Medical Grand Rounds

Parkland Memorial Hospital

September 4, 1980

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DALLAS COUNTY HOSPITAL DISTRICT

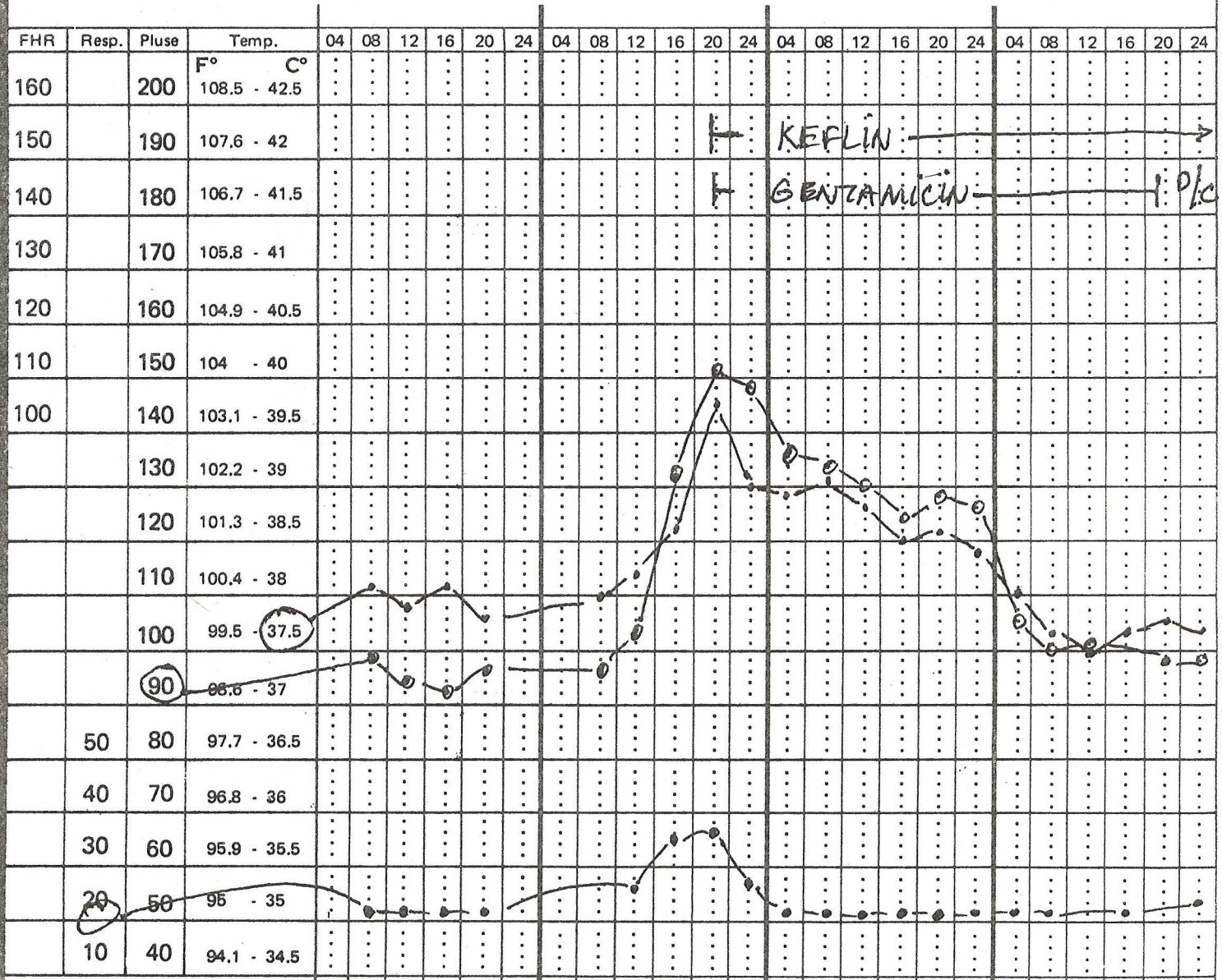
DALLAS, TEXAS

DEPARTMENT OF NURSING SERVICE

GRAPHIC CHART

DATE		8/31	9/1	9/2	9/3
Days After	Admission	4		6	
	Operation				

PREVENTION AND TREATMENT OF GRAM-NEGATIVE ROD BACTEREMIA



BLOOD PRESSURE																							
	110/80	105/80		120/85			120/80	110/80	110/75	110/80	100/60	95/60	100/85	100/60	105/65	110/70	110/80	110/70	108/80		120/85		120/80

WEIGHT				Kg. GMS.						Kg. GMS.						Kg. GMS.						Kg. GMS.	
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OUTLINE

COVER: Typical changes in vital signs during Gram-negative bacteremia. The useful graphics sheet was available on Parkland medical wards until 1979.

ACKNOWLEDGMENT: to Marguerite M. Ray, for her patience and care in the preparation of the handout.

Gram-negative bacteremia is an important cause of morbidity and mortality in the United States today. Recent estimates in large teaching hospitals indicate that Gram-negative bacteremia occurs in over 1% of all hospital admissions (7), and that the incidence of this disease has steadily increased over the last twenty years (4,7). In spite of improvements in antibiotics and other therapeutic modalities, moreover, the fatality rate of Gram-negative bacteremia has remained rather constant (25-40%) (7).

This review will focus on recent developments which may allow improvement in both prevention and therapy of Gram-negative bacteremia. Emphasis will be placed on the new, innovative, and often controversial aspects of prevention and therapy. Many features of this subject were discussed in Dr. James Smith's excellent Medical Grand Rounds on September 24, 1977 and will only be summarized here.

For simplicity, Gram-negative bacteremia will be divided into 3 stages:

I. Entry of bacteria into the blood.
II. Clearance of bacteria from the blood.
III. Host inflammatory response.

At each stage, we shall consider the microbial and host factors which influence outcome and the measures which may be taken to improve host survival.

I. ENTRY OF BACTERIA INTO THE BLOOD (PREVENTION OF GRAM-NEGATIVE BACTEREMIA)

A. Microbial factors

The Gram-negative aerobic rods which most commonly cause bacteremia in man are *Escherichia coli*, *Pseudomonas aeruginosa*, and members of the *Tribes Klebsiellae* (Klebsiella, Enterobacter, Serratia) and *Proteeae* (Proteus species)(1). *E. Coli*, the predominant Gram-negative aerobe in the fecal flora, is also the Gram-negative organism most commonly isolated from blood; to a large extent this association reflects the frequent appearance of *E. coli* in abdominal sepsis, cholecystitis, and pyelonephritis. The organisms listed above are not considered to be pathogenic in the normal host. This discussion will not include the Gram-negative pathogens (such as neisseria, salmonella and shigella) which may also cause bacteremia in man.

1. Factors which influence gastrointestinal colonization by Gram-negative bacteria. In a given individual, the composition of the fecal flora is remarkably constant over time (15). It is usually not possible to replace a resident *E. coli* strain, for example, with another strain of *E. coli*, and most of the other bacteria cited above are infrequently found in feces from normal human subjects (1). Most Gram-negative rod bacteremias are caused by the "normal" flora, yet under certain circumstances non-resident bacteria may colonize the bowel; several studies have linked stool colonization to subsequent Gram-negative bacteremia (or other infection), both in neutropenic (16,17) and non-neutropenic (18) patients. Two factors appear to be important in the establishment of fecal colonization by "invaders:"

   (a). Bacterial attachment (adherence) factors. Adherence of certain *E. coli* strains to human buccal epithelial cells is inhibited by mannose or concanavalin A, a mannose-binding lectin (19). This binding is probably mediated by small, hairlike bacterial surface projections called pili (20).

   Johanson and his coworkers found that oropharyngeal colonization with Gram-negative rods in seriously ill, hospitalized patients correlated with the ability of *P. aeruginosa* and *K. pneumoniae* to adhere *in vitro* to the patients' buccal epithelial cells--epithelial cells from colonized patients appeared to have exposed binding sites for these Gram-negative bacteria. Concanavalin A inhibited bacterial attachment, suggesting that the adherence mechanism might be similar to that described for *E. coli* above. Trypsin treatment of the epithelial cells increased bacterial attachment (21). In subsequent studies, these workers have obtained evidence that adherence of bacteria to buccal mucosal cells, and thus presumably colonization of the oropharynx, correlates with increased levels of proteolytic activity in saliva. The obvious interpretation of this finding is that the proteolytic activity exposes bacteria-binding sites on epithelial cells.

Other bacterial surface structures which allow gut colonization include the K88 antigen of *E. coli*, a plasmid-controlled surface protein which is required for attachment of certain pathogenic strains to pig intestinal mucosa (upper small intestine) (12).

(b). The resident bacterial flora. The normal flora seems to have an important role in preventing overgrowth by newly introduced bacteria (15). Pseudomonads, for example, are present in the feces in less than 10-20% of normal subjects. Since pseudomonads are commonly present in food and water, the absence of these organisms from the normal fecal flora implies that the normal host possesses mechanisms for resisting colonization. Fecal carriage rates may increase 5-fold or more in severely ill patients, and fecal carriage of *P. aeruginosa* appears to be prolonged by concurrent administration of an antibiotic such as ampicillin (22,23).

The ability of normal flora to prevent colonization of the GI tract by new organisms is known as "colonization resistance." For example, as little as 10 to 100 E. coli can colonize the intestine of a germ-free mouse, whereas greater than 10^6 organisms are required to colonize the intestine of a mouse which has "normal flora." (24), Suppression of the bowel flora with antibiotics again allows colonization with a small number of E. coli.

It appears that the anaerobic bowel flora are largely responsible for this effect. Eradication of aerobes with a drug such as nalidixic acid, which does not eliminate the anaerobes, does not impair colonization resistance in the mouse model (10).

2. Factors which influence bacterial growth at local sites. It has been recognized for many years that bacteria require iron for growth; recently attention has been focused on the mechanisms by which bacteria acquire iron from the environment, and several iron-binding compounds have been identified (siderophores). A clear role for these factors in pathogenesis has not been demonstrated, though there is suggestive evidence from several sources (summarized in 25, 26).

3. Factors which influence mucosal invasion by Gram-negative bacteria. Little is known about these factors, and the available information relates to invasive organisms (shigella, salmonella) and not to the non-invasive bacteria under consideration here. The latter organisms usually invade because of breaks in a normal mucosal barrier. In other words, they often get a "free ride" along plastic or rubber tubes.

B. Host factors which prevent entry of bacteria into blood.

1. Mechanical barriers. The most obvious host defense mechanisms for the prevention of Gram-negative bacteremia are anatomical: the skin and the normal mucosa of the GI tract effectively resist invasion by the organisms which are the subject of this discussion.

The normal pulmonary clearance mechanisms (mucociliary blanket, local phagocytes) and the normal bladder "wash-out" also normally prevent the establishment of local Gram-negative bacterial infections. Breaches in these anatomical barriers, obviously, may provide Gram-negative bacilli with direct access to local tissues and/or blood. Obstruction often contributes to the initiation of bacteremia.

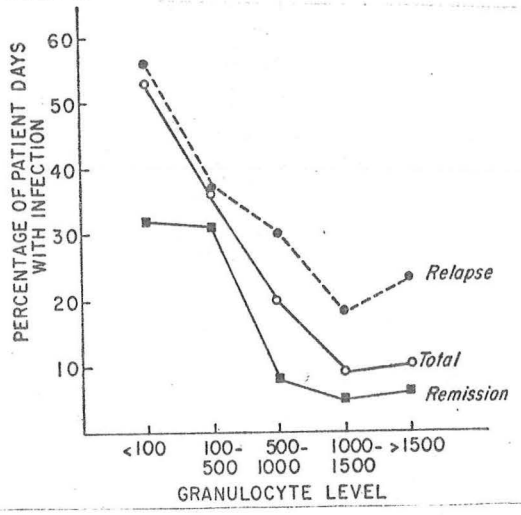


Fig. 1.

Relationship between total granulocyte count and frequency of infection in patients with acute leukemia (in relapse and in remission). From reference 27.

2. Local phagocytes. A role for polymorphonuclear leukocytes (PMNs) in "policing" the gut mucosa has been claimed, though the evidence for this is rather indirect. It is known that PMNs are extruded into the bowel lumen in normal man. It is also apparent that in man, neutropenia predisposes to Gram-negative bacteremia and that often a local source of infection is not apparent (11,27). In several studies, there has been a close correlation between the serotypes of Gram-negative bacteria isolated from blood and stool of the same patients (mainly *P. aeruginosa* and *K. pneumoniae* (16,28). It is generally assumed that bacteremia originates in the GI tract in such patients, possibly because of small mucosal ulcerations.

E. Ziegler has recently described an animal model of Pseudomonas sepsis which appears to mimic the features of Pseudomonas sepsis in man (29)). When inoculated into the conjunctival sac of a normal rabbit, *P. aeruginosa* is relatively innocuous, causing little local inflammation. In the rabbit rendered neutropenic by nitrogen mustard, however, a similar instillation of *P. aeruginosa* into the conjunctival sac leads to a virulent local infection (conjunctivitis, cellulitis) with rapid onset of bacteremia. Pathologic sections show extensive invasion of vessel walls by the bacteria. In this model, mucosal "protection" by neutrophils appears to be critical, although local micro-ulceration cannot be excluded. It should also be noted that *P. aeruginosa* appears to be much more virulent in this model than other Gram-negative rods--this correlates well with the striking association of GI colonization and bacteremia with *P. aeruginosa* in several studies of neutropenic patients (16,28).

3. Other local defenses. Levels of lactoferrin (competes with bacteria for iron) and immunoglobulin in local secretions probably contribute to "mucosal immunity" to Gram-negative rods; this is better established in infants than in adults (30). Though secretory antibody (IgA) binds bacterial antigens in the mucosal glycocalyx (30) and may thus interfere with the attachment of Gram-negative bacteria to the gut mucosa, deficiency of IgA has rarely (if ever) been associated with recurrent Gram-negative bacteremia (31).

C. Prevention

1. Catheter and ventilator care. Approximately 45-70% of patients who develop Gram-negative bacteremia acquire the disease in the hospital (7,32). The importance of careful management of urinary and intravenous catheters cannot be overemphasized. Similarly, much care is required to prevent infection by mechanical devices (blood pressure transducers, mechanical ventilators) and by contamination of intravenous fluids.

Table 1. *Risk of Secondary Nosocomial Bacteremias from Underlying Infections with Gram-Negative Rods*

Underlying site of infection	Rate of Gram-Negative Rod Infection: In underlying site (per 10,000 discharges)[a]	In blood, deriving from underlying site (per 10,000 discharges)[b]	Risk of secondary bacteremia
Respiratory tract	30.9	0.77	2.5%
Cutaneous	9.1	0.19	2.1%
Urinary tract	117.4	1.40	1.2%
Surgical wound	48.4	0.51	1.1%

[a] From summary data of the National Nosocomial Infections Study (NNIS) for 1970 through 1972.
[b] From NNIS data, 1970 through 1973.

from reference 33

The above data from CDC surveillance indicate a low but definite risk of bacteremia associated with different hospital-acquired infections at different local sites. In recent studies from two different institutions, however, less than half of the nosocomial bacteremias could be attributed to a recognized source such as the urinary

tract, an intravenous catheter, or pneumonia associated with the use of ventilation equipment (34). Moreover, in a study at Grady Memorial Hospital (32), McGowan found that only 8% of the total number of nosocomial bacteremias (7 of 91) occurred following an apparent variation from hospital infection-control guidelines. Similarly, Johns Hopkins workers estimated that optimal hospital infection-control measures could prevent only 25% of their nosocomial bacteremias (35).

These discouraging analyses came from institutions which already had active infection-control programs; there are no such estimates from hospitals which do not have personnel interested in preventing hospital infections. Although the Center for Disease Control is currently conducting a large study designed to test the efficacy of hospital infection-control programs, ample evidence exists that the current guidelines for urinary and intravenous catheter care, for example, are effective in preventing nosocomial infections (2). The problem appears to be that some patients acquire bacteremia even when these guidelines are observed, and many acquire nosocomial bacteremia from sites which are not subject to preventive measures. or from unknown sites of origin (38% in the Hospkins study) (34).

2. Measures to decrease bacterial flora at local sites.

(a) Polymyxin aerosol--prevention of oropharyngeal colonization with *P. aeruginosa*.

In an attempt to reduce the frequency of *P. aeruginosa* pneumonia in an intensive care unit, polymyxin B (2.5 mg/kg/day, divided into q4h doses) was given by aerosol spray into the oropharynx or, in intubated patients, into the tracheal tube. The incidence of Pseudomonas colonization of the upper airway was reduced during polymyxin treatment (as compared with 2 month cycles in which patients received a saline placebo), and the incidence of Pseudomonas pneumonia was also reduced (36,37). On the other hand, there was no difference in mortality in polymixin--and placebo-treated groups. Although polymyxin-resistant flora did not emerge during the initial studies, continuous use of polymyxin spray for a 7 month period was associated with a striking increase in both colonization and pneumonia caused by polymyxin-resistant bacteria (38).

Although local administration of polymyxin might be worthwhile in the individual patient at high risk of Pseudomonas pneumonia, prolonged use of this approach should be avoided in units such as intensive care wards.

(b) Topical burn wound therapy. There is good evidence that topical agents reduce the incidence of burn wound sepsis. There are 3 agents currently in general use: mafenide acetate (Sulfamylon)

is very effective but is associated with metabolic derangements and pulmonary complications in patients with large burns. Silver sulfadiazine cream is therefore preferred by some authorities (2). Silver nitrate (0.5% soaks) is the least expensive and is also widely used. These agents are effective in reducing (but not abolishing) colonization with Gram-negative rods, including *P. aeruginosa*, and their use has been associated with significant reductions in mortality due to overwhelming infection.

(c) Oral, non-absorbable antibiotics

Table 2

PROSPECTIVE STUDIES OF ORAL, NON-ABSORBABLE ANTIBIOTICS FOR PREVENTION OF GRAM-NEGATIVE INFECTIONS IN LEUKOPENIC PATIENTS

Study	Date	Regimen Used	No. of Patients		Episodes of Bacteremia		Ref.
			Antibiotics	Control	Antibiotics	Control	
Schimpff	1975	GVN	19	21	7 (37%)	16 (76%)	39
Storring	1977	FRACON	46	49	6 (13%)	18 (37%)	40
Levine	1973	GVN	38	28	6 (15%)	4 (14%)	41

Comments:

Schimpff -- data are from two arms of a 3-arm study. Authors emphasize importance of actual ingestion of the antibiotics, describe taste as "dreadful."

Storring -- patients also received pathogen-free food. Simple reverse isolation.

Levine -- overall serious infection rates were 17/38 (45%) in antibiotic-treated group, 16/28 (57%) in controls (not different statistically). Treated group was shown to have a reduction in fecal flora.

Several studies have found that the administration of oral, non-absorbable antibiotics can reduce the frequency of significant Gram-negative infection in neutropenic patients (39,40). Most American studies have used combinations of gentamicin, vancomycin, and nystatin (GVN). Another regimen (framycetin sulphate [neomycin B], colistin, and nystatin [Fracon]) has been used in England; compared with controls not receiving the combination, treated patients had less Gram-negative bacteremia, fewer febrile days while neutropenic, and fewer ano-rectal infections (40). In contrast, other groups have not found significant benefit to accrue from prophylactic oral

non-absorbable antibiotics (41,42). A major problem with these regimens is patient compliance, as the mixtures taste awful and are poorly tolerated by patients; success with this regimen may reflect close supervision of the administration of the drugs (10).

These drugs also may select resistant flora: in one study (43), 16 of 70 patients undergoing GVN prophylaxis were colonized with gentamicin-resistant bacteria. Most of these organisms were resistant to gentamicin at the time of patient acquisition, while 3 became resistant while GVN was being ingested. Infection caused by gentamicin-resistant Gram-negative rods occurred in 5 of the patients. Unfortunately, control studies were not performed in persons not receiving GVN, so the importance of these data is uncertain. Other studies have also reported significant colonization with gentamicin-resistant organisms in persons receiving GVN (44).

Most workers agree that oral, non-absorbable antibiotic prophylaxis is most useful in patients who have profound (<100/ul) neutropenia associated with cytotoxic chemotherapy (11). It is also apparent that these regimens may prolong short-term survival (39), though the long-term survival rates are influenced ultimately by the achievement of remission and/or cure. Discontinuation of the oral regimen has been followed by serious Gram-negative infection in several patients (39,43), suggesting that oral antibiotics should be continued until the patient recovers from granulotopenia. Efforts to improve the taste of the preparations are needed, as is a less expensive regimen (GVN costs over $100. per day).

(d) Trimethoprim-sulfamethoxasole. During a double-blind trial of TM-SMX for the prevention of *Pneumocystis carinii* pneumonia in children with leukemia, workers in Memphis made the interesting observation that the incidence of Gram-negative infections was also reduced in children receiving the drug (45). Subsequently, 2 groups of workers found that low-dose TM-SMX (2 tablets BID) significantly reduced the incidence of Gram-negative infections and the number of febrile days in adults who experienced neutropenia following cytotoxic chemotherapy (46,47). The beneficial effect was accompanied by a reduction in the fecal flora of Gram-negative aerobes; the acquisition of resistant flora (in particular, *P. aeruginosa* and candida) was uncommon. TM-SMX appears to cause greater reduction in fecal flora than TM alone. The frequency with which resistant flora have emerged has differed in different centers.

TM-SMX initially eradicates fecal aerobes, but with prolonged use may also suppress anaerobic flora (49). The advantage of "colonization resistance" may thus be lost, contrary to the expectation of some workers (10). Drugs with a more selective effect on aerobes include nalidixic acid and some experimental agents (10).

Note: (1) The Memphis study is the only trial which was performed double-blind using placebo-treated controls (45). Neutropenia, per se, was not evaluated in assessing the results.

2) Patients (both TM-SMX treated and controls) in the London study (47) received oral non-absorbable antibiotics (framycetin, colistin, and nystatin). Thus the effect of TM-SMX alone would be hard to evaluate from these data.

3) Although TM-SMX resistant bacteria were isolated from only a small number of patients in the original study by the Winnipeg group (46), a subsequent study by one of the authors found that 73% of patients treated with TM-SMX had resistant organisms isolated from stool at least once during the study (48). See also ref. 50.

TM-SMX has been well-tolerated by most patients. The selective suppression of aerobic flora seems less likely to lead to superinfection than the "total suppression" oral antibiotic regimens. Until further data support the efficacy and safety of this approach, however, it should be considered experimental.

(e) Isolation.

As noted above, most infections in neutropenic patients arise from the patients' own flora. Prevention of colonization by hospital organisms is the goal of isolation, and there is evicence that this modality may be effective.

Table 3., From Ref. 51.

Determination	Treatment group[a]	
	PI	W
No. of evaluated hospital courses	20	23
No. of infections (per 100 hospital days)		
Probable	10 (2.28)	15 (2.45)
Definite[b]	20 (4.58)	16 (2.61)
Overall	30 (6.86)	31 (5.07)
Mean no. of infections per course	1.5	1.4
Sites of infection (no. per 100 days)		
Bacteremia	10 (2.29)[c]	4 (.65)
Oropharyngitis	5 (1.15)	3 (.49)
Tracheobronchitis	1 (.23)	3(.49)
Pneumonia	8 (1.83)	11 (1.80)
Anorectal	1 (.23)	2 (.33)
Other soft tissue	3 (.69)	4 (.65)
Urinary tract	1 (.23)	2 (.33)
Other	1 (.23)	2 (.33)
Definite infections caused by:		
Gram-negative bacilli (*P. aeruginosa*)	10 (2)	7 (–)
Staphylococcus aureus	2	–
Other bacteria or parasites	9	6
Fungi	2	4
Percent of days with fever >38.5°C	34.5	30.0
Outcome		
Discharged alive (%)	11 (55)	15 (65)
Died of infection (%)	8 (40)	8 (35)
Leukemic remission achieved in ANNL[d] (%)	6 (43)	5 (42)

[a] As defined in the text. Prophylactic oral nonabsorbable antibiotics were not used.
[b] Documented microbiologically.
[c] $P < 0.05$.
[d] Acute nonlymphocytic leukemia.

1) Reverse isolation. Although simple reverse isolation (single room, visitors wear gowns and masks, wash hands) is almost universally practiced, there are studies which suggest that it is of limited value. Maki and Nauseef (U. Wisconsin) compared reverse isolation (20 patients) with ward care (double or four-bed room) (23 patients). There were actually fewer bacteremias and overall Gram-negative infections in the patients in the routine ward care group (51). Schimpff (52) found similar results in a study which added oral non-absorbable antibiotics to both reverse isolation and ward-care regimens.

PI = protective isolation
W = ward

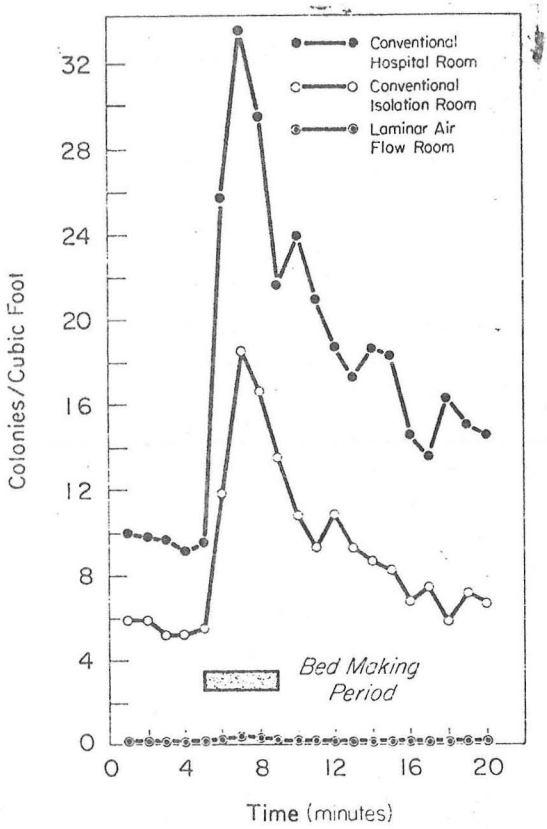


FIG. 3 *Air contamination in laminar airflow room and conventional isolation and hospital rooms measured by slit-samplers placed 3 ft inside the room entrances. Curve for laminar airflow room is mean of four experiments, others are mean of two experiments.*

2) Laminar flow rooms. The goal of laminar flow technology is reduction in the microbial content of room air. Success is indicated by air-sampling surveys.

←←Figure 2--from reference (53).

In a few studies, the use of laminar flow rooms, in combination with oral, non-absorbable antibiotics, has been associated with reductions in both colonization with Gram-negative bacteria and significant infections.

Table 4

PROSPECTIVE COMPARISONS OF LAMINAR FLOW ROOM ISOLATION WITH ORAL NON-ABSORBABLE ANTIBIOTICS

Study	Date	Oral regimen	Number of patients		Episodes of Gram-negative bacteremia			Reference
			LFR + AB	AB alone	LFR alone	LFR + AB	AB alone	
Levine	1973	GVN	22	38		2 (9%)	6 (16%)	41
Schimpff	1975	GVN	24	19		4 (17%)	7 (37%)	39
Schimpff	1978	GVN	11	21		2 (18%)	6 (28%)	52

Notes:

Levine - overall serious infection rates were 3/22 (14%) for LFR + AB and 17/38 (45%) for AB alone ($p = .06$). No difference seen in remission rate or duration.

Schimpff (1975) - only "total bacteremias" reported. Both LFR + AB and AB alone groups had higher remission rates and longer median survival than unprotected ward patients.

Schimpff (1978) - Laminar flow with a Med-Aire filtration unit - Bedside air filtration "Total bacteremias" reported. Patient acquisition (colonization) of pathogens was the same in both groups. A third group (n = 10) with oral antibiotics plus reverse isolation had similar results. The authors attribute failure to show a difference to the high general standard of air quality in their hospital.

These differences were not striking, however. Since Gram-negative bacteremia most often arises from the GI tract in such patients, oral-non-absorbable antibiotics may be more important than laminar-flow isolation for the prevention of this complication. In fact, in one study which found a difference in the overall infection rate in patients given care in a laminar flow room, with oral antibiotics, as compared with patients who only received oral antibiotics, the difference could be explained by skin infections and pneumonia:

Table 5

	LFR + AB	AB alone
Number of patients	22	38
Number with Gram-neg bacteremia*	2	6
Number with skin infections	3	11
Number with pneumonia (no bacteremia)	1	10
Total infections**	8 (36%)	30 (79%)

* Includes pneumonia with bacteremia
** Also includes herpes simplex, UTI

From reference 41

The role of laminar flow isolation, when used in conjunction in conjunction with oral antibiotics, appears to be the prevention of infections related to skin and pulmonary colonization by hospital organisms (54); Gram-negative bacteremia should be prevented only insofar as pneumonia and cellulitis caused by Gram-negative organisms can be prevented.

3. Measures to bolster host defenses

(a) Lithium carbonate. Lithium carbonate administration is commonly associated with an elevation in peripheral blood neutrophil numbers. It is thought to cause an expansion in the neutrophil mass with no decrement in neutrophil function (55). In a small series of patients who underwent cytotoxic chemotherapy for small-cell bronchogenic carcinoma, lithium carbonate administration was associated with significant reductions in the incidence of infection and fever (56). In contrast to *in vitro* predictions (57), repeat courses of lithium appear to be effective in elevating neutrophil counts (56).

Lithium carbonate may enhance the production of granulocyte colony-stimulating factor, and it has been argued that leukemic cells, as well as normal cells, may respond to colony-stimulating factor *in vitro* (57a). Moreover, the benefit of lithium therapy would seem to be greatest in patients who do not have severe marrow granulocyte aplasia, since a certain minimal marrow "reserve" must be present to be stimulated (58). The role of lithium carbonate may thus be limited to patients who undergo cytotoxic chemotherapy for conditions other than acute leukemia--such as the patients with bronchogenic carcinoma discussed above. In such groups, lithium may be a simple and effective way to reduce the incidence of infection, including Gram-negative bacteremia. Although it has been argued that the lower risk of infection may allow more intensive chemotherapy (56), this has not been shown. Indeed, if the above argument is correct, greater reduction of marrow granulocyte reserves should minimize the ability of lithium to stimulate neutrophil production. Further studies of this problem are clearly needed.

Side effects of lithium include weakness, anorexia, nausea and vomiting--these may be difficult to distinguish from the side effects of chemotherapy itself. It is necessary to monitor lithium levels to avoid the more serious neurological side effects (ataxia, slurred speech, confusion, seizures, and/or coma have been noted).

(b) Neutrophil transfusions.

Prophylactic infusions of neutrophils have been used primarily in patients who have received bone marrow transplants. Experience with this approach indicates that it is complicated, expensive, and associated with significant risks. Two studies are of interest:

(1) Clift et al (59) (Seattle) gave WBC transfusions to bone marrow recipients whose granulocyte counts declined to 200/mm^3 post-transplant. Daily transfusions were given, using the same related donor. During the 1st 21 post-transplant days, there were two local infections and no septicemias in 29 transfused patients, while 7 local infections and 10 septicemias developed among the 40 controls. Approximately half of the controls and transfused patients were receiving antibiotic therapy at the time of randomization in the study; there was no difference in the incidence of infection in these groups. Moreover, there was no difference in the mortality in the two groups (all causes).

(2) Winston et al(60) (Los Angeles) gave prophylactic WBCs to bone marrow recipients whose granulocyte counts decreased in 500/mm^3 or less post-transplant. Other differences from the above study: use of more WBC/day, concomitant use of oral, non-absorbed

antibiotics in both transfused and control groups (GVN), and use of donors who were not necessarily histocompatible (as measured by leukocyte cytotoxicity tests) with the recipient. The rate of infections in transfused and control groups was similar, with the exception that CMV infection occurred more commonly in the transfused patients. These workers have abandoned prophylactic granulocyte transfusions and argue for the use of non-absorbable antibiotics plus aggressive antibiotic management of febrile episodes.

Other problems with prophylactic WBC transfusion include cost and (in the absence of an HLA-compatible donor) alloimmunization. The cost-effectiveness of prophylactic transfusion has been analyzed by Rosenshein (61), who estimated that the cost of this modality was some $85,000 per life-year of survival. (p. 31). The problem of allo-immunization is demonstrated in the study of Mannoni (62); it may limit the subsequent use of therapeutic transfusions in such patients.

Young (1) has pointed out that many prospective WBC recipients lack opsonins (substances that "prepare" bacteria for ingestion by phagocytes). He suggests that provision of opsonins, by active or passive immunization, may enhance the functional effect of transfused WBCs. There is experimental evidence in dogs which supports this view (64).

See also section on therapeutic WBC transfusions below.

Table 6. STUDIES OF PROPHYLACTIC TRANSFUSION* (Modified from Ref 61)

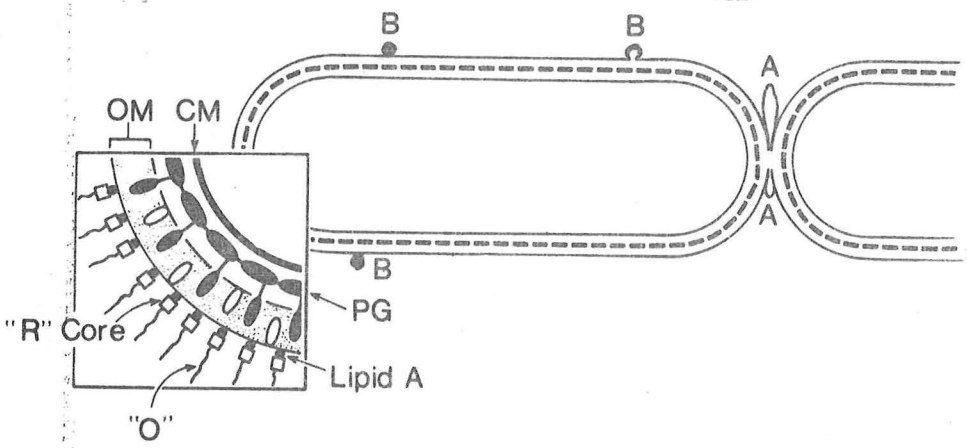
Study	Entry Criteria			Transfusion Data			Results (Infected/Total)		
	Neutrophils $X10^6$/Liter	Patient Population	Collection	Donor Source	PMN Dose $X10^9$/Day	Selected End Point	Control No. Patients	Transfused No. Patients	Ref.
Clift et al	200	Marrow-transplant recipients	FL or CFC	Family, marrow donor	22(FL,15.7 (CFC)/day	Positive blood culture or local infection	17/40	2/29	(59)
Ford	500	Adults with acyte leukemia	IFC,starch, steroids	Related unrelated	15/every other day	Documented infection	6/11	3/13	(63)
Mannoni et al	500	Adults with acute leukemia	IFC and starch	--	10/day until Day 12 of aplasia	Major infection	11/28	1/21	(62)
Winston	500	Marrow-transplant recipients	CFC	Related unrelated	12/day	Documented infection	7/17	9/19	(60)

*PMN denotes polymorphonuclear leukocytes, FL filtration leukapheresis, CFC continuous-flow centrifugation, and IFC intermittent-flow centrifugation.

(c) Immunoprophylaxis

Antibiodies to Gram-negative bacteria appear to serve two beneficial functions for the host: they facilitate phagocytosis (i.e., act as opsonins) and act as antitoxins. Antibodies to capsular polysaccharides are critical for effective opsonization of encapsulated cells. while antibodies to cell wall lipopolysaccharide (endotoxin) may facilitate phagocytosis (more efficiently in non-encapsulated organisms) or act as antitoxins, neutralizing the biological activities of endotoxin.

Figure 3 - From Reference 65



This simplified schematic of the gram-negative bacterial cell wall in cross section shows its three layers (outer membrane (*OM*), peptidoglycan (*PG*), cytoplasmic membrane (*CM*)) with lipopolysaccharide (*LPS*) located in the outer membrane. Lipid A, the toxic moiety, is thought to be integrated into the membrane, linked by a bridge of R-core sugars to the O polysaccharide. Cell surface components such as pili, flagellae, and the capsular polysaccharide are not shown.

(1) Passive Immunization.

(a). Cross-reactive antibodies. An innovative and rather promising approach to passive immunization has been developed by Ziegler, Braude and their colleagues at San Diego. They have shown that an antiserum to Gram-negative bacterial lipopolysaccharide (endotoxin) can neutralize many of the biological effects of endotoxins from different, unrelated Gram-negative organisms. This broad spectrum of protection is possible because the structure of the toxic lipid A moiety and the attached core sugars is almost identical in endotoxins from many Gram-negative bacteria. They have used as a vaccine the J5 mutant of E. coli 0111; this mutant is unable to synthesize O chains or to make complete R-core, so that it has "deep" R-core sugars exposed on the surface. See also page 46.

They prepared human J5 antiserum by vaccinating healthy young men with boiled J5 bacterial cells. After showing that the antiserum was able to protect neutropenic rabbits from lethal Pseudomonas bacteremia, they have undertaken two large clinical trials. First, they have used the serum to treat patients with Gram-negative bacterial shock (see discussion below on page 46. Second, they have studied the role of prophylactic J5 antiserum in the prevention of Gram-negative bacteremia in neutropenic patients. Preliminary results of this trial (see Table 6), suggest that the frequency of febrile episodes may be reduced by the antiserum, thus providing indirect evidence that these episodes are related to endotoxemia. The number of patients with bacteremia was too small to allow analysis of this feature of the trial. A second phase of the trial is nearing completion.

Table 7 *Clinical trial of prophylactic J5 antiserum in neutropenic patients*

Factor	Nonimmune serum	J5 antiserum
Patients	7	9
Neutropenic episodes	12	15
Days of neutropenia	180	194
Gram-negative bacteremias	2	0
Incidence of febrile days unexplained by gram-positive or fungal infections	44%	18% ($P < 0.0005$)

From Ref. 66

(b) Specific immunization. Passive immunization might also employ antiserum to specific pathogens, e.g. *P. aeruginosa*. The obvious problem with this approach is the fact that surface antigens of these bacteria are heterogeneous--*P. aeruginosa* has 16 recognized serotypes, *E. coli* has over 120, etc. In virtually every animal study, however, passive immunization with specific homologous antibodies has been more protective than passive immunization with cross-reactive antibodies (such as J5).

One major problem with passive immunization is the necessity, using currently available techniques, for intramuscular injection of the antiserum. Intravenous administration of antibody-containing fractions of human serum is associated with a risk of anaphylaxis or serum sickness unless precautions are taken. Efforts are currently being made to develop purification techniques which will allow i.v. administration of such preparations. Another future prospect is the development of both cross-reactive (J5) and specific antibodies using hybridoma techniques for monoclonal antibody production.

(2.) Active immunization.

(a) Cross-reactive vaccination. Many Gram-negative bacterial antigens are shared by other Gram-negative and Gram-positive bacteria. Immunization with one strain may induce antibodies which cross-react with antigens on other bacteria. For example, approximately 25% of the *Klebsiella pneumoniae* blood isolates in one study had capsular polysaccharide antigens which cross-reacted with the polysaccharides contained in the current pneumococcal vaccine (67). It has also been possible to induce antibodies to certain pathogens (e.g., *Hemophilus influenzae b*) by colonizing adults with non-pathogenic bacteria (*E. coli* K100) which have immunochemically identical capsular polysaccharides. It is possible that this approach may have some benefit: as a "spin-off" of pneumococcal vaccination, for example.

(b.) Specific vaccination. Successful active immunization to prevent Gram-negative sepsis requires (1) knowledge of the likely pathogen(s) (2) identification of high-risk patients prior to the onset of infection, so that antibody production has time to take place. Such a situation is the burn center with a high rate of sepsis associated with particular pathogens. Recently, a polyvalent *Pseudomonas aeruginosa* vaccine was tested in burn centers in England and India (69). The results were very encouraging: vaccinated patients had greatly reduced risk of bacteremia and death (from both Pseudomonas and, interestingly, Klebsiella), compared with alternate-patient controls. The vaccine contained representative antigens from each of the 16 *Pseudomonas aeruginosa* serotypes. It should be noted that many of the patients in this study did not receive topical antimicrobial therapy. The experimental setting was thus different from the usual situation in U.S. burn centers (see above, page 6). A similar vaccine, using a smaller number of serotypes, was somewhat effective in preventing Pseudomonas infections in cancer patients (68). Because the major antigen in these preparations in LPS, the vaccines themselves are toxic and often produce impressive local reactions.

4. Recommendations for prevention of infection in neutropenic patients

Bolster host defenses.

Reverse the underlying condition and thereby reverse the state of granulocytopenia.

Avoid prophylactic granulocyte transfusions except in a research setting where HLA-identical donors are available.

Await further evaluation of lithium carbonate-induced granulocytosis, passive immunization. Pneumococcal vaccine reasonable, pseudomonas vaccines experimental.

Reduce traumatic and invasive procedures.

Avoid intravenous catheters.

Insert butterfly needles aseptically, change dressing daily, and remove needle after 48 h.

Change all bottles and tubing every day and change all tubing after blood product administration.

Avoid urinary catheters except for shock or urinary obstruction.

Exert pressure to skin site following bone marrow aspiration, fingersticks, and venipunctures to prevent blood extravasation.

Ensure that all medical instruments and devices are appropriately cleaned or sterile as indicated before use.

Reduce acquisition of potential pathogens.

Develop an enforceable program of hospital housekeeping utilizing phenolic antiseptics, double-bucket mop technique, and daily antiseptic cleaning of all horizontal surfaces and bathroom fixtures.

Insist upon adequate handwashing by both patient and staff.

Utilize a low microbial diet and ensure that the water supply is satisfactory.

Place the patient in a single room to reduce direct cross-contamination with other patients.

Suppress microbes.

Teach and insist upon good patient hygiene including complete daily bath and shampoo and careful handwashing with providone-iodine or chlorhexidine preparation.

Encourage good dental hygiene including brushing and flossing to reduce dental plaque.

"Consider oral nonabsorbable antibiotics such as gentamicin, vancomycin, and nystatin only for patients who will be markedly granulocytopenic (<100 per microliter) for prolonged periods (>10 days). Utilize only if there is adequate nursing, pharmacy and medical support to assist in rigid compliance and adequate microbiologic laboratory assistance to observe surveillance cultures as one means of monitoring compliance and detecting acquisition of potential pathogens resistant to the selected oral antibiotics. As a general rule, these agents should be used under conditions of at least semi-isolation to reduce the chance for acquisition of a resistant pathogen."

Consider the use of trimethoprim-sulfamethoxazole plus nystatin or nalidixic acid plus nystatin as promising measures but ones which are as yet not fully evaluated as to efficacy and toxicity. The concept of selective microbial suppression, while intriguing, must still be considered investigational.

These recommendations are modified slightly from those of Schimpff (11).

II. CLEARANCE OF BACTERIA FROM THE BLOOD.

The studies of Rogers, Beeson and others demonstrated that clearance of injected bacteria from the blood of experimental animals takes place in three stages (70):

1. Early, rapid phase (I). Bacteria are removed by the liver and spleen; the splanchnic trapping of a given microbe is remarkably constant in different animal hosts. Phagocytes play an important role in this phase.

2. Disappearance or low-level persistence of bacteremia (II). The explanation for persistence of low levels of Gram-negative rods in the blood is uncertain. Re-entry from splanchnic sites has been noted, and multiplication within phagocytes has been suggested.

3. Resurgence of bacteremia (III).

Fig. 4, From Ref. 70

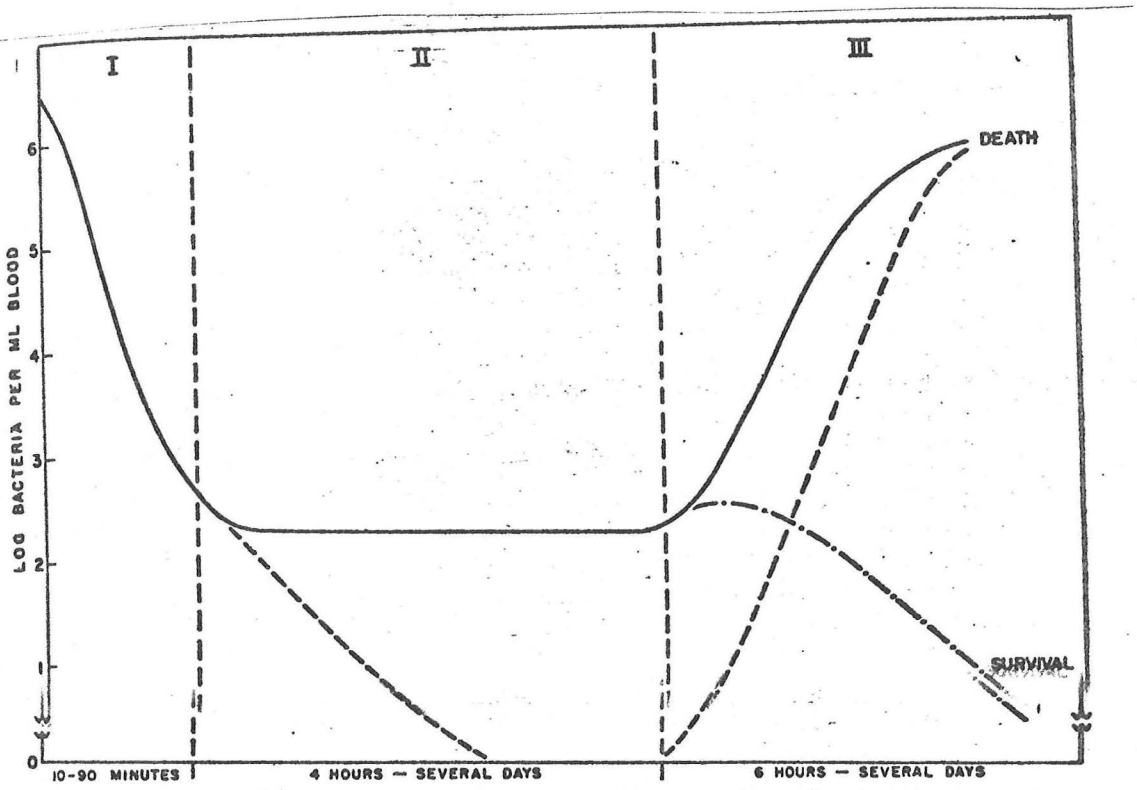


Figure 1. **A schema of blood stream clearance as reflected by blood cultures. In phase I, bacteria disappear rapidly from the circulation. During phase II, bacteremia may disappear or persist at low levels. Depending on the virulence of the bacterium under study, resurging bacteremia or sterilization of the blood stream may be observed in phase III.**

These early studies showed that the initial rapid phase of clearance occurs despite starvation, irradiation, neutropenia, shock, experimental diabetes, renal failure, etc. (70). Maximal reticulo-endothelial clearance capacity can, however, be altered by most of these stresses, increasing the likelihood of persistence or resurgence of bacteremia.

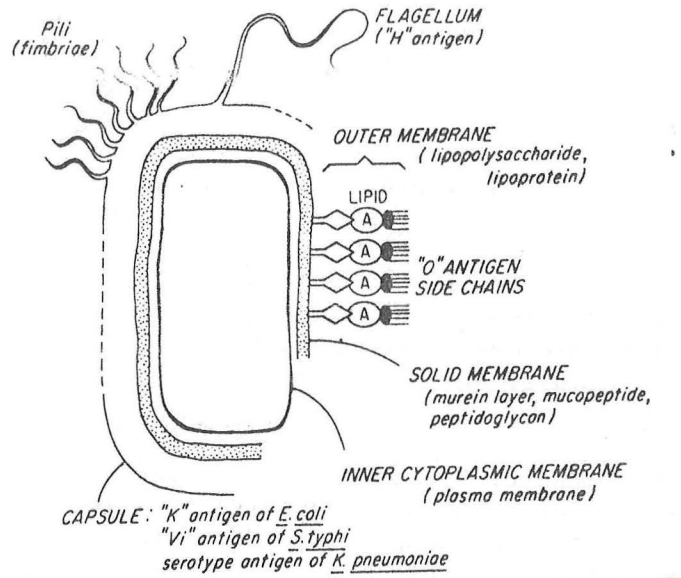


Fig. 5 from Ref. 1

Cross-section of typical Gram-negative rod, showing important structures.

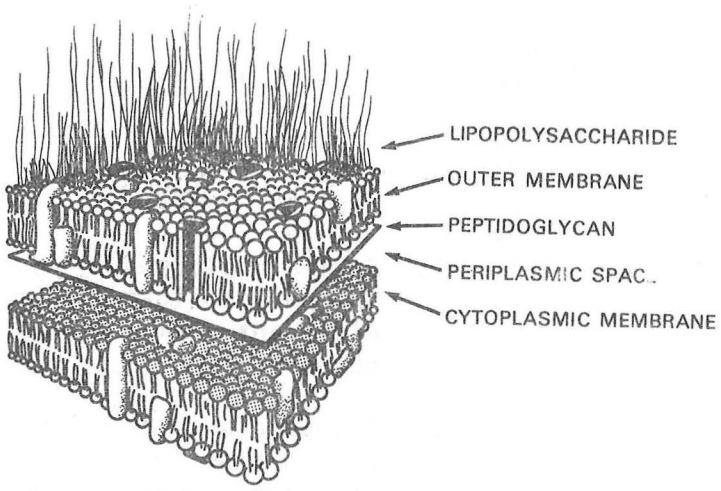


Fig. 6

Gram-negative cell envelope. Note projection of the endotoxin polysaccharide chains from the cell surface. Capsule is not shown.

A. Microbial factors which resist clearance.

1. Capsular polysaccharides. Most Gram-negative rods isolated from human blood have a (negatively-charged) polysaccharide capsule (see Fig. 5 above). It is known that this capsule may prevent phagocytosis by PMNs and macrophages *in vitro* (71), and the capsule may also make the bacterium resistant to killing by normal human serum (72).

In certain clinical conditions, specific capsular antigens (K antigens) seem to predominate. For example, in neonatal *E. coli* meningitis, K1 capsular antigen has been found in 85% of *E. coli* isolated from cerebrospinal fluid and morbidity and mortality in K1 meningitis were significantly greater than in meningitis caused by *E. coli* non-K1 strains (73). Several attempts have been made to correlate either the presence of a capsule or specific capsular types with the risk of Gram-negative bacteremia or shock (7,74). It has not been possible to demonstrate convincingly that the presence of a capsule influences the severity of Gram-negative sepsis, or that specific capsular types are more likely than others to cause bacteremia. The capsular types found in blood generally reflect those present in stool.

2. Cell wall lipopolysaccharides (endotoxin).

Gram-negative bacteria which lack the O antigen side chains of LPS (and are therefore "rough") are more susceptible to killing by human serum and are cleared more rapidly than O antigen-containing cells from the blood of experimental animals. Such

"rough" bacteria are also more efficiently phagocytosed by PMNs than are "smooth" Gram-negative bacteria which have a complete LPS structure , and they are more susceptible to cationic bactericidal leukocyte proteins (75). However, not all "smooth" bacteria have the same ability to resist phagocytosis and to survive in normal serum. So the O antigen appears to be useful, but not sufficient, for survival in the host. In one recent series of patients with Gram-negative bacteremia, certain *E. coli* O types were said to be associated with a high frequency of fatal outcome (7). The differences were small, however, and multifactorial analysis was not employed.

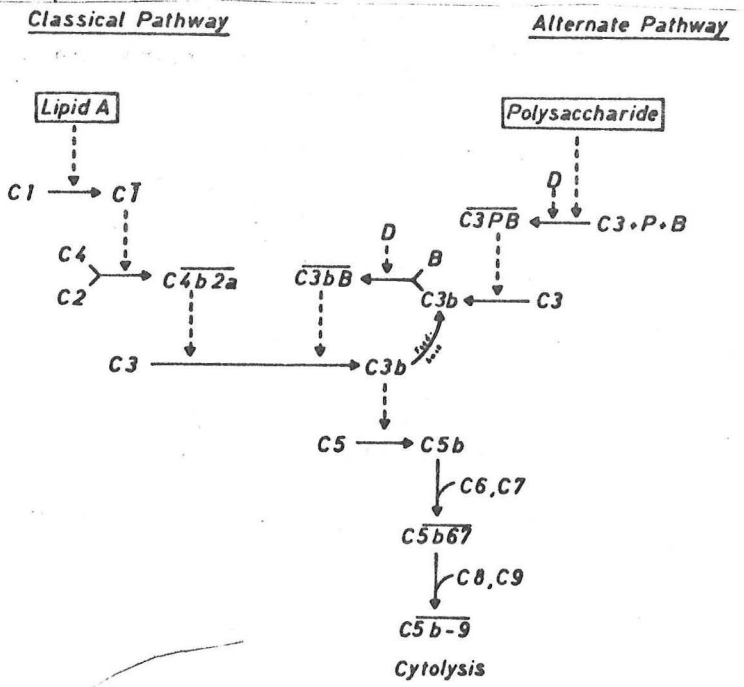
B. Host defenses which enhance bacterial clearance.

1. Phagocytosis. Destruction of Gram-negative bacteria by host phagocytes may be the most critical host defense mechanism, as evidenced by the profound inability of neutropenic patients to eliminate Gram-negative bacteria from the blood. The role of opsonins is key:

   (a) Antibody + complement. Both components appear necessary for phagocytosis of encapsulated strains of *E. coli* (71). Anticapsular antibody is not as efficient in opsonization as antibody plus complement, but antibody is essential for fixation of complement to the bacterial surface. The classical complement pathway is primarily responsible (C3b). (77)

   (b) Complement. Unencapsulated (K^-) *E. coli* may be phagocytosed by complement alone, without specific antibody. Complement is probably fixed following activation by cell-surface lipopolysaccharide (endotoxin); the alternative pathway is most important. See Ref. 81

Fig. 7, from ref. 76



Activation of complement by the lipid A portion and the polysaccharide portion of lipopolysaccharide.

(c) "Surface" phagocytosis. Originally described by Barry Wood (78), this phenomenon has been best studied using the pneumococcus. There is some evidence that Gram-negative bacteria may also be engulfed by phagocytes which nudge them against the surface of a blood vessel or sinusoid (78). This mechanism does not require antibody or complement and may possibly be important in the non-immune host. Few studies or surface phagocytosis have been performed in recent years, and some authorities now doubt its importance.

One group found that many patients who developed Gram-negative bacteremia had ineffective serum opsonins at the time blood cultures were positive (79); this has recently been disputed (80). There is also preliminary evidence that opsonin deficiency may contribute to the failure of some patients to respond to granulocyte transfusions (82).

The mechanisms by which phagocytes degrade ingested bacteria are poorly understood (reviewed in 83).

2. Serum killing. Most Gram-negative bacteria isolated from the gut or from urine are killed *in vitro* by normal human serum (85a). Isolates from blood, in contrast, are usually serum-resistant.

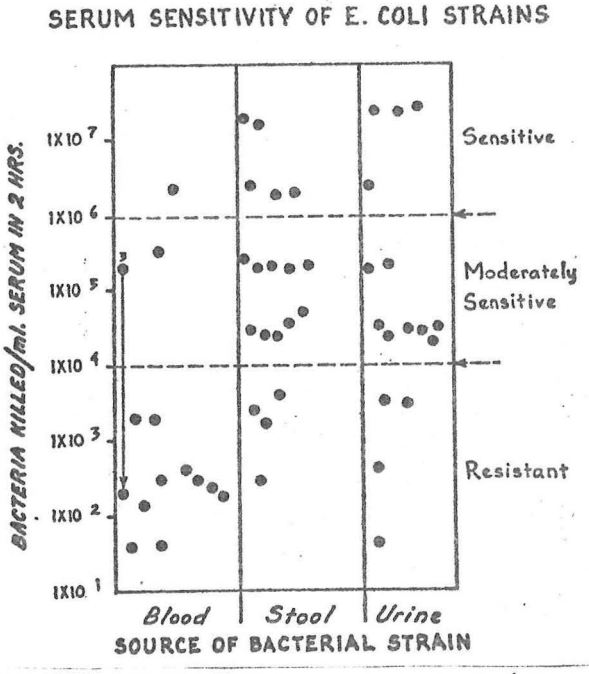
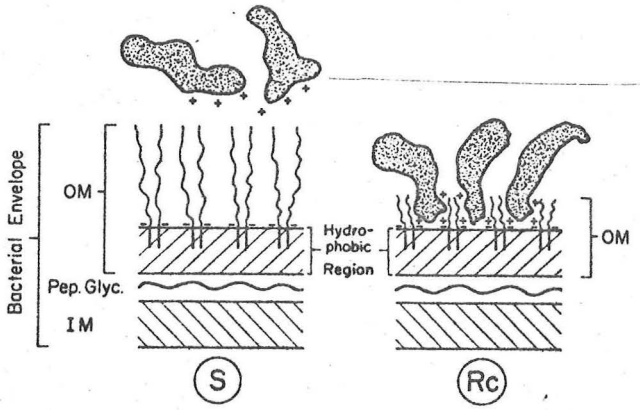


Fig. 8, from ref. 85a

This killing is mediated by complement (85)--both the classical pathway (activated by antigody-antigen complexes) and the alternate pathway (activated directly by cell wall LPS). Bacterial surface structures are thought to contribute to serum

resistance: (a) by shielding certain antigens, preventing their interaction with host antibody, thereby hindering activation of complement by the classical pathway, or (b) by shielding cell surface LPS, preventing direct activation of the alternate pathway. The presence of a capsular polysaccharide has correlated with serum resistance in studies using collections of clinical isolates (85,86). As noted above, Gram-negative bacteria which lack the LPS O antigen are more susceptible to serum killing; this correlates with the observation that complement activation is carried out by lipid A; the O polysaccharide extends from the bacterial surface into the aqueous environment, thus possibly masking the underlying lipid A.

Fig. 9



Interaction of host molecules with lipid A is influenced by the length of the O polysaccharide chain. Longer O chains prevent access of complement and antibody to cell surface in smooth (S) organisms, while in their absence (rough [R] cells) such access is possible. Modified from reference 75 OM = outer membrane

Finally, recent evidence suggests that serum resistance can be plasmid-mediated. The responsible factor appears to be a cell envelope protein which is also responsible for surface exclusion (the reduced ability of plasmid-carrying bacteria to act as effective recipients in conjugation) (87). Interestingly, the plasmid which was used in these studies also carries multiple antibiotic resistance genes, and the authors hypothesize that the presence of both antibiotic resistance and "serum resistance" genes on such plasmids may possibly make their bacterial hosts more virulent--in addition to making them antibiotic resistant.

Although serum resistance appears to be an important bacterial virulence factor, it should be noted that some 15% of clinical blood isolates are serum-sensitive. The reason(s) for this finding are uncertain. At least in one study, serum-resistant *E. coli* were associated with a higher frequency of shock and/or death than were serum-sensitive *E. coli* (86).

3. Nutritional competition. Early in bacterial infection, serum iron levels usually decrease. The mechanism for this change is thought to involve lactoferrin, the potent iron-binding protein found in specific granules of PMN. Lactoferrin complexes with free iron and also removes iron from serum transferrin. The Fe-lactoferrin complex is taken up rapidly by the reticuloendothelial system (25).

As noted earlier, Gram-negative bacteria require iron for growth, and many possess effective iron-scavenging mechanisms (26). The actual importance of iron-deprivation as an antibacterial mechanism *in vivo* is uncertain. There are clinical conditions, however, which indicate that the presence of free hemoglobin greatly favors bacterial growth and survival (for example, the propensity of patients with hemolytic anemia to to develop salmonella bacteremia (25). It also is possible that the hemolysis which occurs in disseminated intravascular coagulation may contribute to bacterial survival and growth. One would anticipate that neutropenic patients might not be able to lower serum iron levels in the normal fashion during bacterial infection, and there is preliminary evidence to this effect (87).

C. Therapy to increase bacterial clearance from the blood.

Most studies of the therapy of Gram-negative bacteremia use death or survival as the end-point. It should be noted that many factors may influence the outcome of Gram-negative bacteremia in man. The following table lists some of the factors cited in the literature:

TABLE 8.

FACTORS INFLUENCING OUTCOME OF GRAM-NEGATIVE BACTEREMIA*

- Underlying disease
- Age
- Site of origin of bacteremia
- Nosocomial infection
- Gram-negative rod isolate (E. coli vs. pseudomonas, e.g.)
  - Certain K antigens
  - Certain O antigens
  - No O antigen
- Prior treatment with antibiotics
- Promptness of antibiotic administration
- Appropriate antibiotic therapy
- Factors related to shock:
  - Low cardiac index
  - Acidosis
- Change in granulocyte count
- Granulocyte transfusions
- Glucocorticoid therapy

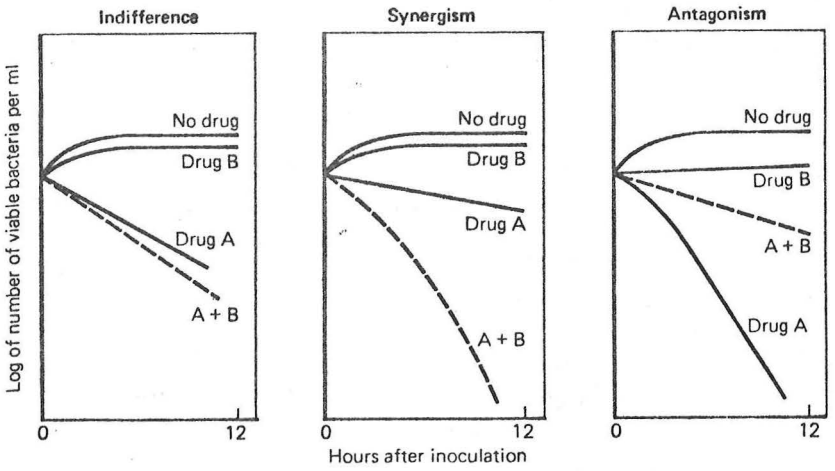
*Cited in one or more clinical study

In virtually every study of this problem, analysis has focused on one (or, at most, a few) of these factors without taking the others into account; there have been very few studies which utilized multifactorial statistical analysis (89). The fact that so many variables may influence outcome mandates that clinical trials not only be randomized and double-blind, but also that the test and control groups be found comparable in the variables mentioned above. No studies of therapy have attempted this. For example, none of the careful studies of different antibiotic regimens cites the interval between onset of clinical symptoms of infection and the initiation of antibiotic therapy, though rapid administration of antibiotics is thought to be an important factor influencing outcome, particularly in neutropenic patients (90).

1. Appropriate antimicrobial therapy.

Most recent clinical series indicate that appropriate antimicrobial therapy has a beneficial effect on patient outcome, regardless of the patient's underlying disease category (8). "Appropriate" in this context refers to the administration of at least one antibiotic to which the patient's isolate is sensitive *in vitro*. There is also circumstantial evidence that *prompt* administration of antibiotics may prevent septic shock (8,90). It is most interesting that reports from several large cancer centers now indicate that the prognosis for survival in neutropenic patients is excellent (greater than 85-90%), provided that marrow recovery occurs (90,96). These results are superior to those reported from general hospitals in patients with *normal* white counts, and they suggest that aggressive, prompt antibiotic administration can indeed improve survival.

Fig. 10, From Ref. 91



Patterns of response to therapy with two antibiotics. The response of bacteria suspended in growth medium to exposure to drug A or B alone is represented by the solid lines. The dashed lines represent the responses to simultaneous administration of the two drugs.

The following comments concern the role of multiple antibiotics in therapy.

a. Indications for synergistic therapy.

Antimicrobial "synergism" refers to a greater-than-additive effect of two antimicrobial agents when used together. (see Fig 10). In practice, analysis has often been less precise: the important clinical question, after all, is whether using two effective agents is better for the patient than using only one--an *additive* benefit might also be useful, provided that additional toxicity and other side-effects were minimal.

The best evidence which favors use of two or more effective antimicrobial agents to treat Gram-negative bacteremia comes from studies in neutropenic patients and is summarized in Table 9 . In all of the analyses cited, improved outcome was found for patients who received antibiotics which acted synergistically *in vitro* toward the patient's blood isolate. Similarly, several groups of workers have found that outcome is better when the blood isolate is sensitive *in vitro* to both agents used to treat the patient, rather than sensitive to only one or neither of the agents (See Table 10). Of the latter studies, only that of Lau (92) tested the drugs for *in vitro* synergism toward the isolates.

It should be noted that many recent series have reported marked differences in clinical outcome which are associated with the *duration of granulocytopenia*. It has become the general observation that bacteremic patients who regain normal neutophil counts have a favorable outcome and that patients whose marrow does not recover often do badly. Many of the attempts to analyze the impact of single vs. combination antimicrobial agents have not factored out such variables as the age of the patient, delay in initiation of therapy, the course of WBC recovery, etc., which are also known to influence outcome.

In *non-neutropenic* cancer patients, use of antibiotics which have synergistic activity toward the patients blood isolate has seemed to be beneficial in several studies by Klastersky and his group (98). The evidence for benefit from antibiotic synergism in patients with normal granulocyte counts is best for *Pseudomonas aeruginosa*. There is a solid basis in animal studies for the use of carbenicillin (ticarcillin) plus an aminoglycoside (gentamicin, tobramycin, or aminokacin) for treating pseudomonas sepsis, and essentially all clinical studies have also supported this conclusion (99). In pseudomonas endocarditis, use of high-dose (8 mg/kg/day) gentamicin or tobramycin, in combination with carbenicillin, appears to be more efficacious than low dose (2.5-5.0 mg/kg/day) (100). One recent study appears to challenge the

TABLE 9.

USE OF SYNERGISTIC ANTIMICROBIAL AGENTS TO TREAT GRAM-NEGATIVE INFECTIONS

Authors	Year	Kind of infection	Kind of patient	Favorable clinical response to combinations of antibiotics: Synergistic*	Not synergistic	Antibiotics
Anderson retrospective	1978 (97)	Bacteremia	Neutropenia cancer	15/25 (60%)	3/16 (41%)	various
Klastersky prospective double blind	1977 (93)	Bacteremia	Cancer patients Not neutropenic	18/24 (75%)	9/22 (41%)	pen G or carbenicillin + amikacin
Lau prospective, randomized	1977 (92)	Bacteremia	Neutropenia cancer	18/22 (82%)	7/16 (44%)	aminoglycoside + carbenicillin
Klastersky	1972 (98)	Various	Cancer, not always neutropenic	80/100 (80%)	52/105 (49%)	various

* *In vitro* effect of 2 antibiotics, used together, exceeds the additive effect of the same antibiotics used independently.

TABLE 10

USE OF COMBINATIONS OF EFFECTIVE*ANTIMICROBIAL AGENTS TO TREAT GRAM-NEGATIVE BACTEREMIA IN NEUTROPENIC PATIENTS

Authors	Year	Infection	Antimicrobial Drugs	Response** if isolate sensitive to: None	One	Both	Patient
Love	1980 (90)	Bacteremia	Various	0/4	6/14	15/20	Cancer, prolonged neutropenia
E.O.R.T.C.	1978 (95)	Bacteremia	Various	0/6	15/32	22/38	Cancer, neutropenia
Keating	1979 (94)	Various	Carbenicillin +	1/6	22/36	21/25	Cancer, neutropenia
Lau	1977 (92)	Bacteremia	Aminoglycoside	0/2	2/7	5/9	Cancer, neutropenia

* Effective=isolate sensitive *in vitro* to agent
** (Clinical response/total treated)

notion that combination therapy is superior (or even effective) for pseudomonas bacteremia in cancer patients, but these authors concede that carbenicillin therapy was delayed by 18-24 hours after the initiation of gentamicin in 2/3 of their patients, and 1/3 of the deaths occurred within 24-36 hours of the suspicion of bacteremia (101). Prompt combination therapy is necessary for optimal recovery rates in pseudomonas sepsis (99). *In vitro* resistance of a given isolate to either carbenicillin or the aminoglycoside suggests that the combination is unlikely to be more effective than the single effective drug alone (102).

b. Choice of empiric regimen - *neutropenic patients.*

In several randomized trials, there has been little difference in the outcome of Gram-negative bacteremia in neutropenic patients treated with various combinations of bactericidal drugs (Table 11). Most authors now recommend a combination of carbenicillin or ticarcillin and an aminoglycoside; the choice of aminoglycoside may often be based on the known susceptibilities of the patient's (or the hospital's) isolates. If staphylococcal infection is suspected, addition of a cephalosporin to the regimen is indicated; this will also provide additional Gram-negative coverage.

c. Choice of aminoglycoside. See reference 103 for recent review.

Characteristics of 72 Patients Given Gentamicin and 74 Patients Given Tobramycin and Compared for Nephrotoxicity.

Characteristic	Gentamicin *	Tobramycin *
Age (yr)	58.0±2.4	58.7±2.2
Initial creatinine level (mg/100 ml) †	1.7±0.2	1.8±0.2
Duration of therapy (days)	5.7±0.3	5.8±0.3
Total dose (g)	1.56±0.10	1.63±0.13
Aminoglycoside levels (μg/ml)		
Before drug	2.8±0.1	2.5±0.3
One hr after administration of drug	5.5±0.2	5.5±0.2
Previous renal disease (no. of patients)	16	19
Urinary-tract infection (no. of patients)	21	14
Concurrent furosemide (no. of patients)	14	15

*Values are mean ±S.E.M.

†To convert to micromoles per liter, multiply by 88.4.

Nephrotoxicity and Auditory Toxicity in Patients Given Gentamicin or Tobramycin.

Toxicity	Gentamicin *	Tobramycin *	P Value
Nephrotoxicity	19/72	9/74	0.025
Auditory toxicity	5/47	5/44	NS †

*Number of patients with toxicity/total number of patients evaluated.

†Not significant.

There is now evidence from a controlled, double-blind trial that tobramycin is less nephrotoxic than gentamicin (104). In this study, nephrotoxicity developed in 19 of 72 (26%) of the patients who received gentamicin and in 9 of 74 (12%) of these who received tobramycin. This difference was statistically significant ($p<0.025$) but there were some problems with the study: (1) more patients in the gentamicin group had elevations in creatinine prior to administration of the drug (see Table); (2) most of the instances of nephrotoxicity were not clinically significant; (3) mean "trough" levels for gentamicin and tobramycin exceeded the recommended

level of 2.0 µg/ml (elevated trough levels have been the best predictor of nephrotoxicity (103). The latter observation may explain the relatively high frequency of nephrotoxicity seen with both agents in this study.

The choice of aminoglycoside for routine use is also influenced by the sensitivity of different Gram-negative bacteria to these agents. At the moment, most isolates of *P. aeruginosa* at Parkland Memorial Hospital and elsewhere are more sensitive to tobramycin than to gentamicin. This greater sensitivity to tobramycin has obvious therapeutic advantages; it also could disappear with widespread use of tobramycin in a given environment.

At the present time, tobramycin should probably be used instead of gentamicin in patients who have risk factors for aminoglycoside nephrotoxicity (advanced age, volume depletion, hypotension, prior renal disease, recent aminoglycoside administration) and in neutropenic patients with presumed sepsis. Use of gentamicin in other clinical situations may possibly postpone the emergence of tobramycin-resistance in *P. aeruginosa*, though this is unproven.

TABLE 11

USE OF ANTIBIOTIC COMBINATIONS FOR THERAPY OF GRAM-NEGATIVE BACTEREMIA IN NEUTROPENIC PATIENTS

Author	Year	Combination Tested	Patients	Favorable Response/ Total Treated	Reference
Klastersky	1975	Tobramycin + ticarcillin	15	47	98
		cephalothin + tobramycin	19	58	
E.O.R.T.C.	1978	Gentamicin + ticar or carb	32	75	95
		Cephalothin + ticar or carb	18	58	
		Cephalothin + gentamicin	22	72	
Lau	1977	Amikacin + carbenicillin	23	65	92
		Gentamicin + carbenicillin	17	59	
Keating (continuous infusion)	1979	Carbenicillin + gentamicin	13	69	94
		Carbenicillin + amilacin	11	91	
Love	1979	Ticarcillin + gentamicin	14	93	96
		Ticarcillin + amikacin	9	78	
		Ticarcillin + netilmicin	12	83	

Empiric amikacin therapy should probably be reserved for seriously ill patients who have recently received aminoglycosides (and who might thus have sepsis caused by organism(s) resistant to the previous drug), or who are hospitalized in a hospital setting known to have highly resistant organisms (such as a burn unit), or who are known to harbor gentamicin-and-tobramycin-resistant Gram-negative rods.

d. Can surveillance cultures help one decide which antimicrobial combination to use? *Pseudomonas aeruginosa* usually is sensitive to carbenicillin but not to cephalosporins, while the reverse is true for *K. pneumoniae*. Knowledge of the presence of one or both of these organisms in a patient's gut flora might guide the choice of agents when the neutropenic patient becomes febrile. In Schimpff's early study, for example, 15 of 22 neutropenic patients who acquired colonization with *P. aeruginosa* developed pseudomonas bacteremia (16). Additional data which support the use of surveillance stool cultures (once or twice a week) comes from the bone marrow transplant service at U.C.L.A. (28):

Relationship between gastrointestinal (GI) colonization and gram-negative rod bacteremia in 23 patients

Condition	No. of patients
GI colonization with gram-negative rod in the 2 weeks preceding bacteremia (oropharynx, stool, perirectal, groin)	24
GI colonization detected on same day as positive blood culture but not before	4
No antecedent GI colonization	5[a]
Not evaluable (no surveillance cultures taken prior to positive blood culture	1
Total bacteremic episodes	34

[a] Two had subsequent documentation in stool, one each had leg ulcers and urinary tract infection, and one showed no source.

Relationship between gastrointestinal colonization and bacteremia in 73 patients ≥15 years of age

Colonization	Total	Bacteremic patients	Episodes of bacteremia
P. aeruginosa			
Colonized	24	10	13
Not colonized	49	1	1
K. pneumoniae			
Colonized	33	8	8
Not colonized	40	0	0
E. coli			
Colonized	36	5	6
Not colonized	37	2	2

These patients were all receiving oral non-absorbable antibiotics (GVN). Stool cultures appeared to be reliable in identifying patients who subsequently developed bacteremia with *P. aeruginosa* and *K. pneumoniae*, but not *E. coli*. Others have found surveillance cultures to be less useful (105), but their data are not convincing. Further studies are needed to establish the usefulness of surveillance cultures in larger groups of patients. Their predictive value should correlate with the frequency of GI-derived bacteremia: in the U.C.L.A. study cited above, almost all of the bacteremia isolates originated in the GI tract.

2. Increasing Neutrophil Number (White Cell Transfusion).

White cell transfusions for the therapy of infected, granulocytopenic patients seem to have become an accepted part of medical practice, although the evidence supporting their use is incomplete and limited to a few studies using small numbers of patients.

The rationale for WBC transfusions is simply that augmentation of neutrophil numbers should allow better phagocytosis and destruction of the organisms causing infection. There is experimental evidence to support this approach, based on studies in animal models of sepsis (105a) and Gram-negative pneumonia (106). The major problem with these animal studies is that they did not address the question which is relevant to the clinical situation: can WBC transfusions improve outcome if used *in addition to* optimal antibiotic therapy and supportive measures? The animal evidence that treatment of pseudomonas pneumonia using gentamicin plus granulocytes is superior to treatment with gentamicin plus carbenicillin, for example, is highly questionable (107).

There is only one randomized trial of WBC transfusion in patients with proven Gram-negative bacteremia as the indication for transfusion. This was a small study (16 transfused patients, 14 controls), conducted in a young population (median age 15). None of 8 control patients with persistent neutropenia survived, while 8 of 12 transfused patients with persistent neutropenia survived and some went into remission from leukemia. If marrow recovery occurred, there was no difference in the survival of transfused and control patients. Granulocytes (collected by either filtration leukophoresis or continuous flow centrifugation) were administered daily until granulocyte counts were greater than $1000/mm^3$, or for 5 or more days with negative blood cultures and 3 days with no fever (111).

There have been 3 other published trials of WBC transfusions which enrolled patients in a randomized fashion (Table 12). In the study of Higby, et.al., proven infection was not required for entry into the study (108). Alavi, et.al. found that patients who did not have proven infection did no better with WBC transfusion than untransfused controls (110). Vogler and Winston noted that post-transfusion WBC counts were higher in children (109); this presumably relates to the higher dose (per kg) that can be given to children.

The most striking feature of these studies is their heterogeneity (Table 12). These groups used different indications for transfusion, different definitions of infection, different methods for preparing granulocytes (and for pre-medicating donors), different criteria for neutropenia, different methods for donor selection, different antibiotic regimens, and different methods for assessing clinical response. Moreover, no study documented the adequacy of antibiotic therapy (blood levels, promptness of administration, antibiotic synergism, etc.). Reaching firm conclusions from these data is extremely difficult.

Nevertheless, all of these studies suggest that WBC transfusions may be a promising addition to the therapy of Gram-negative bacteremia (and other infections, particularly pneumonia) in certain neutropenic patients. There are several remaining problems:

a. Identification of the patients most likely to benefit from transfusion. It is generally agreed that granulocyte transfusions really benefit only those patients who do not experience spontaneous marrow recovery. There are no good guidelines for identifying such patients in advance; the European cancer study group (E.O.R.T.C.) is currently evaluating a set of criteria for this purpose (112)

More important, it is critical now to establish the indications for granulocyte transfusions for specific infections. The numbers of patients studied to date do not allow such recommendations. It appears that WBC transfusions may be beneficial for patients with Gram-negative bacteremia, as discussed above, but the role of this modality in treating soft tissue infections, Gram-positive infections, and pneumonia is less certain.

b. Improvement and standardization of the methodology for collecting white cells. Most workers agree that continuous flow centrifugation is preferable to filtration leukophoresis for harvesting white cells, and that at least 10^{10} cells per transfusion are necessary. Moreover, it is generally agreed that transfusions must be continued once or twice daily for several days for a clinical effect to be observed. There seems to be less agreement concerning the need for pre-medication of the donor (with corticosteroids) to elevate the cell yield. Alloimmunization seems to have been a significant problem only when transfusions were used for prolonged periods to prevent infection (62).

c. Reduction in cost. A single granulocyte transfusion at Parkland Memorial Hospital currently costs $265. A recent study estimated that the cost of therapeutic WBC transfusion was $14,982 per life-year (61).

d. Should WBC transfusions be combined with passive immunization? As discussed above, phagocytes ingest bacteria most efficiently if specific antibody is present. There is evidence in animals that immunization (passive or active) enhances survival in neutropenic dogs with Pseudomonas sepsis (113) or Pseudomonas pneumonia (114); the benefit of passively administered IgG, gentamicin, and WBCs was superior to that of gentamicin and WBCs without IgG (115). Moreover, there is preliminary evidence that opsonin deficiency has an adverse influence on outcome in neutropenic patients who receive WBC transfusions (82).

The difficulties associated with developing an immune serum which would contain antibodies to many O or K antigens of Gram-negative rods are discussed earlier (page 16). The cross-reacting antiserum to R-core LPS (J5 antiserum) apparently functions primarily as an antitoxin; its potential role as an opsonin is less certain.

TABLE 12

STUDIES OF THERAPEUTIC WBC TRANSFUSION*

Study	Year	Entry Criteria		Transfusion Data			Results (Deaths/Total)		Ref.
		Neutrophils $X10^6$/Liter	Relation to Infection	Collection	PMN Dose$X10^9$/ No. of Days	Survival End Point Selected	Control	Transfused	
							No. of patients		
Higby et al	1975	500	>2 days on antibiotics without response	FL and steroids	35/4	Day 20	8/12	1/11	108
Vogler & Winton	1977	500	3 days after infection demonstrated	CFC,starch, steroids	29/7	Day 22	9/13	7/17	109
Alavi et al	1977	250	36 hr after clinical onset of infection	FL and steroids	50/7	Day 21	9/19	3/14	110
Herzig et al	1977	1000	Positive blood culture	CFC,FL	4-17/m^2/9	Day 17 or 21†	9/14	4/16	111

*PMN denotes polymorphonuclear leukocytes, FL filtration leukapheresis, and CFC continuous-flow centrifugation.
†The median time "on study" was 17 days in the transfused group, and 21 days in the controls.

3. Recommendation: granulocyte transfusions. It seems likely that white cell transfusions will benefit patients with prolonged neutropenia and antibiotic-unresponsive Gram-negative bacteremia. Neutropenic patients with antibiotic-unresponsive Gram-negative bacterial pneumonia or soft tissue infections may possibly also be benefited. It is important that clearer guidelines for granulocyte transfusion be developed before this therapy becomes widely misused.

For recent reviews, see references 116 and 117.

III. THE HOST INFLAMMATORY RESPONSE TO GRAM-NEGATIVE BACTEREMIA

This section deals with the complex, poorly understood host abnormalities which often occur during Gram-negative bacteremia. There is a large literature which relates these abnormalities to the toxicity of Gram-negative endotoxin, the lipopolysaccharide molecule discussed previously. Definitive proof of this connection is lacking, yet the "endotoxin hypothesis" serves as a useful framework for examining various approaches to therapy of these abnormalities. Lewis Thomas has described the endotoxin-host interaction quite vividly:

> "It is the information carried by the bacteria that we cannot abide.
>
> "The gram-negative bacteria are the best examples of this. They display lipopolysaccharide endotoxin in their walls, and these macromolecules are read by our tissues as the very worst of bad news. When we sense lipopolysaccharide, we are likely to turn on every defense at our disposal; we will bomb, defoliate, blockage, seal off, and destroy all the tissues in the area. Leukocytes become more actively phagocytic, release lysosomal enzymes, turn sticky, and aggregate together in dense masses, occluding capillaries and shutting off the blood supply. Complement is switched on at the right point in its sequence to release chemotactic signals, calling in leukocytes from everywhere. Vessels become hyperreactive to epinephrine so that physiologic concentrations suddenly possess necrotizing properties. Pyrogen is released from leukocytes, adding fever to hemorrhage, necrosis, and shock. It is a shambles.
>
> "All of this seems unnecessary, panic-driven. There is nothing intrinsically poisonous about endotoxin, but it must look awful, or feel awful, when sensed by cells. Cells believe that it signifies the presence of gram-negative bacteria, and they will stop at nothing to avoid this threat."(13)

A. <u>The endotoxin hypothesis:</u> that Gram-negative bacterial lipopolysaccharide (LPS, endotoxin) is responsible for eliciting the host inflammatory response to Gram-negative bacteria.

1. The gram-negative bacterial cell envelope contains a lipopolysaccharide which has 3 distinct structural regions. Lipid A, which is apparently intercalated into the lipid bilayer of the outer membrane, is the toxic moiety. The R-core region, discussed above because of its role in cross-reactive immune therapy, connects lipid A to a polysaccharide moiety (O antigen) which is non-toxic but which confers serologic identity to different Gram-negative strains. Some of the LPS in the outer membrane is released as the bacteria grow *in vitro*, and this is probably also true *in vivo* (unproven). LPS located in the cell wall is also toxic though the relative toxicities of cell-free and cell-associated LPS have not been defined.

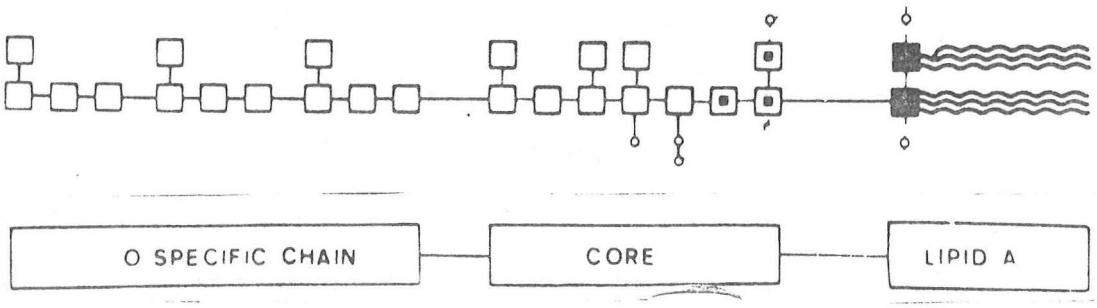


TABLE 13

BIOLOGICAL ACTIVITIES OF GRAM-NEGATIVE ENDOTOXIN: *IN VITRO* ASSAYS

µg/ml LPS*	Activity Measured
100	Rabbit *platelet* aggregation (Muller-Berghaus, 1978) Human *leukocyte* thromboplastic activity (Lerner, Goldstein, Cummings, 1971) Mouse *B-cell* differentiation to antibody production (Kearney, Cooper, Lawton, 1976)
10	Mouse *B-cell* proliferation (Anderson, Melchers et al, 1973) Human factor XII activation (Morrison and Cochrane, 1974) Inhibition of human C1 (Loos, Bitter-Suermann, Dierich, 1974); Activation of human complement (Morrison and Kline, 1977)
1	Mouse *fibroblast* glucose utilization (Ryan and McAdam, 1977) Human *leukocyte* lysosomal enzyme release (Bannatyne et al, 1977)
.1	Mouse *macrophage* lymphocyte-activating factor (Rosenstreich, Vogel, et al, 1978)
.01	Human *macrophage* colony-stimulating factor-release (Ruscetti and Chervenick, 1974)
.001	Mouse *macrophage* PGE_2 release (Rosenstreich, Vogel, et al, 1978)
.0001	
.00001	Human *macrophage* B-cell activating factor (Wood and Cameron, 1978) Human *macrophage* tissue factor generation (Rickles and Rick, 1977)
.000001	*Limulus* amoebocyte lysate gelation

* Different LPS preparations were used in these studies; the criteria for purity also differed. Most amounts refer to lyophilized dry weight.

When LPS stimulation of a given activity occurred over a range of LPS concentrations, the lowest concentration producing a significant response was chosen.

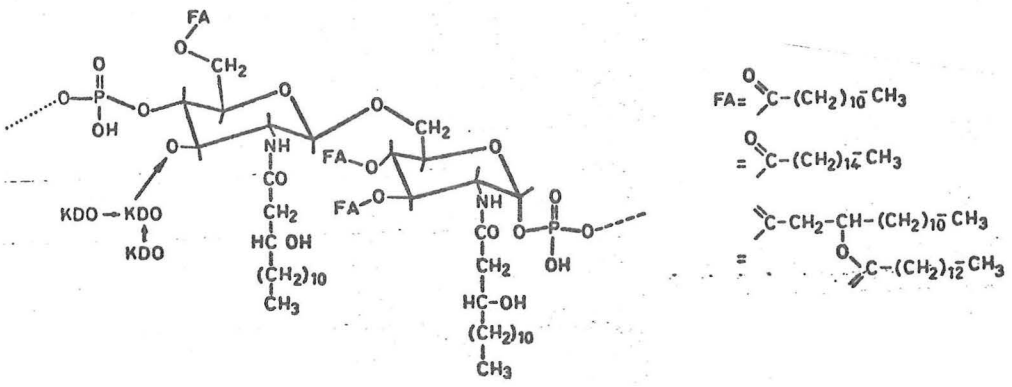


Fig. 12

Structure of lipid A, the toxic moiety of endotoxin

2. The toxicity of LPS is truly remarkable: it has an extremely diverse array of biological activities, both *in vitro* and *in vivo*.

The mechanisms by which LPS brings about these changes are uncertain. Both cellular and humoral (fluid-phase) interactions have been described. It is known, for example, that LPS interacts directly with Hageman factor (Factor XII) and complement factors ($C1_q$) *in vitro* (118,119). The amounts of LPS required to produce demonstrable changes in these studies were very large, however--much larger than the amounts likely to circulate during sepsis. (Table 13).

Most workers now take the view that LPS exerts its toxicity by stimulating host cells--in particular, macrophages (3, 3a). There are several lines of evidence which support this view:

a. A remarkable series of experiments has been conducted using the C3H/HeJ mouse, a strain which possesses a single gene mutation (acquired sometime between 1960 and 1965) that produces hyporesponsiveness to most known biological effects of endotoxin. If C3H/HeJ mice are lethally irradiated and then adoptively transfused with bone marrow cells from histocompatible (but endotoxin responsive) mice, they become sensitive to a number of the effects of endotoxin. The reverse experiment also works (120). Further studies suggest that macrophages are the responsible cells.

b. As noted in the above Table 13, macrophages are generally more sensitive to LPS *in vitro* than PMNs. The amounts of LPS required to stimulate macrophages in the studies cited were very low--probably in the range which might occur during sepsis (recall that most patients with Gram-negative bacteremia have less than 10 bacteria per ml of blood (6,8); this would represent less than 1 nanogram of LPS per ml). It should be noted, however, that many of the *in vitro* responses require hours for detection and that cell preparation methods may influence these results.

c. Although evidence is incomplete, it is known that LPS can stimulate macrophages to release pyrogens (121), "tissue factor" (activates extrinsic limb of clotting system) (122), colony stimulating factor (123) and prostaglandins (124). Studies are needed to determine the actual range of mediators released by macrophages in response to LPS stimulation. In addition, both PMNs and macrophages release mediators during phagocytosis which they do not release simply in response to exogenous LPS (125). Does the presence of LPS on the ingested particle (Gram-negative bacterium) make a difference in the amount and/or range of mediators released? Surprisingly, there appears to be little information on this subject. There is interesting evidence, however, that endotoxin "prepares" macrophages to release plasminogen activator when they subsequently undertake phagocytosis (126).

3. Many of the clinical manifestations of Gram-negative bacteremia are mimicked by endotoxin injection in man (Table 14).

TABLE 14

EFFECTS OF SMALL DOSES (5 ng/kg) OF BACTERIAL ENDOTOXIN GIVEN INTRAVENOUSLY IN MAN

	Reference
Leukocytosis	(127)
Fever	(127)
Fall in serum iron	(127)
Increase in cortisol	(127)
Increase in plasma bradykinin	(128)

Lower doses of endotoxin are required to produce these effects in man than in most other animals, including subhuman primates (133).

There is now evidence that antiserum to endotoxin R-core antigens may prevent febrile episodes in neutropenic patients (see above, p.15). This observation suggests that fever in such patients may relate to endotoxemia (with or without bacteremia).

4. The toxicity of LPS may be modified by other bacterial components, particularly "LPS-associated protein," a poorly defined material which is extractable with LPS using certain methods (3).

5. There are host-defense mechanisms which eliminate or detoxify entotoxin.

   a. Antitoxins. According to McCabe and his co-workers, patients who have pre-formed antibodies to either the O or R-core antigens of their infecting (bacteremic) strain are more likely to survive than those who do not have such antibodies (129). It appears that most people have antibodies to R-core antigens, although the methods for detecting these antibodies give rather disparate results (130), and the method used by McCabe was less sensitive than other methods in subsequent use. While antibodies to capsular (K) and O antigens are effective as opsonins, there is also evidence that antibodies to O and R-core antigens of LPS may act as antitoxins, capable of neutralizing such endotoxin-related phenomena as DIC and the Schwartzman phenomenon in experimental animals (131).

b. Detoxification mechanisms. Following i.v. injection into experimental animals, purified endotoxin appears to:
(1) activate fluid-phase systems (clotting complement)
(2) bind to circulating platelets and white cells
(3) undergo disaggregation and become associated with plasma lipoproteins, particularly high density lipoprotein (HDL)
(4) be taken up by the RES (liver, spleen), where it is probably degraded.

It should be noted that i.v. injection of LPS is exceedingly artificial, as the concentrations attained at the site of injection are many-fold greater than the concentrations likely to occur *in vivo*. This artifact may account for many of the immediate phenomena observed after bolus LPS injection (see below, page 40).

There is evidence that HDL-associated LPS is less toxic than "free" LPS (132); the clinical importance of this observation is uncertain, though the presence of circulating (yet inactive) HLD-LPS complexes might explain the presence of "endotoxemia" in certain patients who do not have clinical symptoms. (65)

c. Stability of proteolytic cascades. The complement, kinin, and clotting pathways comprise a complicated, interlocking system of proteolytic enzymes. A stimulus which activates one component may lead to activation of others. The activity of these enzymes is held in check by a number of serum protease inhibitors.... (C1 esterase inhibitor, anti-thrombin III, α_2-macroglobulin, α_2-antiplasmin, α_1-antitrypsin). These inhibitors have received little attention by students of Gram-negative sepsis, but they probably have an important role in "stabilizing" the proteolytic cascades following perturbations by LPS or LPS-induced cellular mediators.

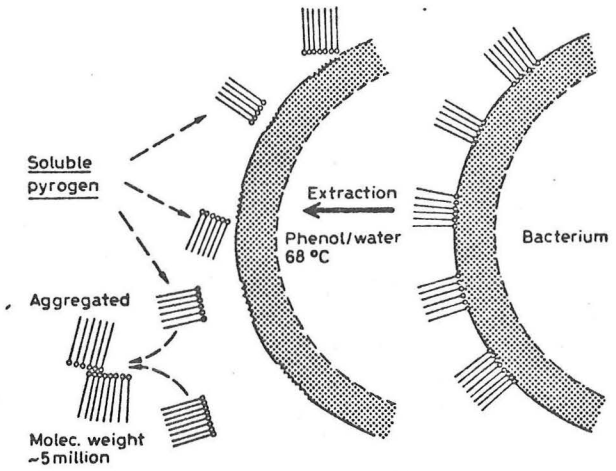
6. Endotoxin hypothesis: reservations.

a. There has not been a convincing demonstration that endotoxin, dissociated from bacteria, is present in blood during Gram-negative bacteria in man. Much recent work on "endotoxemia" has utilized the Limulus lysate test, a very sensitive assay (as little as 10 picograms CPS/ml can be detected) with unproven specificity. Results using this test on plasma samples have been conflicting (14, 141).

It is, of course, possible that the host inflammatory response to endotoxin is generated solely by bacterial cell-associated LPS, and not at all by "free" endotoxin which is shed by the bacteria into the blood. Most of the available evidence suggests that this is not the case, though the question is far from settled. Quantitative blood cultures have found less than 10^3 Gram-negative rods/ml of blood in patients with sepsis, and this number is not enough to cause a positive Limulus test.

b. Purified endotoxin, used for most experimental studies of endotoxin activity, may not accurately resemble the "natural" endotoxin which is shed from the bacterial surface during infection. The most popular extraction procedure involves treatment with 45% phenol-water at 65-70°C. This procedure generates large molecular aggregates of LPS:

FIGURE 13, from ref. 133



Phenol/water extraction of *Enterobacteriaceae* (scheme).

It is known that disruption of purified LPS into smaller aggregates greatly alters (diminishes) its biological activity, at least in certain test systems (9). Moreover, there is evidence that the endotoxin which is shed by Gram-negative bacteria during growth *in vitro* takes the form of small micelle-like structures which contain phospholipid in addition to LPS. The activities of purified LPS and "naturally" shed LPS are currently being compared.

It should also be noted that it is extremely difficult to produce two lots of endotoxin which are physicochemically and biologically identical. Commercially available endotoxin preparations (Difco) are contaminated by significant amounts of nucleic acid and protein. Comparison of endotoxin activities using different LPS preparations is hazardous.

c. Animal models have generally been unsatisfactory.

(1) The sensitivity of animals to endotoxin varies enormously (127). Man is one of the most sensitive species. Extrapolation of the results of LPS administration to animals to the human clinical situation is extremely risky.

(2) Animal models.

(a) Bolus injection of LPS. This is obviously not the way in which LPS is released into the bloodstream during Gram-negative infections. Such studies probably emphasize the interactions between LPS and plasma proteins (complement, clotting factors). Even continuous infusions of LPS create concentration gradients which probably do not exist during natural infections.

(b) Bolus injection of bacteria. This approach is closer to the situation seen in infection. Unfortunately, a large bolus of bacteria contains a large bolus of endotoxin: the acute changes seen in animals given big doses of Gram-negative bacteria are probably caused by the endotoxin challenge. Another problem is the requirement for very large inoculums to produce sustained bacteremia in most animals: 10^9 or more organisms in most animals studied.

Another objection to these studies is that the animals have almost always been healthy--unlike most patients who develop Gram-negative sepsis. Few workers have tried to develop models in which some "host defect" is present. One such model, the neutropenic rabbit which develops spontaneous sepsis if the GI tract is colonized with Gram-negative bacteria, has not been used for studies of the pathophysiology of sepsis. Perhaps the neutropenic-rabbit-Pseudomonas conjunctivitis-bacteremia model, developed recently by Ziegler (29), will be useful for this purpose.

B. Alternative hypothesis.

It should be noted that Gram-negative bacteria also have other toxic products, particularly exotoxins and extracellular enzymes. These have been studied most extensively for *P. aeruginosa*. This bacterium releases several extracellular enzymes (elastase, collagenase, phospholipase C [134]) and, in addition, produces at least one exotoxin. This "exotoxin A" resembles diptheria toxin in many ways, including its mechanism of action within the cell (ADP-ribosylation of elongation factor 2 [135]). Most (80%) clinical isolates make this toxin, and patients who have bacteremia with toxin-producing strains usually make antibodies to the toxin (136). Indeed, there is some evidence that patients who have antibodies to exotoxin A at the time of onset of *P. aeruginosa* bacteremia may have improved survival rates (137). Nevertheless, the role of this toxin in disease is uncertain.

The other extracellular enzymes of *P. aerugonosa* may possibly be important factors which determine the virulence of this organism in the neutropenic host, but this is also uncertain at present (29).

C. Host inflammatory responses during Gram-negative bacteremia in man.

Although much effort has been devoted to an analysis of the interactions between Gram-negative bacilli and the human host, our understanding of this problem is in its infancy. This should not be surprising: there is extreme variability in the underlying diseases of patients who develop Gram-negative bacteremia, the interplay of host inflammatory responses and microbial factors is exceedingly complex, and a single group of investigators is simply unable to measure more than a few of the relevant variables which may contribute to this interplay. The following account is a summary of the major changes which have been recognized in certain host parameters during Gram-negative bacteremia in man.

1. Leukocytosis. Leukocytosis is a common feature of Gram-negative infection. It can be produced in man by doses of LPS which are insufficient to cause fever (127), and endotoxin challenge has been used as a method for determining marrow granulocyte stores in a variety of clinical states (127). The mechanism of LPS-induced leukocytosis is unknown.

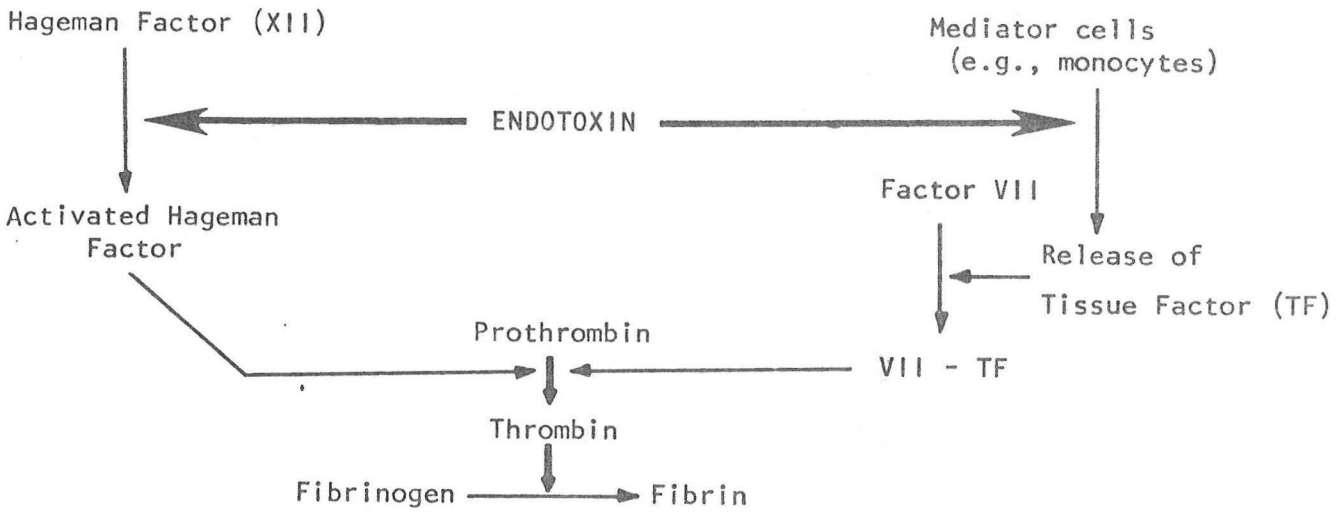
2. Coagulation abnormalities. Alterations in clotting parameters have been commonly described in patients with Gram-negative rod bacteremia. The genesis of these abnormalities is obviously extremely complex and difficult to dissect in clinical studies.

   a. Thrombocytopenia. Endotoxin sticks to platelets and probably induces platelet aggregation--the mechanism is obscure, and the endotoxin concentrations required to produce aggregation *in vitro* are quite high (138). Thrombocytopenia during sepsis is thought to be caused by platlet destruction, aggregation, or by "immune" mechanisms (139,140). The role of complement in the thrombocytopenia of sepsis is controversial (3); much lower doses of LPS are required to produce thrombocytopenia in man than are necessary for producing detectable changes in complement components (142). In one preliminary study, human volunteers given two small doses of LPS experienced thrombocytopenia after the second dose; the thrombocytopenia could not be inhibited by doses of aspirin which effectively blocked LPS-induced fever (142). So a prostaglandin-mediated mechanism may not be responsible.

On the other hand, there is recent evidence that endotoxin shock is associated with elevations in thromboxane B_2 levels in the rat (165). Perhaps this potent platelet-aggregating agent will have a role in producing the thrombocytopenia seen during sepsis in man; there have been no direct studies of this.

b. Activation of clotting factors. LPS will complex with factor XII (Hageman factor) *in vitro* (118), but the amounts of LPS required to produce a functional defect are large. Although primary activation of XII provides an attractive mechanism for activation of clotting, kinin, and complement systems during sepsis (see Figure 14), there is no evidence that a direct interaction of LPS with factor XII occurs *in vivo*.

FIGURE 14



Two possible mechanisms for activation of clotting by endotoxin

It now seems more likely that LPS produces clotting alterations in clotting factors by primarily interacting with host cells. *In vitro* low doses of LPS induce both granulocytes (142a) and macrophages (122) to release a thromboplastin-like material which activates the extrinsic pathway of coagulation. Although there is no evidence for such a mechanism *in vivo*, the idea that endotoxin may activate the clotting system via stimulating the release of mediators from target cells such as macrophages is attractive. Another possible mechanism involves endothelial damage, producing local activation of clotting and platelet accumulation (143, 144, 145). The recent observation that rabbit endothelial cells in culture can activate Hageman factor is consistent with such a mechanism (146).

Still another mechanism for the production of coagulation defects may involve granulocytic proteases which activate or destroy clotting factor proteins.

c. Disseminated intravascular coagulation (DIC). In this syndrome, consumption of platelets and factors II, V, and VIII significantly exceeds production rates, resulting in levels less than those required for hemostasis. At the same time fibrinolysis is activated; plasmin cleaves fibrin into fibrin "split products" and also may activate the complement cascade. The pattern of laboratory abnormalities thus includes (153):

Prolonged prothrombin time
Prolonged partial thromboplastin time (variable)
Prolonged thrombin time
Thrombocytopenia
Decreased levels of factors II, V, VIII, and fibrinogen
Presence of fibrin split products
Evidence for red cell fragmentation (blood smear)

Common complications of DIC include bleeding, renal dysfunction, liver dysfunction, and CNS alterations; in one study from Israel, bleeding in 20% of patients with DIC was thought to be caused by some other process, and shock was said to be attributable to DIC in only 40% (147). On the other hand, many patients with septic shock also have DIC (8,152), and some workers feel that reversal of shock may improve the coagulation abnormalities (148). It is obviously important (1) to distinguish DIC from clotting abnormalities caused by other processes--liver disease, vitamin K deficiency, etc., (2) to recognize other causes of bleeding (such as GI hemorrhage) in patients with DIC, (3) to remember that there are several *non-infectious* causes of DIC (149), and (4) to identify & correct the cause or DIC whenever possible.

There is suggestive evidence that DIC occurs infrequently in *neutropenic* patients with Gram-negative bacteremia (150). This observation, if correct, is interesting in view of the experimental data in animals which relates DIC (or the Shwartzman reaction) to neutrophils (151).

3. Consumption of complement components. As discussed above (page 21), complement is important for enhancing phagocytosis and for mediating lysis of serum-sensitive Gram-negative rods. Depletion of complement components during Gram-negative bacteremia has been documented, although levels were depressed only in patients who were in shock at the time of study (154). Whether complement depletion is a precursor or consequence of hypotension in these patients is not known; moreover, no such study has incorporated control patients with other forms of shock.

Complement activation by purified endotoxin *in vitro* requires very large LPS concentrations (see Table, page 35); endotoxin (lipid A) may interact directly with $C1_q$, activating the classical pathway (119), and LPS also can activate the alternative pathway (119). Alternative pathway components have most often been depleted in patients with septic shock, while classical pathway components have been normal (154).

4. Shock: Kinins, endorphins, prostaglandins.

Study of the hemodynamic changes which occur in man during Gram-negative bacteremia has been difficult. The clinical opportunity for prospective evaluation of patients before, during, and after the onset of bacteremia is presented by patients who undergo urologic manipulation, particularly transurethral resection (155).

TABLE 15

Bacteremia and urinary-tract infection (UTI) in patients who had four types of urinary-tract manipulation.

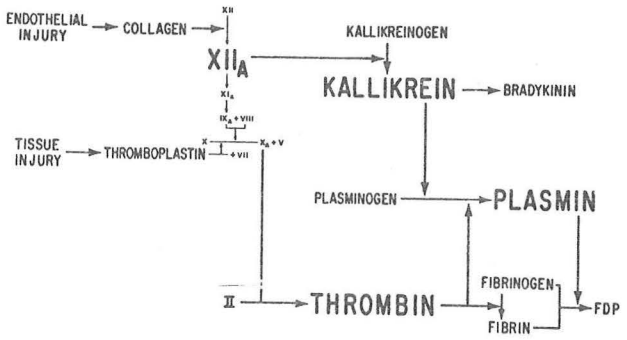
Procedure (no. studied)	Patients with bacteremia		
	No. (%)	Pre-operative UTI*	Same species, blood and urine*
Transurethral resection of prostate (77)	24 (31)	16	15
Cystoscopy (81)	14 (17)	8	5
Urethral dilatation (67)	16 (24)	8	5
Urethral catheterization (75)	6 (8)	2	2

* Number of patients with indicated finding.

Gunnar and his collaborators studied 8 patients who developed Gram-negative bacteremia following GU instrumentation. After surgery, as compared with the day before, these patients had an increase in cardiac output, a fall in systemic vascular resistance (SVR), and a fall in central venous pressure. Two of the patients developed hypotension; in these patients systemic vascular resistance was markedly decreased and cardiac output was increased, particularly when volume infusion was given. These findings were interpreted as evidence for the release of a vasodilator, with an increase in cardiac output in response to this vasodilation (156,157).

The same group of workers found that significant decreases in both prekallikrein and systemic vascular resistance occurred in five patients who developed endotoxemia (as measured by the Limulus lysate test) but did not have hypotension. They noted that kinin generation may be associated with vasodilation (fall in SVR) without detectable hypotension. Other workers have found that levels of kallikrein inhibitor and prekallikrein were also decreased in normotensive volunteers with typhoid fever (158) as well as in patients with septic shock (159). A small group of patients with hemorrhagic hypotension had normal values (160).

FIGURE 15



Low doses of LPS cause elevations in bradykinin levels in human plasma (128). Although these low doses do not provoke hypotension, they do cause granulocytopenia and fever. Many possible mechanisms for kinin activation by endotoxin have been postulated (summarized in 161). These include activation of factor XII, a known initiator of kinin generation, and stimulation of PMNs or macrophages.

Other possible mediators of shock during Gram-negative bacteremia include endorphins and prostacyclins. The available evidence for these is based on animal and/or *in vitro* studies.

Endogenous opiates (endorphins) are released in response to stress, and even small doses are able to depress blood pressure (162). There is evidence in rats that the hypotension may be mediated by a serotonergic pathway (163). Naloxone, the opiate antagonist, blocks or reverses the hypotension (see page , below).

The evidence for a role for prostaglandins in Gram-negative shock is rather preliminary.

a. Stimulation of isolated cells by LPS. Human monocytes and murine macrophages release prostaglandins (PGE, P6F); this release is greatly increased by stimulation with LPS and is blocked by indomethacin (124). Collagenase production by guinea pig macrophages is stimulated by LPS; this effect can be blocked by indomethacin, and PGE_2 and PGE_1 were thought to be responsible mediators (164). In contrast, prostaglandin release by PMNs appears to be unaffected by LPS

although these cells do release prostaglandins when stimulated to phagocytose zymosan (125) and similar release of PG may also accompany phagocytosis of bacteria.

b. Elevated prostaglandin levels in animals given bolus LPS injections. Elevated PGE and PGF levels were found in portal and renal vein blood following bolus injection of LPS into dogs (166). Similar results have been obtained in other species. Interestingly, $PGF_{2\alpha}$ levels increased in portal venous blood following splanchnic artery occlusion in dogs (167), indicating that hypotensive (ischemic) stimuli other than LPS may also increase prostaglandin levels.

High thromboxane (B_2) and PGE levels were found in rats given large doses of LPS (165). In rabbits and baboons, LPS injection induces elevations in 6-keto $PGF_{1\alpha}$, a stable metabolite of prostacyclin, the potent vasodilator (168).

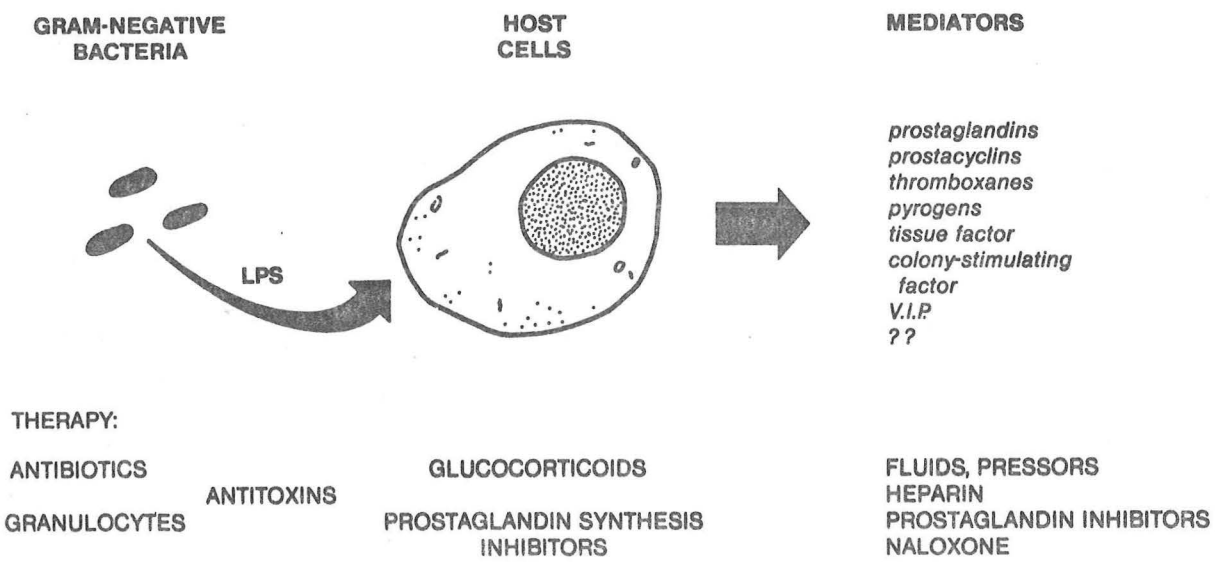
D. Therapy to block or reverse the host inflammatory response.

It is theoretically possible to intervene at three different points in the host inflammatory response to Gram-negative bacteria:

(1) before the bacteria (or LPS) interact with host cells
Examples: antibiotics, antibodies to LPS, granulocytes

(2) to inhibit the lipopolysaccharide-cell interaction
Examples: glucocorticoids, prostaglandin synthesis inhibitors

(3) to block the effects of mediators released by LPS-stimulated cells
Examples: prostaglandin antagonists, heparin, fluids, pressors, naloxone



This formulation is obviously an over-simplification and is intended merely to provide a framework for discussion. The following comments concern the available evidence for the use of therapeutic agents to combat this phase of Gram-negative sepsis.

1. Passive immunization.

As discussed above (page 14), antiserum to the R-core of *E. coli* 0111 LPS functions as an antitoxin in experimental animals, neutralizing many of the effects of endotoxin. Because the R-core region is shared by many Gram-negative organisms, this antiserum appears to have potential for cross-reactive passive immunization. The San Diego workers have given human antiserum (to the J5 mutant of *E. coli* 0111, discussed on page 14) to patients with Gram-negative bacterial shock. The study was double-blind, using pre-immunization serum as the control. Their results suggest that this approach may benefit patients in septic shock. (169)

DEATHS FROM BACTEREMIA IN PATIENTS GIVEN HUMAN SERUM

J5 antiserum	5/37 (14%)
Nonimmune serum	12/46 (26%)
	(p = 0.16)*

* Chi-square method

RECOVERY FROM PROFOUND SHOCK*

J5 antiserum	9/11 (82%)
Nonimmune serum	2/7 (29%)
	(p = 0.024)†

* Requiring pressors for >6 hours
† Chi-square method

The study was continued and the code has been broken again this summer: the results have not been announced.

Whether or not this mode of therapy is beneficial, these workers have shown that it is possible to perform a double-blind therapeutic trial in desperately ill patients. This fact should encourage future studies of the other modalities discussed in this section.

2. Glucocorticoids

a. Possible mechanisms. The available evidence indicates that glucocorticoids inhibit endotoxin activity at the level of host cells.

(1) "Stabilization" of lysosomes. Glucocorticoids may prevent the release of lysosomal enzymes in endotoxin-treated animals (170).

(2) Effects on endothelial cells. Balis (171) subjected rhesus monkeys to a continuous endotoxin infusion. The observed sequelae included margination, degranulation, and fragmentation of leukocytes, the appearance of fibrin in hepatic sinusoids, and the appearance of DIC. Methylprednisolone (30 mg/kg q6h) prevented all of these effects.

Endotoxin-induced detachment and lysis of bovine aortic endothelial cells *in vitro* has been studied by Harlan (144), who found that these effects could be prevented by incubation of the endothelial cells in 0.1 mM hydrocortisone.

(3) Inhibition of the release of mediators by host cells.

It seems likely that glucocorticoids interfere with the release of membrane phospholipids from which fatty acid substrates for the cyclo-oxygenase system are derived (173). Recently it has been shown that steroids induce the synthesis of a factor which blocks phospholipase A_2. This soluble "second messenger" appears to be a protein, as its synthesis is inhibited by cycloheximide (174).

(a) Macrophages, monocytes. Glucocorticoids inhibit the release of several enzymes from stimulated macrophages. Werb (172) found that 1 nM dexamethasone decreased by 50% the release of plasminogen activator from human blood monocytes. Secretion of elestase and collagenase, but not lysozyme, was similarly inhibited by glucocorticoids. Werb has shown that lymphocytes and macrophages contain glucocorticoid-binding proteins with specificity of binding for cortisol, corticosterone, and related synthetic steroids.

(b) Neutrophils. Glucocorticoids decrease the release of thromboxanes by phagocytosing neutrophils (125). Human PMNs appear to release thromboxanes as a result of cell-surface stimulation, independent of phagocytoxis.

(4) Inhibition of polymorphonuclear leukocyte aggregation. Jacob and his colleagues have developed an interesting hypothesis to explain the PMN aggregation which occurs in such syndromes as hemodialysis neutropenia, Purtscher's ischemic retinopathy, and the adult respiratory distress syndrome (ARDS). They showed that PMNs aggregate when exposed to $C5_a$ *in vitro*. Methylprednisolone inhibits this aggregation (175). They also found that intra-arterial injection of injection of zymosan-activated plasma into rats induced PMN clumps which could be visualized in mesenteric vessels. The PMN clumping and microvascular occlusion seen in this model were prevented by methylprednisolone pretreatment.

The role of complement-mediated neutrophil aggregation in Gram-negative bacteremia remains to be discovered. Profound complement activation, such as that produced *in vitro* by zymosan, has been noted only in patients with shock (see above, page 43).

(5) Prevention of hypoglycemia. Hypoglycemia is a consistent finding in experimental animals which are allowed to die with either endotoxemia or Gram-negative bacteremia. In contrast, there is little evidence that hypoglycemia is an important problem during Gram-negative sepsis in man (176). Glucocorticoids prevent this feature of endotoxemia in experimental animals.

b. Evidence from animal models

Much of the animal research on this problem is confused and irrelevant to the clinical problem. This is particularly true of studies which use bolus injections of endotoxin (see above, page 40), since this model is exceedingly artificial. Bolus injections (or continuous infusions) of Gram-negative bacteria provide a somewhat better model; not surprisingly, steroids do not have much effect on the outcome of this model, since they do not kill the bacteria (177)! There is now evidence in 3 kinds of animals that methylprednisolone, used in combination with an effective antibiotic, may prevent death from Gram-negative sepsis (177,178,179).

The most interesting and suitable animal model has been developed by Greisman (179). They inject mice with an LD_{100} of Gram-negative bacteria. After carefully timed intervals, aminoglycosides are given, at doses which were predetermined to produce survival. With progressive delay in initiation of antibiotic therapy, mortality increases from 0 to 100%. They then select delay intervals so that 50 - 70% mortality results. The experimental therapy can then be tested to determine if it will prevent mortality in addition to that prevented by antibiotics alone. The results of glucocorticoid administration in this model were significant (beneficial) but not very impressive. Methylprednisolone or dexamethasone, given in large doses, reduced the mortality not prevented by antibiotics alone, but this beneficial effect could be observed only if the steroids were given before, or at the time of, antibiotic administration.

None of the animal models accurately reflects the clinical situation. Volume replacement and vasopressor therapy are not features of any published model. Moreover, all of the animals used in these studies have been previously healthy- unlike most patients who develop Gram-negative rod bacteremia.

c. Evidence from clinical trials

There are three published studies which examined the efficacy of glucocorticoids in human Gram-negative bacteremia, using a prospective, controlled study design (see Table on the following page).

The Bennett study is noteworthy because it was a placebo-controlled double-blind cooperative study which involved investigators in three different cities. It demonstrated that "it is possible to conduct controlled double-blind studies by multiple hospital groups even in such acute and dramatic situations as life-threatening infections."

No benefit from (low dose) hydrocortisone was noted for Gram-negative bacteremia or for the other bacterial infections studied.

The Klastersky study found no benefit using betamethasone (1 mg/kg/day). This steroid is similar to dexamethasone in its anti-inflammatory potential and pharmacology (181).

The study by Schumer has received much attention because the results suggest that high-dose, bolus glucocorticoid therapy (30 mg/kg methylprednisolone once or twice) greatly improves survival in septic shock. The reported results are indeed impressive, but the study had important defects:

(1) the definition of shock ("falling blood pressure")was not rigorous

(2) death was attributed to the treatment episode if the patient died as long as four weeks later

(3) volume replacement and pressor therapy were not monitored or standardized

(4) adequacy of antibiotic therapy was not shown (blood levels, bacterial sensitivities, etc.); regimen was changed during the study

(5) study was not performed blind

TABLE 16 Controlled prospective studies of glucocorticoid therapy for Gram-negative bacteremia in man

Study	Year	Steroid Preparation	Placebo	Placebo-treated		Steroid-treated		Ref.
				Total	No. dead	Total	No. dead	
Bennett	1963	Hydrocortisone 100 mg/day X3	yes	4	2	5	3	(180)
Klastersky	1971	Bethamethasone 1 mg/kg/day	yes	39*	16	46*	18	(182)
Schumer	1976	Methylprednisolone (30 mg/kg) or Dexamethasone (3 mg/kg)	yes	86	33	86	9	(183)

* includes infections other than bacteremia

Summary: At the present time the available clinical data do not convincingly support the use of corticosteroids in patients with Gram-negative bacteremia and shock. There is no published evidence that bolus high-dose steroids are harmful, however, and Schumer's results should prompt a careful study of this mode of therapy (184).

3. Prostaglandin synthesis inhibitors; antagonists

The evidence which supports the use of these agents in the treatment of Gram-negative sepsis is based on animal studies. Pre-treatment with aspirin or indomethacin significantly reduces mortality in dogs given large doses of LPS intravenously (185). In this model, inhibitors of prostaglandin synthesis appear to have their major impact on the initial phase of endotoxin shock--i.e., on the profound reduction in arterial blood pressure and cardiac output which occurs within minutes of LPS administration. This phase may have little relevance to the events which occur in man, as it probably results from local high concentrations of LPS. Moreover, in other species indomethacin and aspirin appear to have little or no influence on the second, lethal phase of endotoxin shock. In fact, the administration of arachidonic acid improved the survival of rabbits given lethal doses of LPS, and sodium meclofenamate, a prostaglandin synthetase inhibitor, abolished this effect (186). This is a confusing situation which highlights the inadequacy of animal models using bolus doses of LPS and the marked species variations in LPS sensitivity.

In the rat, pretreatment with imidazole, a thromboxane synthetase inhibitor, prevented both elevation in thromboxane B_2 levels after LPS challenge and death of the animals (165). Similarly, pretreatment with 13-azaprostanoic acid, a thromboxane antagonist, reduced mortality from endotoxic shock in this model. In this model, endotoxin appears to induce increased synthesis of thromboxane A_2, and pharmacologic prevention of this increase, or antagonism of thromboxane, improved survival. The rat is very insensitive to LPS, however (the dose used in these experiments was 20 mg/kg!).

In the baboon, LPS induced a transient elevation in thromboxane B_2 and a more prolonged elevation in 6-keto $PGF_{1\alpha}$, a stable metabolite of prostacyclin. Indomethacin, given 1 hour after the LPS dose (and after thromboxane levels had declined to near-normal), prevented the rise in 6-keto $PGF_{1\alpha}$ as well as the subsequent hypotension. The authors argued that prostacyclins, as vasodilators, are more likely mediators of endotoxic shock than thromboxanes, which are potent vasoconstrictors (168). Other workers have also found that indomethacin prevents the hemodynamic consequences of bolus LPS injection in the baboon, even when the indomethacin is given 1-2 hours after the LPS (187). The baboon is also reactively insensitive to endotoxin, however, and the relevance of these findings to clinical sepsis is uncertain.

4. Heparin therapy for DIC

There is strong experimental animal and clinical evidence that DIC can be terminated by heparin treatment (188,189). On the other hand, there is very little evidence that heparin therapy prolongs or improves survival in patients or animals with Gram-negative sepsis (153). Although there has been no prospective, randomized trial of heparin therapy for this

disorder, enthusiasm for its use has decreased in recent years (190). The following approach is acceptable (148):

a. If the patient is normotensive and is not bleeding but has DIC, no therapy for DIC is usually necessary. If needed, vitamin K replacement should be given. Most important, the process which is causing DIC should be corrected.

b. If the patient is hypotensive and has DIC, reversal of hypotension will often be associated with correction of the DIC.

c. If the patient has DIC and is bleeding, exclude other reasons for blood loss (ulcer, varices, etc.). Reverse hypotension. Replace blood as required. Plasma and platelets should be given if DIC does not correct and the patient has not had thrombotic manifestations.

d. Administration of heparin is controversial and probably unnecessary if the above measures are taken. It should probably be used if the patient does have thrombotic manifestations of DIC.

Little attention has been given to the prophylactic use of heparin during sepsis. By enhancing antithrombin III activity, heparin might help "stabilize" the clotting cascade, thereby preventing DIC.

5. Naloxone therapy in shock.

Endogenous opiates (endorphins) are released in response to stress, and even small doses of opiates are able to depress blood pressure (162). Naloxone, which blocks the opiate receptor, increased blood pressure and prevented death in rats with experimental hypovolemic shock. A similar reversal of hypotension was seen in rats with endotoxic shock, but no improvement in survival was noted (191). The effect of naloxone is stereospecific; (-)naloxone reversed hypotension while (+) naloxone did not. In a canine model of endotoxic shock, naloxone (2 mg/kg i.v. bolus followed by 2 mg/kg/hr as i.v. infusion) prevented or reversed hypotension (192). Survival was significantly improved in naloxone-treated animals.

The usual dose of naloxone, administered to antagonize exogenous opiates, is 0.4 - 1.2 mg i.v. Much higher doses have been given to both animals and humans with no evidence of toxicity (193).

A pilot study to examine the efficacy of Naloxone in reversing hypotension in human patients with Gram-negative bacterial shock is currently in progress. Like other measures designed to counteract the effect of circulating mediators, this mode of therapy clearly is adjunctive, to be used only in combination with optimal conventional therapy.

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