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Depression of *in Vivo* Clearance Function of Hepatic Macrophage Complement Receptors following Thermal Injury[1] (41896)

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Abstract. Previous studies have implicated a role for impaired hepatic macrophage blood clearance function in the increased susceptibility to infection caused by experimental thermal injury. The present study evaluated *in vivo* hepatic macrophage complement receptor clearance function as a possible factor contributing to impaired hepatic clearance after thermal injury. Rat erythrocytes treated with anti-erythrocyte serum (EA) were used as the test particle in rats. EA were rapidly removed from the circulation primarily by the liver and hepatic uptake of EA was greatly depressed in animals rendered C3 deficient by treatment with cobra venom factor. Thermal injury caused a large depression in the hepatic uptake of EA. It was shown that the depression in the binding of EA to hepatic macrophages was not due to decreased hepatic blood flow, decreased serum complement levels, or increased fluid phase C3b. Also, the depression of the hepatic uptake of EA incubated with serum prior to injection (EAC) was not different from that of EA after thermal injury. On this basis it was concluded that the impairment in binding of EA to the macrophages was at the cellular level and represented a depression in complement receptor clearance function. Additional studies showed that the injection of erythrocyte stroma, as a model of intravascular hemolysis, also depressed *in vivo* hepatic macrophage complement receptor clearance function. This latter finding suggests that the intravascular hemolysis caused by thermal injury may contribute to the depression of macrophage receptor function. The depression of hepatic macrophage complement receptor clearance function may contribute to the impaired bacterial clearance and increased susceptibility to infection following experimental thermal injury.

Erythrocytes coated with antibody and/or complement have been used extensively *in vitro* to demonstrate the presence and function of Fc and C3b receptors on the surface of macrophages (1). Similarly coated syngeneic erythrocytes have also been used *in vivo* to assess macrophage receptor function (2–7). Erythrocytes coated with C3b are rapidly removed from the blood primarily by binding to hepatic complement receptors (2–4). However, since most of these erythrocytes are not phagocytized, the action of C3b inactivator can release them back into the blood. Erythrocytes coated with enough IgG to activate complement are also rapidly cleared from the blood by binding to hepatic complement receptors, but subsequently interact with Fc receptors and are phagocytized. Erythrocytes coated with insufficient IgG to activate complement are removed from the blood primarily by Fc receptors on splenic macrophages (3). Therefore, these test particles can be used to evaluate hepatic complement or splenic Fc receptor clearance function.

Thermal injury is well known to cause a severe depression of host defense in animals (8, 9). The increased susceptibility to infection was shown to be associated with depressed hepatic clearance and killing of bacteria (10, 11). Other studies have shown that the blood clearance of test particles that bind to undefined receptors on hepatic macrophages was depressed following thermal injury (12–14). It was also shown that thermal injury depressed the *in vitro* phagocytosis of yeast cells by alveolar and peritoneal macrophages (15). The present study extended these observations by evaluating *in vivo* hepatic complement receptor clearance function after experimental thermal injury. Antibody-treated erythrocytes (EA) that activated complement were used as the test particle. The effect of depressed complement levels and hepatic blood flow on the hepatic binding of the EA was determined.

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Additionally, the effect of intravascular hemolysis, as modeled by the injection of erythrocyte stroma, on hepatic complement receptor clearance function was determined. This latter experiment was carried out because previous studies have shown that the intravascular hemolysis caused by thermal injury may contribute to the depression of hepatic clearance and host defense caused by this form of injury (12, 16, 17).

Methods. *Preparation of antisera-treated erythrocytes (EA).* Inbred, male Sprague–Dawley rats weighing 200–250 g were used for all experiments. Blood was collected in ACD (USP-B) and the erythrocytes (E) were washed three times in phosphate buffered saline (PBS, 0.9% NaCl, and 5 m*M* phosphate buffer, pH 7.4). E were labeled with ^{51}Cr and treated with rabbit anti-rat red blood cell antisera. Antisera were obtained from US Biochemical Corporation or from New Zealand white rabbits that had received 15×10^{10} rat E in six injections (half of each injection was given ip and half iv) over 2 weeks. For antisera treatment, packed E were diluted to a concentration of 2×10^9/ml in PBS containing 1% gelatin. The heat-inactivated antisera was diluted 1:100 in a volume of PBS–gelatin equal to the volume of E suspension and then added slowly (3 min) to the E with constant gentle stirring. The mixture was incubated at 37°C for 30 min and the antisera-treated erythrocytes (EA) were washed twice in PBS. Clearance characteristics of EA were unchanged for 24 hr after preparation, and in the present study EA were used 18–24 hr after preparation. There was some variation in the hepatic uptake of different preparations of EA in controls, therefore each experiment was completed with a single preparation of EA. The possible effect of gelatin in the solution used to prepare EA on the clearance characteristics of EA was determined by incubating E in PBS or gelatin–PBS exactly as with EA preparation except that antisera was not added. The clearance of these E was evaluated in control rats.

Additional experiments were carried out using E treated with IgG (EIgG) or IgM (EIgM) fractions of red blood cell antiserum. The IgG and IgM fractions were separated using gel filtration (Sephacryl 200) and verified by immunodiffusion against appropriate antibodies. Small amounts of IgG present in the IgM fraction were removed with protein A.

Clearance of EA from the blood. Animals were anesthetized with sodium pentobarbital (30 mg/kg iv) and blood samples were taken from a cannulated carotid artery. E and EA were injected at a dose of 2.9×10^8/100 g. In an initial study, blood radioactivity was monitored over 2 hr and in separate groups of animals localization of E or EA in the liver, spleen, and lungs was determined at 1, 10, and 120 min after injection. In all subsequent experiments, blood radioactivity was followed for 10 min and organ localization was determined 10 min after injection. Organ localization data were expressed as the percentage of the injected dose per organ (%ID/organ). Body weights of control and experimental animals were never significantly different and none of the interventions caused a change in liver weight.

Effect of thermal injury on the clearance of EA. Thermal injury consisted of a 30-sec scald in 90°C water on the dorsum under ether anesthesia. The burn involved 26–28% of the body surface area (18). Sterile saline was given ip (10% body wt at the time of injury. This fluid therapy reduced 24-hr mortality from 80% to less than 10% with this injury model. EA clearance was determined 30 min or 3 hr after injury.

Effect of erythrocyte stroma on the clearance of EA. Erythrocyte stroma was injected to simulate intravascular hemolysis in otherwise normal animals. Erythrocyte stroma was prepared from lysed rat erythrocytes as previously described (17). Stroma was pelleted at 1000*g*, suspended in an equal volume of PBS and injected at a dose of 0.5 ml/100 g. Clearance of EA was determined 30 min after injection of the stroma.

Effect of decreased hepatic blood flow on the clearance of EA. Since the liver is the principal organ for the clearance of EA a sufficient decrease in hepatic blood flow could decrease the clearance of EA (19). Hepatic blood flow was measured using the fractional clearance technique (20). Gelatinized lipid emulsion was used as the test particle and was injected at a dose of 0.5 mg/100 g (21). At this dose, the rate-limiting factor in the clearance of this test particle is hepatic blood flow and not hepatic

phagocytic function (21). Blood samples were taken every 30 sec for 3 min from a cannulated artery in anesthetized animals. The clearance rate constant which is the proportion of the blood volume cleared of the test particle per min was multiplied by the blood volume to give the hepatic blood flow. Blood volume was estimated by extrapolating the clearance line to zero time and assuming even distribution of the test particle throughout the vascular compartment.

Hepatic blood flow was decreased in normal animals by acute hemorrhage. Mean arterial blood pressure was reduced to 35 mm Hg over 2–3 min by withdrawing 1-ml blood every 30 sec. Hepatic blood flow or EA clearance was determined beginning 5 min after the start of blood withdrawal. Blood pressure was monitored during EA clearance and additional blood was removed or saline injected to maintain blood pressure at 35 mm Hg. Hepatic blood flow was also measured after thermal injury and injection of erythrocyte stroma.

Effect of decreased complement levels on the clearance of EA. Complement levels were depressed by the injection of purified cobra venom factor (CVF). CVF was injected ip at doses of 20, 2, and 1 units/100 g 18 hr prior to the determination of CH_{50} and C3 levels as well as the clearance of EA. CH_{50} levels were determined using the method of Mayer (22) and C3 was determined with the radial immunodiffusion technique using rabbit anti-rat C3 IgG (US Biochemical) (23). Complement levels were also determined following thermal injury and the injection of erythrocyte stroma.

In order to further delineate the role of complement in the clearance of EA following thermal injury, EA incubated in serum prior to injection (EAC) were used as the test particle. Immediately after preparation, EA were incubated in fresh rat serum (1×10^9 EA/ml) for 10 min at 37°C. The EAC were washed twice and used within 2 hr. EAC but not EA showed strong agglutination in the presence of rabbit anti-rat C3 IgG (US Biochemical). Clearance of EA and EAC was determined 3 hr after thermal injury. A single preparation of EA was used for this experiment.

In order to determine if any fluid phase C3b was present in the blood following thermal injury or injection of erythrocyte stroma or injection of CVF plasma samples from these animals were analyzed by crossed immunoelectrophoresis against rabbit anti-rat C3 IgG (US Biochemical). Fresh plasma samples collected in disodium EDTA were used and the electrophoresis was carried out with Tris–tricine buffer containing 5 m*M* disodium EDTA.

Interaction of EA with serum fibronectin. The potential interaction of EA with serum fibronectin was evaluated by determining if fibronectin was depleted from serum following incubation with EA. E or EA were incubated in duplicate samples of rat serum at concentrations of 1.2×10^7, 1.2×10^8, and 1.2×10^9 EA/ml serum for 15 min at 37°C. The EA were then removed from the serum and the fibronectin concentration was determined using the electroimmunoassay method (24).

Statistical analysis. The Student's *t* test was used for two sample comparisons and a single- or two-factor analysis of variance was used for multiple-sample comparisons. The Neuman–Keul test was used with the analysis of variance. The confidence level was placed at 95% for all experiments.

Results. *Clearance of EA from the blood of normal animals.* EA were rapidly removed from the blood during the first 10 min after injection (Fig. 1). Clearance half-time was not

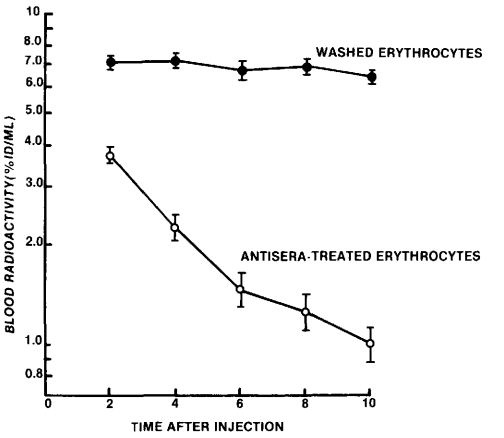

FIG. 1. Blood levels of washed erythrocytes and antisera-treated erythrocytes over 10 min after injection. Values are expressed as the percentage of the injected dose (%ID) per ml blood and are the means ± SE with six animals per group.

calculated because the clearance of EA from the blood was not monoexponential. Between 10 and 120 min after injection, blood levels of EA decreased progressively but at a much slower rate (data not shown). Hepatic localization of EA increased rapidly during the first 10 min after injection but had not increased further at 120 min (Fig. 2). Hepatic uptake accounted for most of the clearance of EA with the spleen and lungs making much smaller contributions. Erythrocytes (E) not treated with antisera were removed from the blood slowly and localization in the liver, spleen, and lungs was 2.18 ± 0.12, 0.90 ± 0.11, and 0.71 ± 0.12% injected dose (ID)/organ, respectively, at 10 min after injection. Organ localization of E incubated in PBS or gelatin-PBS were within 1%ID/organ of each other and none were higher than the localization of nonincubated E. Mean recovery of injected radioactivity in the liver, spleen, lungs, and blood (assuming 6% body wt blood volume) was 89–110% for all experiments. Radioactivity present in the plasma at 10 min after injection was less than 4%ID indicating that there was little intravascular lysis of the EA. Since most of the hepatic uptake of EA had occurred by 10 min after injection, organ localization of EA was determined at this point in all subsequent experiments.

E treated with an equal titer of the IgG fraction of anti-erythrocyte serum had virtually identical blood clearance and organ localization characteristics as EA in normal animals. E treated with the IgM fraction of the antiserum showed similar initial blood clearance and organ localization characteristics up to 10 min after injection (hepatic localization was 78.9 ± 4.1%ID at 10 min). However, thereafter the EIgM slowly returned to the circulation and by 120 min after injection hepatic localization was 11.3 ± 0.9%ID.

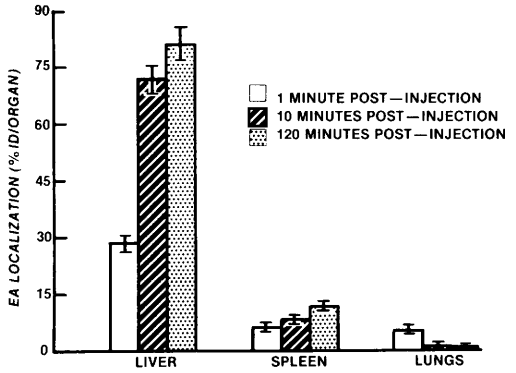

FIG. 2. Organ localization of antisera-treated erythrocytes (EA) at 1, 10, and 120 min after injection. EA were injected at a dose of 2.9 × 10^8/100 g and the data is expressed as the percentage of the injected dose (%ID) per organ. There were significant differences ($P < 0.05$) between the 1- and 10-min values for the liver and lungs. Values are the means ± SE with six animals per group.

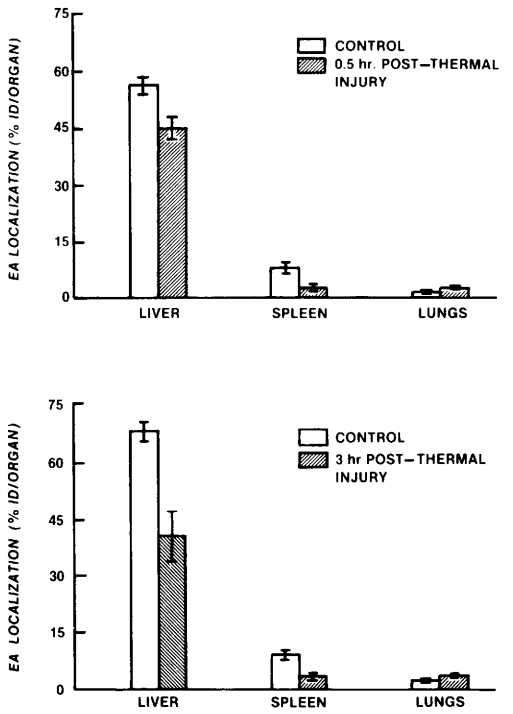

FIG. 3. Effect of thermal injury on the organ localization of antisera-treated erythrocytes (EA). Thermal injury was induced under anesthesia 0.5 hr (upper panel) or 3 hr (lower panel) before the injection of EA (2.9 × 10^8/100 g). Organ localization of EA was determined 10 min after injection and is expressed as the percentage of the injected dose (%ID) per organ. There was a significant ($P < 0.05$) decrease in liver and spleen localization and an increase in lung localization of EA at both time points after injury. Values are the means ± SE with six animals per group.

Effect of thermal injury on the clearance of EA. Hepatic uptake of EA was depressed 19% at 30 min and 42% at 3 hr after injury (Fig. 3). Splenic localization was depressed and pulmonary localization was increased at both times after injury. Organ localization of EA

TABLE I. EFFECT OF ERYTHROCYTE STROMA ON THE ORGAN LOCALIZATION OF ANTISERA-TREATED ERYTHROCYTES (EA)[a]

	Liver (%ID/organ)	Spleen (%ID/organ)	Lungs (%ID/organ)
Control	49.43 ± 4.86	9.73 ± 0.82	2.15 ± 0.15
Erythrocyte stroma	24.39* ± 4.07	4.29* ± 1.41	3.48* ± 0.44

[a] EA were injected 30 min after the injection of erythrocyte stroma (0.5 ml/100 g). Organ localization of EA (2.9×10^8/100 g) was determined 10 min after injection and is expressed as the percentage of the injected dose per organ. Values are the means ± SE with six animals per group.

* $P < 0.05$.

was unchanged in control animals that received the same fluid therapy given to the injured animals. Blood levels of EA at 10 min after injection tended to be higher in injured animals than in controls (3 hr postinjury: 51.6 ± 12.0%ID, control: 22.1 ± 5.5%ID; 30 min postinjury: 50.2 ± 4.8%ID, control: 37.7 ± 4.7%ID). Blood volume of injured animals was assumed to be decreased 24% as previously demonstrated for this type of injury (12). Intravascular lysis of EA was not increased in injured animals.

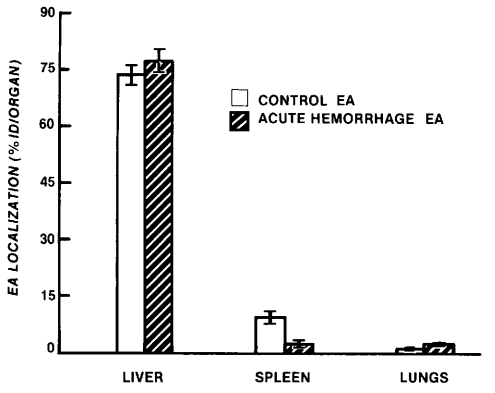

FIG. 4. Effect of acute hemorrhage on the organ localization of antisera-treated erythrocytes (EA). Mean arterial blood pressure was lowered to 35 mm Hg within 2–3 min. EA (2.9×10^8/100 g) were injected 5 min after the start of hemorrhage. Organ localization of EA was determined 10 min after injection. Blood pressure was monitored and maintained at 35 mm Hg during the 10 min EA clearance period. There was a significant ($P < 0.05$) decrease in splenic localization and increase in lung localization of EA. Values are expressed as the percentage injected dose (%ID) per organ and are the means ± SE with six animals per group.

Hepatic localization of EIgG and EIgM was depressed 44.4% and 40.8%, respectively, at 3 hr after thermal injury.

Effect of erythrocyte stroma on the clearance of EA. Erythrocyte stroma caused a 51% decrease in the hepatic uptake of EA (Table I). Splenic localization was decreased and pulmonary localization was increased. Blood levels of EA in animals receiving stroma were increased at 10 min after EA injection reflecting the lower hepatic and splenic uptake of EA.

Effect of decreased hepatic blood flow on the clearance of EA. Lowering arterial blood pressure to 35 mm Hg by acute hemorrhage did not depress the hepatic uptake of EA (Fig. 4). Splenic localization was depressed and pulmonary localization was increased. Acute hemorrhage depressed hepatic blood flow to 38% of control (Table II). This was similar to the depression observed at 30 min and 3 hr after thermal injury. Erythrocyte stroma and CVF did not affect hepatic blood flow. Since acute hemorrhage caused a depression in hepatic blood flow that was at least as great as that caused by the other experimental interventions and did not affect the hepatic uptake

TABLE II. CHANGES IN HEPATIC BLOOD FLOW AFTER ACUTE HEMORRHAGE, THERMAL INJURY, INJECTION OF ERYTHROCYTE STROMA, AND INJECTION OF COBRA VENOM FACTOR[a]

	Hepatic blood flow (ml/min/g)	Percentage of control value
Control	2.14 ± 0.12	—
Acute hemorrhage	0.82 ± 0.07*	38.3 ± 3.9
Thermal injury (30 min postinjury)	0.77 ± 0.08*	35.9 ± 2.0
Thermal injury (3 hr postinjury)	0.79 ± 0.07*	37.0 ± 3.8
Erythrocyte stroma (0.5 ml/100 g)	2.03 ± 0.21	94.9 ± 11.2
Cobra venom factor (20 units/100 g)	1.84 ± 0.18	86.0 ± 9.7

[a] Hepatic blood flow was determined using the fractional clearance technique 30 min after the injection of erythrocyte stroma, and 18 hr after cobra venom factor. Hemorrhaged animals were studied beginning 5 min after the mean arterial blood pressure was lowered to 35 mm Hg. Values are the means ± SE with 12 animals in the control group and 6 animals per experimental group.

* $P < 0.05$ compared with control group.

TABLE III. CHANGES IN COMPLEMENT LEVELS AFTER THE INJECTION OF PURIFIED COBRA VENOM FACTOR, THERMAL INJURY, AND INJECTION OF ERYTHROCYTE STROMA[a]

	C3	CH_{50}
Cobra venom factor		
20 units/100 g	8.8 ± 1.5*	<10
2 units/100 g	16.3 ± 0.9*	<10
1 unit/100 g	57.6 ± 8.4*	43.1 ± 9.1*
Thermal injury		
30 min postinjury	82.3 ± 5.1*	62.9 ± 6.2*
3 hr postinjury	55.0 ± 4.0*	37.2 ± 5.3*
Erythrocyte stroma	76.8 ± 3.7*	72.2 ± 9.5*

[a] Cobra venom factor was injected ip 18 hr prior to the measurement of serum C3 and CH_{50} levels. Erythrocyte stroma was injected (0.5 ml/100 g) 30 min prior to the determination of complement levels. Values are expressed as percentage of control and are the means ± SE with six animals per group.

* $P < 0.05$.

of EA, depression of hepatic blood flow did not contribute to the depression of hepatic uptake of EA under the experimental conditions studied.

Effect of decreased complement levels on the clearance of EA. Complement C3 and CH_{50} levels following the injection of CVF, thermal injury, and injection of particulate material are shown in Table III. The 20-unit/100 g dose of CVF was effective in producing very low levels of C3, and the 1-unit/100 g dose produced complement levels that were lower than or similar to those caused by the other experimental interventions. Following thermal injury, the levels at 3 hr were lower than at 30 min. Erythrocyte stroma caused a 35% decrease in CH_{50} and a 10% decrease in C3 levels.

The 20-unit/100 g dose of CVF caused a 61% depression in the hepatic uptake of EA, the 2-unit/100 g dose caused a 34% depression, and the 1-unit/100 g dose did not depress the hepatic uptake of EA (Table IV). Splenic and pulmonary localization of EA were not affected by CVF. Thus, the 1-unit/100 g dose of CVF did not depress the hepatic uptake of EA but did depress C3 and CH_{50} levels at least as much as thermal injury or injection of erythrocyte stroma. These results indicate that the depression in complement levels caused by thermal injury or injection of erythrocyte stroma were not great enough to cause the depression in the hepatic uptake of EA.

EA incubated in serum prior to injection (EAC) were used to evaluate macrophage complement receptor clearance function after thermal injury (Table V). In controls EAC showed a greater hepatic uptake ($P < 0.05$) and a smaller splenic uptake ($P < 0.05$) than EA. At 3 hr after thermal injury, hepatic and splenic uptake of EAC was depressed and pulmonary localization was increased. The absolute magnitude of changes in organ localization of EA and EAC following thermal injury were not different (two-factor analysis of variance). Therefore, the depression in hepatic uptake of EA following thermal injury was not due to inadequate *in vivo* opsonization of the EA.

The results of the crossed immunoelectrophoresis against anti-rat C3 are shown in Fig. 5. The electrophoretic pattern of plasma from animals subjected to thermal injury was identical to that of plasma from control animals,

TABLE IV. EFFECT OF PURIFIED COBRA VENOM FACTOR ON THE ORGAN LOCALIZATION OF ANTISERA-TREATED ERYTHROCYTES (EA)[a]

	Liver (%ID/organ)	Spleen (%ID/organ)	Lungs (%ID/organ)
Control	61.28 ± 3.56	8.13 ± 1.43	2.09 ± 0.26
Cobra venom factor (20 units/100 g)	23.87 ± 1.31*	8.28 ± 1.02	2.26 ± 0.17
Control	67.84 ± 4.19	8.85 ± 0.59	0.60 ± 0.09
Cobra venom factor (2.0 units/100 g)	44.63 ± 8.71*	7.83 ± 0.32	0.89 ± 0.15
Control	70.04 ± 2.21	8.19 ± 0.52	3.25 ± 0.61
Cobra venom factor (1.0 unit/100 g)	66.21 ± 3.06	7.30 ± 0.43	3.00 ± 0.14

[a] Clearance of EA was determined beginning 18 hr after ip injection of cobra venom factor. Organ localization of EA (2.9×10^8/100 g) was determined 10 min after injection and is expressed as the percentage of the injected dose per total organ. Values are means ± SE with six animals per group.

* $P < 0.05$ compared to the respective control group.

TABLE V. EFFECT OF THERMAL INJURY ON THE ORGAN LOCALIZATION OF ANTISERA-TREATED ERYTHROCYTES (EA) AND EA INCUBATED IN SERUM PRIOR TO INJECTION (EAC)[a]

	Liver (%ID/organ)	Spleen (%ID/organ)	Lungs (%ID/organ)
Control (EA)	67.96 ± 2.77	10.27 ± 1.29	1.26 ± 0.14
Thermal injury (EA)	46.73 ± 5.69*	2.72 ± 0.38*	3.40 ± 0.61*
Control (EAC)	95.19 ± 3.57	7.33 ± 0.58	1.14 ± 0.11
Thermal injury (EAC)	79.72 ± 4.51*	3.33 ± 0.43*	2.40 ± 0.21*

[a] EA or EAC were injected at a dose of 2.9×10^8/100 g 3 hr after thermal injury. Organ localization was determined 10 min after EA or EAC injection and is expressed as the percentage of the injected dose (%ID) per organ. Values are the means ± SE with six animals per group.

* $P < 0.05$ compared with the respective control.

except that the total amount of C3 was less in the plasma from the injured animals. At 30 min after the injection of 0.5 ml/100 g of erythrocyte stroma there was a small additional peak in the electrophoretic pattern suggesting that some fluid phase C3b was present. Serum treated *in vitro* with CVF (0.6 unit/ml, incubated at 37°C for 30 min) was included as a positive control (Fig. 5). This concentration of CVF is similar to the concentration that would be present immediately following the iv injection of 2 units CVF/100 g. Crossed immunoelectrophoresis of plasma samples taken 18 hr after the ip injection of 20, 2, or 1 unit CVF/100 g showed no indication that any fluid-phase C3b was present (data not shown).

Interaction of EA with serum fibronectin. Following the incubation of EA in serum, the serum fibronectin concentration was decreased 10% with 1.2×10^7 EA/ml serum, 0% with 1.2×10^8 EA/ml, and 6% with 1.2×10^9 EA/ml. The lowest concentration of EA used was that which would be expected in the blood immediately following the injection of 2.9×10^8 EA/100 g.

Discussion. The EA employed in the present study were rapidly removed from the blood by the liver with much smaller amounts localized in the spleen and lungs. Depletion of C3 with CVF caused a substantial reduction in the hepatic uptake of EA, and we have previously demonstrated that most of this effect of CVF can be reversed by incubation of the EA in serum prior to injection (25). Additionally, we have shown that CVF did not depress the hepatic uptake another test particle (formalinized sheep red blood cells) (17). Treatment of E with the IgG fraction of the antisera employed resulted in EIgG with clearance characteristics that were very similar to those of the EA. E treated with the IgM fraction were rapidly taken up by the liver but subsequently released. These findings were consistent with previous studies that have demonstrated that the initial hepatic uptake of antibody-coated E is due to interaction with complement receptors and that phagocytosis

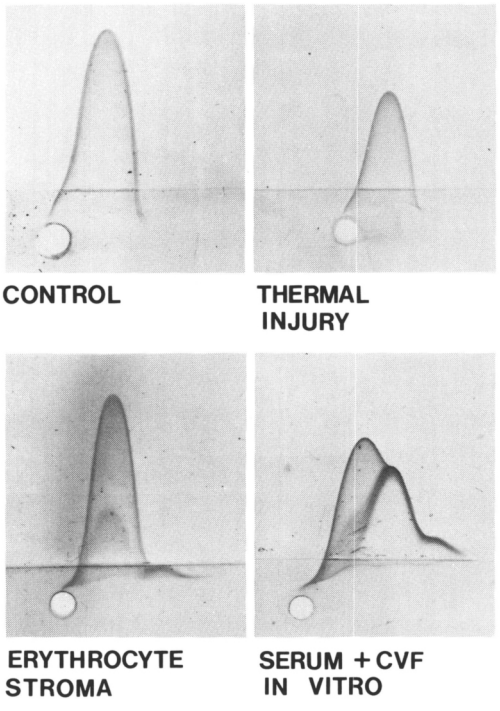

FIG. 5. Representative crossed immunoelectrophoresis patterns of plasma or serum from rats against rabbit anti-rat C3. Plasma samples were obtained from controls, 3 hr after thermal injury, or 30 min after injection of 0.5 ml/100 g erythrocyte stroma. Serum from a control animal was incubated with 0.6 unit CVF/ml for 30 min at 37°C.

requires the presence of IgG and if IgG is not present C3b inactivator will release the E back into the blood (2–4). Therefore, the rapid hepatic uptake of the EA employed in the present study was probably mediated by EA binding C3b in the blood and then interacting with complement receptors on the hepatic macrophages. The lack of lysis of the EA in the blood was probably due to the low lytic activity of complement for homologous erythrocytes (26). *In vitro* studies did not implicate a role for serum fibronectin in the clearance of EA.

Thermal injury caused a depression in the hepatic uptake of EA. Lowering hepatic blood flow by acute hemorrhage and depression of complement with CVF to levels similar to those caused by thermal injury did not affect the hepatic uptake of EA. Therefore, the reduction in hepatic uptake of EA was not due to the depression in hepatic blood flow or complement levels caused by the injury. Additionally, the failure to detect any fluid phase C3b in plasma after thermal injury makes it unlikely that the depressed EA clearance was due to competition of fluid phase C3b for the receptors. Finally, the depression of the hepatic uptake of preopsonized EA (EAC) was not different from that of EA after thermal injury. On this basis it is concluded that the depression in hepatic uptake of EA after thermal injury was at the level of binding to the hepatic macrophages and represented a depression in complement receptor clearance function. The methods employed did not allow the determination of which complement receptor (C3b, iC3b, or C3d) was principally effected by thermal injury. While the present study indicates that complement receptor clearance function is depressed following thermal injury, it is possible that a deficit in general phagocytic mechanisms contributed to the observed response.

Thermal injury also caused a depression of the splenic uptake of EA. Since the magnitude of the depression was similar to that caused by acute hemorrhage it is possible that this was due to a depression of splenic blood flow. The degree of acute hemorrhage employed has been shown to cause more than a 90% reduction in splenic blood flow (27). An additional contributing factor may have been the splenic uptake of damaged erythrocytes (28).

Lung localization of EA was increased after thermal injury and injection of erythrocyte stroma. The amount of EA present in the lungs due to the blood remaining in the lungs was less than 20% based on 0.073 ml blood/g lung tissue (17). While the lung localization of EA was never greater than 3%ID, the small increase in lung uptake of EA could have represented binding to complement receptors on marginated neutrophils (29).

Erythrocyte stroma was injected as a model of the intravascular hemolysis that occurs following thermal injury. The stroma did not cause a sufficient depression of hepatic blood flow or C3 levels to account for the depression of EA uptake by the liver. The detection of some fluid phase C3b in plasma after stroma injection indicates the possibility that this C3b could have been competing with the EA for C3b receptors. Whatever the mechanism, these results indicate that hemolysis could contribute to the depression of complement receptor clearance function following thermal injury. Since intravascular hemolysis occurs at the time of thermal injury (12) the depression of receptor function at 30 min after injury may represent the depression caused by the hepatic uptake of the erythrocyte debris. Additionally, the effect of erythrocyte stroma on macrophage receptor function may represent one of the mechanisms for the effects of stroma on phagocytic function and host defense to shock and infection (12, 16, 17).

The present study has demonstrated that the *in vivo* clearance function of hepatic macrophage complement receptors is depressed following thermal injury. This depression of complement receptor function may contribute to the increase in susceptibility to infection in animals by impairing the clearance of bacteria from the blood (10, 11). While binding to complement receptors does not normally lead directly to phagocytosis it is apparent that complement receptors are important in the binding of particulates to macrophages so that other factors may bring about phagocytosis. Depression of neutrophil function is important in the increased susceptibility of burned patients to infection. However, since opsonization of bacteria is not consistently depressed (30, 31) impaired hepatic macrophage receptor function may also be important. Since antibody-treated erythrocytes have been used to evaluate macrophage receptor clearance function in patients (5–7), this approach could be used in burned patients.

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1. McKeever PE, Spicer SS, Surface receptors on mononuclear macrophages. In: Carr I, Deams WT, eds. The Reticuloendothelial System: A Comprehensive Treatise. New York, Plenum, Vol 1:pp161–258, 1980.
2. Brown DL, Lachmann PJ, Dacie JV. The *in vivo* behavior of complement-coated red cells: Studies in C6-deficient, C3-depleted and normal rabbits. Clin Exp Immunol **7**:401–422, 1970.
3. Frank MM, Schreiber AD, Atkinson JP, Jaffe CJ. Pathophysiology of immune hemolytic anemia. Ann Int Med **87**:210–222, 1977.
4. Pappas MG, Nussenzweig RS, Nussenzweig V, Shear HL. Complement-mediated defect in clearance and sequestration of sensitized, autologous erythrocytes in rodent malaria. J Clin Invest **67**:183–192, 1981.
5. Jaffe CJ, Vierling JM, Jones EA, Lawley TJ, Frank MM. Receptor specific clearance of the reticuloendothelial system in chronic liver diseases: Demonstration of defect in C3b-specific clearance in primary biliary cirrhosis. J Clin Invest **62**:1069–1077, 1978.
6. Frank MM, Hamburger MI, Lawley TJ, Kimberly RP, Poltz PH. Defective reticuloendothelial system Fc-receptor function in systemic lupus erythematosus. N Engl J Med **300**:518–523, 1979.
7. Hamburger MI, Moutsopoulos HM, Lawley TJ, Frank MM. Sjogren's syndrome: A deficit in reticuloendothelial system Fc-receptor specific clearance. Ann Int Med **91**:534–538, 1979.
8. Alexander JW. Effect of thermal injury upon the early resistance to infection. J Surg Res **8**:128–137, 1968.
9. McRipley RJ, Garrison DW. Increased susceptibility of burned rats to *Pseudomonas aeruginosa.* Proc Soc Exp Biol Med **115**:336–338, 1964.
10. McRipley RJ, Garrison DW. Effect of burns in rats on defense mechanisms against *Pseudomonas aeruginosa.* J Inf Dis **115**:159–170, 1965.
11. DiMaio A, DiMaio D, Jacques L. Phagocytaosis in experimental burns. J Surg Res **21**:437–448, 1976.
12. Loegering DJ. Hemolysis following thermal injury and depression of reticuloendothelial system phagocytic function. J Trauma **21**:130–134, 1981.
13. Schildt BE. Function of the RES after thermal and mechanical trauma in mice. Acta Chir Scand **136**:359–364, 1970.
14. Rittenbury MS, Hanback LD. Phagocytic depression in thermal injuries. J Trauma **7**:523–538, 1967.
15. Loose LD, Turinsky J. Macrophage dysfunction after burn injury. Infect Immun **26**:157–162, 1979.
16. Loegering DJ. Intravascular hemolysis and RES phagocytic and host defense functions. Circ Shock **10**:383–395, 1983.
17. Loegering DJ, Kaplan JE, Grover GJ. Effect of erythrocyte stroma on reticuloendothelial system phagocytic function. J Lab Clin Med **102**:231–239, 1983.
18. Turinsky J, Patterson SA. Proximity to a burn wound as a new factor in considerations of postburn insulin resistance. J Surg Res **26**:171–174, 1979.
19. Normann SJ. Reticuloendothelial system function. V. Studies on the correlation between phagocytic rate and liver blood flow. J Reticuloendothel Soc **12**:473–484, 1972.
20. Dobson EL, Jones HB. The behavior of intravenously injected particulate material. Acta Med Scand **144**:5–71, 1952.
21. Saba TM, Di Luzio NR. Surgical stress and reticuloendothelial function. Surgery **65**:802–807, 1969.
22. Mayer MM. Complement and complement fixation. In: Kabat EA, Mayer MM eds. Experimental Immunochemistry. Springfield, Ill., Thomas, pp131–240 1961.
23. Mancini G, Carbonara AO, Heremans, JF. Immunochemical quantitation of antigens by single radial immunodiffusion. Immunochem **2**:235–256, 1965.
24. Blumenstock FA, Weber P, Saba TM, Laffin R. Electroimmunoassay of alpha-2-opsonic protein during reticuloendothelial blockade. Amer J Physiol **232**:R80–R87, 1977.
25. Loegering DJ, Cuddy BG, Blumenstock FA. Evaluation of *in vivo* RES receptor function following thermal injury using antibody-treated red blood cells. Circ Shock **10**:267, 1983.
26. Hansch GM, Hammer CH, Vanguri P, Shin ML. Homologous species restriction in lysis of erythrocytes by terminal complement proteins. Proc Natl Acad Sci USA **78**:5118–5121, 1981.
27. Sapirstein LA, Sapirstein EH, Bredemeyer A. Effect of hemorrhage on the cardiac output and its distribution in the rat. Circ Res **8**:135–148, 1960.
28. Grover GJ, Loegering DJ. Effect of splenic sequestration of erythrocytes on splenic clearance function and susceptibility to septic peritonitis. Infect Immun **36**:96–102, 1982.
29. Lanser ME, Saba TM. Neutrophil-mediated lung localization of bacteria: A mechanism for pulmonary injury. Surgery **90**:473–481, 1981.
30. Bjornson AB, Altemeier WA, Bjornson HS. The septic burned patient. A model for studying the role of complement and immunoglobulins in opsonization of opportunist micro-organisms. Ann Surg **189**:515–527, 1979.
31. Bjornson AB, Altemeier WA, Bjornson HS, Tang T, Iserson ML. Host defense against opportunist microorganisms following trauma. I. Studies to determine the association between changes in humoral components of host defense and septicemia in burned patients. Ann Surg **188**:93–101, 1978.

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