Endotoxin-induced lung injury in mice: structural, functional, and biochemical responses

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Rojas, Mauricio, Charles R. Woods, Ana L. Mora, Jianguo Xu, and Kenneth L. Brigham. Endotoxin-induced lung injury in mice: structural, functional, and biochemical responses. Am J Physiol Lung Cell Mol Physiol 288: L333–L341, 2005. First published October 8, 2004; doi:10.1152/ajplung.00334.2004.—Acute lung injury is usually a complication of sepsis, and endotoxin treatment of mice is a frequently used experimental model. To define this model and to clarify pathogenesis of the lung injury, we injected with 1 mg/kg endotoxin ip and measured pulmonary function, pulmonary edema, serum concentrations of cytokines and growth factors, and lung histology over 48 h. During the first 6 h, tidal volume and minute volume increased and respiratory frequency decreased. Serum concentrations of cytokines showed three patterns: 10 cytokines peaked at 2 h and declined rapidly, two peaked at 6 h and declined, and two had biphasic peaks at 2 and 24 h. Growth factors increased later and remained elevated longer. Both collagen and fibronectin were deposited in the lungs beginning within hours of endotoxin and resolving over 48 h. Histologically, lungs showed increased cellularity at 6 h with minimal persistent inflammation at 48 h. Lung water peaked at 6 h and gradually decreased over 48 h. We conclude that intraperitoneal administration of endotoxin to mice causes a transient systemic inflammatory response and transient lung injury and dysfunction. The response is characterized by successive waves of cytokine release into the circulation, early evidence of lung fibrogenesis, and prolonged increases in growth factors that may participate in lung repair.

acute lung injury; acute respiratory distress syndrome; cytokines; growth factors; lipopolysaccharide; animal model

ACUTE LUNG INJURY (ALI) is a clinical syndrome associated with respiratory dysfunction often as a complication of sepsis and with ~50% mortality (16). The pathophysiology of ALI involves inflammation (11) with diffuse alveolar damage, increased capillary permeability, interstitial and alveolar edema, influx of circulating inflammatory cells, and formation of hyaline membranes.

Because the most common cause of ALI in humans is sepsis, administration of gram-negative bacterial endotoxin lipopolysaccharide (LPS) has been used as an animal model of sepsis-related lung injury in several species (5, 7, 13, 14, 25). Endotoxin is a biologically active component of the gram-negative bacterial cell wall that exists as complexes of LPS and protein. Low-dose exposure to LPS activates macrophages (21) and polymorphonuclear cells, presumably aimed at eliminating the toxic agent, whereas higher doses cause tissue injury. Although virtually all mammalian species react to endotoxin, species sensitivity is highly variable (22).

Although LPS is often used as a stimulus for lung injury in mice, the pathophysiology of this model has not been thoroughly described. To maximize pathogenetic information from this model and permit comparisons to data from human studies, it is important to delineate the time course of the biochemical, functional, and structural alterations induced by endotoxin. To accomplish those goals, we conducted studies in C57BL/6 mice. We measured body weight, pulmonary function (plethysmograph), total lung water (wet-dry lung weight), serum concentrations of cytokines and growth factors, and lung histology over 48 h after intraperitoneal injection of 1 mg/kg LPS. Endotoxin caused a transient loss of body weight over the first day after which weight returned toward baseline. Endotoxin-induced alterations in breathing pattern [decreased Penh (a dimensionless number thought to be related to lung resistance), increased relaxation time, increased minute ventilation, increased tidal volume, and decreased end inspiratory pause] reached a maximum at 4 h and were significantly different from control animals at that time. Accumulation of the total amount of water in the lung began to increase by 2 h after endotoxin, peaked at 6 h, and remained elevated at 48 h. Deposition of fibronectin and collagen in the lungs began within a few hours of endotoxin administration, reached its peak at 24 h, and was largely resolved by 48 h. Endotoxin caused increased serum concentrations of all of the cytokines and growth factors that were measured, but there were distinct temporal patterns for different groups. Macrophage inflammatory protein (MIP)-1α, IL-6, IL-4, IL-3, IL-12, IFN-γ, IL-10, IL-5, IL-1β, and IL-1α reached peak serum concentrations at 2 h after endotoxin and then declined rapidly; this response was followed by an increase in regulated upon activation normal T-expressed and presumably secreted (RANTES) and KC that reached its zenith at 6 h. Both TNF-α and IL-17 showed a bimodal response with an early peak at 2 h and a secondary peak at 24 h. The pattern of increases in serum concentrations of growth factors [FGF, granulocyte colony-stimulating factor (G-CSF), and VEGF] was shifted to the right in time, tending to peak later and remaining elevated for longer times.

We conclude that intraperitoneal administration of endotoxin to mice in doses that have been commonly used as a model of inflammation in the lung causes a transient systemic inflammatory response and transient lung injury and dysfunction. The response is characterized by successive waves of cytokine release into the circulation, early evidence of lung fibrogenesis that rapidly resolves, and prolonged increases in growth factors that may participate in lung repair. We specu-
late that the rapid resolution of endotoxin-induced lung injury in mice is a consequence of a robust reparative response that terminates inflammation and mobilizes endogenous progenitor cells.

MATERIALS AND METHODS

Experimental animals. Six- to eight-week-old C57BL/6 male mice were used in all the experiments (Jackson Laboratories, Bar Harbor, ME). Animals were randomly divided into endotoxin or PBS (control) groups. Before inoculation mice were weighed and a blood sample was collected. All of the animals were maintained in the Division of Animal Resources at Emory University, an American Association for Accreditation of Laboratory Animal Care-approved facility. All animal protocols were reviewed and approved by the Institutional Animal Care and Use Committee.

LPS administration. Mice were inoculated intraperitoneally with 1 mg/kg of endotoxin (LPS) prepared from Escherichia coli O111:B6 (Sigma, St. Louis, MO). In vivo measurements (body weight and plethysmographic determinations of lung function) were made at intervals up to 96 h after administration of endotoxin, and groups of animals were killed at 0, 2, 6, 24, and 48 h. Lungs were collected and used for histological analysis and determination of wet-dry ratio. Blood samples were collected from each animal before death and used to quantify cytokine and growth factor production. Control groups of animals were treated according to exactly the same protocol except that they received an intraperitoneal injection of phosphate-buffered saline (PBS) instead of endotoxin.

Whole body plethysmography. Mice were placed unrestrained and sedated in a cylindrical plethysmograph chamber (~5-in tall, 5-in diameter; Buxco, Wilmington, NC) made of clear plastic. Mice were transferred directly from their cage into the chamber and allowed to acclimate to the chamber for 50–60 min. Measurements were saved into a computer file for future analysis. From the 14 different parameters that can be generated by the machine, we focused on the ones that showed clear differences between control and endotoxin-treated animals. Mice in the chamber showed normal exploratory behavior. This method allows serial observations in the same animal over time, avoids invasive procedures or physical restraint, and avoids anesthetics.

Determination of pulmonary edema. Lungs were removed from mice, placed in a tared microcentrifuge tube, and weighed. Lungs were then desiccated under a vacuum (Vacufuge; Eppendorf, Westbury, NY) at 25°C and weighed again. The wet lung mass was divided by the dry lung mass to give the wet-dry ratio, which is the fraction of the wet lung weight that was water.

Histopathology. To harvest the lungs, we cannulated the trachea and fixed the lungs by inflation with 4% paraformaldehyde. After overnight fixation, tissue was embedded in paraffin, sectioned, and stained. Hematoxylin and eosin (H & E) stains were used to determine morphology and inflammatory infiltrate. Mallory’s phosphotungstic acid hematoxylin (PTAH) stain was used to detect collagen deposition and fibronectin deposition was determined by immunohistochemical staining using a monoclonal antibody against fibronectin followed by a secondary antibody conjugated with Texas red (Molecular Probes, Eugene, OR). To assess fibroin deposition quantitatively, we took photographs through an Olympus EX41 fluorescence microscope (Olympus America, Melville, NY) with an Olympus MagnaFire camera, and the quantity of fibronectin staining was determined in pixels from five images at ×40 magnification per slide with Scion Image software. Similar methods were used to determine the amount of collagen deposition except that light photomicrographs were analyzed since we detected collagen by standard histochemistry.

Analysis of cytokines and growth factors. We measured cytokines and growth factors in serum samples obtained at different times following administration of either endotoxin or PBS. Samples were analyzed with a multiplex bead immunoassay (Luminex, Austin, TX). This assay is a novel technology that can measure up to 100 different biological markers simultaneously by using microspheres labeled with unique fluorophores in a single well of a filter-bottomed ELISA plate. To analyze the blood samples, we used a multiplex kit (Bio-Rad, Hercules, CA) that detects IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-10, IL-12(p40), IL-12(p70), IL-17, IFN-γ, KC, MIP-1α, RANTES, and TNF-α. To determine the levels of the different growth factors in the same samples we used a separate multiplex kit (Biosource, Camarillo, CA) that detects FGF-b, VEGF, and G-CSF. The protocols in both kits are similar. Well filters were washed, and 50 μl of 1:1 diluted sample were applied to each well. Antibody-coated beads specific for the different cytokines were added to the wells and incubated for 2 h at room temperature in a shaker. After incubation, the plate was washed twice with a manifold washer. Biotinylated antibodies against the cytokines were added to the reaction and incubated for 1 h. Afterward, we detected the cytokine-antibody complexes by adding streptavidin coupled to phycoerythrin. We determined the number of positive complexes by reading each sample in a Luminex XXP platform. Data was analyzed with MasterPlex 1.2 software from MiraiBio, and concentration was expressed in pg/ml.

Statistical methods. For comparisons between groups, paired Mann-Whitney tests or unpaired t-tests with or without Welch correction and one-way analysis of variance were used (P values < 0.05 were considered significant). We used GraphPad Prism and GraphPad InStat statistical packages to make these calculations.

RESULTS

Body weight. Within the first hours after administration of endotoxin, animals became lethargic and showed reduced activity. As shown in Fig. 1, during the first 24 h, the weight of animals that received endotoxin decreased ~20%. Weight gradually returned to normal by 4 days after endotoxin. During the same period of time the weight of animals inoculated with PBS was unchanged from baseline.

Measurements of lung water. Pulmonary edema is a hallmark of ALI, and the gold standard for measurement of edema is the amount of water in the lungs. Figure 2 shows the wet-dry weight ratio (uncorrected for residual blood) for both control and LPS-treated animals at different time points after endotoxin administration. Edema began to appear at 2 h after endotoxin, reached a peak at 6 h, and then gradually decreased.

![Fig. 1. Body weights of mice given endotoxin (n = 3) and control (n = 3) mice over 96 h after treatment (weights are normalized to baseline weight in each animal). Mice given endotoxin lost weight rapidly over the first 24 h, and then weight gradually returned toward baseline. *Significantly different between control and endotoxin groups.](http://ajplung.physiology.org/)
Even 48 h after endotoxin administration, lung water was still on average 10% greater than in control animals. The whole body plethysmograph that we used measures a number of variables related to breathing pattern and respiratory function, many of which were affected by endotoxin administration. As shown in Table 1, at 4 h after endotoxin, the time at which the maximum effect was seen, there were significant differences between endotoxin-treated and control animals for Penh (a dimensionless number thought to be related to airway resistance), relaxation time, minute ventilation, tidal volume, end inspiratory pause, and respiratory frequency. All of these changes in function were transient and had returned to normal by 48 h after endotoxin administration. These changes in breathing pattern are likely due to both the systemic inflammatory response and to the local inflammation and edema in the lungs.

**Histology and immunohistochemistry.** Photomicrographs of H & E-stained sections of lung at baseline and 6, 24, and 48 h after administration of endotoxin are shown in Fig. 3. At 6 h, there was vascular congestion and a general increase in cellularity, predominantly due to the presence of neutrophils. These changes were more pronounced at 24 h after endotoxin, and at that time thickening of the alveolar septae was especially apparent. By 48 h, there were residual inflammatory cells and some alveolar wall thickening, but these changes were resolving.

To gain information about alterations in matrix proteins in the lungs in this model, we stained sections for collagen (Mallory’s PTAH) and immunostained sections for fibronectin using a fluorescent system. In both cases, we determined the amount of matrix protein by measuring the intensity of the staining quantitatively. Figure 4 shows photomicrographs of lung sections immunostained for fibronectin at baseline and at several time points after endotoxin administration; a graph of the quantitative data is also shown in the figure. Fibronectin deposition began very early after endotoxin administration and reached peak at 24 h, at which time the amount of fibronectin was significantly greater than that in control lungs. By 48 h after endotoxin, lung fibronectin content had returned to baseline values.

Figure 5 shows photomicrographs of sections of lung stained for collagen at baseline and 24 and 48 h after endotoxin along with a graph of the quantitation of staining intensity. Collagen was increased at 24 h and was largely resolved by 48 h.

**Serum concentrations of cytokines and growth factors.** At five different time points after administering endotoxin, we analyzed serum for the presence of an array of cytokines using an analytic system that permits measurements of a large number of cytokines in the same serum samples. Figure 6 summarizes the data for 15 cytokines in the serum of control animals and that of animals that received endotoxin. Serum concentrations of all of these cytokines increased transiently after endotoxin administration, but there were specific temporal patterns of the responses for different groups of cytokines as shown in Fig. 6. Concentrations of IL-5, IL-1β, IL-1α, MIP-1α, IL-6, IL-9, IL-3, IL-12, IFN-γ, and IL-10 all reached peak concentrations at 2 h after endotoxin administration and then declined. Concentrations of RANTES and KC were slightly elevated by 2 h, reached a peak value at 6 h, and decreased to baseline by 24 h. TNF-α and IL-17 showed a bimodal response with a peak at 2 h and a second peak at 24 h, returning to baseline by 48 h.

### Table 1. Effects of endotoxin treatment compared with control animals on several variables describing breathing pattern

<table>
<thead>
<tr>
<th>Time after Endotoxin</th>
<th>Group</th>
<th>Penh</th>
<th>Relaxation Time, s</th>
<th>Minute Volume, ml/min</th>
<th>End Inspiratory Pause, ms</th>
<th>Tidal Volume, ml</th>
<th>Frequency, breaths/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 h</td>
<td>Control</td>
<td>0.45±0.01</td>
<td>0.06±0.0</td>
<td>118.32±12.4</td>
<td>4.72±0.0</td>
<td>0.26±0.0</td>
<td>447±22.0</td>
</tr>
<tr>
<td>4 h</td>
<td>Control</td>
<td>0.43±0.03</td>
<td>0.06±0.0</td>
<td>118.32±12.4</td>
<td>4.72±0.0</td>
<td>0.26±0.0</td>
<td>447±22.0</td>
</tr>
<tr>
<td>6 h</td>
<td>Control</td>
<td>0.35±0.01</td>
<td>0.09±0.0</td>
<td>42.39±3.0</td>
<td>5.06±0.0</td>
<td>0.18±0.0</td>
<td>420±5.7</td>
</tr>
<tr>
<td>8 h</td>
<td>Control</td>
<td>0.39±0.01</td>
<td>0.14±0.0</td>
<td>44.72±2.2</td>
<td>4.3±0.0</td>
<td>0.24±0.0</td>
<td>323±1.9</td>
</tr>
<tr>
<td>12 h</td>
<td>Control</td>
<td>0.41±0.01</td>
<td>0.15±0.0</td>
<td>39.11±2.7</td>
<td>5.0±0.0</td>
<td>0.17±0.0</td>
<td>305±1.6</td>
</tr>
<tr>
<td>24 h</td>
<td>Control</td>
<td>0.43±0.01</td>
<td>0.15±0.0</td>
<td>41.63±0.2</td>
<td>4.16±0.0</td>
<td>0.24±0.0</td>
<td>198±1.7</td>
</tr>
<tr>
<td>48 h</td>
<td>Control</td>
<td>0.56±0.0</td>
<td>0.09±0.0</td>
<td>62.52±4.7</td>
<td>5.13±0.0</td>
<td>0.2±0.0</td>
<td>263±9.2</td>
</tr>
<tr>
<td>48 h</td>
<td>Endotoxin</td>
<td>0.56±0.0</td>
<td>0.11±0.0</td>
<td>51.85±0.41</td>
<td>4.14±0.0</td>
<td>0.24±0.0</td>
<td>217±1.6</td>
</tr>
<tr>
<td>48 h</td>
<td>Endotoxin</td>
<td>0.56±0.0</td>
<td>0.12±0.0</td>
<td>77.88±0.9</td>
<td>4.42±0.0</td>
<td>0.25±0.0</td>
<td>315±9.2</td>
</tr>
<tr>
<td>48 h</td>
<td>Endotoxin</td>
<td>0.56±0.0</td>
<td>0.09±0.0</td>
<td>56.6±6.5</td>
<td>4.73±0.0</td>
<td>0.23±0.0</td>
<td>236±18.6</td>
</tr>
<tr>
<td>48 h</td>
<td>Endotoxin</td>
<td>0.41±0.01</td>
<td>0.08±0.0</td>
<td>106.6±5.7</td>
<td>5.0±0.0</td>
<td>0.25±0.0</td>
<td>409±10.1</td>
</tr>
</tbody>
</table>

Results are means ± SE from 4 different experiments with 2 mice in each group (total n = 8 in each group). The data for 6 variables that were significantly different between endotoxin-treated and control animals are shown at 0, 2, 4, 6, 24, and 48 h after endotoxin. Marked changes in breathing pattern likely resulted from both systemic inflammatory response and inflammation and edema in the lungs. *Differences between endotoxin and control groups are significant (P < 0.05) for the variables at different time points.
Figure 7 summarizes the serum concentrations of three growth factors that could play important roles in lung repair in endotoxin treated and control animals over 48 h after endotoxin administration. Concentrations of FGF-b, VEGF, and G-CSF all increased after endotoxin. In addition, the growth factor response tended to occur later and to be more prolonged than the cytokine response; concentrations of all three factors remained elevated at 24 h.

DISCUSSION

Sepsis is the most common clinical setting in which ALI develops and bacterial endotoxin is implicated as an important toxin precipitating lung injury. Endotoxin is an LPS component of bacterial cell walls that has a large number of potent biological actions, including ALI, when administered to animals. Although virtually all animal species respond to endotoxin, there are marked differences in susceptibility among and within species (3, 6, 13, 14, 17, 20, 41).

Numerous investigators have used endotoxin-treated mice as a model of lung inflammation and injury (15, 24, 28). Compared with some other species (e.g. swine, sheep), mice are highly resistant to endotoxin so that large doses of the toxin are necessary to cause a response in the lungs (40). In addition, the lung injury produced by endotoxin administration in mice is mild and transient even when lethal doses of the toxin are given (33). Despite those possible objections, the mouse model is especially attractive because of the catalogue of genetically manipulated mice available for identifying important events in the pathogenesis of lung injury (30, 32, 38).

Although numerous studies of endotoxin effects in mice are reported in the literature, few studies provide a comprehensive characterization of the physiological, structural, and biochemical effects. Kabir and colleagues (18) measured lung water, histology, and lung levels of a panel of inflammatory cytokines during the early phase of the endotoxin response in mice given lethal doses of endotoxin. But we wished to characterize responses to more commonly used endotoxin doses and to include both the acute and recovery phases of the response. We conducted such a study on the assumption that a more complete description of the response would provide a sounder basis for interpreting effects of interventions (or altered effects in genetically manipulated animals) and because we thought integration of such data would provide additional insights into the pathogenesis of the endotoxin response.

In humans a clinical constellation of findings called the systemic inflammatory response syndrome (SIRS) defines a population at risk for ALI (29); SIRS is attributed to systemic release of an array of proinflammatory cytokines (35). Responses of mice to endotoxin include lethargy, weight loss, and an acutely increased release of a host of inflammatory cytokines. This systemic inflammatory response is presumably similar to that which occurs in septic humans. As reported by others (18), we found that endotoxin caused mild and transient lung injury. Pulmonary function was altered early, reaching a maximum at 4 h after endotoxin, and then returning gradually to normal over the next day. Histological sections primarily showed infiltration of inflammatory cells and thickening of alveolar septae that began at 2 h, peaked at
Fig. 4. Intensity of fibronectin staining determined quantitatively using Scion Image software (A) and immunofluorescent staining for fibrinogen (stained with Texas red) in mouse lungs before and at several time points after endotoxin administration (B). The sections are shown at low power (magnification ×20). Fibrinogen began to increase as early as 3 h and by 24 h was elevated significantly above baseline values. Increased fibrinogen deposition was no longer detectable at 48 h after endotoxin. *Time point at which the value was significantly different from the control value.

Fig. 5. Mallory’s phosphotungstic acid hematoxylin-stained sections of mouse lung before and at 24 and 48 h after endotoxin administration (collagen stained brown, magnification ×100; bottom) *Time point at which the value was significantly different from the control value and collagen staining determined quantitatively using Scion Image software (top). Expression of collagen in the lung is increased 24 h after systemic endotoxin and is declining by 48 h (arrows, bottom).
24 h, and was resolving by 48 h. Pulmonary edema as determined by lung wet-to-dry weight ratios was apparent by 2 h, peaked at 6 h, and then gradually declined, but lung water remained elevated even at 48 h. The early and transient alterations in lung function are consistent with the time course of inflammatory cytokine release and pulmonary edema in our studies and as reported in studies of lethal murine endotoxemia (12, 18). The fact that the time of maximum edema came 6 h after endotoxin is also identical to the time course of edema in the reported studies of lethal endotoxemia (18). The degree of inflammatory cytokine release and pulmonary edema in our studies and as reported in studies of lethal murine endotoxemia (12, 18). The fact that the time of maximum edema came 6 h after endotoxin is also identical to the time course of edema in the reported studies of lethal endotoxemia (18). The degree of

Fig. 6. Time course of serum concentrations of a panel of cytokines grouped according to their kinetics (groups with similar kinetics are outlined by bold boxes). Concentrations of all of the cytokines increased after endotoxin and the temporal patterns were characterized by successive waves of cytokine release. MIP, macrophage inflammatory protein; RANTES, regulated upon activation normal T-expressed and presumably secreted. *Points at which the differences between endotoxin and control values were significant ($P < 0.05$).
edema is not severe and appears to be primarily restricted to the interstitium since histological sections did not show alveolar flooding. Lung function returns to normal before complete resolution of either the edema or the inflammatory lung infiltrate, both of which persisted to a degree even at 48 h.

In the human syndrome there is a “fibroproliferative phase” of ALI that has been thought to begin subsequent to the acute inflammation (23, 26). However, there is considerable biochemical and histological evidence that fibrogenesis likely begins very early and either resolves if the patient recovers or progresses to extensive and fatal fibrosis (23, 31). For example, Chesnutt and coworkers (10) reported increased levels of type III procollagen peptide (a marker of collagen synthesis) in bronchoalveolar lavage fluid of patients with ALI within 24 h of intubation. We found that endotoxin-induced lung injury in mice resulted in increased deposition of both fibrinogen and collagen that began within hours of endotoxin administration, reached a peak at 24 h, and slowly resolved over 48 h. This finding is consistent with the conclusion that increased accumulation of matrix proteins is an early event in the response to injury that resolves promptly when conditions favor repair as opposed to progression of the injury.

We found that mice responded to sublethal endotoxemia with release into the circulation of many of the same cytokines that have been implicated in the pathogenesis of ALI in humans. Serum cytokine concentrations could be grouped into three temporal patterns as shown in Fig. 6: primary (peak at 2 h and rapid decline), secondary (peak at 6 h and rapid decline), and biphasic (a peak at 2 h and again at 24 h). The cytokines with the primary pattern include IL-1, IL-6, and IFN-γ, all of which have been implicated in ALI in humans and animal models (19). This pattern was similar to that of the alterations in pulmonary function and pulmonary edema, both of which began as early as 2 h and reached a peak at 4–6 h after endotoxin administration. There was a secondary peak in concentrations of RANTES and KC (the murine analog of IL-8) at 6 h with a rapid decline to baseline by 24 h. Histological sections showed infiltration of the lung with inflammatory cells (predominantly neutrophils) by 6 h that increased by 24 h and then was resolving by 48 h (see Fig. 3). If KC is a principal neutrophil chemoattractant (2, 4), it would be expected that the cellular response would trail chemoattractant concentrations, and the temporal relationships we observed are consistent with that idea. This conclusion assumes that serum concentrations reflect concentrations in the lungs. Serum concentrations of TNF-α and IL-17 showed a biphasic pattern. TNF-α has been strongly implicated in the pathogenesis of lung injury (27), and this cytokine can have many of the physiological effects characteristic of acute tissue injury (42). The early pulmonary edema and alterations in lung function after endotoxin in the mouse model were likely contributed to by TNF-α since it can cause both pulmonary dysfunction and pulmonary edema (9). The later peak occurred coincidently with the maximum neutrophil accumulation in the lungs and with the peak accumulation of fibronectin and collagen. It is possible that TNF-α contributed to those responses as well, either as a primary or a secondary effect (34).

Serum concentrations of all three of the growth factors that we measured increased and remained elevated longer than the cytokines (see Fig. 7). VEGF concentrations increased by 2 h and peaked at 6 h but remained substantially elevated at 24 h. G-CSF also increased some at 2 and 6 h but reached peak concentrations at 24 h and remained slightly elevated at 48 h. FGF-b was not increased until 6 h and remained substantially elevated at 24 h. The pattern of FGF-b response vis-à-vis fibrinogen and collagen deposition in and disappearance from the lungs is consistent with a role for this factor in the transient fibrogenesis that we observed (8, 36). Both VEGF and G-CSF can mobilize progenitor cells from bone marrow pools (1, 37). A growing body of evidence, including studies in a mouse endotoxin model, indicates that mobilization of stem cells is important to the repair process (39). In our studies, both VEGF and G-CSF were markedly elevated at 24 h after endotoxin, and the tissue injury largely resolved between 24 and 48 h, a pattern consistent with a role for these growth factors in initiating repair.

In summary, systemic administration of a sublethal dose of endotoxin to mice caused the rapid onset of a systemic inflammatory response characterized by constitutional symptoms and a flood of cytokines released into the circulation. There was a rapid onset of ALI characterized by altered respiratory function, interstitial pulmonary edema, inflammatory cell accumulation, and deposition of fibronectin and collagen in the lungs. The lung injury was transient, largely resolving by 48 h. Serum cytokine concentrations could be grouped into three temporal patterns, primary, secondary, and biphasic, that associated different cytokines with different lung injury responses. Serum concentrations of growth factors generally increased later and remained elevated longer than the cytokines. The pattern of FGF concentrations was consistent with a role in transient...
fibrogenesis, and the pattern of VEGF and G-CSF concentrations was consistent with a role in initiating repair. We speculate that the rapid resolution of endotoxin-induced lung injury in mice is a consequence of a robust reparative response that terminates inflammation and mobilizes endogenous progenitor cells.

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