Adoptive transfer of acute lung injury

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Moxley, Michael A., Tracey L. Baird, and John A. Corbett. Adoptive transfer of acute lung injury. Am J Physiol Lung Cell Mol Physiol 279: L985–L993, 2000.—In this study, we describe a novel adoptive transfer protocol to study acute lung injury in the rat. We show that bronchoalveolar lavage (BAL) cells isolated from rats 5 h after intratracheal administration of lipopolysaccharide (LPS) induce a lung injury when transferred to normal control recipient rats. This lung injury is characterized by increased alveolar-arterial oxygen difference and extravasation of Evans blue dye (EBD) into lungs of recipient rats. Recipient rats receiving similar numbers of donor cells isolated from healthy rats do not show adverse changes in the alveolar-arterial oxygen difference or in extravasation of EBD. The adoptive transfer-induced lung injury is associated with increased numbers of neutrophils in the BAL, the levels of which are similar to the numbers observed in BAL cells isolated from rats treated for 5 h with LPS. As an indicator of BAL cell activation, donor BAL cell inducible nitric oxide synthase (iNOS) expression was compared with BAL cell iNOS expression 48 h after adoptive transfer. BAL cells isolated 5 h after LPS administration expressed iNOS immediately after isolation. In contrast, BAL cells isolated 48 h after adoptive transfer did not express iNOS immediately after isolation but expressed iNOS following a 24-h ex vivo culture. These findings indicate that the activation state of donor BAL cells differs from BAL cells isolated 48 h after adoptive transfer, suggesting that donor BAL cells may stimulate migration of new inflammatory cells into the recipient rats lungs.

ACUTE RESPIRATORY DISTRESS SYNDROME (ARDS) is a severe form of acute lung injury (14) characterized by respiratory failure associated with abnormal gas exchange, pulmonary infiltrates, and alterations in surfactant composition and function (13, 32). The essential precipitating events leading to the cellular and fluid infiltration that characterize acute lung injury have not been clearly defined. ARDS can result from direct or indirect insults, resulting in localized inflammation in the lung (7). Associated with this inflammatory process is the local production of proinflammatory mediators (such as cytokines, prostaglandins, and free radicals), and these mediators appear to further influence lung inflammation and tissue damage. A number of studies have identified increased levels of proinflammatory mediators such as tumor necrosis factor-α (TNF-α) (4, 20, 35, 38, 44), interleukin (IL)-8 (56), IL-1β, and IL-6 (18, 27, 56) in the lungs of ARDS patients and in animal models of this disease. Importantly, the degree of polymorphonuclear neutrophil (PMN) activation or infiltration appears to correlate with the levels of TNF-α, IL-6, and IL-8 contained in the lavage fluid and plasma of ARDS patients, and bronchoalveolar lavage (BAL) levels are increased compared with cytokine levels in healthy volunteers (4). Additional evidence for cytokine participation in lung inflammation includes the attenuation of lipopolysaccharide (LPS)- or IL-1-induced lung injury by the administration of IL-1 receptor antagonist in rats (47). In addition, neutralizing TNF antibodies prevent lung injury associated with periparient syndrome (19, 46), and administration of IL-8 monoclonal antibodies prevents endotoxemia-induced ARDS (56). Other mediators such as interferon-γ (IFN-γ) (36, 37) and products of arachidonic acid metabolism are increased in animal models of acute lung injury as well as in human studies of ARDS (11, 33).

Recently, attention has focused on the potential role of nitric oxide free radical (NO·) in mediating tissue damage and stimulating lung inflammation during the development of ARDS. Increased levels of NO have been detected in the exhaled gas in a rat model of lung inflammation due to sepsis (43). In addition, NO has been shown to inhibit type II cell metabolism and surfactant synthesis in vitro (15, 28). Importantly, lung injury induced by immune complexes (29), ischemia (21) or paraquat (2) involves NO· or its metabolites as indicated by increased levels of nitrate and nitrite in perfusate or lavage, the attenuation of each of these injuries by NO synthase (NOS) inhibitors, and the reversal of these protective effects by L-arginine. We have recently reported that lung injury induced by N-nitroso-N-methylurethane (NNMU) is dependent on NO· production. Administration of aminoguanidine, a selective inducible NOS (iNOS) inhibitor, significantly attenuates NNMU-induced alterations in gas exchange, decreased surfactant phospholipid protein ratio, elevated surface tension, and neutrophilic infiltration into the alveolar space in rat lungs (5, 6).

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While numerous inflammatory mediators appear to participate in lung inflammation and injury, the cellular source(s) of these mediators and the manner by which these mediators induce lung damage have yet to be elucidated. In this study, we describe a novel animal model of lung injury that should allow for the identification of individual components (such as soluble mediators and infiltrating cell types) of the inflammatory response and the determination of how these mediators stimulate lung inflammation. Using an adoptive transfer approach, infiltrating lung cells isolated from LPS-treated rats are transferred to a normal, healthy control rat. The recipient animals show evidence of altered gas exchange and extravasation of plasma 48 h after transfer.

**MATERIALS AND METHODS**

**Animals.** In all experiments, pathogen-free, male Sprague-Dawley rats (250–350 g) obtained from Harlan Sprague Dawley (Indianapolis, IN) were used. At least 1 wk before experiments, heparin-coated polyethylene catheters were inserted into the left carotid artery. The catheter was exteriorized at the base of the skull. The catheter was plugged with a stainless steel pin and used for the collection of blood samples for arterial blood gas (ABG) determinations. The rats were individually housed in plastic cages on self-watering racks in semi-barrier rooms and fed normal rat chow ad libitum. In the studies described, donor rats for the adoptive transfer experiments were placed into one of three groups: untreated, saline instilled, or LPS instilled. Five hours after LPS or vehicle instillation, animals were killed, and cells were collected by BAL (8 × 8 ml). The recovered BAL cells were then either placed in culture as controls and analyzed as described above or instilled into recipient rats. Forty-eight hours after cells were instilled, BAL cells were recovered from the recipient rats by lavage. Analysis of the recovered cells is described below. All common chemical reagents were obtained from Sigma Chemical (St. Louis, MO) unless otherwise noted.

**Statistics.** Statistical significance was calculated using one-way ANOVA with Bonferroni post hoc analysis. The data presented are means ± SE.

**Intratracheal LPS instillation.** Donor animals were anesthetized by inhalation of halothane (Halocarbon Laboratories, River Edge, NJ). The animals were placed on a surgical board at an angle of ~45° with the head supported by an elastic cord placed over the upper incisors. The vocal chords were visualized with a modified otoscope. Saline or LPS (serotype 0111:B4, 10 mg/ml) was administered using a stainless steel right-angled miniature nebulizer (Penn-Century, Philadelphia, PA) (50, 52). LPS was administered at a dose of 15 mg/kg.

**Alveolar-arterial oxygen difference analysis.** Arterial blood samples (0.7 ml) were collected directly into a heparin-flushed 1.0-ml syringe via the indwelling arterial catheter from spontaneously breathing, unanesthetized rats. ABGs were measured using a Radiometer ABL 520 (Radiometer, Copenhagen, Denmark) blood microsystem. Oxygen and carbon dioxide partial pressures (P O2 and P CO2, respectively) from ABG determinations were used to calculate the alveolar-arterial oxygen difference. The alveolar P O2 (P A O2) was calculated using the alveolar gas equation, and the arterial P O2 (P A O2) was obtained from ABG determinations. Values are corrected for body temperature determined at the time blood was drawn (22). The respiratory quotient and the inspired O2 fraction were assumed to be constant at 0.8 and 0.21, respectively.

**Collection of inflammatory cells.** Five hours after LPS or vehicle instillation and after blood samples were drawn and ABGs determined, the lungs were removed, and ~50 ml of BAL were collected from each isolated rat lung in successive 8-ml aliquots. Briefly, rats were anesthetized with pentobarbital sodium (65 mg/kg body wt ip), the trachea was exposed, and a tracheostomy tube was inserted. The lungs were perfused through the right ventricle of the heart with a buffered salt solution (125 mM NaCl, 5 mM KCl, 2.5 mM Na2HPO4, 17 mM HEPES, 10 µg/ml gentamicin, and 1 mg/ml dextrose, pH 7.4) to remove blood cells from the pulmonary vasculature. The intact lungs were then lavaged with 8-ml aliquots of the buffered salt solution. The lavage was centrifuged at 300 g for 15 min at 4°C. The supernatant was reserved for the isolation of a crude surfactant pellet. The cell pellet was resuspended, the total volume was brought to 50 ml with the buffered salt solution, and the suspension was centrifuged again at the same relative centrifugal force. Donor cells were resuspended in a total of 5 ml and the total viable cell number was determined by trypan blue exclusion. The cells were then resuspended in the salt solution such that recipient rats received 38 × 106 inflammatory cells in a total volume of 1 ml while under halothane anesthesia. Aliquots of the cell suspension were centrifuged onto glass slides and stained using a modified Wright’s stain procedure (Diff-Quik; Baxter Scientific) for differential cell counts.

In adoptive transfer experiments, the cells were collected by lavage 48 h after cell instillation. The cells were centrifuged and washed as described above. The cell pellet was resuspended in Ham’s F-12 nutrient mixture (GIBCO BRL) culture medium containing 10% FCS, gentamicin (50 µg/ml), streptomycin (1 mg/ml), and penicillin (100 U/ml), and aliquots were taken for cell counting and differential counts.

**Phospholipid and protein analysis.** The BAL cell-free supernatant was centrifuged at 48,000 g for 1 h to obtain a crude surfactant pellet (CSP) that was resuspended in 300 µl of 154 mM NaCl and 5 mM CaCl2 (5, 6, 16). An aliquot was taken and the protein concentration was determined by a modified Lowry assay (25, 31). Phospholipid concentration was determined (16) on an aliquot of CSP extracted according to Bligh and Dyer (3).

**Analysis of CSP surface activity.** Phospholipid concentration of the CSP was determined as noted above, and the pellet was diluted to a final phospholipid concentration of 1.5 µmol/ml. Surface tension at minimum bubble size was determined on a pulsating bubble surfactometer after 5 min at 37°C, 50% surface area compression, and 20 cycles/min (6, 8, 16). Dynamic surface tension <10 mN/m indicates functional pulmonary surfactant in a crude surfactant pellet.

**Analysis of nitrite and nitrate in cell culture media.** Freshly isolated BAL cells were seeded onto a 24-well tissue culture plate at 300,000 viable cells/well in 500 µl of Ham’s F-12 culture medium containing 10% FCS, gentamicin (50 µg/ml), streptomycin (1 mg/ml), and penicillin (100 U/ml). To designated wells, 1 mM aminoguanidine, 10 µg/ml LPS, and/or 1 µM actinomycin D was added. Cells were cultured at 37°C, 85% humidity, and 10% CO2 for 24 h. After 24 h, the culture medium was collected and centrifuged, and the supernatant was stored at −20°C for nitrate and nitrate determination. The remaining cell pellets were stored at −20°C for Western blot analysis of iNOS protein. Following enzymatic conversion of nitrate to nitrite by nitrate reductase (12), total nitrite was determined by the Griess assay (26). Total nitrite concentration was based on a sodium nitrate standard curve.
Western blot analysis for iNOS. For culture samples, 25 μl of 5× sample buffer (0.5 M Tris-HCl, 2.0% β-mercaptoethanol, 10% SDS, and 0.5% bromphenol blue; pH 6.6) were added to 300,000 cells in 100 μl of saline, and the cells were vortexed and boiled for 10 min. Protein was separated by SDS gel electrophoresis and transferred to a nitrocellulose membrane overnight (Bio-Rad Mini Trans-Blot cell). Detection of iNOS protein was performed by enhanced chemiluminescence (Amersham) using rabbit anti-mouse iNOS (Cayman Chemicals) at a dilution of 1:2,000 and horseradish peroxidase-conjugated donkey anti-rabbit (Jackson Immunological Research) at a dilution of 1:6,000 (5, 6). IL-1β-stimulated (1.0 U/ml, 24 h) RIN-m5F cells were used as an iNOS-positive control (17).

Extravasation of Evans blue dye into the alveolar space. Untreated animals, animals receiving cells from normal donors, or animals receiving cells from LPS-treated animals were killed 48 h after cell instillation. One hour before death, 20 mg/kg Evans blue dye (EBD) was administered via a tail vein (45). The rats were anesthetized with pentobarbital sodium, a 0.6-ml blood sample was taken from the inferior vena cava, and the rats were exsanguinated as described above. The lungs were instilled with a single 8.0-ml volume of the balanced salt solution, and the lavage was collected. The BAL was centrifuged as described above and the fluid was lyophilized. The dried lavage was resuspended in 0.8 ml of water and extracted according to Bligh and Dyer to partition the dye into the aqueous phase to avoid interference from surfactant in lavage (51). EBD concentration was determined spectrophotometrically on 200-μl aliquots in 96-well microtiter plates at 620 nm using a standard curve prepared in Bligh and Dyer upper phase. Plasma concentrations of the dye were determined using a standard curve prepared in saline. The tissue content of EBD was determined by homogenizing lyophilized lung tissue in 10 ml of formamide. Following overnight extraction, the tissue was centrifuged at 3,900 g. EBD concentration of the supernatant was determined spectrophotometrically at 620 nm using a standard curve prepared in formamide. Lavage content is expressed as a percentage of the plasma concentration, and tissue content of EBD is expressed as micrograms per lung.

RESULTS

Total cells recovered after adoptive transfer. To more fully characterize the role of inflammatory cells in the development of ARDS, we have developed a novel adoptive transfer model in which cells from LPS-injured rats are transferred to normal animals intratracheally. In these experiments, we first determined the total number of inflammatory cells in recipient lungs 48 h after adoptive transfer. Each recipient rat received −38 × 10⁶ donor cells isolated from either control rats or rats 5 h after intratracheal LPS instillation. This number of cells is comparable to the number recovered from animals 5 h after LPS administration (data not shown). Figure 1A shows that the total number of cells recoverable from recipient rats receiving cells from LPS-treated donors 48 h after instillation was of the same order of magnitude as the number of cells delivered to each recipient animal (45.9 ± 11.6 × 10⁶ vs. 37.8 ± 10⁶, respectively). Importantly, the number of cells recovered from recipient rats receiving cells from normal donors was not different from animals receiving saline (11.0 ± 3.6 × 10⁶; Fig. 1A) or no treatment (7.7 ± 0.8 × 10⁶; data not shown), although these animals received 38.3 × 10⁶ donor cells. These results show that rats receiving cells from normal donors were able to clear the donor cells from the lung over the 48-h period. However, rats receiving cells from LPS-treated donors did not clear these inflammatory cells.

Differential cell counts of bronchoalveolar cells after adoptive transfer. To gain a more thorough understanding of the types of cells present in healthy animals and in the injury model, differential cell counts were performed. As shown in Fig. 1B, cells recovered by BAL of healthy animals were almost entirely macrophages. BAL cells recovered from donor rats treated for 5 h with LPS were 24.6% macrophages and 73.4% neutrophils. Importantly, 31.9 ± 7.4% of the BAL cells isolated from recipient rats 48 h after transfer of LPS-treated donor cells were PMNs. In contrast, cells re-
covered from recipient rats receiving normal donor cells were 81.5 ± 3.2% macrophages and 5.6 ± 1.2% neutrophils, a cellular composition comparable to the populations observed in saline-treated rats and in untreated rats (93.4 and 97% macrophages, respectively). These results indicate that the cell population recovered after adoptive transfer, like that seen after 5 h of LPS exposure, contained a significant percentage of neutrophils in the lung.

Alveolar-arterial oxygen difference after adoptive transfer. As an indicator of lung function, ABGs of recipient rats 48 h after adoptive transfer of BAL cells isolated from control and LPS-treated rats were determined, and the alveolar-arterial oxygen difference was calculated. Five hours of LPS treatment produced an alveolar-arterial oxygen difference indicative of alterations in gas exchange associated with lung injury (Fig. 2A). The difference observed in recipient rats receiving 37.8 × 10⁶ cells from LPS-treated donors was increased 13-fold compared with that in the control rats and rats receiving donor cells (38.3 × 10⁶) isolated from untreated donors. While this increase did not achieve statistical significance, the level of impairment in gas exchange did approach the value used to define significant deterioration of gas exchange (25 mmHg) (1, 16). Alveolar-arterial oxygen difference from animals receiving cells from normal donors was not different from that in saline-treated animals (Fig. 2A) or untreated animals (data not shown).

Effects of adoptive transfer on pulmonary surfactant. To determine the effects of adoptive transfer on pulmonary surfactant, a crude surfactant pellet was isolated from cell-free lavage, and the phospholipid protein ratio of the CSP was determined. Adoptive transfer of cells from control donor or from LPS-treated donor rats had no effect on the phospholipid protein ratio of recipient rat surfactant 48 h after transfer (Fig. 2B). However, 5 h after LPS treatment of LPS donor rats, the phospholipid protein ratio was significantly increased compared with that observed in CSP isolated from control donor rats. Thus adoptive transfer of inflammatory cells does not appear to affect the quality of pulmonary surfactant over the 48 h.

Consistent with normal phospholipid protein ratio, surfactant function is not altered in rats receiving BAL cells isolated from LPS-treated donor rats compared with animals receiving control BAL cells from control rats. As seen in Fig. 2C, the minimum surface tension was well below 10 mN/m in all recipient groups examined, indicative of normal surface activity in each group of rats.

Activation state of BAL cells before and after adoptive transfer. To determine the activation state of BAL cells before and after adoptive transfer, BAL cell production of NO and expression of iNOS were examined. For these experiments, BAL cells were isolated from recipient rats 48 h after the transfer of 38 × 10⁶ cells isolated from control or LPS-treated donor rats. The cells were then cultured for 24 h in the presence and absence of LPS or actinomycin D, and iNOS expression and NO- production were determined. Also, samples were prepared for SDS gel electrophoresis immediately after isolation. As shown in Fig. 3, following a 24-h ex vivo culture, BAL cells isolated from rats treated with LPS for 5 h produced high levels of NO- (Fig. 3A) and expressed iNOS (Fig. 3B). iNOS expression appears to be stimulated in vivo because the subsequent addition of LPS during the ex vivo 24-h culture did not further enhance iNOS expression above the level of iNOS expressed immediately after isolation. In addition, the transcriptional inhibitor actinomycin D did not inhibit iNOS expression during the 24-h ex vivo culture. Cells
isolated from saline-treated animals did not express iNOS (either immediately after isolation or following the 24-h culture) or produce measurable amounts of NO following a 24-h culture. However, treatment with LPS (10 μg/ml) during this 24-h culture of control cells elicited an ~10-fold increase in NO production. BAL cells isolated from recipient rats 48 h after adoptive transfer of BAL cells isolated from LPS-treated donors produced NO at levels similar in magnitude to those produced by control BAL cells treated for 24 h with LPS or untreated BAL cells isolated from rats treated with LPS for 5 h as determined by nitrite/nitrate determination. Consistent with NO production, these cells expressed high levels of iNOS following the 24-h culture. Although the BAL cells isolated from rats treated for 5 h with LPS expressed iNOS, BAL cells isolated 48 h after adoptive transfer no longer expressed iNOS immediately after isolation (preculture condition). However, BAL cells isolated 48 h after adoptive transfer expressed iNOS following a 24-h culture, and iNOS expression was prevented by actinomycin D. Importantly, these findings suggest that BAL cells isolated 48 h after adoptive transfer were activated in vitro, and this is most likely due to the release of soluble mediators that stimulated iNOS expression during the ex vivo culture. Consistent with these findings, BAL cells isolated 48 h after transfer of control donor BAL cells expressed iNOS and produced NO only when treated in vitro with LPS.

Effects of adoptive transfer on alveolar plasma transudation. Forty-eight hours after instillation of BAL cells isolated from normal donors or from animals treated with intratracheal LPS for 5 h, recipient rats received 20 mg/kg of EBD. After 1 h, the animals were killed, a blood sample was obtained, and the lungs were lavaged one time, with the lavage fluid recovery at 75.8 ± 0.8% for untreated controls, 70 ± 2.6% for animals receiving cells from LPS-treated donors, and 75.8 ± 1.4% for animals receiving cells from normal donors. The lung was then perfused and the plasma, lavage, and tissue concentrations of EBD were determined. There was no significant difference in the plasma concentration of EBD in the three groups (297 ± 9.3, 335 ± 9.6, and 317 ± 15.9, respectively). However, there was a significant increase in the concentration of EBD, expressed as a percentage of plasma concentration, in the alveolar lavage of recipient rats receiving donor cells from LPS-treated animals compared with that in untreated controls or rats receiving normal donor cells (Fig. 4A). Consistent with lung damage, the concentration of EBD was significantly greater in the adoptive transfer lung tissue (Fig. 4B) after lavage and perfusion of the vasculature, indicative of extravasation of the dye. As shown in Fig. 4C, even after lavage and perfusion, areas of infiltration of EBD into the air spaces were visible in the adoptive transfer lung (left lung in Fig. 4C). These results indicate that adoptive transfer of inflammatory cells from LPS-injured lungs influenced movement of fluid from the pulmonary vasculature into the airways.

The data presented in Figs. 1–4 clearly demonstrate that adoptive transfer of inflammatory cells from LPS-injured lungs to normal animals produced a lung injury. However, the possibility exists that this could result from the carryover of LPS in the lavage. To demonstrate that these effects on iNOS expression and NO production did not result from the carryover of LPS in the lavage from donor animals, two different experiments were performed. In the first experiment, animals received a dose of LPS that we calculated to be equivalent to the amount of LPS bound by the inflammatory cells present in the lavage. These calculations
were based on the studies of Stamme and Wright (42), which indicated that alveolar macrophages could bind 6,620 pg/10^6 cells. The number of donor cells in our adoptive transfer experiments would thus bind 250 ng of LPS, assuming that the binding of LPS to macrophages and neutrophils is similar. Forty-eight hours after administration of 250 ng of LPS, the animals were lavaged, and the isolated cells were placed in culture. As can be seen in Fig. 5A, NO production by these cells was threefold lower than in cells exposed to LPS in vitro, and iNOS expression was not detectable either before or after culture, except in the presence of LPS added in vitro (Fig. 5B). In the second study, rats received 15 mg/kg LPS as described and were lavaged 5 h after LPS administration. The lavage supernatant was separated from the inflammatory cells and combined with 10× culture medium. Four hundred thousand RAW 264.7 cells were seeded onto a 24-well plate and incubated in medium containing 0, 25, 50, 100, and 200 μl of lavage supernatant. As can be seen in Fig. 6, NO production and iNOS expression by RAW cells were not significantly stimulated by the presence of the lavage supernatant from LPS-treated animals. In a similar experiment, RAW cells were incubated in the presence of lavage supernatant (1, 10, 50, 10, or 200 μl) or LPS ranging in concentration from 1 × 10^-7 to 10 μg/ml, and NO production was determined after 24 h. When the response of the RAW cells to the lavage supernatant is compared with the LPS dose-response curve, 200 μl elicited a response equivalent to <0.1 μg/ml of LPS. This indicates that the concentration of LPS in the lavage supernatant was <250 ng/ml, which was shown not to induce iNOS expression or NO production in isolated BAL cells (Fig. 5). These results...
Numerous animal models have been developed to examine the various etiologies that can lead to the development of acute lung injury (ALI) (53). These include pneumonia-induced lung injury (48, 49), acid aspiration (9, 10, 24), endotoxin infusion (34, 38, 39), bacteremia (30), intratracheal endotoxin administration (52, 55), and administration of lung toxins such as NNMU (1, 16) and 3-methylindole (23, 54). One characteristic of human ARDS encompassed into each of these models is the infiltration of inflammatory cells into the interstitium and the alveolar space of the lung (7). These inflammatory cells, as well as resident type II pneumocytes and perhaps other resident cells, are capable of producing a number of proinflammatory mediators. The role of each of these inflammatory molecules in the development of ALI and ARDS, as well as their cellular sources, has long been an area of intense investigation. In applying adoptive transfer of lung disease in this study, we have developed a model system that will allow us to dissect the role of each cell type in the development of ALI and ARDS.

In this study, we show that in rats receiving cells from control donors, the number of cells remaining in the lung 48 h after transfer was similar to the number of cells recovered from untreated rats or rats receiving saline alone. In the control adoptive transfer rats, the majority of the cells were macrophages, with a limited number of infiltrating PMNs. This finding is consistent with the cell population in saline-treated rats and indicates that the majority of the cells that were transferred were cleared from the lung within 48 h. After adoptive transfer of cells from LPS-treated rats, the number of cells remaining in the lung was of the same order of magnitude as the number of cells transferred. This cell population 48 h after transfer of cells from LPS-treated donor rats contained significant numbers of PMNs (31%), the level similar to the percentage of PMNs found in the BAL of rats treated for 5 h with LPS (47.5%). These results indicate that after adoptive transfer, the cell population is comparable to that seen following LPS exposure, as well as demonstrating an increase in the number of neutrophils, consistent with the cellular composition observed in ARDS and in animal models of this syndrome.

In examining indicators of lung function, adoptive transfer had no apparent effect on the quality or function of pulmonary surfactant, as indicated by normal phospholipid protein ratios and normal surface activity. The alveolar-arterial oxygen difference was not significantly different from either saline-treated controls or adoptive transfer controls. However, the difference was increased severalfold above the control groups and was 43% of that seen in LPS-treated animals. These results indicate that there was a trend toward alterations in gas exchange in the adoptive transfer experiments. The lack of significant effect on indicators of lung function suggests that unlike the aerosol delivery of LPS, the liquid bolus delivery of cells does not produce as global an injury because of uneven distribution of these cells. Gas exchange in uninjured areas of the lung may be sufficient to keep the alveolar oxygen difference at levels lower then that seen in the other models of ALI or ARDS. It has been observed that lung injury, as indicated by edema formation, can be dissociated from hypoxemia in ARDS models (40, 41).

The cells recovered from animals 48 h after receiving cells from normal donors produced basal levels of NO in culture, and this level was enhanced sixfold following LPS treatment in vitro. Cells from LPS-treated animals produced much higher levels of NO; which resulted from iNOS expression in vivo, as indicated by iNOS expression detected immediately after isolation. Importantly, the activation state of the BAL cells isolated from rats treated for 5 h with LPS was significantly different from the activation state of BAL cells isolated from rats 48 h after adoptive transfer of donor cells isolated from LPS-treated rats. This was reflected in the in vivo stimulation of iNOS expression in BAL cells isolated 5 h after LPS administration, while BAL cells isolated 48 h after adoptive transfer did not express iNOS immediately after isolation. These cells indicate that carryover of LPS does not contribute to lung injury induced by adoptive transfer of BAL cells.
required a 24-h ex vivo culture period to express iNOS. These findings suggest 1) that donor BAL cells from LPS-treated rats are not the same as the cells isolated 48 h after adoptive transfer or 2) that BAL cells isolated 48 h after adoptive transfer of donor cells from LPS-treated rats are the same cells, but these cells no longer express iNOS. While both these hypotheses are possible, we favor the hypothesis that the adoptively transferred BAL cells (isolated from LPS-treated donors) stimulated additional inflammatory cells to migrate to the lung, and it may be that this influx of inflammatory cells mediates lung injury. However, future studies using vital dyes will be required to determine whether the new inflammatory cells migrate to the lung and whether these cells mediate tissue damage in the adoptive transfer model of lung injury.

Of concern in these studies is that the lung damage observed in the adoptive transfer experiments could result from LPS carryover in the lavage. However, experimental evidence does not support this conclusion. First, iNOS expression differs under the various conditions. Following adoptive transfer, BAL cells do not express iNOS protein immediately after lavage but do after 24 h of ex vivo culture. Cells isolated 5 h after tracheal instillation of LPS express iNOS immediately following isolation as well as after a 24-h ex vivo culture. Similarly, immediately following isolation, cells obtained 48 h after LPS instillation also express iNOS (data not shown). Second, based on the results of Stamme and Wright (42), we calculated that the number of cells that were instilled would bind ~250 ng of LPS, which could become available in the lung. However, BAL cells isolated 48 h after the administration of 250 ng of LPS did not express iNOS either before or after a 24-h ex vivo culture or produce significant amounts of NO unless exposed to LPS in vitro. Third, treatment of RAW 264.7 cells in culture with increasing concentrations of the lavage supernatant did not result in iNOS expression or significant NO production following a 24-h incubation. Furthermore, using the RAW 264.7 cells as a biological assay for LPS, we determined that the lavage supernatant obtained 5 h after LPS administration contained 200 ng/ml of LPS in the lavage supernatant. This is <250 ng/ml, which we showed did not induce iNOS expression or NO production in cells isolated 48 h after LPS instillation. Taken together, these data make it unlikely that lung injury induced by adoptive transfer is due to LPS that may be carried over during transfer.

The results presented in this study indicate that it is possible by adoptive transfer of inflammatory cells to induce lung injury in the rat. Using this new animal model, it should be possible to independently determine the contribution of each of the inflammatory cell types in lung injury by the selective transfer of macrophages and PMNs, either from injured animals or activated in vitro. Using this approach, it will be possible to isolate inflammatory cells from lungs injured by different etiologies as well as from specific transgenic or knockout mice and adoptively transfer these cells to normal control animals, enabling us to assess the contributions of these cell types and/or inflammatory mediators to the development of lung injury.

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REFERENCES
INFLAMMATORY CELL-INDUCED ACUTE LUNG INJURY


38. Simons RK, Junger WG, Loomis WH, and Hoyt DB. Acute lung injury in endotoxemic rats is associated with sustained circulating IL-6 levels and intrapulmonary CINC activity and neutrophil recruitment—role of circulating TNF-α and IL-β. Shock 6: 39–45, 1996.


