SEPTIC SHOCK CONFERENCE
MOLECULAR AND CELLULAR MECHANISMS
OF SEPTIC SHOCK
BETHESDA, MARYLAND
29 February - 1 March 1988

Sponsored by the
Naval Medical Research and Development Command
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Naval Medical Command
National Capital Region

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Molecular and Cellular Mechanisms of Septic Shock

Sepsis and septic shock are a major focus of the Naval Medical Research Institute because of the importance of septic shock as a complication of treating combat casualties. Additionally, the civilian research community has a strong interest in septic shock research because of its complications in many serious diseases. Research investigators from this command, the National Institutes of Health, universities, and the pharmaceutical industry discussed recent research advances and treatment potentials in three broad areas, including alterations in cell receptor-linked signal transduction in septic shock, the role of bacterial endotoxin in sepsis and shock, and the action of lymphokines in septic and endotoxic shock. The conference provided a forum for investigators interested in the significant combat casualty wound complication of septic shock to meet and exchange ideas. A book covering the proceedings of the conference will be published and available in July 1988.
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Program
SEPTIC SHOCK CONFERENCE
Molecular and Cellular Mechanisms of Septic Shock

All sessions, including the poster session will be held in the Naval Medical Command, National Capital Region Theater, Bethesda, MD.

MONDAY, 29 FEBRUARY 1988

0800-0830 Introduction: Dr. Adam McKee, Head, Casualty Care Research Department, Naval Medical Research Institute.
Welcome: CAPT K. Sorensen, Commanding Officer, Naval Medical Research Institute.
Opening remarks: RADM R. P. Caudill, Deputy Director of Naval Medicine.

0830-1200 TOPIC A: HOW RECEPTOR ALTERATIONS EXPLAIN CLINICAL ASPECTS OF SEPTIC SHOCK. Moderator: De-Naw Chuang, Ph.D.

0840-0910 Overview: Vascular alpha adrenergic receptors and signal transduction. William D. Matthews, Ph.D.

0910-0940 Role of the endothelium in modulating vascular adrenergic receptor actions. Virginia M. Miller, Ph.D.

0940-0950 Discussion

0950-1010 Coffee Break

1010-1040 Alterations in vascular and hepatic alpha adrenergic receptors and signal transduction in sepsis. Bryan L. Roth, M.D., Ph.D.

1040-1045 Discussion

1045-1115 Modifications of adrenergic and vasopressin receptor-linked lipid metabolism during endotoxemia. Judy A. Spitzer, Ph.D.

1115-1120 Discussion

1120-1145 Methodologies for drug discovery based on receptor technologies. Stafford McLean, Ph.D.

1145-1200 Discussion

1200-1300 Lunch
1300-1330 Role of tumor necrosis factor in sepsis. Bruce Beutler, M.D.

1330-1400 Overview: Endotoxin biosynthesis - role of intermediates in activation of protein kinase C. C. Christian Raetz, M.D., Ph.D.

1400-1600 Workshop: New approaches to understanding pathophysiology and devising treatment approaches for sepsis and septic shock.
Moderator: Bryan L. Roth, M.D., Ph.D. Presentations by members of the Casualty Care Research Department, Naval Medical Research Institute.

1. Receptors for lipopolysaccharide (LPS) on liver cells. James J. Parenč, Ph.D.

2. Immunological approach of septic shock research. Che-Hung Robert Lee, Ph.D.

3. Effect of macrophage inhibition in carrageenan and glucosamine-induced sensitivity to low-dose endotoxin. Lyn J. Yaffe, M.D.

4. Bradykinin (BK) and BK antagonists effects on endothelial cell (EC) phosphatidylinositol metabolism, implications for septic shock. Thor B. Nielsen, Ph.D.

5. Possible role of bacterial adherence and bacterial adhesins in sepsis and septic shock. Taffy J. Williams, Ph.D.

6. Endotoxemia and sepsis alter splenic and hepatic protein kinase C receptors. James B. Hermiller, M.D.


1550-1600 Coffee Break

1600-1800 Poster Presentations

1800-1930 Recess

1930-2300 Conference Dinner. Speaker: RADM. J.S. Cassels, Commander, Naval Medical Command. F. F. Waters Caterers, Banquet Suite, 1225 Nebel Street, Rockville, MD. A bus will pick up attendees at the main entrance to the Medical Command at 1900.
TUESDAY, 1 MARCH 1988

0830-0920 Poster Presentations

0920-1200 TOPIC B: ROLE OF ENDOTOXIN IN CAUSE AND TREATMENT OF SEPTIC SHOCK. Moderator: John Spitzer, M.D.

0925-1000 Human monoclonal antibodies to endotoxin: Potential therapy for sepsis. Matthew Pollack, M.D.

1010-1020 Discussion

1020-1040 Altered control of carbohydrate metabolism in endotoxemia. John Spitzer, M.D.

1110-1115 Discussion

1115-1200 Effects of endotoxin(s) on human hemodynamics: Potential protective effects of lipid A analogues. Joseph Parillo, M.D.

1200-1300 Lunch

1300-1630 TOPIC C: LYMPHOKINES IN SEPTIC SHOCK: POTENTIAL FOR THERAPY.

1310-1345 Cytokines and the metabolic pathophysiology of sepsis. James Filkins, Ph.D.

1345-1350 Discussion

1350-1435 Synergy between tumor necrosis factor and interleukin-1. Charles Dinarello, M.D.

1435-1440 Discussion

1440-1500 Coffee Break

1500-1530 Role of lymphokines in altering receptor mediated vascular contraction in sepsis. Thomas McKenna, Ph.D.

1535-1605 Role of lymphokines in altering hepatic metabolism in sepsis. Frank B. Cerra, M.D.

1605-1610 Discussion

1610-1630 Summary and conclusions
List of Speakers

Bruce Beutler M.D.: Investigator, Howard Hughes Medical Institute, University of Texas Health Sciences Center, 5323 Harry Hines Blvd. Dallas, TX 75235-9050.

Joseph Carcillo, M.D.; Assistant Professor of Anesthesiology and Child Health and Development, George Washington University; Attending Physician, Department of Critical Care Medicine, Children's Hospital National Medical Center, 1111 Michigan Avenue, N.W., Washington, DC 20010.

Frank B. Cerra M.D.: Professor, Department of Surgery, University of Minnesota Medical School, 420 Delaware Street, S.E. Minneapolis, MN 55455.


Charles Dinarello M.D.: Associate Professor, Department of Infectious Diseases, Tufts University Medical Center, 136 Harrison Ave. Boston, MA 02111.

James Filkins Ph.D.: Chairman, Department of Physiology, Loyola University School of Medicine, 2160 S. First Street, Maywood, IL 60153.

James B. Hermiller M.D.: Principal Investigator, Stop 15, Naval Medical Research Institute, Bethesda, MD 10814-5055.

Che-Hung R. Lee, Ph.D.: Principal Investigator, Stop 42, Naval Medical Research Institute, Bethesda, MD 10814-5055.

William D. Matthews Ph.D.: Director, Investigative Toxicology, Smith, Kline and French Labs, 709 Swedeland Road, Swedeland, PA 19406.


Adam E. McKee, D.V.M.: Head, Casualty Care Research Department, Naval Medical Research Institute, Bethesda, MD 10814-5055.

Thomas McKenna Ph.D.: Principal Investigator, Stop 15, Naval Medical Research Institute, Bethesda, MD 20814-5055.

Virginia Miller, Ph.D.: Department of Physiology, Mayo Medical School and Mayo Graduate School of Medicine, 200 First Street S.W., Rochester, MN 55905.

Thor B. Neilsen, Ph.D.: Principal Investigator, Stop 42, Naval Medical Research Institute, Bethesda, MD 10814-5055.
James B. Parent, Ph.D.: Principal Investigator, Stop 42, Naval Medical Research Institute, Bethesda, MD 10814-5055.

Joseph Porillo M.D.: Director, Department of Critical Care Medicine, Building 10, National Institutes of Health, Bethesda, MD 10814.

Matthew Pollack M.D.: Professor, Department of Medicine, F. Edward Hebert School of Medicine, Uniformed Services University School of Health Sciences, Bethesda, MD 20814-4799.

Christian Raetz M.D.: Ph.D.: Professor, Department of Biochemistry, University of Wisconsin School of Medicine, Madison, WI 53706.

Bryan L. Roth M.D.: Ph.D.: Principal Investigator, Stop 15, Naval Medical Research Institute, Bethesda, MD 20814-5055.

John J. Spitzer M.D.: Head, Department of Physiology, Louisiana State University Medical Center, 1901 Perdido Street, New Orleans, LA 70112.

Judy A. Spitzer Ph.D.: Professor, Department of Physiology, Louisiana State University Medical Center, 1901 Perdido Street, New Orleans, LA 70122.

Taffy J. Williams, Ph.D.: Head, Metabolic Research Division, Stop 42, Naval Medical Research Institute, Bethesda, MD 10814-5055.

Lyn J. Yaffe, M.D.: Research Area Manager, Combat Casualty Care, Naval Medical Research and Development Command, Bethesda, MD 20814-5044

Meeting Coordinated by Louise Salmon, Meetings Manager, American Institute of Biological Sciences, 730 11th Street NW, Washington, DC 20001-4584. Tel: 101/628-1500.
Conference Speaker Abstracts
Cachectin (tumor necrosis factor) is a macrophage hormone originally isolated as a mediator of shock and wasting, and as a cytolysis agent. It is now clear that this protein, produced in great abundance in response to lipopolysaccharide (LPS) and certain other invasive stimuli, is a central mediator of inflammation and metabolic disturbances as they occur in the setting of invasive disease. Cachectin biosynthesis seems to be controlled at several levels. In response to LPS, cachectin gene transcription is enhanced 3-fold over the rate observed in resting cells. However, cachectin mRNA levels increase by 100-fold or more, and cachectin protein biosynthesis increases more than 1000-fold. Thus, much of the control of cachectin gene expression appears to occur at a post-transcriptional level. The 3'-untranslated region of cachectin cDNA contains a conserved element consisting entirely of A and T residues (the "TTATATAT" sequence). This element is also found in many other cytokine and proto-oncogene cDNAs and appears to confer message instability. We are presently attempting to isolate the ribonucleases responsible for this instability.
Role of lymphokines in altering hepatic metabolism in sepsis.

Abstract not received.
Charles Dinarello M.D.: Associate Professor, Department of Infectious Diseases, Tufts University Medical Center, 136 Harrison Ave. Boston, MA 02111. Synergy between tumor necrosis factor and interleukin-1.

Abstract not received.
FILKINS, JAMES P. Department of Physiology, Loyola University of Chicago at the Medical Center, Maywood, IL 60153. Cytokines and the metabolic pathophysiology of septic shock.

The pathogenesis of cell and metabolic failure in septic shock is linked to the release and/or action of peptide mediators (cytokines) from cells of the inflammatory and immune systems — especially monokines from the mononuclear phagocytes. Prior studies from this laboratory described two monokines that altered glucoregulation in septic shock: MILA or macrophage insulin-like activity and MIRA or macrophage insulin-releasing activity. Current studies indicate that interleukin-1 (IL-1) has direct effects on glucose metabolism in epididymal fat pads similar to MILA. IL-1 also exerted insulin-releasing activity in the ex vivo isolated perfused pancreas similar to MIRA. In contrast, tumor necrosis factor (TNF) was less potent than IL-1 as a glucoregulatory monokine. Thus, IL-1 may mediate the hyperinsulinism of sepsis by both direct insulin-like action and by enhancement of insulin secretion. In this fashion, IL-1 may underwrite the metabolic pathophysiology of septic shock. (Supported by Grant HL 31163).

Abstract not received.
MCKENNA, THOMAS M.,*, AND WOLFGANG A. W. TITIUS. Surgical Research Division, Naval Medical Research Institute, Bethesda, MD 20814 and Bundeswehrzentralkrankenhaus, Chirurgische Abteilung, Koblenz, West Germany. Role of monokines in altering adrenergic receptor-mediated vascular contraction in sepsis.

Vascular contractile responses to agents that induce contraction are diminished in aortas isolated from septic rats. The aortic tissue manifests subnormal maximal contraction, in vivo, after receptor (norepinephrine, NE, and vasopressin) or KCl-mediated activation. Stimulation of peritoneal macrophages in vivo by sterile silica particles also results in diminished contraction to NE by subsequently isolated aortas. Incubation of aortas in medium conditioned by endotoxin-stimulated human monocytes suppresses contractile ability; treatment of the conditioned medium with antibody to interleukin-1 (IL-1) prevents the suppression. Incubation of aortas with recombinant IL-1 or tumor necrosis factor (TNF, cachectin) results in dose-dependent suppression of contraction to NE. Induction of IL-1 mediated suppression does not require vascular endothelium, and is not ameliorated by treatment by indomethacin, but can be completely prevented by inhibition of protein synthesis by cyclohexamide or actinomycin D. Addition of phorbol 12,13-dibutyrate to aortas from septic rats or to monokine-treated aortas causes maximal contraction similar to that of tissue from control rats.

We conclude that IL-1 and TNF are likely mediators for vascular insensitivity to catecholamines associated with sepsis, that the monokine induced lesion in vascular contraction is generalized to other receptor- and nonreceptor-mediated contractile stimuli, and that the inhibition of contraction is not simply attributable to cellular damage by the monokines.
MCLEAN, STAFFORD. Central Research, Pfizer, Inc., Groton, CT 06340.

Drug discovery based on receptor binding methods.
The presentation will be an overview of drug development strategies that may be
employed depending on the degree of knowledge about the biochemistry and
receptors underlying the disease. The focus will be on development of new
therapeutic agents based on receptor binding methodology. The advantages and
limitations of receptor binding will be discussed. Mechanisms of receptor
regulation such as tachyphylaxis, competitive and noncompetitive inhibition,
and receptor interactions with second messenger systems will also be
discussed.
MILLER, VIRGINIA M.,* AND PAUL M. VANHOUTTE. Department of Biophysics, Mayo Clinic and Foundation, Rochester, MN 55905. Role of the endothelium in modulating vascular adrenergic receptor actions.

Since the description by Furchgott and his colleagues in 1980, of the essential role of the endothelium in mediating relaxations to acetylcholine, it has been shown to release relaxing substances under resting or basal conditions as well as in response to stimulation by a variety of agents, including circulating hormones (catecholamines), products released from aggregating platelets, and changes in blood flow. Endothelium-derived relaxing factor(s) act as a functional antagonist to contractility agents of the vascular smooth muscle. Therefore, the ability of the endothelium to modulate an excitatory signal depends on the efficacy of the agonist-receptor interaction. Endothelium-derived relaxing factor(s) initiate relaxation of the vascular smooth muscle through activation of the guanylate cyclase system. In addition, at least one other factor is released from endothelial cells which hyperpolarizes vascular products of the metabolism of arachidonic acid through cyclooxygenase. Chronic exposure of the endothelium to alterations in blood flow, oxygen tension, and hormones, as well as pathological conditions, affect the expression of endothelium-dependent responses.
Joseph Parillo M.D.: Director, Department of Critical Care Medicine, Building 10, National Institutes of Health, Bethesda, MD 10814. Effects of endotoxin(s) on human hemodynamics: Potential protective effects of lipid A analogues.

Abstract not received.
Human monoclonal antibodies to endotoxin: Potential therapy for sepsis.

Abstract not received.
Christian Raetz M.D., Ph.D., Professor, Department of Biochemistry, University of Wisconsin School of Medicine, Madison, WI 53706. Overview: Endotoxin biosynthesis - role of intermediates in activation of protein kinase C.

Abstract not received.
Alterations in hepatic and aortic phospholipase-C coupled receptors and signal transduction in rat intraperitoneal sepsis. Alterations in alpha-l-adrenergic activity in intraperitoneal sepsis and endotoxemia in liver and cardiovascular tissue are well described (see Chernow and Roth, Circ Shock, 1986 for review). Until recently, the mechanism for this apparent tachyphylaxis has been unknown. We proposed that receptor-mediated signal transduction might, in part, be responsible for this insensitivity. We found that each step of the alpha-l-adrenergic receptor cascade, including receptor number, phosphoinositide hydrolysis, calcium mobilization and protein phosphorylation, was decreased in rat intraperitoneal sepsis. These results imply that decrements of receptor-mediated signal transduction might be responsible for the adrenergic insensitivity found in sepsis and endotoxemia.
SPITZER, JOHN J.*, GREGORY J. BAGBY, CHARLES H. LANE, AND KAROLY MESZAROS. Louisiana State University Medical Center, New Orleans, LA 70112. Altered control of carbohydrate metabolism in endotoxemia. Altered carbohydrate homeostasis is one of the hallmarks of endotoxemia. The factors responsible for the changes, however, are not clearly understood. In order to clarify the altered control of glucose metabolism, we have studied the effects of an approximately LD-25 dose of Escherichia coli endotoxin (ET) in conscious, unrestrained rats. ET markedly increased arterial glucose concentration, glucose Ra, recycling and metabolic clearance rate (MCR). Eicosanoids did not seem to be responsible for the altered glucose homeostasis, since blocking the cyclooxygenase and lipoxygenase pathways with indomethacin or BW 755C did not prevent the ET-induced changes in carbohydrate metabolism, although it eliminated the early hypotensive response. In contrast, combined alpha plus beta adrenergic blockade prevented ET-induced increases in glucose concentration, Ra and recycling, but not MCR. Infusion of human recombinant tumor necrosis factor caused an increase in glucose Ra, implying that leukocytic mediators may also play a role in eliciting the metabolic alterations. ET also elevated glucose uptake by skeletal muscle, and by several organs that are rich in mononuclear phagocytes (liver, spleen, intestine, skin, lung). Thus, the activation of the immune system by ET may, in part, be responsible for the increased glucose clearance and metabolism to lactate. Although inducible gluconeogenic enzyme activity may be decreased by ET, hepatic gluconeogenesis is increased due to the elevated precursor concentration delivered to the liver. These studies indicate that ET altered glucose metabolism through modulators of the immune system and through catabolic hormones.
SPITZER, JUDY A.,* Louisiana State University, Medical Center, New Orleans, LA 70112. Modifications of adrenergic and vasopressin-linked lipid metabolism in endotoxemia.

We previously demonstrated alterations in hepatic vasopressin (VP) and alpha,-adrenergic receptor-effector mechanisms in chronic endotoxemia (Spitzer, J.A., Turco, E.R., Deaciuc, I.V., Roth, B.L. Progr. in Clin. Biol. Res. 235A:401-418, 1987, New York, Liss). Subsequent studies were directed toward changes in rat hepatocyte lipid content and metabolism after 30h of continuous infusion of a nonlethal dose of Escherichia coli endotoxin (ET) via an implanted osmotic pump. Changes in membrane phospholipid (PL) composition (increase in sphingomyelin and phosphatidylserine, decrease in phosphatidylcholine) were consistent with previously documented functional perturbations. Modulation of PL metabolism in ET cells included a faster and higher incorporation of [2-3H]glycerol into phosphatidic acid followed by a shift toward the synthesis of triglyceride and phosphatidylinositol (PI). Additionally, VP (10-11)-induced diglyceride (DG) accumulation was delayed and reduced 50% in ET cells. Studies with [1-14C]arachidonic acid (AA) revealed impaired activation/acylation mechanisms in ET cells resulting in decreased AA content in PI and increased amounts of [1-14C]AA remaining in the free fatty acid pool. Thus, (1) adjustments in lipid metabolic flux seem to compensate for catabolic processes known to be triggered by ET and/or fasting, (2) the diminished DG signal generation for VP receptor-linked transduction mechanisms is likely to underlie some concomitant functional impairments, and (3) defective acylation of AA may contribute to cellular metabolic perturbations by affecting the turnover of PI, a molecule involved in signal transduction, and leading to increased availability of AA for eicosanoid synthesis. (Supp. by NIH grants GM 32654 and GM 30312).
Workshop Speaker Abstracts
CARCILLO, JOSEPH A.*, B.L. ROTH‡, AND CHRISTOPHER J. HOUGH.

Department of Anesthesiology and Child Health, George Washington, University, Washington, D.C.; Surgical Research Division and Biochemistry Division, Naval Medical Research Institute, Bethesda, MD 20814. Endotoxin-derived lipopolysaccharide (LPS) induces c-myc mRNA expression in rat hepatic and vascular tissue. Molecular biology allows investigation of the role of nuclear function in disease and therapeutics. The mechanism of LPS-induced pathology may be more clearly delineated with greater understanding of the molecular effects of this important mediator of sepsis. We investigated the role of LPS in c-myc mRNA expression in hepatic and vascular tissue. C-myc mRNA expression was analyzed from the liver tissue of male Sprague-Dawley rats after intraperitoneal (IP) LPS injection, or from aortae that had been treated with LPS and 5% fetal calf serum in a physiologic organ bath preparation. C-myc mRNA expression was assayed with Northern Blot analysis and a [32P] human c-myc probe. In aorta, 10 μg/ml LPS induced expression at the 2-hr and 6-hr time points, while 100 μg/ml LPS induced increased expression from 1 through 6 hr. In liver, 10 mg/kg IP LPS induced c-myc mRNA expression at 2 hr, while 20 mg/kg IP LPS induced increased expression at the 1-hr time point. These results suggest that LPS can induce c-myc mRNA expression in hepatic and vascular tissue in a dose-dependent time responsive manner.
HERMILLER, James B.,*, I. DEUCIUC+, J.P. MEHEGAN, J.A. SPITZER+, AND B.L. ROTH. Surgical Research Division, Naval Medical Research Institute, Bethesda, MD 20814, and Department of Physiology, Louisiana State University, Medical Center, New Orleans, LA.

Endotoxemia and sepsis alter hepatic and splenic protein kinase C receptors.

Protein kinase C (PKC) is essential for the signal transduction of a diverse group of extracellular messengers. Endotoxemia and sepsis have been shown to profoundly perturb the proximal portions of PKC-linked pathways, resulting in a down-regulation of PKC-coupled receptors and an attenuation of phosphoinositide hydrolysis and synthesis. We postulated that endotoxemia and sepsis might directly affect PKC as well. We developed a technique that allowed an analysis of in situ hepatic and splenic PKC receptors. Using quantitative receptor autoradiography of (3H)-phorbol-12,13-dibutyrate (PDBu) binding, we noted a dramatic alteration in the regional distribution of bound PDBu within the liver and spleen of treated animals. In particular, the “speckled” pattern of PDBu binding noted in control rats was absent or greatly decreased in endotoxin-infused and septic animals. These results suggest that endotoxemia and sepsis either decrease the level of PKC or lower the phorbol-binding affinity of PKC.
LEE, CHE-HUNG,*, AKINDELE O. JOHNSON, ROBERT BROWN, AND LADONNA WILKERSO. Metabolic Research Division, Naval Medical Research Institute, Bethesda, MD 20814. Immunological approach of septic shock research.

Murine monoclonal antibodies (mAb) to lipopolysaccharide (LPS) of Escherichia coli J5 and its lipid A have been generated for studies of the diagnosis, prevention, and treatment of Gram-negative sepsis. The isotypes of these mAb were determined to be IgG or IgM. Results from SDS-polyacrylamide gel electrophoresis and immunoblotting experiments indicated that these mAb recognized the antigenic determinants on lipid A. In enzyme-linked immunosorbent assay (ELISA) using assay plates coated with various lipid A, rough-LPS, and smooth-LPS, the mAb show cross-reactivity with these endotoxins of various B(-) bacteria such as E. coli, Salmonella, Serratia, etc., presumably through binding to the lipid A portion of the structure. A reverse single radial immunodiffusion (rSRID) method is developed for detection of antibodies to LPS and lipid A. With endotoxin or lipid A incorporated into the agar of the immunodiffusion plates, the antibodies will diffuse from the well and form concentric rings of precipitation. This method renders the possibility of fast screening for the presence of anti-LPS or anti-lipid A in the samples and is used in the present studies. For detection of the presence of endotoxin, combination of the use of the mAb and a reagent dye was carried out in the agglutination type of reaction. It was found that the dye reagent is specific to endotoxin and B(-) bacteria in double diffusion and bacterial staining, respectively. In this test, the dye agglutinates in the presence of the coexisting mAb and endotoxin. Using this method, we were able to screen the cerebrospinal fluids (CSF) of the meningitis patients and single out the ones that have B(-) bacteria infection. Further studies are required and in progress to develop and formulate this method so that it can be used to quantitate the amount of LPS in the sample.
NIELSEN, THOR B.,* AND DAVID K. WOOD. Metabolic Research Division, Naval Medical Research Institute, Bethesda, MD 20814. Bradykinin (BK) and antagonists effects on endothelial cell (EC) phosphatidylinositol metabolism, implications for septic shock.

The potent vasodilator BK may be a factor in shock, but the action of BK on the endothelium is unclear. We used a bovine aortic EC line, strain 787, to investigate stimulation of phosphatidylinositol turnover and kinin binding. Confluent EC were incubated for 4 hr at 37°C with 1 μCi/0.5 ml of [3H]-inositol. Potential antagonists (2 x 10^-6 M final) and L-Cl (0.01 M final) were added. After 15 min, BK was added for 60 min. The reaction was terminated by replacing the supernatant with cold ethanol-HCl. [3H]-Inositol phosphate was isolated by chromatography. Among several analogs of BK tested only one, dArg-frg-Pro-Hyp-Gly-Thi-Ser-dPhe-Thi-frg, inhibited stimulation by 10^-4 M BK. The antagonist des-arg^1-leu^7-BK did block stimulation by 10^-4 M BK. Kinin binding to receptors was studied with analogs of BK labeled with ^125I on tyrosine residues at the amino terminal position (TIK), the five position (T5BK) or the eight position (T8BK). EC were trypsinized, washed with 0.1mM PMSF in PBS and then frozen. Each μg EC protein bound about 12.4 pg TIK, 4.9 pg T5BK and 5.8 pg T8BK, which was blocked by 7 μg/ml BK. Thus both metabolic and binding experiments are consistent with dual modes of BK action on EC. We are probing differential modulation of these pathways during sepsis.
PARENT, JAMES B.,* Metabolic Research Division, Naval Medical Research Institute, Bethesda, MD 20814. Core-specific receptors for lipopolysaccharide (LPS) on liver cells.

The liver is the major organ mediating clearance of LPS from the blood stream. To determine if hepatocytes have receptors for LPS, we have studied the binding of seven S-form LPS (isolated from S. illinois, S. montevideo, and E. coli strains 4, 9, 32, 75, and 86) and also the binding of five R-form LPS isolated from S. minnesota rough mutants (Ra, Rb2, Rc, Rdi, and Re) deficient in the biosynthesis of their complete (smooth) LPS polysaccharide. LPS was extensively purified and labeled with $^{14}$I to 1-5 pCi/pg using Wood's reagent.

All seven of the S-form LPS tested bind to hepatocytes via specific receptors since binding is: (1) competitive with excess cold LPS, (2) saturable (max binding from 20-200 ng LPS/10,000 cells), and (3) high affinity (1/2 max binding at 1-2 ng LPS/ml). Of the five R-form LPS tested, only LPS isolated from Ra and Rb2 mutants (and wild type) demonstrate specific binding to liver cell receptors. These results suggest that liver cell receptors recognize the LPS core region and that high affinity binding requires LPS maturation beyond the incomplete core oligosaccharide found in LPS isolated from Rc mutants. A variety of sugars were tested as inhibitors of LPS binding and only D-Man at 20 mM was a potent inhibitor of binding of all seven S-form LPS to hepatocytes. Since several of the LPS tested lack D-Man in their glycan structure, we hypothesize that liver cell receptors recognize, in part, L-glycero-D-mannoheptose in the LPS core.
Yaffe, L., A. Berning, B. Hook, and K. Kujawa. Pathophysiology Division, Naval Medical Research Institute, Bethesda, MD 20814.

Effect of macrophage inhibition in carrageenan and galactosamine-induced sensitivity to low-dose endotoxin.

We have examined the influence of macrophage inhibitors in two lethal, low-dose lipopolysaccharide (LPS) model systems. Procedures used include low-dose LPS sensitivity induced by a 24-hour pretreatment of mice with 1 mg intraperitoneal carrageenan followed by 0.5-2 μg LPS, as well as an 8-10 mg D-galactosamine intraperitoneal administration immediately followed by low-dose LPS. Trypan blue (12 mg/mouse), thought to block the release of macrophage-derived tumor necrosis factor (TNF), failed to prevent the lethal effects of LPS in either carrageenan or D-galactosamine treated 8- to 12-week-old Balb/c mice. However, in vivo administered silica particles, known to be cytotoxic for macrophages, clearly prevented the lethal effects of LPS. These results suggest that LPS lethality can be prevented by macrophage cytotoxic agents, though an inhibitor thought to be specific for the release of TNF failed to provide in vivo protection against LPS lethality.
WILLIAMS, TAFFY,* SUSAN GARTNER, LYNNE HOBAN, JOSEPH NEVOLA, THOMAS MCKENNA, TIMOTHY MORRISON, ANNIE STATON, JOHN LEUDERS, AND DAVID RUESCH.
Naval Medical Research Institute, Bethesda, MD 20814. Possible role of bacterial adherence and bacterial adhesins in sepsis and septic shock.

Both bacterial adherence and bacterial adhesin proteins play an important role in nosocomial infections, but their relationships to sepsis have yet to be examined. An in vitro study of bacterial adherence/adhesins is being examined using E. coli strain 2699 (O6:K13), a pathogenic strain expressing type 1 pili. These studies consist of measuring 1) the binding of ¹⁴C-labeled bacteria to various rat tissues and 2) the production of interleukin-1 by elicited macrophages stimulated with type 1 pili. At present, the data suggests that bacterial adherence may play a role in the establishment of wound infections and the bacterial adhesins may affect production of mediators involved in septic shock.
Poster Session
List of Posters and Poster Presentors


3. Bottoms, Gerald D., Susan Gimarc, Victor Butto, Gordon Coppoc, and Gary Lantz. Purdue University, School of Veterinary Medicine, West Lafayette, IN 47907. Plasma concentrations of endotoxin following jugular or portal injections of endotoxin, and intestinal ischemia.

4. Dunn, Charles W., Jureta W. Horton, and Paula B. Walker. Department of Surgery, University of Texas Southwestern Medical Center, Dallas, TX 75235-9031. Immunostimulant plus broad spectrum antibiotic enhance survival in fecal peritonitis.

5. Gartner, Susan L., Thomas M. McKenna, John Leuders, David Reusch, Annie Staton, Timothy Morrison, and Taffy J. Williams. Metabolic Research Division, Naval Medical Research Institute, Bethesda, MD 20814. Type I pili from E. coli stimulate interleukin-1 production in rat peritoneal macrophages.


10. Kier, Ann B. University of Cincinnati Medical School, Cincinnati, OH 45267. Hageman factor (factor XII) deficiency in cats results in a significantly decreased localized Shwartzman reaction.


BANKEY, PAUL,*, WHEN YEN WANG, RAVINDER SINGH, ANN CARLSON, AND FRANK CERRA. Department of Surgery, University of Minnesota, Minneapolis, MN 55455. Platelet activating factor (PAF) primes macrophage for lipopolysaccharide (LPS) signaled tumor necrosis factor (TNF) release mechanism by rapid increase in intracellular calcium.

Recent investigation has linked membrane inositol phospholipid hydrolysis and resultant intracellular calcium \([Ca^{++}]_{i}\) flux with macrophage activation. We have been interested in the role of this signal transduction pathway in macrophage cytokine production. Elicited peritoneal macrophages were stimulated with PAF alone, LPS alone, or the combination. Resulting changes in intracellular calcium and TNF production were assayed using the fluorescent probe Indo-1 and L929 cell lysis, respectively. PAF induced a rapid change in intracellular calcium to levels from 110nM to greater than 1µM within 5 minutes, while LPS-treated cells showed no change in resting \([Ca^{++}]_{i}\) over the same time period. PAF alone did not trigger TNF activity at concentrations that stimulated calcium flux; however, treatment prior to LPS triggering (100ng/ml) increased subsequent TNF activity from 2+/−2 units to 27+/−4 units. Additional studies have assessed phosphatidylinositol turnover (PI) and inositol triphosphate (IP-3) production by PAF and LPS using \(^{3}H\)-inositol and anion exchange chromatography. These results indicate that PAF, but not LPS, is capable of stimulating PI turnover and production of IP-3. We conclude from these studies that PAF is capable of priming the macrophage and that this effect may be signaled through inositol phospholipids.
BERSTEN, ANDREW,*, MOSHE HERSCH, ANDE NEAL, MICHAEL TROSTER, ALBERT DRIEDER, FRANK RUTLEDGE, AND WILLIAM SIBBALD. Victoria Hospital, P.O. Box 5375, The University of Western Ontario, London, Canada N6A 4G5.

Myocardial injury despite adequate oxygen transport in a model of sepsis.

The etiology of the depression in ventricular contractility characterizing sepsis is unclear. To examine the hypothesis that myocardial injury in sepsis results from ischemia, myocardial oxygen transport (MDo2) was assessed in 8 sheep before and 48-72 hours after induction of nonhypotensive sepsis by cecal ligation and perforation (CLP). Subsequently, biopsies of LV were taken from a similar group of animals 48 hours after sham laparotomy, or at 24-hour intervals post-CLP for morphological examination. Results (mean ± SD, *p<.05 paired t-test) The CI increased following CLP (4.1 ± 0.2 to 6.8 ± 0.9 L/min/M²*), while mean BP was unchanged. Morphologically, the LV displayed diffuse injury characterized by interstitial and cellular edema, contraction bands, mitochondrial degeneration, and positive PTAH staining. Concurrently, LVO2 consumption rose (9.7 ± 2.1 to 18.3 ± 4.8 ml/100g/min*) as did LV flows (105.8 ± 32.1 to 246.0 ± 98.1 ml/100g/min*). LV endo/epi ratios also rose (1.2 ± 0.1 to 1.28 ± 0.14), while LV lactate metabolism was unchanged. Conclusion Despite an apparently adequate increase in MDo2, diffuse tissue injury characterized normotensive sepsis in this model. While not excluding ischemia, this study suggests other mechanisms may cause the myocardial injury.
Endotoxin (LPS) was quantitated in canine plasma using the Limulus amebocyte lysate (LAL) chromogenic testing procedure. The assay was validated for sensitivity (10 pg/ml), recovery (90-110%), intra-assay precision (CV=5.5%), inter-assay precision (CV=10%), and stability of diluted, heat-treated, frozen samples (at least 8 wk). Analysis of canine plasma samples following sublethal IV (jugular or portal) injections of LPS revealed a rapid phase of clearance (T 1/2, <2 min) followed by a prolonged plateau of LPS just above baseline. Plasma LPS increased from undetectable amounts to > 100 pg/ml within 30 min following ischemia due to hemorrhage and to > 600 pg/ml by 2 hr following ischemia due to gastric dilation-volvulus (GDV). The LAL-chromogenic procedure is sensitive and reliable for detecting plasma LPS. LPS has a short initial T 1/2 with a rapid clearance following jugular injection and a faster clearance following portal injection. High plasma LPS occurred following intestinal ischemia due to hemorrhage or GDV. This indicates that LPS leaks into the circulation following intestinal ischemia and supports the concept that endotoxemia is a component of hemorrhagic shock and GDV.
DUNN, CHARLES W., JURETA M. HORTON, AND PAULA B. WALKER. Department of Surgery, University of Texas Southwestern Medical Center, Dallas, TX 75235-9031. Immunostimulant plus broad spectrum antibiotic enhance survival in fecal peritonitis.

Previous studies showed 24-hr pretreatment with an immunostimulant muramyl dipptide (MDP) alone enhanced survival in a peritonitis rat model. However, no data is available regarding concomitant use of antibiotics and MDP. This study used MDP (3 g/gm) in combination with Cefoxitin (15 mg/kg) in a human fecal peritonitis rat model. The fecal inoculum consisted of process human stool with predominant organisms being E. coli 2x10^6 org/ml, E. cloacae 3x10^7 org/ml, B. ovatus 1x10^6 org/ml, and Clostridia sp. 1x10^7 org/ml. Sprague-Dawley rats (103) were divided into four groups and received a .2cc fecal inoculum. Blood cultures (BC) were obtained at 4 and 24 hr post-inoculum and all animals were followed for 7 days and sacrificed.

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Treatment (Txt)</th>
<th>BC 4 hr</th>
<th>BC 24 hr</th>
<th>Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>28</td>
<td>Control, no txt</td>
<td>3.4</td>
<td>4.6</td>
<td>1(3%)</td>
</tr>
<tr>
<td>II</td>
<td>26</td>
<td>Antibx at inoc</td>
<td>2.4</td>
<td>3.8</td>
<td>14(54%)</td>
</tr>
<tr>
<td>III</td>
<td>25</td>
<td>24 hr pretxt MDP + Antibx</td>
<td>3.4</td>
<td>3.9</td>
<td>12(48%)</td>
</tr>
<tr>
<td>IV</td>
<td>24</td>
<td>24 hr pretxt MDP + Antibx at inoc</td>
<td>1.19</td>
<td>3.4**</td>
<td>24(100%)**</td>
</tr>
</tbody>
</table>

*IV < I,II,III, p < 0.05. **I,II,III,IV, NS. ***I < II,III < IV, p < 0.05

This study demonstrates an additive effect of 24-hr pretreatment with MDP and systemic antibiotic in a fecal peritonitis rat model. Clinical application of MDP may be as a prophylactic agent in surgical procedures with high septic risk.
SARTNER, SUSAN L.,*, THOMAS M. MCKENNA, JOHN LEUDERS, DAVID REUSCH,
ANNIE STATON, TIMOTHY MORRISON, AND TAFFY J. WILLIAMS. Metabolic
Research Division, Naval Medical Research Institute, Bethesda, MD
20814. Type I pili from E. coli stimulate interleukin-1 production
in rat peritoneal macrophages.

Surface components of Gram-negative bacteria, such as pili, may interact with
host cells and alter pathogenesis. We isolated and purified a mannose-specific
Type I pili from E. coli. Various concentrations of pili were incubated
with rat peritoneal macrophages and the supernatants were analyzed for
interleukin-1 (IL-1) by the thymocyte stimulation assay. We report that pili
increase IL-1 production in a dose-dependent manner.

<table>
<thead>
<tr>
<th>Condition</th>
<th>IL-1, pg/ml ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.9 ± 0.9</td>
</tr>
<tr>
<td>Pili, 0.05 ug/ml</td>
<td>110 ± 40</td>
</tr>
<tr>
<td>Pili, 0.5 ug/ml</td>
<td>420 ± 140</td>
</tr>
<tr>
<td>Pili, 5.0 ug/ml</td>
<td>850 ± 210</td>
</tr>
</tbody>
</table>

Endotoxin contamination was estimated by GLC analysis for glucosamine using
representative endotoxins as a standard. Estimates indicate pili contained
no more than 3.9% endotoxin. Experiments using commercially available lipo-
 polysaccharide (LPS) indicate this level of LPS could not, by itself, account
for the enhanced IL-1 production. We conclude that bacterial components such
as pili, acting alone or with LPS, may alter host immune responses.
Bacterial infusions are commonly used to produce an animal model of sepsis for therapeutic trials. In this study, we examine whether Escherichia coli intra-peritoneal infusion in an awake porcine model of septic shock is representative of the clinical hemodynamic septic syndrome. Three dose ranges of live bacteria were utilized: 1-2x10^10 bacteria/kg (Group A), 3-5x10^10 bacteria/kg (Group B), and 10-15x10^10 bacteria/kg (Group C). Awake animals were monitored over a 24-hour period following infusion. All animals exhibited lethargy, tachypnea, tachycardia, temperature instability, and positive blood cultures within 2 hours of bacterial infusion. Marked pulmonary hypertension with elevated pulmonary and systemic vascular resistances occurred early, followed by a 50 percent decrease in cardiac output. Group C animals developed overwhelming hypodynamic shock and died within 7 hours. At 24 hours, Group A animals had become normodynamic (cardiac output 5.2 versus control 4.9 L/min). Group B animals demonstrated hypotension and a low SVR, becoming clearly hyperdynamic with fluid resuscitation (cardiac output 6.4, at 28 hours, versus control of 4.8 L/min). We conclude that E. coli models of septic shock must be carefully characterized to hemodynamic status, as great variation can appear with bacterial dose and time sequence.

Lack of demonstrable humoral antibodies against *H. influenzae* type b in children has been associated with increased incidence of meningitis. The importance of antibody is also a factor in adults, since military recruits without antibody to *N. meningitidis* are more likely to develop disease. A latex agglutination test to detect anti-endotoxin antibodies in serum or cerebrospinal fluid (CSF) was developed using solubilized lipid A for sensitization of polybead-carboxylate latex in borate buffer at pH 8.2. The sensitized latex was stabilized by addition of fatty acid-free bovine albumin to a final concentration of 0.5 w/v percent. Testing done on 69 children's CSF random samples, suspected of exposure to Gram-negative bacterial infection, identified 54 samples with readily visible agglutination against a dark background, while 15 negative samples appeared uniformly turbid. False positive reactions, often caused by rheumatoid factors, were ruled out by rheumafex assay. Identical results were obtained by reversed single radial immunodiffusion assay (rSRID) for antiendotoxin detection in agarose gels. The easy-to-perform assay requires no equipment and can be performed in 3 to 5 minutes. Thus, the highly sensitive agglutination test will be a valuable screening tool for detecting total antiendotoxin present in immunocompromised patients. This assay can also be used to monitor the pharmacokinetics of anti-endotoxin therapy in bacteremia.
In recent years, growing medical costs have prompted a distinct trend toward simplified diagnostic immunoassay procedures that require minimal time and equipment to perform. A new JLN endotoxin reagent was used to develop a highly sensitive and specific agglutination assay for detecting Gram-negative bacterial endotoxin in biological and nonbiological fluids. The test is performed by mixing a test sample with JLN endotoxin reagent on a microvue card (18 mm circle), followed by rotation on a clinical rotator at 130 ± 2 RPM or by hand. A positive reaction is visualized after 5 minutes by a slight color change and the presence of colored precipitate. The JLN reagent-based assay detected Escherichia coli reference standard endotoxin (EC-5, U. S. Food and Drug Administration) in the range of less than 1 endotoxin unit (less than 0.1 ng/ml). The assay is useful for endotoxin detection in cerebrospinal fluid (CSF). It should be suitable for rapid diagnosis of Gram-negative bacterial meningitis as well as aid in the subsequent management and therapy of patients. Detection and correlation of endotoxin levels with various diseases, such as septicemia, are now being studied.
KANG, YUAN-HSU,*, LORRITA P. WATSON, ROBERT WILLIAMS, AND MACK HOLT.
Pathophysiology Division, Naval Medical Research Institute, Bethesda,
MD 20814. Effect of bacterial endotoxin on Ca^{2+}-ATPase and calmodulin
in rat hepatocytes.

Our previous studies indicate that bacterial endotoxin, lipopolysaccharide
(LPS), increases intracellular calcium associated with endoplasmic reticulum
and mitochondria in rat hepatocytes. It is known that Ca^{2+}-ATPase, an ATP-
driven Ca^{2+} pump, and calmodulin (CAM), an intracellular calcium receptor, are
involved in the regulation of intracellular calcium. The present study was
designed to examine the effect of LPS on the activity of Ca^{2+}-ATPase and CAM in
rat hepatocytes. Livers were fixed by perfusion with 3% paraformaldehyde
24 hours after I.V. administration of 10 mg/kg LPS (E. coli, 0111:B4) and then
processed for ultracytochemical localization of Ca^{2+}-ATPase and immunostaining
of CAM. Results showed that Ca^{2+}-ATPase activity in the hepatocytes of
endotoxic rats was distinctly decreased on the plasma membranes surrounding
bile canaliculi and spaces of Disse as compared to those in the controls. The
hepatocytes of endotoxic rats were more intensely stained for CAM than those of
controls. These LPS-induced changes in Ca^{2+}-ATPase activity and CAM are
consistent with the increase of intracellular calcium in endotoxic hepatocytes.
Hageman factor (factor XII) deficiency in cats results in a significantly decreased localized Shwartzman reaction.

In vitro, Hageman factor (HF) can be activated by endotoxin. The classical localized Shwartzman reaction (LSR) was produced in domestic cats by a preparative intradermal (ID) injection of 0.4 mg endotoxin (0111:B4) per kg body weight, followed 18 hours later by a provocative intravascular injection of the same dose of endotoxin. Blood samples were collected at 0, 1, 2, 4, 18, 19, 20, 22, 48, 72, 120, 144, and 240 hours after the first injection, and were analyzed for a complete blood count, platelet count, HF activity, and total serum protein, fibrinogen, and fibrinogen degradation product concentration. Skin biopsies were taken for quantitative histopathologic evaluation from the endotoxin ID injection sites at 1, 2, and 4 hours after the second endotoxin injection. Cats genetically deficient in HF had a decreased magnitude of change in platelet count, neutrophil count, and fibrinogen concentration as compared to cats having normal HF activity. There was a statistically significant decrease in the histopathologic severity the LSR skin lesions in the HF deficient cats, including a decrease in fibrin deposition, thrombi formation, necrosis, inflammatory cell accumulation, vasculitis, and hemorrhage. Thus the absence of HF in this model system had a protective effect against endotoxin-induced lesions.
MAZUSKI, JOHN E.,*, MARIASTELA ORTIZ, HOWARD C. TOMLE, AND FRANK B. CERRA. Department of Surgery and Biochemistry, University of Minnesota, Minneapolis, MN 55455. Direct control of hepatocyte protein synthesis by endotoxin: pretranslational regulation of a 23 kD secretory protein.

The principal regulators of hepatic acute phase protein synthesis are felt to be various monokines, although a few of these proteins may also be controlled by glucocorticoids. We have recently identified a 23 kD secretory protein which is synthesized by cultured murine hepatocytes in response to the monokine interleukin-1, glucocorticoid hormones and lipopolysaccharide (LPS). This latter effect appears to represent a direct action of endotoxin on hepatocytes, since it occurs in the absence of significant monokine release by these cultures. The present experiments indicate that regulation of this protein is at the mRNA level. RNA isolated from livers of mice stimulated in vivo with LPS was subjected to in vivo translation using the reticulocyte lysate system. Translation of RNA from LPS-treated mouse liver, but not from control mouse liver, gave rise to a 23 kD polypeptide very similar to that secreted by hepatocytes. Translation of RNA isolated from cultured hepatocytes treated in vitro with LPS produced an identical 23 kD polypeptide. These experiments indicate that the regulation of this 23 kD protein, both in vivo and in vitro, is primarily at the RNA level. At present, efforts are under way to obtain a cDNA clone corresponding to this protein.
NEVOLA, JOSEPH J.,*, LYNNE D. HOBAN, AND TAFFY J. WILLIAMS.
Naval Medical Research Institute, Bethesda, MD 20814.
In vitro adherence of a pathogenic strain of Escherichia coli to selected rat tissues.

bacterial adherence to exposed tissues may be the first step in the pathogenesis of wound infection. The specificity of such bacterial adherence to whole tissue is undetermined. We investigated in vitro adherence of E. coli strain 2699 (O6:K13), a pathogenic strain which expresses type 1 pili. 14C-labeled E. coli was found to adhere to rat tissues in this order: shaved skin > abdominal muscle > calcium hydroxide/calcium thioglycolate treated skin > kidney. Pretreatment of muscle and skin with alpha-methyl-D-mannopyranoside, a pili-binding inhibitor, reduced the levels of adhesion.
It has been proposed that in septic shock two groups of patients with differing oxygen utilization exist. One group exhibits high oxygen consumption and a lower mortality. The other group exhibits decreased oxygen consumption that is proportional to delivery and has a higher mortality. *Escherichia coli* strain B7 (086:K61) was infused in the intraperitoneal space of male yucatan minipigs to induce sepsis. Two dose ranges were used: 1-2 $\times 10^{10}$ bacteria/kg (Group A) and 3-5 $\times 10^{10}$ bacteria/kg (Group B). The animals exhibited clinical manifestations of sepsis and positive blood cultures 2 hr post infusion. On the following day, surviving animals were studied without the influence of anesthesia. All animals were clinically ill with continued positive blood cultures. Group A demonstrated normodynamic cardiovascular function with an increased oxygen utilization (282 mL O$_2$/min versus control 248 mL O$_2$/min) and a higher point of critical oxygen delivery (680 mL O$_2$/min versus control 600 mL O$_2$/min). Group B demonstrated hyperdynamic cardiovascular function with deficient oxygen utilization, dependent on flow (127 mL O$_2$/min increasing to 281 mL O$_2$/min with increasing cardiac output). These animals simulate observed clinical abnormalities in oxygen utilization in septic shock and should allow comparison of treatment modalities to increase tissue oxygen consumption.