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Anti-endotoxin vaccines
Back to the future

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Abbreviations: LPS, lipopolysaccharide; GNB, gram-negative bacteria; IVIG, intravenous immunoglobulin; MDR, multidrug-resistant; OMP, outer membrane protein; CGL, core glycolipid; dLPS, detoxified LPS; CLP, cecal ligation/puncture; WRAIR, Walter Reed Army Institute of Research; BPI, bactericidal/permeability-increasing protein

Gram-negative bacterial (GNB) infections are a leading cause of serious infections both in hospitals and the community. The mortality remains high despite potent antimicrobials and modern supportive care. In the last decade invasive GNB have become increasingly resistant to commonly used antibiotics, and attempts to intervene with novel biological therapies have been unsuccessful. Earlier studies with antibodies directed against a highly conserved core region in the GNB lipopolysaccharide (LPS, or endotoxin) suggested that this approach may have therapeutic benefit, and led to the development of a subunit vaccine that has progressed to phase 1 clinical testing. Since only a few serogroups of GNB cause bacteremia, O-specific vaccines had been developed, but these were not deployed because of the availability of other therapeutic options at the time. Given the likelihood that new antibiotics will not be soon available, the development of vaccines and antibodies directed against endotoxin, both O and core antigens, deserves a "second look".

Introduction

Although gram-negative bacteria (GNB) had been isolated from the blood of patients for decades, it was not until 1951 that the clinical syndrome of gram-negative bacterial sepsis was first described.[1] Nevertheless, infections caused by GNB were not a significant clinical problem until landmark reports by Rogers and by Finland heralded the rise of GNB infections in hospitalized patients.[2,3] At that time several drugs were available to treat these infections and shortly thereafter, new aminoglycoside and extended spectrum β lactam antibiotics came into widespread clinical use.

Since then, multidrug-resistant (MDR) GNB have become an increasingly important cause of invasive infection in the United States. In data submitted to the CDC databases (National Healthcare Safety Network [NHSN], National Nosocomial Infections Surveillance [NNIS] systems), over the last decade the prevalence of carbapenem-resistant *Enterobacteriaceae* increased from 1.2% in 2001 to 4.2% of isolates in 2011, with *Klebsiella pneumoniae* (KP) becoming the most resistant (1.6% to 10.4%).[4] Resistance is emerging even in outpatient settings.[5] With the ease of intercontinental travel, highly resistant GNB harboring mobile genetic elements such as NDM-1 that were first isolated in developing countries are being "imported" to developed countries.[6,7] These multidrug-resistant GNBs, labeled "nightmare bugs" by the director of the CDC,[8] necessitate the use of toxic, less effective, "last resort" antibiotics such as polymixin/colistin, often in combination with other antibiotics. This has resulted in prolonged hospital length of stays, increased costs and increased morbidity and mortality. Ineffective treatment of these infections may lead to dissemination and sepsis, where the mortality has stubbornly remained above 20% over the last 3 decades. These antibiotic-resistant bacteria have raised concerns that there will be no effective means of treating these infections. During the past 10 years there has been a steady decline in the number of antibiotics submitted for approval to the FDA, with only 2 new antibiotics approved in the past 2 years, and those approved have been analogs of previously approved classes of antibiotics.[9] Thus, there is little likelihood that new antibiotics will be available in the near term.

Given the fact that despite potent antibiotics and advances in supportive care, mortality rates from sepsis remain high, there have been ongoing efforts to provide adjunctive care that may improve outcome. Such efforts include therapies directed toward the host by either enhancing host immune responses, or measures designed to attenuate the excessive innate immune responses characteristic of sepsis. Such therapies may "overshoot" the mark and sufficiently impair the host immune response that renders the host susceptible to secondary infections, as is reported for patients on anti-TNFα therapy for rheumatoid arthritis.[10] Another approach is to direct interventions toward the pathogen, typically with vaccine-induced antibodies or more recently, monoclonal antibodies. Historically, these efforts have targeted virulence factors required by the pathogen to evade host defenses and establish infection, primarily bacterial capsular polysaccharides, lipopolysaccharide (LPS, endotoxin), and toxins.[11-13] More recently, in silico studies have identified other immunogenic proteins on the bacterial surface, often without clearly defined

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virulence characteristics, as antigens for inclusion in vaccines.[14] Antibodies may be actively induced with vaccines or delivered passively as immune or hyperimmune gamma globulin for intravenous use (IVIG). The pathogen-directed approach has the advantage of not compromising the host immune system, but may not be feasible if a patient cannot respond to a vaccine or if a hyperimmune preparation is not available for the pathogen.

Anti-Endotoxin Antibody Approaches to Sepsis

With advances in our understanding of the structure of LPS in the 1960s, it was clear that the O-polysaccharide (O "side chain") was immunodominant such that immunization of animals with bacteria of a specific serotype would induce antibodies directed predominantly against that particular O polysaccharide. Administration of anti-O antibodies protected animals against lethal infection with the homologous strain.[16] In a critical experiment, Braude reported that an experimental infection with *E. coli* in the joint of rabbits led to fever and leukocytosis despite the absence of circulating bacteria. Administration of antibodies against the O polysaccharide of the *E. coli* infecting the knee resulted in resolution of both fever and leukocytosis. Braude concluded that LPS from the *E. coli* in the joint entered the circulation and was responsible for the generalized symptoms and that antibody directed against the endotoxin could protect the animal.[16] Although this experiment suggested that anti-endotoxin antibodies may be therapeutically useful, it was believed that the multiplicity of serotypes within *E. coli* and *Pseudomonas aeruginosa* strains, among others, would preclude any translation of this observation into a useful therapy.

Concurrently, other work demonstrated that for a bacterial organism to survive in the bloodstream, it must evade complement lysis and other serum factors ("serum-resistant"), a property not shared by most gram-negative bacteria.[17,18] Subsequent seroepidemiologic studies revealed that among clinical bacteremic isolates, only a relatively few O serotypes could be identified. Thus, for example, ~12 *E. coli*, 4 *Klebsiella*, and 7 *P. aeruginosa* O types accounted for the majority of bacteremic isolates with these bacterial species.[19-22] This recognition led investigators at the Walter Reed Army Institute of Research (WRAIR) in collaboration with investigators at the former Swiss Serum and Vaccine Institute to develop multivalent LPS-based vaccines for *E. coli* and *P. aeruginosa*, as well as a 23-valent capsular polysaccharide-based vaccine for *Klebsiella*. The *E. coli* vaccine was a 12-valent vaccine in which the O polysaccharides were conjugated to *P. aeruginosa* exotoxin A that was well-tolerated and immunogenic in phase 1 human testing.[19,22] The multi-valent *P. aeruginosa* and *Klebsiella* vaccines were also immunogenic and well-tolerated in phase 1 testing, even when given together.[23,24] In a pilot study, these vaccines were administered to 10 patients within 72 h of their arrival at the Shock Trauma Center at the University of Maryland for treatment of traumatic injuries. The antibody response, first measured at 14 d after administration, demonstrated a robust antibody response similar to that seen with historic controls.[24]

The polyvalent *P. aeruginosa* and *Klebsiella* vaccines were used to generate hyperimmune plasma enriched in antibodies against the serotypes contained in the vaccines.[25] The plasma was processed into an IVIG and used in a VA Cooperative Study whose goal was to determine whether infusion of this hyperimmune IVIG would prevent bacteremic infection caused by serotypes of *Klebsiella* and PA included in the vaccine.[26] Each patient received a single infusion of 150 mg/kg of IVIG or albumen upon entry into the ICU and was then followed for the duration of their stay in the ICU. The study was terminated after including nearly 3000 patients because there were too few bacteremic cases to test the hypothesis. It was assumed that some patients admitted to the ICU without infection would, in fact, have had an infection incubating at the time of study entry. This group was prospectively identified in the protocol and analyzed separately. Among the ~100 patients that were incubating infections with the targeted gram-negative bacterial pathogens there was a strong trend by Cox analysis toward improvement in the IVIG-treated group during the first week in the ICU that was no longer apparent at the second week. This is consistent with the hypothesis that when given as treatment, hyperimmune IVIG may have had a beneficial effect, but by the second week the amount of antibody had fallen below an effective therapeutic level.

Anti-Core Endotoxin-Specific Antibodies

Early work on the structure of endotoxin revealed that following immunization or infection, the antibody response was directed primarily against the immunodominant O polysaccharide and provided serotype-specific antibodies.[15,27,28] Mutant GNB that lacked the O polysaccharide exhibited a core structure that was considered to have highly conserved epitopes capable of inducing antibodies that recognized a wide range of *Enterobactericeae*. Studies by Braude et al. with a mutant of *E. coli* O111:B4, (J5, Rc core structure) and by McCabe et al. with an Re mutant of *S. minnesota* demonstrated that *Enterobacteriaceae* that lack O polysaccharide unmasked the core structures to the immune system, and that the antibody response that followed infection with either of these strains recognized a wide spectrum of heterologous organism and were protective in animal models of infection.[29-33] A clinical trial was performed by Ziegler et al. in which either pre- or post-immune sera of healthy volunteers immunized with a killed, whole bacterial cell J5 vaccine was administered to patients in hospitals diagnosed with sepsis. Patients with GNB bacteremia who received the post-immunization sera were more likely to survive their septic episode than patients who received the pre-immune sera, and this significant effect became more pronounced in patients with hypotension and even more so with profound shock[34] (**Table 1**). This was the first successful clinical study of adjuvant therapy in sepsis. Shortly thereafter Baumgartner and colleagues prophylactically administered anti-J5 plasma or controls to patients entering an intensive care unit.[35] While receipt of the anti-J5 plasma did not prevent acquisition of GNB infections, it did improve survival in patients who developed shock and lethal shock. This beneficial

effect was more pronounced in patients entering the ICU after abdominal surgery.

Subsequent studies intending to confirm these positive findings were unsuccessful, however. A Swiss–Dutch J5 Study Group immunized donors with the J5 killed bacterial vaccine, harvested the plasma, and processed it into a purportedly J5-enriched IVIG; however, this reagent was unsuccessful in ameliorating sepsis.[36] Another study screened plasma from outdated blood for high titers of anti-core (Re, not Rc core structure) LPS.[37] This product also failed to improve outcome in septic patients. Finally a French group reported that administration of plasma enriched in J5 antibodies did not protect children with meningococcal sepsis.[38]

One plausible hypothesis to explain the findings in these studies is that the anti-core endotoxin antibody level was subtherapeutic. Further analysis of the "hyperimmune" IVIG in the Swiss–Dutch study revealed that the anti-J5 antibody level increased only 2-fold before the plasma was fractionated into IVIG.[36] The pooled plasma from the blood of donors was unable to prevent sepsis, but when the anti-Re antibody levels were measured at 2 d, the levels were <50% of levels obtained at 2 h post-infusion.[37] When anti-J5 antibody levels were measured at 6 h after infusion to pediatric patients with meningococcal sepsis, there was no increase over baseline.[38] Thus in these three "negative" studies, there were either inadequate levels of anti-core endotoxin antibodies infused initially, or perhaps during sepsis the anti-core endotoxin antibodies were consumed, resulting in inadequate antibody levels.

Multiple studies have established a relationship between the level of anti-core glycolipid (CGL) antibody at the onset of sepsis and outcome.[39-41] Further, a decrease in circulating anti-CGL antibody during a septic episode predicted a poor outcome.[42] Schedel et al. reported that the maintenance of "adequate levels" of immunoglobulin enriched in IgM having anti-core LPS specificity led to a decrease in circulating LPS levels and increased survival.[43]

Lipid A is responsible for the endotoxicity of LPS. Consequently, MAbs against this moiety were developed for use in the therapy of sepsis. The passive administration of MAbs to lipid A (HA1A and E5) did not demonstrate any therapeutic benefit.[44,45] This should not have been surprising since earlier studies with polyclonal anti-lipid A antibodies did not show any benefit,[46] perhaps because lipid A is buried within the bacterial membrane and unavailable for antibody binding.

Detoxified J5 LPS Vaccine for the Prevention and Treatment of Sepsis

With the failure of many passive anti-endotoxin antibody therapy studies in the adjunctive treatment of sepsis, attention turned to the emerging field of anti-cytokine and anti-inflammatory mediator therapy. The role of cytokines such as TNFα and IL-1β in the pathogenesis of sepsis was being elucidated and interventions designed to neutralize the activities of these and other cytokines were tested without success.[47-49] In addition, treatments targeting the coagulation system and bactericidal/permeability-increasing protein (BPI), an endogenous, neutrophil-derived, anti-LPS protein, were undertaken.[50-52]

Table 1. J5 Anti-serum reduces mortality from gram-negative bacteremia

Treatment group			
Patient group	**Non-immune serum**	**J5 anti-serum**	***P* value**
Blood culture positive	38/100 (38)	22/91 (24)	0.041
-With hypotension	34/66 (52)	20/62 (32)	0.028
-In profound shock	26/34 (76)	17/37 (46)	0.009
Blood cultures negative	4/6 (44)	1/12 (8)	0.080

Adapted from reference 34.

Table 2. Anti-J5 serum contains antibody to J5 LPS and lipid A, but affinity purified IgG antibody has minimal anti-lipid A

ELISA titers in O.D. units				
Sample description	**J5 LPS**	**Lipid A**	***P. aeruginosa* LPS**	**Survivors/ total tested**
Anti-J5 serum	4822	2872	29	9/19
Purified IgG	3468	2406	5	13/20
J5 LPS-specific IgG	1558	84	0	6/8
Non-J5 LPS-specific IgG	278	1100	0	4/13

*All 25 animals treated with IgG from pre-immune serum died. Rabbits were immunized with the J5dLPS/OMP vaccine and the harvested anti-J5 serum cycled through a protein G-Sepharose column as previously described (53). The eluted purified IgG ("purified IgG") was then cycled through a J5 LPS-EAH Sepharose 4B affinity column to which J5 LPS was bound. The non-adsorbed fraction was designated "non-J5 LPS-specific IgG" and the eluted adsorbed antibody was designated "J5 LPS-specific IgG." The various fractions were then tested in a neutropenic rat model of sepsis in which the fractions were administered (9 mL/kg) i.v. at the onset of sepsis and survival followed. Control animals were given normal saline. The antibody levels against J5 LPS, lipid A or the LPS of the bacterial challenge strain (*P. aeruginosa* 12.4.4) were measured in each of the fractions.

Given the success of the initial Ziegler study of J5 antisera, my colleagues and I at WRAIR sought to make a vaccine from the LPS of the J5 mutant of *E. coli* O111:B4, which was considered to be the critical antigen in the killed bacterial vaccine, but never formally proven. Moreover, in the original Ziegler study, it was not clear whether it was the J5-induced antibodies in the plasma that provided the protection, and if so, whether they were directed against the J5 LPS. The hemagglutinin titers of the infused J5 antisera did not significantly correlate with patient survival. Consequently, after obtaining the original J5 strain from Elizabeth Ziegler, we immunized rabbits with a heat-killed J5 mutant vaccine, purified the serum antibodies first over a protein A column to isolate the IgG and then passed the protein A eluate (IgG) over a J5 LPS affinity column.[53] These IgG fractions (total IgG antibody, non-J5 LPS IgG [affinity column pass-through] and J5 LPS-specific IgG [affinity column eluate]) were evaluated for levels to J5 LPS, lipid A and to *P. aeruginosa* LPS from a strain to be used in a neutropenic rat model of experimental sepsis[54] (**Table 2**). Rats were administered a clinical isolate of *P. aeruginosa* by gavage and temperature monitored. At the first onset of fever (usually around day 5), rats were given unfractionated

J5 antisera, the non-J5-specific IgG, the J5 LPS-specific IgG, or pre-immune IgG and survival followed. Animals receiving the J5LPS-specific IgG had a 75% (6/8) survival compared with no survival (0/25) among rats who received pre-immune sera. Rats treated with the non-J5 LPS-specific IgG fraction had a 30% survival (4/13). These studies established that (1) J5 LPS was a relevant immunogen in the J5 vaccine; (2) IgG antibody could mediate the protection, but that (3) other antigens could provide some therapeutic benefit. The protection was not attributable to either induction of antibodies to lipid A or to *P. aeruginosa* LPS. Follow-up studies showed that protection was highly dependent on the amount of anti-J5 IgG passively infused, consistent with the hypothesis that earlier failures with J5 antisera could have been attributable to inadequate circulating antibody levels either through antibody consumption or inadequate initial dose.

Having shown in this study that the protection from a whole bacterial vaccine could be attributable to IgG antibodies directed against the J5 LPS, we set out to develop an *E. coli* O111:B4, J5 LPS subunit vaccine. Immunization of human subjects with either the whole killed *E. coli* J5 or *S. minnesota* Re vaccines by Ziegler and McCabe respectively resulted in unacceptable local reactions to the vaccine. In order to make the vaccine better tolerated, we removed (detoxified) the ester-linked fatty acids from the LPS with alkali treatment. Initial immunogenicity studies with the detoxified LPS (dLPS) alone in mice revealed a poor antibody response. Consequently, we added the outer membrane protein (OMP) from group B *N. meningitidis* to the LPS.[55] This non-covalently complexed J5dLPS/OMP vaccine was highly immunogenic in murine studies and protected neutropenic rats from lethal infection when given actively or when vaccine-induced antibodies were given passively at the onset of fever.[56]

Based on these findings, a phase 1 clinical trial was conducted with the vaccine prepared under cGMP conditions. This vaccine was safe and well-tolerated in human subjects who compared the local reactogenicity as similar to that experienced with influenza vaccines; however, while the vaccine was highly immunogenic in rabbits, mice, and rats, it elicited only 3-fold increase in antibody in humans.[57] Consequently, we began studies of this vaccine with adjuvants. When the vaccine was administered to mice with alum, there was a 2-fold increase in geometric mean titer compared with the response with vaccine alone. The combination of vaccine and the TLR9 adjuvant CpG (short, unmethylated, single stranded synthetic DNA sequence comprised of cytosine- and guanine-triphosphate nucleotides linked by phosphorothioate), however, resulted in a 6-fold increase in antibody levels. Surprisingly, when the vaccine was given with CpG and alum, the antibody response was lower than that observed with vaccine alone.[58] Consequently, subsequent studies were performed with CpG.

Active immunization with the J5dLPS/OMP vaccine with and without CpG protected mice against lethal polymicrobial sepsis in a cecal ligation/puncture (CLP) model of intraabdominal sepsis.[58] Mice were immunized with the vaccine alone, vaccine with CpG, or given saline with CpG. CLP was then performed 60 d after the third immunization. To control for the effect of CpG we also included a group of mice that underwent CLP within 6 d of the last dose of saline plus CpG. In the absence of immunization, animals that received the CpG 60 d prior to CLP all died. Mice that received either the vaccine alone or vaccine with CpG had >95% survival. In contrast to mice that were given CpG 60 d before CLP, mice that underwent CLP within 6 d of the last dose of CpG had 80% survival. Thus, an innate stimulatory adjuvant could provide short-term protection.

No protective effect was observed in earlier clinical studies with passive immunization with J5 antisera or IVIG[36-38]; however we speculated that either inadequate levels or consumption of antibody may have contributed to the lack of protection reported. We observed that in the CLP model, there was specific consumption of anti-J5 IgG. We compared serum levels of total IgG, OMP-specific IgG, and J5-specific IgG before and 48 h after CLP. The decrease in J5 LPS-specific IgG was significantly greater than the loss in total and OMP-specific IgG. In contrast, in the absence of administration of J5 dLPS/OMP vaccine the level of decrease of J5-specific IgG was similar to that of total IgG alone.[58] These data are consistent with the hypothesis that during sepsis, there is IgG catabolism, but more importantly, a loss of J5-specific IgG which binds to bacteria that are then cleared from the circulation. With ongoing infection, in the absence of sufficient IgG provided, antibody consumption will lead to inadequate levels of J5-specific IgG to affect a beneficial outcome. The J5dLPS/OMP vaccine is currently undergoing another phase 1 clinical trial in combination with CpG.

Other Anti-Endotoxin Vaccines/Antibodies

In addition to the early studies with whole bacterial vaccines performed by Braude and McCabe, Gaffin also reported that antisera generated from a whole bacterial vaccine comprised of multiple core LPS phenotypes might provide protection in non-human primates and humans.[59,60] As vaccine technology improved, additional core LPS-specific vaccines were developed. One was composed of the oligosaccharide core LPS of *E. coli* R1, R2, R3, and J5 (Rc) structures as well as *Salmonella* Ra each linked to a tetanus toxoid carrier protein.[61,62] In another formulation, the Ra core LPS structures from multiple mutants were incorporated into a multilamellar liposomal preparation[63,64]; however, to my knowledge none of these vaccines have progressed to human clinical trial. A non-toxic conjugate of polymixin B, which neutralizes endotoxin, covalently linked to human immunoglobulin G protected against lethal LPS-mediated sepsis in mice when given prophylactically.[65] More recently, a mAb, WN1 222-5, was found to recognize the *E. coli* and *Salmonella* LPS inner core epitope(s); however, since it lacked reactivity with other enteric bacilli, it was not further developed.[66-68]

Functional Activity of Anti-Core LPS Antibodies

In the J5 vaccine clinical trial the investigators never clearly established if the protective moiety in the plasma was antibody nor did they demonstrate in earlier studies the mechanism of action of a core glycolipid antibody.[34] It has been widely assumed that any antibody to endotoxin must neutralize the endotoxic

Table 3. Target populations that may benefit from active immunization with anti-endotoxin vaccine

Target populations	Rationale
Occupations at high risk of trauma	Police, firemen, military, helicopter pilots, loggers, fishermen at risk of wound sepsis
Patients with "leaky" gut (HIV, coronary bypass surgery, radiation injury, chemotherapy)	Endotoxemia contributes to morbidity
Patients undergoing elective GI and GU surgery	Higher risk of infectious complications
Patients undergoing immunosuppression	Cancer patients, transplant recipients, rheumatoid arthritis
Acutely injured or burned patients; ICU patients	High risk of sepsis. Patients do respond to immunization*
All patients at hospital discharge	High likelihood for readmission over next 5 years**

*Acutely traumatized patients as well as those admitted to ICUs respond to active immunization.[24,75] **Patients admitted to hospitals are more likely to have recurrent hospitalizations over next 5 years.[69] High-risk hospitalized populations may be immunized once they have stabilized, for example at hospital discharge, with booster doses given as outpatients.

activity of the molecule. In the case of antibody generated by the J5dLPS/OMP vaccine, it was not able to neutralize Limulus activity in a turbidometric assay. Rather, the antibody did bind to a diverse array of GNB (but not *S. aureus*), and promoted the clearance of bacteria from the circulation.[55]

Immunization Strategies

Anti-endotoxin vaccines for the prevention and treatment of gram-negative bacterial infections, including those caused by multidrug-resistant pathogens, would target either hospitalized patients or those patients at significant risk of acquiring these infections. An anti-endotoxin vaccine could be used for the preparation of hyperimmune globulins for passive administration to actively infected patients, as was done in the original Ziegler study. Such a vaccine could also be used to actively immunize subsets of patients at risk of developing sepsis. Unlike "universal" vaccines that are administered to the population at large according to well-established protocols, vaccines for nosocomial infections (e.g., *C. difficile*, MRSA, gram-negative bacterial infections) would target subsets of patients at risk.[69] The need to consider strategies to prevent sepsis has been highlighted in a number of recent studies that document the lingering consequences of sepsis: increased risk of death from other causes up to 5 years after a septic episode; the development of cognitive and functional disabilities, the requirement for substantial ongoing long-term care and the poor quality of life.[70-74] These considerations suggest that "surviving sepsis" is not good enough. Rather there is a need to prevent sepsis or to prevent its progression to severe sepsis. This would necessitate the development of strategies to target patient populations and define their responsiveness to vaccines.

It is possible to identify target populations that may benefit from active immunization with an immunogenic anti-endotoxin vaccine (**Table 3**). In addition, patients admitted to hospitals have a higher likelihood of re-admission during the next 5 years.[69] Consequently, one strategy might consider immunization of patients just before discharge, with booster doses to be given as an outpatient. We previously immunized 10 acutely injured patients within 72 h of injury, and found that these 10 patients responded to experimental *Klebsiella* and *Pseudomonas* vaccines as well as historical controls.[24] More recently, immunization of patients in a medical intensive care unit were reported to mount an antibody response.[75] Thus, it appears that active immunization of acutely ill patients might be feasible. The immune responsiveness of various populations must be better defined before such active immunization strategies can be implemented.

Conclusions

Given the prevalence of infections caused by multiantibiotic-resistant bacteria and the paucity of effective antimicrobials, anti-endotoxin antibodies merit a second look in the prevention/treatment of sepsis. Both O-specific and core glycolipid antibody approaches are feasible. In order to avoid the confusing results of previous studies, and given the likelihood of antibody consumption, particularly during fulminant sepsis, antibody levels must be carefully monitored. Given the sequelae among sepsis survivors, prevention of sepsis with active immunization would be preferable to passive therapy. Active immunization strategies for anti-endotoxin vaccines and other "nosocomial vaccines" must be developed.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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