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Phagocytic and Bactericidal Activities of Pulmonary Macrophages following Sublethal Traumatic Shock[1] (39524)

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Phagocytosis of microorganisms and particulate matter by the alveolar macrophage is a pivotal mechanism utilized by the pulmonary reticuloendothelial system (RES) to inactivate pathogens invading the lower respiratory tract (1). The facilitation of phagocytosis by humoral opsonins as well as the postphagocytic stimulation of macrophage metabolism are well-known parameters of macrophage function and have recently been emphasized as to their importance in resistance of the lung to bacterial challenge. Several studies have demonstrated the existence and importance of opsonic antibodies found in respiratory secretions which are able to promote phagocytosis of *Pseudomonas aeruginosa*, a virulent lung pathogen in the compromised host (2, 3). Postphagocytic stimulation of respiration and glucose metabolism by lung macrophages has been linked to H_2O_2 production and peroxidative metabolism, thus delineating the formation of an effective bactericidal agent following bacterial ingestion by the alveolar macrophage (4). To what extent the phagocytic and metabolic activities of alveolar macrophages are altered acutely by injury has not been intensely investigated although of obvious importance in assessing pulmonary defense mechanisms against bacterial infection during a post-traumatic period.

The role of the systemic RES as a critical cellular defense mechanism in the host's response to shock and trauma has been intensely investigated (5-7). These studies have demonstrated that the macrophage population of the liver (i.e., Kupffer cell) undergoes phasic changes following nonlethal shock or trauma manifested by early functional depression and subsequent RE recovery and stimulation. In contrast to this acute depression in hepatic phagocytic activity following injury, there appears to be an inverse response by the lung with increased pulmonary localization of blood-borne test colloids within 1 hr after the onset of trauma or injury (8, 9). The potential clinical importance of this pulmonary response to injury is further emphasized by the fact that a similar pattern exists following surgery (10) as well as during the course of metastatic spread following malignant tumor cell challenge (12). Thus, intravenous challenge of animals with viable tumor cells or test colloids during a period of postoperative Kupffer cell depression is manifested by delayed clearance from the blood and increased localization in the lung (10-12). The intent of the present study was to evaluate alveolar macrophage phagocytosis, serum opsonic responsiveness, and postphagocytic bactericidal activity following standardized whole-body sublethal trauma in order to define the status of the pulmonary macrophage system acutely after injury. A bacterial challenge of *Pseudomonas aeruginosa* was utilized to assess lung macrophage phagocytosis. The trauma model was a sublethal traumatic shock produced by Noble-Collip drum (NCD) trauma. This model was chosen due to its reproducibility and previous use to investigate systemic RES function *in vivo* (9).

Methods. Male Sprague-Dawley rats weighing 300-350 g were anesthetized by intraperitoneal injection of sodium pentobarbital (2 mg/100 g) and used in all studies. The Noble-Collip drum (NCD) trauma was performed at 40 rpm for 300 revolutions and all rats were anesthetized prior to trauma. This shock model resulted in less

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than 2% mortality and has been previously documented (7, 9) as a sublethal trauma procedure. Alveolar macrophages (AM) were harvested prior to and following trauma by a modification of the method described by Myrvik (13). Inferior vena cava puncture was used for serum collection, and macrophages were obtained by repeated lung lavage with 0.2% EDTA in 0.15 *M* saline, pH 7.4, at 37° via the exposed trachea. Cell pellets were washed twice in Hanks' balanced salt solution without glucose (HBSS) at a pH of 7.4 and at 4°. Cell differentials were obtained by Wright-Geimsa staining techniques, and cell viability was determined by the exclusion of 1% trypan blue. Total cell counts were performed in duplicate by routine hemacytometry.

Bacterial phagocytosis of ^{14}C-labeled *P. aeruginosa* was measured by a modification of the method described by De Chatelet *et al.* (14). A stock culture of *P. aeruginosa* was incubated overnight at 37° in 50 ml of trypticase soy broth containing 50 μCi of uniformly ^{14}C-labeled L-amino acid mixture (New England Nuclear Corp., Boston, Mass.). The resulting ^{14}C-labeled *P. aeruginosa* was washed four times in 0.9% saline at 4°, and the final bacterial pellet was diluted with HBSS to a concentration of 10–15 $\times$ 10^8 bacteria/ml and stored at 4° until use. *In vitro* phagocytosis was initiated by the addition of 2.0 ml of the alveolar macrophage suspension (5–10 $\times$ 10^6 cells/ml) in HBSS to 0.5 ml of the ^{14}C-labeled *P. aeruginosa* suspension (5–10 $\times$ 10^8 bacteria/ml) followed by supplementation with 12.5% rat serum in a total volume of 4.0 ml. Flasks were incubated at 37° in a metabolic bath shaking at 60 rpm. Incubations were terminated by the rapid addition of 5.0 ml of 0.9% saline at 4° and centrifuged at 500*g* for 10 min at 4°. Cell pellets were washed twice in 0.9% saline to remove noningested bacteria and the resulting cells digested in 0.5 ml of 0.2 *N* NaOH for 4 hr at 80°. The cell digest was neutralized with 0.1 ml of 5% acetic acid and duplicate aliquots were added to 10 ml of Scintiverse (Fisher Scientific Co., Rochester, N.Y.). Samples were counted in an Isocap 300 liquid scintillation system (Amersham/Searle, Arlington Heights, Ill.) and phagocytic uptake was expressed as counts per minute of ^{14}C-labeled *P. aeruginosa* 15 min/10^7 alveolar macrophages.

The *in vitro* bactericidal activity of alveolar macrophages was determined by adding 2.0 $\times$ 10^7 alveolar macrophages obtained either prior to or 60 min following trauma to flasks containing 10% fresh rat serum and 2.0 $\times$ 10^7 *P. aeruginosa* in a total volume of 5.0 ml. Flasks containing serum and bacteria without macrophages served as background controls. All flasks were incubated at 37° in a metabolic shaking bath at 60 rpm and aliquots were removed and centrifuged at 500*g* for 10 min at 4°. Serial dilutions of the supernatants were incubated utilizing standard pour-plate colony counting techniques for 48 hr at 37° and the viable extracellular bacteria counted. Pellets of the alveolar macrophages were washed in sterile saline and resuspended in sterile distilled H_2O for 30 min in order to lyse the macrophages, and the viable intracellular bacteria were determined. Phagocytosis was expressed as the percentage of extracellular viable bacteria remaining at each incubation interval as compared to control flasks containing no alveolar macrophages. Bactericidal activity was expressed as the percentage of the phagocytized bacteria that remained viable.

The rate of oxygen uptake by macrophages was measured with a Clark-type electrode utilizing a Gilson oxymeter (Gilson Medical Electronics, Middletown, Wis.). O_2 consumption was measured at 37°, in HBSS containing 5.5 m*M* glucose, before and after the addition of heat-killed (30 min, 90°) *P. aeruginosa* preincubated in rat serum for 30 min at 37°. Oxygen uptake was expressed as micromoles of O_2 utilized per 60 min per 10^6 macrophages. The mean $\pm$ the standard error of the mean was calculated for all studies. All nonpaired analyses were made utilizing the Student's *t* test, and a confidence limit of 95% was used for significance.

Results. Table I demonstrates the recovery yields for alveolar macrophages from control and post-traumatic rat lungs at both the acute (60-min) and 24-hr interval. Repeated lavage of rat lungs yielded 12.6 $\pm$

0.97×10^6 cells/rat in nontraumatized controls; $6.4 \pm 0.4 \times 10^6$ cells/rat at 60 min post-trauma; and $7.5 \pm 0.5 \times 10^6$ cells/rat at 24 hr post-trauma. All lung cell populations demonstrated greater than 85% viability with mononuclear phagocytes comprising 90% of the isolated cell population. The yield (approximately 50%) was consistently and significantly ($P < 0.05$) less at both the 60-min and 24-hr post-trauma levels as compared to controls, but no difference was detected between the 1-hr and 24-hr trauma groups.

Phagocytosis of ^{14}C-labeled *P. aeruginosa* by macrophages lavaged from control rat lungs and from rat lungs 1.0 hr following nonlethal traumatic injury is represented in Table II. Bacterial phagocytosis by alveolar macrophages 1.0 hr following trauma in the absence of exogenous serum was significantly elevated when compared to control macrophages. With the addition of normal serum, bacterial phagocytosis was again greater by macrophages obtained at 60 min post-trauma. Furthermore, a stimulation of phagocytosis was obtained by both control and trauma macrophages with serum addition. The addition of serum from the traumatized rats obtained at 60 min postshock did not differ from control serum in its ability to enhance phagocytosis of *P. aeruginosa* by either control or trauma AM, indicating no difference in the opsonic capabilities of serum following NCD trauma, at least with respect to bacterial phagocytosis by lung macrophages. This latter comparative study evaluating the opsonic capacity of normal and 60-min post-trauma serum with respect to bacteria phagocytosis was critical since previous findings (7, 9) demonstrated a profound acute post-trauma opsonic deficiency in terms of nonbacterial phagocytosis. In order to determine if the pulmonary macrophage response was transient or would be sustained, a similar cellular comparison was performed at 24 hr postinjury. Table III illustrates bacterial phagocytosis by lung macrophages harvested 24 hr following sublethal trauma. In contrast to the stimulation of phagocytosis demonstrated 1 hr following trauma, bacterial phagocytosis by alveolar macrophages 24 hr post-traumatic injury was no longer elevated when compared to nontraumatized control cells in the presence or absence of serum opsonins. Again, however, both control and trauma cells respond with an increased bacterial phagocytosis in the presence of serum.

TABLE I. RECOVERY YIELDS OF ALVEOLAR MACROPHAGES AS INFLUENCED BY TRAUMATIC INJURY.

Experimental groups	Animals per experimental group	Number of alveolar cells recovered[a] per rat ($\times 10^6$)
Pretrauma controls	60	12.6 ± 0.97[b]
Post-trauma (1 hr)	60	6.4 ± 0.40
Post-trauma (24 hr)	24	7.5 ± 0.50

[a] Rat lungs were repeatedly lavaged *in situ* with 0.2% EDTA in saline at 37° with a total volume of 20–30 ml.

[b] Data are expressed as the mean ± SEM. The 1- and 24-hr post-trauma yields are significantly ($P < 0.05$) less than controls.

The acute stimulation of phagocytosis by lung macrophages following traumatic injury warranted further investigations into the function of post-trauma cells, and Table IV demonstrates the oxygen consumption of control and 1-hr post-trauma alveolar macrophages in both the resting state and following the phagocytosis of opsonized heat-killed *P. aeruginosa*. Oxygen consumption was significantly elevated ($P < 0.05$) in the trauma alveolar macrophages when compared to control cells in the resting condition. Following the addition of opsonized heat-killed bacteria, macrophage oxygen consumption was stimulated 60% in control AM and 30% in post-trauma AM.

In order to correlate further this apparent acute hyperphagocytic state by the alveolar macrophages to injury with other parameters of host defense, bacterial killing was also studied. Figure 1 demonstrates the *in vitro* bactericidal activity of alveolar macrophages harvested either prior to or 1 hr post-sublethal trauma. Bacterial count determinations after 30 and 60 min of incubation indicated a significant acute increase in the ability of macrophages from traumatized animals to kill ingested bacteria. Control AM were able to phagocytize 1.4×10^7 *P. aeruginosa* by 30 min, of which less than

TABLE II. PHAGOCYTOSIS OF ^{14}C-Labeled *Pseudomonas aeruginosa* BY ALVEOLAR MACROPHAGES HARVESTED ACUTELY (1 hr) FOLLOWING TRAUMATIC INJURY.

Source of alveolar macrophage cell population[a]	Number of experiments	Bacterial phagocytosis[b] (cpm/15 min/10^7 AM)		
		No serum	Normal serum	Trauma serum
Pretrauma controls	6	2007 ± 104	4915 ± 38[c]	4886 ± 341[c]
Post-trauma (1 hr)	6	3021 ± 333[d]	5995 ± 109[c]	6097 ± 293[c]

[a] Alveolar macrophages, 10–20 × 10^6, were incubated with ^{14}C-labeled *Pseudomonas aeruginosa* at a bacteria-to-cell ratio of 100:1 in a total volume of 4.0 ml at 37° at 60 rpm ± 12% fresh rat serum. Each flask was supplemented with an average of 53,000 cpm (injected dose = ID) of labeled bacteria.

[b] Data are expressed as the mean ± SEM.

[c] Significantly different from no serum ($P < 0.01$).

[d] Significantly ($P < 0.05$) different from pretrauma control levels of bacterial phagocytosis.

TABLE III. PHAGOCYTOSIS OF ^{14}C-LABELED *Pseudomonas aeruginosa* HARVESTED 24 HOURS FOLLOWING TRAUMATIC INJURY

Source of alveolar macrophage cell population[a]	Number of experiments	Bacterial phagocytosis[b] (cpm/15 min/10^7 AM)	
		No serum	Normal serum
Pretrauma controls	6	11625 ± 734	17984 ± 300[c]
Post-trauma (24 hr)	12	11376 ± 386	16955 ± 606[d]

[a] Alveolar macrophages, 10–20 × 10^6 were incubated with ^{14}C-labeled *Pseudomonas aeruginosa* (activity/flask = 300,000 cpm) at a bacteria to cell ratio of 100:1 in a total volume of 4.0 ml at 37°, 60 rpm ± 12% fresh rat serum. Each flask was supplemented with an average of 130,000 cpm (injected dose = ID) of labeled bacteria.

[b] Data are expressed as the mean ± SEM.

[c] Significantly different from no serum controls ($P < 0.05$).

[d] Significantly ($P < 0.05$) different from pretrauma control levels of bacterial phagocytosis.

30% were killed. In contrast, the post-trauma cells ingested 1.7 × 10^7 *P. aeruginosa* and killed greater than 50% of the ingested bacteria.

Discussion. These findings indicate that, rapidly following sublethal trauma, bacterial phagocytosis, oxygen consumption, and bactericidal activity by the pulmonary macrophage as tested *in vitro* are stimulated. Furthermore, bacterial phagocytosis is augmented in post-trauma alveolar macrophages in the absence of serum opsonins indicating a nonhumoral element, possibly a metabolic event, in the macrophage phagocytic activation. In fact, phagocytosis of *P. aeruginosa* remained elevated in alveolar

TABLE IV. EFFECT OF *Pseudomonas aeruginosa* PHAGOCYTOSIS ON O_2 CONSUMPTION BY ALVEOLAR MACROPHAGES HARVESTED PRIOR TO AND FOLLOWING (1 hr) TRAUMATIC INJURY.

Experimental model[a]	Number of experiments	O_2 consumption[b] (μmoles O_2/60 min/10^6 AM)	
		Control macrophages (mean ± SE)	Post-trauma macrophages (mean ± SE)
Resting (no bacteria)	6	5.9 ± 0.2	8.7 ± 1.0
Stimulated (with bacteria)	4	9.5 ± 0.4	11.3 ± 1.1

[a] Each flask contained 10 × 10^6 macrophages in HBSS supplemented with 5.5 m*M* glucose and incubated at 37°. The stimulated model was supplemented with 5 × 10^7 heat-killed *P. aeruginosa* previously opsonized in 10% fresh serum.

[b] Data are expressed as mean ± SEM.

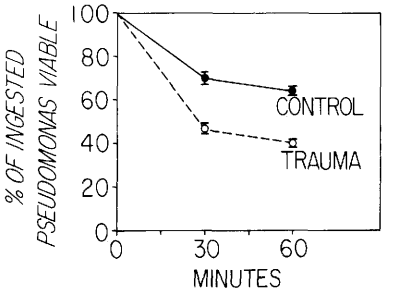

FIG. 1. The *in vitro* bactericidal activity of control (●) and 1-hr post-trauma (○) alveolar macrophages. In each flask 2.0 × 10^7 macrophages were incubated with 2.0 × 10^7 *Pseudomonas aeruginosa* in the presence of 10% rat serum at 37°. Bactericidal activity is expressed as the percentage of the calculated phagocytized bacteria that remained viable at each incubation interval. Each group consists of nine experiments with three determinations at each time interval (0, 30, 60 min).

macrophages harvested from animals 1 hr after trauma in the presence of serum opsonins, which augmented bacterial phagocytosis twofold in both populations. This stimulatory opsonic effect of serum is in close agreement with studies demonstrating a doubling of lung RE cell phagocytosis of albumin–paraffin oil emulsion particles in the presence of serum factors (15). However, the opsonic potential of serum obtained 1 hr post-trauma did not differ from control serum in its ability to enhance bacterial phagocytosis, suggesting no alterations in blood-borne bacterial opsonic factor levels in the early post-trauma period. Recent studies have indicated that hepatic RES function is maximally depressed 1–2 hr post-NCD trauma as manifested by a significant decline in the *in vivo* phagocytic index *K* value for colloid clearance. This decrease in systemic RE function has been shown to be mediated by opsonic deficiency with respect to nonbacterial phagocytosis (7, 9). The present data suggest, however, a distinct difference in pulmonary macrophage function as compared to hepatic Kupffer cell phagocytosis in the early postinjury period. Recently, Dressler and Skornik (16), utilizing a model of burn injury, reported a 27% killing efficiency by control rat AM for *P. aeruginosa* by 30 min at a bacteria to cell ratio of 50:1 and comparable to our control killing efficiency of 30% at a bacteria to cell ratio of 1:1. Furthermore, these same authors demonstrated a sustained increase in the phagocytic and bactericidal activities of rat AM following burn injury, thus lending support to the concept of pulmonary RES activation following injury. However, it appears from the present study that alveolar macrophage activation is a rapid phasic event in response to traumatic injury and appears to return to control pretrauma levels by 24 hr, at least with respect to the parameter of phagocytosis.

This temporal pattern again correlates in an inverse manner with the functional state of the Kupffer cell since it manifests phagocytic recovery after this type of trauma by 24 hr (9).

Although a wide spectrum of environmental and endogenous agents has been shown to alter the phagocytic and metabolic activities of the lung macrophage (17–19), the metabolic activities of this cellular population during the pathogenesis of lung injury or trauma have not been explored. Increases in the postphagocytic O_2 consumption and glucose oxidation in the isolated alveolar macrophage have been reported to reflect stimulation of the HMPS pathway and H_2O_2 metabolism (4, 20). In this context, our observations demonstrate a significantly elevated O_2 consumption in the resting state in post-trauma cells. In addition, the elevated postphagocytic respiratory rate indicates a metabolic basis for the stimulation of bacterial phagocytosis in the lung macrophage following NCD trauma. The precise mechanism whereby the stimulation in O_2 consumption and H_2O_2 generation are linked to bactericidal activity in AM is presently not defined; however, Paul *et al.* (21) have recently demonstrated peroxidase–H_2O_2 activity in AM homogenates, thus delineating one potent antimicrobial system which may exist within the AM.

Rapid stimulation of macrophage phagocytosis and metabolism would be a distinct advantageous cellular defense mechanism in the lung, preventing pulmonary infection by opportunistic organisms such as *P. aeruginosa* acutely following trauma. However, one must consider the findings as presented in this study that, while on a cell-to-cell basis, there is an apparent acute activation following injury, the total number of cells recoverable was significantly reduced at both periods after trauma. Hyperphagocytosis by lung macrophages acutely following injury suggests that the previous observations of increased pulmonary localization of particulate matter following surgery in temporal association with Kupffer cell phagocytic dysfunction may not represent a nonspecific event as previously suggested (9–12). This may reflect a compensatory response by extrahepatic macrophages to compensate for the hepatic RE depression or it may be mediated at the local level by the presence of tissue debris and denatured protein generated as a result of the trauma (7). In either event, the relationship of these findings to the previously documented pulmonary localization of blood-borne cellular and noncellular particulate matter following trauma (7) warrants investigation.

Summary. The *in vitro* phagocytic and

bactericidal responses of rat alveolar macrophages were investigated following sublethal traumatic shock. Phagocytosis of ^{14}C-labeled *Pseudomonas aeruginosa* by lung macrophages was elevated 1 hr post-trauma in the absence of bacterial opsonins. This response was transient with a return to normal by 24 hr. Bacterial phagocytosis by control and trauma alveolar macrophages was doubled in the presence of serum obtained either prior to or following trauma. In association with this acute phagocytic activation, resting O_2 consumption as well as bactericidal activity was stimulated in isolated alveolar macrophages harvested at 60 min post-traumatic injury. In contrast, the recoverable yields of lung macrophages following traumatic injury were significantly reduced at both the 1-hr and 24-hr postinjury period. Alveolar macrophage phagocytosis and metabolism are thus rapidly and transiently activated following host defense mechanisms following sublethal trauma. This response is in direct contrast to the previously documented acute depression of the hepatic Kupffer cell following traumatic shock and may represent a compensatory response of the lung RES during periods of hepatic RES dysfunction.

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