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α-2-Glycoprotein Opsonic Deficiency after Trauma[1] (39865)

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Depression of the phagocytic clearance activity of the reticuloendothelial system (RES) has been observed following traumatic shock and correlated with failure to survive the shock episode (1-3). Recent studies have suggested that this hepatic Kupffer cell clearance dysfunction is mediated, in part, by a depletion of an α-2-glycoprotein which has opsonic activity (3). Levels of this α-2-globulin opsonic protein appear to be intimately related to RES phagocytic function under both physiological and pathological conditions with respect to the removal of circulating foreign colloidal and denatured particulate matter (4-7). While previous studies have clearly delineated a deficiency in plasma opsonic activity following trauma (3, 6), such studies have been lacking in identifying whether or not an inhibitory factor exists in the post-trauma period which may be exerting a suppressing influence on opsonic activity. In addition, a clear identification of the comparative cellular (Kupffer) versus humoral (opsonin) involvement in the RE deficit has not been investigated, especially as it is influenced by varying levels of traumatic shock. The present study was designed to test the hypothesis that the post-traumatic RE depression is a reflection of a humoral opsonin deficit in the phagocytic promoting capacity of plasma rather than an intrinsic cellular deficit as expressed by the hepatic Kupffer cells. In addition, the potential participation of an RES-inhibitory substance, as well as acidosis and hypoxia in the post-trauma period, was also studied. Thus, the present investigation compared *in vitro* hepatic Kupffer cell phagocytosis by liver slices obtained from normal rats and rats following trauma at a time of RE depression as influenced by plasma from normal rats or post-trauma rats. The time interval selected was 60 min following graded levels of trauma, an interval previously associated with significant RE depression (2, 3). The potential inhibitory effect of post-trauma plasma was evaluated on both *in vivo* and *in vitro* RE activity and the effect of acidosis and hypoxia on hepatic phagocytosis was evaluated *in vitro*.

Materials and methods. Nonfasted male Sprague-Dawley rats weighing 200-300 g were used. The trauma model utilized was graded Noble-Collip drum trauma (8) at 40 rpm under sodium pentobarbital (2 mg/100 g) anesthesia (2). This model was approximately nonlethal (<5% mortality) with 300 revolutions and lethal (>95% mortality within 24 hr) with 700 revolutions, with an LD_{50} of approximately 500 revolutions (3).

In vitro hepatic Kupffer cell phagocytosis was evaluated with a previously described tissue-slice technique (9). Liver slices prepared with a Stadie-Riggs tissue slicer were incubated with 2000 μg of gelatinized ^{131}I-labeled RE test lipid emulsion, which is actively phagocytized by Kupffer cells in 3 ml of heparinized (100 USP units) media containing 1 ml of plasma (unless otherwise specified) and 2 ml of Krebs-Ringer phosphate solution (pH 7.4) under a gas phase of 95% O_2 and 5% CO_2. Following 30 min of incubation at 37° with oscillation, the liver slices were removed, washed in isotonic saline, and analyzed isotopically for particle uptake. Kupffer cell phagocytosis was expressed in terms of micrograms uptake of test colloid per 100 mg of liver tissue (μg/100 mg). In experiments to evaluate the effects of hypoxia and/or acidosis on hepatic

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phagocytosis, the gas phase was air and 5% CO_2 and the pH was reduced to 7.1. This gas phase represents extreme hypoxia despite near normal pO_2 due to the lack of oxygen-carrying capacity in the media (i.e., no hemoglobin).

In vivo RE function was evaluated by the colloid clearance technique (10). Rats were injected intravenously with the radioiodinated test lipid emulsion (50 mg/100 g) and the rate of phagocytosis of the test colloid from the blood was monitored by isotopic analysis of five serial 0.1-ml aliquots of whole blood obtained from the tail at 2-min intervals. The global phagocytic index (K) was determined as previously described (3, 10) from the best-fit exponential clearance curve determined with a digital computer. At 15 min following colloid injection, the liver was excised, washed, and analyzed for phagocytic uptake.

The test lipid emulsion was prepared as an anhydrous base with glycerol, [^{131}I]-glyceryl trioleate, and lecithin homogenized in a blender in a 10:10:1 ratio by weight. For *in vivo* use, a 10% suspension of the anhydrous lipid base was prepared using a 0.3% gelatin-supplemented 5% dextrose and water solution (pH 7.4) as the diluent. For *in vitro* use, a 1% suspension in a 0.1% gelatin-supplemented 5% dextrose and water (pH 7.4) solution was used (9).

Results. The *in vitro* assay system was utilized employing various combinations of control plasma (nontrauma) and post-trauma plasma with liver slices obtained from normal animals and liver slices obtained from animals at 60 min following trauma. This allowed for the comparative evaluation of the relative contribution of humoral and cellular factors in the RE depression (Fig. 1). As can be seen in Fig. 1, at all levels of trauma both the normal and post-trauma hepatic RE cells are capable of normal phagocytic uptake when incubated in normal plasma but not in plasma obtained from the shock animals. Phagocytosis in "shock plasma" is significantly ($P < 0.01$) depressed following 200 revolutions and maximally depressed by 89% after 700 revolutions. These data suggest that plasma obtained from RE-depressed rats following traumatic shock is deficient in ability to support phagocytosis by hepatic Kupffer cells.

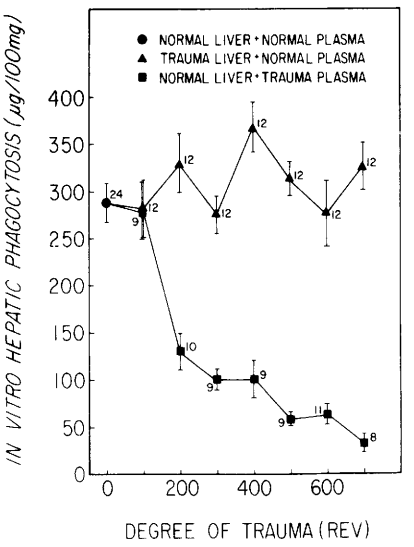

FIG. 1. Comparative humoral and cellular aspects of Kupffer cell phagocytosis following graded trauma. NCD trauma was at 40 rpm under sodium pentobarbital (2 mg/100 g) anesthesia. Hepatic phagocytic uptake following 30 min of incubation is expressed as micrograms of the initial 2000-μg dose phagocytized per 100 mg liver-slice weight. Mean ± standard error of the mean is presented with the number of incubation samples indicated in parentheses. ●, Normal liver slices and normal plasma; ▲——▲, liver slices removed after various levels of graded trauma (100–700 rev) incubated in normal plasma; ■——■, plasma obtained after various levels of graded trauma (100–700 rev) used as a medium for normal liver slices.

Since the humoral nature of RE depression observed in the results above does not distinguish between lack of a phagocytosis-supporting substance or the presence of a phagocytosis-depressing factor, studies were designed to determine the existence of a passively transferable RES-depressing factor during the post-trauma period in association with RE depression. Donor plasma was obtained from nontraumatized rats, and from rats receiving 300 and 500 revolutions of trauma, 60 min following traumatization. The plasma was injected at a dose of 1 ml/100 g into nontraumatized recipients which were evaluated 15-min post-treatment (passive plasma transfer) in terms of RE clearance and hepatic colloid uptake capacity. Additional experiments were performed in which the effect of adding plasma obtained

from traumatized animals (300 revolutions) to normal plasma *in vitro* was evaluated in order to provide both an *in vitro* and *in vivo* test for the existence of an RE-depressant factor.

As seen in Table 1, passive transfer of "trauma plasma" into nontraumatized animals had no significant effect as compared to normal plasma with respect to influencing RE function evaluated as global phagocytic index (K), or hepatic localization of colloid.

The effect of trauma plasma on the phagocytosis-supporting capacity of various concentrations of normal plasma is shown in Fig. 2. The phagocytic activity increases with increasing volume of normal plasma, with a significant correlation ($P < 0.01, r = 0.96$) to the regression line described by the formula: $Y = 3.81\ X + 5$, where Y is opsonic activity as micrograms per 100 mg and X equals precentage of normal plasma in the incubation media. Similarly, when increasing concentrations of normal plasma are added to the media already containing 1 ml of plasma obtained from animals 60 min after sublethal trauma (300 revolutions), the total phagocytic-supporting capacity again increases, with a highly significant ($P < 0.01, r = 0.98$) linear correlation to the regression line described by $Y = 3.87\ X + 107$. The nearly identical slopes of these two regression lines (3.87 versus 3.81) clearly indicate that the ability of normal plasma to support *in vitro* hepatic phagocytosis, as well as the ability of hepatic phagocytes to respond to the phagocytic stimulus of normal plasma, is identical in the presence or absence of "trauma plasma." These data do not support the existence of a humoral phagocytic-depressing substance.

An additional experiment was performed to evaluate the effect of adding increasing concentrations of trauma plasma to a constant volume of normal plasma (Table 2). It is observed that the addition of 0.5, 1, 1.5, and 2.0 ml of trauma plasma did not alter the basal level of phagocytic stimulation afforded by 1.0 ml of normal plasma. Regression analysis revealed no dependence whatsoever ($P > 0.9, r = 0.07$) of the level of

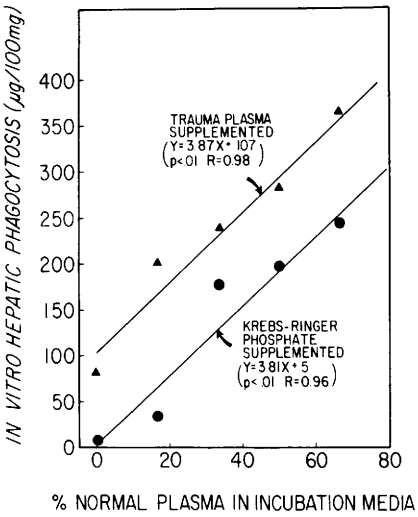

FIG. 2. Absence of an RE depressant factor post-trauma as detected by effect of trauma plasma on *in vitro* hepatic phagocytosis in the presence of normal plasma. Values are micrograms of the initial 2000-μg dose phagocytized per 100 mg liver-slice weight. Trauma plasma was obtained from rats 60 min after 300 revolutions of Noble-Collip drum trauma and added at a concentration of 33.3% in indicated "trauma plasma-treated" group. Control assays were supplemented with equal volumes of Krebs-Ringer phosphate. Each line consists of 15 experimental determinations, with three samples per point.

TABLE I. PHAGOCYTIC INDEX[a] AND *in Vivo* HEPATIC PHAGOCYTOSIS[b] IN NONTRAUMATIZED RATS FOLLOWING PASSIVE TRANSFER OF PLASMA FROM NORMAL OR TRAUMATIZED RATS.[c]

Source of plasma for transfer	Phagocytic index (K)	Hepatic colloid uptake	
		% ID/g	% ID/TO
Normal	0.096 ± 0.003	4.85 ± 0.32	51.08 ± 2.32
Post-trauma (300 rev)	0.099 ± 0.005	5.29 ± 0.29	51.95 ± 2.17
Post-trauma (500 rev)	0.099 ± 0.005	4.66 ± 0.38	54.11 ± 2.77

[a] K was determined from slope of clearance curve of 50 mg/100 g of gelatinized RE test lipid emulsion.

[b] Hepatic uptake is expressed as percentage of the injected dose (% ID) phagocytized 15 min following systemic injection of test particles on both a per gram (g) and per total organ (TO) basis. Means ± standard error of the mean are presented.

[c] A total of four to six rats was in each recipient group, and test plasma was pooled in equal-sized groups either prior to (normal) or 60 min post-traumatic shock. RE function was evaluated at 15 min post-plasma transfer (IV).

phagocytic activity on the volume of trauma plasma in the incubation media. These data similarly do not support the existence of a humoral phagocytic-depressing substance in the plasma of the post-trauma animals.

As can be seen in Table 3, conditions of hypoxia and/or acidosis, at levels designed to stimulate or surpass the conditions in the post-trauma hepatic circulation, had no influence on the level of *in vitro* hepatic phagocytosis. The replacement of 95% O_2–5% CO_2 with air–5% CO_2 or the altering of the pH from 7.4 to 7.1, alone or in combination, did not alter the phagocytic activity in either plasma or Krebs–Ringer phosphate solution.

Discussion. Previous findings have documented the involvement of the reticuloendothelial system in the ability to survive severe stress such as trauma and circulatory shock (1–3, 6, 11, 12). RE function deteriorates after trauma and there is a positive correlation between host survival and the functional state of this cellular system (1–3). Since a major activity of the RES is the clearance of blood-borne particulate material, and since circulating levels of α-2-opsonic protein are of importance in the regulation of this clearance function, alteration in opsonic activity may modulate the RE response to trauma.

Temporal correlation between changes in RE phagocytic function and circulating opsonin activity has been previously demonstrated (3, 6). Additionally, it has been shown that systemic administration of antiserum prepared to purified opsonic protein resulted in a decrement in both circulating opsonic activity and RE clearance activity accompanied by a severe decrease in the resistance to traumatic shock (12). The present study extends these findings in that a large opsonic deficit was demonstrated but no hepatic tissue phagocytic deficit was ob-

TABLE II. EFFECT OF INCREASING VOLUME OF TRAUMA PLASMA ON *in Vitro* HEPATIC PHAGOCYTOSIS IN THE PRESENCE OF A CONSTANT VOLUME OF NORMAL PLASMA.

Volume[a] of normal plasma (ml)	Volume[a] of trauma plasma[b] (ml)	Number of incubation samples	Hepatic phagocytisis	
			μg/100 mg[c]	% Control
1	0	9	381 ± 34	100
1	0.5	8	415 ± 18	109
1	1	10	501 ± 42	131
1	1.5	10	433 ± 30	114
1	2	10	361 ± 38	95
0	0	4	2 ± 1	1

[a] Volumes of normal and trauma plasma are out of a total volume of 3 ml. Differences in volume are adjusted with Krebs–Ringer phosphate solution.

[b] Trauma plasma was obtained from rats 60 min following exposure to 300 revolutions of Noble–Collip drum trauma (sublethal).

[c] *In vitro* hepatic phagocytosis is expressed as micrograms dose of colloid phagocytized per 100 mg of liver slice over a 30-min incubation period.

TABLE III. EFFECT OF HYPOXIA AND ACIDOSIS ON *in Vitro* HEPATIC PHAGOCYTOSIS.

Volume of plasma[a] (ml)	Media pH	Gas phase composition	Number of incubation samples	Hepatic phagocytosis	
				μg/100 mg[b]	% Control
1	7.4	95 % O_2–5% CO_2	14	166 ± 22	100
1	7.4	Air–5% CO_2	14	185 ± 26	111
1	7.1	95% O_2–5% CO_2	15	176 ± 21	106
1	7.1	Air–5% CO_2	14	155 ± 23	93
0	7.4	95% O_2–5% CO_2	3	8 ± 2	5
0	7.4	Air–5% CO_2	3	10 ± 1	6
0	7.1	95% O_2–5% CO_2	3	10 ± 2	6
0	7.1	Air–5% CO_2	3	8 ± 1	5

[a] Volume of plasma in a total volume of 3 ml with Krebs–Ringer phosphate solution.

[b] *In vitro* hepatic phagocytosis is expressed as micrograms dose of colloid phagocytized per 100 mg of liver slice over a 30-min incubation period.

served, even following severe (LD_{100}) trauma, which corresponds to profound *in vivo* RE depression (2, 3).

The current study found no evidence for the existence of a RE-depressing substance circulating following traumatic shock. Blattberg and Levy (13–15) reported the existence of a transferable RE-depressing substance following hemorrhagic shock in the dog and intestinal ischemic shock in the rat and dog. A prerequisite for the appearance of this substance which has been partially isolated and characterized appears to be an ischemic splanchnic vasculature. In agreement with the present study, Saba (16) was unable to transfer an inhibitory factor during postoperative RE depression and Schildt (17) was unable to transfer such a depressant factor postburn during a period of phagocytic depression. Furthermore, hypoxia and acidosis did not impair hepatic phagocytosis.

Thus, the present data extend previous findings on the importance of a humoral factor or circulating α-2-opsonic glycoprotein in governing Kupffer cell phagocytosis (3–7) and demonstrates a significant depression in RES humoral control following traumatic shock which is quite distinct from the apparent phagocytic stability manifested at the hepatic Kupffer cellular level. It is suggested that an opsonic insufficiency rather than a humoral phagocytosis-depressant substance is responsible for post-traumatic RE depression. The recent isolation of this α-2-glycoprotein and its partial biochemical characterization (5, 7) may provide a means to passively administer this protein following trauma in an attempt to circumvent RE depression.

Summary. The present study evaluated the relative humoral and cellular contribution to post-traumatic reticuloendothelial phagocytic depression in an *in vitro* phagocytic system. It was found that there is a severe depression of the humoral α-2-glycoprotein support of phagocytosis but no hepatic RES cellular deficit after severe trauma. It was additionally determined that transfer of plasma from traumatized animals into normal recipient animals and into the *in vitro* phagocytic system does not result in phagocytic depression, nor do hypoxia and acidosis impair phagocytosis *in vitro*. It is concluded that the post-traumatic reticuloendothelial phagocytic depression is primarily mediated by diminished humoral support rather than by cellular dysfunction and that the humoral deficit is a lack of opsonic activity rather than a phagocytic-depressant substance or hypoxic or acidotic conditions.

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