S E P T I C S H O C K

Medical Grand Rounds
Anthony R. Dal Nogare, M.D.
January 24, 1991

"I suspect that the host is caught up in mistaken, inappropriate, and unquestionably self-destructive mechanisms by the very multiplicity of defenses available to him, defenses which do not seem to have been designed to operate in net coordination with each other. The end result is not defense, it is an agitated, committee-directed, harum-scarum effort to make war..."

Lewis Thomas, The Immunopathology of Inflammation, 1971

INTRODUCTION

There are an estimated 200,000 cases/year of septic shock in the United States, and about 50% of these patients, or 100,000 people/year, die. Septic shock affects all types of medical patients and is the commonest type of shock treated by internists. Thus, it is important for internists to be knowledgeable about it's diagnosis and treatment. Additionally, the prevalence of sepsis in hospitalized patients appears to have significantly increased over the past decade. Data from the CDC's National Hospital Discharge Survey show a 139% increase in the discharge diagnosis of sepsis from 1977 to 1987 (1). The increase was especially marked (162%) for patients over 65 years of age. It is likely that the increased sepsis rate reflects an increased number of chronically ill or immunocompromised patients who are being kept alive longer by improved medical therapy and who are also put at greater risk for sepsis by invasive medical procedures and devices. Therefore septic shock is, at least in part, a disease of medical progress, and it will continue to be a major problem.

Over the past ten years important advances in elucidating the mechanisms of septic shock have occurred, and this review will focus largely on this new information. In a broad sense, septic shock represents an extreme example of the normal inflammatory response run amuck. The systemic effects of sepsis are largely caused by the host's own endogenous cytokines, which are usually beneficial when limited to discrete tissue infection sites, but are often lethal when they enter the circulation. The major targets of circulating cytokines are leukocytes, endothelial cells, and the heart. Other than initiating the production of inflammatory mediators, the infecting microbe plays a minor role. Because inflammatory cytokines are central to pathogenesis, and because future therapies will depend on interfering with the effects of these cytokines, they will be emphasized in this discussion.

CLINICAL FEATURES OF SEPTIC SHOCK

THE SEPSIS SYNDROME

Prior to the development of overt shock, most patients exhibit a number of signs which alert physicians to the possibility of impending shock, and such patients are frequently said to appear septic. Objective criteria for recognizing septic patients were established recently by the Methylprednisolone Study Group and are listed on Table 1 (2).

TABLE 1

SEPSIS SYNDROME CRITERIA

1. Clinical evidence of infection
2. Temperature > 101°F or < 96°F
3. Respiratory rate > 20/min.
4. Pulse > 90/min.
5. One or more manifestations of inadequate organ function:
   - Altered Mental Status
   - PaO2 < 72 mm Hg
   - Elevated lactate level
   - Oliguria (less than 30 cc/hr)

These criteria were used to prospectively identify 191 patients, of whom 113, or 64%, subsequently developed shock. Positive blood or tissue cultures were not required, and only 45% of the patients had positive blood cultures. There were no differences in either the clinical characteristics or the laboratory values of blood culture negative and positive patients, and shock developed with similar frequency in both groups. Furthermore, mortality rates were similar (30 versus 36%) for the non-bacteremic and bacteremic shock patients. Thus, it is apparent that objective evidence of infection is often absent in septic patients and bacteremia is not necessary for shock. Similar findings have been noted by other investigators (3). The fact that bacteremia is frequently absent in septic shock is less surprising when one considers animal studies in which the induction of an inflammatory response by intraperitoneal injection of sterile zymosan reproduced the physiologic and pathologic changes of septic shock (4).

Other pertinent findings of the methylprednisolone study were that shock complicated gram positive bacteremia as often as gram negative bacteremia and that shock usually occurred rapidly after the onset of the sepsis syndrome; 85% of the patients developing shock did so within 24 hours. Other studies have shown that shock more often complicates aerobic gram negative bacteremia; in one study of 500 bacteremic patients, 26% of patients with aerobic gram negative bacteremias developed shock, compared to 12% of patients with gram positive bacteremias (5). Patients with sepsis usually have an acute respiratory alkalosis and, if a Swan Ganz catheter is in place, they will usually have an elevated cardiac index (CI, normal range 2.5-4.0 $l/min/m^2$), a low or low normal systemic vascular resistance (SVR, normal range 800-1600 $dynes \cdot sec/cm^5$), and a slight fall or no change in blood pressure (6).

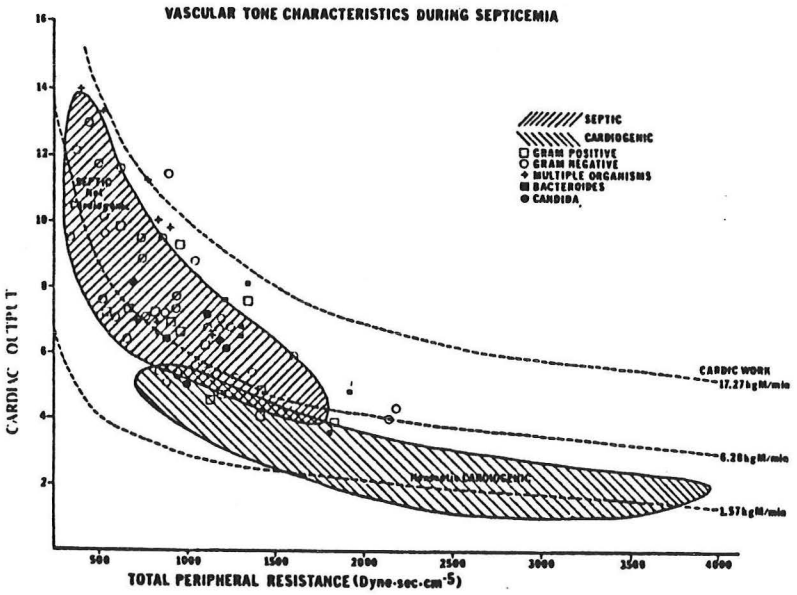
SEPTIC SHOCK

Septic shock is defined as a mean blood pressure of < 60 mm Hg in a patient with clinical evidence of an infection. Septic

shock is classified as a distributive type of shock, which means that total blood flow is normal but flow to metabolically active tissues is inadequate. Other types of distributive shock are neurogenic, anaphylactic, and endocrinologic (adrenal insufficiency). The diagnosis of septic shock is usually not difficult, although both cirrhosis and thiamine deficiency cause a hemodynamic pattern similar to that of sepsis.

The major hemodynamic characteristic of septic shock is a low SVR, which is diagnostically helpful because it usually overlaps minimally with SVR values of patients in hypovolemic or cardiogenic shock (Figure 1). Patients are almost always tachycardic and have a normal or high CI. Several reports of septic shock patients published in the 1960's and 1970's suggested that there were two hemodynamic types of septic shock.

FIGURE 1

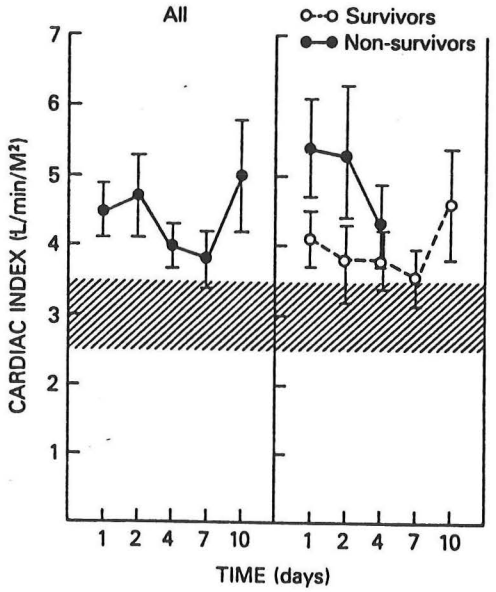


Crit. Care Med. 8:58, 1980.

Patients with warm septic shock had a high CI, while those with cold septic shock had a low CI, and it was thought that warm shock commonly evolved into cold shock and that patients with low CI had significantly higher mortality (7-9). However, fluid administration to these patients was guided by central venous pressure measurements, which correlate poorly with the pulmonary capillary wedge pressure (PCWP) of septic patients (10), and septic shock patients often require large amounts of fluid for initial resuscitation, due to their increased capillary permeability and low intravascular volume (11, 12).

In most recent series septic shock patients have been treated with vigorous volume repletion to maintain a PCWP of >12 mm Hg, and several investigators have shown that in volume repleted patients the CI remains elevated, even within hours of death (12-14). Typical hemodynamic data from one recent series is shown (Figure 2). Thus, the concept of a low CI form of shock is erroneous and likely reflected intravascular volume depletion. Although most hemodynamic studies have been performed on patients with aerobic gram negative bacterial infections, identical hemodynamic alterations are present in patients infected with gram positive bacteria, anaerobes, and fungi, a finding which supports the importance of host derived cytokines, rather than microbial products, as common mediators (15).

FIGURE 2



Ann. Int. Med. 100:485, 1984.

Although all types of patients may develop septic shock, it occurs most commonly in elderly patients with genitourinary, abdominal, or thoracic infections. Both the frequency of shock as a complication of bacteremia, and the mortality of shock, correlate positively with increasing age (5). Many of the characteristic signs may be absent in elderly patients, in whom tachycardia and altered mental status may be the only findings (16). Review of four series of bacteremic septic shock reveals that about 60% were caused by aerobic gram negative bacteria, 20-40% by gram positive bacteria, and the remainder by anaerobes (Bacteroides fragilis) or fungi (Candida albicans) (5, 6, 15, 17). In addition to its cardiovascular effects, septic shock affects multiple other organs (Table 2).

TABLE 2

NON-CARDIOVASCULAR EFFECTS OF SEPTIC SHOCK

SYSTEM	ABNORMALITY	REFERENCE
CNS	Encephalopathy, agitation	(18)
GI	Cholestatic jaundice	(19)
	Gastric erosions in 100% (gross bleeding rare)	(20)
BLOOD	Early neutropenia, then neutrophilia	(18)
	DIC, especially with gram negative bacteremia	(21)
	Thrombocytopenia in 50%	(22)
RENAL	Proteinuria	(18)
	Focal proliferative glomerulonephritis	(23)
	Acute tubular necrosis	(24)
METABOLIC	Hyper, hypoglycemia	(18, 25)
	Skeletal muscle proteolysis	(26)
	Hypertriglyceridemia	(27)
	Lactic acidosis	(28)
PULMONARY	Respiratory alkalosis	(29)
	↑ A-a O_2 gradient	(18)
	Respiratory muscle fatigue/failure (terminal)	(18)

Although most septic shock patients are febrile hypothermia may be present (especially in the very young and very old) and usually predicts a poor prognosis (2, 5). In a series of 85 patients presenting off the street with hypothermia (temperature < 35C°), the presence of a low SVR and high CI was a helpful clue to an infectious etiology; all of the bacteremic patients had SVR of < 800 dynes·sec/cm^5, whereas none of the nonbacteremic patients had an SVR of < 1100 (30).

Several observations, in addition to the frequent presence of lactic acidosis, suggest that cellular oxygen utilization is impaired in septic shock. Normally, oxygen consumption ($\dot{V}O_2$) is independent of oxygen delivery ($\dot{D}O_2$, which is the cardiac output X hemoglobin concentration X % O_2 saturation) until $\dot{D}O_2$ reaches low levels (8-10 ml O_2/kg/min in normal anesthetized humans) (31). In septic shock, $\dot{D}O_2$ is normal or high and increasing $\dot{D}O_2$ is frequently associated with an increased $\dot{V}O_2$, a phenomenon which has been called supply dependency of $\dot{V}O_2$. The phenomenon occurs most often in septic shock patients in lactic acidosis, in whom increasing $\dot{D}O_2$ both increases $\dot{V}O_2$ and decreases lactic acid levels (32-34). Associated findings in these patients include normal (> 35 mm Hg) mixed venous oxygen tensions (PVO_2), low arterial-venous oxygen content differences, and low O_2 extraction ratios. Because the PVO_2 is often normal in septic shock, it should not be used as an index of normal tissue oxygenation.

Three possible explanations for abnormal oxygen utilization are the presence of arterial-venous shunts, diversion of blood flow to metabolically quiescent tissues, or impaired oxygen extraction. Based on experimental and clinical data, arterial-venous shunts are not present (35, 36). Measurement of regional blood flow in a dog model of bacterial lipopolysaccharide (LPS) induced shock showed that marked (50-90%) decreases in splanchnic and renal blood flow occurred while flow to resting skeletal muscle increased by 50-100% (37). Supply dependency of $\dot{V}O_2$ can be induced in dogs by intravenous administration of 15 micron polystyrene microspheres or live bacteria (38, 39). To extrapolate these results to human septic shock, it is likely that a combination of both decreased flow to visceral organs (due to abnormal vasoregulation) and impaired oxygen extraction (due to capillary occlusion by leukoemboli and fibrin deposits) explains the frequent occurrence of lactic acidosis, normal PVO_2 values, and supply dependency of $\dot{V}O_2$ in the face of a normal or elevated cardiac output.

NATURAL HISTORY

As noted, the mortality of septic shock is high and averages 50%. About 75% of deaths occur within hours to days of shock onset and are due to persistent hypotension which is refractory to treatment. The other 25% of deaths occur days to weeks after patients have been successfully treated for hypotension and are due to the development of multiple organ failure (40). Respiratory failure, manifest as the adult respiratory distress syndrome or ARDS, usually develops first, followed by renal, hepatic, or hematologic failure. Septic shock is one of the commonest causes of ARDS (41). Survival varies inversely with the number of failed organ systems, and few patients survive after three organs fail. Multiple organ failure is a common cause of prolonged intensive care unit stays, and the average cost of caring for one such patient has been calculated at $85,000. Multiple organ failure probably occurs due to an initial endothelial injury which occurs during shock and impairs visceral organ perfusion, leading to reduced substrate delivery and eventual organ failure. Persistent bacterial infections are commonly present in patients with multiple organ failure. The infections are often gram negative bacterial, are from ongoing sepsis or a new infection, and may be clinically occult; abdominal and thoracic infections are common in the latter group (42, 43).

PATHOLOGY

The major findings of septic shock, which are present to some extent in most tissues, are cell necrosis, tissue hemorrhage and edema, and fibrin deposition (44). The lungs are the most heavily involved organ and typically show diffuse interstitial edema and polymorphonuclear leukocyte (PMN) accumulation.

Centrilobular necrosis involving more than 25% of liver mass is common, as are mucosal erosions of the gastrointestinal epithelium. Evidence of gross myocardial injury (i.e. infarction) is unusual. However, several investigators have reported ultrastructural changes in canine and primate hearts obtained six to 48 hours after gram negative bacteremic shock. The observed changes included interstitial edema, capillary endothelial cell swelling, PMN accumulation, and myocyte necrosis (45-47).

CARDIOMYOPATHY OF SEPTIC SHOCK

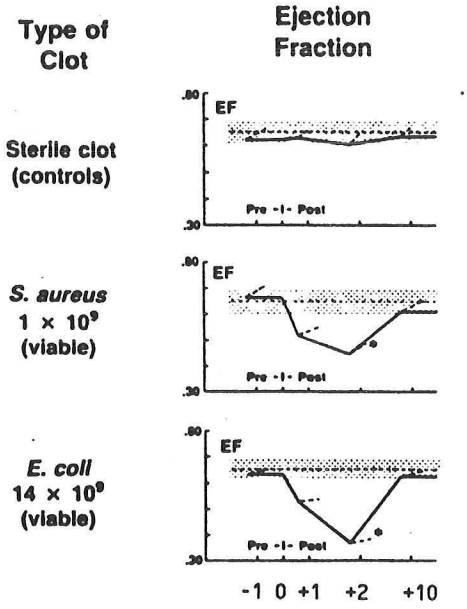
Although the CI in septic shock is often above normal, it is not high enough to maintain an adequate blood pressure. Given a typical septic shock SVR of 600 dynes·sec/cm^5, a CI of 4.8 L/min/m^2 will result in an inadequate mean blood pressure of 60 mm Hg, while a CI of 6.0 L/min/m^2 will result in a normal mean pressure of 80 mm Hg. Since a normal heart should be capable of a 6 liter CI, it is clear that ventricular performance is relatively impaired in septic shock. A hemodynamic study of 50 septic shock patients in 1973 found that many patients failed to increase CI after intravenous volume challenges sufficient to increase PCWP by 19%, suggesting impaired contractility (17). A similar study of fifty normotensive septic patients showed that 50% failed to augment CI after volume infusion (10).

To investigate cardiac performance in a controlled fashion, investigators at the NIH have developed a dog model of septic shock. Bacteria within fibrin clots are placed intraperitoneally, resulting in bacteremia, hypotension, and gradual recovery over seven days. The model reproduces the low SVR-high CI pattern of human septic shock and is a considerable advance over prior animal models, which used large intravenous boluses of live bacteria or LPS to induce a low CI, high SVR shock very dissimilar from septic shock (48). Using this realistic dog model, the following results have been obtained.

1. Viable Escherichia coli, Staphylococcus aureus, or formalin fixed Escherichia coli all produced the same hemodynamic pattern of a low SVR, high CI. LPS was not detectable in the circulation of Staphylococcus aureus infected dogs, thus ruling out entry of LPS into the blood from gut lesions (49). These results reinforce clinical studies showing that all types of infection, with or without bacteremia, produce identical hemodynamic changes.

2. The left ventricular ejection fraction fell within 24 hours of infection, then gradually returned to normal over ten days (Figure 3).

FIGURE 3

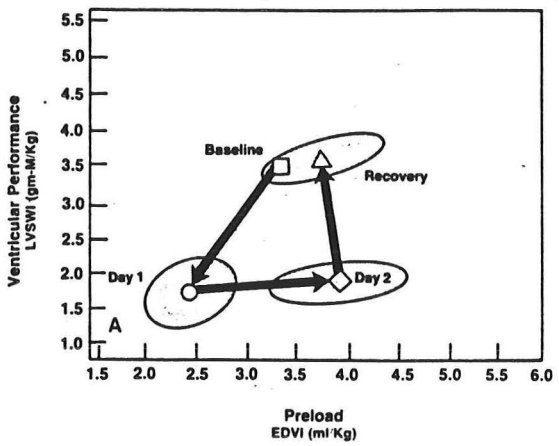


J. Clin. Invest. 83:246, 1989.

3. Ventricular performance was assessed by measuring the left ventricular stroke work index (LVSWI) versus preload (measured as the end diastolic volume index, EDVI) (Figure 4). Preload was maximized on day 2 of shock by infusion of large (80 ml/kg) volumes of saline solution. As shown on the figure, LVSWI was less than normal on day 1 and day 2, when the EDVI had been increased to normal by volume replacement. With recovery, the animals ventricular performance returned to baseline (50). These results show that maximizing preload had very little effect on cardiac output.

FIGURE 4

LEFT VENTRICULAR PERFORMANCE BEFORE, DURING, AND AFTER SEPSIS



J. Clin. Invest. 78:265, 1986.

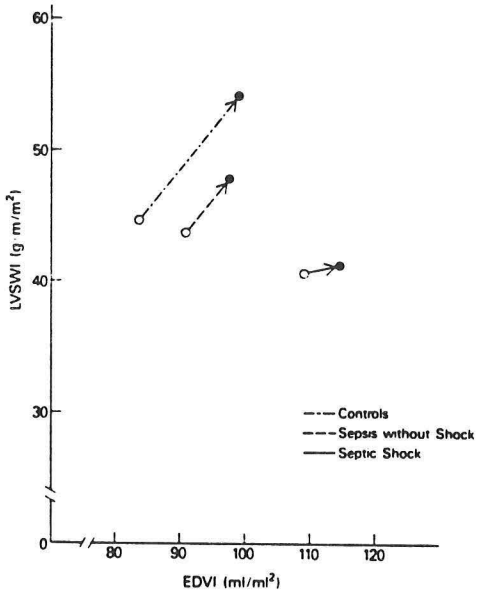
4. Endomyocardial biopsies, performed 48 hours after clot implantation when the ejection fraction had decreased from 71 to 42%, showed PMN accumulation, edema, and myocyte necrosis (47).

In summary, these animal experiments have shown that impaired ventricular performance, manifested by a low LVSWI and ejection fraction, occurs routinely in the course of septic shock and may be a transient phenomenon.

Similar careful hemodynamic studies have been performed on septic shock patients by the same group of NIH investigators. Patients have been treated by a standard protocol of volume infusion (to maintain a PCWP of > 12 mm Hg) and dopamine (titrated to a mean blood pressure of 60 mm Hg). Serial data on 48 patients have been collected. Impaired left ventricular performance has been noted, with septic shock patients, but not normotensive septic patients, having lower LVSWI which increase minimally after volume challenge (Figure 5) (51). Differences in afterload, vasoactive drugs, or the presence of pre-extant heart disease did not explain impaired contractility. Subnormal left and right ventricular ejection fractions were noted as well, and ejection fractions returned to normal as patients recovered (52).

FIGURE 5

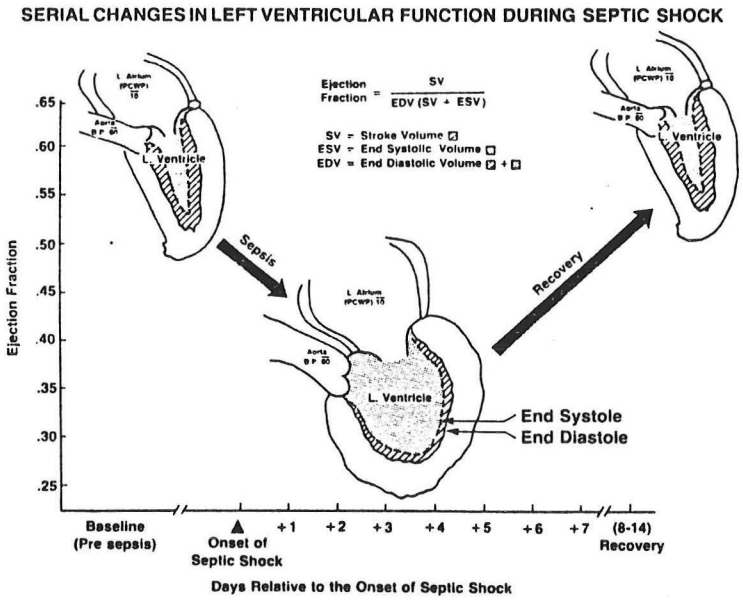
VENTRICULAR DYSFUNCTION IN SEPTIC SHOCK



Chest 93:908, 1988.

Radionuclide scans showed that left ventricular dilatation occurred 1-2 days after shock onset, with marked increase of both end diastolic and end systolic volumes (53). The increased ventricular volumes allowed a near normal stroke volume despite the low ejection fractions, and left ventricular dilatation was noted to be transient, ventricular volume returning to normal as patients recovered (Figure 6). These results show that impaired contractility and a dilated cardiomyopathy are present in human septic shock.

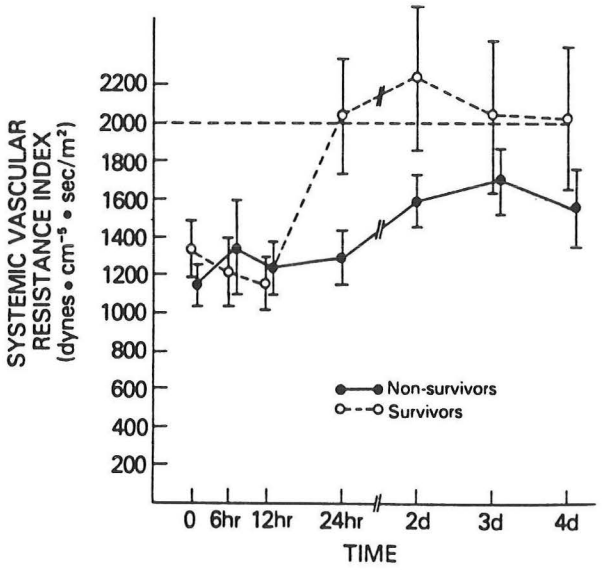
FIGURE 6



Clinical Research 38:208, 1990.

When hemodynamic data from survivors and nonsurvivors were compared, both groups were indistinguishable at shock onset. However, within 24 hours there were clear differences between the two groups, with survivors having higher SVR, lower heart rates, and lower CIs (Figure 7). Thus, the ability to rapidly recover vascular tone and return SVR to normal levels is apparently important for survival. Interestingly, the 1-3 day post shock ejection fractions of survivors were much lower than nonsurvivors, most of whom had normal ejection fractions; the likely explanation is that the nonsurvivors low SVR decreased left ventricular afterload enough to normalize their ejection fractions.

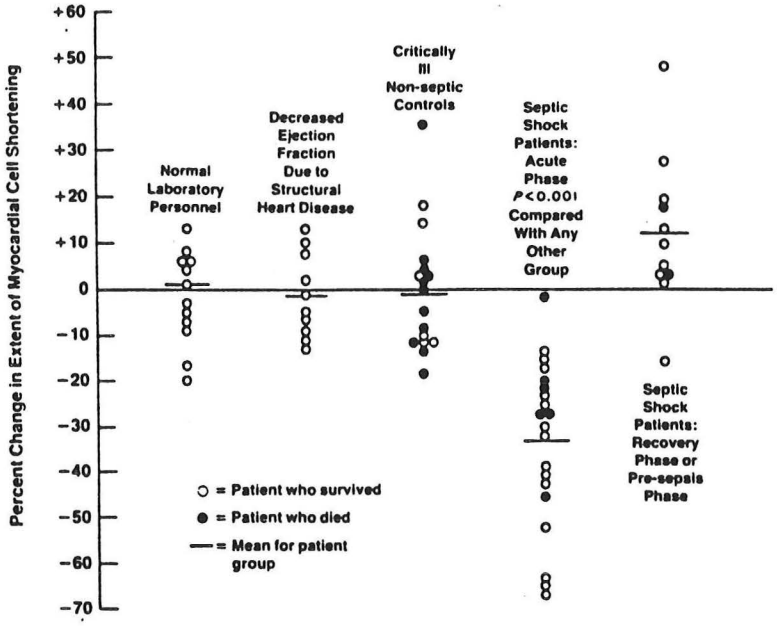
FIGURE 7



Crit. Care Med. 15:926, 1987.

What causes the cardiomyopathy of septic shock? Coronary circulation appears to be adequate, as judged by above normal coronary artery flow, normal myocardial oxygen consumption, and net myocardial lactate extraction even in patients with low ejection fractions (54). The existence of a circulating myocardial depressant factor (MDF) was first suggested by Lefer in 1967. A substance in the plasma of animals in hypovolemic shock was found to cause a marked (60%) decrease in tension development by isolated papillary muscle preparations. The MDF originated from the splanchnic circulation and was partially characterized as a < 1 kD molecular weight peptide (55, 56). A similar low molecular weight protein with MDF activity has been detected in serum of septic shock patients, using a rat myocyte bioassay. Serum from twenty septic shock patients caused a 34% mean decrease in both the extent and velocity of myocyte shortening (Figure 8), and there was an inverse correlation between individual patients MDF activity and ejection fraction (57, 58). Patients with cardiac disease, critically ill patients without sepsis, and septic patients not in shock served as controls, and MDF activity was absent in all. It is interesting to speculate that human MDF activity might originate from the gastrointestinal tract, since pancreatic and gut blood flow is decreased in animal septic shock models, in hypovolemic shock MDF originates from the pancreas, and MDF positive patients usually have lactic acidosis, suggesting that tissue ischemia is associated with MDF release. Inflammatory cytokines and LPS have also been tested for their effect on myocardial function, using

FIGURE 8



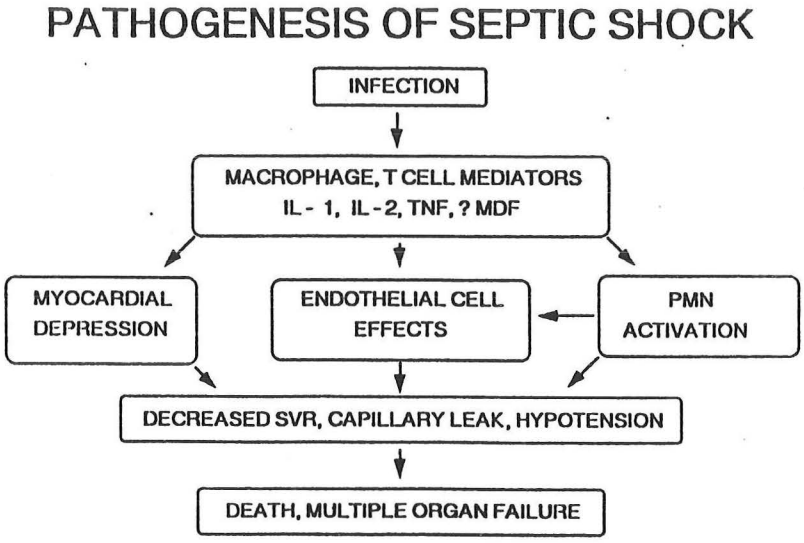
J. Clin. Invest. 76:1546, 1985.

the <u>in</u> <u>vitro</u> rat myocyte assay. High concentrations of LPS (200 μg/ml), IL-1 (1,000 units/ml), and IL-2 (1,000 units/ml) had no effect, but low concentrations of tumor necrosis factor (TNF) (5 ng/ml) depressed contraction by 24% (59). However, it is unlikely that TNF accounted for much of the MDF activity measured in patients, since the molecular weight of TNF is 17 kD. Thus, at the present time the identity of MDF remains an enigma.

PATHOGENESIS

The subsequent discussion will generally follow the outline (Figure 9) with particular emphasis on the role played by the endothelium, both as a target for cytokines and as a source of additional mediators.

FIGURE 9



BACTERIAL PRODUCTS AND SEPTIC SHOCK

Macrophages become activated by phagocytosing opsonized bacteria via macrophage Fc and C3b receptors (60, 61). Fibronectin, a large molecular weight serum glycoprotein, is also capable of opsonizing bacteria (62). In addition to these general mechanisms of macrophage activation, bacteria also contain substances capable of specifically stimulating cytokine release from macrophages and lymphocytes.

GRAM POSITIVE BACTERIA - The gram positive bacterial cell wall contains peptidoglycans, including the immunostimulatory muramyl dipeptide (63). Picogram concentrations of pneumococcal cell wall elicit interleukin 1 (IL-1) production by human monocytes, and higher concentrations cause tumor necrosis factor (TNF) release as well (64). Both Staphylococcus aureus and Streptococcus pyogenes produce structurally homologous toxins (S. aureus enterotoxins A, B, C, D, E, toxin shock syndrome toxin 1 (TSST-1), S. pyogenes exotoxins A, B, C) which bind to class II MHC molecules (particularly DR) on antigen presenting cells (65, 66). T cells bind to the MHC-toxin complex and are activated, resulting in a polyclonal T cell stimulation and cytokine release. In vitro, picogram toxin concentrations cause prolonged (3-6 day) release of TNF and interleukin 2 (IL-2) by human blood mononuclear cells (67, 68). Rabbits receiving microgram amounts of TSST-1 rapidly (within hours) develop characteristic hemodynamic changes of septic shock and have measurable circulating TNF (69). Humans infected with toxin producing S. aureus and S. pyogenes develop typical septic shock hemodynamics (70). Thus, both exotoxins and cell wall components of gram positive bacteria can activate mononuclear phagocytes and T cells, resulting in IL-1, IL-2, and TNF release.

GRAM NEGATIVE BACTERIAL LIPOPOLYSACCHARIDE (LPS) - LPS is a major factor when gram negative bacterial infections are complicated by shock. Most of the hemodynamic effects of LPS are now considered to be secondary to LPS-induced synthesis and release of TNF from macrophages. The most convincing evidence for this comes from studies of C3H/HeJ mice, which cannot make TNF and tolerate large LPS doses without ill effects (71). Macrophages are extraordinarily sensitive to LPS, synthesizing large (1-2% of total protein synthesis) amounts of TNF when exposed to small amounts of LPS (72). In an *in vivo* rabbit model, 10 μg LPS caused release of 130 μg TNF into the circulation (73).

In addition to its role as an agonist for TNF production, LPS also has a number of direct effects which may be important. LPS activates complement via the alternate pathway (74). *In vitro*, endothelial cells treated with ng LPS concentrations release prostacyclin, develop increased permeability, and have decreased thrombomodulin activity in conjunction with increased tissue factor activity (75, 76). Tissue factor activates factor VII and initiates blood clotting via the extrinsic pathway. LPS also promotes coagulation by activating factor XII (77), and the procoagulant effects of LPS, in association with LPS-induced release of plasminogen activator inhibitor and decreased thrombomodulin activity, explain the association of gram negative sepsis with DIC (78). LPS has a number of direct effects on PMNs, including increased adhesiveness (via expression of CD11/CD18 on PMN membrane) and priming of PMNs for oxygen radical release (79, 80). Enhanced PMN adhesiveness may explain the neutropenia which commonly occurs during septic shock and which is not mediated by TNF (73).

A number of investigators have given bolus LPS injections (4 ng/kg) to normal human volunteers and measured subsequent hemodynamic and biochemical changes. The earliest effect was a rapid rise and fall in TNF levels, which returned to the normal undetectable level within 4 hours. Tachycardia, fever, increased CI, decreased SVR, and a 20% fall in blood pressure were present 2 hours after LPS, persisted for several hours, and were accompanied by a fall in left ventricular ejection fractions at 4 hours. Biochemical changes included an early rise in circulating plasminogen activator activity, followed by a prolonged increase in plasminogen activator inhibitor activity, epinephrine, and ACTH levels. Circulating PMN elastase-α1PI complexes were present, and evidence of subclinical pulmonary impairment, manifested by an increased alveolar to arterial oxygen tension gradient and increased alveolar epithelial permeability, was present (81-84). Taken together, these results indicate that even small amounts of LPS cause TNF release and produce, in a mild form, many of the characteristic abnormalities of septic shock as well as PMN activation. It is important to realize that circulating LPS can be present without gram negative bacteremia; a recent study of LPS levels in the blood of 100 consecutive septic shock patients found 43% had detectable LPS, 46% of the

LPS positive patients had negative blood cultures, and LPS positive patients had lower SVR, ejection fractions, were more often in lactic acidosis, and developed ARDS and renal failure more frequently than LPS negative patients (85). Thus, circulating LPS indicates a poor prognosis in septic shock.

ENDOGENOUS MEDIATORS OF SEPTIC SHOCK

Available evidence indicates that three host derived mediators - TNF, IL-1 and IL-2 - cause many of the manifestations of septic shock, either directly or by the generation of secondary mediators such as prostaglandins and platelet activating factor. Although each mediator will be reviewed separately, it is important to keep in mind that in vivo, these mediators undoubtedly interact with each other (and with bacterial products) in complex ways. Thus the clinical phenomenon of septic shock is probably due to more than the effects of individual mediators.

TUMOR NECROSIS FACTOR - Since TNFs structure was determined in 1985 there have been a plethora of investigations of its effects. As noted previously, LPS is a potent stimulus for TNF production but gram positive bacteria, viruses, and even Mycobacteria also stimulate macrophage TNF production (86, 87).

In Vitro Effects - TNF, at concentrations similar to those achievable in vivo, affects a number of cells (Table 3).

TABLE 3

IN VITRO EFFECTS OF TNF

CELL	EFFECT	REFERENCE
PMN	↑ adherence to endothelial cells	(88)
	↑ oxygen radical, enzyme release	(89, 90)
skeletal muscle	↓ transmembrane potential	(91)
adipocyte	↓ lipoprotein lipase activity	(92)
macrophage	↑ IL-1, PAF, PGE_2 release	(93, 94)
hepatocyte	↑ actute phase protein synthesis	(95)
	↓ albumin synthesis	(85)
endothelial	↑ platelet activating factor	(96)
	↑ tissue factor, ↓ thrombomodulin	(97)
	↑ prostacyclin release	(98)
	↑ adhesion (ICAMs, ELAMs) proteins	(99)
	↑ interleukin 1 release	(100)
cardiac	↓ contractility	(59)

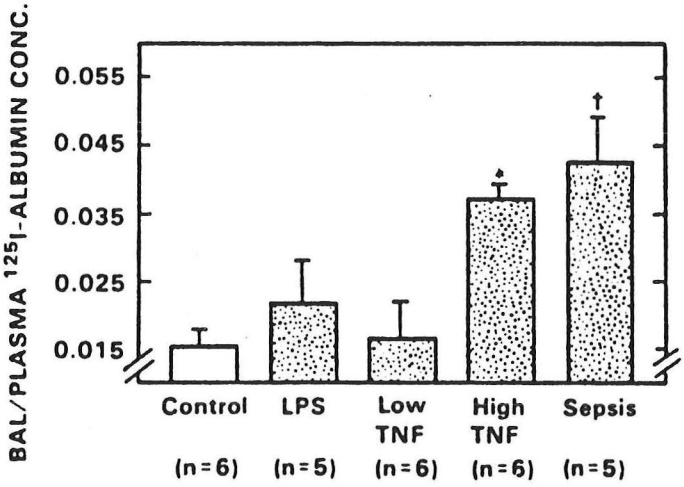
The large number of endothelial cell effects is particularly significant in light of the major role played by the endothelium in septic shock (vide infra). The time required for TNF effects is variable; PMNs and cardiac myocytes are affected within minutes, platelet activating factor and prostacyclin release

requires 1-3 hours, and interleukin 1 release, endothelial cell adhesion proteins, and the other effects require 6 hours to days to occur. TNF also damages human endothelial monolayers, causing cells to round up and develop endothelial gaps, but this effect requires 3-4 days of TNF exposure (101).

Animal Studies - The most convincing evidence for TNFs role in septic shock comes from animal experiments, in which similar results have been found when TNF (in ng/kg doses) was given intravenously to rats, guinea pigs, and dogs. The response to infused TNF includes hypotension, metabolic acidosis, hemoconcentration, hypoxemia, neutropenia, and frequently death. Pathologic changes are similar to those found in patients dying from sepsis and include pulmonary edema and hemorrhage, diffuse fibrin deposits, and accumulation of PMNs in multiple organs (102-104). Increased skeletal muscle lactic acid production has been noted in dogs, and increased pulmonary capillary permeability, evidenced by increased lung wet weights and increased flux of ^{125}I albumin from the circulation into alveoli, has been demonstrated in guinea pigs (Figure 10). The hemodynamic pattern present in dogs following a bolus (60 μg/kg) TNF injection is similar to that of human septic shock, including a high CI, low SVR, and depressed ejection fraction (104). Thus, TNF reproduces the hemodynamic, biochemical, pathological, and capillary permeability changes which are typical of septic shock.

FIGURE 10

EFFECT OF TNF ON PULMONARY CAPILLARY PERMEABILITY

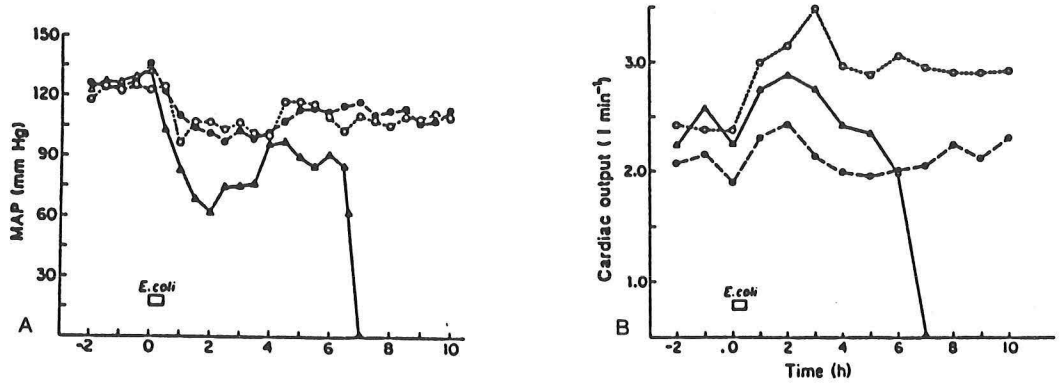


Amer. Rev. Respir. Dis. 137:1366, 1988.

Additional evidence of TNFs central role in septic shock comes from experiments with rabbits and baboons in which pretreatment with anti-TNF monoclonal antibodies (mAbs), followed by intravenous challenge with either live bacteria, LPS, or TNF, completely prevented the metabolic and hemodynamic changes of septic shock (Figure 11) (73, 105).

FIGURE 11

PROTECTIVE EFFECT OF ANTI-TNF MABS IN BABOONS FOLLOWING E. COLI SEPSIS



Circles represent animals pretreated with anti-TNF Mab

Crit. Care Clinics 5:42, 1989.

TNF In Humans - For obvious reasons, large doses of TNF have not been administered to humans, but TNF has been given therapeutically to cancer patients. Patients receiving more than 150 $\mu g/m^2$ intravenous TNF frequently developed, within hours of TNF administration, fever, tachycardia, and a fall in blood pressure (92). Muscle protein catabolism and hypertriglyceridemia were noted as well. As previously discussed, small amounts of LPS result in the rapid appearance of circulating TNF (but not IL-1) (82), and TNF has been detected in the blood of patients with falciparum malaria and meningoccocal infection (106, 107). In the latter study, of the 79 patients tested, 18 had TNF in their blood; 50% of hypotensive patients had detectable TNF versus only 12% of normotensive patients, there was a strong inverse correlation between the white blood cell count and the TNF level, and all patients with TNF levels > 100 picogram/ml died. Although not conclusive, the data suggests an association between TNF and shock in a naturally occurring human disease.

TNF and Secondary Mediators - Although it is certain that TNF is a very important mediator of shock, it is less certain that TNF directly causes shock. IL-1, prostaglandins, and

platelet activating factor (PAF) are formed in response to TNF and may be of pathophysiologic importance. Both TNF and IL-1 cause endothelial cell PAF release, and the effects of the two cytokines are additive (96). Minute PAF concentrations increase vascular permeability, and PAF has potent hemodynamic effects, but animal studies have shown that intravenous PAF causes hypotension, an elevated SVR, and decreased CI, which is not the pattern of septic shock (108, 109). Rats injected with TNF or LPS developed bowel necrosis (associated with significantly increased amounts of PAF in the intestines) and decreased renal blood flow, and both of these changes were absent when the animals were pretreated with specific PAF antagonists (110, 111). Thus, PAF may be an important secondary mediator of bowel and renal injury. TNF induces synthesis of vasodilatory prostaglandins of the E and I series, and indomethacin, given one hour before or one hour after a large intravenous TNF dose, completely blocked metabolic acidosis, shock, and death in rats (112). Similar results have been reported in dogs pretreated with ibuprofen and given LPS (113). The role of prostaglandins in hypotension will be subsequently discussed in more detail.

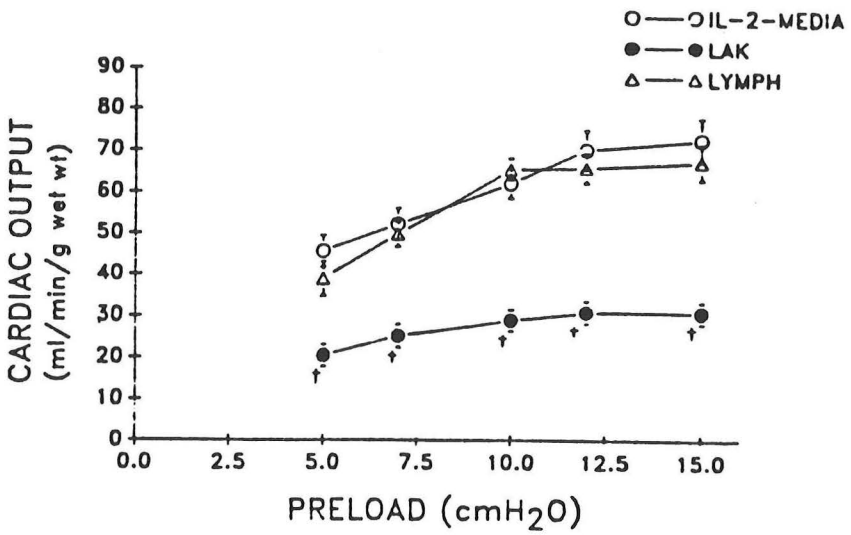
INTERLEUKIN 1 - IL-1 is released from macrophages and endothelial cells by TNF, and LPS and gram positive bacterial toxins cause macrophages to release IL-1 (114). Small amounts of IL-1 increase endothelial tissue factor and plasminogen activator inhibitor activity, and IL-1 treated rabbits have fibrin deposits on aortic endothelium (115). IL-1 stimulates endothelial prostacyclin production and induces skeletal muscle proteolysis via a PGE_2 regulated process (116-118). Synergy between TNF and IL-1 has been demonstrated for dermal inflammatory reactions and shock (119). Rabbits, given 5 ng/kg doses of TNF and IL-1 (doses which, when given separately, had no effect), developed a low SVR shock (120). Evidence that IL-1 and TNF are regulated in a coordinated fashion is that the MRNA for both contains a unique TTATTTAT octamer sequence in the 3′ untranslated end. The sequence is also found in interferon and fibronectin message and probably regulates cytokine synthesis by affecting message stability (121). IL-1 was detectable in baboon blood after gram negative bacteremia, but IL-1 levels increased late, after hypotension occurred (122) IL-1 was not detected in humans after LPS, and large (1 mg/kg) IL-1 doses did not produce any hemodynamic changes in dogs (82, 48). The available evidence suggests that IL-1 plays an ancillary role in septic shock, IL-1 production being stimulated by TNF and IL-1 potentiating many of TNF's effects.

INTERLEUKIN 2 (IL-2) - As reviewed earlier, many gram positive bacterial toxins cause lymphocyte IL-2 release. IL-2, either alone or with the concomitant infusion of lymphokine activated killer cells, has been given as a cancer therapy, and the resultant toxicity has provided insight into septic shock. About 65% of patients treated with IL-2 have developed hypotension, which was associated with increased vascular permeability (measured by ^{125}I albumin egress from blood) and some fatalities

(123). Serial hemodynamic study of these patients revealed typical septic shock features, with low SVRs, high CIs, and low ejection fractions (124). In vitro, IL-2 causes natural killer cells (a type of circulating lymphocyte) to adhere to and damage human endothelial cells (125). Experiments with an isolated rat heart model have shown that IL-2 by itself, even at very high concentrations (10,000 units/ml), does not affect ventricular performance. However, supernatants from lymphocytes treated ex vivo with IL-2 markedly impaired cardiac function, decreasing cardiac output by more than 50% over a wide preload range (Figure 12) (126). Thus, it is probable that some factor secreted by IL-2 activated lymphocytes, and not IL-2 itself, causes toxicity;

FIGURE 12

EFFECT OF SUPERNATANTS FROM IL-2 STIMULATED LYMPHOCYTES (LAK) ON CARDIAC FUNCTION



J. Clin. Invest. 86:848, 1990.

TNF is the likely culprit since IL-2 activated cells are known to release TNF and TNF has been detected in the blood of IL-2 treated patients (127). It sould be noted that all of the IL-2 effects noted in this section required IL-2 concentration of > 1,500 units/ml, and it is not known if such high concentrations occur in the course of naturally occurring infections.

SEPTIC SHOCK AND THE ENDOTHELIUM

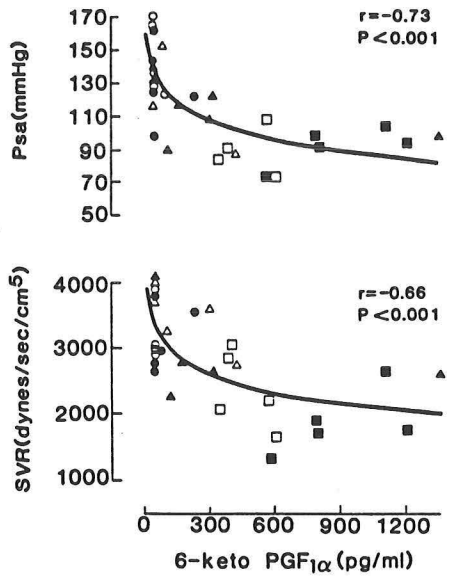
Circulating cytokines affect both leukocytes and the endothelial lining of blood vessels. Recent investigations have made it clear that endothelium is a metabolically active tissue with important regulatory functions, including control of vascular tone and traffic of circulating cells into tissue (128).

In this section the role of endothelial cells in septic shock will be reviewed.

Endothelial prostaglandin metabolism - The major prostaglandin produced by endothelial cells is prostacyclin (PGI_2), although small amounts of PGE_2 are also synthesized (129). Both substances are vasodilators and are active in the normal regulation of splanchnic and renal blood flow. Picomolar TNF or IL-1 concentrations caused cultured human umbilical vein or aortic endothelial cells to release PGI_2, and the effect was markedly potentiated (i.e. a 10 fold increase) when thrombin was present (116). The latter finding is particularly interesting because TNF and IL-1 also increase endothelial tissue factor activity and thus promote thrombin formation. Endothelial cells release PGI_2 within 3 hours of TNF exposure, whereas a 24 hour IL-1 exposure is required before PGI_2 release occurs (97).

Circulating PGI_2 is not normally detectable, and clinical experience with PGI_2 infusions has shown it to be a powerful vasodilator; doses of 10-15 ng/kg cause hypotension and a low SVR (130). PGI_2 acts by increasing cAMP levels in vascular smooth muscle. Several studies have indirectly implicated prostaglandins in shock by demonstrating that pretreatment of animals with aspirin, indomethacin, or ibuprofen protected against death from subsequent challenge with intravenous LPS (112, 113, 131, 132). More direct evidence comes from animal experiments measuring PGI_2 blood levels. Dogs given 150 μg/kg LPS developed hypotension temporally associated with elevated levels of thromboxane B_2 (the stable metabolite of thromboxane A_2, a vascoconstrictor prostaglandin) and 6 keto PGF1α (the stable metabolite of PGl_2) (133). Loss of the normal pulmonary vasoconstrictor response to alveolar hypoxia and angiotensin II was noted. Indomethacin pretreatment prevented hypotension and maintained normal pulmonary vasoregulation, but treatment with imidazole, a specific thromboxane synthetase inhibitor, had no effect, which suggests that PGI_2 was the active prostaglandin. In baboons infused with live E. coli, TXB_2 levels rose and fell rapidly, while PGI_2 levels peaked two hours after bacteremia and remained elevated for four hours (134). Hypotension, low SVR, and metabolic acidosis occurred at the time of peak PGI_2. Dogs given intratracheal Pseudomonas aeruginosa developed pneumonia, sepsis, hypotension, and a low SVR. Six keto PGF1α levels rose to high levels (900 pg/ml), TXB_2 did not increase, and there was a good correlation between the 6 keto PGFIα level and either blood pressure or SVR (Figure 13) (135). One investigator has

FIGURE 13



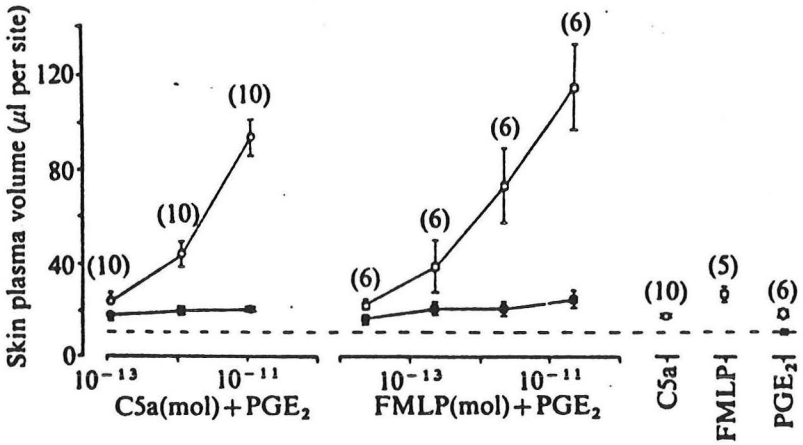
Amer. Rev. Respir. Dis. 137:703, 1988.

tested patients for PGI_2 (136). PGI_2 was undetectable in normal controls, elevated in septic shock patients who survived (median value 30 pg/ml), and markedly elevated in nonsurvivors (median 229, range 31-21,998 pg/ml). Thus, a large body of experimental and clinical evidence implicates PGI_2, produced by arteriolar endothelial cells in response to TNF and thrombin, as causing vasodilatation and the low SVR of septic shock.

In addition to hemodynamic effects, vasodilator prostaglandins are important effectors of inflammatory reactions. This has been elegantly demonstrated in experiments measuring inflammation in skin after intradermal injection of PMN chemotaxins (FMLP, C5a) with or without PGE_2 or PGI_2 (137, 138). Little effect was seen when either chemotaxin or prostaglandin alone was administered, but giving both resulted in marked edema, hemorrhage, and PMN accumulation (Figure 14). Neutropenia prevented the response, and vasoconstrictor prostaglandins had no effect. The results indicate that PMNs and prostaglandins cooperate to produce inflammation; PMNs are necessary to injure vessels and increase permeability, and prostaglandins dilate arterioles and increase blood flow, thus increasing movement of cells and plasma from the vascular space into tissue.

FIGURE 14

EFFECT OF CHEMOTAXINS AND PROSTAGLANDINS ON DERMAL INFLAMMATION



Solid squares are from neutropenic rabbits.

Nature 289:647, 1981.

Nitric Oxide - Nitric oxide (NO) is another important regulator of vascular smooth muscle which is produced by endothelial cells. In humans, blocking NO synthesis causes a rapid increase in blood pressure, suggesting that NO plays an important role in maintaining vascular smooth muscle in a basal relaxed state (128). LPS causes cultured bovine aortic endothelial cells to rapidly (within minutes) release NO, and mice given intraperitoneal LPS have significantly increased blood and urine nitrate levels (139, 140). Since LPS administration to humans causes a decreased SVR within 1 hour and blood pressure within 2 hours, and both LPS and TNF require a minimum of 3 hours to induce PGI_2 release, it is possible that NO mediates vasodilatation early during septic shock. Addition of thrombin or arachidonic acid to endothelial cells causes coupled release of both NO and PGI_2 (141). Since NO causes vasodilatation by an entirely different mechanism than PGI_2 (NO increases smooth muscle cGMP), coupled release *in vivo* might result in marked vasodilatation and hypotension. However, there is as yet no direct evidence implicating NO in septic shock.

Endothelial cell adhesion molecules - In addition to their other effects, inflammatory cytokines increase expression of adhesion molecules on both endothelial cells and PMNs. Investigation into the molecular mechanisms of cell-cell adhesion is a burgeoning field, and at the present time there are four families of

adhesion molecules - the integrins (LFA 1, CD11/CD18), the immunoglobulin superfamily (ICAM-1 and 2, NCAM, VCAM), cadherins, and the LEC-CAMs (ELAM-1, GMP 140) (142). The post-capillary venules are the site of adhesion molecule expression. A number of *in vitro* and *in vivo* experiments have determined the following:

1. ICAM 1 is expressed at low levels on normal human endothelial cells, but ELAM-1 and GMP140 are not. GMP140 is stored preformed in the Weibel-Palade bodies of endothelial cells but ELAM-1 and ICAM-1 expression requires protein synthesis (143, 144).

2. The ligand for ICAM 1 is CD11/CD18, a molecule stored preformed in the specific granules of PMNs (145). The ligand for both ELAM-1 and GMP140 is a sialylated glycoprotein which is similar to the Lewis blood group antigen and is normally present on the PMN surface (146). Thus, in the basal state one member of each pair of adhesion molecules is not present on the cell surface, and PMN-endothelial cell adhesion is weak.

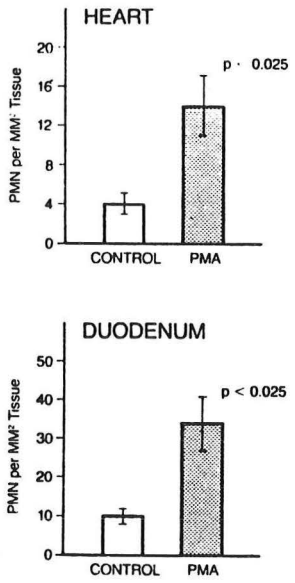
3. Small amounts of LPS, TNF, and IL-1 increase surface levels of all the adhesion molecules (except for the sialylated glycoprotein PMN ligand for ELAM-1 and GMP140) (147-149). Thrombin stimulates GMP 140 expression (150). Increased expression of CD11/CD18 and GMP 140 occurs within minutes, since both are stored preformed. ICAM-1 levels rise slowly but remain elevated for days, whereas ELAM-1 levels increase within two hours but ELAM-1 is absent twenty-four hours after a stimulus.

4. *In vivo*, immunochemical staining of human and baboon skin biopsies after intradermal injection of TNF or substances eliciting delayed hypersensitivity reactions has confirmed that ICAM-1 and ELAM-1 are expressed; PMNs were prominently clustered along ELAM-1 positive venules (151, 152).

PMN-endothelial cell interactions - The presence of a redundant system of adhesion molecules ensures that during inflammation PMNs adhere to venular endothelium, and PMN margination explains the neutropenia of septic shock. PMNs from septic shock patients are more adherent *in vitro*, and adding septic shock plasma to normal PMNs causes them to become more adherent (153). In addition to increasing adhesion molecule expression, TNF, IL-1, and LPS cause human endothelial cells to synthesize and release a 1.8 Kd protein which is chemotactic for PMNs, and TNF and IL-1 have also been shown to facilitate PMN movement across endothelial monolayers by a separate (and as yet unexplained) mechanism (154, 155). The net effect is an increase in the number of PMNs adherent to endothelium and in tissue; this has been quantified in an animal model of shock and ARDS in which

large numbers of PMNs were present in heart, liver, and duodenal biopsies (Figure 15) (156). Adherent PMNs are activated and are significantly more efficient at damaging underlying cells, either by releasing granule enzymes or producing toxic oxygen radicals (157-160). Thus, the final pathway of septic shock involves leukocytes, clogging capillaries and entering tissue to inflict irreversible endothelial and visceral organ damage.

FIGURE 15



Amer. Rev. Respir. Dis. 139:1024, 1989.

OTHER MEDIATORS

Both complement and kinins have been implicated as hypotensive agents. Gram negative bacteria activate complement via the alternate pathway, and two studies have found low levels of C3, C5, C6, and C9 in gram negative bacteremic shock, as compared to normal levels in normotensive bacteremic patients (77, 161). However, animals given intravenous cobra venom factor, which activated 91% of total complement, do not develop hypotension, and prior complement depletion did not protect animals from shock after an intravenous LPS challenge (162, 163). LPS is also capable of activating Factor XII <u>in vitro</u>, which could potentially result in kallikrein formation and subsequent generation of bradykinin, a vasodilator which causes increased vascular permeabilty. Compared to normotensive bacteremic patients, patients with bacteremic shock have been shown to have low levels of Factor XII and pre-kallikrein, suggesting that bradykinin formation occurred (164). Small intravenous LPS doses given to normal humans resulted in five fold bradykinin elevations, but no change in blood pressure (165). Thus, a

causal role for either complement or bradykinin in septic shock is uncertain.

THERAPY OF SEPTIC SHOCK

Patients developing septic shock should have appropriate cultures obtained and should be carefully examined for a tissue site of infection; if there is no obvious site then occult pelvic, abdominal, and thoracic abscesses should be considered. Empiric antibiotics need to be given promptly, although even with appropriate antibiotics the mortally of septic shock is discouragingly high. One review of 1,186 cases of gram negative bacteremia found that rapid (within 24 hours) administration of an antibiotic active against the blood isolate had no effect on mortality (166). One explanation for this seemingly paradoxical observation comes from an animal model of gram negative bacterial peritonitis, in which gentamicin administration resulted in large (eight fold) increases in both total and free LPS blood levels (167). Adequate volume resuscitation is important for reversing hypotension, as septic shock patients are frequently intravascular volume depleted from increased capillary permeability and may require several liters (or more) of fluid (168). Lastly, vasopressors should be given to maintain a mean blood pressure > 60 mm Hg. Dopamine should be infused and, if there is no response to maximal dopamine doses, norepinephrine added (169). If an adequate blood pressure is achieved but the patient remains in lactic acidosis, some authorities suggest adding an inotropic agent (dobutamine), as there is evidence that increasing cardiac output will reverse lactic acidosis (32, 170).

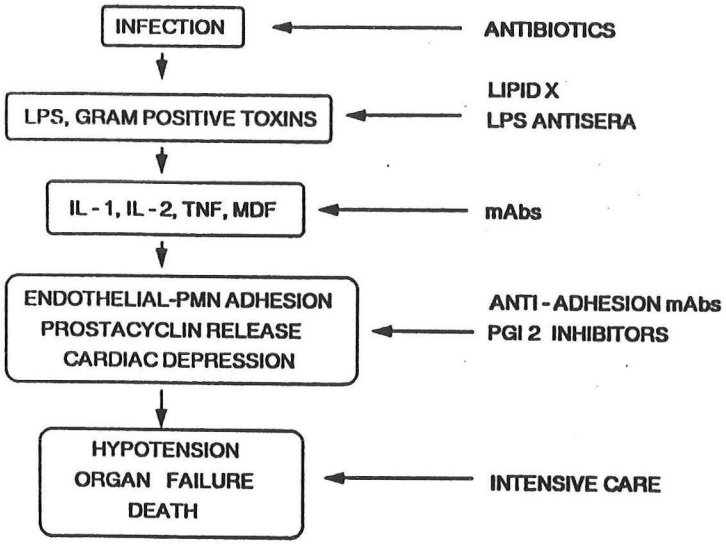
Clinical trials of three new therapies have been performed over the past ten years. In an uncontrolled series of nine patients naloxone, given in 0.4 mg increments up to a total dose of 8.0 mg, resulted in a > 20 mm Hg rise in systolic blood pressure (171). Another uncontrolled study of 12 patients found that naloxone had no effect on blood pressure and four patients developed serious side effects, including decreased blood pressure in two and a seizure in one (172). A placebo controlled study found that both saline and naloxone injections caused minor, clinically insignificant elevations of blood pressure and no difference in mortality (173). Thus, naloxone should not be used in septic shock. Because of experiments showing that corticosteroid pretreatment protected animals from septic shock, two large scale, randomized and blinded trials of corticosteroid therapy in humans have been performed. Both studies were well designed and treated patients rapidly (within two hours of sepsis onset) with large (75-120 mg/kg/24 hours) doses of methylprednisolone. There were no differences in mortality between treatment groups, and the number of patients developing shock and surviving shock were identical. A significant increase in the incidence of complicating infections in the corticosteroid group was noted (174, 175). These results indicate that even when given as early as possible corticosteroids are not helpful.

In contrast to naloxone and corticosteroids, administration of polyclonal antibodies against the Lipid A core moiety of LPS has proven efficacious, even when given after shock onset. Two hundred and twelve severely ill patients with clinical evidence of gram negative infections were randomly treated with either a lipid A antisera (prepared by immunizing normal volunteers with a mutant, Lipid A rich LPS) or nonimmune sera. Sixty-six percent of patients in both groups were hypotensive, and patients treated with lipid A antisera had significantly less mortality (22% versus 39% for the controls) (176). Patients who had been in shock > 6 hours prior to receiving sera also had lower mortality (46% versus 76%). In a subsequent study of surgical ICU patients thought to be at risk for gram negative sepsis, lipid A antisera significantly reduced both the incidence and mortality of septic shock (177). A third study found that lipid A antisera had no effect on the development of organ failure, mortality, or shock reversal in 71 patients with gram negative bacteremia and shock. However, the patients had been in shock for a mean of 12 hours prior to receiving the antisera (178). In addition to lipid A antisera, polyclonal human IgG LPS antisera significantly prevented organ failure, DIC, and death and also shortened hospital stay when given to young obstetric patients in septic shock (179).

Although the experience with lipid A antisera is encouraging, the therapy is obviously limited to gram negative infections, preparation of the antisera is tedious, and animal experiments have not shown that lipid A antisera prevents mortality or blocks TNF production (180, 181). Lipid X is a nontoxic monosaccharide lipid A precursor. Lipid X has been shown to block LPS priming of human PMNs for oxygen radical release, and administration of Lipid X either before or four hours after LPS protects animals from mortality and tissue injury (182, 183). The ability of Lipid X to protect even after a four hour interval makes it attractive for clinical use.

Based on the pathogenic schema outlined earlier, Figure 16 identifies potential future therapies for septic shock. Many of these are already under active investigation; for instance, monclonal antibodies (mAbs) to PMN CD11/CD18 adhesion molecules prevented PMN tissue influx, CSF protein accumulation, brain edema, and death in a rabbit model of bacterial meningitis (184).

FIGURE 16



In the future, it is probable that specific treatment targeted to PMN and endothelial cell adhesion molecules, TNF, IL-1, and prostaglandins will become available. It is likely that the efficacy of any therapy will depend on early application, before serious endothelial and visceral organ injury has occurred. Thus, identification of patients of risk for the development of sepsis, and close observation of such patients for early signs of the septic syndrome and shock, will continue to be of paramount importance.

REFERENCES

1. Increase in National Hospital Discharge Survey Rates for Septicemia - United States, 1979-1987. Morb and Mort Weekly Reports 39:31-34, 1990.

2. Bone RC, Fisher CJ, Clemmer TP, Slotman GJ, Metz CA, Balk RA, The Methylprednisolone Severe Sepis Study Group: Sepsis Syndrome: A valid clinical entity. Crit Care Med 17:389-393, 1989.

3. Meakins JL, McLean APH: The Surgical Intensive Care Unit. Surg Clin N Am 60:117-132, 1980.

4. Goris RJA, Boekholtz WKF, van Bebber IPT, Nuytinck JKS, Schillings PHM: Multiple-organ failure and sepsis without bacteria: An experimental model. Arch Surg 121:897-901, 1986.

5. Weinstein MP, Murphy JR, Reller LB, Lichtenstein KA: The clinical significance of positive blood cultures: A comprehensive analysis of 500 episodes of bacteremia and fungemia in adults. II. Clinical observations, with special reference to factors influencing prognosis. Rev Infect Dis 5:54-68, 1983.

6. Abraham E, Shoemaker WC, Bland RD, Cobo JC: Sequential cardiorespiratory patterns in septic shock. Crit Care Med 11:799-803, 1983.

7. Siegel JH, Greenspan M, Del Guercio LRM: Abnormal vascular tone, defective oxygen transport and myocardial failure in human septic shock. Ann Surg 165:504-516, 1967.

8. Hess ML., Hastillo A, Greenfield LJ: Spectrum of cardiovascular function during gram-negative sepsis. Prog Cardiovasc Dis 23:279-298, 1981.

9. Nishijima H, Weil MH, Shubin H, Cavanilles J: Hemodynamic and metabolic studies on shock sssociated with gram negative bacteremia. Medicine 52:287-294, 1973.

10. Weisel RD, Vito L, Dennis RC, Valeri CR, Hechtman HB: Myocardial depression during sepsis. Am J Surg 133:512-520, 1977.

11. Parker MM, Parrillo JE: Septic shock: Hemodynamics and pathogenesis. JAMA 250:3324-3327, 1983.

12. Parrillo JE, Parker MM, Natanson C, Suffredini AF, Danner RL, Cunnion RE, Ognibene FP: Septic shock in humans: Advances in the understanding of pathogenesis, cardiovascular dysfunction, and therapy. Ann Int Med

113:227-242, 1990.

13. Parker MM, Shelhamer JH, Natanson C, Alling DW, Parrillo JE: Serial cardiovascular variables in survivors and nonsurvivors of human septic shock: Heart rate as an early predictor of prognosis. Crit Care Med 15:923-929, 1987.

14. Cunnion RE, Parrillo JE: Myocardial dysfunction in sepsis. Crit Care Clin 5:99-118, 1989.

15. Wiles JB, Cerra FB, Siegel JH, Border JR: The systemic septic response: Does the organism matter? Crit Care Med 8:55-60, 1980.

16. Holloway WA, Reinhardt J: Septic shock in the elderly. Geriatrics 39:48-54, 1984.

17. Winslow EJ, Loeb HS, Rahimtoola SH, Kamath S, Gunnar RM: Hemodynamic studies and results of therapy in 50 patients with bacteremic shock. Am J Med 54:421-432, 1973.

18. Harris RL, Musher DM, Bloom K, Gathe J, Rice L, Sugarman B, Williams TW, Young EJ: Manifestations of sepsis. Arch Int Med 147:1895-1906, 1987.

19. Zimmerman HJ, Fang M, Utili R: Jaundice due to bacterial infection. Gastroenterology 77:362-374, 1979.

20. Schuster DP, Rowley H, Feinstein S: Prospective evaluation of the risk of upper gastrointestial bleeding after admission to a medical intensive care unit. Am J Med 76:623-630, 1984.

21. Colman RW, Robboy SJ, Minna JD: Disseminated intravascular coagulation: A reappraisal. Ann Rev Med 30:359-374, 1979.

22. Poskitt TR, Poskitt PKF: Thrombocytopenia of sepsis. Arch Int Med 145:891-894, 1985.

23. Beaufils M, Morel-Maroger L, Sraer JD: Acute renal failure of glomerular origin during visceral abscesses. N Engl J Med 295:185-189, 1976.

24. Rasmussen HH, Ibels LS: Acute renal failure. Am J Med 73:211-218, 1982.

25. Miller SI, Wallace Jr. RJ, Musher DM, Septimus EJ, Kohl S, Baughn RE: Hypoglycemia as a manifestation of sepsis. Am J Med 68:649-654,1980.

26. Baracos V, Rodemann HP, Dinarello CA, Goldberg AL: Stimulation of muscle protein degradation and prostaglandin E_2 release by leukocytic pyrogen (Interleukin-1). N Engl J Med 308:553-558, 1983.

27. Nordenstrom J, Carpentier YA, Askanazi J, Kinney JM: Free fatty acid mobilization and oxidation during total parenteral nutrition in trauma and infection. Ann Surg 198:725-735, 1983.

28. Kreisberg RA: Lactate homeostasis and lactic acidosis. Ann Int Med 92:227-237, 1980.

29. Simmons DH, Nicoloff J, Guze LB: Hyperventilation and respiratory alkalosis as signs of gram negative bacteremic shock. JAMA 174: 2196-2199, 1960.

30. Morris DL, Chambers HF, Morris MG, Sande MA: Hemodynamic characteristics of patients with hypothermia due to occult infection and other causes. Ann Int Med 102:153-157, 1985.

31. Cain SM: Supply dependency of oxygen uptake in ARDS: Myth or reality? Am J Med Sci 288:119-124, 1984.

32. Vincent JL, Roman A, De Backer D, Kahn RJ: Oxygen uptake/supply dependency: Effects of short-term dobutamine infusion. Am Rev Respir Dis 142:2-7, 1990.

33. Danek SJ, Lynch JP, Weg JG, Dantzker DR: The dependence of oxygen uptake on oxygen delivery in the adult respiratory distress syndrome. Am Rev Respir Dis 122:387-395, 1980.

34. Haupt MT, Gilbert EM, Carlson RW: Fluid loading increases oxygen consumption in septic patients with lactic acidosis. Am Rev Respir Dis 131:912-916, 1985.

35. Archie Jr JP: Anatomic arterial-venous shunting in endotoxic and septic shock in dogs. Ann Surg 186:171-176, 1976.

36. Wright CJ, Duff JH, McLean APH, MacLean LD: Regional capillary blood flow and oxygen uptake in severe sepsis. Surg Gynecol Obstet 132:637-644, 1971.

37. Van Lambalgen AA, Bronsveld W, Van Den Bos GC, Thijs LG: Distribution of cardiac output, oxygen consumption and lactate production in canine endotoxin shock. Cardiovasc Res 18:195-205, 1984.

38. Landau SE, Alexander RS, Powers SR, Stratton HH, Goldfarb RD: Tissue oxygen exchange and reactive hyperemia following microembolization. J Surg Res 32:38-43, 1982.

39. Nelson DP, Beyer C, Samsel RW, Wood LDH, Schumacker PT: Pathological supply dependence of O_2 uptake during bacteremia in dogs. J Appl Phys 63:1487-1492, 1987.

40. Cerra FB: The multiple organ failure syndrome. Hospital

Practice, August 15, 99-106, 1990.

41. Fowler AA, Hamman RF, Good JT, Benson KN, Baird M, Eberle DJ, Petty TL, Hyers TM: Adult respiratory distress syndrome: Risk with common predispositions. Ann Int Med 98:593-597, 1983.

42. Seidenfeld JJ, Pohl DF, Bell RC, Harris GD, Johanson WG: Incidence, site, and outcome of infections in patients with the adult respiratory distress syndrome. Am Rev Respir Dis 134:12-16, 1986.

43. Bell RC, Coalson JJ, Smith JD, Johanson WG: Multiple organ system failure and infection in adult respiratory distress syndrome. Ann Int Med 99:293-298, 1983.

44. McGovern VJ: Shock revisited. Pathol Ann 19:15-36, 1984.

45. Coalson JJ, Archer LT, Benjamin BA, Beller-Todd BK, Hinshaw LB: A morphologic study of live escherichia coli organism shock in baboons. Exp Molec Pathol 31:10-22, 1979.

46. Coalson JJ, Hinshaw LB, Guenter CA, Berrell EL, Greenfield LJ: Pathophysiologic responses of the subhuman primate in experimental septic shock. Lab Invest 32:561-569, 1975.

47. Natanson C, Cunnion RE, Barrett DA, Peart KW, Danner RL, Conklin JJ, Macvittie TJ, Walker RI, Ferrans VJ, Parrillo JE: Reversible myocardial dysfunction in a canine model of septic shock is associated with myocardial microcirculatory damage and focal neutrophil infiltration. Clin Res 34:639A, 1986.

48. Natanson C: Studies using a canine model to investigate the cardiovascular abnormality of and potential therapies for septic shock. Clin Res 38:206-214, 1990.

49. Natanson C, Danner RL, Elin RJ, Hosseini JM, Peart KW, Banks SM, MacVittie TJ, Walker RI, Parrillo JE: Role of endotoxemia in cardiocvascular dysfunction and mortality. J Clin Invest 83:243-251, 1989.

50. Natanson C, Fink MP, Ballantyne HK, MacVittie TJ, Conklin JJ, Parrillo JE: Gram-negative bacteremia produces both severe systolic and diastolic cardiac dysfunction in a canine model that simulates human septic shock. J Clin Invest 78:259-270, 1986.

51. Ognibene FP, Parker MM, Natanson C, Shelhamer JH, Parrillo JE: Depressed left ventricular performance: Response to volume infusion in patients with sepsis and septic shock. Chest 93:903-910, 1988.

52. Parker MM, McCarthy KE, Ognibene FP, Parrillo JE: Right

ventricular dysfunction and dilatation, similar to left ventricular changes, characterize the cardiac depression of septic shock in humans. Chest 97:126-131, 1990.

53. Parker MM, Shelhamer JH, Bacharach SL, Green MV, Natanson C, Frederick TM, Damske BA, Parrillo JE: Profound but reversible myocardial depression in patients with septic shock. Ann Int Med 100:483-490, 1984.

54. Cunnion RE, Schaer GL, Parker MM, Natanson C, Parrillo JE: The coronary circulation in human septic shock. Circulation 73:637-644, 1986.

55. Lefer AM, Cowgill R, Marshall FF, Hall LM, Brand ED: Characterization of a myocardial depressant factor present in hemorrhagic shock. Am J Physiol 213:492-498, 1967.

56. Lefer AM, Martin J: Origin of myocardial depressant factor in shock. Am J Physiol 218:1423-1427, 1970.

57. Parrillo JE, Burch C, Shelhamer JH, Parker MM, Natanson C, Schuette W: A circulating myocardial depressant substance in humans with septic shock. J Clin Invest 76:1539-1553, 1985.

58. Reilly JM, Cunnion RE, Burch-Whitman C, Parker MM, Shelhamer JH, Parrillo JE: A circulating myocardial depressant substance is associated with cardiac dysfunction and peripheral hypoperfusion (lactic acidemia) in patients with septic shock. Chest 95:1072-1080, 1989.

59. Hollenberg SM, Cunnion RE, Lawrence Marion, Kelly JL, Parrillo JE: Tumor necrosis factor depresses myocardial cell function: Results using an in vitro assay of myocyte performance. Clin Res 37:528A, 1989.

60. Cohn ZA: The activation of mononuclear phagocytes: Fact, fancy, and future. J Immunol 121:813-816, 1978.

61. Nathan CF, Murray HW, Cohn ZA: The macrophage as an effector cell. N Engl J Med 303:622-626, 1980.

62. Czop KL, McGowan SE, Center DM: Opsonin-independent phagocytosis by human alveolar macrophages: Augmentation by human plasma fibronectin. Am Rev Respir Dis 125:607-609, 1982.

63. Noso Y, Parant M, Parant F, Chedid L: Production of tumor necrosis factor in nude mice by muramyl peptides associated with bacterial vaccines. Cancer Res 48:5766-5769, 1988.

64. Riesenfeld-Orn I, Wolpe S, Garcia-Bustos JF, Hoffmann MK, Tuomanen E: Production of interleukin-1 but not tumor necrosis factor by human monocytes stimulated with

pneumococcal cell surface components. Infect Immun 57:1890-1893, 1989.

65. White J, Herman A, Pullen AM, Kubo R, Kappler JW, Marrack P: The Vβ-specific superantigen staphylococcal enterotoxin B: Stimulation of mature T cells and clonal deletion in neonatal mice. Cell 56:27-35, 1989.

66. Marrack P, Kappler J: The staphylococcal enterotoxins and their relatives. Science 248:705-711, 1990.

67. Fast DJ, Schlievert PM, Nelson RD: Toxic shock syndrome-Associated staphylococcal and streptococcal pyrogenic toxins are potent inducers of tumor necrosis factor production. Infect Immun 57:291-294, 1989.

68. Uchiyama T, Kamagata Y, Yan X, Kohno M, Yoshioka M, Fujikawa H, Igarashi H, Okubo M, Awano F, Saito-Taki T, Nakano M: Study of the biological activities of toxic shock syndrome toxin-1: II. Induction of the proliferative response and the interleukin 2 production by T cells from human peripheral blood mononuclear cells stimulated with the toxin. Clin Exp Immunol 68:638-647, 1987.

69. Ikejima T, Okusawa S, van der Meer JWM, Dinarello CA: Induction by toxic-shock-syndrome toxin-1 of a circulating tumor necrosis factor-like substance in rabbits and of immunoreactive tumor necrosis factor and interleukin-1 from human monounuclear cells. J Infect Dis 158:1017-1025, 1988.

70. Cone LA, Woodard DR, Schlievert PM, Tomory GS. Clinical and bacteriologic observations of a toxic shock-like syndrome due to Streptococcus pyogenes. N Engl J Med 317:146-149, 1987.

71. Michalek SM, Moore RN, McGhee JR, Rosenstreich DL, Mergenhagen SE: The primary role of lymphoreticular cells in the mediation of host responses to bacterial endotoxin. J Infect Dis 141:55-62, 1980.

72. Epstein FH: Cachectin: More than a tumor necrosis factor. N Engl J Med 316:379-385, 1987.

73. Mathison JC, Wolfson E, Ulevitch RJ: Participation of tumor necrosis factor in the mediation of gram negative bacterial lipopolysaccharide-induced injury in rabbits. J Clin Invest 81:1925-1937, 1988.

74. Fearon DT, Ruddy S, Schur PH, McCabe WR: Activation of the properdin pathway of complement in patients with gram-negative bacteremia. N Engl J Med 292:937-940, 1975.

75. Moore KL, Andreoli SP, Esmon NL, Esmon CT, Bang NU: Endotoxin enhances tissue factor and suppresses

thrombomodulin expression of human vascular endothelium in vitro. J Clin Invest 79: 124-130, 1987.

76. Meyrick BO, Ryan US, Brigham KL: Direct effects of E. coli endotoxin on structure and permeability of pulmonary endothelial monolayers and the endothelial layer of intimal explants. Am J Pathol 122:140-151, 1986.

77. Morrison DC, Cochrane CG: Direct evidence for Hageman factor (Factor XII) activation by bacterial lipopolysaccharides (Endotoxins). J Exp Med 140:797-809,1974.

78. Kreger BE, Craven DE, McCabe WR: Gram-negative bacteremia: IV. Re-evaluation of clinical features and treatment in 612 patients. Am J Med 68:344-355, 1980.

79. Dal Nogare AR, Yarbrough Jr WC: A comparison of the effects of intact and deacylated lipopolysaccharide on human polymorphonuclear leukocytes. J Immunol 144:1404-1410, 1990.

80. Guthrie LA, McPhail LC, Henson PM, Johnston RB: Priming of neutrophils for enhanced release of oxygen metabolites by bacterial lipopolysaccharide: evidence for increased activity of the superoxide-producing enzyme. J Exp. Med. 160:1656-1671, 1984.

81. Suffredini AF, Fromm RE, Parker MM, Brenner M, Kovacs JA, Wesley RA, Parrillo JE: The cardiovascular response of normal humans to the administration of endotoxin. N Engl J Med 321:280-287, 1989.

82. Michie HR, Manogue KR, Spriggs DR, Revhaug A, O'Dwyer S, Dinarello CA, Cerami A, Wolff SM, Wilmore DW: Detection of circulating tumor necrosis factor after endotoxin administration. N Engl J Med 318:1481-1486, 1988.

83. Suffredini AF, Harpel PC, Parrillo JE: Promotion and subsequent inhibition of plasminogen activation after administration of intravenous endotoxin to normal subjects. N Engl J Med 320:1165-1172, 1989.

84. Suffredini AF, Shelhamer JH, Neumann RD, Brenner M, Parrillo JE: Intravenous endotoxin(E) to normal humans causes gas exchange abnormalities and increased alveolar epithelial permeability. Clin Res 36:511A, 1988.

85. Elin RJ, Hosseini JM, Schlesinger T, Reilly JM, Parrillo JE: Endotoxin determinations in 100 patients with septic shock. Clin Res 36:453A, 1988.

86. Moreno C, Taverne J, Mehlert A, Bate CAW, Brealey RJ, Meager A, Rook GAW, Playfair JHL: Lipoarabinomannan from

Mycobacterium tuberculosis induces the production of tumour necrosis factor from human and murine macrophages. Clin Exp Immunol 76:240-245, 1989.

87. Kunkel SL, Remick DG, Strieter RM, Larrick JW: Mechanisms that regulate the production and effects of tumor necrosis factor. Crit Rev Immunol 9:93-111, 1989.

88. Gamble JR, Harlan JM, Klebanoff SJ, Vadas MA: Stimulation of the adherence of neutrophils to umbilical vein endothelium by human recombinant tumor necrosis factor. Proc Natl Acad Sci 82:8667-8671, 1985.

89. Larrick JW, Graham D, Toy K, Lin LS, Senyk G, Fendly BM: Recombinant tumor necrosis factor causes activation of human granulocytes. Blood 69:640-644, 1987.

90. Klebanoff SJ, Vadas MA, Harlan JM, Sparks LH, Gamble JR, Agosti JM, Waltersdorph AM: Stimulation of neutrophils by tumor necrosis factor. J Immunol 136:4220-4225, 1986.

91. Tracey KJ, Lowry SF, Beutler B, Cerami A, Albert JD, Shires GT: Cachectin/tumor necrosis factor mediates changes of skeletal muscle plasma membrane potential. J Exp Med 164:1368-1373, 1986.

92. Starnes Jr HF, Warren RS, Jeevanandam M, Gabrilove JL, Larchian W, Oettgen HF, Brennan MF: Tumor necrosis factor and the acute metabolic response to tissue injury in man. J Clin Invest 82:1321-1325, 1988.

93. Camussi G, Bussolino F, Salvidio G, Baglioni C: Tumor necrosis factor/cachectin stimulates peritoneal macrophages, polymorphonuclear neutrophils, and vascular endothelial cells to synthesize and release platelet-activating factor. J Exp Med 166:1390-1404, 1987.

94. Bachwich PR, Chensue SW, Larrick JW, Kunkel SL: Tumor necrosis factor stimulates interleukin-1 and prostaglandin E_2 production in resting macrophages. Biochem Biophys Res Comm 136:94-101, 1986.

95. Perlmutter DH, Dinarello CA, Punsal PI, Colten HR: Cachectin/tumor necrosis factor regulates hepatic acute-phase gene expression. J Clin Invest 78:1349-1354, 1986.

96. Bussolino F, Camussi G, Baglioni C: Synthesis and release of platelet-activating factor by human vascular endothelial cells treated with tumor necrosis factor or Interleukin 1a. J Biol Chem 263:11856-11861, 1988.

97. Nawroth PP, Stern DM: Modulation of endothelial cell hemostatic properties by tumor necrosis factor. J Exp Med 163:740-745, 1986.

98. Kawakami M, Ishibashi S, Ogawa H, Murase T, Takaku F, Shibata S: Cachectin/TNF as well as interleukin-1 induces prostacyclin synthesis in cultured vascular endothelial cells. Biochem Biophys Res Comm 141:482-487, 1986.

99. Pober JS, Cotran RS: Cytokines and endothelial cell biology. Physiol Rev 70:427-451, 1990.

100. Nawroth PP, Bank I, Handley D, Cassimeris J, Chess L, Stern D: Tumor necrosis factor/cachectin interacts with endothelial cell receptors to induce release of interleukin 1. J Exp Med 163:1363-1375, 1986.

101. Stolpen AH, Guinan EC, Fiers W, Pober JS: Recombinant tumor necrosis factor and immune interferon act singly and in combination to reorganize human vascular endothelial cell monolayers. Am J Pathol 123:16-24, 1986.

102. Stephens KE, Ishizaka A, Larrick JW, Raffin TA: Tumor necrosis factor causes increased pulmonary permeability and edema: Comparison to septic acute lung injury. Am Rev Respir Dis 137:1364-1370, 1988.

103. Tracey KJ, Beutler B, Lowry SF, Merryweather J, Wolpe S, Milsark IW, Hariri RJ, Fahey III TJ, Zentella A, Albert JD, Shires GT, Cerami A: Shock and tissue inury induced by recombinant human cachectin. Science 234:470-474, 1986.

104. Natanson C, Eichenholz PW, Danner RL, Eichacker PQ, Hoffman WD, Kuo GC, Banks SM, MacVittie TJ, Parrillo JE: Endotoxin and tumor necrosis factor challenges in dogs simulate the cardiovascular profile of human septic shock. J Exp Med 169:823-832, 1989.

105. Tracy KJ, Fong Y, Hesse DG, Manogue KR, Lee AT, Kuo GC, Lowry SF, Cerami A: Anti-cachectin/TNF monoclonal antibodies prevent septic shock during lethal bacteraemia. Nature 330:662-666, 1987.

106. Grau GE, Taylor TE, Molyneux ME, Wirima JJ, Vassalli P, Hommel M, Lambert P: Tumor necrosis factor and disease severity in children with falciparum malaria. N Engl J Med 320:1586-1591, 1989.

107. Waage A, Halstensen A, Espevik T: Association between tumour necrosis factor in serum and fatal outcome in patients with meningococcal disease. Lancet I:355-357, 1987.

108. Kenzora JL, Perez JE, Bergmann SR, Lange LG: Effects of acetyl glyceryl ether of phosphorylcholine (platelet activating factor) on ventricular preload, afterload, and contractility in dogs. J Clin Invest 74:1193-1203, 1984.

109. McManus LM, Pinckard RN, Fitzpatrick FA, O'Rourke RA, Crawford MH, Hanahan DJ: Acetyl glyceryl ether phosphorylcholine: intravascular alterations following intravenous infusion into the baboon. Lab Invest 45:303-307, 1981.

110. Wang J, Dunn MJ: Platelet-activating factor mediates endotoxin-induced acute renal insufficiency in rats. Am Physiolog Soc 253:F1283-F1289, 1987.

111. Sun X, Hsueh W: Bowel necrosis induced by tumor necrosis factor in rats is mediated by platelet-activating factor. J Clin Invest 81:1328-1331, 1988.

112. Kettelhut IC, Fiers W, Goldberg AL: The toxic effects of tumor necrosis factor in vivo and their prevention by cyclooxygenase inhibitors. Proc Natl Acad Sci 84:4273-4277, 1987.

113. Jacobs ER, Soulsby ME, Bone RC, Wilson FJ, Hiller FC. Ibuprofen in canine endotoxin shock. J Clin Invest 70:536-541, 1982.

114. Ikejima T, Dinarello CA, Gill DM, Wolff SM: Induction of human interleukin-1 by a product of Staphylococcus aureus associated with toxic shock syndrome. J Clin Invest 73:1312-1320, 1984.

115. Nawroth PP, Handley DA, Esmon CT, Stern DM: Interleukin 1 induces endothelial cell procoagulant while suppressing cell-surface anticoagulant activity. Proc Natl Acad Sci 83:3460-3464, 1986.

116. Zavoico GB, Ewenstein BM, Schafer AI, Pober JS: IL-1 and related cytokines enhance thrombin-stimulated PGI_2 production in cultured endothelial cells without affecting thrombin-stimulated von Willebrand factor secretion or platelet-activating factor biosynthesis. J Immunol 142:3993-3999, 1989.

117. Rossi V, Breviario F, Ghezzi P, Dejana E, Mantovani A: Prostacyclin synthesis induced in vascular cells by interleukin-1. Science 229:174-176, 1985.

118. Baracos V, Rodemann HP, Dinarello CA, AL Goldberg: Stimulation of muscle protein degradation and prostaglandin E_2 release by leukocytic pyrogen (interleukin-1): A mechanism for the increased degradation of muscle protein during fever. N Engl J Med 308:553-558, 1983.

119. Movat HZ, Burrowes CE, Cybulsky ML, Dinarello CA: Acute inflammation and a Shwartzman-like reaction induced by interleukin-1 and tumor necrosis factor. Am J Pathol

129:463-476, 1987.

120. Okusawa S, Gelfand JA, Ikejima T, Connolly RJ, Dinarello CA: Interleukin 1 induces a shock-like state in rabbits. J Clin Invest 81:1162-1172, 1988.

121. Caput D, Beutler B, Hartog K, Thayer R, Brown-Shimer S, Cerami A: Identification of a common nucleotide sequence in the 3'-untranslated region of mRNA molecules specifying inflammatory mediators. Proc Natl Acad Sci 83:1670-1674, 1986.

122. Hesse DG, Tracey KJ, Fong Y, Manogue KR, Palladino MA, Cerami A, Shires GT, Lowry SF: Cytokine appearance in human endotoxemia and primate bacteremia. Surg Gynecol Obstet 166:147-153, 1988.

123. Rosenberg SA, Lotze MT, Mule JJ: New approaches to the immunotherapy of cancer using interleukin-2. Ann Int Med 108:853-864, 1988.

124. Ognibene FP, Rosenberg SA, Lotze M, Skibber J, Parker MM, Shelhamer JH, Parrillo JE: Interleukin-2 administration causes reversible hemodynamic changes and left ventricular dysfunction similar to those seen in septic shock. Chest 94:750-754, 1988.

125. Aronson FR, Libby P, Brandon EP, Janicka MW, Mier JW: IL-2 rapidly induces natural killer cell adhesion to human endothelial cells. J Immunol 141:158-163, 1988.

126. Sobotka PA, McMannis J, Fisher RI, Stein DG, Thomas JX: Effects of interleukin 2 on cardiac function in the isolated rat heart. J Clin Invest 86:845-850, 1990.

127. Gemlo BT, Palladino MA, Jaffe HS, Espevik TP, Rayner AA: Circulating cytokines in patients with metastatic cancer treated with recombinant interleukin 2 and lymphokine-activated killer cells. Cancer Res 48:5864-5867, 1988.

128. Vane JR, Anggard EE, Botting RM: Regulatory functions of the vascular endothelium. N Engl J Med 323:27-36, 1990.

129. Oates JA, FitzGerald GA, Branch RA, Jackson EK, Knapp HR, Roberts LJ: Clinical implications of prostaglandin and thromboxane A_2 formation. N Engl J Med 319:689-698, 1988.

130. Guadagni DN, Ikram H, Maslowski AH: Haemodynamic effects of prostacyclin (PGI_2) in pulmonary hypertension. Br Heart J 45:385-388, 1981.

131. Halushka PV, Wise WC, Cook JA: Studies on the beneficial effects of aspirin in endotoxic shock. Am J Med 74:91-96, 1983.

132. Cook JA, Wise WC, Halushka PV: Elevated thromboxane levels in the rat during endotoxic shock. J Clin Invest 65:227-230, 1980.

133. Hales CA, Sonne L, Peterson M, Kong D., Miller M, Watkins WD: Role of thromboxane and prostacyclin in pulmonary vasomotor changes after endotoxin in dogs. J Clin Invest 68:497-505, 1981.

134. Camporesi EM, Oda S, Fracica PJ, Griebel JA, Shook JE, Piantadosi CA, Watkins WD: Eicosanoids and the hemodynamic course of live Escherichia coli-induced sepsis in baboons. Circulatory Shock 29:229-244, 1989.

135. Hanly PJ, Sienko A, Light RB: Role of prostacyclin and thromboxane in the circulatory changes of acute bacteremic Pseudomonas Pneumonia in dogs. Am Rev Respir Dis 137:700-706, 1988.

136. Halushka PV, Reines HD, Barrow SE, Blair IA, Dollery CT, Rambo W, Cook JA, Wise WC: Elevated plasma 6-keto-prostaglandin F_{1a} in patients in septic shock. Crit Care Med 13:451-453, 1985.

137. Wedmore CV, Williams TJ: Control of vascular permeability by polymorphonuclear leukocytes in inflammation. Nature 289:646-650, 1981.

138. Issekutz AC, Movat HZ: The effect of vasodilator prostaglandins on polymorphonuclear leukocyte infiltration and vascular injury. Am J Pathol 107:300-309, 1982.

139. Salvemini D, Korbut R, Anggard E, Vane J: Immediate release of a nitric oxide-like factor from bovine aortic endothelial cells by Escherichia coli lipopolysaccharide. Proc Natl Acad Sci 87:2593-2597, 1990.

140. Stuehr DJ, Marletta MA: Mammalian nitrate biosynthesis: Mouse macrophages produce nitrite and nitrate in response to Escherichia coli lipopolysaccharide. Proc Natl Acad Sci 82:7738-7742, 1985.

141. de Nucci G, Gryglewski RJ, Warner TD, Vane JR: Receptor-mediated release of endothelium-derived relaxing factor and prostacyclin from bovine aortic endothelial cells is coupled. Proc Natl Acad Sci 85:2334-2338, 1988.

142. Rosen SD: The LEC-CAMs: An emerging family of cell-cell adhesion receptors based upon carbohydrate recognition. Am J Respir Cell Mol Biol 3:397-402, 1990.

143. Bevilacqua MP, Stengelin S, Gimbrone MA, Seed B: Endothelial leukocyte adhesion molecule 1: An inducible

receptor for neutrophils related to complement regulatory proteins and lectins. Science 243:1160-1165, 1989.

144. Bevilacqua MP, Pober JS, Mendrick DL, Cotran RS, Gimbrone Jr MA: Identification of an inducible endothelial-leukocyte adhesion molecule. Proc Natl Acad Sci 84:9238-9242, 1987.

145. Bainton DF, Miller LJ, Kishimoto TK, Springer TA: Leukocyte adhesion receptors are stored in peroxidase-negative granules of human neutrophils. J Exp Med 166:1641-1653, 1987.

146. Phillips ML, Nedelman, Nedelman E, Gaeta FCA, Perez M, Singhal AK, Hakomori S, Paulson JC: ELAM-1 mediates cell adhesion by recognition of a carbohydrate ligand, sialyl-Le^x. Science 250:1130-1134, 1990.

147. Dustin ML, Rothlein R, Bhan AK, Dinarello Ca, Springer TA: Induction by IL 1 and Interferon-y: Tissue distribution, biochemistry, and function of a natural adherence molecule (ICAM-1). J Immunol 137:245-254, 1986.

148. Smith CW, Rothlein R, Hughes BJ, Mariscalco MM, Rudloff HE, Schmalstieg FC, Anderson DC: Recognition of an endothelial determinant for CD18-dependent human neutrophil adherence and transendothelial migration. J Clin Invest 82:1746-1756, 1988.

149. Pober JS, Gombrone a, Lapierre LA, Mendrick DL, Fiers W, Rothlein R, Springer TA: Overlapping patterns of activation of human endothelial cells by interleukin 1, tumor necrosis factor, and immune interferon. J Immunol 137:1893-1986, 1986.

150. Geng J, Bevilacqua MP, Moore KL, McIntyre TM, Prescott SM, Kim JM, Bliss GA, Zimmerman GA, McEver RP: Rapid neutrophil adhesion to activated endothelium mediated by GMP-140. Nature 343:757-760, 1990.

151. Munro JM, Pober JS, Cotran RS: Tumor necrosis factor and interferon-y induce distinct patterns of endothelial activation and associated leukocyte accumulation in skin of Papin anubis. Am J Pathol 135:121-133, 1989.

152. Cotran RS, Gimbrone, Bevilacqua MP, Mendrick DL, Pober JS: Induction and detection of a human endothelial activation antigen in vivo. J Exp Med 164:661-666, 1986.

153. Venezio FR, Westenfelder GO, Phair JP: The adherence of polymorphonuclear leukocytes in patients with sepsis. J Infect Dis 145:351-357, 1982.

154. Strieter RM, Kunkel SL, Showell HJ, Remick DG, Phan SH, Ward PA, Marks RM: Endothelial cell gene expression of a

neutrophil chemotactic factor by TNF, LPS, and IL-1. Science 243:1467-1469, 1989.

155. Moser R, Schleiffenbaum B, Groscurth P, Fehr J: Interleukin 1 and tumor necrosis factor stimulate human vascular endothelial cells to promote transendothelial neutrophil passage. J Clin Invest 83:444-455, 1989.

156. Mizer LA, Weisbrode SE, Dorinsky PM: Neutrophil accumulation and structural changes in nonpulmonary organs after acute lung injury induced by phorbol myristate acetate. Am Rev Respir Dis 139:1017-1026, 1989.

157. Weiss SJ: Tissue destruction by neutrophils. N Engl J Med 320:365-376, 1989.

158. Movat HZ, Wasi S: Severe microvascular injury induced by lysosomal releasates of human polymorphonuclear leukocytes. Am J Pathol 121:404-417, 1985.

159. Weiss SJ, Regiani S: Neutrophils degrade subendothelial matrices in the presence of alpha-1 proteinase inhibitor. J Clin Invest 73:1297-1303, 1984.

160. Smedly LA, Tonnesen MG, Sandhaus RA, Haslett C, Guthrie LA, Johnston Jr RB, Henson PM, Worthen GS: Neutrophil-mediated injury to endothelial cells. J Clin Invest 77:1233-1243, 1986.

161. Kalter ES, Daha MR, ten Cate JW, Verhoef J, Bouma BN: Activation and inhibition of Hageman factor-dependent pathways and the complement system in uncomplicated bacteremia or bacterial shock. J Infect Dis 151:1019-1025, 1985.

162. Webster RO, Larsen GL, Mitchell BC, Goins AJ, Henson PM: Absence of inflammatory lung injury in rabbits challenged intravascularly with complement-derived chemotactic factors. Am Rev Respir Dis 125:335-340, 1982.

163. Ulevitch RJ, Cochrane CG, Bangs K, Herman CM, Fletcher JR, Rice CL: The effect of complement depletion on bacterial lipopolysaccharide (LPS)-induced hemodynamic and hermatologic changes in the rhesus monkey. Am J Pathol 92:227-240, 1978.

164. Mason JW, Kleeberg U, Dolan P, Colman RW: Plasma kallikrein and Hageman factor in gram-negative bacteremia. Ann Int Med 73:545-551, 1970.

165. Kimball HR: Endotoxin induced kinin production in man. Proc Soc Exp Biol Med 139:1078-1081, 1971.

166. Bryan CS, Reynolds KL, Brenner ER: Analysis of 1,186

episodes of gram-negative bacteremia in non-university hospitals: The effects of antimicrobial therapy. Rev Infect Dis 5:629-638, 1983.

167. Shenep JL, Mogan KA: Kinetics of endotoxin release during antibiotic therapy for experimental gram-negative bacterial sepsis. J Infect Dis 150:380-388, 1984.

168. Parrillo JE: Septic shock in humans: Clinical evaluation, pathogenesis, and therapeutic approach. In: Crit Care, 2nd edition, Shoemaker W, ed., W. B. Saunders Co., Philadelphia, PA, 1006-1024, 1989.

169. Meadows D, Edwards JD, Wilkins RG, Nightingale P: Reversal of intractable septic shock with norepinephrine therapy. Crit Care Med 16:663-666, 1988.

170. Astiz ME, Rackow EC, Falk JL, Kaufman BS, Weil MH: Oxygen delivery and consumption in patients with hyperdynamic septic shock. Crit Care Med 15:26-28, 1987.

171. Peters WP, Johnson MW, Friedman PA, Mitch WE: Pressor effect of naloxone in septic shock. Lancet 1:529-532, 1981.

172. Rock P, Silverman H, Plump D, Kecala Z, Smith P, Michael JR, Summer W: Efficacy and safety of naloxone in septic shock. Crit Care Med 13:28-33, 1985.

173. DeMaria A, Craven DE, Heffernan JJ, McIntosh TK, Grindlinger GA, McCabe WR: Naloxone versus placebo in treatment of septic shock. Lancet II:1363-1365, 1985.

174. The Veterans Administration Systemic Sepsis Cooperative Study Group: Effect of high-dose glucocorticoid therapy on mortality in patients with clinical signs of systemic sepsis. N Engl J Med 317:659-665, 1987.

175. Bone RC, Fisher CJ, Clemmer TP, Slotman GJ, Metz CA, Balk RA: A controlled clinical trial of high-dose methylprednisolone in the treatment of severe sepsis and septic shock. N Engl J Med 317:653-658, 1987.

176. Ziegler EJ, McCutchan JA, Fierer J, Glauser MP, Sadoff JC, Douglas H, Braude AI: Treatment of gram-negative bacteremia and shock with human antiserum to a mutant Escherichia coli. N Engl J Med 307:1225-1230, 1982.

177. Baumgartner J, McCutchan JA, Van Melle G, Vogt M, Luethy R, Glauser MP, Ziegler EJ, Klauber MR, Muehlen E, Chiolero R, Geroulanos S: Prevention of gram-negative shock and death in surgical patients by antibody to endotoxin core glycolipid. Lancet II: 59-63, 1985.

178. Calandra T, Glauser MP, Schellekens J, Verhoef J, and the

Swiss-Dutch J5 Immunoglobulin Study Group: Treatment of gram-negative septic shock with human IgG antibody to Escherichia coli J5: A prospective, double-blind, randomized trial. J Infect Dis 158:312-318, 1988.

179. Lachman E, Pitsoe SB, Gaffin SL: Anti-lipopolysaccharide immunotherapy in management of septic shock of obstetric and gynaecological origin. Lancet I:981-984, 1983.

180. Greisman SE, Johnston CA: Failure of antisera to J5 and R595 rough mutants to reduce endotoxemic lethality. J Infect Dis 157:54-64, 1988.

181. Baumgartner JD, Heumann D, Gerain J, Weinbreck P, Grau GE, Glauser MP: Association between protective efficacy of anti-lipopolysaccharide (LPS) antibodies and suppression of LPS-induced tumor necrosis factor a and interleukin 6. J Exp Med 171:889-896, 1990.

182. Danner RL, Joiner KA, Parrillo JE: Inhibition of endotoxin-induced priming of human neutrophils by Lipid X and 3-Aza-Lipid X. J Clin Invest 80:605-612, 1987.

183. Proctor RA, Will JA, Burhop KE, Raetz CRH: Protection of mice against lethal endotoxemia by a Lipid A precursor. Infect Immun 52:905-907, 1986.

184. Tuomanen EI, Saukkonen K, Sande S, Cioffe C, Wright SD: Reduction of inflammation, tissue damage, and mortality in bacterial meningitis in rabbits treated with monoclonal antibodies against adhesion-promoting receptors of leukocytes. J Exp Med 170:959-969, 1989.