Response of the Opsonic and Phagocytic System to Trauma in Adrenalectomized Animals[1] (37284)

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Host defense alterations following surgical trauma have been repeatedly demonstrated (1–3). In this regard, surgical trauma impairs resistance to both bacterial infection and tumor growth (1–3). While the factors mediating this response following trauma remain to be delineated, failure of the reticuloendothelial system (RES), a host defense mechanism (4–6) as studied after celiotomy (3), abdominal surgery (7, 8), extracorporeal circulation (9), cutaneous burn (10), and skin transplantation (11), suggest macrophage dysfunction as a potential pathway in the genesis of impaired host defense following trauma.

RE function, especially intravascular phagocytosis, is intimately related to the presence of specific plasma proteins called "opsonins" whose interaction with a foreign or denatured surface appears to be a determinant of active phagocytosis (12, 13). This humoral control on intravascular phagocytosis is fundamental to the RE depression following surgical or burn trauma in which a depletion of plasma opsonic activity exists (3, 12, 14). Furthermore, restoration of the hepatic RE clearance mechanism is related to a recovery of opsonin levels (3, 14).

A rapid elevation of plasma 17-hydroxycorticosteroids exists during and following trauma (15). Furthermore, excessive steroid administration will induce a state of RE depression (16). While the basis for phagocytic depression following steroid treatment is not known, it appears to be related to the initial attachment or adsorption phase of phagocytosis (16), an event which is also conditioned by opsonic protein interaction (12). These findings suggest that a functional relationship may exist between adrenal activation and opsonin depletion following trauma and the response of the RES. In an attempt to evaluate this concept, the response of the phagocytic and the opsonic system to surgical trauma in normal and bilaterally adrenalectomized animals was evaluated.

Methods. Male Holtzman rats (175–250 g) maintained on Rockland Lab-Tek chow and tap water *ad libitum* were used. Adrenalectomy was performed through sterile 2.5 cm bilateral flank incisions under light ether anesthesia, and sham-adrenalectomized control animals were treated in an identical fashion with bilateral exposure of the adrenal glands. Postoperatively, the adrenalectomized, sham-adrenalectomized, and control nonoperated rats were maintained with food *ad libitum* and 0.9% NaCl as the drinking fluid for 24 hr prior to experimental evaluation of their opsonic and phagocytic response to a subsequent "standard surgical trauma" procedure.

The standard surgical trauma procedure executed under light ether anesthesia consisted of a sterile 5 cm midline laparotomy coupled with a 1.5 cm incision of the upper small intestine followed by immediate closure of both the enterotomy and abdominal incision. This surgical trauma procedure has been previously utilized (3), and all animals were mobile within 5–10 min following surgery.

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The intravascular phagocytic capacity of the RES was evaluated by means of the colloid clearance technique (3, 8, 12) utilizing a gelatinized radioiodinated lipid emulsion which is removed from the vascular compartment by fixed RE cells especially the hepatic Kupffer cells. Its rate of disappearance from the circulation has been used in animal and human studies as a measure of intravascular phagocytosis (12). The lipid emulsion having a specific activity of 0.01 μCi/mg lipid base was injected intravenously at a dose of 50 mg/100 g body weight. Following injection, the level of colloid retention in the blood was determined by radioassay with a Nuclear-Chicago Auto-Gamma system as previously described (12, 22). Blood colloid concentration levels were plotted semilogarithmically against time (min) and the global phagocytic index (K) was determined (12) from the expression:

$$K = \frac{\log C_1 - \log C_2}{T_2 - T_1};$$

where C_1 and C_2 represent the blood concentration at time T_1 and T_2, respectively (17). In order to clearly delineate the functional state of the RES at the 24 hr post-adrenalectomy point prior to the standard surgical trauma, the liver, lung and spleen were taken 15 min following colloid injection and analyzed by radioassay for tissue localization of the test colloid (3, 8). Tissue uptake of the colloid in relationship to the injected dose (ID) was calculated on both a per gram (% ID/g) and total organ (% ID/TO) basis.

The plasma opsonic activity was determined before and after the standard surgical trauma using an *in vitro* tissue slice bioassay which has been used to evaluate the opsonic activity in both animal and human studies (3, 12–14). In this technique, the opsonic activity of plasma is evaluated in terms of its ability to support Kupffer cell phagocytosis utilizing an *in vitro* assay system composed of a 200–400 mg liver slice, 1.0 ml test plasma, 2.0 ml of Krebs–Ringer phosphate (pH = 7.4), 100 USP units of heparin and a 2000 μg dose of gelatinized (0.1%) ^{131}I test lipid emulsion (13, 14). All samples were incubated with oscillation for 30 min under a gas phase of 95% O_2 and 5% CO_2, and uptake was expressed as the percentage of the 2000 μg particle dose (ID) phagocytized/100 mg wet liver tissue weight (% ID/100 mg).

Means and standard errors were calculated for all experimental groups, and statistical significance was determined by the t test with placement of the confidence level of 95%.

Results. Presented in Table I, is the response of the reticuloendothelial system in terms of phagocytic activity by major RE cell containing organs (liver, lung, spleen) 24 hr after adrenalectomy or sham-adrenalectomy as compared to nonoperated controls. As shown, there is a definite hyperactivity of the macrophage system at the tissue level in terms of colloid uptake, in both the sham-adrenalectomized and adrenalectomized animals. The liver was the major critical compartment reflecting this increased uptake capacity, with a 43 and 50% stimulation of phagocytosis on a per gram basis, respectively, in the sham-adrenalectomized and adrenalectomized animals. In terms of total organ localization, the livers in the sham-adrenalectomized and adrenalectomized rats removed, respectively, an additional 13.7 and 22.6% of the injected dose (ID) in excess of that cleared by the normal controls at 15 min postinjection (66.28%). Total liver uptake in the adrenalectomizeds rats was 11% greater than that manifested by the sham-adrenalectomized. As would be expected on the basis of previous observations (12), the profound stimulation of hepatic clearance activity in the adrenalectomized rats was reflected in a significant ($p < 0.05$) decrease in extrahepatic uptake, *i.e.*, lung and spleen.

Having defined the basal levels of *in vivo* tissue uptake activity in the three experimental models (nonoperated, sham-adrenalectomized, adrenalectomized), studies were then performed to ascertain the response of the RES to the standard surgical trauma procedure in the absence of the adrenals. Since a maximum RE depression exists at 60 min posttrauma in the normal animal (3),

TABLE I. Effect of Sham-Adrenalectomy and Adrenalectomy on Hepatic, Splenic and Pulmonary Phagocytosis of Intravenously Injected Test Colloid.

Exptl[a] parameter	Nonoperated[b] controls (9)	Sham-adrenalectomized[b] animals (6)	Adrenalectomized[b] animals (6)
Liver			
% ID/g	7.97 ± 0.90	11.40 ± 0.70	12.02 ± 0.80
% ID/TO	66.28 ± 4.42	79.95 ± 0.46	88.90 ± 2.26
Lung			
% ID/g	1.66 ± 0.78	2.10 ± 0.61	0.84 ± 0.42
% ID/TO	2.12 ± 0.99	2.29 ± 0.67	1.07 ± 0.48
Spleen			
% ID/g	6.75 ± 0.76	5.64 ± 0.55	2.76 ± 0.36
% ID/TO	4.86 ± 0.74	3.41 ± 0.46	1.91 ± 0.11

[a] Tissue distribution was evaluated at 15 min postinjection utilizing a 50 mg/100 g dose of the lipid emulsion. Data are expressed as percentage of the injected dose phagocytized per gram (% ID/g) and per total organ (% ID/TO).

[b] Means ± SE are presented with the number of experiments in parentheses. Sham-operated and adrenalectomized rats were tested at 24 hr postsurgery.

this time interval was selected for the response end-point. Presented in Fig. 1, is the RE clearance activity expressed as the global phagocytic index K in animals prior to the standard surgical trauma (24 hr point), and at 60 min after the surgical trauma procedure (25 hr point). Adrenalectomy as well as sham-adrenalectomy resulted in an enhanced phagocytic activity by the RES. This was especially evident in the bilaterally adrenalectomized rats at 24 hr which revealed a clearance half-time ($t/2$) of 3.18 ± 0.36 min compared to a mean 10.13 ± 1.66 min $t/2$ in normal, nonoperated controls. As shown, by 60 min postsurgical trauma all experimental models manifested a subsequent RES failure response to trauma. In this regard, there was a significant ($p < 0.05$) 40.5, 20.4, and 51.9% decrease in the K value in the normal, sham-adrenalectomized, and adrenalectomized rats, respectively. These findings suggest that the presence of the adrenals is not a prerequisite to the early RE depression response to trauma.

Since previous data (3, 14) indicate that the early posttrauma RE depression response is mediated by a fall in the circulating opsonin activity, and since a hepatic hyperphagocytosis can be induced by elevation of the opsonin levels (12), studies were then performed to determine the role of

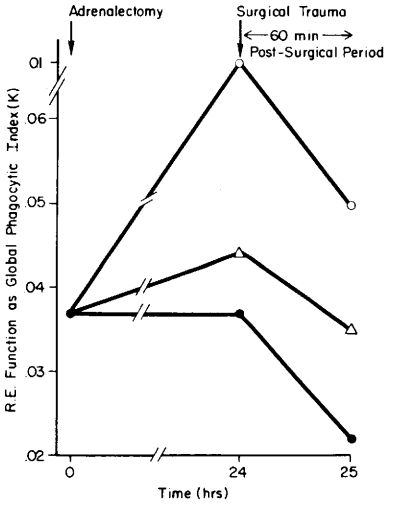

FIG. 1. Effect of standard surgical trauma (laparotomy plus intestinal surgery) on reticuloendothelial function expressed as the global phagocytic index K in previously nonoperated (●), sham-adrenalectomized (△) and adrenalectomized (○) rats. Each point represents the mean of 6–9 experiments. A 60 min postsurgical trauma end point (25 hr) was studied. In all three experimental models the 60 min posttrauma K value (25 hr) was significantly ($p < 0.05$) lower than pretrauma levels (24 hr).

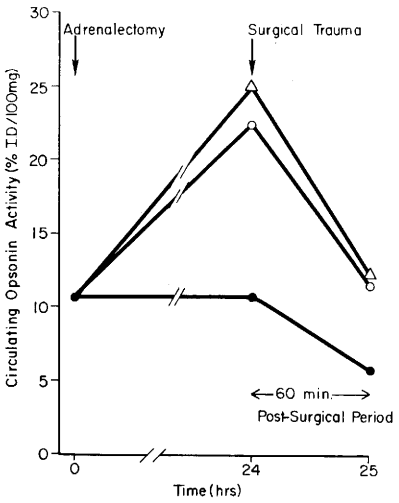

FIG. 2. Effect of standard surgical trauma (laparotomy plus intestinal surgery) on circulating opsonin levels in previously nonoperated (●), sham-adrenalectomized (△), and adrenalectomized (○) rats. Each point represents the mean of approximately 13 determinations. In all three experimental models, the 60 min posttrauma circulating opsonin level (25 hr) was significantly ($p < 0.001$) decreased as compared to pretrauma levels (24 hr).

plasma opsonin activity alterations in the observed RE functional changes. Presented in Fig. 2, is the circulating plasma opsonic activity following adrenalectomy and prior to the standard surgical trauma (24 hr point) as well as at 60 min posttrauma (25 hr point). As shown, the RE hyperactivity in both the sham-adrenalectomized and adrenalectomized rats, respectively, is related to a highly significant ($p < 0.001$) elevation of the opsonin levels at this time which exceeds 100% in both groups. However, by 60 min post-standard surgical trauma, there was a rapid decrease in the circulating opsonic activity in all groups. The similarity of the magnitude of this opsonin depletion response in the early 60 min trauma period was supported by the fact that the percentage depletion was 46.2, 51.1, and 48.2% in the controls, sham-operated, and adrenalectomized animals, respectively. The time course and magnitude similarity of the opsonic system response in all groups suggests that the posttrauma hypoopsonemic state is not mediated via an adrenal response to the applied stress. However, the profound RE stimulation in the adrenalectomized rats at 24 hr (Fig. 1) to a level greater than would be expected on the basis of the opsonin changes (Fig. 2) suggests an associated RE cellular alteration in the absence of the adrenals.

Discussion. The reticuloendothelial system (RES) is an important physiologic mechanism of systemic host defense (5, 10, 12). Recent attempts to define the physiologic parameters that modulate the systemic clearance capacity of fixed RE cells have provided abundant evidence to focus on the role of plasma opsonin activity as a critical determinant of intravascular phagocytosis especially with reference to the hepatic Kupffer cell clearance mechanism (12).

Reticuloendothelial failure following trauma has been reported in both animal and human models (3, 7–10, 14). In this regard, a state of RE failure develops following abdominal surgical trauma (3, 18), burn injury (10, 19), mechanical trauma (17), and skin transplantation (11). While the measured macrophage response is conditioned by the duration of injury, experimental model, and test colloid utilized, a typical pattern emerges from the majority of findings. This pattern is one of an early transient profound depression followed by a recovery and phase of hyperphagocytosis by 24–48 hr in close relationship with the changes in the circulating opsonin activity (3).

The effect of adrenal cortical steroid administration on RE function has been extensively studied (16, 20, 21). While these findings are, in part, contradictory, the bulk of evidence suggests that low steroid doses will stimulate and large pharmacological doses will induce a functional RE depression. Wiener *et al.* (16), demonstrated failure of particle attachment to Kupffer cells following administration of cortisone acetate, and Schildt and Low (17) reported that administration of cortisone or ACTH in amounts to simulate posttrauma plasma levels exerted a depressing effect on phagocytosis. While these findings suggest the possibility of an adrenal response to trauma as a factor mediating the early RE depression, the

present findings, demonstrating opsonic deficiency and RE failure following trauma in both the sham-adrenalectomized and bilaterally adrenalectomized animals, imply that the presence of the adrenal is not a prerequisite for this response. However, the present data do not rule out the potential direct role of ACTH on either the humoral (opsonin) or cellular (macrohpage) components of the system.

Enhanced intravascular phagocytosis in both the sham-adrenalectomized and adrenalectomized rats at 24 hr postoperative preparation supports recent findings on the pattern of opsonic and phagocytic activity over a 4 day postoperative period (3). In this regard, an early depression followed by RE hyperactivity at 24 hr is consistently observed (3, 8, 14). However, hepatic hyperphagocytosis in the adrenalectomized rats at 24 hr (Fig. 1) was in excess of that anticipated on the basis of the opsonin levels (Fig. 2). It may be due to an increased sensitivity of the Kupffer cells to an opsonic stimulus upon withdrawal of the continual steroid influence. In this sense, steroids may play a "permissive" role in the regulation of the RES with their presence serving as a "physiologic" brake on excessive RE hyperactivity.

Reticuloendothelial depression after trauma has important clinical implications. The central role of opsonic protein depletion in this response coupled with recent isolation and purification of this factor which is an alpha-2-globulin (22) should provide a mechanism to circumvent this response via opsonin administration during surgery. While the basis for opsonin depletion after trauma remains to be found, the depletion of opsonic protein upon systemic entrance of foreign particles as well as the interaction of opsonic protein with effete tissue (12) suggests that opsonic protein(s) may be either sequestered at the site of injury or utilized in the vascular clearance of denatured proteins and damaged tissue.

Summary. Adrenalectomy and sham-adrenalectomy resulted in a significant stimulation of the reticuloendothelial system (RES) at the 24 hr postoperative period. This response was primarily reflected in an enhanced vascular clearance of test colloids and an associated hepatic hyperphagocytosis. The physiological factor apparently mediating this response was the presence of a "hyperopsonemia" with an associated alteration of Kupffer cell function mediated at the cellular level in the adrenalectomized animals. Evaluation of the physiologic response of both the opsonic system and RE system to surgical trauma in the bilaterally adrenalectomized rats revealed that the "hypoopsonemia" and RE failure response during the early posttrauma interval (60 min) was not dependent on the presence of the adrenal glands. However, the data does support the concept that the opsonic or humoral deficit is related to the posttrauma RE depression. It is suggested that the hyperfunctional state of the hepatic RE system resulting from the experimental withdrawal of steroids by adrenalectomy may indicate that steroids have a regulatory role in RE function. Thus, host defense failure following surgical trauma may be related to depression of the opsonic and RE system, a response that does not necessitate the presence of the adrenals.

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