INFECTION AND IMMUNITY, Oct. 1979, p. 157-162
0019-9567/79/10-0157/06$02.00/0

Vol. 26, No. 1

Macrophage Dysfunction After Burn Injury

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Received for publication 13 July 1979

The phagocytic and microbicidal activities of alveolar and peritoneal macrophages were evaluated 4 and 24 h after a full-thickness scald burn of 26 to 28% body surface area in anesthetized rats. The contribution of serum factors to the macrophage functions was studied concurrently. The phagocytic activity of alveolar macrophages obtained 4 or 24 postburn was reduced approximately 65% below control values when they were incubated in media containing autologous serum and approximately 45% (below controls) when they were incubated in media containing normal (control) serum. A similar, although not as marked, decrease in the phagocytic activity of peritoneal macrophages was also demonstrated. Serum obtained from rats 4 or 24 h postburn had a significant suppressive effect on the phagocytic activity of control alveolar, but not peritoneal, macrophages. The intracellular microbicidal activity of peritoneal macrophages obtained at the postburn intervals and incubated in media containing either autologous or control serum was unaltered from control values. However, alveolar macrophages obtained 24 h, but not 4 h, postburn had a significant (approximately 80%) increase, above control values, in their microbicidal activity. Serum obtained 24 h, but not 4 h, postburn stimulated control alveolar macrophage killing ability. These data indicate that thermal injury induces a defect in the phagocytic activity rather than the microbicidal activity of macrophages. This phagocytic alteration is mediated, in part, by serum.

Mortality that occurs after burn injury is closely related to the extent of full-thickness destruction of skin. The actual cause of death is not related to the biological effect of thermally killed skin but rather to the metabolic and bacterial consequences of a large, open wound (5). Indeed, it has long been recognized that burned patients and animals exhibit heightened susceptibility to infections (19, 35). This is not due merely to the presence of overwhelming numbers of bacteria on the burned surface since it occurs much too rapidly after injury and affects not only the skin but other organ systems as well. In fact, increased susceptibility to infection can be demonstrated within a few minutes of burning (1), at a time when the burn wound is normally still sterile (30). If germfree mice, which have a low mortality after thermal injury, are "normalized" by the introduction of *Escherichia coli* into their gastrointestinal tracts, their mortality after burn injury approaches that of conventional mice (23). It is suggested that some defect in defense mechanisms against infection is brought about by thermal injury.

Pneumonitis has been reported to be the most common cause of postburn death (33) and has been attributed to an impaired host resistance. Using the burned rat as a model of altered host resistance, Dressler and Skornik (9) showed that susceptibility of the lung to sepsis is directly related to host resistance and specifically to the ability of the lung to clear an aerosolized bacterial insult.

An integral component of pulmonary host defense is the alveolar macrophage, which has been suggested to undergo phasic functional alterations after thermal injury in the rat (10). These alterations have been suggested to account, in part, for the enhanced pulmonary susceptibility to infection postburn. However, Smith and Goldman (34), using *Candida* sp. as a test particle, previously demonstrated a functional impairment in peritoneal macrophages after burn injury in mice. These studies (9, 10, 34) suggest that macrophage dysfunction that occurs after burn injury may involve macrophage populations other than alveolar.

The present study was conducted to compare the influence of thermal injury on functional characteristics of two distinct populations of macrophages, i.e., alveolar and peritoneal. Macrophage function was assessed by determining phagocytosis and intracellular microbicidal activity. The contribution of postburn serum fac-

tors to macrophage function was evaluated 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-night photoperiod was maintained. All animals were fasted overnight prior to the study.

Burn injury. A full-thickness scald burn 26 to 28% of body surface area was produced by immersing the dorsum of ether-anesthetized rats into a 90°C water bath for 30 s. A control group was anesthetized with ether but not burned.

Cell isolation. Peritoneal macrophages were isolated by saline lavage of the peritoneal cavity. Three 15-ml amounts of sterile isotonic saline were injected into the peritoneal cavity of ether-anesthetized rats, and the peritoneal cavity was then vigorously massaged for 2 min prior to harvesting of the peritoneal 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 resuspended in HBSS. The peritoneal lavage fluid contained approximately 80% macrophages, 5% lymphocytes, and 5% mast cells with >90% viability as determined by trypan blue dye exclusion. Wright's stain was used for the differential cell count, and macrophages were enumerated by uptake of neutral red.

Alveolar macrophages were obtained by pulmonary lavage using isotonic saline. Three 10-ml amounts 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 resuspended in HBSS. A differential cell count using Wright's stain and uptake of neutral red revealed that the suspension contained approximately 90% macrophages and 5% small lymphocytes with a >95% viability as determined by trypan blue dye exclusion.

Phagocytic and microbicidal studies. The technique used to assess the phagocytic and microbicidal activity of murine phagocytic cells is an adaptation (21) of a light microscopic procedure initially described by Schmid and Brune (31), who used human peripheral blood neutrophils. Briefly, a phagocyte-yeast (*Saccharomyces cerevisiae*) ratio of 1:3 was utilized. Sterile capped plastic test tubes (12 by 75 mm) which had 0.20 ml of a solution containing 25.6 mg of methylene blue/100 ml of HBSS, 0.25 ml of the cell suspension in HBSS, 0.25 ml of yeast in serum, and 0.10 ml of HBSS were incubated at 37°C in a Dubnoff shaking water bath for 30 min. Preliminary studies conducted to adapt the method of Schmid and Brune (31), which used human cells, to a murine system revealed optimal activity at 30 min. This time was then chosen as the time for measurement. The tubes were centrifuged at 312 × *g* for 6 min at 4°C after removal from the water bath. The supernatant was discarded, and the cell pellet was resuspended in 20 μl of HBSS. A wet mount was prepared for light microscopic examination, and a minimum of 200 phagocytic cells were counted per preparation. Dead yeast cells stained blue with the methylene blue whereas live yeast remained unstained. The percentage of macrophages which contained at least two yeast cells was used as an index of the phagocytic activity. The total number of yeast cells, live and dead, ingested per 100 phagocytic cells was tabulated and expressed as the phagocytic capacity. The total number of dead yeast cells, i.e., those which stained blue, within the macrophages was counted and expressed as a function of the cellular microbicidal activity. All phagocytic and microbicidal studies were conducted in duplicate.

Statistical evaluation. Data are presented as the percent change from the control response. Each bar in Fig. 1–3 represents the mean percent change of the experimental parameters compared with a mean control value. The animals from which normal cells and serum were obtained were used as controls for each parameter, and the mean of these values provides a base line for comparison of experimental, i.e., burned, animals. Significance was determined by the Student *t* test and is noted with an asterisk where $P < 0.05$.

RESULTS

Alveolar macrophages obtained either 4 or 24 h postburn manifested a reduction of approximately 65% in their phagocytic activity when they were incubated in media containing autologous serum obtained at the same postburn time intervals. When these cells were incubated in control serum, the reduction in phagocytic activity was only approximately 45%, suggesting a contribution of the burn serum to the impaired phagocytic activity of the alveolar macrophages. Furthermore, when control alveolar macrophages were incubated in media containing serum obtained from rats 4 or 24 h postburn, a reduction of approximately 25%, below control values, in their phagocytic activity was noted. Peritoneal macrophages obtained from the rats either 4 or 24 h after burn injury also demonstrated a significant impairment in their phagocytic activity, and serum from burned animals contributed to this reduction. However, a selectivity in the alteration induced by the burn serum is suggested since no alteration in phagocytic activity was noted when control peritoneal cells were incubated in media containing serum from the burned rats (Fig. 1).

In contrast to the profound impairment in

phagocytic activity which was demonstrated in the alveolar and peritoneal macrophages obtained from burned rats, their phagocytic capacity was significantly increased at 24 h but not 4 h after burn injury (Fig. 2). A contribution of serum factors to this enhanced phagocytic capacity was noted in peritoneal macrophages but not in alveolar macrophages.

At 4 h postburn no alteration in the intracellular microbicidal activity of either alveolar or peritoneal macrophages incubated in autologous or control serum was observed. In addition, serum obtained at 4 h after burn injury did not have any influence on the microbicidal activity of normal control cells. However, alveolar, but not peritoneal, macrophages obtained 24 h postburn manifested approximately a 90% increase over control responses in their microbicidal function. This enhancement was observed when the cells were incubated in media containing autologous serum obtained 24 h postburn or control serum. Also, control alveolar macrophages incubated in the serum obtained 24 h postburn had a 75% increase above control values in their intracellular killing ability (Fig. 3).

The alveolar and peritoneal macrophage cell yield obtained at 24 h postburn was consistently below the yield obtained from control animals; however, this difference was not statistically significant.

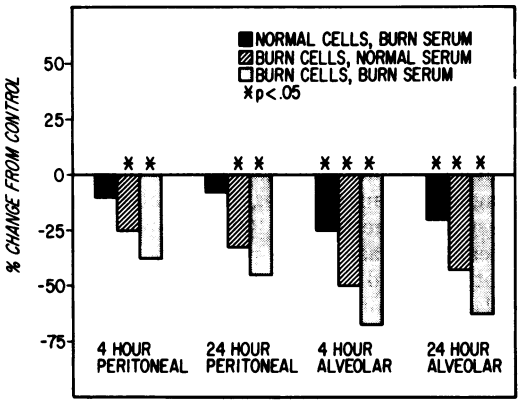

FIG. 1. *Phagocytic activity of peritoneal and alveolar macrophages 4 and 24 h after scald burn. The percentage of macrophages containing two or more yeast cells was used as the index of phagocytic activity. Macrophages were incubated with yeast at 1:3 ratio for 30 min. The yeast cells were opsonized in burn or control serum for 30 min at 37°C; final serum concentration in the media was 20%. Controls consisted of normal cells and control serum.*

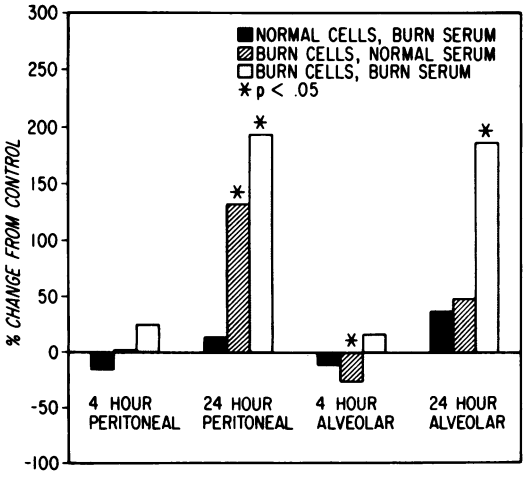

FIG. 2. *Phagocytic capacity of peritoneal and alveolar macrophages 4 and 24 h after scald burn. The total number of yeast cells ingested per 100 phagocytic cells was used as the index of phagocytic capacity.*

DISCUSSION

Although death from invasive burn wound sepsis has been significantly reduced with the introduction of topical antimicrobial therapy (25), systemic and pulmonary bacterial infection persists as the main cause of death in thermally injured patients (28). The infections are most commonly due to opportunistic organisms, e.g., *Pseudomonas aeruginosa, Klebsiella aerobacter,* or *Candida albicans* (26), and are further complicated by severe metabolic and endocrine alterations (J. Turinsky, L. D. Loose, and T. Saba, J. Burns, in press). A direct relationship between the surface area of the burn and susceptibility to infection has been shown (20).

The increased susceptibility to infection has been attributed to immunological changes which often accompany thermal injury. Immunological responsiveness after thermal injury has been

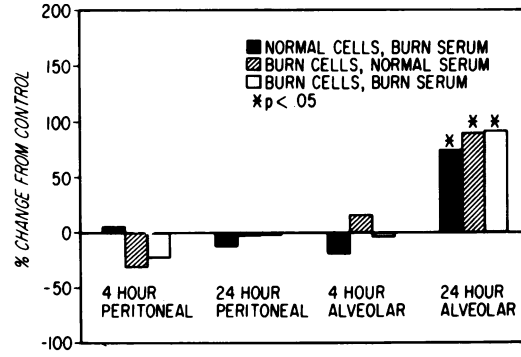

FIG. 3. *Microbicidal activity of peritoneal and alveolar macrophages 4 and 24 h after scald burn. Microbicidal activity was measured as the total number of dead yeast cells (those which stained positive with methylene blue) within the macrophages.*

characterized by a depressed reactivity to skin-test antigens, delayed graft rejection, defective in vitro lymphocyte-mediated responses, and diminished graft versus host reaction (24), but relatively normal in vivo antibody responses; an enhancement in antibody formation has been reported (29). Total hemolytic complement (CH_{50}) levels do not appear to be depressed after thermal injury (4, 8); however, a consumption of components of the classical and/or alternate pathways of complement activation has been shown (4). Associated with an impaired cell-mediated immune responsiveness in the burn patient is an apparent T-to-B cell shift in the peripheral blood lymphocyte population (7, 18, 38). Similar shifts in T and B lymphocyte numbers have not been observed in burned mice (24).

Although extensive studies regarding postburn neutrophil phagocytic and bactericidal activity (2, 3, 12, 14, 22, 25), chemotaxis (13, 36), oxygen consumption (16), and chemiluminescence (17) have been conducted, a similar evaluation of postburn macrophage function has not been done. Since macrophages are integral cell types in immune responses and in host defense, a determination of their role in the postburn sepsis is essential. This is especially important with regard to the alveolar macrophage, which is the principal cell type involved in pulmonary host defense and whose possible dysfunction may contribute to postburn pulmonary infections.

The results of the present study, which evaluated the in vitro phagocytic and microbicidal activity of pulmonary alveolar and peritoneal macrophages obtained 4 and 24 h postburn, suggest that the increased pulmonary susceptibility to infection may perhaps be due not to an impaired alveolar macrophage microbicidal activity but rather to an alteration in the phagocytic process mediated, in part, by serum components. At 24 h postburn the pulmonary alveolar macrophages demonstrated a significant increase in their microbicidal activity. An increase in the killing ability, and phagocytic activity, of postburn alveolar macrophages was also reported by Dressler and Skornik (9) in rats exposed to a scald burn of 20% of body surface area. Similar results were also observed in subsequent parabiotic studies (15). However, since penicillin and streptomycin were added to the bacteria-macrophage cultures and since these antibiotics have been demonstrated to enter macrophages (6), the contribution of the antibiotics to the observed increase in macrophage microbicidal activity cannot be determined. In the present study, the observed increase in killing ability that was demonstrated in alveolar macrophages at 24 h postburn was not observed in peritoneal macrophages at either 4 or 24 h postburn or in alveolar macrophages at 4 h after burn injury. Smith and Goldman (34), using peritoneal macrophages obtained from scald-burned mice 24 h postburn, also demonstrated no alteration in the ability of the cells to promote a change in the uptake of neutral red by phagocytized *C. albicans*.

In contrast to the enhanced microbicidal activity seen in alveolar macrophages obtained 24 h postburn, the phagocytic activity of peritoneal and alveolar macrophages was significantly depressed at 4 and 24 h postburn. Serum components contributed, in part, to the depressed phagocytic activity. Serum from burned animals suppressed alveolar but not peritoneal macrophage phagocytic activity.

In an evaluation of the postburn macrophage phagocytic capacity, i.e., the total number of yeast cells ingested per 100 macrophages, a significant increase in the phagocytic capacity of peritoneal and alveolar macrophages obtained at 24 h, but not 4 h, postburn was noted. Serum factors contributed to the enhanced phagocytic capacity of alveolar macrophages to a greater extent than with the peritoneal macrophages. No significant influence of burn serum on control alveolar or peritoneal macrophage phagocytic capacity was observed. Smith and Goldman (34) similarly did not observe any alteration in the phagocytic capacity of peritoneal macrophages obtained 6 days after scald-burn injury to mice.

The demonstration that "phagocytic capacity" of both cell types is increased at 24 h postburn and "phagocytic activity" is suppressed suggests the presence of two distinct cell populations. A small hyperphagocytic cell population could account for the enhanced phagocytic capacity but may be too small a population, numerically, to impact on the phagocytic activity. It is recognized that this distinction between phagocytic activity and capacity is arbitrary; however, these may be additional parameters which may be used to evaluate functional heterogeneity among macrophages (37). The two different populations may, perhaps, be two different states of maturation of the cells.

In addition to the differences observed between the two macrophage populations obtained from the thermally injured animals, the distinct influence of the burn serum on the cellular functions is of equal importance. At the present time, the identification of the serum factors influencing macrophage function cannot be ascertained. Previous investigators have postulated the presence of a burn toxin (32) or *P. aeruginosa* exotoxin (27, 30) as causal factors in postburn host defense alterations. More recently, Ninnemann

et al. (26) demonstrated that serum from burned patients suppressed phytohemagglutinin-induced blastogenesis in normal human lymphocytes. They were able to block this suppressive effect by using an immunoglobulin G fraction in postrecovery serum. However, alterations in opsonins (11) and endocrine parameters (Turinsky, Loose, and Saba, J. Burns, in press) may be of equal importance.

Further studies to delineate the mechanism of macrophage alterations after burn injury as well as identification of serum factors mediating these changes are essential. Also, the contribution of macrophage dysfunction to the observed alterations in cell-mediated immunity are important to further our understanding of postburn alterations in host defense.

ACKNOWLEDGMENT

This research was supported in part by Public Health Service grant GM-22825 from the National Institute of General Medical Sciences to J.T.

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