Pathophysiologic Glucocorticoid Elevations Promote Bacterial Translocation after Thermal Injury

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Thermal injury results in transient elevations of plasma glucocorticoids and promotes translocation of bacteria from the gut to the mesenteric lymph nodes (MLN) in rats. Translocated organisms are quickly cleared following uncomplicated thermal injury. However, subsequent burn wound infection, in temporal association with sustained pathophysiologic elevations of plasma corticosterone, results in the continued presence of enteric bacteria in the MLN. To study the role of sustained pathophysiologic steroid elevations in the mediation of this prolonged bacterial translocation, Wistar rats were randomly placed in groups receiving one of the following: (i) a 30% total body surface area scald injury with placement of a subcutaneous corticosterone pellet, (ii) a 30% total body surface area scald and a sham pellet implanatation, (iii) a sham burn and a corticosterone pellet implantation, or (iv) a sham burn and a sham pellet implantation. The animals were sacrificed on days 1 and 4 after injury, and cultures of the MLN, as well as the liver and spleen, were taken. Implantation of corticosterone pellets resulted in sustained elevations of plasma corticosterone compared with controls not receiving corticosterone pellets, similar to results seen in association with injury and infection. These pathophysiologic elevations were associated with the prolonged presence of organisms in the MLN (90% of burned rats with implanted corticosterone pellets versus 25% of rats with uncomplicated burns on postburn day 4; $P < 0.01$), but only in the presence of burn injury. Pathophysiologic glucocorticoid elevations did not lead to progression of translocation to the viscera or blood. Thus, the pathophysiologic glucocorticoid response contributes to the translocation of enteric bacteria and their prolonged presence in the MLN after systemic injury.

Thermal injury promotes the translocation of viable enteric bacteria from the gut to the mesenteric lymph nodes (MLN) in rats (8). Such bacterial translocation occurs only transiently after uncomplicated thermal injury, with translocated enteric organisms cleared from the MLN by day 4 postburn (5). However, burn wound infection complicating thermal injury is associated with prolonged bacterial translocation from the gut and failure to clear translocated organisms, as evidenced by the presence of enteric bacteria in the MLN beyond day 7 postburn (5).

Bacterial translocation after acute thermal injury appears to result from an ischemic insult to the intestinal barrier occurring secondary to a transient decrease in splanchnic blood flow (13). However, recent evidence suggests that the more chronic injury resulting from subsequent burn wound infection does not alter intestinal blood flow, implying that the prolonged bacterial translocation occurring during sepsis is mediated by pathophysiologic mechanisms other than ischemia (4a).

Burn wound sepsis in rats produces sustained and progressive pathophysiologic elevations of plasma glucocorticoids, up to 20-fold higher than baseline by postburn day 7, while an equivalent noninfected thermal injury produces no significant glucocorticoid response beyond the first 24 h (12). The purpose of the present study was therefore to determine the role of a sustained glucocorticoid response after thermal injury in the mediation of the prolonged bacterial translocation that we had previously observed following thermal injury complicated by burn wound infection. To this end, sustained glucocorticoid elevations were induced after thermal injury without infection in rats by administration of exogenous corticosterone.

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MATERIALS AND METHODS

Male Wistar rats (body weight, 291 ± 2 g) were randomly placed into four groups receiving one of the following: (i) a 30% total body surface area scald injury with placement of a corticosterone pellet as described below (Burn + Cort) (n = 19), (ii) a 30% total body surface area scald injury with sham pellet implantation (Burn) (n = 16), (iii) a sham burn with placement of a corticosterone pellet (Sham + Cort) (n = 19), or (iv) a sham burn with sham pellet implantation (Sham) (n = 16). Animals were housed in individual cages in a temperature-controlled room with alternating 12-h light and dark cycles and acclimated for a minimum of 4 days prior to study. Animals were allowed free access to water and standard laboratory chow, but as in our previous use of this model (5, 12), no additional fluid resuscitation was given.

Experimental burn injuries were induced by the method of Walker and Mason (15). Briefly, animals were anesthetized with intraperitoneal sodium pentobarbital (35 mg/kg of body weight). Shaved dorsal surfaces were then exposed to 95°C water for 10 s through a plastic template designed to produce a 30% body surface area full-thickness burn. Sham-burned controls underwent identical procedures with immersion in

21°C water. Animals were then allowed to fully recover from anesthesia before being returned to their cages.

Corticosterone pellets were prepared in advance by gently heating pure corticosterone (Sigma Chemical Co., St. Louis, Mo.) until it melted. One-hundred-milligram portions were then molded into pellets of approximately 6 to 7 mm in diameter and allowed to cool and solidify. Before recovery from anesthesia after the burn or sham burn injury, a 1-cm incision was made on the dorsal surface of the neck, and one 100-mg corticosterone pellet was implanted into a small subcutaneous pocket. The wounds were then closed with stainless steel clips. Rats receiving sham pellet implantation underwent an identical surgical procedure without placement of pellets.

This method of exogenous corticosterone administration by subcutaneous pellet implantation has previously been reported by Richardson et al. to produce reliable elevations of plasma corticosterone through postburn day 5 (R. P. Richardson, C. D. Rhyne, A. Kumar, H. de Riesthal, A. C. Antonacci, and S. E. Calvano, Abstr. Proc. Am. Burn Assoc., 21st Annu. Meet., p. 74, 1989; R. P. Richardson, M. A. Marano, Y. Fong, L. L. Moldawer, A. E. Barber, H. Wei, S. E. Calvano, S. F. Lowry, and A. C. Antonacci, Abstr. Proc. Am. Burn Assoc., 22nd Annu. Meet., p. 127, 1990). These authors have reported corticosterone levels in plasma of 24.1 μg/dl in sham-burned rats receiving corticosterone pellets versus 6.8 μg/dl in sham-burned rats without corticosterone pellets on postburn day 5, and levels of 18.9 and 5.4 μg/dl in burned rats with and without corticosterone pellets, respectively, on the same day ($P < 0.01$ for values in animals with corticosterone pellets versus the corresponding controls by analysis of variance.

On days 1 and 4 after injury, tail rein blood samples were obtained for plasma corticosterone determinations at 8 a.m., prior to any other manipulations. Plasma from tail vein blood samples was separated by centrifugation and stored at −70°C until study. Plasma corticosterone determinations were performed by a radioimmunoassay as described by Keith et al (6). Results were reported in micrograms per deciliter, with a lower limit of detection of <1 μg/dl.

Specimens of MLN, liver, spleen, blood, cecal contents, and burn wounds were obtained for culture for evidence of bacterial translocation after sacrifice by anesthetic overdose by modifying the methods of Maejima et al. (9) as follows to include quantitative burn wound cultures. Each rat was immediately placed on a sterile field, and its ventral surface was cleansed with 70% isopropyl alcohol. By using sterile instruments, the skin was opened and the peritoneum was also cleansed with alcohol. The peritoneal cavity was then entered, and 1.0 ml of caval blood (approximately 5% of the circulating blood volume), the entire MLN chain, and sections of liver and spleen were collected by sterile techniques; each specimen was placed in 5 ml of brain heart infusion broth. Weights of tissue specimens for culture were determined by subtraction of the weight of culture tubes and broth alone from the weight of the tubes and broth with tissue for each sample. Full-thickness burn wound biopsy specimens (1 cm²) were also collected for culture and placed in 5 ml of brain heart infusion broth, and their weights were determined as described above. All tissue specimens were homogenized in brain heart infusion broth with sterilized Teflon-coated grinding rods (catalog no. 08-414-14D; Fisher Scientific, Co., Pittsburgh, Pa.). Cecal contents were collected, with 0.5 ml placed in 9.5 ml of brain heart infusion broth, and serial dilutions were performed in sterile saline. Aliquots (200 μl each) from each broth culture were addi-

tionally plated on both tryptic soy agar with 5% sheep blood and MacConkey agar plates, allowing enumeration of the number of gram-negative CFU recovered per gram of tissue cultured. This method of reporting quantitative culture results as CFU per gram of tissue has previously been employed in similar studies of bacterial translocation by us and others (1, 5, 7–9). All cultures were incubated at 37°C and examined at 24 and 48 h for bacterial growth. The presence of any bacterial CFU constituted a positive qualitative culture in this study. Identification of bacterial species cultured was performed by standard microbiologic methods.

After the removal of specimens for culture, the entire small bowel from the ligament of Trietz to the ileocecal valve was excised, and any remaining mesentery was carefully dissected away. The bowel contents were removed by gentle saline irrigation, and the total small bowel weight was determined and normalized for body weight.

All data are reported as mean ± standard error of the mean (SEM). Means and standard errors of the means for quantitative MLN cultures were determined from only those animals with cultures positive for bacterial growth. Statistical analysis of comparisons between controls and study groups was performed by chi-square analysis with Yates correlation for qualitative culture results and by one-way analysis of variance with Newman-Keuls multiple range testing for body and organ weights, quantitative MLN culture results, log quantitative cecal culture results, and plasma corticosterone measurements (17).

RESULTS

The implantation of corticosterone pellets in uninfected burned rats produced significant elevations of plasma corticosterone (Table 1) similar to the pathophysiologic increases previously observed in burned and infected rats (12). Corticosterone elevations were sustained only in the rats with corticosterone pellet implantation by postburn day 4, when plasma levels remained five to six times greater than those seen in either burned or sham-burned rats not receiving pellets.

Body weight changes over the study period are shown in Fig. 1. Both sham and burn injuries were associated with mild loss of body weight by day 1 after injury, possibly because of temporary decreases in food intake following anesthesia. Although the burned rats implanted with corticosterone pellets had greater losses of body weight by day 1 than all other groups, the differences were not statistically significant. By day 4, however, the combination of burn injury and pathophysiologic corticosterone elevations was associated with further, significant ($P < 0.05$) weight loss compared with all other groups. This marked loss of body mass closely parallels that reported for animal models of burn wound sepsis (5, 12).

The results of cultures of the MLN are shown in Table 1. Burn injury was associated with a high incidence (75% in the Burn group and 100% in the Burn + Cort group; $P < 0.01$ versus the Sham group) of translocation of enteric bacteria to the MLN by postburn day 1. The translocating organisms were predominantly *Escherichia coli*, with rare *Klebsiella pneumoniae* and *Proteus mirabilis*. By day 4, only 25% of rats with uncomplicated burn injury had evidence of bacterial translocation. In contrast, 90% of the rats implanted with corticosterone pellets continued to have MLN positive for growth of enteric bacteria on day 4 ($P < 0.01$). Sham burns, either with or without corticosterone pellet implantation, failed to produce significant bacterial translocation on either

TABLE 1. Results of plasma corticosterone determinations and qualitative and quantitative cultures

Day	Group	Corticosterone concn (μg/dl)	No. of rats with bacteria in MLN/no. in group	No. of CFU/g of MLN[a]	No. of CFU (10^6/ml of cecal contents[b]
1	Sham	1.6 ± 0.6^c	0/8		2.8 ± 1.4
	Sham + Cort	30.0 ± 1.7	3/10	242 ± 192	1.9 ± 1.7
	Burn	14.3 ± 2.2	$6/8^d$	295 ± 188	8.4 ± 4.1
	Burn + Cort	22.4 ± 1.1	$9/9^e$	903 ± 316	7.5 ± 3.5
4	Sham	2.2 ± 0.7	0/8		6.5 ± 2.6
	Sham + Cort	13.5 ± 2.1^f	1/10	413	2.7 ± 2.0
	Burn	2.0 ± 0.9	2/8	$1,013 \pm 380$	6.9 ± 3.4
	Burn + Cort	9.6 ± 1.0^f	$9/10^g$	467 ± 137	6.1 ± 3.4

[a] Mean ± SEM for organisms recovered from animals with positive cultures.
[b] Mean ± SEM for facultatively anaerobic gram-negative bacteria.
[c] P <0.05 versus all groups on day 1 by analysis of variance and Newman-Keuls test.
[d] P <0.01 versus Sham on day 1 by chi-square and Yates tests.
[e] P <0.01 versus Sham and Sham + Cort on day 1 by chi-square and Yates tests.
[f] P <0.05 versus Sham and Burn on day 4 by analysis of variance and Newman-Keuls test.
[g] P <0.01 versus all groups on day 4 by chi-square and Yates tests.

day 1 or day 4 after injury. Neither burn injury alone nor burn injury with corticosterone pellet implantation was associated with progression of translocating organisms to the abdominal viscera or to the blood, as cultures of these tissues were consistently negative for bacterial growth.

Quantitative cultures of the cecal contents failed to demonstrate any significant differences in numbers of facultatively anaerobic gram-negative bacteria among groups (Table 1), suggesting that the increased incidence of bacterial translocation in Burn + Cort rats occurred without cecal bacterial overgrowth. No evidence of burn wound infection was demonstrated, as burn wound cultures resulted in only scant growth of predominantly gram-positve species. Further, no burn wound grew more than 1,000 organisms per gram of tissue, and no significant differences between the Burn and the Burn + Cort rats existed.

Acute burn injury was associated with significant (P < 0.01 for Burn and Burn + Cort versus Sham and Sham +

Cort, respectively) loss of small bowel mass on day 1 compared with sham burns (Fig. 2), and this loss of bowel mass did not seem to be affected by the presence of the corticosterone pellet. By day 4, burned rats without pellets demonstrated a return of small bowel mass to normal, while burned rats with pellets failed to show evidence of recovery (P < 0.05 for Burn + Cort versus Burn). Food intake levels were similar among all groups and therefore did not account for these findings.

DISCUSSION

It is now well established that thermal injury promotes the translocation of viable bacteria from the gut to the MLN in animal models (1, 5, 8, 9, 13). Such bacterial translocation occurs transiently, with enteric organisms cleared from the MLN by postburn day 4 (5). It has subsequently been shown that the superimposition of infection after thermal injury
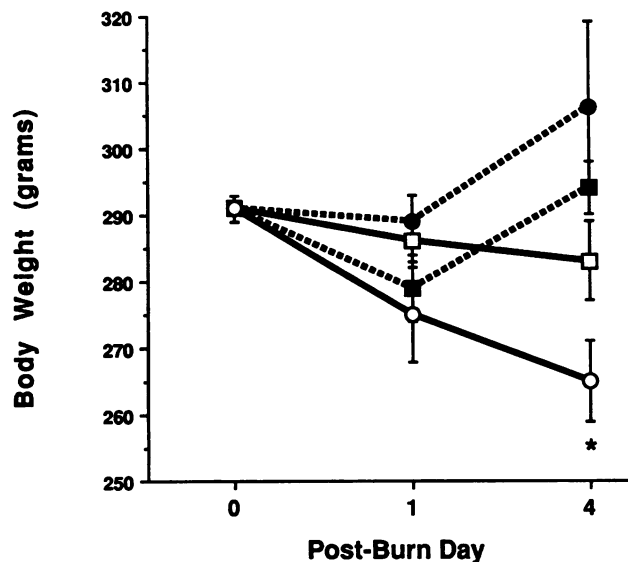

FIG. 1. Body weight by groups over time. Burned rats receiving corticosterone pellets (Burn + Cort) had significantly greater loss of body weight (*; P <0.05) than all other groups on postburn day 4. Symbols: ●, Sham; □, Sham + Cort; ■, Burn; ○, Burn + Cort. Error bars indicate SEM.
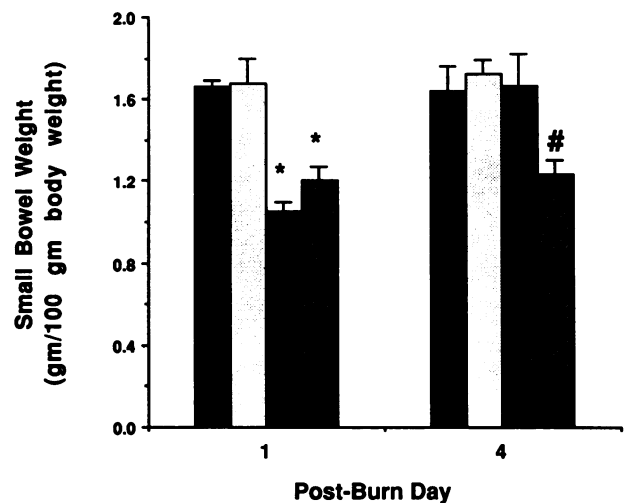

FIG. 2. Small bowel weight normalized for body weight by groups over time. Burned rats (Burn and Burn + Cort) had significant losses of bowel mass on postburn day 1, (*; P <0.01), compared with unburned groups (Sham and Sham + Cort). By day 4, burned rats receiving corticosterone pellets (Burn + Cort) continued to have significantly less small bowel mass (#; P <0.01) than all other groups. Symbols: ■, Sham; ▨, Sham + Cort; ▨, Burn; ▨, Burn + Cort. Error bars indicate SEM.

produces prolonged bacterial translocation, resulting in the continued presence of enteric organisms in the MLN through postburn day 7 and greater numbers of enteric organisms per gram of MLN tissue (5). Progression of enteric bacteria to the abdominal viscera and ultimately to the bloodstream is also seen during burn wound sepsis.

Bacterial translocation after thermal injury appears to be the result of ischemic changes within the intestine associated with decreased splanchnic blood blow (13) and activation of tissue xanthine oxidase (7). However, some evidence suggests that the continued translocation associated with subsequent infection after thermal injury may occur independently of intestinal ischemia (4a; W. G. Jones, A. E. Barber, J. P. Minei, T. J. Fahey, R. Inamdar, G. T. Shires III, and G. T. Shires, Abstr. Proc. Am. Burn Assoc., 22nd Annu. Meet., p. 34, 1990). Thus, bacterial translocation, at least in association with distant infection after injury, may be mediated by mechanisms other than ischemia.

A variety of stresses, including thermal injury and infection, induce a pathophysiologic glucocorticoid response, resulting in increased circulating levels of corticosteroids (3, 10). In rats, a 30 to 50% total body surface area full-thickness scald burn produces acute elevations in plasma corticosterone limited to day 1 after injury (S. e. Calvano, B. C. Organ, A. Kumar, and A. C. Antonacci, Abstr. Proc. Am. Burn Assoc., 19th Annu. Meet., p. 1, 1987). The superimposition of burn wound sepsis in this model, however, results in a sustained pathophysiologic glucocorticoid response lasting until the death of the animal, with circulating levels of corticosterone up to 20 times greater than baseline by postburn day 7 (12). Such increased systemic corticosteroids may alter host immunocompetence by suppressing inflammatory responses (11) and by altering the numbers, subsets, and distributions of circulating and tissue-bound lymphocytes (2).

Despite these far-reaching effects, the role of pathophysiologic elevations of steroid levels in bacterial translocation remains unclear. Pharmacologic dosages of the corticosteroid prednisone (dosages similar to those used in the chemotherapy of certain malignancies) have been demonstrated to produce bacterial translocation in rat models (1). Although such studies are important in understanding the potential for bacterial translocation after chemotherapy, they do not emulate the corticosteroid response that occurs after trauma. These pharmacologic dosages may produce supraphysiologic circulating corticosteroid levels many times greater than the pathophysiologic levels that result from even severe injury and injection. Additionally, in the rat the active physiologic corticosteroid is corticosterone, making extrapolation of the results of pharmacologic manipulations with cortisol analogs to the posttrauma setting even more difficult. Thus, the purpose of the present study was to elucidate the relationship of pathophysiologic levels of corticosterone in the rat to bacterial translocation in order to determine if the prolonged presence of enteric organisms in the MLN following the combination of injury and infection might be mediated by a sustained glucocorticoid response.

Previous experimental work has demonstrated that implantation of corticosterone pellets in the subcutaneous tissues of rats reliably produces elevations in plasma corticosterone that are sustained for up to 5 days (Richardson et al., Abstr. Proc. Am. Burn Assoc. 1989; Richardson et al., Abstr. Proc. Am. Burn Assoc. 1990). The levels of plasma corticosterone achieved by pellet implantation in the present study were up to 15 times greater than baseline levels in unstressed rats on day 1 after placement and remained

approximately 5 times greater than baseline levels by day 4. Such elevations are similar to the pathophysiologic elevations seen in the same strain of rats after thermal injury and infection (12). Burn + Cort rats had slightly lower plasma corticosterone levels on both days 1 and 4 compared with the Sham + Cort controls; this difference may be secondary to vasoconstriction and reduced subcutaneous blood flow after the burn injury.

A 30% scald burn produced bacterial translocation to the MLN in 15 of 17 rats on day 1 after injury (Table 1), with no significant differences noted in translocation frequencies (100 versus 75%), or the numbers of organisms per gram of MLN tissue (903 ± 316 for Burn + Cort and 295 ± 188 for Burn). Translocating organisms were exclusively facultatively anaerobic gram-negative bacteria (E. coli, P. mirabilis, and K. pneumoniae), similar to observations in other reports (1, 5, 8, 9). By day 4, plasma corticosterone levels in burned rats not receiving corticosterone pellets had returned to normal and culture of the MLN resulted in growth of bacteria in only 2 of 8 rats. In contrast, 9 of 10 Burn + Cort rats continued to have positive MLN cultures on day 4 in association with sustained pathophysiologic levels of plasma corticosterone. However, despite this high rate of positive MLN cultures, the abdominal viscera and blood in these rats remained sterile, suggesting that some mechanism other than corticosterone elevation is responsible for systemic spread of translocating organisms.

Deitch and Berg (4) noted that neither acute nor chronic stress in the absence of tissue injury promotes bacterial translocation. This observation is supported by the present study, as implantation of corticosterone pellets without a scald burn resulted in elevation of plasma corticosterone levels in the pathophysiologic range but failed to result in bacterial translocation to the MLN.

The continued presence of enteric organisms in the MLN of Burn + Cort rats on day 4 may result from either ongoing translocation of bacteria from the gut, the failure of host defenses to kill previously translocated organisms, or both. The presence of elevated plasma corticosterone was associated with failure of recovery of small bowel mass after burn injury (Fig. 2), similar to that reported for burned and infected rats (5). This prolonged intestinal atrophy may prevent restoration of the gut mucosal barrier, resulting in continued bacterial translocation.

Recently, thermal injury in rats was demonstrated to result in increased numbers of lymphocytes in the lymph nodes draining the burn wound, while total lymphocyte numbers in the MLN and other lymphoid compartments significantly decreased (14). It may be postulated that infection of the burn wound may lead to further recruitment of lymphocytes from compartments distant from the wound, including the MLN. Such an effect may be mediated in part by the pathophysiologic glucocorticoid response to injury and infection. Depletion of mesenteric nodal lymphocytes may then be associated with a concomitant attenuation of the ability of the MLN to contain and control bacteria translocating from the gastrointestinal tract through inadequate antibody production, bacterial opsonization, and impaired antigen processing and presentation. Depletion or dysfunction of macrophages in the bowel wall and mesenteric lymph nodes may also occur in association with pathophysiologic elevations of glucocorticoids after injury. Such a failure of MLN function is consistent with the mechanisms of translocation of intestinal bacteria recently proposed by Wells et al. (16).

Thus, the pathophysiologic increases in plasma corticos-

terone seen after the combination of thermal injury and infection in rats can be reproduced by the implantation of corticosterone pellets. Such pathophysiologic elevations of plasma corticosterone in the presence of injury appear to promote sustained bacterial translocation to the MLN but do not lead to increased translocation to the viscera or blood. After injury, therefore, the pathophysiologic glucocorticoid response may contribute to bacterial translocation, but other mechanisms appear to be necessary for the systemic spread of translocating organisms.

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