Depression of the Respiratory Burst in Alveolar and Peritoneal Macrophages After Thermal Injury

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The resting O2 consumption of alveolar and peritoneal macrophages obtained from rats at 4 and 24 h after thermal injury was unaltered from control values. However, when heat-killed Pseudomonas aeruginosa or polystyrene latex particles were added to the cell suspensions to initiate phagocytosis, a significant depression in the respiratory burst accompanying the phagocytic event was demonstrated. The addition of phorbol myristate acetate, used to maximize the respiratory response, was ineffective in elevating, to control values, the respiratory burst of macrophages obtained from burned animals. The deficit was only, in part, serum mediated since the responses could not be restored to control values even when the cells from the burned animals were vigorously washed with control serum and incubated with control serum. The contribution of a burn serum factor, which was non-dialyzable, heat stable at 56°C but not at 65°C, and insensitive to pronase treatment, must be considered. These data indicate that thermal injury results in macrophage metabolic alterations which are mediated, in part, by a burn serum factor. Furthermore, the data suggest that pulmonary alveolar macrophages are more sensitive to thermal injury than peritoneal macrophages. Serum factors contributed, in part, to this observed impairment in the respiratory burst as indicated by: (i) an approximate 50% reversal of the impairment by control serum, and (ii) an approximate decrease of 50 to 80% in the control alveolar macrophage respiratory burst when serum from the thermally injured rats was added to the culture medium.

The rate of oxygen uptake of phagocytic cells is increased above the basal rate during the phagocytosis of nontoxic particles. A concomitant increase in lactate production and a direct stimulation of the oxidative pathway for glucose metabolism during phagocytosis also occurs (20). Phagocytic stimulation of respiration and glucose metabolism in alveolar macrophages has been linked to H2O2 production and peroxidative metabolism, thus delineating the formation of an effective bactericidal agent after bacterial ingestion by the alveolar macrophage (6). In this regard, the alveolar macrophage serves as the major cellular defense system in the lung (8).

Functional alterations in the alveolar macrophages have been suggested to be responsible for the increased incidence of pulmonary infections after thermal injury. Since phagocytosis is an integral step in the induction of the primary immune response (5) and in protection against bacteria (3), viruses (16), and protozoa (22), the present study was conducted to evaluate the influence of thermal injury on phagocytosis vis-a-vis the determination of the respiratory burst accompanying the phagocytic event. The influence of serum components on oxygen utilization by alveolar and peritoneal macrophages after thermal injury was studied concurrently.

MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats (190 to 210 g) were used throughout. Purina lab chow and water were provided ad libitum, and a 12/12—day-to-night—photoperiod was maintained. All animals were starved overnight before the study.

Burn injury. A 26 to 28% body surface area burn was produced by immersing the dorsum of ether-anesthetized rats in a 90°C water bath for 30 s. A control group was anesthetized with ether alone. Alveolar and peritoneal macrophages were isolated either 4 or 24 h after thermal injury. Serum was harvested at the same time periods. Cells and serum obtained from thermally injured animals will hereafter be referred to as burn cells or burn serum.

Cell isolation. Peritoneal macrophages were isolated by saline lavage of the peritoneal cavity. Three 15-ml samples of sterile isotonic saline were injected into the peritoneal cavity of ether-anesthetized rats, and the cavity was then vigorously massaged for 2 min before harvesting of the lavage fluid. This procedure was conducted three times for each animal, and all three peritoneal aspirates were then pooled and kept on ice. The pooled lavage fluid from each animal was then centrifuged at 200 × g for 5 min at 4°C in...
siliconized conical centrifuge tubes. Contaminating erythrocytes were removed by hypotonic lysis for 15 s with distilled water. The cell suspension was returned to isotonicity by the addition of 2× Hanks balanced salt solution (HBSS). The suspension was centrifuged again at 200 × g for 5 min at 4°C, and the cell pellet was suspended in HBSS. A differential cell count using Wright stain and neutral red revealed that the peritoneal lavage fluid contained approximately 80% macrophages, 5% lymphocytes, and 5% Mast cells with >90% viability as determined by trypan blue dye exclusion.

Alveolar macrophages were obtained by pulmonary lavage with isotonic saline. Three 10-mL samples of isotonic saline were infused into the lungs of ether-anesthetized rats through a tracheal cannula. The lungs were massaged in situ for 2 min, and the pulmonary lavage fluid was then withdrawn through the cannula. This procedure was conducted three times for each animal, and all three pulmonary aspirates were pooled and kept on ice. The pooled lavage fluid was centrifuged at 200 × g for 5 min at 4°C in siliconized conical centrifuge tubes. The cell pellet was suspended in HBSS. A differential cell count using Wright stain and neutral red revealed that the suspension contained approximately 90% macrophages and 5% small lymphocytes with >90% viability as determined by trypan blue dye exclusion.

Oxygen consumption. Oxygen consumption of peritoneal and alveolar macrophages was measured as previously described (14) with a Gilson recording oxygen graph equipped with a Clarke oxygen electrode. A constant temperature of 37°C was maintained in the chamber. Resting O₂ consumption was measured for 5 min, after which phagocytosis was initiated by the addition of either heat-killed opsonized Pseudomonas aeruginosa added at a bacteria/cell ratio of 100:1 or polystyrene latex (1.099 μm; Coulter Electronics, Inc.) at a particle/cell ratio of 1,000:1. The bacteria and latex particles were opsonized in serum from control or burned rats for 30 min at 37°C before their addition to the macrophage suspensions. A final concentration of 20% serum from either control or burn animals was used in the cell suspension. The O₂ consumption of the phagocytizing cells was measured for 15 min. The respiratory burst (ΔQO₂) observed after the addition of the bacteria or latex particles was determined by the difference in the slopes of the lines representing resting O₂ consumption versus the phagocytizing O₂ consumption.

In additional studies, the respiratory burst was enhanced with phorbol myristate acetate (PMA) in an attempt to elicit a maximal response. PMA (Consolidated Midland Corp.) was prepared in dimethyl sulfoxide and diluted in the appropriate medium for each experiment. Dimethyl sulfoxide, in comparable concentrations, did not alter the respiration of either population of macrophages.

Statistical evaluation. Data are presented as the mean ± standard error of the mean. Significance was determined by the Student t test, and an asterisk denotes where P < 0.05. For statistical comparisons, we used the control cells incubated with control serum as the control group against which all comparisons were made.

RESULTS

The resting O₂ consumption of either alveolar or peritoneal macrophages isolated 4 or 24 h after thermal injury was unaltered from control values (Table 1). Furthermore, the addition to the culture medium of serum (20%) obtained from rats after thermal injury did not alter the resting O₂ utilization of control macrophages.

However, a significant decrease in the respiratory burst of alveolar and peritoneal macrophages obtained 4 and 24 h postburn was observed when the cells were presented with a phagocytizable particle, i.e., either heat-killed P. aeruginosa or polystyrene latex (1.099 μm). Both particles had been opsonized in either burn or control serum for 30 min at 37°C before addition to the cell suspensions (Table 2). The addition of the serum-opsonized particles resulted in a final serum concentration of 20% in the cell suspension.

Serum factors contributed, in part, to the observed impairment in the respiratory burst of both cell types since the addition of control serum to the burn cells partially restored their respiratory burst. However, the burst still did not approximate that observed with the control group (Table 2). Also, washing the burn cells three times in HBSS plus 20% control serum did not restore their respiratory burst.

When control alveolar macrophages were incubated in 20% control serum and allowed to phagocytize either heat-killed P. aeruginosa or latex particles which had been opsonized in control serum, they manifested a respiratory burst of approximately 20%. However, when the control alveolar macrophages were incubated in 20% serum did not restore their respiratory burst.

| Table 1. Oxygen consumption of resting alveolar and peritoneal macrophages after thermal injury |
|---------------------------------|-----------------|-----------------|
| Incubation medium               | O₂ consumption  |
|                                 | Alveolar macrophages | Peritoneal macrophages |
|                                 | at post-burn:       | at h post-burn:       |
|                                 | 4                | 24               | 4               | 24               |
| Control cells + control serum   | 52 ± 6.3         | 58 ± 6.1         | 40 ± 3.3        | 36 ± 4.6         |
| Control cells + burn serum      | 50 ± 4.7         | 46 ± 7.7         | 45 ± 5.2        | 37 ± 5.9         |
| Burn cells + control serum      | 43 ± 5.8         | 53 ± 4.0         | 38 ± 3.5        | 39 ± 4.4         |
| Burn cells + burn serum         | 61 ± 7.4         | 51 ± 7.5         | 40 ± 5.2        | 41 ± 4.9         |

Resting O₂ consumption of postburn macrophages was measured with a Clarke O₂ electrode and calculated as micromoles of O₂ consumed per hour per 10⁶ cells. Data are expressed as the mean ± standard error of the mean; n = 8. Differences were not statistically significant. Cell-serum combinations used cells and serum obtained at identical time periods.
burn serum and presented with particles opsonized in burn serum, a respiratory burst of 10% was observed (Table 2). This deleterious effect of burn serum was not observed when peritoneal macrophages were used. Washing both cell populations (alveolar and peritoneal) in 50% control serum and then exposing them to the burn serum elicited the same response as was seen without the washing procedure.

To maximize the respiratory burst, PMA suspended in dimethyl sulfoxide was added to the cell suspensions (Table 3). An approximate twofold increase (as compared to non-PMA-containing preparations, Table 2) in the respiratory burst of alveolar and peritoneal macrophages was elicited when the control cells were incubated in media containing PMA. The concentration of PMA used to maximize the response was 200 μg/ml for both cell types. The response of the burn cells, incubated in either control or burn serum, was also increased by PMA, but the response was still significantly lower than control cells. The burn cells did not respond in any significantly greater magnitude to higher concentrations of PMA.

Since the possibility existed that the impaired respiratory burst of burn cells and the burn serum-mediated deficit in control cells were either an opsonin deficiency or a burn toxin or both, an initial attempt was made to delineate certain characteristics of the burn toxin. The burn serum obtained at 4 to 24 h postburn and, after its dialysis, thermostability, and enzyme sensitivity determination were tested against control alveolar macrophages, since this appeared to be the most sensitive cell type. P. aeruginosa was used as the test particle. The toxic, i.e., respiratory burst-depressing characteristics, are that the substance is: (i) non-dialyzable, (ii) unaltered by pronase, and (iii) heat labile when heated at 56°C for 30 min but heat-stable when heated at 65°C for 30 min.

### DISCUSSION

Alveolar and peritoneal macrophages obtained 4 and 24 h after thermal injury had no alterations in their resting O2 utilization when incubated in media containing either control serum or serum from burned rats (Table 1). However, when the burn cells were presented with a phagocytizable particle, e.g., heat-killed P. aeruginosa or polystyrene latex spheres, a significant depression in the respiratory burst was observed. The decrease in the respiratory burst was greater in the alveolar macrophages than in the peritoneal macrophages (Table 2). A suppression of the respiratory burst has also been reported in pulmonary alveolar macrophages obtained after traumatic shock (9), in peripheral blood polymorphonuclear leukocytes isolated from burn patients (10), and in alveolar macrophages exposed to heavy metals (14). Profound differences in the chemiluminescent response of alveolar and peritoneal macrophages and the subsequent inhibition by superoxide dismutase or Na+ benzoate have also been observed (1).

The response of pulmonary alveolar macrophages versus peritoneal macrophages to thermal injury and other insults (12–14) may be due, in part, to their differences in metabolism. In general, it is believed that alveolar macrophages derive their energy, especially for phagocytosis,
mainly from oxidative phosphorylation, whereas peritoneal macrophages derive their energy mainly from glycolysis (11). This contention is supported by: (i) the overall metabolic patterns of cells from the two sites, (ii) high levels of cytochrome oxidase and succinic dehydrogenase and low levels of pyruvate kinase and phosphofructokinase (consistent with aerobic energy metabolism) in alveolar macrophages and low levels of cytochrome oxidase and high levels of pyruvate kinase and phosphofructokinase (consistent with anaerobic energy metabolism) in peritoneal macrophages. Also, pulmonary alveolar macrophages (unlike other phagocytic cells) show no Crabtree effect (depression of cellular respiration by glucose), and particle ingestion is diminished in the presence of inhibitors of oxidative metabolism. Histologically, alveolar macrophages have more numerous and larger cytoplasmic inclusions, lysozyme, acid phosphatase, and B-glucuronidase than peritoneal macrophages (11).

The addition of serum from the thermally injured animals to the cell suspension did not alter the resting O2 consumption, which suggests the absence of a burn toxin (21). However, a contribution of serum factor(s) to the decreased respiratory burst is suggested by the demonstration that the burst was reduced less when the cells from burned animals were incubated in media containing normal serum than in media containing burn serum. If a reduction in postburn opsonins (7) was responsible for the depression in the respiratory burst, then the use of normal serum, containing a normal level of opsonins, should have resulted in a respiratory burst comparable to controls. Since the burst was still less than control values, the results suggest a cellular defect. Furthermore, a cell specificity is suggested by the lack of influence of the burn serum on the control peritoneal macrophage respiratory burst. One recent study by Constantian (4) has shown a direct correlation between the presence of serum suppressor factors, suggested to be polypeptides, and the occurrence of septic episodes. Ninnemann et al. (17) have shown that survival associated with therapeutic allografts applied in the treatment of grade IV thermal injuries is directly related to the immunosuppressive activity of patient sera to phytohemagglutinin-induced blastogenesis of normal lymphocytes in vitro. Subsequent studies (18) have demonstrated that this suppressive activity can be blocked by an immunoglobulin G (IgG) fraction in postrecovery serum. The presence of a suppressive burn serum factor which inhibited the normal respiratory burst of macrophages, as seen in the present study, may explain, in part, the impaired phagocytic activity of postburn macrophages as previously reported (15). Preliminary characterization of such a factor indicated that it is non-dialyzable, which suggests a molecular weight of >8,000, and pronase insensitive, suggesting that it is not a classical opsonin.

The membrane-active agent, PMA, has been shown to promote a marked stimulation of the respiratory burst of neutrophils and cause a release of enzymes of the secondary granules of neutrophils (2). As recently demonstrated by Bass et al. (2), PMA can be used to dissociate the respiratory burst of neutrophils from their chemotactic activity. In the present study, PMA was used to maximize the respiratory burst of the macrophages. As shown in Table 3, an enhanced respiratory burst could be elicited by burn macrophages in the presence of PMA, but the burst was still significantly less than that observed in controls.

Therefore, the mechanism whereby thermal injury impairs the respiratory burst of alveolar and peritoneal macrophages obtained after burn injury encompasses both a serum and cell component and may be related to the profound endocrine and metabolic changes which occur after thermal injury (J. Turinsky, L. Loose, T. Saba, Burns, in press).

### TABLE 3. Effect of PMA on the respiratory burst (ΔQO2) of alveolar and peritoneal macrophages after thermal injury

<table>
<thead>
<tr>
<th>Incubation medium</th>
<th>Respiratory burst (% ΔQO2) of:</th>
<th>Alveolar macrophages at h post-burn:</th>
<th>Peritoneal macrophages at h post-burn:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>24</td>
</tr>
<tr>
<td>Control cells +</td>
<td>44 ± 4.5</td>
<td>42 ± 6.1</td>
<td>85 ± 7.9</td>
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<td>control serum</td>
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<td>91 ± 12.7</td>
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<td>12 ± 2.3</td>
<td>63 ± 10.8</td>
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<tr>
<td>burn serum</td>
<td></td>
<td></td>
<td>78 ± 6.9</td>
</tr>
<tr>
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<td>7 ± 1.5*</td>
<td>28 ± 3.3*</td>
</tr>
<tr>
<td>control serum</td>
<td></td>
<td></td>
<td>24 ± 3.5*</td>
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<tr>
<td>Burn cells + burn</td>
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<td>1 ± 0.3*</td>
<td>15 ± 2.0*</td>
</tr>
<tr>
<td>serum</td>
<td></td>
<td></td>
<td>18 ± 2.3*</td>
</tr>
</tbody>
</table>

*The respiratory burst of alveolar or peritoneal macrophages was assessed with P. aeruginosa (bacteria/cell ratio of 100:1) as a phagocytizable particle. The pseudomonad was opsonized in the appropriate serum for 30 min at 37°C and was added to the cell suspension so that the final serum concentration was 20%. PMA was prepared in dimethyl sulfoxide (preliminary studies demonstrated that at the concentrations of dimethyl sulfoxide used, it induced no alteration in respiration) and added at the same time as the bacteria. Initial dose-response studies indicated that maximal stimulation of either cell type occurred at a dose of 200 μg/ml; therefore, this dose was chosen as the stimulatory dose. All data are presented as the mean ± standard error, with an asterisk denoting significance at P < 0.05; n = 7 in all groups.
ACKNOWLEDGMENT

This study was supported in part by Public Health Service grant GM-22825 to J.T. from the National Institutes of Health.

LITERATURE CITED


