Mechanisms of Pulmonary Edema Induced by Tumor Necrosis Factor-α

Denise C. Hocking, Patricia G. Phillips, Thomas J. Ferro, and Arnold Johnson

We tested the hypothesis that human recombinant tumor necrosis factor-α (TNF) promotes pulmonary edema by neutrophil-dependent effects on the pulmonary vasculature. The isolated guinea pig lung was perfused with phosphate-buffered Ringer’s solution with or without human neutrophils. The infusion of neutrophils (9×10⁶ total) into lungs isolated after the in vivo administration of TNF (3.2×10⁶ units/kg) resulted in weight gain (+1.95±0.311 g versus −0.053±0.053 g in control) and an increase in the lung (wet-dry)-to-dry weight ratio (8.3±0.5 versus 6.0±0.2 in control), indicating the formation of pulmonary edema. The neutrophil-dependent pulmonary edema induced by TNF was associated with a combination of increased capillary permeability (capillary filtration coefficient [Kf,c], 0.170±0.048 g/min/cm H₂O/g at 30 minutes versus 0.118±0.008 g/min/cm H₂O/g at baseline) and increased pulmonary capillary pressure (Pp, 12.8±0.8 cm H₂O at 60 minutes versus 6.0±0.3 cm H₂O at baseline). The Pp increase was mediated by thromboxane A₂ (TXA₂) because the TXA₂ synthetase inhibitor Dazoxiben (0.5 mM) prevented the effect (Pp, 6.7±0.6 cm H₂O at 60 minutes with Dazoxiben) and thromboxane B₂ (TXB₂) levels were increased in the pulmonary venous effluent (5.24±599 pg/ml at 60 minutes versus 60±13 pg/ml at baseline). Studies using WEB-2086 (37 μM), a platelet activating factor (PAF) receptor antagonist, indicated that PAF mediated the increased vascular permeability (Kf,c, 0.107±0.014 g/min/cm H₂O/g at 30 minutes using WEB-2086) and, in part, the increased Pp (Pp, 8.4±0.7 cm H₂O at 60 minutes using WEB-2086). In addition, alterations of endothelial peripheral actin bands were noted after TNF administration. The data indicate that TNF induces neutrophil-dependent pulmonary edema associated with increased Pp (mediated by TXA₂ and PAF), increased Kf,c (mediated by PAF), and changes in endothelial peripheral actin bands. (Circulation Research 1990;67:68–77)

Tumor necrosis factor-α (TNF) is a monokine produced by monocytes and macrophages in response to endotoxin, interleukin-2, and mitogens.1-3 TNF induces many immune functions, including endothelial secretion of interleukin-1,4,5 endothelial procoagulant activity,6,7 and neutrophil (PMN) activation.8,9 TNF is a mediator of endotoxemia because 1) TNF infusion induces an endotoxemia-like state in animals,1 2) passive immunization against TNF can prevent the multiple organ failure seen after endotoxin challenge,3 3) genetic deficiency of TNF production inhibits the development of gram-negative shock,8 and 4) the anatomic evidence of injury and edema seen in the lungs and other organs in TNF-treated mice is similar to that seen in endotoxemia.1

The mechanism of TNF-induced pulmonary edema is not clear. TNF directly increases pulmonary endothelial permeability to protein in vivo11 and induces the rearrangement of human endothelial monolayers in vitro.12 TNF may also promote pulmonary edema by increasing pulmonary capillary pressure.11 Platelet activating factor (PAF) may mediate TNF-induced pulmonary edema because 1) TNF stimulates the release of PAF from endothelial cells, macrophages, and PMN,13 and 2) PAF can induce pulmonary vasoconstriction and increases in vascular permeability.14 Arachidonic acid metabolites such as thromboxane A₂ (TXA₂) may play a role in the pathogenesis of TNF-induced pulmonary edema because cyclooxygenase inhibition partially blocks the hemodynamic and other effects of TNF in vivo.11,15
The role of PMN in the pathogenesis of TNF-induced pulmonary edema is also unclear, but several of the in vitro activities of TNF indicate that PMN may mediate this effect. TNF can 1) activate PMN to release oxygen radicals, 2) increase PMN adherence to the endothelium, 3) stimulate endothelial cells to produce a PMN chemotactic factor, and 4) induce endothelial cell lysis by activated PMN. Pulmonary edema mediated by TNF in vivo in the guinea pig is prevented by prior PMN depletion, whereas pulmonary edema occurs despite prior PMN depletion in sheep.

We postulated that TNF promotes pulmonary edema predominantly by PMN-dependent effects on the pulmonary vasculature. We tested this hypothesis using the isolated guinea pig lung perfused with phosphate-buffered Ringer's solution with or without human PMN. The TNF (3.2x10^5 units/kg) was administered in vivo 18 hours before study to allow sufficient time for presumptive endothelial-derived PMN chemotactic and adherence factors to be expressed. The role of arachidonic acid metabolites and PAF was evaluated because these factors may mediate the effects of TNF. We also studied the effects of TNF on perfused lung endothelial actin filament morphology because peripheral actin bands are thought to play a role in paracellular permeability by stabilizing intercellular junctional proteins, and changes in the peripheral actin bands coincide with increases in vascular permeability.

**Materials and Methods**

*Isolated Perfused Lung Preparation*

The isolated perfused lung studies were performed as previously described. In brief, Hartley guinea pigs (300–500 g; Charles River Laboratories, Wilmington, Mass.) were anesthetized with sodium pentobarbital (5 mg/100 g i.p.; Abbott Laboratories, Chicago, Ill.), and the trachea was cannulated. The chest was opened by median sternotomy, and 700 units/kg heparin (Invenex, Chagrin Falls, Ohio) was administered by intracardiac puncture injection. The lungs and heart were excised and suspended from a counter-weighted beam balance attached to a force transducer (model 100DCT, Shaevitz, Pennsauken, N.J.). The airway pressure was maintained at 1 cm H2O with 95% O2 and 5% CO2 throughout the experiment. The pulmonary artery and left atrium were cannulated, and the left atrial pressure was set at 4 cm H2O. Lung perfusion (6 ml/min/100 g body wt) was maintained by a peristaltic roller perfusion pump (model 1215, Harvard Apparatus, Millis, Mass.). Recirculation of the perfusate (300 ml) was begun after the venous effluent was clear of cellular elements. The perfusate consisted of a phosphate-buffered Ringer's solution with 5.55 mM dextrose and 0.5% bovine serum albumin (fraction V, Sigma Chemical Co., St. Louis, Mo.). The system was maintained at a constant temperature of 37°C, and the pH was maintained between 7.30 and 7.40. The pulmonary artery pressure (Ppa) was monitored using a pressure catheter (PE-50) that was inserted into the pulmonary artery cannula and connected to a pressure transducer (model P10EZ, Spectramed, Oxnard, Calif.). The pulmonary venous pressure (Ppv) was monitored similarly except that the pressure catheter was inserted into the left atrial catheter. The Ppa, Ppv, and change in weight were recorded continuously (model 3000S recorder, Gould Instruments, Cleveland, Ohio). Rarely, lungs were excluded from study because a spontaneous increase in Ppa or weight occurred during the 15-minute baseline perfusion period following lung isolation.

**Pulmonary Capillary Pressure, Pulmonary Arterial Resistance, and Pulmonary Venous Resistance**

The pulmonary capillary pressure (Pcp) was estimated using the double occlusion method. The arterial inflow and venous outflow lines were simultaneously occluded using in-line solenoid valves, and the Pcp was estimated by measuring the subsequent equilibrium pressure. The Pcp estimate was used to determine both the pulmonary arterial resistance (Ra) and the pulmonary venous resistance (Rv) from the equations Ra = (Ppa−Ppc)/Q and Rv = (Ppv−Ppc)/Q, where Q equals flow.

**Pulmonary Capillary Filtration Coefficient**

The pulmonary capillary filtration coefficient (Kfc), a measure of pulmonary vascular permeability to water, was estimated using the method of Drake et al. After an isogravimetric period, the Ppv was elevated by 4 cm H2O for 5.5 minutes, and the resulting increase in lung weight was recorded. To evaluate changes in interstitial volume, the late component (from 2 to 5.5 minutes after Ppv elevation) of weight change was analyzed. The rate of change in weight was extrapolated back to the time of the initial Ppv elevation using the antilog of the y intercept of the linear regression line. The Kfc was calculated by dividing the extrapolated rate of weight change by the change in Pcp and the dry lung weight.

**Lung (Wet-Dry)-to-Dry Weight Ratio**

At the end of the experimental period, the lungs were removed, cleared of all extrapulmonary tissue, and weighed (wet weight). After the lungs were dried at 75°C for 3 days, they were again weighed (dry weight), and the lung (wet-dry)-to-dry weight ratio (W/D weight ratio) was calculated.

**Tumor Necrosis Factor-α**

TNF (highly purified recombinant human TNF-α from Escherichia coli, courtesy of Dr. Abla Creasey, Cetus Corporation, Emeryville, Calif.) having a specific activity of 24x10^6 units/mg was used. The preparation contained less than 0.8 pg of endotoxin per 10^6 units of TNF activity by standard limulus assay. TNF (3.2x10^6 units/kg) was injected intraperitoneally 18 hours before lung isolation. To control for endotoxin contamination and possible effects of the
TNF vehicle, TNF was heated to 90°C (a temperature that does not inactivate endotoxin)27 for 45 minutes. After heating, no residual TNF activity was noted using the standard L929 fibroblast lysis assay.28

Preparation of Neutrophils

Human PMN were isolated from venous blood obtained from healthy donors as described.29 The blood was collected in tubes containing EDTA (0.1 M) and layered onto neutrophil isolation medium (NIM, Los Alamos Diagnostics, Los Alamos, N.M.). The blood-NIM was centrifuged at 400g for 30 minutes at room temperature. The PMN-rich layer was removed and pooled in a 50-ml conical tube with equal volume of Hanks’ balanced salt solution without calcium and magnesium (HBSS, GIBCO Laboratories, Grand Island, N.Y.). The PMN were then centrifuged at 300g for 10 minutes at room temperature. The supernatant was discarded, and the remaining red blood cells were removed by gentle lysis by adding hypotonic (0.2% NaCl) phosphate-buffered saline to the cells for 15 seconds followed by the immediate addition of hypertonic (1.6% NaCl) phosphate-buffered saline. After centrifugation (300g, 5 minutes, room temperature) and removal of the supernatant, PMNs were resuspended in 1 ml HBSS containing 5.5 mM glucose. PMNs were counted and diluted in HBSS containing 5.5 mM glucose to give a final concentration of 3×10⁶ PMN/ml. Greater than 98% of the cells counted were PMNs by light microscopy. PMN viability was always greater than 97% by trypan blue exclusion.

Dazoxiben

To test the role of TXA₂ in the response to TNF, Dazoxiben (Pfizer, Groton, Conn.), a specific inhibitor of TXA₂ synthetase,30 was used. The Dazoxiben was added to the perfusate before the baseline period to achieve a final concentration of 0.5 mM. We have previously shown this dose to inhibit TXA₂-dependent increases in Pₚc in the isolated perfused lung.22

WEB-2086

To assess the role of platelet activating factor (PAF) in the response to TNF, WEB-2086 (Boehringer Ingelheim, Ridgefield, Conn.), a specific inhibitor of the PAF receptor, was used.31 WEB-2086 was added to the perfusate before the baseline period to achieve a final concentration of 37 μM. This dose of WEB-2086 has been shown to block the hemodynamic responses to PAF.31

Control Studies

Control group (n=7). The lung isolation and 15-minute baseline perfusion period were as described above. The Pₚa, Pₚc, change in weight, and Kₑc were measured at baseline and at 30 and 60 minutes.

Tumor Necrosis Factor-α In Vivo Studies

TNF group (n=8). TNF was administered as outlined above. The lungs were otherwise treated as in the control group.

TNF+Dazoxiben group (n=5). Guinea pigs were treated as in the TNF group. After lung isolation, Dazoxiben was added to the perfusate as described above. The lungs were then treated as in the TNF group.

Dazoxiben group (n=3). This group was studied to control for possible independent effects of Dazoxiben. Dazoxiben was added to the perfusate before the baseline period as outlined above, and the lungs were treated as in the control group.

TNF+WEB-2086 group (n=6). Guinea pigs were treated as in the TNF group. After lung isolation, WEB-2086 was added to the perfusate as described above. The lungs were then treated as indicated in the TNF group.

WEB-2086 group (n=3). This group was studied to control for possible independent effects of WEB-2086. WEB-2086 was added to the perfusate before the baseline period as outlined above, and the lungs were treated as in the control group.

Heat-inactivated TNF group (n=3). These experiments were performed identically to the TNF group, using TNF that had been heat-inactivated at 90°C for 45 minutes.28

Neutrophil Studies

PMN group (n=6). Lung isolation and baseline measurements were obtained after a 10-minute period of stable lung weight, and PMNs (9×10⁶ suspended in 3.0 ml HBSS containing 5.5 mM glucose) were infused into the lung over 5 minutes via the pulmonary artery port. Measurements were taken as in the control group.

TNF+PMN group (n=10). Guinea pigs were administered TNF as in the TNF group. The lungs were then isolated and treated as in the PMN group.

TNF+WEB-2086+PMN group (n=11). The protocol for this group was similar to the TNF+WEB-2086 group. PMN were then infused and measurements obtained as in the PMN group.

WEB-2086+PMN group (n=3). This group was studied to control for possible independent effects of WEB-2086. WEB-2086 was added to the perfusate before the baseline period as outlined above, and the lungs were treated as in the PMN group.

TNF+Dazoxiben+PMN group (n=8). The protocol for this group was similar to the TNF+Dazoxiben group. PMN were then infused and measurements obtained as in the PMN group.

Dazoxiben+PMN group (n=3). This group was studied to control for possible independent effects of Dazoxiben. Dazoxiben was added to the perfusate before the baseline period as outlined above, and the lungs were treated as in the PMN group.
Radioimmunoassay of Arachidonic Acid Metabolites

Lung venous effluent samples were collected at 0, 30, and 60 minutes for measurement of thromboxane B2 (TXB2), the stable product of TXA2, and 6-ketoprostaglandin-F1α (6-keto-PGF1α), the stable product of prostacyclin. Concentrations were determined using a double-antibody radioimmunoassay as described.22

Perfused Lung Pulmonary Artery Endothelial Actin Filament Morphology

The perfusion fixation technique previously described by Forssman et al32 was used. Isolated lungs were prepared as indicated above. At the end of the experimental period, the lungs were perfused for 3 minutes with a rinse solution (0.9% NaCl, 2.5% polyvinylpyrrolidone, 0.025% heparin, and 0.5% procaine HCl), followed by a 15-minute perfusion with fixative (2.0% formaldehyde, 0.1% picric acid, and 50 mM sodium cacodylate, pH 7.4). After fixation, the lungs were briefly rinsed with phosphate-buffered saline containing calcium. The pulmonary artery was then cut open lengthwise and pinned on dental wax. After three 5-minute washes with phosphate-buffered saline, the tissue was permeabilized with −20°C acetone for 5 minutes, followed by three additional 5-minute washes with phosphate-buffered saline. Specific staining of actin filaments was performed by incubating the tissue segment with rhodamine phalloidin (Molecular Probes, Eugene, Ore.) for 60 minutes at 37°C. Cells were visualized using an Olympus IM2 inverted microscope equipped for epifluorescence and photographed on Kodak Tri-X Pan film at ×1,235 magnification.

Statistics

The two-way analysis of variance with repeated measures for mixed design33 was used to compare the results of different time points (columns) and different experimental groups (rows). If variance among rows or columns was noted, Scheffe’s test with correction for multiple comparisons33 was used to determine significant differences between specific points within the rows or columns.

Results

Pulmonary Effects of Neutrophils After Administration of Tumor Necrosis Factor-α

Increases in both the lung weight and Ppc were noted in the TNF+PMN group at 60 minutes compared with baseline and also compared with the heat-inactivated TNF+PMN, TNF, and PMN groups at 60 minutes (Figure 1). The TNF and PMN were synergistic, as opposed to additive, in promoting weight gain. Small increases in the Ppc and lung weight were noted in the TNF group at 60 minutes compared with baseline (Figure 1). Small increases in lung weight at 60 minutes compared with baseline, without significant increases in Ppc were noted in the heat-inactivated TNF+PMN and PMN groups (Figure 1). No differences in the baseline Ppc measurements were noted among the groups (Figure 1).

The use of either Dazoxiben or WEB-2086 prevented the increase in Ppc and lung weight seen in the TNF+PMN group (Figure 2). No differences in the baseline Ppc measurements were noted among the groups (Figure 2).

Pulmonary Effects of Administration of Tumor Necrosis Factor-α

Increases in the Ppc and lung weight were noted in the TNF group at 60 minutes compared with baseline and the heat-inactivated TNF group (Table 1). The addition of either Dazoxiben or WEB-2086 to the perfusate inhibited the increase in Ppc and lung weight seen in the TNF group (Table 1). No differences in the baseline Ppc measurements were noted among the groups (Table 1).

Lung (Wet-Dry)-to-Dry Weight Ratio

An increase in the lung W/D weight ratio was noted in the TNF+PMN group compared with the other groups (Table 2). The addition of either
Dazoxiben or WEB-2086 to the circulating perfusate prevented the increase (Table 2).

**Pulmonary Arterial and Venous Resistance**

An increase in $R_s$ was noted in the TNF+PMN group at 60 minutes compared with baseline and the other groups (Table 3). The use of either Dazoxiben or WEB-2086 prevented the increase in $R_s$ seen in the TNF+PMN group (Table 3). There were no significant changes in the $R_s$ in any of the groups (Table 3).

**Table 2. Effect of Tumor Necrosis Factor and Neutrophils on Lung (Wet-Dry)-to-Dry Weight Ratio**

<table>
<thead>
<tr>
<th>Group</th>
<th>W/D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.0±0.2</td>
</tr>
<tr>
<td>TNF+PMN</td>
<td>8.3±0.5*</td>
</tr>
<tr>
<td>Heat-inactivated TNF+PMN</td>
<td>6.0±0.3</td>
</tr>
<tr>
<td>TNF+Dazoxiben+PMN</td>
<td>5.9±0.2</td>
</tr>
<tr>
<td>TNF+WEB-2086+PMN</td>
<td>6.6±0.4</td>
</tr>
<tr>
<td>TNF</td>
<td>6.5±0.3</td>
</tr>
<tr>
<td>Heat-inactivated TNF</td>
<td>5.8±0.1</td>
</tr>
<tr>
<td>PMN</td>
<td>6.4±0.5</td>
</tr>
</tbody>
</table>

Values are mean±SEM. W/D, (wet-dry)-to-dry weight ratio; TNF, tumor necrosis factor-α; PMN, neutrophil.

*Different from all other groups ($p<0.05$).

**Pulmonary Capillary Filtration Coefficient**

Increases in the $K_{fe}$ were noted at 30 minutes compared with baseline, but not at 60 minutes, in both the TNF+Dazoxiben and TNF+Dazoxiben+PMN groups (Table 4). The $K_{fe}$ was unchanged in the TNF+WEB-2086, TNF+WEB-2086, Dazoxiben+PMN, Dazoxiben, and control groups (Table 4). The $K_{fe}$ was measured only in the control group and in groups in which Dazoxiben or WEB-2086 were used because prolonged periods of stability in lung weight (which are required for the measurement24) were present only in these groups.

**Lung Effluent Arachidonic Acid Metabolites**

Levels of TXB$_2$ were increased in the TNF+PMN group at 60 minutes compared with baseline and the other groups (Table 5). Smaller increases were noted in TXB$_2$ levels in the TNF, PMN, and TNF+WEB-PMN groups at 60 minutes compared with baseline (Table 5). In the control, TNF+Dazoxiben+PMN, and TNF+WEB-2086+PMN groups, there were no changes in TXB$_2$ levels (Table 5). Levels of 6-keto-PGF$_1α$ were increased in the TNF+PMN group at 60 minutes compared with the other groups, and in all groups at 60 minutes compared with baseline (Table 5).

**Pulmonary Artery Endothelial Actin Filament Morphology**

The characteristic appearance of normal pulmonary artery endothelium stained with rhodamine

**Table 1. Effect of Tumor Necrosis Factor on Pulmonary Capillary Pressure and Change in Lung Weight**

<table>
<thead>
<tr>
<th>Group</th>
<th>Pulmonary capillary pressure (cm H$_2$O)</th>
<th>Change in lung weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline 30 min 60 min</td>
<td>30 min 60 min</td>
</tr>
<tr>
<td>Control</td>
<td>5.9±0.4 5.4±0.3 5.9±0.3</td>
<td>-0.010±0.060 -0.053±0.053</td>
</tr>
<tr>
<td>TNF</td>
<td>5.8±0.3 6.0±0.3 8.3±0.7*</td>
<td>-0.012±0.066 0.409±0.179*</td>
</tr>
<tr>
<td>Heat-inactivated TNF</td>
<td>4.9±0.1 5.1±0.1 5.4±0.3</td>
<td>-0.019±0.065 -0.073±0.074</td>
</tr>
<tr>
<td>TNF+Dazoxiben</td>
<td>4.9±0.1 5.2±0.2 5.4±0.2</td>
<td>-0.080±0.040 -0.052±0.060</td>
</tr>
<tr>
<td>TNF+WEB-2086</td>
<td>4.7±0.1 4.6±0.2 5.5±0.2</td>
<td>-0.047±0.066 -0.021±0.076</td>
</tr>
</tbody>
</table>

Values are mean±SEM. TNF, tumor necrosis factor-α.

*Different from the respective baseline value ($p<0.01$) and from the heat-inactivated TNF, TNF+Dazoxiben, TNF+WEB-2086, and control groups ($p<0.01$).
TABLE 3. Effect of Tumor Necrosis Factor and Neutrophils on Arterial and Venous Resistance

<table>
<thead>
<tr>
<th>Group</th>
<th>Arterial resistance</th>
<th>Venous resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline 60 min</td>
<td>Baseline 60 min</td>
</tr>
<tr>
<td>Control</td>
<td>0.072±0.012 0.073±0.007</td>
<td>0.056±0.006 0.086±0.015</td>
</tr>
<tr>
<td>TNF+PMN</td>
<td>0.068±0.016 0.108±0.019</td>
<td>0.075±0.008 0.339±0.54*</td>
</tr>
<tr>
<td>Heat-inactivated TNF+PMN</td>
<td>0.043±0.018 0.052±0.021</td>
<td>0.073±0.008 0.117±0.004</td>
</tr>
<tr>
<td>TNF+Dazoxiben+PMN</td>
<td>0.088±0.008 0.079±0.014</td>
<td>0.060±0.007 0.110±0.027</td>
</tr>
<tr>
<td>TNF+WEB-2086+PMN</td>
<td>0.070±0.013 0.068±0.008</td>
<td>0.051±0.008 0.164±0.031*</td>
</tr>
<tr>
<td>TNF</td>
<td>0.061±0.009 0.078±0.007</td>
<td>0.070±0.010 0.078±0.028</td>
</tr>
<tr>
<td>Heat-inactivated TNF</td>
<td>0.075±0.015 0.086±0.024</td>
<td>0.040±0.007 0.061±0.027</td>
</tr>
<tr>
<td>PMN</td>
<td>0.087±0.008 0.064±0.014</td>
<td>0.064±0.010 0.108±0.023</td>
</tr>
</tbody>
</table>

Values are mean±SEM in cm H2O/ml/min. TNF, tumor necrosis factor-α; PMN, neutrophil.
*Different from the respective baseline value (p<0.05).
†Different from all other groups (p<0.01).

phallodin, a cobblestone pattern with intact peripheral actin bands, was seen in the control group (Figure 3A). In the TNF+PMN group (Figure 3B), focal areas of abnormal morphology were noted, characterized by marked ruffling of peripheral actin bands, less regular orientation of endothelial cells, and occasional cell shape changes. Abnormalities similar to those seen in Figure 3B were noted in the TNF group. The use of Dazoxiben or WEB-2086 did not prevent these abnormalities (morphology similar to Figure 3B). No abnormalities were noted in the PMN, Dazoxiben, and WEB-2086 groups (morphology similar to Figure 3A).

Discussion

In this study, we have shown that TNF administered in vivo sensitizes the lung to the development of PMN-dependent edema, as increases were noted in both lung weight and lung W/D weight ratio in the TNF+PMN group compared with the TNF and PMN groups. The pulmonary edema was not due to nonspecific effects of the TNF vehicle or contamination with endotoxin because heat-inactivated TNF did not cause similar changes. The increases in lung weight noted in the TNF and PMN groups were not associated with increases in the lung W/D weight ratio, and therefore may have been due to either increases in vascular volume or pulmonary edema. A small amount of edema may have been present in these groups because decreases in vascular volume might occur in association with the increases in Rv noted in this study, and because the lung W/D weight ratio is a specific but insensitive indicator of pulmonary edema.34

The pulmonary edema in the TNF+PMN group was mediated in part by vasoconstriction because the increase in lung weight was associated with an increase in Ppc and reduction of the increase in Ppc using Dazoxiben prevented the increase in lung weight despite an increase in the Ktc. The increases in Ppc were predominantly due to venous constriction because Rv was increased, whereas Rh did not change significantly. TXA2 was a mediator of the increase in Ppc in the TNF+PMN group because the TXB2 concentration of lung effluent was increased, and treatment of the lung with Dazoxiben inhibited the increases in both Ppc and TXB2 levels. PAF also promoted increases in Ppc but probably did so by inducing TXA2 release35,36 because pretreatment of the lung with WEB-2086 inhibited the increases in both Ppc and TXB2 levels. Increases in 6-keto-PGF1α levels were noted in all groups, indicating constitutive release by the isolated lung of prostacyclin. The elevations in Ppc in the TNF+PMN group occurred despite the increased release of prostacyclin, a vasodilator.22

Increased pulmonary vascular permeability was also a factor in the pathogenesis of the edema noted

TABLE 4. Effect of Tumor Necrosis Factor and Neutrophils on Pulmonary Capillary Filtration Coefficient

<table>
<thead>
<tr>
<th>Group</th>
<th>Pulmonary capillary filtration coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline 30 min 60 min</td>
</tr>
<tr>
<td>Control</td>
<td>0.111±0.013 0.104±0.016 0.109±0.018</td>
</tr>
<tr>
<td>TNF+Dazoxiben+PMN</td>
<td>0.118±0.008 0.170±0.048* 0.153±0.020</td>
</tr>
<tr>
<td>TNF+WEB-2086+PMN</td>
<td>0.107±0.016 0.107±0.014 N.D.</td>
</tr>
<tr>
<td>TNF</td>
<td>0.122±0.025 0.220±0.079* 0.150±0.022</td>
</tr>
<tr>
<td>TNF+WEB-2086</td>
<td>0.136±0.012 0.137±0.008 0.128±0.018</td>
</tr>
<tr>
<td>Dazoxiben+PMN</td>
<td>0.126±0.018 0.131±0.020 0.151±0.020</td>
</tr>
<tr>
<td>Dazoxiben</td>
<td>0.119±0.022 0.098±0.014 0.111±0.028</td>
</tr>
</tbody>
</table>

Values are mean±SEM in g/min/cm H2O/g dry lung wt. TNF, tumor necrosis factor-α; PMN, neutrophil; N.D., not done because lungs were not isogravimetric.
*Different from respective baselines (p<0.05).
TABLE 5. Effect of Tumor Necrosis Factor and Neutrophils on Thromboxane B\(_2\) and 6-Ketoprostaglandin-F\(_{1\alpha}\) in Pulmonary Venous Effluent

<table>
<thead>
<tr>
<th>Group</th>
<th>TXB(_2) (pg/ml)</th>
<th>6-Keto-PGF(_{1\alpha}) (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline 60 min</td>
<td>Baseline 60 min</td>
</tr>
<tr>
<td>Control</td>
<td>49±25 49±30</td>
<td>112±12 612±21*</td>
</tr>
<tr>
<td>TNF+PMN</td>
<td>60±13 5,244±599*</td>
<td>184±27 1,101±17*†</td>
</tr>
<tr>
<td>TNF+Dazoxiben+PMN</td>
<td>33±21 76±15</td>
<td>108±24 686±198*</td>
</tr>
<tr>
<td>TNF+WEB-2086+PMN</td>
<td>25±20 307±29*</td>
<td>98±21 441±209*</td>
</tr>
<tr>
<td>TNF</td>
<td>21±6 190±23*</td>
<td>86±13 305±10*</td>
</tr>
<tr>
<td>PMN</td>
<td>34±4 284±135*</td>
<td>77±11 422±47*</td>
</tr>
</tbody>
</table>

Values are mean±SEM. TXB\(_2\), thromboxane B\(_2\); 6-keto-PGF\(_{1\alpha}\), 6-ketoprostaglandin-F\(_{1\alpha}\); TNF, tumor necrosis factor-α; PMN, neutrophil.

*Different from respective baseline values (p<0.05).
†Different from all other groups (p<0.05).

in this study because \(K_{oc}\) was increased in the TNF+Dazoxiben+PMN group. \(K_{oc}\) was increased at 30 minutes but not at 60 minutes, indicating either reversible increases in vascular permeability or decreases in lung compliance and/or vascular surface area, which could have prevented continued increases in \(K_{oc}\) despite persistent increases in vascular permeability.\(^{34}\) PAF was a mediator of the increases in vascular permeability because \(K_{oc}\) was not increased in the TNF+WEB-2086+PMN group. This result is consistent with previous studies indicating that PAF can increase pulmonary vascular permeability during endotoxemia.\(^{14}\) Despite the increases in \(K_{oc}\), minimal weight gain was noted in the TNF+Dazoxiben+PMN and the TNF+Dazoxiben groups. The lack of weight gain may be explained by the absence of a significant driving pressure (i.e., an increased \(P_{pc}\)) for edema formation, which minimized the effect of the increased \(K_{oc}\).\(^{37,38}\)

The cellular source of the PAF and TXA\(_2\) remains unclear. Cooperation between the TNF-activated lung and circulating PMN may be required because PAF and TXA\(_2\) were more physiologically active in the TNF+PMN group than in either the TNF or PMN groups. Likely sources of PAF in our system include endothelial cells, macrophages, and PMN because these cells release PAF upon stimulation by TNF.\(^{13}\) Possible sources of TXA\(_2\) include circulating PMN\(^{39}\) and parenchymal lung cells such as macrophages,\(^{40}\) epithelial cells,\(^{41}\) and endothelial cells.\(^{42}\) The release of TXA\(_2\) may be stimulated from these cells by TNF,\(^{39}\) PAF,\(^{40}\) or other factors. In our study, TXA\(_2\) may have been released predominantly by PAF because WEB-2086 markedly inhibited TXA\(_2\) release. However, a small component of PAF-independent TXA\(_2\) secretion was present because the inhibition of TXA\(_2\) release with WEB-2086 was not complete.

FIGURE 3. Changes in pulmonary artery endothelial actin filament morphology in the tumor necrosis factor (TNF)+neutrophil (PMN) group. Panel A: Rhodamine phalloidin staining in the control group shows the characteristic appearance of normal endothelium, a cobblestone pattern with intact peripheral actin bands. Panel B: The endothelium in the TNF+PMN group shows focal areas of abnormal morphology characterized by marked ruffling of peripheral actin bands, less regular orientation of endothelial cells within the monolayer, and occasional cell shape changes. Peripheral bands in an individual cell are denoted by twin arrows; magnification ×1,235. A pattern similar to panel B was seen in the TNF, TNF+Dazoxiben+PMN, and TNF+WEB-2086+PMN groups (not shown). A pattern similar to panel A was seen in the PMN, Dazoxiben, and WEB-2086 groups (not shown).
Alterations in the endothelial peripheral actin bands were noted in the TNF+PMN group. The peripheral band abnormalities may play a role in the increased capillary permeability seen in the TNF+PMN group because similar cytoskeletal changes are associated with endothelial permeability increases in vitro\(^1\), and because the changes were not due to altered vascular tone, as the use of Dazoxiben prevented increases in \(P_{\text{er}}\), but not the morphological changes. The changes in peripheral actin bands may act as a permissive cofactor that renders the endothelium susceptible to the permeability-increasing effect of PAF\(^1\) because PAF mediated the increased capillary permeability, and PAF has no independent effect,\(^43\) or only a small one,\(^38\) on permeability in normal isolated perfused lungs.

The in vivo effects of TNF played a role in the pathogenesis of the pulmonary edema in the TNF+PMN group. Vasoconstriction mediated by both TXA\(_2\) and PAF resulted from in vivo TNF treatment because both \(P_{\text{er}}\) and TXB\(_2\) levels were increased in the TNF group, and the increases in \(P_{\text{er}}\) were inhibited using either Dazoxiben or WEB-2086. Increases in pulmonary vascular permeability were also noted after in vivo TNF treatment. The permeability increases were mediated by PAF because \(K_{\text{er}}\) was significantly increased in the TNF+Dazoxiben group but not in the TNF+WEB-2086 group. TNF may have contributed to the permeability increases by altering peripheral actin bands. The peripheral band abnormalities noted in the TNF+PMN group were not prevented using either Dazoxiben or WEB-2086 and were also seen in the TNF group. These data suggest that TNF induces the changes in vivo, independent of ex vivo TXA\(_2\), PAF, or PMN. However, a role for these potential mediators in vivo has not been eliminated.

The mechanisms of the effects of TNF in vivo remain uncertain. The effects could have resulted from the direct action of TNF on the endothelium or other tissues.\(^11\)-\(^13\) In addition, TNF may have primed the lung for the actions of PMN, possibly as a chemotactic,\(^17\) adherence-promoting,\(^16, 44, 45\) and activating\(^8\) agent for PMN because TNF and PMN were synergistic (as opposed to additive) in promoting lung weight gain in the TNF+PMN group. The inflammatory milieu that may have been created by TNF in vivo could have resulted in pulmonary edema in vivo, with subsequent clearing by pulmonary lymphatics and local and blood-borne anti-inflammatory and tissue-protecting mechanisms, resulting in an “activated” but minimally edematous lung at the time of excision.

Our data indicate that PMNs promote pulmonary edema in TNF-treated lungs because increases in lung weight and lung W/D weight ratio were noted in the TNF+PMN group compared with the TNF group. PMN promoted edema by increasing the \(P_{\text{er}}\) (probably by the release of TXA\(_2\)) in the presence of TNF- and PAF-mediated permeability increases, rather than by directly increasing permeability because 1) \(P_{\text{er}}\) and TXB\(_2\) levels were increased in the TNF+PMN group compared with the TNF group, 2) \(K_{\text{er}}\) was not significantly increased in the TNF+Dazoxiben+PMN group compared with the TNF+Dazoxiben group, and 3) changes in endothelial morphology occurred similarly in the TNF+PMN and TNF groups. In contrast, Varani et al\(^18\) showed that pretreatment (18 hours) with human recombinant TNF-\(\alpha\) (50 ng/ml) in vitro enhanced the susceptibility of vascular endothelial cells to lysis by PMN. However, the PMNs used by Varani et al were activated by phorbol myristate acetate or C5a, making comparison with our data difficult.

Previous studies addressing the role of PMN in the pathogenesis of TNF-induced pulmonary edema have been inconclusive. Stephens et al\(^19, 46\) demonstrated that pulmonary edema occurs 8 hours after the injection of human recombinant TNF-\(\alpha\) (1.4\(\times\)10\(^6\) units/kg i.v.) in the guinea pig. The edema was associated with increases in the permeability index, peripheral leukopenia, and increases in the number of PMN per alveolus. Cyclophosphamide-induced PMN depletion prevented the edema response,\(^19\) implicating PMN in the vascular effects of TNF. Horvath et al\(^11\) showed that the infusion of TNF-\(\alpha\) (6.5\(\times\)10\(^6\) units total, which is approximately 2\(\times\)10\(^5\) units/kg over 30 minutes) in sheep resulted in pulmonary edema associated with increases in \(P_{\text{er}}\) and capillary permeability. In contrast to the work of Stephens et al,\(^19\) the vascular effects of TNF noted by Horvath et al\(^11\) were not prevented by hydroxyurea-induced PMN depletion, suggesting that the effects occurred independent of circulating PMN. However, interpretation of the results of PMN depletion studies is made difficult by the possible independent effects of the agents used to deplete PMN and the persistence of PMN in the lung despite bloodstream PMN depletion.\(^19\)

The results of this study indicate that PMNs mediate edema in lungs isolated from TNF-treated guinea pigs. The edema is associated with permeability increases (by the pulmonary release of PAF) and vasoconstriction (by the pulmonary release of PAF and TXA\(_2\)). TNF mediates changes in endothelial peripheral actin bands independent of ex vivo TXA\(_2\), ex vivo PAF, or exogenous PMNs.

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**References**


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43. Imai T, Vercelloti GM, Moldow CF, Jacob HS, Weir EK: Pulmonary hypertension and edema induced by platelet-activating factor in isolated, perfused rat lungs are blocked by BN52021. J Lab Clin Med 1988;111:211–217


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