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Pulmonary Microvascular Injury Induced by *Pseudomonas aeruginosa* Cytotoxin in Isolated Rabbit Lungs

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The effects of *Pseudomonas aeruginosa* cytotoxin on the pulmonary microvasculature were studied in blood-free, perfused, isolated rabbit lungs. Cytotoxin was administered to the recirculating Krebs Henseleit albumin (1%) buffer during two consecutive 30-min-perfusion phases (phases 1 and 2) at a concentration of 13 μg/ml, followed by a third perfusion phase (phase 3) without toxin. After perfusion phases 2 and 3, the capillary filtration coefficient ($K_{f,c}$) and vascular compliance were determined gravimetrically from two-step microvascular pressure increments under zero-flow conditions. Cytotoxin caused a continuous release of K^+ and lactate dehydrogenase, which started within the first 5 min and amounted to about 50% of the total lung cellular K^+ and 5 to 7% of the total lactate dehydrogenase by the end of the experiment. The toxin caused the continuous generation of prostaglandin I_2, which was detectable in the perfusates of all perfusion phases at maximum values five times above the control values and which was measured in the bronchoalveolar lavage fluid at the end of the experiment. Thromboxane generation in toxin-treated lungs did not significantly exceed that of control lungs or of lungs with mechanically induced edema. Cytotoxin caused a gradual increase in pulmonary vascular resistance, to maximum values 2.5 times above the control, starting within 1 min; the increase was partially reversible after washout of the toxin. After a lag period of 20 to 30 min, the lungs gained weight, amounting to a mean gain of 9.1 g at the end of the experiments. After perfusion phases 2 and 3, an almost fourfold increase in $K_{f,c}$, which was not reversible after washout of the toxin, was measured, whereas the values of vascular compliance were not altered. We conclude that pseudomonal cytotoxin may be an important factor in the pathogenesis of prolonged microvascular injury, encountered in states of *P. aeruginosa* sepsis or acute lung failure with secondarily acquired *P. aeruginosa* pneumonia.

Clinical studies have repeatedly shown that bacterial sepsis is the most consistent factor associated with the acute respiratory distress syndrome (ARDS) of the adult (16, 22, 24, 33, 39, 57). During the past 30 years, *Pseudomonas aeruginosa* has become an increasingly important pathogen in septicemia and septic organ failure, including that of the lung (1, 2, 13, 32, 38). Moreover, in the course of ARDS, primarily started by different underlying diseases, lung injury is often complicated by the invasion of tissue by bacteria and by nosocomial pneumonia, for which *P. aeruginosa* was frequently found to be responsible and which is associated with high mortality (12, 19, 23, 38, 52, 53). Bacterial colonization and microvascular lung injury appear to be related in a vicious circle: increased rates of clearance of circulating *P. aeruginosa* from the lungs and a steady increase of the number of bacteria in serial lung biopsy specimens have been demonstrated in experimental models of ARDS (7, 49, 51), whereas the infusion of live *Pseudomonas* organisms itself is known to mimic ARDS in sheep and pigs (5, 7, 18). A rise in pulmonary artery pressure (PAP) and a prolonged increase in lung vascular permeability were especially noted in those experiments; these are purportedly the key alterations in the pathogenesis of ARDS. These lung microvascular lesions have been closely associated with pulmonary sequestration and stimulation of circulating granulocytes. A careful review of the role of granulocytes in increased pulmonary permeability edema shows, however, that in animal models with infusion of bacteria, intrapulmonic neutrophil trapping accounts for part, but not all, of the noted increase in lung vascular permeability (17). In general, a multitude of insults leads to pulmonary granulocyte entrapment, but in several models this may be temporally related to rather than causative of the noted lung lesions (17, 33, 59). There are thus several reasons to search for bacterial products that might contribute to lung microvascular injury independent of granulocytes. Concerning *P. aeruginosa*, different factors have been implicated as important pathogens, and one of these is an acidic cytotoxic protein with a molecular mass of 25,100 daltons (26, 42), from which destructive effects on most eucaryotic cells were observed (30). No enzymatic activities are known for this protein, and it probably acts primarily on target cell membranes to create transmembrane lesions, which allow the passage of small molecules (3, 27–30, 41, 43, 54). In the present study, the effects of *P. aeruginosa* cytotoxin on the pulmonary microvascular bed were studied in blood-free, perfused, isolated rabbit lungs. When administered to the perfusion fluid, the toxin caused a moderate and partially reversible increase in vascular resistance and a delayed but irreversible increase in the capillary filtration coefficient ($K_{f,c}$) up to four times greater than base-line levels. These alterations were both preceded and accompanied by a marked release of K^+ greater than that of lactate dehydrogenase (LDH) and by an augmented generation of prostaglandin I_2 (PGI_2). Thus, cytotoxin appears to be an important factor of *P. aeruginosa*, which may initiate or amplify lung microvascular injury in states of *P. aeruginosa* sepsis and lung tissue invasion. This work was presented in part at the 7th World Congress on Animal, Plant, and Microbial Toxins, Brisbane, Australia, July 1982 [Toxicon **20**(Suppl. 3):257–260].

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MATERIALS AND METHODS

Source of materials. Thromboxane B_2 (TxB_2) was graciously supplied by Ono Pharmaceutical (Osaka, Japan). 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-PG$F_{1\alpha}$) was obtained from Sigma Chemical Co. (Munich, Federal Republic of Germany). Rabbit anti-TxB_2 and anti-6-keto-PG$F_{1\alpha}$, as well as bovine albumin (92% purity; reduced in free fatty acids to <5 μg/g), were purchased from Paesel (Frankfurt, Federal Republic of Germany). [^{3}H]TxB_2 and [^{3}H]6-keto-PG$F_{1\alpha}$ were obtained from New England Nuclear Corp. (Dreieich, Federal Republic of Germany). All other biochemicals were obtained from E. Merck AG (Darmstadt, Federal Republic of Germany) and were of p. a. quality.

***P. aeruginosa* cytotoxin.** Cytotoxin from *P. aeruginosa* was prepared as described previously by Lutz (30). The preparation contains no lipid and no carbohydrate moieties, and no enzymatic activities could be measured. The absence of protease, lipase, lecithinase, alkaline phosphatase, sphingomyelinase, and phospholipase C and D activities especially has been shown (30). There is only one protein band upon sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Portions of the toxin were stored at −20°C in phosphate-buffered saline, pH 7 (0.01 M sodium phosphate, 0.1 M sodium chloride).

Radioimmunoassay of TxB_2 and 6-keto-PG$F_{1\alpha}$. Thromboxane A_2 and PGI_2 were assayed serologically from the perfusion fluid and from the bronchoalveolar lavage fluid as their stable hydrolysis products, TxB_2 and 6-keto-PG$F_{1\alpha}$, as described previously (38). The limit of detection was 5 pg for TxB_2 and 10 pg for 6-keto-PG$F_{1\alpha}$, and 50% binding occurred at 50 pg for TxB_2 and at 170 pg for 6-keto-PG$F_{1\alpha}$. Cross-reactivity with other prostaglandins was <0.05% in both assays.

LDH was measured photometrically, and K^+ was measured by flame photometry by standard methods.

Model of isolated lungs—general procedure. The isolated-lung model has been described previously (45, 46, 48; W. Seeger, H. Radinger, and H. Neuhof, submitted for publication). Briefly, rabbits of either sex (body weight of between 2.2 and 2.6 kg; mean weight, 2.35 kg) were deeply anesthetized with 60 to 90 mg of pentobarbital per kg of body weight and were anticoagulated with 1,000 U of heparin per kg. The lungs were excised while being perfused with ice-cold Krebs Henseleit albumin buffer (KHAB) through cannulas in the pulmonary artery and the left atrium. The buffer contained 132.8 mM NaCl, 4.3 mM KCl, 1.1 mM KH_2PO_4, 24.1 mM $NaHCO_3$, 2.4 mM $CaCl_2$, 1.3 mM $MgPO_4$, 240 mg (wt/vol) of glucose per 100 ml, and 1 g (wt/vol) of albumin per 100 ml. Ice-cold perfusion fluid was used to slow down possible biochemical events stimulated by the onset of artificial circulation. The lungs were then placed in a temperature-equilibrated housing chamber at 0°C, freely suspended from a force transducer. As soon as the artificial perfusion was started, the lungs were ventilated with 4% CO_2–17% O_2–79% N_2. After equilibration with this CO_2-containing gas mixture, the pH of the perfusion fluid ranged between 7.35 and 7.45. The temperatures of the perfusion fluid and of the chamber were then raised to 37°C within 30 min, and the lungs were recirculatingly perfused during another 30-min steady-state period with a pulsatile flow of 100 ml/min. At constant flow, alterations of PAP are interchangeable with alterations of vascular resistance. The alternate use of two separate perfusion circuits, each containing 180 ml of KHAB, allowed the repetitive exchange of perfusion fluid. Pulmonary venous pressure was set at 0 mm Hg (1 mm Hg = 133.3 Pa), referenced at the hilum. Perfusion pressure, ventilation pressure, and the weight of the isolated organ were registered continuously. Lungs selected for the study were those that (i) had a homogeneous white appearance without signs of hemostasis or edema formation, and (ii) had lost lung weight during the phase of temperature increase and were completely isogravimetric in the 37°C steady-state period. Random examination of these lungs by light microscopy revealed virtually no adherence of erythrocytes, platelets, or leukocytes to the vascular wall and no evidence of interstitial edema or alveolar flooding.

Gravimetric estimation of the $K_{f,c}$ and vascular compliance. The $K_{f,c}$ of a vascular bed represents the hydraulic conductivity of the (micro)vascular walls times the surface area. It is thus a convenient parameter of the "leakiness" of the capillary bed, allowing the extravasation of fluid. The $K_{f,c}$ values were determined from the slope of lung weight gain ($\Delta W/\Delta t$) induced by a sudden increase in microvascular pressure (9–11, 50, 58; G. M. Greenberg, W. R. Henderson, D. L. Luchtel, R. J. Guest, and R. K. Albert, Am. Rev. Respir. Dis. **131**:420; Seeger et al., submitted); such a step rise in capillary pressure is followed by an initial phase of rapid weight gain (ΔW), which is generally accepted to represent vascular volume increase and is used for the calculation of vascular compliance, followed by a slower phase of weight gain due to filtration of fluid into extravascular spaces. The steepness of the slower phase is used for the calculation of $K_{f,c}$. The use of KHAB with only 1% albumin minimizes osmotic buffering, which interferes with the pressure-step-induced fluid filtration, and avoids alterations of vascular permeability, described for isolated organs perfused in the total absence of circulating protein (20, 21). The determinations of $K_{f,c}$ and vascular compliance were performed under zero-flow conditions to avoid any ambiguity in the height of the capillary filtration pressure. Immediately after stoppage of the perfusion flow, both the catheters to the pulmonary artery and to the left atrium were successively connected to KHAB reservoirs at 0 cm H_2O (60 s), 10 cm H_2O (60 s), 20 cm H_2O (30 s), −10 cm H_2O (60 s), and again at 0 cm H_2O (30 s). Thereafter, the perfusion fluid was exchanged, and the flow was again increased to 100 ml/min within 5 min. Thus, the entire maneuver of $K_{f,c}$ measurement lasted about 10 min and was performed without interruption of ventilation. $K_{f,c}$ was calculated from the rate of lung weight gain ($\Delta W/\Delta t$) during the slow phase of fluid filtration, which was taken to begin 15 s (25, 35, 56) after onset of the sudden microvascular pressure increments (Δ0 to 10 cm H_2O and Δ10 to 20 cm H_2O), and was expressed in cm^3 per cm H_2O per s per g (wet weight) of the lung $\times 10^{-4}$. The vascular compliance (40), which is the change in the vascular volume per microvascular pressure step, was determined from the initial, rapid phase of ΔW induced by the Δ0 to 10 and Δ10 to 20 cm H_2O pressure increments. The rapid weight change was read by extrapolating the slope of the slower phase of ΔW to the zero time of pressure increment.

The total ΔW induced by one maneuver of hydrostatic challenge was determined by the difference in lung weight between the two phases of 0 microvascular pressure.

Protocol of stimulation. In 12 isolated lungs, cytotoxin was administered to the KHAB in a concentration of 13 μg/ml in two consecutive 30-min-perfusion phases (Fig. 1, phases 1 and 2). After a stop-flow maneuver for the determination of $K_{f,c}$ and vascular compliance (7 out of 12 lungs), another perfusion phase without cytotoxin followed (Fig. 1, phase 3). This was interrupted when the the total ΔW exceeded 8 to 12 g (range, 15 to 30 min), and was followed by a second

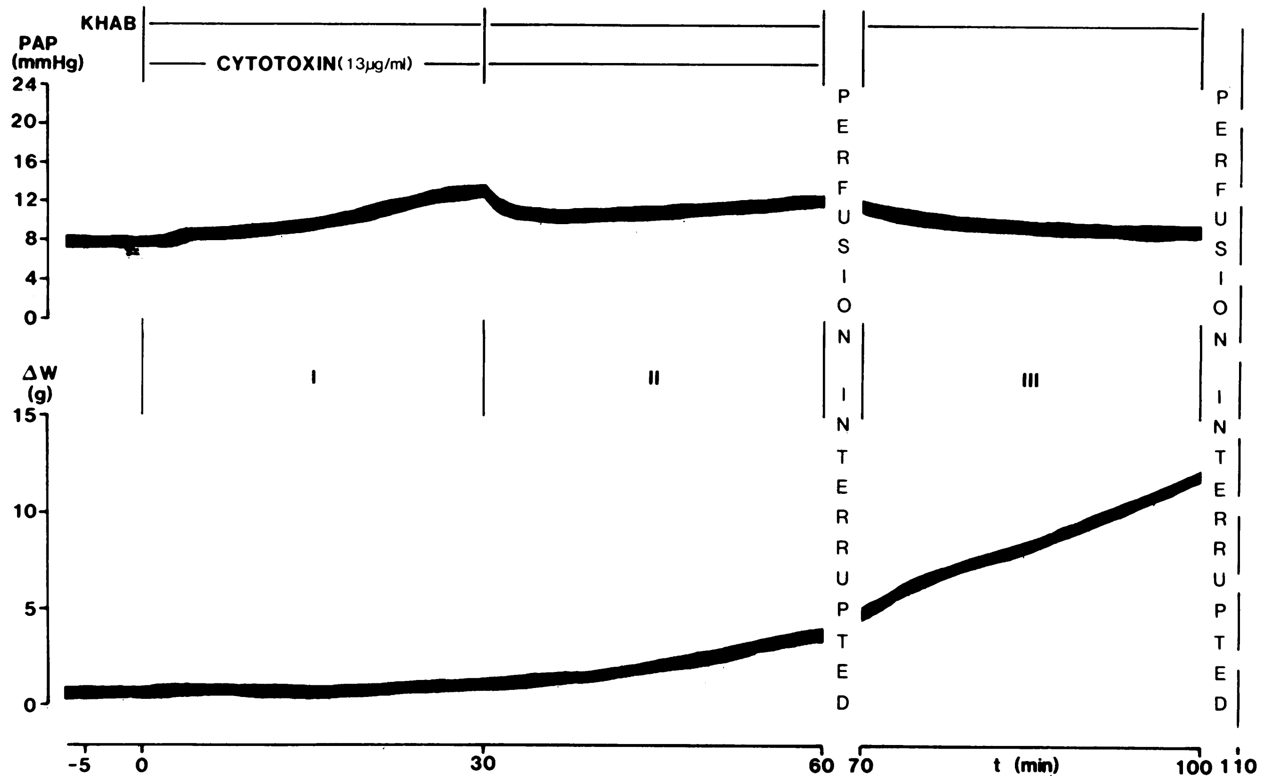

FIG. 1. PAP and ΔW after application of *Pseudomonas* cytotoxin. The toxin was administered to KHAB during two 30-min perfusion phases (1 and 2; the bars indicate change of perfusion fluid). After a stop-flow maneuver for the measurement of $K_{f,c}$, another perfusion phase without toxin was performed (phase 3). The modest and partially reversible increase in PAP and the marked ΔW are evident.

stop-flow maneuver. Five control lungs underwent the same time schedule and stop-flow procedure without application of the toxin.

In five separate experiments without toxin, edema formation was induced mechanically (after termination of the steady-state period) by elevating the left atrial pressure to values between 20 and 35 cm H_2O. This procedure took 10 to 25 min before a total increase in lung weight of 10 to 20 g was achieved (measured during intermittent phases of 0 left atrial pressure), mimicking the ΔW that the cytotoxin-treated lungs showed at the end of the experiment.

Bronchoalveolar lavage. After termination of the experiments, the bronchoalveolar trees of the lungs were lavaged twice with 30 ml of saline as described previously (46). The total recovery of lavage fluid was about 90%.

Statistical methods. Data were analyzed by the two-tailed Student *t* test for unpaired samples.

RESULTS

Control lungs. The control lungs showed virtually no change in PAP and lung weight throughout the experiments (Fig. 1; Table 1). The values of $K_{f,c}$ and vascular compliance (Table 2) corresponded to those previously described for this model (Seeger et al., submitted) and were in the same range as the values given from other laboratories for similar isolated-lung preparations (0.8 [31], 1.2 [58], 3.4 [37], and 4.7 cm^3 per cm H_2O per s per g [wet weight] of the lung $\times 10^{-4}$ [D. F. Kern, C. Kilven, and A. G. Malik, Microvasc. Res. 27:248]). The lungs showed only minimal release of LDH and no release of K^+ into the recirculating buffer (Table 3). There was very little generation of TxB_2 detectable after 30 min of recirculating perfusion. Some release of 6-keto-$PGF_{1\alpha}$

TABLE 1. PAP and ΔW after application of *P. cytotoxin* in isolated lungs[a]

Phase/time postapplication (min)	Cytotoxin-treated lungs (n = 12)[b]		Control lungs (n = 5)	
	PAP (mm Hg)	ΔW (g)	PAP (mm Hg)	ΔW (g)
0	7.9 ± 1.5	0	6.4 ± 0.7	0
1				
2	8.7 ± 1.5		6.4 ± 0.6	
10	10.8 ± 1.8[c]		6.4 ± 0.6	
30	15.0 ± 2.6[c]	0.3 ± 1.0	7.1 ± 1.4	0
2				
2	13.5 ± 1.8[c]		6.9 ± 0.8	
10	15.0 ± 4.1[c]		7.3 ± 0.8	
30	19.0 ± 7.9[d]	1.8 ± 1.3	7.5 ± 1.0	0
3				
2	19.3 ± 9.4[e]		8.0 ± 0.9	
10	17.8 ± 8.2[e]		8.0 ± 0.9	
15–30[f]	17.8 ± 8.1[e]	9.1 ± 5.5[d]	8.0 ± 0.9	<1

[a] The table gives the mean ± standard deviation of PAP and ΔW before (0 min) and at different times after (2, 10, and 15 to 30 min) the onset of each perfusion phase.

[b] *Pseudomonas* cytotoxin was administered to the perfusion fluid at a concentration of 13 µg/ml during phases 1 and 2.

[c] $P < 0.001$.

[d] $P < 0.01$.

[e] $P < 0.05$ (versus control, by the two-tailed Student *t* test for unpaired samples).

[f] Perfusion phase 3 was stopped in the cytotoxin group when the total lung weight exceeded 8 to 12 g. Therefore, the last values of PAP and ΔW between 15 and 30 min after onset of this phase are given.

TABLE 2. $K_{f,c}$ and vascular compliance after application of *Pseudomonas* cytotoxin in isolated lungs[a]

Stop-flow phase/Δ microvascular pressure	Cytotoxin-treated lungs (n = 7)[b]			Control lungs (n = 5)		
	$K_{f,c}$	Vascular compliance (ml)	$\Delta W_{stop\ flow}$ (g)	$K_{f,c}$	Vascular compliance (ml)	$\Delta W_{stop\ flow}$ (g)
1						
Δ0–10	6.9 ± 4.0	5.1 ± 0.6		3.2 ± 1.2	5.9 ± 1.7	
Δ10–20	18.0 ± 7.0[c]	4.8 ± 0.7	3.4 ± 2.9[d]	4.7 ± 0.9	5.4 ± 1.0	0.4 ± 0.6
2						
Δ0–10	11.7 ± 8.4	5.8 ± 1.0		3.3 ± 1.0	6.6 ± 1.9	
Δ10–20	18.5 ± 5.5[c]	4.7 ± 0.6	5.6 ± 1.7[e]	4.8 ± 1.8	5.0 ± 1.0	0.4 ± 0.4

[a] The table gives the mean ± standard deviation of $K_{f,c}$ (cm^3 per cm H_2O per s per g [wet weight] of the lung × 10^{-4}) and vascular compliance, detected from the microvascular pressure steps Δ0 to 10 and Δ10 to 20 cm H_2O in two stop-flow maneuvers. $\Delta W_{stop\ flow}$ gives the net ΔW induced by the two pressure steps.
[b] *Pseudomonas* cytotoxin had been administered to the perfusion fluid at a concentration of 13 μg/ml in two preceding 30-min perfusion phases.
[c] $P < 0.01$.
[d] $P < 0.05$ (versus control, by the two-tailed Student t test for unpaired samples).
[e] $P < 0.001$.

was measurable 10 and 30 min after the onset of a new perfusion phase, which corresponds to previous investigations with the present model (44, 45, 47).

Application of *P. aeruginosa* cytotoxin. Perfusion with cytotoxin containing KHAB resulted in a gradual rise of PAP to a maximum of about 2.5 times greater than that of the control (Fig. 1; Table 1). The increase in vascular resistance started within the first 5 min of phase 1 and was partially reversible in phase 3 in the absence of toxin. There was virtually no ΔW within the first 30 min of perfusion, and only a moderate ΔW (mean, 1.8 g) was found after 60 min of perfusion in the presence of toxin. The first stop-flow maneuver performed at this time did, however, already reveal a twofold increase of $K_{f,c}$ determined from a hydrostatic challenge of 10 cm H_2O and a fourfold increase of $K_{f,c}$ determined under a hydrostatic stress of 20 cm H_2O (Table 2). Corresponding to this marked increase in vascular permeability, there was a steady increase in lung weight in perfusion phase 3, even though the PAP decreased. The second stop-flow maneuver again detected an approximately fourfold increase in the capillary filtration coefficient. The values of vascular compliance did not differ between the

TABLE 3. Release of LDH, potassium (ΔK^+), 6-keto-$PGF_{1\alpha}$, and TxB_2 into the recirculating perfusate after application of *Pseudomonas* cytotoxin in isolated lungs[a]

Phase/time post-application (min)	Cytotoxin-treated lungs (n = 12)[b] Release of:				Control lungs (n = 5) Release of:			
	LDH (U/1)	ΔK^+ (mM)	TxB_2 (pg/ml)[c]	6-keto-$PGF_{1\alpha}$ (pg/ml)[c]	LDH (U/l)	ΔK^+ (mM)	TxB_2 (pg/ml)	6-keto-$PGF_{1\alpha}$ (pg/ml)
1								
2	10 ± 5	0.05 ± 0.02	<25	149 ± 161				
5	23 ± 9	0.12 ± 0.09	35 ± 10	238 ± 201				
10	35 ± 12	0.26 ± 0.14	39 ± 16	274 ± 192			<25	170 ± 83
20	47 ± 15	0.48 ± 0.21	36 ± 19	361 ± 142				
30	57 ± 15[d]	0.67 ± 0.24[d]	43 ± 31	487 ± 278[e]	13 ± 3	<0.1	60 ± 14	200 ± 43
2								
2	12 ± 5	0.14 ± 0.12	<25	227 ± 209				
5	19 ± 3	0.23 ± 0.15	41 ± 38	298 ± 171				
10	24 ± 5	0.31 ± 0.17	46 ± 41	361 ± 147[f]			<25	148 ± 79
20	33 ± 7	0.43 ± 0.17	48 ± 40	540 ± 204				
30	44 ± 11[d]	0.52 ± 0.16[d]	52 ± 40	1,103 ± 609[f]	11 ± 2	<0.1	51 ± 42	237 ± 78
3								
2	13 ± 5	0.12 ± 0.12	32 ± 14	289 ± 191				
5	18 ± 6	0.18 ± 0.15	32 ± 8	405 ± 137				
10	24 ± 6	0.23 ± 0.16	35 ± 12	604 ± 408[e]			<25	125 ± 79
20	33 ± 11	0.27 ± 0.15	42 ± 18	832 ± 264				
30	36 ± 11[f]	0.26 ± 0.08[f]	64 ± 25	1,043 ± 372[f]	11 ± 3	<0.1	72 ± 18	222 ± 91

[a] The table gives the mean ± standard deviation at different times (2, 5, 10, 20, and 30 min) after the onset of each perfusion phase.
[b] *Pseudomonas* cytotoxin was administered to the perfusion fluid at a concentration of 13 μg/ml during phases 1 and 2.
[c] TxB_2 and 6-keto-$PGF_{1\alpha}$ were determined in 7 out of 12 experiments with application of cytotoxin. In phase 3, perfusion was stopped in the cytotoxin group when the total ΔW exceeded 8 to 12 g; therefore, only (n = 5) lungs remained at 20 min, and (n = 3) lungs remained at 30 min in this group.
[d] $P < 0.001$.
[e] $P < 0.05$ (versus control, by the two-tailed Student t test for unpaired samples.
[f] $P < 0.01$.

control lungs and the cytotoxin-treated lungs in both stop-flow maneuvers.

Cytotoxin application caused a continuous release of K^+ and LDH into the recirculating KHAB. Both events started within the first 5 min and continued during perfusion phases 1 and 2 in the presence of toxin, as well as during perfusion phase 3, performed in the absence of toxin (Table 3). Summing up the amounts of K^+ and LDH released into the three perfusates gives a mean of 0.26 mmol and 25 U, respectively. The total K^+ and LDH contents of lungs in the size used (determined after complete rinsing of the vascular bed) ranged from 0.45 to 0.52 mmol and 350 to 500 U, respectively. Thus, about 50% of the lung cellular K^+ and about 5 to 7% of the lung cellular LDH was released into the circulating buffer after pseudomonal cytotoxin exposure.

In contrast to the control lungs, there was some TxB_2 generation detectable 10 min after cytotoxin application. This ranged only slightly above the detection limit, however, and the TxB_2 levels measured in the three perfusates after 30 min of recirculation showed no difference between the cytotoxin-treated lungs and the control lungs. In contrast, there was a marked and continuous release of 6-keto-$PGF_{1\alpha}$ into the three perfusates of the cytotoxin-treated lungs, up to maximum values five times above that of the control. As with K^+ and LDH release, PGI_2 formation was already detectable within the first 5 min after cytotoxin application.

In seven cytotoxin-treated lungs, in the five control lungs, and in the five separate lungs with mechanically induced edema, thromboxane and PGI_2 were measured in the bronchoalveolar lavage fluid at the end of the experiments. The TxB_2 values were 721 ± 320 (cytotoxin-treated), 418 ± 157 (mechanically induced edema), and 43 ± 28 pg/ml (control) (mean ± standard deviation). The corresponding values of 6-keto-$PGF_{1\alpha}$ were 3,680 ± 1,810, 1,614 ± 408, and 147 ± 73 pg/ml. With both parameters, the edematous lungs thus differed significantly from the control lungs ($P < 0.01$ and $P < 0.001$, respectively). Additionally, the amount of 6-keto-$PGF_{1\alpha}$ released from the cytotoxin-treated lungs into the lavage fluid significantly exceeded the amount found in the lungs with mechanically induced edema ($P < 0.05$).

DISCUSSION

The effects of *P. aeruginosa* cytotoxin on the pulmonary microvascular bed observed in the present study can be categorized under three aspects: (i) evidence for lung cell membrane damage, (ii) induction of PGI_2 generation, and (iii) increase of vascular resistance and permeability.

Evidence for lung cell membrane damage. *P. aeruginosa* cytotoxin effects have been studied on a variety of eucaryotic cells, including granulocytes (3, 43), endothelial cells (54, 55), liver and hepatoma cells (14), splenocytes (34), and tumor cells (27–29). The earliest symptom of toxification in these cells is an increased permeability of the plasma membrane to small ions and molecules, such as K^+, Na^+, Ca^{2+}, glucose, ATP, and ruthenium red. The loss of cellular K^+ especially may amount to a total of greater than 70%. Swelling of the cells due to the colloid osmotic pressure of the cellular macromolecules is assumed to cause subsequent larger membrane defects, allowing the loss of larger molecules such as LDH or protein-bound ^{51}Cr after an initial lag period. The K^+ loss noted in the present study, which amounts to nearly 50% of the total lung cellular K^+ within 90 min, is well in accordance with these results from isolated cells. There is also a clear quantitative gap between K^+ loss and LDH release; LDH release amounts to only 5 to 7% of the total lung cellular LDH during the time of the experiment. In contrast to the findings from isolated cells, however, no time gap was seen between K^+ release and LDH release, but the time courses of both were nearly parallel over the 90-min period. This may be partly explained by the limited accessibility to cells within an intact organ structure after acute toxin application; both the time course of toxin access to the different cells and the time course of cellular events upon toxin attack superimpose and may thus obscure discrete differences in the sequence of cellular events. The K^+ losses and LDH losses in the present study clearly precede the edema formation and are not the consequence of tissue hydration. The first signs of edema formation start only after 20 to 30 min; severe pulmonary edema caused mechanically or by other agents, such as arachidonic acid, is not accompanied by any release of these cellular markers (45). A similar K^+ release in isolated lungs has been noted after the application of staphylococcal alpha-toxin (45). This toxin oligomerizes on target membranes to form annular transmembrane pores with an inner diameter of 2 to 3 nm (15). Owing to this limited inner pore size, the release of K^+ was not accompanied by any release of LDH in the isolated lung experiments with administration of staphylococcal alpha-toxin. It is not clear from the available data why the same degree of K^+ loss, indicative of a similar degree of primary membrane attack, is accompanied by significant LDH release as an indicator of colloid osmotic cell swelling after addition of *Pseudomonas* cytotoxin but not *Staphylococcus* alpha-toxin. It must kept in mind, however, that the total amount of LDH release was only about 10% of the total K^+ loss. Differing toxin accessibility to the different lung cells may be relevant, and the cytotoxin-induced LDH release may originate only from types of lung cells not affected by alpha-toxin. Cytotoxin-treated pulmonary endothelial cells in vitro revealed marked K^+ loss without any LDH release over an incubation period of 75 min (54).

Induction of PGI_2 generation. In addition to their K^+ loss, these cells generated large amounts of PGI_2. Accordingly, there was marked and continuous release of PGI_2 into the three perfusates of the cytotoxin-treated lungs in the present study to maximum values five times above that of the control. This PGI_2 generation is not a secondary event caused by ΔW or increase in PAP, since it was already detectable within the first 5 min, when these physical parameters were unaltered or only minimally altered. The PGI_2 levels in the bronchoalveolar lavage fluid, determined at the end of the experiment, were found to be about 25 times above that of the control. These levels significantly exceeded those found in the lungs in which the same amount of edema was induced purely mechanically (values about 10 times above that of the control). The marked PGI_2 generation induced by cytotoxin is indicative of toxin attack on the endothelial cells in the present study. This is in accordance with experiments with rats, in which deposition of cytotoxin in the endothelial cells of nearly all organs, including the lung, could be demonstrated immunohistologically (60). Correspondingly, a significant release of angiotensin-converting enzyme, an endothelial cell marker, into the lung lymph fluid could be demonstrated during *Pseudomonas* bacteremia in sheep (18). Though PGI_2 generation in the lung was detected early in the present study, it cannot account for the other described cytotoxin effects. Application of high doses of PGI_2 in isolated rabbit lungs does not increase vascular resistance or permeability and does not cause K^+ loss or LDH loss (48). The time course and the maximum amount of PGI_2 released into the perfusate after cytotoxin administration were similar to those observed after application of

staphylococcal alpha-toxin in the same model system (45). In contrast to alpha-toxin, however, only small and insignificant levels of thromboxane were detectable in the perfusion fluid after cytotoxin administration, and the markedly increased amounts of TxB_2 found in the bronchoalveolar lavage fluid (about 17 times above that of the control) did not differ significantly from those found in the lungs with mechanically induced edema (10 times above that of the control). The cellular origin of thromboxane in blood-free isolated lungs is still unsettled, and different accessibility or sensitivity of the responsible cell(s) to cytotoxin and to alpha-toxin may be responsible for the noted difference.

Increase of vascular resistance and permeability. In rabbit lungs, toxins such as eledoisin, melittin, and staphylococcal alpha-toxin cause a severalfold acute increase in pulmonary vascular resistance via stimulation of the pulmonary arachidonic acid cascade and formation of the vasoconstrictive agent thromboxane A_2 (45, 47). In contrast, the pressure response evoked by pseudomonal cytotoxin was only moderate and, at least in part, reversible. The very low levels of TxB_2 in the perfusion fluid argue against a major contribution of this agent to the pressure increase. The predominating microvascular response to *P. aeruginosa* cytotoxin was a fourfold increase in the $K_{f,c}$, which was detectable after a lag period of 20 to 30 min and was not reversible during a subsequent perfusion phase in the absence of the toxin. In contrast to $K_{f,c}$, the values of vascular compliance were not altered. This strongly suggests that the capillary surface area was not significantly increased (36), and thus the severalfold rise of $K_{f,c}$ appears to be entirely due to an increased hydraulic conductivity of the pulmonary microvessels, i.e., an increased leakiness of the capillary bed, allowing extravasation of fluid. The time course of pressure response and $K_{f,c}$ increase and the severity of the alteration of lung vascular permeability after application of cytotoxin are reminiscent of the effects described for *P. aeruginosa* bacteremia in sheep (5, 7, 18, 49). The present results are also in accordance with recent experiments in intact rabbits, in which *P. aeruginosa* cytotoxin infusion caused an early increase in lung vascular resistance, pulmonary edema formation, and severe disturbances of pulmonary gas exchange (H. Neuhof, E. Meier, A. Reichwein, W. Seeger, and F. Lutz, manuscript in preparation). The effects of *P. aeruginosa* or its cytotoxin in these two animal species are thus similar to those repeatedly described after application of endotoxin in animal models (4). Endotoxin, however, is ineffective in isolated lungs perfused in the absence of plasma and circulating cells (6; W. Seeger, unpublished data), whereas it is a salient feature of the present study that the microvascular lesions were evoked by cytotoxin in the primary absence of circulating leukocytes and plasma components such as complement. As the cytotoxin is known to be also effective on granulocytes (3, 43), these alterations might even be amplified in the presence of lung leukocyte sequestration. The concentration of cytotoxin used in the present study is similar to that usually used in other systems (range, 1 to 60 μg/ml). This concentration is probably higher than can be expected in the circulation in vivo, even in states of severe *P. aeruginosa* sepsis. It has to be kept in mind, however, that lung tissue invasion by *P. aeruginosa* will create local toxin concentrations far exceeding those in the systemic circulation. Thus, pseudomonal cytotoxin may be an important factor in the pathogenesis of prolonged pulmonary microvascular injury, encountered in states of *P. aeruginosa* sepsis or in the course of ARDS with secondarily acquired *P. aeruginosa* pneumonia.

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