Prevention of Liver Reticuloendothelial Systemic Host Defense Failure After Surgery by Intravenous Opsonic Glycoprotein Therapy

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Depression of the reticuloendothelial system (RES) was observed in rats following operative trauma consisting of laparotomy and jejunal enterotomy. This RES depression was manifested as a significant impairment in the phagocytic clearance of intravenously injected blood-borne test colloid mediated by a decline in hepatic Kupffer cell phagocytosis. Reticuloendothelial systemic host defense depression was correlated with a significant decline in bioassayable and immunoreactive opsonic α_2SB glycoprotein concentration over a 1–3 hr postoperative period with rebound elevation in opsonic activity by 24 hr postsurgery. Intravenous administration of purified opsonic α_2SB glycoprotein at the end of the operation prevented postoperative opsonic deficiency and restored normal hepatic RES phagocytic function. These studies coupled with previous observations in patients following surgery or after severe multiple trauma suggest that reticuloendothelial depression during and after operation mediated by opsonic deficiency may lead to a precarious imbalance between systemic host defense and the dissemination of blood-borne foreign and effete particulate matter such as injured platelets, fibrin microaggregates and immune complexes. Immunoreactive serum opsonic α_2SB glycoprotein determinations may serve as a valuable index of hepatic RE clearance capacity and opsonin therapy may potentially be a selective means to augment systemic host defense.

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PREVIOUS STUDIES HAVE DOCUMENTED a profound depression of the RES in animals after surgery, hemorrhage and whole-body trauma.[4,12,21,32,33,45] Parallel observations in patients following major elective surgery or multiple trauma also reveals a similar impairment of RES phagocytic activity.[14,38,39,40] The phagocytic depression in man and experimental animals appears to be due to a humoral opsonic deficiency as expressed by consumption of a nonimmunoglobulin alpha-2-glycoprotein.[6,7,21,34,40] In an analogous manner, "consumptive opsoninopathy" with reference to specific immunoglobulin depletion has been demonstrated in surgical and burn patients with sepsis and correlated with decreased antibacterial immunity.[2,3]

Recently, the α-2-globulin modulating hepatic phagocytic uptake of nonbacterial test particulates has been isolated and purified from rat and human serum.[1,6,7,8] It is a large molecular weight glycoprotein[6,8,10,43] which readily binds to and opsonizes foreign particulates.[6,31] Its affinity for binding to abnormal and foreign surfaces has suggested the term alpha-2-surface binding glycoprotein (α_2SB glycoprotein) in reference to this macroglobulin[9,10] and hepatic Kupffer cells are especially sensitive to its regulatory influence[1,7,21,35,36] which correlates with the liver's role as a major determinant of systemic particulate clearance.[31,36]

The recent isolation of the opsonic α_2SB glycoprotein from rat and human serum coupled with the development of an immunoassay to quantify its serum concentration[7,9,10] provides the opportunity to evaluate the effectiveness of intravenous opsonic therapy during surgery as a means to prevent postoperative opsonic deficiency. In the present study, the ability for intravenous opsonin therapy to circumvent postoperative opsonic α_2SB glycoprotein depletion and associated RES depression was investigated in experimental rats. Bioreactive and immunoreactive opsonic α_2SB glycoprotein levels were used to monitor opsonin replacement and RES function was quantified by colloid clearance.

Materials and Methods

Animals and Surgical Procedure

Male Sprague-Dawley rats (250–300 g) maintained on lab chow and tap water *ad libitum* were used in

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This research was supported by USPHS Grants GM-21447 from the Institute of General Medical Sciences and CA-16011 from the National Cancer Institute.

Submitted for publication: November 18, 1977.

0003-4932-78-0800-0142-0115

all experiments. The surgical trauma procedure consisted of a 5 cm sterile laparotomy coupled with jejunal enterotomy (1.5 cm) under ether anesthesia followed by immediate closure of the intestinal and abdominal incisions. Animals were mobile within 15 min following surgery and allowed access to food and water throughout the experimental period. Control sham operated rats were similarly handled and anesthetized.

Reticuloendothelial Phagocytic Evaluation

Reticuloendothelial function with reference to phagocytic activity was evaluated by a colloid clearance technique[31] with the use of the gelatinized I^{131} "RE test lipid emulsion."[21,23,36,42] This clearance technique has been used previously in the experimental evaluation of phagocytic activity in animals and man[31] and the selective localization of this test colloid in RE cells, especially the hepatic Kupffer cells, has been confirmed by electronmicroscopy.[42] The test emulsion was prepared from an anhydrous base of ^{131}I-labelled triolein (Mallinckrodt Nuclear, St. Louis, MO), glycerol, and alcohol-soluble soya lecithin, mixed by high-speed blenderization in a ratio of 10/10/1 by weight, respectively.[21,32,36,39] Prior to use, the anhydrous lipid base was supplemented with 0.3% gelatin (N.B.C.) containing sterile 5% dextrose and water solution previously adjusted to pH 7.4. The final RE test emulsion with a 10% lipid base concentration was incubated with oscillation at 37° for 20 min prior to intravenous injection.

The rate of vascular clearance of the test emulsion, expressed as either the "phagocytic index" (K) or half-time (t/2) was used as a measure of RE phagocytic activity.[21,23,24,32,37] In this procedure, the emulsion having a maximum specific activity of 0.03 μC/mg lipid base was injected intravenously at a dose of 50 mg per 100 g of body weight and serial 0.1 ml aliquots of whole blood were collected from the cut tail at two minute intervals and analyzed for ^{31}I colloid concentration. Postinjection blood levels of the colloid, expressed as the per cent of the injected dose circulating per millimeter of blood (%ID/ml), were plotted similogarithmically against time in min and the phagocytic index (K) as well as the half-time (t/2) for colloid clearance was calculated.[21,32,36] The phagocytic index K is classically calculated[31] from the expression:

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

where C_1 and C_2 represent the blood colloid concentration at times T_1 and T_2, respectively. Alternatively, the phagocytic index (K; min^{-1}) which is the rate constant of the exponential disappearance curve can be calculated by least squares fit of the five serial blood concentration determinations (%ID/ml) to an exponential against time for each individual rat. This was done for each clearance with a DEC-10 computer to validate each individual half-time (t/2) determination. Tissue distribution of the test particles in random duplicate aliquots of liver, lungs, spleen and femoral bone marrow was evaluated on a per gram (%ID/g) and total organ (%ID/TO) basis at ten minutes after injection, and bone marrow compartment size was estimated as previously described.[21,32,36] All tissue samples were washed in cold isotonic saline to remove residual blood radioactivity prior to isotopic analysis.

Bioassayable Plasma Opsonin Determinations

The opsonic activity of normal and postoperative plasma was evaluated with an *in vitro* tissue slice bioassay method.[6,12,20,21,32,36] Livers obtained from normal rats were sliced to a weight of 200–300 mg with a Stadie-Riggs tissue slicer and incubated in a medium containing 0.75 ml of experimental plasma, 2.25 ml of Krebs-Ringer phosphate buffer (pH = 7.4), 100 USP units of heparin (Upjohn) and 2 mg of the gelatinized ^{131}I-RE test lipid emulsion (1.0% emulsion with 0.1% gelatin was used in the *in vitro* bioassay). All tissue slices were incubated under a gas phase of 95% O_2 and 5% CO_2 with oscillation (60 cycles/min) in a Dubnoff metabolic shaker at 37° for 30 minutes. Following incubation, the liver slices were washed in cold isotonic saline, weighed, and analyzed for ^{131}I colloid uptake. The plasma opsonic activity was evaluated in terms of its ability to increase hepatic Kupffer cell phagocytosis expressed as the per cent of the supplemented 2.0 mg dose (%ID) phagocytized per 100 mg of liver tissue.[32,36,40] This response is due to the opsonic α-2-globulin coating the test particles and thus augmenting their recognition and uptake by the Kupffer cells.[1,6,7,36] This technique has been previously used to evaluate plasma or serum opsonic activity in animals and man under a variety of experimental conditions.[6,12,20,23,34,36,40]

Blood and tissue ^{131}I radioactivity was determined with a Nuclear-Chicago auto gamma crystal scintillation system equipped with a two inch sodium iodide crystal. All samples were counted in duplicate with independent standards in each experiment. The data were statistically analyzed with Student's t-test, placing the confidence level at 95%.

Isolation and Purification of Opsonic α_2SB Glycoprotein (Sequential Steps)

Serum collection. For obtaining fresh serum, blood was drawn *via* the inferior *vena cava* from unfasted, ether anesthetized male Sprague-Dawley rats (200–300 mg) using plastic syringes rinsed with 0.1 M EDTA to

prevent fast clotting.[6,10] The blood was allowed to clot for 60 min at room temperature and then centrifuged (4°) at 5000 × g for 20 minutes. Serum obtained was maintained at 4° prior to and during fractionation.

Ammonium sulfate precipitation. The pooled serum was diluted with three volumes of ice-chilled distilled water containing 2-mercaptoethanol (ME) in a final concentration of 0.01% (v/v) and then subjected to ammonium sulfate precipitation.[6,10] Solid $(NH_4)_2SO_4$ was added under stirring to the diluted chilled serum to a final concentration of 35% saturation (20.9 g $(NH_4)_2SO_4$/100 ml diluted serum). Half of the salt was added directly and allowed to equilibrate for 15 min prior to addition of the remaining salt over a 30 min period. After equilibration for 1.0 hr at 4° the suspension was centrifuged at 8000 × g for 20 min at 0°, and the sediment was dissolved in cold distilled water containing 0.1% ME, using a volume equal to the original volume of serum. This was dialyzed overnight against 30–40 volumes of 0.053 M Tris-citrate buffer (pH 8.4) at 4°.

Preparative free-flow electrophoresis. The dialyzed fraction was further fractionated using high voltage free-flow electrophoresis (Brinkman FF-1). Electrophoresis was conducted at an operating temperature of 5° with a separating buffer composed of 0.053% M Tris-citrate (pH 8.4) and an electrode rinse buffer of 0.240 M Tris-citrate. Electrophoresis was conducted at 120–140 mA and 2,600 volts with a buffer flow rate of 115 ml/hr and sample application rate of 3 ml/hr. After plotting of the elution profile, the α-2-globulin peak was pooled and dialyzed against 25 volumes of 55% saturated $(NH_4)_2SO_4$ in phosphate buffered saline containing 0.01%–2 ME (pH 7.4) at 4° for 6.5 hours. The suspension was centrifuged at 8000 × g for 20 min and the sedimented protein was collected and stored at −78° under N_2. The isolated protein will stimulate phagocytosis and can be maintained in the lyophilized state with retention of biological activity.[1,6] This fraction with an approximately 90-fold purification[6,10] was used in the therapy experiments. Further purification to an antigenically homogeneous fraction can be achieved by application of Sepharose 4B gel filtration followed by gel filtration on Sepharose 6B and the level of purity has been confirmed by polyacrylamide gel electrophoresis[6,10] as well as electroimmunoassay.[7] Purified α_2SB glycoprotein was used in the immunization procedure to develop monospecific antibody for the immunoassay.

Antiserum Preparation

Antiserum to the isolated opsonic protein was prepared in a manner similar to that described by Blumenstock et al.[7] One milligram of the opsonic protein in 1.0 ml phosphate buffered saline was combined with 1.0 ml of complete Fruends adjuvant by emulsification. The immunization course consisted of a weekly injection of 0.1 ml of this emulsion into each footpad of male New Zealand white rabbits (3–5 kg) for four weeks. At the time of the fourth injection an additional 0.5 ml of the emulsion was injected into each thigh muscle of the rabbit. The rabbits were bled one week after the final injection at peak levels of antiopsonic activity.

Preparation of Immunoadsorbant

The immunoadsorbant used to remove antibodies developed against contaminating antigens for preparation of monospecific antiserum was prepared using opsonin deficient serum obtained from colloid induced RE-blockade rats as recently described.[7,36] In this procedure, aqueous glutaraldehyde at a concentration of 2.5% (wt/wt) was added dropwise to the opsonin deficient serum while stirring at room temperature in a ratio of 0.26 ml of glutaraldehyde per ml of serum. The serum glucaraldehyde mixture was allowed to stand for a minimum of 3–4 hr or preferably overnight at room temperature. The insolubilized serum was dispersed in two volumes of distilled water using a 10 ml disposable tissue culture pipette. The gel was then centrifuged at 4000 × g for ten minutes and the supernatant was aspirated and discarded. The final gel was washed twice with distilled water. Continued washing was performed with 0.1 M phosphate buffer, pH 7.4, until the supernatant had zero absorbance at 280 nm. The antiopsonin antiserum was then adsorbed to yield monospecific antiserum by adding 1.5 g of the immunoadsorbant to 10 ml of antiserum with mixing at room temperature for two hours. The mixture was then centrifuged at 105,000 × g for 20 min and the supernatant antiserum was collected and readsorbed again in an identical manner. It takes two to five repeat adsorptions to render the antisera distinctly monospecific as verified by immunoelectrophoresis.[7]

Electroimmunoassay Quantification of Immunoreactive α_2SB Glycoprotein In Serum

Electroimmunoassay or "rocket" immunoelectrophoresis was used to quantitate opsonin levels.[7] Agarose (Seakem) was dissolved in 0.07 M barbital buffer (pH 8.6 and 2 mM Ca lactate) to a concentration of 1% by careful heating to 100° while stirring. The 1% agarose was then transferred to a 63° water bath and warmed monospecific antiserum was mixed with the agarose to yield a final antiserum concentration of 0.6% (dependent on the antiopsonin titer of the antiserum). A

15 ml volume of the agarose-antiserum solution was then pipetted onto a clear, warm, level 5 × 10 cm glass plate to yield a gel thickness of about 1.8 mm. After the agarose had hardened, (about 15–20 min) 3 mm wells were cut in the agarose plate one cm apart and 1.5–2 cm from one edge of the plate. The experimental serum to be assayed for immunoreactive opsonic α_2SB glycoprotein was collected via the tail vein under light ether anesthesia. All blood samples were allowed to clot for a minimum of 30 min prior to centrifugation to obtain serum. The serum was diluted to 10% and 10 μl was added to each well. The samples were then moved electrophoretically toward the anode at a voltage of 7.5 V/cm at 4° for 22 hr using an LKB multiphore system. The plates were then washed overnight at 37° in 0.15 M saline with several changes.

The electroimmunoassay plates were then pressed and dried and subsequently stained for three minutes in 0.5% Coomassie Blue R-250.[7] The stain consisted of 5 g of Coomassie Brilliant Blue R-250 in a solution of 450 ml of 95% ethanol and 100 ml of glacial acetic acid. A volume of 450 ml of 95% ethanol and 100 ml of glacial acetic acid. A volume of 450 ml of distilled water was added to the filtered stain in the ethanol-acetic acid solution and mixed. Slides were destained by washing three to four times in a solution of 95% ethanol-acetic acid-water (5/2/9) for three to four minutes per wash, and allowed to dry. The rocket heights were used as a quantative index of immunoreactive α_2SB glycoprotein concentration. Rocket heights were recorded in millimeters and a double reciprocal standard plot (1/mm vs 1/% serum) was defined with a DEC-10 computer using a standard rat serum (470 μg/ml at varying concentrations (2–20%). This curve was used for quantification of serum immunoreactive α_2SB glycoprotein levels in μg/ml.[7,10]

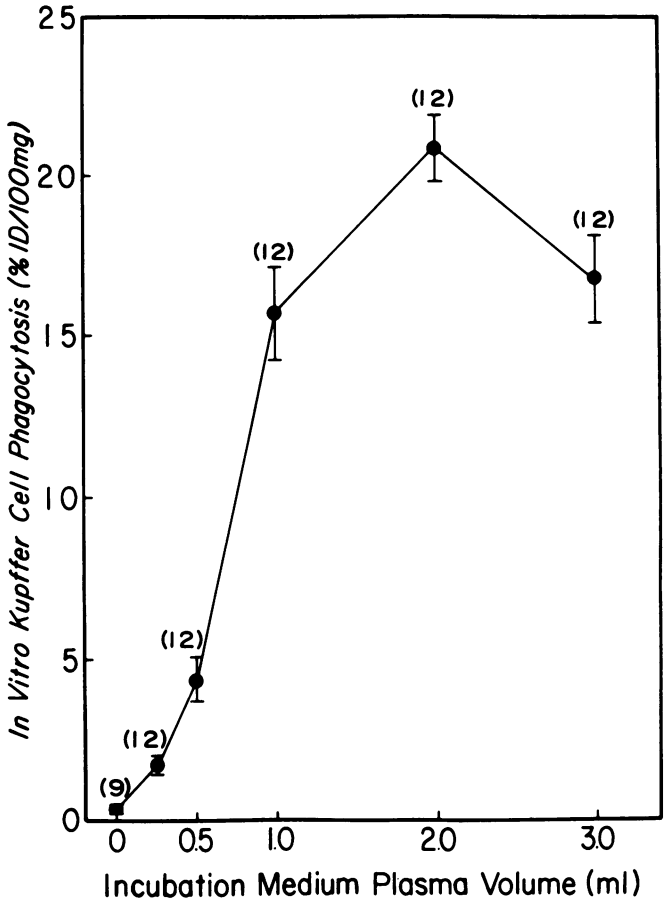

FIG. 1. Augmentation of *in vitro* hepatic Kupffer cell phagocytosis of the gelatinized I^{131} RE test lipid emulsion by fresh rat plasma. Each point represents the mean ± SE of the mean with the number of samples in parentheses. Data is expressed as per cent added dose phagocytized per 100 mg liver (%ID/100 mg) after a 30 min incubation period. Each flask was supplemented with 100 USP units of heparin. In this experiment, varying volumes of plasma (0, 0.5, 1, 2, or 3 ml) were evaluated. Krebs Ringer phosphate buffer used as the diluent to maintain a constant final incubation volume of 3.0 ml.

Results

The data in Figure 1 demonstrates the significant influence that plasma has on hepatic Kupffer cell phagocytosis using the tissue slice bioassay. In 3.0 ml of the artificial medium, which was Krebs Ringer phosphate without plasma, minimal phagocytosis of the test colloid was observed. In contrast, with increases in the volume of plasma added to the incubation medium (with a parallel decrease in Krebs Ringer phosphate volume to maintain a total volume of 3.0 ml) liver slice uptake of the radiolabelled test colloid increases significantly ($p < 0.05$) and was approximately 25-fold greater than that observed in buffer. Maximal phagocytosis is usually observed at approximately 50% plasma concentration *i.e.*, 1.5 ml plasma and 1.5 ml of Krebs Ringer phosphate. The steep portion of the sensitivity curve to the opsonic influence was at a plasma concentration of approximately 25% (Fig. 1). This concentration of plasma was used in the liver slice bioassay to detect increases or decreases in bioassayable plasma activity as reflected in the Kupffer cell colloid uptake.

Presented in Figure 2 is the bioassayable opsonin activity of plasma obtained at various intervals after operative intervention of laparotomy plus jejunal enterotomy. A significant ($p < 0.05$) deficiency in opsonin activity was observed over the one to three hour postoperative surgery. The preoperative period control level of 13.50 ± 0.96 %ID/100 mg declined by 46% at two hour postsurgery to a level of 7.34 ± 0.85 %ID/100 mg. A rebound increase in the activity was apparent at 24 hr as reflected in a level approximately 40% above

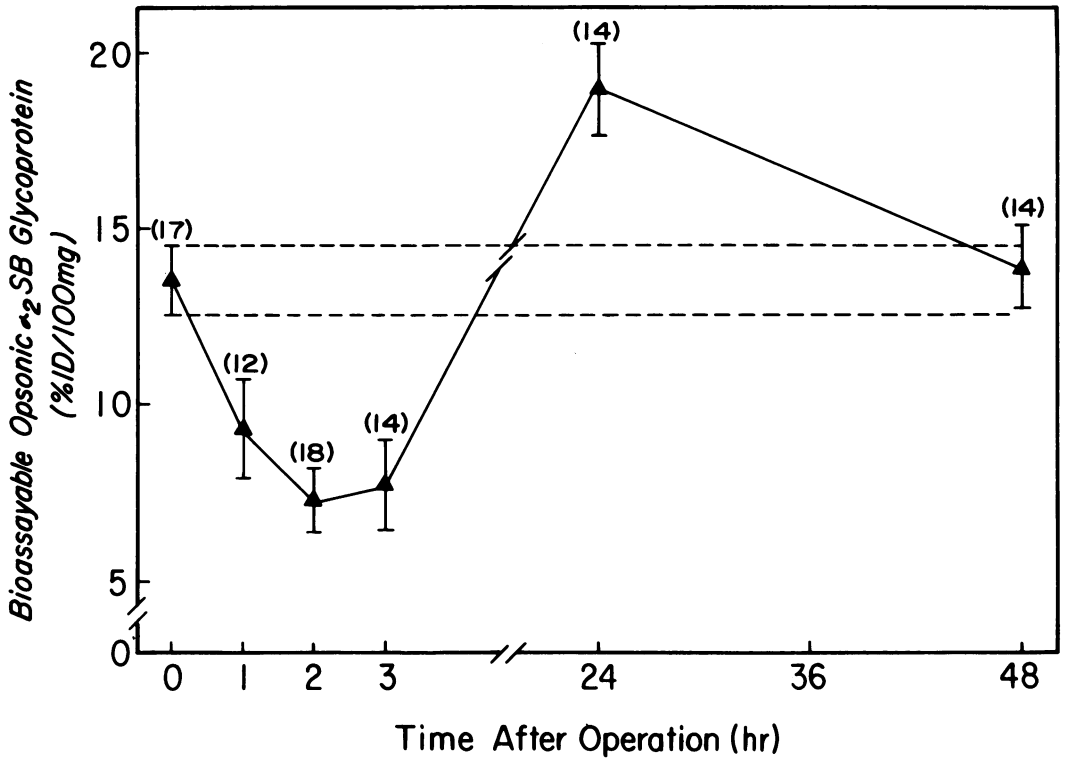

FIG. 2. Bioassayable plasma opsonic activity as a function of time following surgical trauma (laparotomy plus jejunal enterotomy). Each point represents the mean ± SE of the mean with the number of incubation samples in parentheses. Data is presented as the per cent of injected dose phagocytized per 100 mg liver tissue (%ID/100 mg) over the 30 min incubation period. Activity at one, two, and three hours was significantly ($p < 0.05$) decreased from preoperative levels with significant elevation ($p < 0.05$) at 24 hr. Experimental plasma was added to the 3 ml of medium in a 25% concentration.

preoperative values. The rate of decline after laparotomy plus jejunal enterotomy was greater than that observed after only midline laparotomy (Saba, unpublished data) which confirms previous studies[32] indicating a correlation between the degree of surgical trauma and the degree of RE phagocytic depression.

The biological activity of the purified protein relative to enhancement of Kupffer cell colloid phagocytosis is presented in Figure 3. In this study the *in vitro* activity was tested with the liver slice bioassay over a dose range of 0.25–3.0 mg of Lowry protein in the purified opsonic fraction per assay. All flasks were supplemented with heparin (100 USP units/flask) in order to allow for expression of the biological activity of the purified protein. The amount of heparin used is far in excess of that required to trigger the reaction[31] but the bioassay is supplemented with this concentration so that it is not rate limiting during the incubation procedure. A marked stimulation of particle phagocytosis was observed and uptake with 3.0 mg of the purified protein fraction was comparable to that observed with approximately 1.0 ml of plasma. As determined from the bioassayable levels and immunoreactive levels after surgery, the protein fraction was given i.v. at a dose of 2.8 mg/100 g of body weight. This was at maximum approximately two times the estimated degree of depletion after surgery.

Presented in Table 1 is the comparative response observed in immunoreactive opsonic α_2SB glycoprotein levels with and without opsonic therapy after operation. Immunoreactive α_2SB glycoprotein concentrations usually decline by 35–40% at two hour postsurgery. Over a 24–72 hr postoperative period, an elevation in the serum level of this protein to at least 50–100% above preoperative values is typically observed. The pattern detected by bioassay is quite comparable to the pattern detected by immunoassay. The intravenous administration of the purified protein at the end of surgery circumvented the opsonic deficiency at all postoperative intervals. Indeed, with replacement calculated at twice the estimated loss there was the anticipated higher than normal ($p < 0.05$) level of opsonic α_2SB glycoprotein in the serum throughout the postoperative period. Figure 4 demonstrates that treatment with opsonic protein restores bioassayable activity to a level higher than control which correlates with the immunoreactive levels as presented in Table 1.

To test whether the administration of the protein following surgery also prevented the RE phagocytic failure, the functional clearance capacity of the RES relative to test colloid removal was evaluated following operative intervention at the two hour period in both nontreated and treated rats (Table 2). A significant ($p < 0.05$) depression in RES clearance of the test colloid was observed by two hour postsurgery as reflected by the 40% decrease in the phagocytic index (K) from

0.025 ± 0.002 to 0.015 ± 0.001. Tissue distribution analysis revealed that the RES systemic clearance depression was primarily due to a 33% decrease in hepatic Kupffer cell phagocytosis ($p < 0.05$) and associated with a slight but significant elevation in pulmonary and bone marrow localization of the blood-borne test particles (Table 2). Intravenous administration of the opsonic α_2SB glycoprotein at the time of surgery reversed the RES clearance depression (Fig. 5) by restoring hepatic Kupffer cell phagocytic function (Table 2). While a decline in per gm particle localization in the lung was observed with therapy, the bone marrow uptake was still elevated. Spleen RE cell uptake of the test particles was comparable in all experimental groups indicating the specificity of the depression response especially with reference to the liver RE phagocytic mechanism.

The overall composite clearance curves in Figure 5 reveals the kinetics of the RES removal of the test particles with and without opsonin therapy. As can be seen, in contrast to the t/2 of 12.65 ± 1.03 min in normal rats, there was a pronounced RES depression ($p < 0.05$) after surgery with a t/2 of 21.97 ± 2.09 min. Opsonin therapy reversed this RE depression as reflected in a particle clearance t/2 of 11.73 ± 0.67 min.

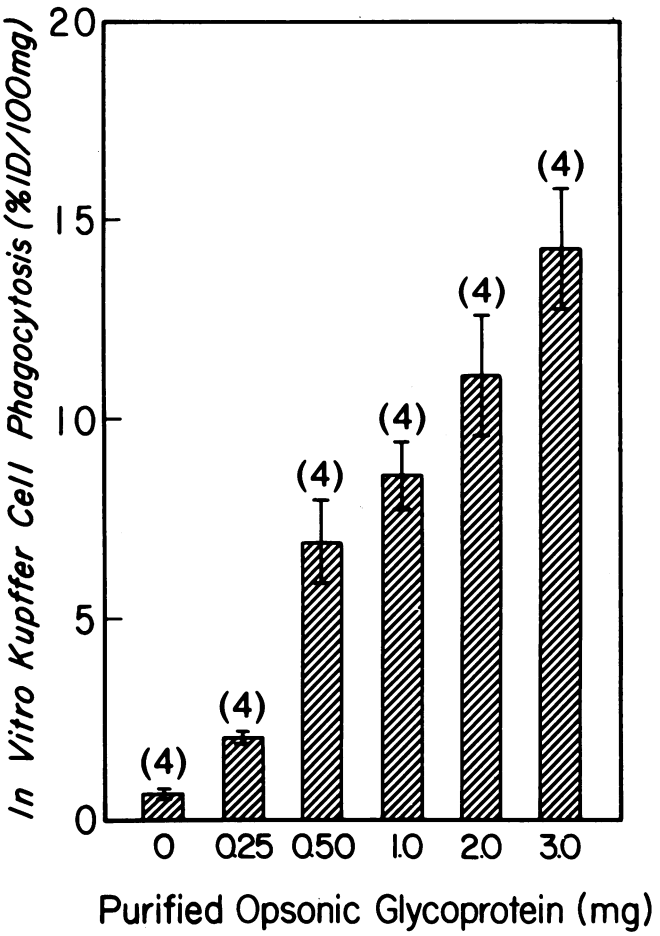

FIG. 3. Biological activity of purified opsonic α_2SB glycoprotein as tested by liver slice bioassay. The isolated protein was added to a plasma free medium of Krebs-Ringer phosphate buffer (pH 7.4) at the doses indicated and supplemented with 100 USP units of heparin per flask. Each point represents the mean ± SE of the mean with the number of samples in parentheses.

Discussion

The reticuloendothelial system (RES) is a major host defense mechanism against infection, tumor growth, and potential microembolization of the microcirculation during intravascular coagulation.[5,18,19,24,27] Additionally, the RES has been implicated as an important system of nonspecific resistance against various forms of stress such as major surgery, severe hemorrhage, burn injury and whole-body trauma.[4,21,32,34,46] A prominent physiologic function of the RES, especially those RE cells in the liver and spleen, is clearance of blood-borne particulate matter as well as effete autologous tissue debris and denatured protein from the vascular compartment.[31,33,34,38,41] Such matter includes bacteria, endotoxin, immune complexes, altered platelets, tumor cells, and microaggregates of fibrin.[19,24,25,29,34,41] This process of "intravascular phagocytosis" or so-called nonspecific "systemic host defense"[31] has been correlated with various parameters of altered resistance since experimentally induced RE depression decreases resistance to experimental shock, sepsis, as well as tumor growth and stimulation of RE clearance will increase resistance to similar insults.[5,23,24,28,31,45,46]

Many investigators have adequately documented the importance of both macrophages and polymorphonuclear leukocytes in both non-specific and specific host defense mechanisms.[2,3,13,31,34] However, in terms of RES removal of circulating particulates the liver is most centrally involved in this "vascular filtering" process primarily due to its extensive RE cell population (Kupffer cells) and its significant vascular perfusion which in man is approximately 25–30% of the cardiac output. This clearance or "filtering" function of the liver is not only of vital importance to cardiovascular integrity but also prevents antigen "overload" to the periphery in the form of gut derived bacterial antigen.

Attempts to quantify the RES removal process in the intact animal have utilized extensively the rate of clearance of various test colloids as a measure of RE function.[31] Such particulates have included colloidal carbon, radiogold, microaggregated denatured human serum albumin and the gelatinized RE-test lipid emulsion. At adequate intravenous dose levels in excess of the "critical colloid dose" (CCD) the rate constant

TABLE 1. *Influence of Intravenous Therapy* with Purified Opsonic Alpha-2-Glycoprotein on Bioassayable and Immunoreactive Opsonic Activity Following Surgery*

Experimental Group	Treatment	Bioassayable Plasma Opsonic Activity†			Immunoreactive Serum Opsonin Concentration†		
		%ID/100 mg (mean ± SE)	μg/100 mg	% Control	Rocket Height (mm)	μg/ml	% Control
Presurgery	None	12.02 ± 0.51	240.4	100	33.4	290.1 ± 25.0	100
Surgical trauma	Saline	6.20 ± 0.73	124.2‡	52‡	28.3	191.4‡ ± 13.5	66‡
Surgical trauma	Opsonin	16.19 ± 0.97	323.8§	128§	36.5	367.5§ ± 56.7	127

* The opsonic α_2SB glycoprotein was given intravenously via the dorsal vein of the penis at a dose of 2.8 mg of the protein fraction/100 g body weight immediately at the end of surgery (laparotomy plus jejunal enterotomy). The protein fraction obtained at the end of free flow separation with subsequent dialysis contains 50% of its Lowry protein, as the opsonic protein. These rats received 1.4 mg of opsonin/100 mg body weight, on the basis of this calculation.

† At two hours postsurgery bioassayable levels were assayed on plasma withdrawn in heparinized syringes and immunoreactive levels were quantified in fresh serum. Bioassayable data are presented as per cent added colloid phagocytized per 100 mg liver tissue (%ID/100 mg) and μg colloid per 100 mg. Normal fresh rat serum was used in the immunoassay to plot the standard curve from which relative immunoreactive levels (μg/ml) were determined. The normal opsonin concentration of the standard serum was 470 μg/ml and the standard curve was sensitive over a range of 94–940 μg of opsonin. For the immunoassay, the presurgery concentration represents the average of all 12 rats prior to surgery. Thereafter, they were split into two groups with six rats per group depending on whether they received the opsonic protein or saline.

‡ Significantly ($p < 0.05$) decreased from presurgery level.

§ Significantly ($p < 0.05$) greater than postsurgery level without opsonin therapy.

for test colloid removal which is called the phagocytic index (K) is an index of RE phagocytic activity[31] and correlates with various parameters of host defense as reflected in antitumor immunity, antibacterial immunity, and nonspecific host resistance to hemorrhagic, endotoxic, and traumatic shock.[4,5,23,31,45,46] Intravenous colloid injection not only challenges the RES but additionally results in a transient but significant state of postcolloidal RE depression called "RE blockade."[31,36] Experimentally induced RE blockade which was originally speculated to reflect cell saturation appears mediated by depletion of opsonic protein as detected by bioassayable and immunoreactive α_2SB glycoprotein levels and the temporal pattern of the opsonic activity correlates with RES function.[7,31,36] Animals in a state of RE blockade manifest decreased resistance to various forms of experimental shock and an increased tendency to develop sepsis after severe hemorrhage.[4,24,27,31,33,45,46] In addition, RE blockade predisposes the host to the development of the generalized Schwartzman response after endotoxin challenge[46] and exaggerates the organ disruption elicited by low grade intravascular coagulation.[27,33,41] These observations suggesting a relationship between RES function and shock resistance are further emphasized by the findings of Olcay et al.[28] who demonstrated that with a variety of liver function tests, only hepatic RE clearance correlated with survival of baboons following portal vein occlusive shock. Indeed, the studies by Kaplan, et al.[23] demonstrate directly that selective RES depression induced experimentally by injection of antiserum to α-2-opsonic protein will acutely undermine resistance and increase mortality to experimental traumatic shock.

From the standpoint of time course, the bioassayable α-2-globulin opsonic activity and associated phagocytic activity declines in experimental animals within the first few hours after surgery,[22,32,34] and is followed by a priod of rebound RE hyperactivity in association with an elevation in the level of this protein by one to two days.[32,34] Similarly, patients following various forms of elective surgery also demonstrate an acute deficiency of bioassayable plasma α-2-globulin opsonic activity within the first 24 hours following operation with recovery by two to three days after surgery.[39] With experimental whole-body trauma, a phase of hepatic RE failure is observed over the one to six hour postinjury interval followed by restoration and subsequent augmentation of RE function within 24–48 hurs.[4,21,33,34] With increased levels of trauma, the degree of depression in animals is more severe[21] and a positive correlation can be demonstrated between survival following experimental shock and RE recovery.[4,28,33] Similarly, patients following severe multiple trauma demonstrate bioassayable α-2-globulin opsonic deficiency with a close correlation between opsonin levels in the post-trauma period and survival.[40] Thus, nonsurviving trauma patients manifest persistent opsonin deficiency throughout their clinical course, while surviving trauma patients manifest an early restoration in activity.[40]

Comparable to surgery and blunt trauma, an α-2-opsonic deficiency is observed in experimental burns as well as in burned patients and correlated to the extent of burn injury.[20,37] These collective observations in various models of experimental and clinical shock have led to the concept that a fundamental disturbance in the opsonic system may be an important factor modulating RES function as well as survival after severe injury.[33]

The role of the phagocytic system in resistance to severe shock and trauma and coexistent sepsis is not a new concept and has been amply emphasized by Alexander, et al., Saba, et al. and others[2,4,23,27,31,34] since the studies by Zweifach.[45,46] However, the basis for this RE failure is only beginning to be understood and an effective therapeutic means to circumvent postoperative as well as posttrauma RE failure in the clinical setting remains to be documented. The chemical compounds that are available to stimulate RE phagocytic activity also result in RE cell or macrophage hypertrophy and hyperplasia[31] and thus induce cellular and organ disturbances which preclude routine clinical use. Additionally, the classical colloid clearance technique to measure RES function in patients[14,38] has limitations in terms of routine clinical applicability.[7,31]

In the present study, an early decline in bioassayable and immunoreactive α_2SB opsonin levels was observed after surgical intervention which was followed by a rebound hyperopsonemia. These determinations by immunoassay confirm and extend our previous observations which were exclusively obtained by bioassay with respect to alterations in this nonimmunoglobulin opsonic protein following surgery[32–34] and trauma.[21,34,40] Indeed, impairment of RE function has now been documented after whole-body multiple trauma, elective surgery, intestinal ischemia, portal occlusive ischemia, burn injury, hemorrhage, and endotoxin challenge.[4,12,14,21,24,32–34,37,38,45,46] Review[33] of these observations indicate that a typical RES systemic response pattern to injury consists of an early decrease in phagocytic activity followed by a phase of rebound hyperphagocytosis. Moreover, the level of immunoreactive opsonic protein appears to parallel the hepatic RE function activity relative to particulate clearance.

The potential clinical importance of RE phagocytic depression after surgery, burn injury, or traumatic shock has been amply reviewed.[33,34] RES failure would result in a precarious imbalance between systemic host defense and the potential development of sepsis as well as nonspecific embolization of vital vascular beds by blood-borne denatured and effete particulate matter. It undoubtedly may have other consequences and could be related to the development of multiple organ failure after trauma especially with reference

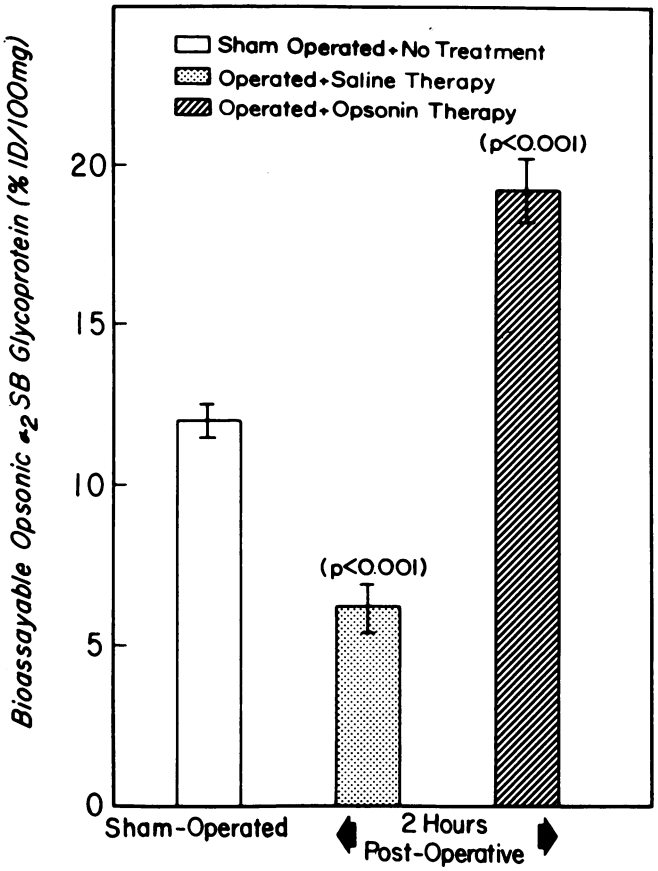

FIG. 4. Bioassayable plasma opsonin activity two hours following surgery with and without intravenous opsonin therapy. The opsonic protein was injected intravenously at a dose of 2.8 mg Lowry protein per 100 gm body weight and controls were given an equivalent volume of the saline. Mean ± SE of the mean are presented. Data is expressed as per cent injected dose phagocytized per 100 mg liver tissue (%ID/100 mg). Each assay was supplemented with 0.75 ml of plasma to yield a concentration of 25%.

to the genesis of posttraumatic pulmonary insufficiency.[27,33] The combination of failure in systemic RES clearance function as revealed in the present study coupled with interference of leukocyte bacterial phagocytosis as demonstrated by Alexander, et al.[2,3] in septic and burn patients would result in a severe "crippling" of nonspecific and specific host defense mechanisms. Both events appear to be related to an opsonic defect or "consumptive opsoninopathy" but the humoral mediators appear to be distinctly different. This could reflect a difference between polymorphonuclear leukocytes and Kupffer cells, but more likely is related to the specificity of the substance phagocytized, *i.e.*, bacterial uptake versus nonbacterial particulate phagocytosis. While its relationship to postoperative[5,11,15,16,26] altered resistance to tumor growth can only be speculated,[32] the recent documentation of decreased survival to tumor cell challenge by rats with postoperative hypoopsonemia[5] warrants further investigation. In either

TABLE 2. *Influence of Intravenous Alph-2-Opsonic Glycoprotein Administration Following Surgery on Postoperative Reticuloendothelial Tissue Uptake of the Test Colloid**

Experimental Group	Phagocytic Index (K)	Tissue Phagocytic Activity†							
		Liver		Lung		Spleen		Bone Marrow	
		%ID/g	%ID/TO	%ID/g	%ID/TO	%ID/g	%ID/TO	%ID/g	%ID/TO
Normal	0.025 ± 0.002	4.18 ± 0.14	42.82 ± 2.00	0.49 ± 0.03	0.61 ± 0.03	7.51 ± 0.65	4.97 ± 0.33	0.25 ± 0.04	1.52 ± 0.26
Surgery (Saline)	0.015‡ ± 0.001	2.80‡ ± 0.26	26.91‡ ± 2.61	0.80‡ ± 0.14	0.98‡ ± 0.19	7.23 ± 0.85	4.61 ± 0.48	0.69‡ ± 0.09	4.23‡ ± 0.50
Surgery (Opsonin)	0.026 ± 0.002	3.71 ± 0.22	36.57 ± 2.25	0.68 ± 0.05	0.86 ± 0.06	8.25 ± 0.71	5.98 ± 0.47	0.70 ± 0.07	4.32 ± 0.47

* RE function was measured by colloid clearance using a test dose of 50 mg/100 g body weight of the ^{131}I gelatinized RE test lipid emulsion injected intravenously into normal rats or at two hours postsurgery.

† Tissue uptake of the test colloid was determined at 15 min post-injection and expressed on both a per gram (%ID/g) and total organ (%ID/TO) basis. Mean ± SE are presented. Each group has six to eight rats.

‡ Significantly ($p < 0.05$) altered from control normal parameters.

case, the basis for the RE phagocytic depression after surgery appears to be an opsonic α_2SB deficiency and the liver RES appears most responsive to this deficiency. The pattern of increased extrahepatic particulate localization with liver depression is similar to that observed after RE blockade, intravascular coagulation and sepsis.[21,33,36]

While opsonic deficiency appears central to the RE depression, the basis for the decline in opsonin levels has not been defined. Accelerated removal of purified ^{125}I labelled opsonic glycoprotein from the blood following surgery in the rat has been documented and a high affinity for sites of tissue injury is apparent.[22,23] Purified opsonic protein binds to exposed collagen as well as fibrin microaggregates and adsorption of serum with such particulate will deplete its opsonic activity (Saba and Kaplan, unpublished data). In the present study, the sequestration of opsonin at areas of surgical incision and tissue injury as quantified previously by isotopic analysis[22,34] coupled with its utilization in the RES removal of blood-borne denatured particulate matter following surgery[7] probably accounts for a significant fraction of the loss observed. However, since the deficiency as quantified by immunoassay (35%) was less than that detected by bioassay (48%), this could reflect some inhibition and/or inactivation of the bio-

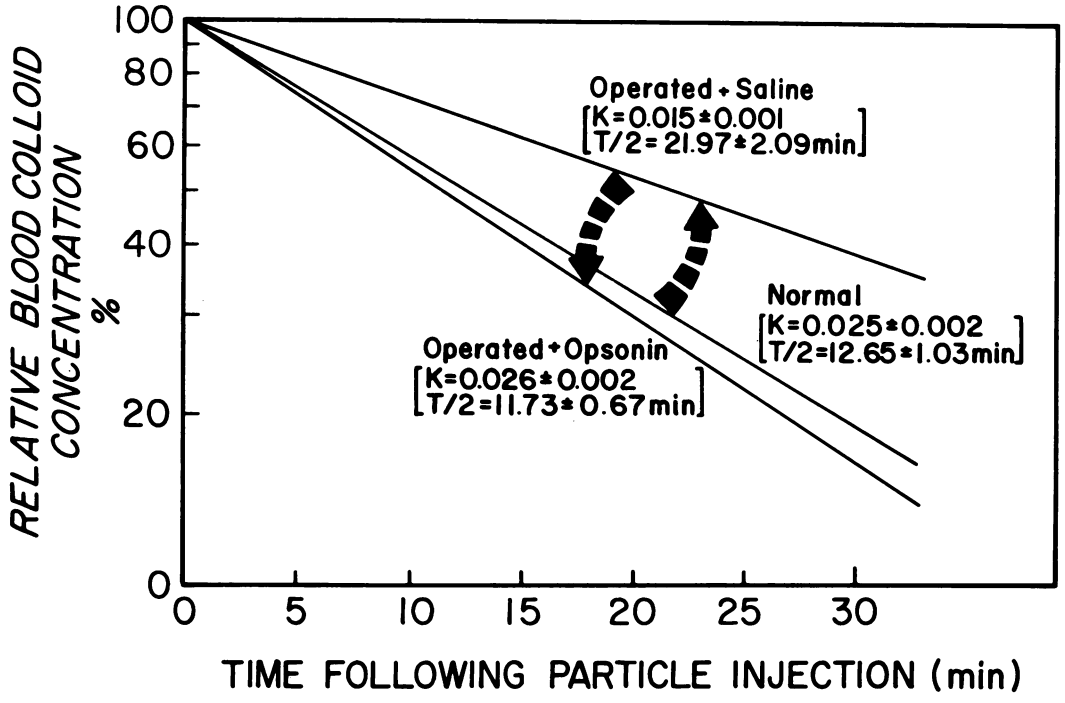

FIG. 5. Reversal of systemic RES phagocytic clearance depression by intravenous opsonin therapy. Data represents composite clearance curve of three experimental groups plotted semilogarithmically. Each group had six to eight rats. Nontreated rats after operation revealed significant RES depression ($p < 0.001$) with total RES recovery with opsonin therapy.

logical activity of this protein by circulating substances released in the postinjury period.

The ability to reverse the opsonin deficiency and circumvent the RES depression was clearly demonstrated in the present study. This protective response was not only reflected in elevated immunoreactive opsonic α_2SB glycoprotein levels at two hours but a clear normalization of RES function. At the tissue level, it was observed that Kupffer cell phagocytic activity which was the area of primary depression was restored to normal by therapy. A similar reversal of colloid induced RE blockade can be accomplished with prior serum particle opsonization with either the purified protein[1] or serum[36] which emphasizes the potential similarities of the mechanism involved in the etiology of RES depression in these two experimental conditions. Thus, the intravenous administration of this alpha-2-glycoprotein or an opsonic rich fraction of plasma may be of specific therapeutic value with respect to supporting the RES during surgery or following blunt body or burn trauma.

Recent studies from this laboratory[35] in septic surgical and trauma patients indirectly support this concept in that they demonstrate reversal of osponic α_2SB glycoprotein deficiency by intravenous administration of cryoprecipitate which is an opsonic rich fraction of human plasma. The opsonic α_2SB glycoprotein has been isolated from rat and human serum and characterized as a glycoprotein with a molecular weight of about 440,000 daltons. It is thermolabile at 60°; is cryoprecipitable in heparinized plasma at 4°; lacks relationship to the complement system; and requires heparin for its particulate binding activity.[6,8,10] Immunologic characterization with monospecific antiserum to other proteins reveals that the protein has no identity with the α_2HS glycoprotein (Alpert and Saba, unpublished data) shown by Van Oss, et al.[44] to be important in bacterial phagocytosis and depleted in trauma patients. In contrast, the α_2SB glycoprotein has immunologic identity and a similar amino acid composition as cold-insoluble globulin[9] which is a concentrated component of plasma cryoprecipitate. These observations coupled with the present data demonstrating ability to reverse RES depression after surgery by intravenous opsonin administration suggests that such an approach may eventually be valuable as a selective means to referse RES clearance failure especially during states of opsonic α_2SB glycoprotein deficiency.

Acknowledgments

The author acknowledges the secretarial assistance of Maryhelen Johnson and Maureen Kaiser. The technical assistance of Eshin Cho, M.S., Research Associate, is also acknowledged.

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