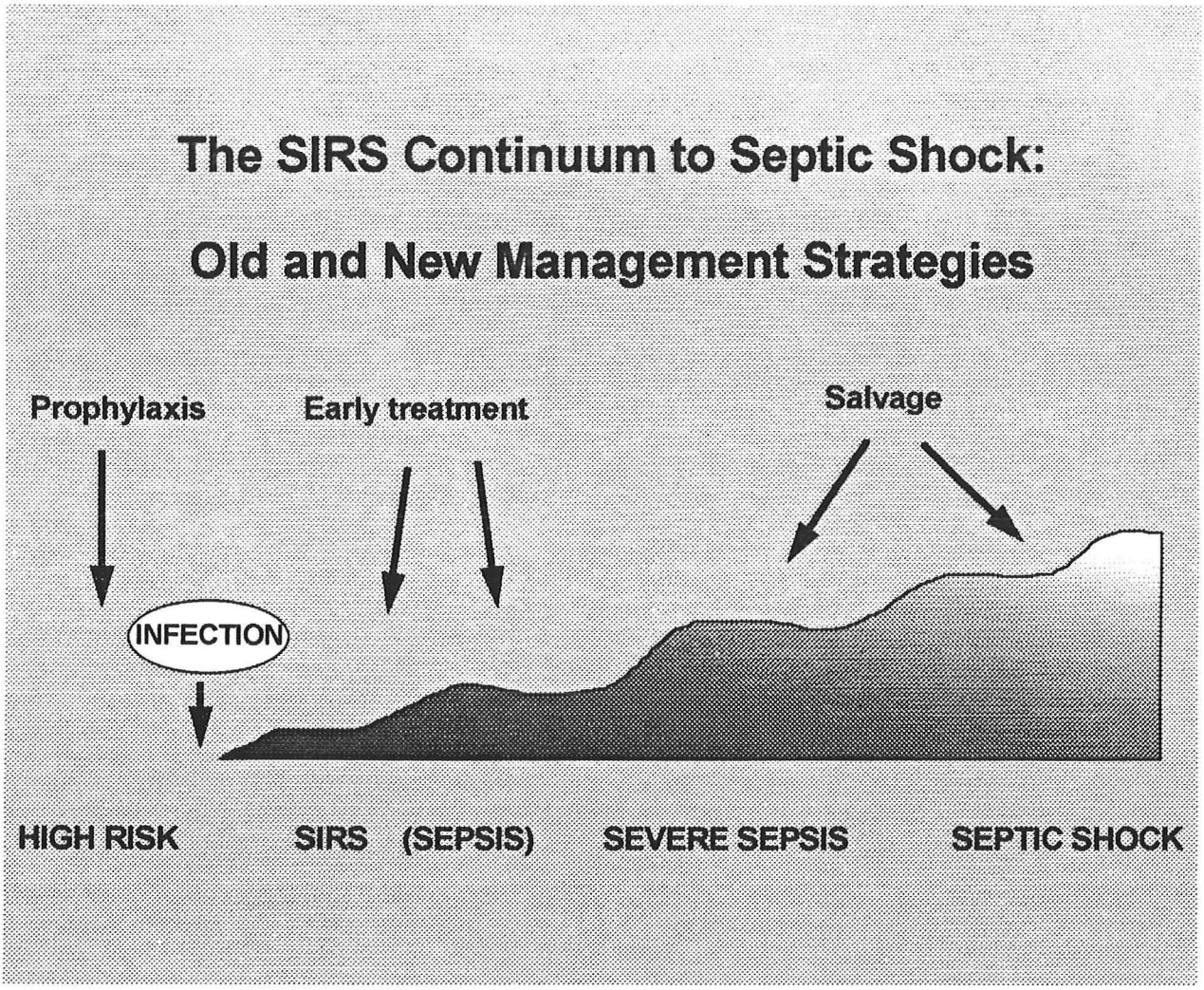

Internal Medicine Grand Rounds

Parkland Memorial Hospital

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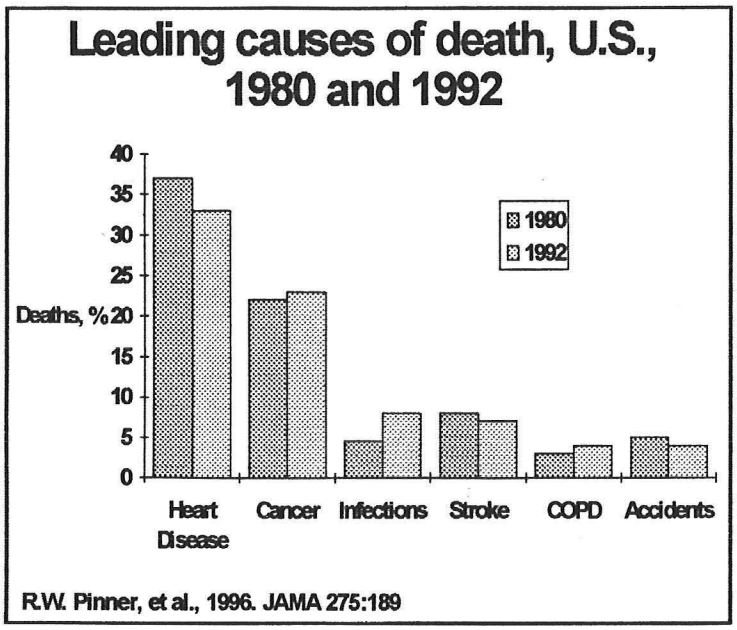
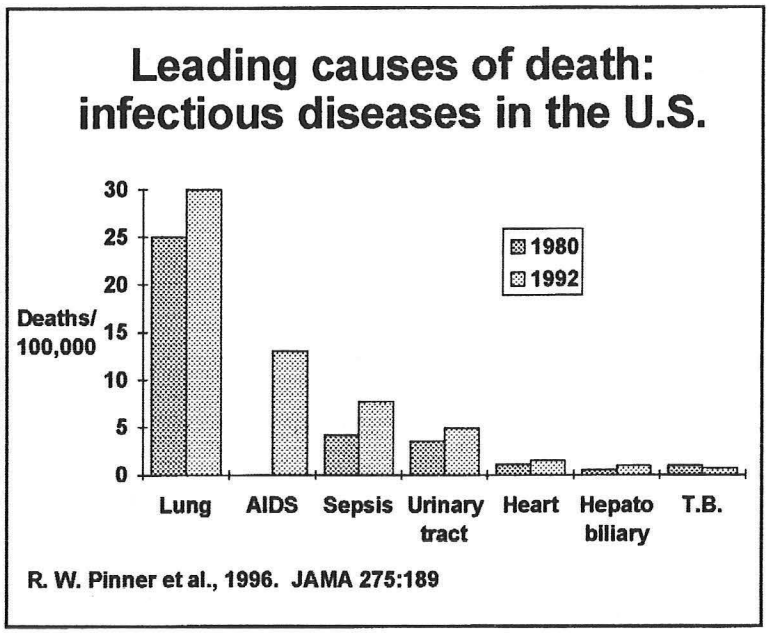
The SIRS Continuum to Septic Shock: Old and New Strategies

According to recent data from the Centers for Disease Control and Prevention, the incidence of severe sepsis has been increasing in the U.S.(1) Only a small fraction of this increase is attributable to patients with AIDS.





The increase in sepsis incidence occurred during an intense industry-driven effort to find a "magic bullet" that could resuscitate patients with severe sepsis or septic shock. When tested in randomized, placebo-controlled clinical trials, however, almost a dozen drugs failed to improve the outcome of septic patients (Table 1). The goal of this Grand Rounds is to re-examine the scientific assumptions that underpinned this effort and to suggest some directions for future research and clinical practice.

Table 1

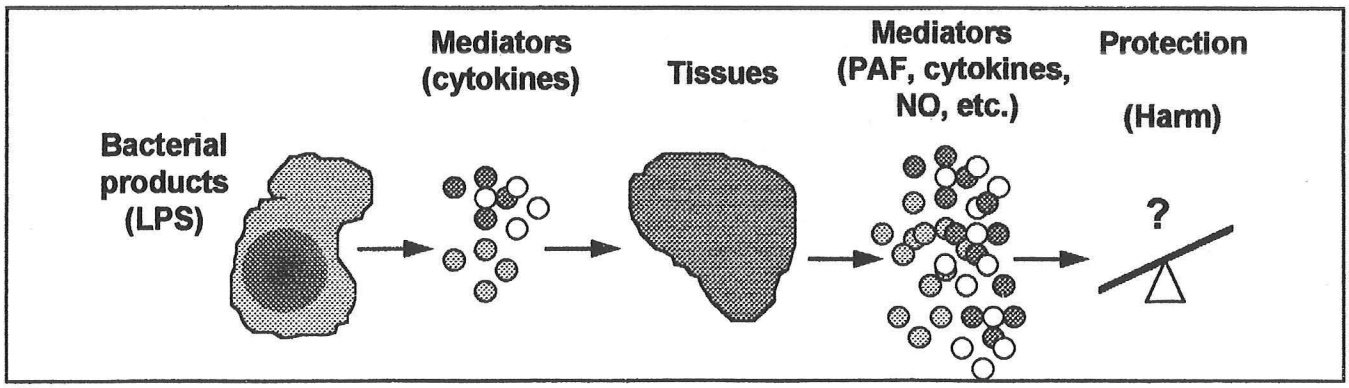
Randomized, placebo-controlled, double-blinded trials in patients with sepsis syndrome

Drug	*Target*	*Entry criteria*	*Pts.*	*Result*	*Comment*	*Year*	*Ref.*
Polyclonal anti-J5 serum	endotoxin	Gram-negative bacteremia	212	Striking reduction in death from septic shock	Entry and end-point criteria difficult to understand	1982	(2)
Polyclonal anti-J5 serum	endotoxin	High risk of gram-negative infection	262	Reduction in death from septic shock	Prophylaxis, not treatment	1985	(3)
J5-IV Ig	endotoxin	septic shock	100	No benefit		1988	(4)
Glucocorticoids	General immunosup-pression	Sepsis syndrome	382	Ineffective; increased incidence of infections	Methylprednisolone, 30 mg/kg q6h x 4	1987	(5)
Glucocorticoids	same	Sepsis syndrome (but normal mental status)	223	Ineffective overall; possibly effective in patients with GN bacteremia	VA Cooperative Study Group	1987	(6)

HA-1A	endotoxin	Sepsis syndrome	200	Reported effective in pts with gram-negative bactermia	End-points changed after reviewing interim analysis (7)	1991	(8)
HA-1A	same	Septic shock	2199	Trial stopped after interim analysis-possible toxicity	More deaths in HA-1A recipients (pts who did not have GN bacteremia), $p < 0.07$	1994	(9)
E5	endotoxin	Sepsis syndrome	486	Reported effective in pts with GN sepsis, not in shock		1991	(10)
E5	same	Sepsis syndrome, no shock	811	Second trial; no effect on mortality	Only published as abstract	1992	(11)
CB0006	TNFα (mAb)	Severe sepsis/ shock	80	Apparent benefit in patients with high TNF blood levels	Phase II study	1993	(12)
BAYx1351	TNFα (mAb)	Sepsis syndrome	564	No reduction in mortality at 28 days	unpublished	1995	
NORASEPT	TNFα (mAb)	Sepsis syndrome	994	Trend: efficacy at 3d, not at 28d; only in pts with shock	Non-significant trend toward toxicity in high dose group	1995	(13)
TNFR	TNFα (soluble receptor)	Septic shock	141	Dose-related toxicity	Dimer of human p80 TNF receptor fused to Fc of IgG1. See studies in human volunteers (14).	1996	unpub.
rhuTNFR:Fc	same	Sepsis syndrome		Lower mortality in severe sepsis, not in refractory septic shock	Dimer of human p55 TNF receptor--fused to Fc from human IgG1	1995	(15)
IL-1Ra	IL-1	Sepsis syndrome	893	No decrease in mortality	May have been effective in pts with high predicted risk of dying. Second study stopped following interim analysis.	1994	(16)
BN52021	PAF receptor antagonist	Sepsis syndrome	262	May be effective in GN bacteremia (not GP)	Benefit only found in post-hoc subgroup analysis	1994	(17)

Other interventions tested in recent phase II or III trials: ketoconazole (18), anti-thrombin III concentrate (19), methylene blue (20), pentoxifylline (21), and an antibody to E-selectin (22).

Simply stated, *sepsis* is an exaggerated host response to microbial invasion. It is the normal inflammatory response careening out of control. Inflammation is an evolutionarily conserved, tightly regulated response that walls off and kills invading microorganisms, responds to tissue injury, and reacts to stress. It is this innate immune system--the rapid response team--



that protects animals from microbes during the time before specific immunity can be acquired. For uncertain reasons, this response can get out of control, often producing injury that far exceeds the damage produced by the invading microbe itself.

Again put in simple terms, the inflammatory response involves several related phenomena: (1) host recognition that microbes have invaded, that tissue has been injured, etc., (2) production/release of soluble molecules, such as proteins (cytokines) and lipids (prostanoids, PAF), that activate the cells (neutrophils, monocytes, lymphocytes, endothelial cells) that mediate the defense reaction, and (3) the production/release of various molecules that damp the response and turn it off.

The definitions used to describe septic patients have evolved during the past 15 years from very loose ("severely ill, with recent deterioriation in the form of sudden high fever or hypothermia...")(2), to more quantitative ("sepsis syndrome"(23)), to the recent consensus definitions that emphasize that the septic response is a continuum from SIRS to septic shock (24)(Table 2). Roger Bone (a former Parkland internal medicine resident and pulmonary fellow) played a major role in this evolution, which has fostered clear communication, given workers in the field a way to standardize entry criteria for clinical trials, and engendered a growing appreciation that the manifestations of the septic response can differ substantially among individuals.

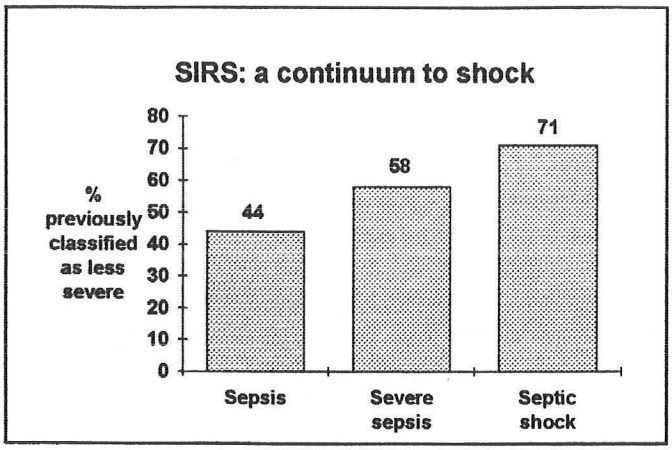
Table 2. Definitions used to describe septic patients (24)

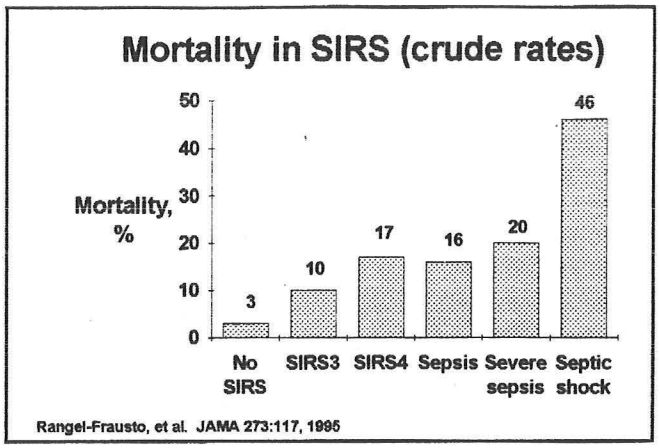
Bacteremia	Presence of bacteria in the blood, as evidenced by positive blood cultures
Septicemia	Presence of microbes or their toxins in blood
Systemic inflammatory response syndrome (SIRS)	Two or more of the following conditions: fever (oral temperature > 38 C) or hypothermia (< 36 C); tachypnea (> 24 breaths/min); tachycardia (heart rate > 90 beats/min); leukocytosis (>12,000/mm^3), leukopenia (< 4,000/mm^3), or > 10% bands. 3 criteria = SIRS3; 4 criteria = SIRS4 Can have a non-infectious etiology (see text)
Sepsis	SIRS that has a proven or suspected microbial etiology
Severe sepsis	Sepsis with one or more signs of organ dysfunction (such as metabolic acidosis, acute encephalopathy, oliguria, hypoxemia, or DIC) or hypotension.
Septic shock	Sepsis with hypotension (arterial BP < 90 systolic, or 40 mm Hg less than patient's normal BP) that is unresponsive to fluid resuscitation, along with organ dysfunction (see severe sepsis).
Refractory septic shock	Septic shock that lasts for more than 1 hour and does not respond to fluid or pressor administration
Sepsis syndrome	Sepsis with organ dysfunction or hypotension; similar to severe sepsis.

The inflammatory response progresses from mild to severe.

Rangel Frausto and others prospectively studied 3708 patients who were admitted to medical and surgical intensive care units at the University of Iowa Hospital (25). Each patient was evaluated daily for SIRS, severe sepsis, and septic shock. Overall, 68% of the ICU patients developed SIRS; of these, 26% developed sepsis, 18% developed severe sepsis, and 4% had septic shock. If one includes patients who had suspected but unproven infection, 61% of the patients who had SIRS had sepsis, 39% developed severe sepsis and 7.6% developed septic shock.

Over time, many patients progressed to more severe stages (Figure). For example, 71% of the patients who developed septic shock had been classified in a less severe category on at least one previous day .





Case fatality rates increased from SIRS (7%), sepsis (16%), severe sepsis (20%), to septic shock (46%). ICU patients who did not develop SIRS had a case fatality rate of 3%.

This was the first large study to confirm that patients progress through the various stages of the septic response, from mild to severe. Although some of the group's methods and data are poorly described in the published article, the study showed that the SIRS continuum can be quantitated prospectively.

Drugs for severe sepsis and septic shock: assumptions used for the recent trials

> Most of the clinical trials tested the hypothesis that antagonizing a *single* host or bacterial molecule can increase *28 day survival* in a diverse, *heterogeneous population* of patients with *severe sepsis or septic shock* while having *no adverse side effects*.

Assumption/evidence/comment:

1. Antagonizing a single molecule can modulate the septic response. With the exception of the glucocorticoid trials, all of the clinical trials in septic patients have tested drugs intended to neutralize a single bacterial or host molecule. For the most part, there was suggestive evidence that this strategy should work.

A. Pro-inflammatory cytokines or other mediators. For the various host pro-inflammatory molecules, supporting evidence principally comes from the ability of specific or selective antagonists to protect animals from endotoxin challenge or bacterial infection. Table 3 summarizes a large number of studies in this area.

Table 3

Host molecules that contribute to the septic process
(Specific inhibitors of these molecules can prevent death from endotoxic shock, or reduce endotoxic injury, in animals.)

Molecule	Class	Inhibitor/antagonist	Animal species	Reference
TNF-α	cytokine	pAb, mAb	mouse - protection rabbit - protection mouse - no protection chimpanzee - blocked fibrinolysis	(26) (27) (28) (29)

		TNF-R (p55 soluble receptor)	rat mouse	(30) (31)
		chlorpromazine, thalidomide (block TNF production)	mouse - benefit mouse - no benefit, worsened outcome in Candida infection	(32) (33)
IL-1ß	cytokine	IL-1Ra (receptor antagonist)	baboon mouse rabbit	(34) (35) (36,37)
IL-6	cytokine	mAb	chimpanzee mouse	(38) (39)
IL-12	cytokine	mAb	mouse	(40,41)
MIP-2	C-X-C chemokine	polyclonal antibody	rat (pulmonary injury)	(42)
Interferon-γ	cytokine	Polyclonal antibody mAb	mouse mouse	(43) (44,45)
PAF	mediator	BN 50739 BN 52021 CL 184005 SRI 63-675 TCV-309 PAF-acetyl hydrolase	rabbit rat mouse pig chimpanzee mouse	(46) (47) (48) (49) (50) (51)
Migration inhibitory factor (MIF)	cytokine	Polyclonal antibody		(52)
D factor (leukemia inhibitory factor)	cytokine	Polyclonal antibody	mouse	(53)
CD18	adhesion molecule	mAb	rabbit	(54,55)
CD14	LPS receptor	mAb	mouse, primate	
Phosphatidic acid	Lipid signalling intermediate	lisofylline - selective inhibitor of PA formation	mouse	(56)
Intracellular Ca++	Multiple actions	Dantrolene	mouse	(57)
Prostanoids	Multiple actions	Indomethacin, NSAIDS		(58)
Clotting factors	procoagulants	protein C (inhibits factors V, VIII...)	baboon	(59)
	tissue factor, factor VII	tissue factor pathway inhibitor; mAb to factor VII/VIIa	baboon, chimpanzee	(60,61)
	factor XII	mAb (blocks hypotension, not DIC)	baboon	(62)
Oxygen free radicals	Multiple actions	superoxide dismutase	mouse	(63,64)

Adenosine kinase	degrades adenosine	GP-1-515	rat	(65)
Nitric oxide	Vasodilator	NNMA, others	various species	(66)(review)

B. Bacterial endotoxin. Neutralizing endotoxin can protect animals from endotoxic death and/or gram-negative bacterial infection. Molecules that neutralize endotoxin may also have other antibacterial actions. For example, antiendotoxin antibodies may promote opsonophagocytosis of gram-negative bacteria (67), while BPI ("bactericidal permeability-increasing protein") enhances bacterial killing (68,69). Antibodies directed to the O-antigen of a particular gram-negative bacterium are highly effective vs. that O serotype, but they lack protective efficacy vs. other gram-negative bacteria. Although many workers believed that certain antibodies to "rough" LPS structures could confer "cross-protection"--i.e., protect animals from challenge with various heterologous bacteria, Greisman and Johnston identified numerous problems with the experiments often touted to support this notion (70). The first genuine cross-protective antibody to endotoxin was described only recently (71), and it binds/neutralizes endotoxins from a relatively narrow spectrum of enterobacteriaceae.

The cross-protective antiendotoxin antibody concept was tested (many would say prematurely and ineffectively) by the clinical trials of two IgM monoclonal antiendotoxin antibodies, HA-1A (Centoxin) and E5. Neither of these antibodies bound a broad range of LPSs in vitro, and neither could be shown reproducibly to protect animals from challenge with diverse endotoxins or gram-negative bacteria (72,73). It wasn't surprising that neither mAb was efficacious when tested in human clinical trials. *The role of endotoxin as a target for sepsis therapeutics remains uncertain.*

- *Summary:* in many different animal challenge models, neutralizing a single component of the inflammatory cascade can be protective. This (along with much experimental evidence (74-78)) argues for exquisite *synergy* among interacting mediators. It also suggests that many different drug interventions might work. With few exceptions, however, the animal studies suggest that, to be effective, the neutralizing drug must be given *before, with, or very shortly after* the inflammatory stimulus.

2. Treatment is effective late in the inflammatory response, after severe sepsis has developed. Most of the recent trials used the "sepsis syndrome" definition to enroll patients. This definition requires evidence for organ dysfunction or hypotension. So only patients with severe sepsis or septic shock were studied. Is this too late in the SIRS continuum?

A. *Antiendotoxin therapy.* Since endotoxin is thought to be a trigger for the inflammatory response, early treatment would seem essential. Several studies have indicated, however, that endotoxin can circulate in the blood of septic patients for days after the initiation of conventional therapy (79), suggesting that even delayed treatment might be effective. There is also evidence that antimicrobial drugs can release endotoxin from bacteria *in vivo* (80-82), even in infected humans (83), arguing for administering anti-endotoxin drugs at the same time that (or before) antimicrobials are initiated (84).

In animal models, delayed treatment with antiendotoxin antibodies is generally much less effective (protective) than early treatment.

Casey et al. (85) studied 97 consecutive patients on a medical service who developed severe sepsis. Endotoxin was detected in the plasma of 89% of these patients. Only 24 had gram-negative bacterial infection, however, and mean endotoxin levels did not distinguish these patients from those with gram-positive bacteremia or no positive cultures. Many critically ill but non-septic patients also had detectable plasma endotoxin levels. Goldie et al. (86) detected plasma endotoxin in 66% of 146 patients with severe sepsis, but there was no relationship between endotoxin concentration and survival. High plasma endotoxin levels correlated with greater mortality in a small series of septic neutropenic patients (87) and in the study performed at the NIH by Danner et al. (79).

B. Anticytokine or antimediator therapy. Patients with severe sepsis or septic shock often have high blood concentrations of pro-inflammatory cytokines, soluble endotoxin receptors (CD14), soluble adhesion molecules, and various inflammatory mediators, and many also have high levels of putative anticytokine molecules such as soluble TNF-R's, IL-1Ra, IL-10, and TGF-ß.

Table 4

Molecules found in higher than normal concentrations in the blood of humans with severe sepsis or septic shock

Molecule	Blood levels correlate with illness severity	Comment	Reference
Proinflammatory molecules			
Tumor necrosis factor-α	yes	High levels persist for days in non-survivors(88-91)	(85,88-90,92,93)
Interleukin-1 β	?	Generally found only in late or more severe sepsis. Not detected in many studies, elevated in some (85).	(85,88,94-96)
Interleukin-2	yes	Levels often undetectable. May be a prognostic indicator in *early* sepsis.	(91,94,97)
Interleukin-6	yes	Best marker for severity; induces acute phase protein production; pro-inflammatory role poorly understood	(85,90,94,95,98-100)
Interleukin-8	yes	Chemotaxin	(96,101)
Interferon-γ	no	levels usually low or not detectable	(88,91,97)
MCP-1, MCP-2	no	Increased MCP-1 with gram positive and negative infection; increased MCP-2 only with gram-positive infection	(102)
Leukemia inhibitory factor (LIF, D-factor)	yes	Meningococcal disease	(103)
Prostaglandins, prostacyclin	?	Diverse data	(104)
Thromboxane(TXB_2)	probably	Small sample (12)	(105)
Platelet activating factor (PAF)	?		(106-108)
Soluble adhesion molecules	yes	sELAM-1, sICAM-1, sVCAM-1; uncertain role in illness	(109)
Lactic acid	yes		(110)
Vasoactive neuropeptides	yes	C-GRP, neuropeptide Y	(111)
Phospholipase A_2	no		(112,113)
Plasminogen activator inhibitor-1	yes (in some studies)	procoagulant	(90,114,115)
Neutrophil elastase	yes	protease	(101,112)
Neopterin	yes	human counterpart of NO?	(116)
CD14, LPS binding protein (LBP)	yes	LPS transfer proteins; the 55 kDa form of CD14 is elevated.	(117,118)
Anti-inflammatory molecules			
Interleukin-10	yes	inhibits TNF production	(96,119-121)

Interleukin-1 Ra	weak association	levels >>those of IL-1	(86,95,122)
Type II IL-1 receptor	?	may be shed from PMNs; inhibits IL-1 activity	(123)
TGF-ß	no	26 patients studied	(124)
Soluble TNFα receptors (p55, p80)	weak association	levels>>those of TNFα. Ability to neutralize TNF in vivo uncertain. Ratio of TNF/TNF-R may be higher in patients with fatal outcome.	(86,96,122,125)
C-reactive protein	yes (weak)	opsonic for some bacteria; stimulates IL-1Ra production	(90,112)
α-MSH	?	unpublished studies (J. Lipton, personal commun.)	
Endothelins	?	small study; E-1 and E-3	(126)

Other molecules predictably elevated: cortisol, epinephrine; interleukin-2 receptor (116). Molecules with lower than normal concentrations in blood of patients with septic shock: several complement components, protein C (negative correlation with mortality)(127), interleukin-6 receptor (negative correlation with IL-6 levels) (128). C-reactive protein levels are lower in patients with fulminant meningococcemia than in those with meningococcal meningitis (129). IL-4 was not detected in patients with fulminant meningococemia (96).

(Note that high levels of **both** pro- and anti-inflammatory molecules are found in patients with severe sepsis/septic shock, and that levels of both may correlate **directly** with mortality risk.)

Although there are important methological issues (such as the relationship between the immunoreactivity and bioactivity of the various molecules assayed (130,131)), the picture that emerges from these data is a Rorschach pattern: a complex melee of molecules that might be interpreted in many ways. Large numbers of pro-inflammatory cytokines mix with (often even larger) numbers of anti-inflammatory molecules in a sort of molecular soup that lacks physiologic meaning. It's not at all clear that adding another anticytokine or antimediator molecule to the soup should make a real difference. It would probably still taste awful.

In fact, most studies of anticytokine drug therapy in animal models also suggest that delayed treatment is likely to be ineffective. One exception may be IL-10, which was more effective in reducing mortality when given 6 hours after, rather than simultaneously with or 6 hours before, induction of experimental peritonitis in mice (132). Prolonged, high levels of TNF-α also correlate with a poor prognosis; there is still hope that neutralizing it will be beneficial.

It is interesting that blood levels of TNF seem to remain elevated in patients who eventually die from the septic episode. At the same time, levels of both the p55 and p75 soluble TNF receptors are elevated, often markedly so. The p75 TNF-R showed dose-related toxicity in a phase II clinical trial, and it has subsequently been shown to prolong the half-life of bioactive TNF in the circulation. It also functions to "pass" TNF to the p55 TNF-R, which is the major receptor for cellular signalling (133,134). So in severely septic patients, could p75 TNF-R actually be pro-inflammatory ?

- *Summary:* there is little pre-clinical or clinical evidence that any drug can *reverse* severe sepsis and/or septic shock.

3. Therapy should be effective in patients with diverse infectious etiologies and underlying diseases. Although some of the trials were directed toward patients with gram-negative sepsis/bacteremia, almost all enrolled patients on an "intention to treat" basis before the microbial etiology of sepsis was identified. The patient populations studied were usually very heterogeneous with respect to covariates such as age, underlying disease, and etiologic microbial agent.

A. Although endotoxin is not infrequently detected in the blood of patients who do not have known gram-negative bacterial infection, antiendotoxin therapies have been evaluated primarily in patients who have cultures positive for

gram-negative bacteria. Since such patients now account for only 40 to 50% of patients with severe sepsis/septic shock, trials of these drugs must be roughly twice as large as trials of anticytokine or antimediator drugs to achieve the same level of statistical validity. One would also imagine that the clinical utility of antiendotoxin drugs will be greatest in (the minority of) patients who have gram-negative bacterial disease.

B. All septic patients do not have the same risk of dying. Many of the recent trials found that patients with urosepsis, for example, have a substantially lower risk of dying than patients with primary infections in other organs (135). A potentially more serious problem for evaluating new drugs is imposed by the patients' underlying disease(s). There is no evidence that patients who have "rapidly fatal" underlying diseases (see (136)) should respond to adjunctive therapy as well as patients with less severe ("ultimately fatal," "non-fatal") diseases. In fact, antiinflammatory therapy may work best in previously normal individuals, such as children with bacterial meningitis (84). In the recent clinical trials, many of the deaths have occurred in patients with rapidly fatal underlying diseases (136). These deaths could possibly obscure a beneficial effect in patients with less serious underlying conditions.

4. *Resuscitating patients from severe sepsis/septic shock can reduce mortality 28 days later*

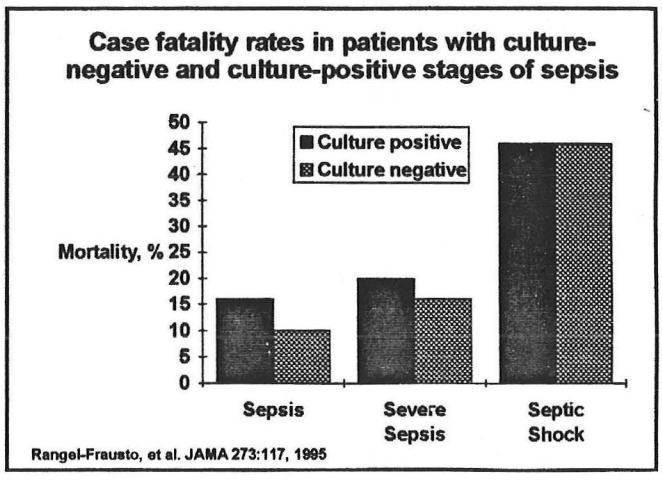
Since the septic response usually runs its course over a few days, a short observation period (7 or 14 days) may be preferable to the recent standard (28 days) for evaluating the impact of new therapies. Patients who develop severe sepsis often experience numerous complications of hospitalization, including suprainfection, GI bleeding, etc. Adequate sample size and randomization should prevent these variables from confounding trial results. On the other hand, the long-term (say, 6 month) mortality in patients with severe sepsis can be very high (137). The longer the observation period, the more likely that no effect of a drug will be found--since the survival curves will eventually converge.

The other issue raised here is the choice of end-point. We expect antibiotics to cure bacterial diseases. Life extension is an obvious consequence in most instances. Perhaps similar expectations are appropriate for anti-sepsis drugs. We should expect them to reverse sepsis, not necessarily to prolong life for long periods of time. So end-points such as reversal of shock, improvement in some index of organ failure, or reduction in ICU stay might be more reasonable indicators of drug efficacy. On the other hand, if these drugs *don't* prevent death, would they really be useful or worth the expense? And as some F.D.A. officials have argued, if a drug doesn't prevent death (i.e., if it doesn't reduce the case-fatality rate so that the confidence limits on the difference between drug and placebo don't overlap zero), how can we be sure that it isn't toxic?

The septic response - changing concepts

- *Local vs. systemic cytokine production.* Cytokines that are produced and have their impact in local sites may be very important. For example, it is possible to protect animals from systemic LPS injection with anti-TNF mAb, whereas the same antibody has little efficacy when the LPS is injected into the intraperitoneal space or trachea (138-140). Similarly, there was no correlation between *serum* TNF and mortality in mice injected *intraperitoneally* with LPS (141). Local vs. systemic TNF levels are also important for the metabolic responses to this cytokine (142). Compartmentalization of the cytokine response may help explain why anti-TNF mAb and soluble TNF inhibitors have been ineffective in clinical trials.

  Neutralizing IL-10, an anti-inflammatory cytokine, *increased* mortality in animals injected i.v. with LPS or with experimental peritonitis (143), but *decreased* mortality in a murine model of *Klebsiella* pneumonia (144), suggesting that the local cytokine networks may be quite different in these local sites. In humans injected i.v. with endotoxin, the lung appears to be insulated from high blood levels of cytokines (145).



- There is now good evidence that *the host inflammatory response to gram-negative and gram-positive bacterial*

infection is very similar (146). Drugs that modulate this response thus potentially could benefit all patients with severe sepsis/shock. In addition, there is now evidence that outcomes are essentially identical in patients at a particular stage of the septic response, whether or not a positive culture is obtained (Figure) (25,147).

- There is no animal model that adequately mimics the human septic response. This imposes an important limitation on the pre-clinical evaluation of drugs. Not only does human physiology differ substantially from that of laboratory animals, but it is also impossible to mimic important variables such as underlying disease (136). Perhaps the burn/trauma/surgery models come closest to resembling a clinical situation.

The future: prevention is more likely to succeed than resuscitation?

> **A revised hypothesis**: severe sepsis and septic shock can be *safely prevented* in *high risk* patients by using measures that (a) prevent the infectious complications of hospitalization and serious illness, (b) provide *prophlaxis* to boost immune defenses and/or damp the inflammatory response, and/or (c) *intevene early* to interrrupt the sepsis cascade *before* it causes organ damage and shock.

In other words, preventing severe sepsis may be a lot easier than treating it. Although it may be possible to prevent severe sepsis in only half (or so) of the patients who would develop it in the hospital, even this would be a very worthwhile achievement. (After all, the most optimistic advocates of the salvage (resuscitation) strategy hope to reduce septic mortality by only 40%. No strategy will work for every patient. Fortunately, we don't have to choose between strategies--the greatest good would be achieved by maximizing *both* prevention and salvage.)

Comments on three components of the hypothesis:

1. Severe sepsis can be prevented. The available data concern the efficacy of perioperative antimicrobial chemoprophylaxis to prevent infection, not with adjunctive (immunomodulatory) measures to prevent severe sepsis/septic shock. On the other hand, there is a provocative theoretical basis for immunoprophylaxis and early intervention.

*A. There is a rationale for **(immuno)prophylaxis** to prevent infection.*

Risk factors for post-operative infection and sepsis. Much useful information on the pathogenesis of the human septic response is found in the surgery literature. This is not surprising: sepsis-associated organ injury is the probable cause of death in over 80% of patients who die more than 7 days after traumatic injury (148). Major surgery or trauma is a finite, quantifiable event that places patients at increased risk for infection and sepsis. Such patients are more clinically homogeneous (at least with respect to the event that put them at risk) than are medical patients. They therefore offer special opportunities for understanding basic mechanisms. The published data suggest strongly that patients at high risk for post-operative or post-trauma infection and sepsis can often be identified by clinical and laboratory data obtained either *before, or shortly after,* surgery or trauma, long before infection is clinically apparent. Although these studies obviously will not apply to all patients who develop severe sepsis, and possibly to few patients on the medicine wards, I think they offer useful clues to basic pathophysiology.

It's important to note that the pathophysiologic state of "severe sepsis" usually is triggered by infection, even in patients whose cultures are negative. Preventing infection should prevent most cases of severe sepsis and septic shock. Factors that predispose patients to infection also increase their risk of developing severe sepsis. It's often hard to separate the two phenomena.

For example, a large study by Christou (McGill, Montreal) found that patients who were anergic to a battery of 5

skin test antigens had a two-fold higher risk of post-operative infection than those who reacted to two or more of the antigens (149). In addition, anergic patients who developed infectious complications had a higher risk of dying than did infected reactive patients. Overall, when compared with patients with 2 or more reactive skin tests, patients who had pre-operative anergy were more than 5-fold more likely to die during the post-operative period.

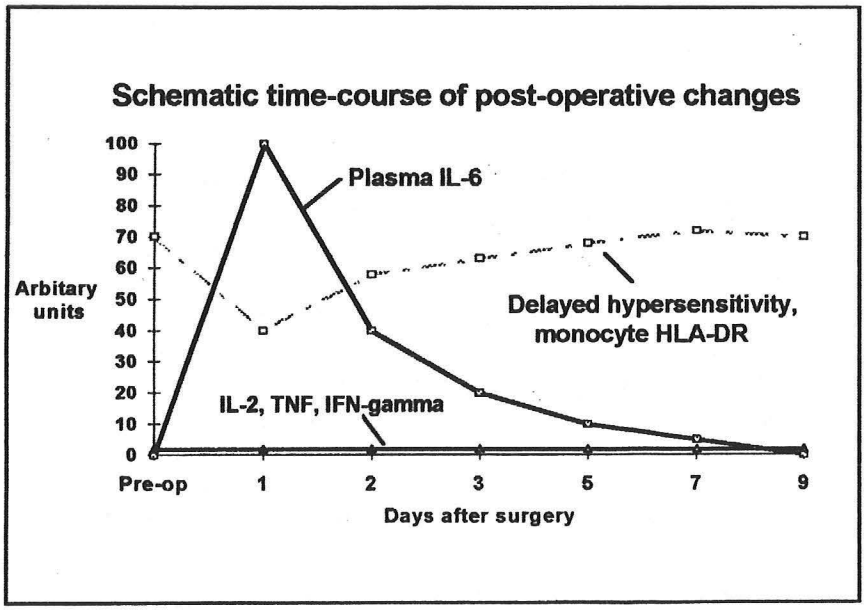
Considering all patients who were tested within 24 hours of admission to the hospital, there was a striking exponential relationship between skin test reactivity (the sum of induration diameters for all 5 tests) and mortality (90% of deaths were attributed to complications of sepsis). Patients who became anergic *after* surgery and remained so for more than 1 week also had a higher risk of sepsis and death (149).

In an earlier study, also from Canada (150), the impact of anergy was even more dramatic: of 42 patients who were anergic or relatively anergic in pre-operative testing, 9 (21%) developed sepsis and 14 (33%) died, compared with 13 episodes of sepsis (4.6%) and 12 deaths (4.3%) in the 322 patients who had normal skin test responses before surgery. Cancer and age did not account for these findings. Other workers have also described striking increases in both post-operative septic complications and death in patients who were anergic (151).

None of these reports used multivariate analysis to evaluate other risk factors, although Christou noted that anergic patients had lower total serum globulin and hemoglobin levels than did reactive patients. Anergy often occurs in individuals with protein-calorie malnutrition and it may reflect aging and many other underlying processes. How it relates to the risk of bacterial infection is uncertain. However, there are laboratory data that give clues.

Post-operative or post-trauma patients who developed infectious complications often have had, when compared to patients who recovered uneventfully:

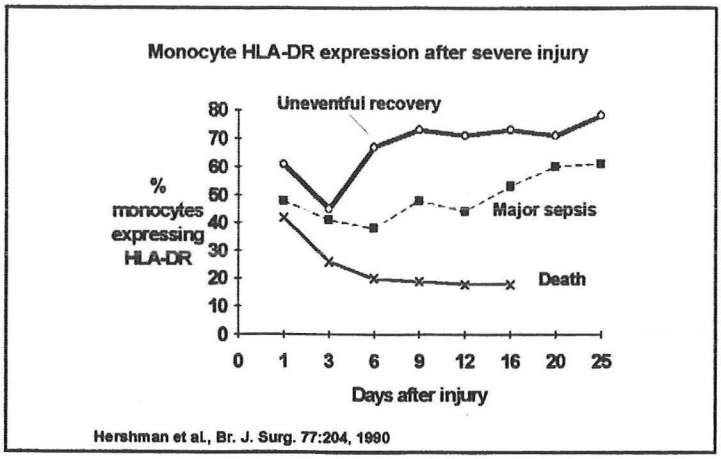
1. Lower pre-operative monocyte HLA-DR expression (one study)(152), greater post-operative decreases in monocyte HLA-DR expression (several studies), and lower LPS-induced monocyte HLA-DR expression (152-155). Among septic patients, the absence of LPS-inducible HLA-DR expression correlated directly with risk of dying (154,156).
2. Greater production (higher or more prolonged blood concentrations) of IL-6 (157), IL-1Ra (158), TNF-R1, and TNF-R2 (159).
3. More prolonged elevations in C-reactive protein (160).
4. Greater increases in neutrophil CD11b (161,162).

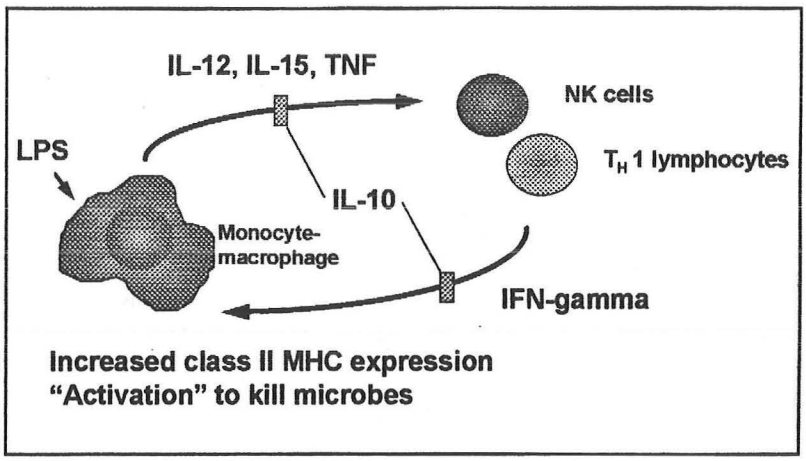


Note the "typical" changes in the scheme at left. In patients who develop post-operative infections, these changes are often exaggerated: higher IL-6, lower HLA-DR expression, more prolonged anergy, etc. (see Figure at upper left on the next page).

In general, these differences are detectable 1 - 4 days after surgery or trauma, well *before* the infectious complications are clinically apparent. Non-uniformity in laboratory methodology and study design makes cross-study comparisons very difficult, yet the observations from different centers are generally consistent.

Is there a plausible explanation for these findings?

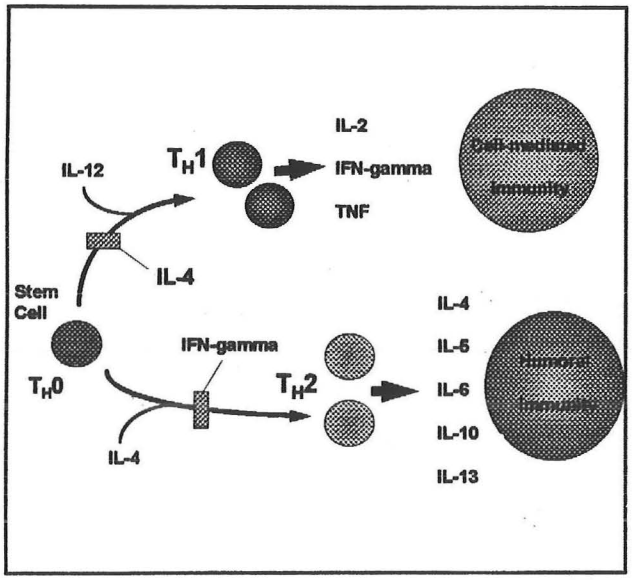




HLA-DR is the most commonly expressed class II HLA molecule on human monocytes. It plays a key role in presenting microbial antigens to T cells and therefore is important for host defense. In response to bacterial molecules like LPS, monocyte-macrophages release TNF-α, IL-12, IL-15 and other factors (41,163) that induce NK cells and T_H1 CD4 cells to release interferon-γ. Interferon-γ then increases HLA-DR expression on monocytes (164), activates monocyte-macrophages to kill bacteria, and enhances production of IL-12, TNF and other cytokines. A positive feed-back loop is formed. (Although this loop is felt to enhance host resistance to infection, it can also contribute to pathology (165): IL-12, TNF-α and IFN-γ play major roles in priming mice for the generalized Shwartzman reaction, a catastrophic cytokine explosion that can be elicited by two precisely-timed doses of endotoxin.) The loop may be modulated by interleukin-10, which can inhibit LPS-induced IL-12 and TNF production and decrease HLA-DR expression (166).

Interleukin-12

- produced by monocytes, macrophages, neutrophils, and dendritic cells
- activates NK cells and Th1 T helper cells
- induces production of interferon-γ
- "jump starts" cell-mediated immunity
- has adjuvant activity; reduces growth of intracellular parasites



T helper cell phenotypes. IL-4 induces naive T cells (T_H0) to differentiate into T_H2 cells (which make IL-4, IL5, IL-10, and IL-13 and participate in humoral immunity), while IL-12 favors differentiation into T_H1 cells (which secrete IL-2, interferon-γ and TNF-α and underpin cellular immunity). IL-4 also stimulates IL-1Ra production while suppressing synthesis of IL-1 (167). When peripheral blood monocytes from traumatized patients have been studied *in vitro*, they have produced more PGE_2 and less interferon-γ, HLA-DR, and IL-1ß than those from normal individuals (155,168,169). Similarly, T cells isolated from trauma patients have produced less IL-2 and more IL-6 than controls when cultured *in vitro* (170). Peripheral blood mononuclear cells (PBMN) isolated from burn and trauma patients released less interferon-γ and more IL-4 than did PBMN from normal controls (169), and Mannick, Rodrick and others (169) suggested that trauma is associated with a shift in circulating CD4 cells from T_H1 to the "immunosuppressive" T_H2-dominant phenotype. This notion fits with many of the

abnormalities observed so far in trauma/burn/post-operative patients: skin test anergy (CMI), reduced interferon-γ production, high IL-4 production, low T cell IL-2 production, low monocyte HLA-DR expression and cytokine production, and high IL-1Ra production. Exactly how this imbalance increases the risk of bacterial infection and severe sepsis is not clear. In keeping with the idea that Th2>Th1 imbalance predisposes to serious infection, however, pretreatment of mice with IL-2 improved survival from *E. coli* peritonitis (171), and IL-12 [in the right dose] greatly improved survival in mouse burn-CLP model. On the other hand, van Deuren et al. (122), found low blood levels of TNF and high concentrations of IL-1Ra and IL-6 in patients with mild meningococcal disease and thought that the dominance of anti-inflammatory proteins could protect them from more severe disease. One would like to compare these results with those from patients who develop the severe, fulminant form of meningococcal disease, but information from the early stages of this rapidly-developing process is not likely to become available.

Post-trauma susceptibility to infection: a helper T cell imbalance?

- reduced interferon -γ, IL-2 production
- high IL-4, IL-6, IL-1Ra, and IL-10 production

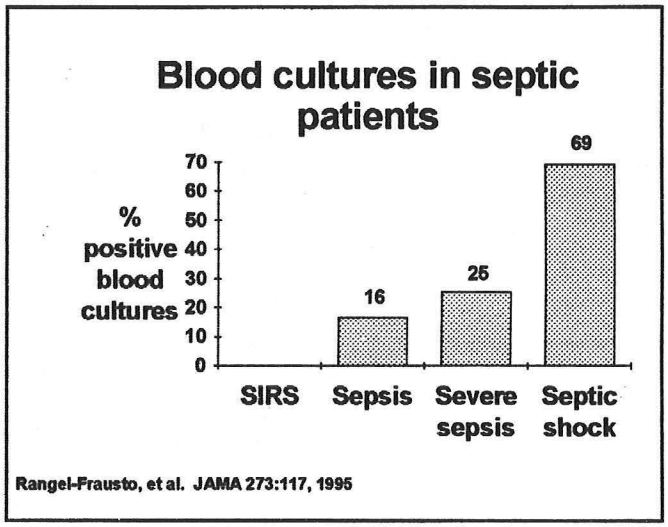
So $T_H2 >> T_H1$

→→Low HLA-DR expression, high C-RP and IL-1Ra production

? increased susceptibility to infection

(In vivo resistance to endotoxic shock can also be induced by pretreating animals with TNF, IL-1, IFNα, G-CSF, LIF, and IL-10 (reviewed in (172)). In most of these examples, resistance has been associated with reduced LPS-induced production of TNF and IL-6 by macrophages. The animal models largely involved bolus injections of LPS or bacteria, so they haven't really reproduced the clinical scenario discussed above.)

These data come from different sources--clinical observation, blood cytokine measurements, and *in vitro* analysis of blood leukocyte function. Remarkably, they all seem to fit with the idea that patients are at increased risk for infection, severe sepsis and/or septic shock because of a *weak, or delayed, pro-inflammatory host response, with a consequently weakened ability to prevent microbial growth.* This state might arise from either impaired production of, say, TNF, IL-12, or IL-15, or excessive production of IL-10 (or IL-4?). Also consistent with this notion is the frequency with which blood cultures are positive in patients with sepsis, severe sepsis, and septic shock: the intensity of the septic response can be related to the magnitude of the microbial burden (Figure). In the Iowa study cited earlier, patients who had 3 or more infections were > 15-fold more likely to develop septic shock than those who had no documented infection.



Risk factors for infection:
Immunoglobulin deficiency/dysfunction
Complement deficiency
Neutropenia, neutrophil dysfunction
Defective cell-mediated immunity

T_H cell imbalance?

Taken together, these data provide a theoretical basis for using immunostimulation to prevent severe sepsis in high-risk post-trauma or post-operative patients. They also suggest parameters that might be used to identify such patients, and they encourage studies to define risk factors and underlying immunopathology in patients with other primary illnesses.

*B. There is a rationale for **intervening early** in high-risk patients who develop SIRS3 or SIRS4.* This strategy would be conceptually similar to the currently accepted approach for managing leukopenic patients who develop fever. Prompt administration of antimicrobials has had a beneficial impact in this setting. As noted above, many different interventions can block the septic response in animal models provided that they are given before, with, or shortly after the bacterial or endotoxic challenge. In patients at high risk for severe sepsis, perhaps early intervention with antimicrobials and an immunomodulatory drug would prevent the physiologic progression from SIRS3/4 to severe sepsis. However, there is a well-established phenomenon that may be important:

The state of leukocyte "tolerance" during severe sepsis. During severe sepsis, both circulating neutrophils (173) and monocytes (174,175) lose their responsiveness to LPS *in vitro*. Although this has been studied in a number of ways, it appears that monocytes lose their normal ability to produce various cytokines (174,175) and to increase HLA-DR expression when stimulated with LPS. LPS-induced IL-1Ra secretion is preserved (176) and mononuclear cells show increased sensitivity to glucocorticoids (177), again suggesting an anti-inflammatory balance. The monocytes of patients who survive regain their ability to respond to LPS, whereas monocytes of non-survivors do not (174). Monocyte hyporesponsiveness to LPS and other agonists also occurs after i.v. endotoxin administration to normal volunteers (178). Unlike monocytes, neutrophils seem to lose LPS responsiveness only during sepsis and not following severe trauma, and the tolerance is specific for endotoxin (they respond normally to *S. aureus*)(173); a labile transcriptional repressor (? IκB) has been suggested (179).

When Randow et al. (176) studied blood monocytes from patients with severe sepsis, 19 of the 24 specimens with low HLA-DR expression (<45% of monocytes) showed significant IL-10 secretion within 4 hrs after LPS stimulation. IL-10 mRNA was detected in freshly isolated monocyes from 18 of these patients. In contrast, IL-10 mRNA was found 4 hr post-stimulation in the monocytes of only 3 of 12 patients with high (>45%) monocyte HLA-DR expression; unlike the monocytes from patients with low HLA-DR expression, monocytes from these patients secreted normal amounts of IL-10 and TNF. These results suggest that, in the HLA-DR-deficient cells, early IL-10 secretion may diminish TNF production (see box) and produce a relative state of LPS hyporesponsiveness. Production of IL-1Ra, which antagonizes IL-1, remains normal. Again, the balance is *anti*-inflammatory!

Interleukin-10

- produced by Th2 (type 2 T helper cells), B cells, macrophages, and keratinocytes
- inhibits cytokine synthesis by Th1 cells (IFN-γ, IL-2, TGF-ß)
- inhibits pro-inflammatory cytokine synthesis (TNF-α, IL-1, IL-12) and class II (HLA-DR) by monocyte-macrophages; stimulates IL-1Ra production
- stimulates B cell differentiation and immunoglobulin synthesis
- when infused in to humans, induces monocyte hyporesponsiveness to LPS stimulation *ex vivo*

IL-10 knockout mice develop chronic enterocolitis and have exaggerated Th1 responses (IFN-γ production).

So there is an interesting paradox: although circulating pro-inflammatory cytokine levels may be extremely high, at least some of the cells that can make these proteins and other mediators seem to be unable to do so when studied *in vitro*. If these *in vitro* findings reflect *in vivo* phenomena, how are such high concentrations of proinflammatory mediators produced in septic patients? Possible sources include (a) non-tolerant cells in inflamed tissues, or in the GI tract, which produces a substantial fraction of circulating TNF (180)(this would be consistent with compartmentalization of the septic response--see above), (b) blood leukocytes that, although unable to react to LPS, respond to other agonists (e.g., PAF) by releasing pro-inflammatory cytokines, and (c) abnormalities that slow mediator clearance from the blood (such as saturation, internalization, or release of cytokine receptors) might also contribute. It should also be noted that although leukocytes are consistently *hypo*responsive *in vitro*, they do make and release cytokines. This level of cytokine production may be sufficient to perpetuate high cytokine levels *in vivo*.

Also unknown are the factors that so profoundly down-regulate leukocyte responsiveness during sepsis--IL-10, TGF-β, IL-4, and α-MSH are currently the best candidates. Interestingly, α-MSH can both inhibit the actions of proinflammatory cytokines on target cells and induce monocytes to produce IL-10 (181).

What is the significance of leukocyte tolerance during sepsis? It might be viewed as a protective mechanism by which

the infected host damps the inflammatory response. Alternatively, it may actually be a form of immunosuppression that increases susceptibility to subsequent infection. In any case, leukocyte tolerance raises the possibility that antiinflammatory interventions (perhaps even as early as SIRS3 or SIRS4) may be ineffective.

- *Summary:* at least in individuals who have sustained major trauma or surgery, a state of immunosuppression seems to increase the risk of infection and a septic response. This information provides a rationale for efforts to boost immunity with non-specific adjuvants. It may also provide a useful model for exploring susceptibility risk in non-traumatized patients.

2) Patients at high risk for severe sepsis can be identified.

1. Patient demographics, clinical signs. (This discussion concerns patients who do not have obvious (traditional) risk factors for bacterial and/or fungal infection - such as neutropenia, hypogamma-globulinemia, AIDS.)

In the prospective epidemiologic study of ICU patients from the University of Iowa (25), 1 in 4 patients admitted to their ICUs developed severe sepsis (culture negative or positive) and 1 in 20 had septic shock (it is not clear from their data how many patients had these syndromes at the time of admission, however). A retrospective study performed in a large referral hospital in The Netherlands (182) found that 28 (36%) of 73 patients who developed sepsis syndrome without shock and 23/41 (56%) of those who developed septic shock were already located in an ICU.

Post-operative infection and sepsis -- ways to identify high-risk patients:

Demographics:	ICU admission
	kind of surgery or trauma
Bedside evaluation:	skin test anergy
	a prognostic scoring system?
Blood tests:	monocyte HLA-DR
	IL-6, C-RP time course
	endocrine parameters?
	cytokine profiles?

Taken together, the available data suggest that ICU admission implies a substantial risk for severe sepsis/septic shock. Much more research is needed to identify those at highest risk, so that prophylaxis and/or early intervention can be maximally cost-efficient and safe.

Other studies suggest that some such risk factors can be defined. For a clinical trial of prophylactic antiendotoxin antiserum in surgical patients, patient selection criteria were able to identify a population that subsequently had a high (8 to 20%) septic mortality (3,183). Most of the enrolled patients had abdominal or pulmonary surgery or multiple trauma. One group in Italy developed a multiparametric test based on delayed hypersensitivity testing and serum protein electrophoretic patterns, claiming a positive predictive value for post-operative sepsis of 76% (184). In another study, severe sepsis developed in 9% of 11,828 patients admitted to ICUs in France(147); risk factors included age >60 years, immunosuppression, medical admission, unscheduled surgery, and chronic liver insufficiency.

It is also likely that prognostic scoring systems could identify patients at high risk for developing severe sepsis. The published systems have all dealt with estimates of mortality risk for patients either admitted to the ICU with severe sepsis (135,147,185-192) or developing *S. aureus* bacteremia in the hospital (190). None has attempted to develop sepsis risk predictions for patients admitted to ICUs with SIRS or no overt signs of inflammation.

Regarding the progression from SIRS to later stages in the continuum, in the Iowa study *58% of patients who developed severe sepsis had had sepsis or SIRS on at least one previous day, and 71% of those who progressed to septic shock had been previously classified as SIRS, sepsis, or severe sepsis.* Subsequent analyses of the data suggest that only 18 - 24% of the patients with SIRS will progress to the next stage. On the other hand, many (> 50%) patients who developed severe sepsis or septic shock had experienced SIRS3 or SIRS4 on at least one previous day in the ICU.

2. *Bedside immunologic evaluation - skin test anergy.* See discussion of skin test anergy above. Despite its value

as a research tool, the usefulness of skin test anergy for predicting post-operative complications is debated. Some workers found skin testing an unreliable method for predicting post-operative morbidity and mortality (193).

3. Laboratory evaluation:

Endocrine values. In addition to the measurements of immune status discussed above, endocrine parameters may also be useful. In a recent study (194)from Edinburgh, Scotland, the following formula was found to predict death in a general (no cardiac or trauma patients) ICU with a power of 0.94:

$$P = 1/(1 + \exp[0.174 \text{ thyrotropin} + 0.568 \text{ thyroxine} - 0.042 \text{ cortisol} - 0.51]$$

where P is the probability of death and standard American units are used for the endocrine values. So nonsurvivors had lower thyrotropin and thyroxine and higher cortisol levels than survivors. Unfortunately, the endocrinologist authors of the study did not indicate the causes of death.

Genotyping. Are there genes that determine whether an individual, once infected with a microbe, will develop severe sepsis/septic shock? If so, genotyping could contribute greatly to estimating risk. Most work so far has focused on the TNF-α gene. Although several studies have failed to identify a promising polymorphism (reviewed in reference (195)), a German group recently reported that septic patients homozygous at the TNFB2 allele had higher plasma TNF-α levels and greater mortality than TNFB1 homozygotes (196). Pociot et al. had found previously that, when compared with TNFB1 homozygotes or heterozygotes, monocytes from TNFB2 homozygotes produced more TNF in response to LPS in vitro, but Derkx and others (197), using similar methods, reported essentially opposite results. A polymorphism in the IL-1Ra gene may be involved in IL-1 and IL-1Ra production (198). This area is likely to receive much attention over the next few years.

3. Safe interventions can be found. Two issues stand out. First, interventions must maintain the normal antimicrobial host defense. Second, side effects must be inconsequential.

The two-edged nature of the inflammatory response is well known. Neutralizing certain cytokines and other mediators increases susceptibility to infections, particularly with intracellular parasites. The obvious concern with the anticytokine and antimediator drugs is that their targets play roles in the normal host response to infection. Low doses of IL-1 and TNFα can actually protect animals from infectious challenge (199-202). Animals that lack TNFR1 (p55 receptor) are hypersusceptible to infection by *Listeria monocytogenes* (203,204); neutralizing TNFα *in vivo* can also increase susceptibility to bacterial infection (31,205,206). TNF is also needed for effective defense from viral infection (207,208). A recent phase II clinical trial found that a soluble p75 TNF-receptor-immunoglobulin fusion protein caused dose-related toxicity in human patients with severe sepsis/septic shock. (In human volunteers given intravenous endotoxin, Suffredini et al. found that a high dose of this protein was less immunosuppressive than a low dose, and that neither dose blocked endotoxin-induced symptoms (14).) Interestingly, the lethal toxicity of TNF in mice depends strongly on the time of day that the cytokine is given: greatest in the early morning and least in the late afternoon and evening (209). How this might impact the administration of anti-TNF drugs is uncertain.

It may be very difficult to determine the "right" dose and the optimal time to administer cytokine-neutralizing drugs to septic patients. And it's not just anticytokine agents: preventing neutrophil adhesion with a mAb to CD18 increased levels of circulating endotoxin and worsened cardiovascular injury in a canine model of gram-negative bacterial infection (210).

Somewhat different considerations apply to antiendotoxin drugs. A major issue with antiendotoxin antibodies has been their specificity for lipid A (LPS). In fact, one monoclonal antibody (Centoxin) was tested in two large clinical trials before its binding properties were fully known. The second trial was stopped when an interim analysis showed a higher fatality rate in recipients of the antibody (9). Bhat and others then reported that the antibody (an IgM with VH4.21 gene usage) binds the erythrocyte i antigen, B-lymphocyte antigens, transferrin, and other molecules (211). In other words, it is a polyreactive autoantibody. In fact, the original trial of the antibody had also shown a trend toward excess mortality in Centoxin recipients who did not have gram-negative bacterial infection (212).

Another antiendotoxin drug is bactericidal permeability-increasing protein (BPI). BPI is a neutrophil granule protein that has high affinity for binding many LPSs (213). It also has bactericidal activity in human blood (69), and it is currently being evaluated in phase I/II clinical trials. Recently, a group in the U.K. found that BPI is a target antigen for many

vasculitis patients who have anti-neutrophil cytoplasmic autoantibodies (ANCA)(214). At the moment, the implications of this finding are uncertain.

An important key to developing safe interventions is careful studies of different doses of each candidate drug in phase II trials.

Preventing severe sepsis and septic shock: 3 strategies

1. Prevent infections in critically ill patients

a. Provide adequate nutrition. Numerous studies have now shown the importance of adequate nutrition for preventing infection in critically ill patients. Two features have received most attention. First, there is now a general consensus that *enteral feeding (TEN) is superior to parenteral nutrition (TPN)* for preventing post-operative septic complications (215-217). In addition to its greater safety, convenience, and lower cost, enteral feeding is thought to maintain the integrity of the gastrointestinal mucosa, thereby preventing translocation of bacteria and their products into the circulation. It also seems to maintain immunocompetence and to reduce cytokine and neutrophil responses to endotoxemia (218,219). A meta-analysis (216) found that significantly fewer high-risk surgical patients experienced septic complications when they received TEN (TEN, 18%, TPN, 35%, $p = 0.01$). In malnourished individuals, anergy may be corrected by TPN (220)--presumably enteral nutrition would also be effective.

The second (and more controversial) issue is the composition of the enteral diet. There are data that suggest the *superiority of diets that contain ω-3 rather than ω-6 fatty acids, supplemental arginine, and nucleotides* (217,221-223). The most recent study (222) compared a commonly used formula (Osmolite, Ross Laboratories) with an experimental formula (Impact, Sandoz). The experimental formula contained supplemental L-arginine, nucleotides (from yeast RNA), selenium, vitamins A and E, and ω-3 fatty acids (from menhaden [fish] oil), while the common use formula had more medium-chain triglycerides and vitamin C. The 296 patients had all experienced an event (trauma, surgery, or new onset of infection) that required admission to a surgical ICU. Enteral feeding was begun within 96 hrs of ICU admission and continued for 7 days. Patients who received the experimental formula had, on average, shorter length of hospital stay; patients who were septic at the time of enrollment had significantly shorter hospital stay and reduced frequency of acquired infections if they received the experimental formula. There was no difference between the groups in overall mortality, but mortality in both groups was significantly lower than predicted by APACHE II scores at the time of ICU admission. The authors attributed the low mortality to the fact that both groups received enteral nutrition--a practice not factored into the original APACHE prognostic scoring scale.

The Medical Grand Rounds handout prepared by Dr. Claibe Yarbrough (September 10, 1992) has an exceptionally comprehensive and clear discussion of these issues, including the effects of ω-3 and ω-6 fatty acids on immune function. Suffice it to say that infusion of ω-6 fatty acids (from linoleic acid), which may be converted to prostaglandin E_2, thromboxane A_2, and leukotriene B_4, may have a significant immunosuppressive effect. ω-3 fatty acids (from linolenic acid) generate different prostanoids that are much less potent (so less immunosuppressive) than those derived from linoleic acid.

A recent study found that patients who developed multiple organ failure following admission to a surgical ICU had significantly lower plasma vitamin C concentrations than those who did not develop organ failure (159). There were no differences in the concentrations of other antioxidants. The patients were followed prospectively; lower vitamin C levels were noted throughout the period of observation.

b. Enforce hospital infection control measures. In particular, improve care of intravascular catheters, including a dedicated team for catheter placement (224). Numerous studies have documented that intravascular catheters greatly increase the risk of hospital-acquired bacteremia (225). CDC guidelines now recommend that peripheral venous catheters be changed every 48 - 72 hours (or within 24 hours after emergency insertion), that lower extremity insertions be avoided, that central lines be placed using full sterile prep (gown, mask, gloves, large sterile drape) even when done in the O.R., and that trained personnel insert vascular catheters whenever possible. Details are provided in the Federal Register (224); a copy may be obtained from the I.D. Division.

2. Prophylaxis to prevent infection, prevent SIRS, or reduce its severity

Here the primary goal is to prevent clinical infection or reduce its severity. Based on the considerations detailed

above, in many instances the best interventions may be immunostimulatory, not anti-inflammatory. There are several candidate drugs.

A. Immunostimulation

a. Lipid A analogs. Monophosphoryl lipid A (Ribi Immunochem) is a purified natural product obtained by alkaline and acid hydrolysis of *Salmonella minnesota* Re 595 LPS (226). It is 10,000-fold less toxic than LPS when infused into humans (227), and it can induce tolerance to endotoxin in both animals and humans (227). In animals, it is an effective adjuvant and it increases non-specific resistance to infection, possibly because it is a potent stimulus for interferon-γ production by NK cells (228). It also can block the hemodynamic effects of endotoxin *in vivo* in rats (229). It therefore has many attractive attributes as a prophylactic drug in patients at high risk for infection and sepsis. Other lipid A analogs are also being evaluated as immunopotentiating and tolerance-inducing agents, including SDZ MRL 953 (230,231), E5531 (232) and DT-5461 (233).

b. Interferon-γ. There have been two clinical trials in surgical/trauma patients. In each trial, patients who had sustained severe trauma were given 100 μg interferon-γ daily. In the first trial (193 patients, 10 day interferon-γ course), no statistically significant differences were found between treatment and placebo groups (234), although there were non-significant trends toward efficacy. In the second trial (416 patients, 21 day interferon-γ administration), interferon-γ recipients experienced significantly fewer deaths related to infection and fewer overall deaths than did patients who received placebo, but the results were dominated by the findings at a single study center (235).

c. GM-CSF. In a mouse model, GM-CSF improved survival when it was given after burn injury and before sepsis was induced by cecal ligation and puncture (236). The effect was attributed in part to restoration of T cell proliferation and IL-2 production. Similar results were obtained in another mouse model (237). In contrast, G-CSF exacerbated lung injury in cyclophosphamide-treated guinea pigs that were challenged with an intratracheal injection of endotoxin (238).

d. PGG-glucan (Betafectin). A multicenter phase II trial recently studied the ability of PGG-glucan to prevent infections in high-risk surgical patients (239). There was a lot of data dredging but it seems that recipients of PGG-glucan may have had fewer serious infections than the placebo recipients. PGG-glucan is a glucose polymer derived from a strain of *Saccharomyces cerevisiae*. It is thought to "stimulate the phagocytes." How it does this is uncertain.

e. NSAIDS. Although more appropriately classified as anti-inflammatory drugs, NSAIDS may be immunostimulatory in the sense that they block production of PGE_2, a prostanoid with potent inhibitory effects on monocyte/macrophage HLA-DR expression and cytokine production. Faist and his colleagues have suggested that combining a cyclooxygenase inhibitor with gamma interferon could repair the post-surgical immunosuppression discussed above. No clinical trial testing this idea has been published.

f. Others: muramyltripeptide (MTP-PE)(240), linomide (quinoline-3-carboxamide)(241), leukemia inhibitory factor (LIF)(172).

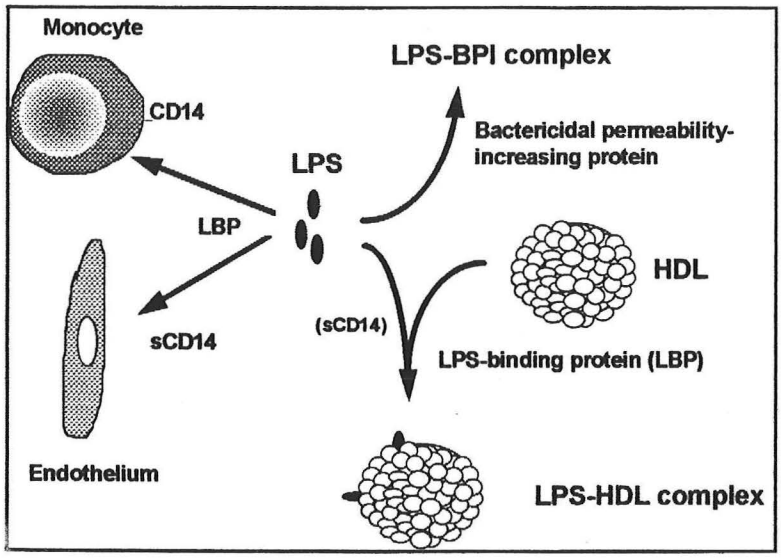
B. Passive neutralization

a. IV immunoglobulin. In a trial conducted in Switzerland and other European countries, intravenous immune globulin reduced the incidence of infection in high-risk post-operative patients (183). In the same trial, globulin enriched with antibodies to the J5 *E. coli* endotoxin determinant had no effect. A smaller multicenter, randomized Italian trial identified patients at risk for post-operative infection and compared prophylaxis with antibiotic alone vs. antibiotic plus i.v. immunoglobulin. There was a significant reduction in post-operative infections in the immunoglobulin + antibiotic group (184).

Studies in animals suggest that multiple mAbs can be used together to prevent *Pseudomonas* infections (242) and that combination prophylaxis with a type-specific anti-O-polysaccharide mAb, an anti-TNF mAb, and polyclonal anti-J5 antiserum may be superior to passive immunization with any of the individual antibodies (243). Affinity-purified anti-J5 antibodies were also effective prophylaxis (244).

The isotype (Fc region) of therapeutic or prophylactic antibodies may be important. A murine-human chimera containing the gamma-4 Fc region was more effective at neutralizing TNF *in vivo* than one containing the gamma-1 Fc region, probably because the latter induced an immune complex-mediated response (245).

b. HDL infusion (246-248). HDL binds endotoxin (LPS) and certain other lipophilic molecules in the circulation. A plasma protein, LPS-binding protein (LBP), transfers LPS to HDL, where the LPS is effectively neutralized (the bioactive lipid A moiety is inserted into the HDL micelle). Soluble CD14 may also shuttle LPS and other lipids to



lipoproteins. Other lipoproteins may also bind LPS. Several recent studies have found that lipoproteins can neutralize LPS in animal models (246,248-251). The NIH group found that reconstituted HDL caused seizures in their dog sepsis model, however (247).

c. Bactericidal permeability-increasing protein (BPI). BPI effectively neutralizes LPS and kills many gram-negative bacteria (252,253). It might be a very effective prophylactic for gram-negative bacterial infections. The major drawback is its short plasma half-life--only 1 to 2 hours. It must be given by constant infusion. If this problem can be solved and safety issues resolved (see above), BPI could be a very useful drug in this setting. It is currently in two phase II trials at UTSW--one in adults who have had hemorrhagic shock, the other in children with meningococcemia.

C. New drug delivery approaches

Cytokine-inducible antidote protein production (gene delivery)(254). This approach is intended to provide recombinant antidote proteins (either pro- or anti-inflammatory proteins, depending upon the circumstances) according to the intensity of a patient's inflammatory response. Promoters for acute phase proteins (C-RP, SAA, etc.) are used to regulate transcription of antidote protein genes, so that expression of the antidote protein will be controlled by the host's acute phase response. The constructs are then inserted into gene delivery vectors which, when administered intravenously, will go to the liver (the site of most acute phase protein synthesis). No antidote protein will be produced unless the individual has an acute phase response. The goal is to boost innate immunity (by producing immunostimulatory proteins such as gamma interferon), or to put a ceiling on the inflammatory response without interfering with its beneficial impact on host defense (as might be possible by producing IL-10). No "proof of principle" data are available at this time.

3. Early intervention (SIRS3 or SIRS4) to prevent the progression to severe sepsis

Very few intervention studies have been done in patients with SIRS3 or SIRS4. Presumably, at this stage the pro-inflammatory arm of the host defense has the upper hand. So anti-inflammatory drugs should be more useful than immunostimulatory ones (but see discussion above regarding leukocyte tolerance). There is a rough precedent in the management of neutropenic patients who develop fever: rapid, empiric antimicrobial therapy has improved survival substantially in these patients. In addition to empiric antimicrobial agents, which would be the cornerstone of any early intervention strategy, there are several drugs that, if given at an early stage, might blunt the SIRS progression to septic shock:

a. Ketoconazole is an imidazole antifungal drug that has a number of unrelated activities such as inhibiting thromboxane synthase, lipoxygenase (255), and nitric oxide synthase (256). In two small studies, administration of ketoconazole (400 mg qd p.o.) to surgical patients with severe sepsis significantly reduced the incidence of ARDS and lowered mortality (18). Prophlactic use of ketoconazole was not associated with toxicity and may have reduced yeast colonization in one study in surgical and trauma patients (257). Larger studies are in progress.

b. Glucocorticoids, given in large doses, did not improve survival from severe sepsis in two large clinical trials. In the VA Cooperative Trial, however, glucocorticoid administration was associated with statistically significant reductions in the frequency of ARDS and coma in one prospectively-designated subgroup (patients with gram-negative bacteremia)(6). In this subgroup, mortality was also 75% lower in the patients who received steroid therapy, but the number of subjects was small. Studies in patients with typhoid fever (258) and in children with *H. influenzae* meningitis also suggest that dexamethasone administration can reduce morbidity, and a large body of experimental and clinical (84) data now suggests that the best time to give the drug is before antimicrobial drugs are administered. Antimicrobials can release endotoxin and other toxic components from bacterial cells (83).

A common feature of the successful glucocorticoid trials has been early drug administration--prior to the development of severe sepsis. The VA steroid trial was carried out in patients who had organ injury, but only in patients who could give

informed consent themselves--patients who had altered mental status and were excluded from the study had a 2-fold higher case fatality rate than those included in the study. It seems likely that the VA investigators inadvertently selected patients with relatively mild (i.e., early) "severe" sepsis. A second feature of the successful glucocorticoid trials has been a relatively low steroid dose. The highest dose, used in the Bone trial (5), was associated with increased risk of infection. Lower doses have generally been well tolerated.

Careful studies in human volunteers indicate that the effects of hypercortisolemia can be very complex (259). When given along with intravenous endotoxin, hydrocortisone blunted the TNF and IL-6 response. When hydrocortisone was given 12 to 144 hours before the endotoxin, however, the TNF and IL-6 responses were greatly increased. Clearly, there is much to be learned about these interactions.

c. Pentoxifylline. This methylxanthine derivative, a phosphodiesterase inhibitor, has been effective in several animal models of acute inflammation and/or sepsis. It blocks TNF and other cytokine production by monocytes. Recently prophylactic pentoxifylline was used to prevent death in a mouse model of burn wound sepsis (260). It restored IL-2 production and reduced the production of inflammatory cytokines in a dose- and time-dependent fashion. Pentoxifylline also inhibited neutrophil activation and clotting abnormalities in chimpanzees challenged with intravenous endotoxin (261,262); TNF and IL-6 increases were blunted while the IL-8 response to endotoxin was normal. When given to humans with septic shock, pentoxifylline decreased plasma TNF levels but did not improve hemodynamics (263).

In humans given i.v. endotoxin, pentoxifylline (given as i.v. infusion) blocked TNF but not IL-6 production, and it had no effect on symptoms (fever, myalgias, chills)(264). In humans with sepsis, administration of pentoxifylline increased plasma concentrations of adhesion molecules (265). The drug was ineffective when used (400 mg p.o. q6h) to reduce transplant-related toxicity after bone marrow transplantation (266). Its prospects as a sepsis drug are uncertain.

d. α-MSH. This 13-amino acid peptide, derived from proopiomelanocortin, is a potent anti-inflammatory hormone (267). In part, it appears to act by inducing monocyte IL-10 production (181). It also may act in an autocrine manner to decrease the effects of pro-inflammatory hormones on macrophages (268). In humans, it is known to be an effective, non-toxic anti-pyretic; its ability to modulate human inflammation is currently under investigation. Interestingly, the C-terminal tripeptide (Lys Pro Val) is the bioactive moiety.

e. NSAIDS. When given prior to an intravenous infusion of endotoxin, ibuprofen prevents most endotoxin-induced symptoms and changes in stress hormones (269) without substantially blocking endotoxin-induced changes in blood pressure and SVR (270). Post-endotoxin blood levels of TNF-α and IL-8 are higher in ibuprofen-pretreated subjects than in individuals who receive no pretreatment, suggesting that cyclooxygenase products (presumably, PGE_2) are important both for mediating certain actions of inflammatory cytokines (such as fever) and for decreasing cytokine production (feedback inhibition)(271,272). The IL-6 response to endotoxin is unaffected by ibuprofen pre-treatment, as is the subsequent increase in C-RP (269). Results of a large multicenter trial of ibuprofen in patients with ARDS should become available soon.

Current Management of Severe Sepsis and Septic Shock: Controlled Clinical Trials

None of the components of standard management has been tested in a controlled trial. This includes such maneuvers as fluid resuscitation and pressor agents. Antimicrobial therapy has been studied indirectly in recent trials of new therapeutic agents, where patients who received the placebo were categorized according to whether or not they received "appropriate" antimicrobial therapy. In the first trial of HA-1A, for example, there was a striking benefit from receiving appropriate antimicrobial therapy (72): patients in the placebo group who received inappropriate antimicrobial therapy had a 69% mortality, while only 27% of those who received appropriate antimicrobial therapy died. Several retrospective studies also suggest that antimicrobial therapy is beneficial (273,274).

Regarding cardiovascular support, the best data have come from studies in a canine model of septic shock (275). In this model, neither antibiotic treatment nor cardiovascular support (fluids, dopamine) was effective when used alone, whereas combined antibiotic and cardiovascular support provided moderately successful treatment.

There is evidence that experienced physicians get better results: in two hospitals which underwent staffing changes, the introduction of specialists was associated with statistically significant improvement in overall survival (276) and in survival from septic shock (277).

Conclusions

1. There is a *pathophysiologic continuum* from mild (sepsis) to severe (septic shock); case-fatality rates increase as the continuum worsens.

2. Most cases of severe sepsis/septic shock occur in patients who are *already hospitalized.* It may be possible to identify patients who have a high risk of developing these syndromes. One underappreciated risk: A state of immunosuppression (T_H imbalance) may predispose many patients to infection and sepsis.
4. *Severe sepsis/septic shock might be prevented in high risk patients* by
   Preventing infectious complications of hospitalization
      Improving nutrition, catheter care, and other hospital infection control measures
      Immunoprophylaxis to reduce the risk of infection
   Intervening early to prevent progression along the sepsis continuum. This is plausible because many patients who develop severe sepsis/septic shock in the hospital will first manifest milder signs/symptoms (SIRS, sepsis).
5. *Research is needed* to identify efficacious, inexpensive, and safe drugs for immunoprophylaxis and/or early intervention; to clarify risk factors so that these interventions can be targeted appropriately; to evaluate the role of immunosuppression as a risk factor; and to define outcome variables and evaluate cost-effectiveness. Since many of the potential drugs are either off-patent or inexpensive, public support will probably be required for these studies.

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