Opsonic α_2 Surface Binding Glycoprotein Therapy During Sepsis

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A pronounced depletion of an opsonic protein for hepatic reticuloendothelial (RE) phagocytosis has been demonstrated in critically ill trauma patients. This opsonic α_2 surface binding (SB) glycoprotein has immunologic identity and a similar amino acid composition to cold insoluble globulin (CIg). Since CIg can be concentrated in cryoprecipitate, it was utilized as a readily available source of opsonic α_2SB glycoprotein for replacement therapy after injury with documented hypoopsonemia. Six septic patients (2 multiple trauma, 2 thermal burn, and 2 intra-abdominal abscess) were studied to test whether cryoprecipitate infusion would restore this humoral component. Pre- and posttherapy opsonin levels were determined by bioassay and electroimmunoassay. In all patients, severe opsonin depletion was reversed following cryoprecipitate infusion. All patients had a rapid improvement in febrile state, normalization of leukocyte levels, and improvement in pulmonary function as evidenced by decreasing requirements for end expiratory pressure at lowered levels of inspired oxygen. One patient was studied more extensively and demonstrated an increase in cardiac output, limb blood flow, total body and limb oxygen delivery, total body and limb oxygen consumption and a progressive decrease in pulmonary shunt fraction. Thus, opsonic α_2SB glycoprotein deficiency can be reversed by cryoprecipitate infusion in critically ill septic injured patients. Replacement of this humoral factor may be an important therapeutic modality in prevention of multiple organ failure, but it should be administered only after documentation of hypoopsonemia in traumatized patients.

A MAJOR CONTROL MECHANISM[8,9,10,21–26] for the recognition of abnormal particulates and their subsequent phagocytic ingestion by reticuloendothelial hepatic Kupffer cells and perhaps other macrophages is a humoral factor referred to as opsonic alpha-2-surface binding (SB) glycoprotein (opsonic α_2SB glycoprotein). Recent studies in man have documented a depression of circulating opsonic α_2SB glycoprotein activity by bioassay following multiple trauma and abdominal operation.[29,30] In these studies, nonsurviving trauma patients demonstrated a very profound depletion with no sustained tendency for recovery of normal opsonic glycoprotein levels, whereas surviving patients had spontaneous improvement in the level of this opsonic protein to normal or above normal levels.[29] Furthermore, documented septicemia was consistently associated with depressed levels of this opsonic protein.

In animal studies there is a close correlation between the presence of opsonic activity as manifested by this α_2 glycoprotein and phagocytic clearance of intravenously injected test colloids.[22,26] RE phagocytic depression in animals following injury, including abdominal surgery, blunt trauma, hemorrhagic shock, and burn injury is directly related to the presence in plasma or serum of opsonically active protein.[24,26] Restoration of normal phagocytic clearance capacity has been associated in all cases with a restoration of this humoral mechanism. Indeed, recent studies on the effects of laparotomy in rats reveal a complete circumvention of postoperative phagocytic depression by the intravenous administration of purified rat opsonic α_2SB glycoprotein.[21]

Purification of human opsonic α_2SB glycoprotein in our laboratory has led to both its biochemical characterization as well as the development of an immunoassay for quantification of its serum level.[8,9,10] Biochemical characterization reveals that it is a large molecular weight glycoprotein with an amino acid composition and immunologic similarity to human cold-insoluble globulin (CIg) or so-called plasma fibronectin.[10,20] While replacement therapy in small animals has been recently demonstrated, one of the major factors limiting replacement of opsonic deficiency in patients following trauma and burn has been the availability of the protein in sufficiently large sterile quantities. However, the common identity of cold-insoluble

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globulin and the opsonic α_2SB glycoprotein suggests that opsonin therapy in patients may be possible by utilization of human plasma cryoprecipitate. In the present study, we tested the hypothesis that cryoprecipitate infusion into septic surgical, burn, and trauma patients would be an effective means of correcting opsonic deficiency as tested by both bioassay and immunoassay.

Methods and Patients Studied

A total of six patients were studied. Two patients had suffered multiple trauma; two patients had major operations for intra-abdominal abscess; and two patients had sustained major thermal burn amounting to either 60 or 75% body surface area. All patients at the time of study had either documented or suspected septicemia, and all had abscess or invasive wound infection. The response to cryoprecipitate therapy was assessed in all patients by the usual clinical criteria. These criteria consisted of pulse rate, temperature, leukocyte count, and mental alertness. Extensive evaluation of pulmonary and cardiovascular hemodynamic endices was done in one of these patients admitted to the Albany Trauma Center. Cryoprecipitate, obtained from ten units of fresh plasma and suspended in a volume of 250 ml, was infused intravenously over a 60 minute period. Circulating levels of opsonic α_2SB glycoprotein was assessed by bioassay (liver slice) and immunoassay (rocket immunoelectrophoresis) prior to infusion and at 0.5, 4, 24 and 48 hours following cryoprecipitate infusion.

Opsonic activity of serum was assessed in terms of its ability to support *in vitro* hepatic Kupffer phagocytosis of test particles.[11,14,15,23,25] In this procedure, liver slices weighing 200–400 mg were prepared from normal rat donors with a Stadie-Riggs tissue slicer. The slices were incubated in a medium containing 1 ml of the experimental human serum, 2 ml of Krebs-Ringer phosphate (pH 7.4), 100 USP units of heparin, and 2 mg of the gelatinized ^{131}I labelled RE test lipid emulsion. This gelatinized RE test lipid emulsion has been previously described and its exclusive uptake by phagocytic cells of the RE system has been documented by light and electron microscopic techniques. All samples were incubated with oscillation in a 25 ml Erlenmeyer flasks under a gas phase of 95% O_2 and 5% CO_2 in a Dubnoff metabolic shaker at 37° for 30 minutes. Following incubation, the liver slices were washed, weighed, and isotopically analyzed for colloid uptake. Opsonic activity was expressed as percentage of the injected 2000 μg colloid dose (ID) phagocytized per 100 mg of liver slice (%ID/100 mg).

Immunoreactive opsonic α_2SB glycoprotein levels were quantitated by electroimmunoassay as recently described by our laboratory.[8,9] The human opsonic protein was isolated by a combination of ammonium sulfate fractionation, high voltage free-flow electrophoresis, and gel filtration on Sepharose 4B as recently described.[9,10] Purity was tested by both gradient polyacrylamide gel electrophoresis and electroimmunoassay using polyspecific antiserum. Antiserum to the isolated opsonic protein was prepared as previously documented.[9] When antiserum is not monospecific, it can be rendered monospecific by absorption with glutaraldehyde cross-linked opsonin depleted human serum accomplished by particle absorption. The monospecific antiserum was then mixed with a 1% agarose gel to yield a final antiserum concentration of 0.6% for use in the electroimmunoassay. Three millimeter wells were cut in agarose coated 5 × 10 inch glass plates at intervals of 1 cm, and 10 μl of diluted 10% experimental serum was added to each well. Samples were electrophoresed toward the anode at a voltage of 7V/cm at 4° for 22 hours. The plates were then stained and washed as previously described.[9] The human serum standard for the assay contained 400 μg/ml of immunoreactive human opsonin. Rocket heights were recorded in millimeters and a double reciprocal standard plot (1/mm versus 1/μg opsonin) was defined with a DEC-10 computer using the standard serum at concentrations of 2–20% to allow for determination of immunoreactive protein in μg/ml.[8]

Arterial Po_2, P_{CO_2} and pH were measured using standard electrodes, calibrated with gases assayed by the micro-Scholander technique. Arterial and mixed venous samples were drawn simultaneously and the physiological shunt fraction was calculated using Berggren's shunt formula[6] at the patients therapeutic level of inspired oxygen fraction (FIO_2). For the determination of physiological dead space, expired gas was mixed in a stirring chamber from which a sample was pumped directly into the blood gas analyzer. Mixed expired gas was assayed for CO_2 and O_2 simultaneously with the sampling of arterial and mixed venous blood for blood gas determinations. The physiological dead space/tidal volume ratio (V_D/V_T) was calculated using the Suwa and Bendixen modifications of the equations of Enghoff and Bohr.[31]

Blood flow to a segment of the leg was measured by venous occlusion impedance plethysmography.[34] A tetrapolar impedance plethysmograph measured electrical impedance of the limb segment at 100 KHz. A blood pressure cuff located proximal to the segment was inflated to a pressure sufficient to occlude venous outflow. The limb segment then increased its volume for several seconds due to the continuing arterial inflow. This volume increase results in an impedance

decrease, allowing an indirect quantitative measurement of arterial inflow. Limb blood flow in ml/min/100 gm after occlusion of venous outflow is calculated by:

$$\text{Flow} = \rho \frac{L^2}{Z_0^2} \times \frac{\Delta Z}{\Delta T}$$

where: ρ = resistivity of the limb tissue assumed to equal 150 ohm cm.
L = the distance between the measuring electrodes in cm.
Z_0 = the total impedance between the measuring electrodes in ohms.
$\Delta Z/\Delta T$ = the change in impedance over time following venous occlusion in ohm/min.

Whole body O_2 consumption ($\dot{V}_{O_2}$) was measured by the direct Fick technique. Oxygen content of arterial and mixed venous blood samples was calculated from the hemoglobin concentration determined spectrophotometrically, and the oxyhemoglobin saturation determined by blood gas slide rule.[28] Cardiac output was determined by dye dilution.

Limb V_{O_2} was determined by analyzing femoral venous and arterial blood samples for their O_2 content and multiplying by limb blood flow obtained by venous occlusion plethysmography.

Case Reports

Case 1. This 66-year-old woman developed lower abdominal pain and fever and gram negative septicemia. Exploratory laparotomy revealed a large pelvic abscess with suspected perforated

TABLE 1. *Clinical Course Before and After Intravenous Cryoprecipitate Therapy in Septic Surgical, Trauma and Burn Patients*

Patient Number	Clinical Parameters* 48 Hr Period Pretherapy	Clinical Parameters* 48 Hr Period Posttherapy
1	Large pelvic abscess with colocutaneous fistula; persistent sepsis. Temperature = 37.9–39.5° Pulse = 88–122/min WBC = 2,700–3,300/mm³	Rapid recovery, no antibiotics needed. Temperature = 36.6–37.2° Pulse = 80–100/min WBC = 4,200–4,700/mm³
2	Traumatic wound of lower extremity; High above-knee amputation; Confused and disoriented; Co-existent sepsis and pulmonary insufficiency. Temperature = 39.5–40.7° Pulse = 130–160/min WBC = 11,600–37,900/mm³ Pao_2 = 88 torr on 10 cm H_2O PEEP and 50% FIO_2	Improved mental status and pulmonary function. Temperature = 37.9–38.8° Pulse = 88–100/min WBC = 13,000–16,500/mm³ Pao_2 = 74 torr on 5 cm H_2O PEEP and 50% FIO_2
3	Multiple trauma; left pleural emphyema; Confused and disoriented; Pulmonary insufficiency; Serratia marcescens cultured from pleural space, urine, and sputum. Temperature = 37.2–38.8° Pulse = 93–130/min WBC = 18,500–22,800/mm³ Pao_2 = 53 torr on 5 cm H_2O PEEP and 40% FIO_2	Improved mental state and pulmonary function. Temperature = 37.2–37.9° Pulse = 80–110/min WBC = 16,600–17,400/mm³ Pao_2 = 117 torr on 5 cm H_2O PEEP and 40% FIO_2
4	Flame burns involving 75% of body surface; positive blood cultures. Temperature = 37.9–39.5° Pulse = 100–140/min WBC = 6,700–9,200/mm³ Pao_2 = 58 torr on 0 cm H_2O PEEP and 60% FIO_2	Improvement in mental status. Temperature = 35.5–37.5° Pulse = 86–104/min WBC = 5,400–6,500/mm³ Pao_2 = 115 torr on 5 cm H_2O PEEP and 40% FIO_2
5	Burn involving 60% of body surface; blood cultures positive. Temperature = 39–39.5° Pulse = 118–160/min WBC = 10,000–16,700/mm³ Pao_2 = 58 torr on 0 cm H_2O PEEP and 40% FIO_2	Improved pulmonary function. Temperature = 36–38.6° Pulse = 110–128/min WBC = 6,500–8,800/mm³ Pao_2 = 128 torr on 0 cm H_2O PEEP and 40% FIO_2
6	Multiple trauma; Delayed massive intra-abdominal hemorrhage; Jaundiced and septic 3 weeks post-injury. Temperature = 37.2–39.5° Pulse = 100–132/min WBC = 23,000–39,500/mm³ Pao_2 = 130.5 torr on 12 cm H_2O PEEP and 45% FIO_2	Improved cardiac and peripheral hemodynamics (Table 2). Temperature = 37.2–39.5° Pulse = 78–140/min WBC = 8,000–39,300/mm³ Pao_2 = 137.9 torr on 12 cm H_2O PEEP and 45% FIO_2

* Values are presented as range of multiple determinations done over 48 hr period before and 48 hr period after therapy.

appendicitis. After appendectomy and drainage of the pelvic abscess, she developed upper gastrointestinal bleeding and required transfusion. Subsequently, an enterocutaneous fistula was identified and four weeks following her first operation, she was transferred to the Albany Medical Center. The cutaneous fistula was identified by sinogram to originate from the sigmoid colon. At this time, the patient showed signs of sepsis with tachycardia, spiking fever, and leukopenia. At operation, a pelvic abscess was drained and a diverting colostomy created with closure of the sigmoid fistula. Cryoprecipitate was transfused intraoperatively. The combination of cryoprecipitate infusion and drainage of her abscess, was followed by an improvement of fever and white count (Table 1). While multiple antibiotics had been used prior to her transfer and operation, no further antimicrobial therapy was given.

Case 2. This 20-year-old man was involved in a vehicular accident resulting in mild cerebral contusion and injuries to his right lower extremity consisting of fractures and extensive soft tissue injury with devitalized skin flaps. Hematuria was present with a normal intravenous pyelogram. In spite of debridement of the lower extremity, he became septic with deterioration in the appearance of the soft tissues of his leg during the first 36 hours following his injury. He was then transferred to Albany Medical Center where smears of the wound demonstrated pleomorphic rods, gram positive rods, and gram positive cocci. He manifested a high fever, marked tachycardia, tachypnea, and severe hypoxia requiring ventilatory support. Debridement of his wounds revealed myonecrosis and an above the knee amputation was performed. Preoperatively, he had been treated with high dose penicillin and tobramycin and this was continued postoperatively. On the first postoperative day, his febrile course and severe respiratory insufficiency persisted with subcutaneous emphysema and erythema noted over the anterior chest wall. Cryoprecipitate therapy was initiated on the second day after operation. Within 24 hours, the patient's pulmonary function and hyperpyrexia improved and he became more alert and responsive (Table 1). A steady modest temperature elevation persisted until eventual closure of the amputation stump.

Case 3. This 58-year-old man was involved in a vehicular accident and sustained a fracture dislocation of left hip, fractured left ulna and left tibia, multiple fractured ribs resulting in a flail segment and bilateral pneumothorax. There was evidence of lung contusion on x-ray within the first 24 hours after injury. Exploratory laparotomy revealed a stellate laceration of the right lobe of his liver and hematoma in the region of the duodenum and pancreas. During the first 24 hours after surgery there was a rapid deterioration of his pulmonary function and he was transferred to the Albany Medical Center. Severe respiratory insufficiency persisted for the first several weeks following injury and this was complicated by a large intrapleural air leak. Mild renal failure developed with creatinine clearance as low as 25 ml/min with oliguria. Four weeks following injury a left pleural empyema was identified and drained. Serratia marcescens was subsequently cultured from the left pleural space, urine and sputum. The patient developed tachycardia, fever, leukocytosis, and persistent respiratory insufficiency. He was treated with cryoprecipitate and during a two day period following treatment, the febrile course and sensorium all improved with relief of the persistent pulmonary insufficiency (Table 1). By the fourth day mechanical ventilatory support was no longer required.

Case 4. This 64-year-old man sustained a 75% body surface burn, 70% full thickness, and evidence of smoke inhalation. He had five documented episodes of septicemia. During these periods he had positive blood cultures, marked alteration in his febrile state, leukocytosis, as well as other signs of sepsis including depressed mental function and failure to accept nasogastric tube feedings. He was dependent upon mechanical ventilation throughout most of his hospital course and his pulmonary function further deteriorated with septic episodes. On two separate occasions, he was treated with cryoprecipitate obtained from 10 units of blood. Following each course of cryoprecipitate therapy, there has been an improvement in the clinical signs of sepsis, pulmonary function and no growth obtained on blood cultures. The patient has completely healed 22 weeks following burn injury.

Case 5. This 52-year-old white woman sustained a 60% flame burn, all full thickness, while lighting a gas stove. She expired eight weeks following her burn injury and during the course of her hospitalization, she had four documented episodes of septicemia. The first episode occurred three weeks following burn injury where blood cultures were repeatedly positive for staphylococcus aureus coagulase positive. At that time, she had hyperpyrexia, intestinal ileus and depressed mental status. After treatment with cryoprecipitate, her temperature decreased, mental status improved, and an improvement in her pulmonary function was observed (Table 1). Later in the course of her illness, she presented a clinical picture compatible with fungal septicemia and eventually succumbed.

Case 6. This 30-year-old man sustained splenic rupture, pelvic fracture, and multiple lacerations as a result of a vehicular accident. In the early postoperative period following splenectomy he became febrile, hypoxic, and required ventilatory support. During a ten day period the fever persisted and hepatic function deteriorated with resulting jaundice. He developed right upper quadrant pain and then hypotension. Exploratory laparotomy revealed a suprahepatic hematoma which was evaluated. No bacteria were subsequently cultured from the hematoma. Eight hours following this operation, the patient was profoundly hypotensive with evidence of massive intra-abdominal hemorrhage. Immediate reoperation revealed bleeding from the celiac axis at the origin of the splenic artery which was ligated. The patient had a cardiac arrest and multiple transfusions were required for resuscitation. There was evidence of dilutional coagulopathy and following this operation, there was extensive hemorrhage from the wound requiring reoperation for control of bleeding. Following these operations, he was admitted to the Trauma Center for further care and study. He developed acute renal failure, followed by spontaneous diuresis and restoration of renal function. His mental function was markedly depressed and his electroencephalogram grossly abnormal. The febrile course persisted with leukocytosis and four weeks following his initial injury blood cultures were positive for *Klebsiella pneumoniae*. This organism was also cultured from the abdominal wound. The white blood count was 39,000 cells/mm^3 and his temperature markedly elevated. Infusion of cryoprecipitate was followed by improvement in his fever, reduction in leukocyte count, as well as the transient but striking pulmonary and cardiovascular improvements as presented in Table 2. His mental status did not improve, and the electroencephalogram reflected brain death. He expired eight weeks following his initial injuries.

Results

The quantification of opsonic α_2SB glycoprotein in plasma cryoprecipitate utilized for intravenous therapy is presented in Figure 1, as detected by electroimmunoassay. The concentration of the protein in normal human serum is usually about 330–360 μg/ml, and an approximate 10–12 fold concentration (4.0 mg/ml) of the protein was routinely detected in plasma cryoprecipitate. In the present study, each of the septic patients was infused intravenously with 250 ml of plasma cryoprecipitate over a 60 minutes interval which provided about 1.0 g of opsonic protein per patient.

TABLE 2. *Cardiovascular and Pulmonary Response to Intravenous Cryoprecipitate Therapy in Multiple Trauma Patient Over a 72 Hour Posttherapy Period*

Parameter	Pretherapy	Time After Intravenous Infusion			
		4 Hr	24 Hr	48 Hr	72 Hr
Pa_{O_2} (mm Hg)	131	126	130	156	99
Physiological Shunt (%)	14.2	12.9	10.5	8.8	6.8
Dead Space (V_D/V_T)	0.377	0.438	0.444	0.475	0.345
Cardiac Output (L/min)	6.97	9.00	6.85	7.90	5.74
Cardiac Index (L/min/sq. m)	3.30	4.27	3.25	3.74	2.72
Total Body O_2 Delivery (ml/min)	1030	1667	995	1203	795
Total Body O_2 Consumption (ml/min)	210	600	273	290	161
Limb Blood Flow (ml/min/100 g tissue)	3.80	10.6	1.49	1.36	2.81
Limb O_2 Delivery (ml/min/100 g tissue)	0.562	1.628	0.216	0.206	0.389
Limb O_2 Consumption (ml/min/100 g tissue)	0.084	0.249	0.053	0.050	0.072
Mixed Venous Saturation (%)	79.6	76.6	72.7	76.8	81.8
Mixed Venous Po_2 (mm Hg)	47.2	45.1	41.8	41.0	44.6

All data points were obtained at identical ventilator settings: FIO_2 = 45%; PEEP = 13 cm H_2O.

Both immunoreactive and bioassayable levels of opsonic α_2SB glycoprotein over a 48 hour period following intravenous cryoprecipitate infusion are presented in Figure 2. There was a simultaneous elevation in both bioassayable serum opsonic activity ($p < 0.05$) and serum immunoreactive opsonic α_2SB glycoprotein ($p < 0.05$) at the ½ and 4 hr interval with return to pretherapy levels by 24–48 hours following cryoprecipitate infusion. The percentage increment in immunoreactive protein was much less than the associated elevation in bioassayable activity as tested by the liver slice assay in each corresponding test sample. For example, the 30–50% elevation in immunoreactive protein which was detected over the 0.5–4.0 hour period, corresponds to an approximate 150% increase in bioassayable activity (Fig. 3). This finding suggests that the *in vivo* functional state of the RES may have been substantially stimulated at the cryoprecipitate dose utilized in these preliminary studies.

The clinical responses in terms of temperature, heart rate, white cell level, and pulmonary function including ventilatory requirements are presented in Table 1. In general, the patients as a group manifested a drop in temperature, normalization of their leukocyte level, improvement in pulmonary function and acute reversal of their septic state early after cryoprecipitate infusion. In an attempt to more critically analyze the responsiveness of these patients to cryoprecipitate, one patient was extensively studied in the Trauma Center with respect to pulmonary function, cardiac performance, and peripheral circulatory hemodynamics (Table 2). In this patient, there was an increase in cardiac output, total body O_2 delivery and O_2 consumption, limb blood flow, as well as limb O_2 delivery and O_2 consumption which was apparent over the early posttherapy period and returned toward base-line by 24 hours. This response during the early posttherapy intervals occurred in association with the elevation of immunoreactive and bioreactive opsonic protein. In contrast to the transient nature of the response relative to cardiac output and limb blood flow, a progressive decline in pulmonary shunt fraction was observed over the three day period.

Discussion

Participation of the reticuloendothelial system in resistance to shock and trauma is not a new concept.[24,33] Hepatic phagocytic depression following hemorrhage, trauma, surgery, and thermal burn has been documented in both animals and man and RE function has been suggested to be of importance to survival following

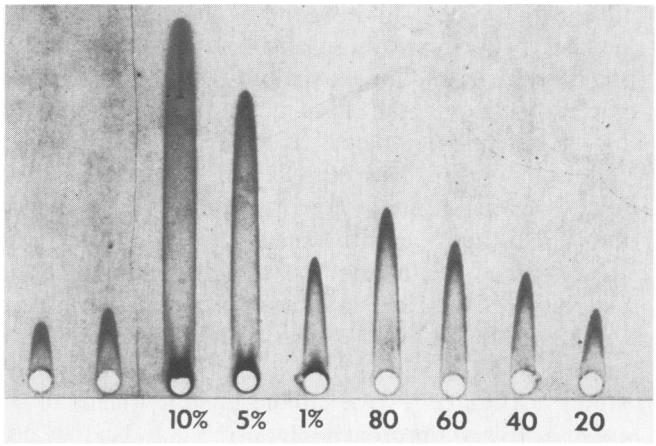

FIG. 1. Immunologic detection of opsonic α_2SB glycoprotein in fresh human plasma cryoprecipitate with the use of rocket immunoelectrophoresis (electroimmunoassay). Each well contained a 10 μl aliquot of either a 1, 5 or 10% dilution of cryoprecipitate. Rocket height was compared to varying quantities of the purified human protein. Isolated protein solutions had an opsonin concentration of either 20, 40, 60, or 80 μg/ml and a 10 μl aliquot of each was tested. Cryoprecipitate typically has an opsonic protein concentration of approximately 4 mg/ml and each patient received 250 ml (about 1.0 g) intravenously over a 60 minutes infusion interval. Percentages refer to cryoprecipitate, numbers refer to μg/ml α_2SB glycoprotein.

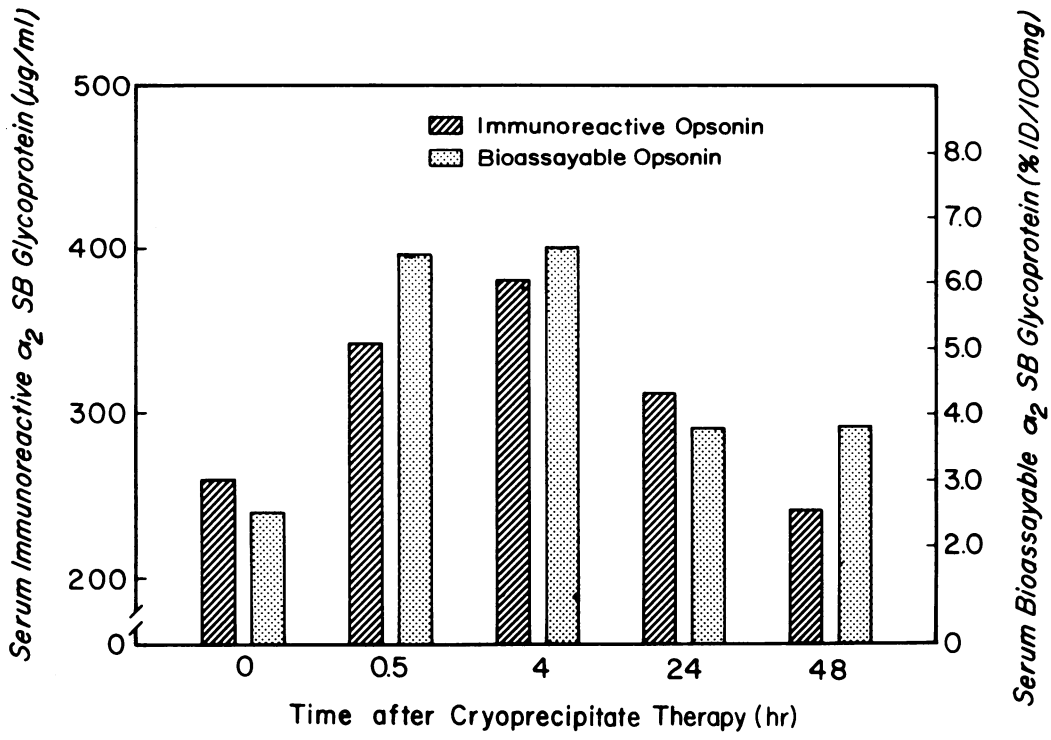

FIG. 2. Temporal response in septicemic patients after cryoprecipitate infusion. Average bioassayable serum opsonin activity and immunoreactive serum opsonic α_2SB glycoprotein concentration prior to and over a 48 hour period after cryoprecipitate infusion. Values presented are the mean of determinations in the six patients. Bioassayable opsonic activity is expressed as per cent of the added 2000 μg colloid dose phagocytized per 100 mg liver tissue. Serum immunoreactive protein is expressed as μg/ml.

major injury.[3,15,24,26,29,30] Many studies dealing with host resistance to sepsis after burn and trauma have emphasized granulocyte function[1,2] as influenced by specific opsonic immunoglobulins and complement in addition to disruption of the immune response. For example, Alexander et al.[1,2] has proposed that phagocytic dysfunction of granulocytes due to opsonic deficiency may be a limiting factor in antibacterial immunity in the septic burn and trauma patient. Thus, the combined functional capability of the polymorphonuclear leukocyte, the blood monocytes, and fixed cells of the reticuloendothelial system[22] may collectively represent a broad-based cellular defense system where levels of relative importance would be dependent on the type of injury and the site or extent of infection.

A noninvasive method to quantify the functional capability of the RES in the clinical setting has yet to be developed. The *in vivo* colloid clearance technique is invasive and its routine applicability for patient studies is limited[22] since colloid injection leads to opsonic α_2SB glycoprotein depletion[8,22,25] and can be correlated with increased sensitivity to various forms of trauma and injury in addition to lowering antibacterial immune defenses.[22,26] Thus, sequential assessment of RE function by colloid clearance has inherent problems in terms of routine clinical applicability. Since opsonic α_2SB glycoprotein activity relates closely to liver phagocytic activity,[15,17,22,25,26] the bioassay[23,25] as well as the immunoassay[8,9] of α_2SB glycoprotein may be a noninvasive index of the functional state of the RES.

Methods to modulate RE function or reverse RE depression in the trauma patient have not been reported. While a variety of chemical and nonspecific immunostimulants are available which can increase liver and spleen phagocytic activity, these agents are not of value for widespread clinical utilization due, in part, to the hypertrophy and hyperplasia of the RE system that is induced.[22] The present data suggests that intravenous administration of opsonic α_2SB glycoprotein, accomplished by cryoprecipitate infusion, may be a selective means to correct opsonic deficiency and augment RE function. Further technical modifications with eventual utilization of purified sterile isolated human opsonic protein[9] in a biologically active form will be an important advancement. Recent development of an affinity chromatographic technique in our laboratory has led to the potential for a rapid and selective means to isolate this protein to antigenic purity (Blumenstock and Saba, unpublished data). This was previously achieved by a combination of ammonium sulfate fractionation, high voltage free-flow electrophoresis, and gel filtration.[8-10]

Our demonstration[9,10] that opsonic α_2SB glycoprotein is similar to cold-insoluble globulin or plasma fibronectin is supported by several observations[20] and is of major biological significance. Both proteins have a molecular weight of about 450,000 daltons and consist of two subunits, each of about 220,000–230,000 daltons held together by disulfide bonds.[10] Both proteins have a similar amino acid composition and Ouchterlony

double diffusion demonstrates their antigenic identity utilizing monospecific antiserum to each respective protein.[10] Cold-insoluble globulin has a high affinity for binding to exposed collagen and injured tissue and nonspecific consumption at sites of tissue injury or areas of ischemia following trauma and shock may be etiologic in its depletion after trauma.[16,20] In addition, potential activation of intravascular coagulation following trauma[7] with the generation of microaggregates of fibrin and injured platelets within the vascular compartment would represent a phagocytic load to the RES resulting in opsonic deficiency.[24] This deficiency may be magnified during septicemia in which circulating immune complexes as well as free bacteria can provide an additional particulate load to the liver and spleen. Previous findings of opsonic α_2SB glycoprotein deficiency in septic trauma patients[29] coupled with the observed association of sepsis and multiple organ failure[12,37] following injury have led us to the hypothesis[24] that RE dysfunction mediated by opsonic deficiency may be a major factor in the etiology of multiple organ failure following trauma.

Recent studies[29,30] in traumatized man are consistent with our animal data.[20] Spontaneous restoration of normal levels of opsonic α_2SB glycoprotein in man after trauma has been associated with survival.[29] In the present study we have augmented circulating opsonic α_2SB glycoprotein levels by the infusion of cryoprecipitate. All patients demonstrated a transient clinical improvement in pulmonary function and a lessening of the septic state following a single therapeutic trial of cryoprecipitate which closely corresponded to the transient reversal of the opsonic deficiency. These preliminary observations of clinical improvement are in agreement with animal data[17,21] but will require more extensive evaluation by a controlled prospective study. The consequences of maintaining a heightened serum level of this protein for a prolonged period of time remains to be determined, but is presently under investigation.

One patient, who was studied in more detail in the Trauma Center, demonstrated substantial increase in cardiac output as well as a decrease in pulmonary shunt fraction with improved limb blood flow and an increase in total body as well as limb oxygen consumption. While the mechanism responsible for the increased cardiac output remains to be determined, one might hypothesize that this may be related to increased Kupffer cell clearance of noxious particulates which may influence cardiac performance. The volume of cryoprecipitate infused and its total protein content would not be expected to increase cardiac output to the degree observed. Particulate localization in peripheral microcirculatory beds may also influence capillary permeability, and regional regulation of perfusion. Enhanced

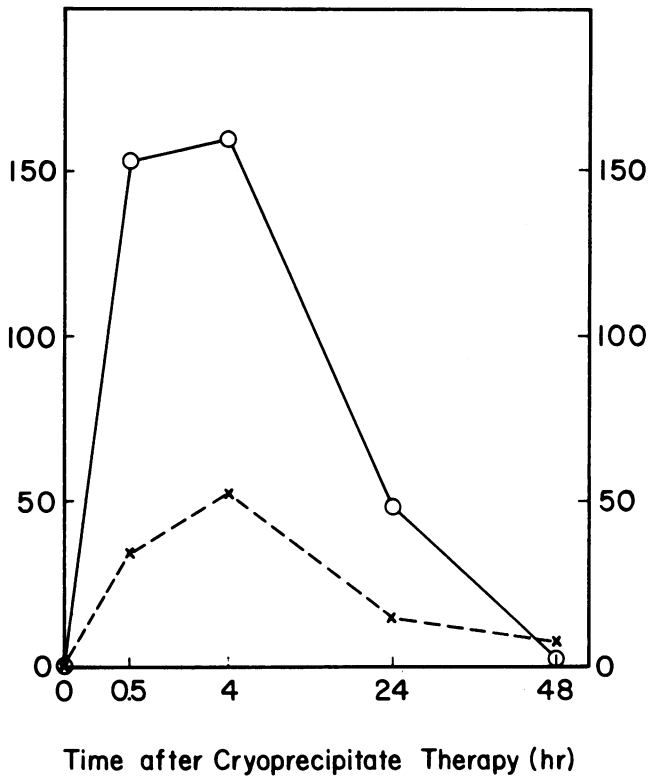

FIG. 3. Quantitative relationship between the posttherapy elevation in immunoreactive opsonic α_2SB glycoprotein as determined by electroimmunoassay and the parallel alteration in bioassayable opsonic activity of serum as tested by *in vitro* liver slice assay using the gelatinized I^{131} RE test lipid emulsion. Values represent the average response in the six patients. Data are expressed as per cent increase observed in each patient from pretherapy values over a 48 hour period after cryoprecipitate administration. Immunoreactive and bioassayable levels were significantly elevated at 0.5 hour ($p < 0.05$) and four hour ($p < 0.05$) but within normal ranges at 24 and 48 hr. O —— O: per cent increase in serum bioassayable Kupffer cell *in vitro* activity. × – – – ×: per cent in serum immunoreactive opsonic α_2SB glycoprotein in patients.

limb and total body oxygen consumption following the infusion of cryoprecipitate as documented in the present studies provides some support for these theoretical considerations, and may reflect hepatic RE "protection" of the periphery.[15,22–24]

Pulmonary function in the postinjury state may be influenced by the functional integrity of hepatic RE clearance mechanisms.[27] RE phagocytic depression has been shown to result in increased localization of blood-borne particulate matter in the lungs.[15,23] Such microembolization may be etiologic in ventilation–perfusion imbalances, increased pulmonary vascular permeability, and the genesis of pulmonary edema. The close correlation of sepsis with posttraumatic pulmonary insufficiency[12,32] in the trauma and burn patient may be, in part, mediated by the impaired RES removal of circulating microaggregates and immune complexes and their subsequent deleterious influence on lung function and peripheral circulatory perfu-

sion.[4,5,7,19,26] Circulating immune complexes are known to be cleared by RE cells, and complement or endotoxin induced leukostasis within the pulmonary vasculature during sepsis may directly disturb the pulmonary microvasculature as well as increase pulmonary capillary permeability leading to permeability edema. Additionally, the septic or endotoxemic state will not only lead to leukostasis and the formation of immune complexes, but this state itself is a potent activator of intravascular coagulation. Phagocytic clearance of products of coagulation such as altered platelets, fibrin, and other activated coagulation factors has been documented.[13,18] Hepatic Kupffer cells have been shown by immunofluorescence to contain fibrin microaggregates[18] following low-grade intravascular coagulation and Kupffer cell clearance of radiolabelled fibrin has been demonstrated.[13] Furthermore, evidence has been provided that the clearance of fibrin degradation products and fibrinogen-fibrin complexes is related to the functional status of the RES.

The findings of this study document the effectiveness of cryoprecipitate infusion as a means to reverse opsonic α_2SB glycoprotein deficiency in critically ill patients. The data further suggest that the maintenance of normal levels of this humoral component, which would augment RES function, may provide an important means of prevention or attenuation of multiple organ system failure in the critically ill, septic, burn or trauma patient.[20]

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Discussion

Dr. Lloyd D. Maclean (Montreal, Quebec): This is a remarkable paper, I think, about a naturally-occurring immune regulatory molecule. It's remarkable for several reasons.

First of all, they have showed a stimulatory effect, whereas most of us working in this field have been able to detect only inhibitors.

They have two quantitative assays, and one of those requires that they purify the opsonic protein, which they have done. Any one of these things, I think, would be enough for a complete discussion.

They have also found a concentrated source for the material, in the form of cryoprecipitate. Finally, they have applied it clinically.

I'd like to show two or three slides, just to put it in perspective, maybe, for some who aren't as familiar with these things.

(Slide) The inflammatory response, the component that they're studying, is over on this side. Opsonic activity is buttering up bacteria, or other particles, so they become attractive to the phagocytes. The Kupffer cell is the particular phagocyte that they're studying.

Several people are working on this side, in chemotaxis. There's a good quantitative assay for intracellular killing. This activity can be quantitated. There isn't too much work going on in here. I'd like to show you two or three slides of activity on this side of the scale.

(Slide) Using this quantitative assay, neutrophils can be taken from trauma patients, put in this grid with this attractant below it and the degree of migration can be accurately quantitated of the neutrophils across this grid.

(Slide) Patients who have been traumatized fall into three groups, generally: a group with minor injuries, who have normal skin tests and don't migrate from the normal range; patients with multiple injuries, two body cavity injuries, with, again, a normal skin test, and finally, people with severe multiple injuries, who have abnormal skin testing, and take about a month to get back to normal.

It looks like this measurement, chemotaxis, (slide) might be a more sensitive index of decreased host resistance than is skin testing.

Finally, as was done here in 14 anergic patients, if one follows them long enough, until they do return, as the skin test returns to normal, so does the chemotaxis. Furthermore, if one takes cells from a normal person and adds the abnormal serum from these anergic patients, those cells will also fail to migrate normally.

I have two or three questions that they might like to speculate on. I'd like to know if they did skin testing on these patients, or others, and if they found this to be helpful. What is the normal response to infection with the opsonic protein? Does a normal person with a controlled infection get a jump in that protein?

I think you did tell us some of the hemodynamic effects, but I'd be interested to know whether in the normal setting, this opsonic protein has a hemodynamic effect.

Dr. Ben Eiseman (Denver, Colorado): I was privileged to review this manuscript in advance. This work is important not because following administration of some plasma fraction several sick septic patients got better. Indeed each had good surgical care and some probably would have otherwise recovered. What is important is the authors' evidence that the glycoprotein fraction, which happily can be isolated relatively easily in the cold precipitated fraction of normal serum, increases opsonization. This is a modern return to a bacteriologic principle whose time, I judge, now has returned.

(Slide) Let me put this in context with our own studies, which for several years have emphasized the etiologic role of remote bacterial sepsis to liver, lung, kidney failure and stress ulcer.

(Slide) Our hypothesis is that such infection produces circulating immune complexes of antigen, antibody and complement and that these particles, if sufficiently large, are caught in the reticular endothelial cells of the liver. If they are smaller than 11S or if the RE cells are saturated or damaged, the complexes slip by the Kupffer cells and land in the lung, kidney or other organs where they cause cellular damage (slide).

This was our hypothesis, and we have evidence that we are correct. In four patients in multiple organ failure and in over 25 rabbits made septic by experimental intraperitoneal abscesses, the immune complexes visualized by immunofluorescent antibodies coating the vasculature of the liver, lungs and kidney in a pattern so far recognized only in glomerular basement membrane disease support the concept presented today that an immune mechanism contributes to organ damage during remote sepsis. The unanswered question remains: How does the opsonin described by the Albany group work?

Dr. William Scovill (Closing discussion): Dr. MacLean, we have not done skin testing in any of these patients, and I think the results of skin testing would have a lot of complex interpretations, which may have very important bearings on future lines of investigation. I think that would be an ideal thing to do.

The questions about opsonic protein levels—that is, the opsonin required for normal Kupffer cell phagocytic function and the influence of infection on these opsonic protein levels—is an important one. There is a suggestion that early infection may result in heightened levels of this opsonic protein, whereas the data I presented in severely traumatized man with documented septicemia, indicated that there was a severe undermining of this opsonic system.

The hemodynamic effects, as I presented in a preliminary fashion in these two patients, are probably not due to the volume of cryoprecipitate infused, since it was so small. It would be appealing to hypothesize an improvement in peripheral microcirculation as a means of improving overall dynamics by the phagocytic ingestion of potentially noxious particulates that are circulating during septic periods.

Dr. Eiseman, we have looked at the question of complement early on, in cruder isolated protein fractions, and have not found complement activity in the crude fractions; and you're quite right, the structure of this protein is not compatible with this being complement.

The further question of what happens to liver blood flow, hepatic sinusoidal blood flow, following the ingestion of a large particulate load is a very interesting one, and one that we have not yet confronted. I think the former model of reticuloendothelial blockade, that induced by large volume colloid injections, was thought at one time to be due to a saturation of the capacity of the Kupffer cell to ingest these particles. Actually, it was shown almost ten years ago by Dr. Saba, one of the coauthors, to be due to a depletion of this humoral component.

I would like to add just one additional comment. We have carefully selected patients for study by first assessing them for the prevailing level of opsonic protein. There are some in vitro data available in our laboratory that demonstrate that superheightened levels of opsonic protein may, indeed, be deleterious due to rapid agglutination of particles in vitro in excess of R.E. activity and thus the potential of pulmonary localization. Therefore, we have carefully limited cryoprecipitate infusion to patients with documented hypoopsonemia, and in this setting an effective response is observed.