after infusion of zymosan-activated plasma (26, 27) or phorbol myristate acetate (28).

Since the GBS toxin induced changes in lung lymph flow and protein clearance were not significantly altered by depletion, it is likely that there are other mediators than those derived from granulocytes affecting the endothelium. The GBS toxin itself might have a direct effect on the endothelium. The possible influence of lymphocytes, macrophages, and other inflammatory cells on the GBS toxin response is not known. Lymphocytes might be involved in the response to GBS toxin, since the fall in blood concentrations of lymphocytes paralleled that of the granulocytes. Lung lymph concentration of lymphocytes fell consistently after GBS toxin administration, which was not explained by increased lymph flow.

It is possible that the observed effect on the GBS toxin response during granulocyte depletion is due to factors other than depletion of granulocytes. An increase in age in the lambs is not a likely explanation for the results, since the response to GBS toxin during the control studies did not vary with age. A direct effect of hydroxyurea itself is possible but we do not consider that to be likely. Studies of repeated administrations of endotoxin to sheep has shown that the endotoxin response was not affected by hydroxyurea treatment until granulocyte depletion was achieved (7, 9). However, Winn *et al.* (29) have recently reported that the increase in lung water observed after high doses of endotoxin was markedly attenuated in granulocyte-depleted goats after pretreatment with hydroxyurea but not after nitrogen mustard, suggesting that hydroxyurea prevents pulmonary edema following endotoxin by a mechanism other than granulocyte depletion. In five of the lambs, we were able to do a partial second control study after spontaneous granulocyte repletion. In this study, the changes in pulmonary arterial pressure and leukocyte counts were similar to what was seen in the first control study, before granulocyte depletion with hydroxyurea was started. This would indicate that tolerance to the toxin is not a likely explanation for the attenuated response in the granulocyte depleted state.

In summary, we found changes in lung mechanics, \dot{V}_{min} , arterial oxygen tension, pulmonary arterial pressure, lung lymph flow, and protein clearance after a type III group B streptococcal toxin infusion in young lambs. Granulocyte depletion with hydroxyurea markedly attenuated alterations in lung mechanics, pulmonary hypertension, hypoxemia, and increased \dot{V}_{min} , but it did not significantly change the increase in lymph protein clearance, an index of vascular permeability to protein or TXB_2 lung lymph levels. The results indicate that granulocytes are involved as mediators of the changes in lung mechanics seen after GBS toxin infusion in young lambs. Granulocytes contribute to the pulmonary hypertension and decrease in arterial oxygenation, but other mediators appear to be responsible for the injury of the vascular endothelium.

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