The issue of anti-endotoxin vaccines:
Back to the future

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Objectives

- Polyvalent O-specific antibodies

- Early studies of anti-core LPS (J5) antibodies: pro/con critique

- Detoxified J5 LPS/OMP vaccine

- The case for active immunization
There has been little change in the mortality from sepsis in the last 20 years

| Mortality (%) |  
|---------------|---
| Ziegler et al (1982) | 38 |
| Angus et al (2001) | 28.6 |
| *Crit Care Med* 2001;29:1303 |

With increasing antibiotic resistance among Gram-positive and -negative bacteria, mortality could increase.
Anti-endotoxin Antibody Approaches to Sepsis

- *O*-specific antibodies directed against polysaccharide chain-considered not to be feasible given the multiplicity of serotypes.

- Anti-core LPS antibodies against a postulated conserved region shared by many GNB.

- Anti-lipid A antibodies directed against that portion of the LPS responsible for toxicity
Gram-negative bacterial lipopolysaccharide (LPS): O-polysaccharide
Polyvalent O-specific antibodies

• Despite multiplicity of serotypes, relatively few O types cause invasive disease in humans: ~12 E. coli, 4 Klebsiella and 7 P. aeruginosa account for majority of bacteremic isolates in serosurveys.

• Multi-valent vaccines have been tested in human phase 1 studies, and hyperimmune IVIG tested in phase 2 study.
Vaccines for Gram-negative bacteria

Polyvalent O-or K-specific vaccines and H-IVIG studied through phase 1 clinical testing.

- *PA*-JCI 1987;80:51
Gram-negative bacterial lipopolysaccharide (LPS): core glycolipid
Core LPS-Specific Antibodies

• Intensive work to assess ability of mutants whose core glycolipid was exposed to the immune system to induce cross-protective antibodies.

• Whole bacterial vaccines derived from *E. coli* O111 Rc chemotype [J5] mutant that lacked a gal-epimerase (Braude), and *S. minnesota* Re mutant (McCabe) extensively studied.

• Both whole killed vaccines given to human subjects.
First Successful Trial of Adjunctive Therapy in the Treatment of Sepsis

TREATMENT OF GRAM-NEGATIVE BACTEREMIA AND SHOCK WITH HUMAN ANTISERUM TO A MUTANT ESCHERICHIA COLI

ELIZABETH J. ZIEGLER, M.D., J. ALLEN MCCUTCHEAN, M.D., JOSHUA FIERER, M.D., MICHEL P. GLAUSER, M.D., JERALD C. SADOFF, M.D., HERNDON DOUGLAS, B.S., AND ABRAHAM I. BRAUDE, M.D.

Abstract In an effort to decrease deaths from gram-negative bacteremia and endotoxin shock, we treated bacteremic patients with human antiserum to endotoxin (lipopolysaccharide) core. Antiserum was prepared by vaccinating healthy men with heat-killed Escherichia coli J5; this mutant lacks lipopolysaccharide oligosaccharide side chains, so that the core, which is nearly identical to that of most other gram-negative bacteria, is exposed for antibody formation. In a randomized controlled trial, patients were given either J5 antiserum or preimmune control serum intravenously, near the onset of illness. The number of deaths in the bacteremic patients was 42 of 109 (39 per cent) in controls and 23 of 103 (22 per cent) in recipients of J5 antiserum (P = 0.011). In those with profound shock, mortality was 30 of 39 (77 per cent) in controls and 18 of 41 (44 per cent) in recipients of J5 antiserum (P = 0.003). We conclude that human antiserum to the lipopolysaccharide core can substantially reduce deaths from gram-negative bacteremia. (N Engl J Med. 1982; 307:1225-30.)
Prophylactic anti-J5 LPS antibody prevents GNB shock and death in surgical patients

<table>
<thead>
<tr>
<th></th>
<th>J5</th>
<th>Control</th>
<th>RR</th>
<th>p</th>
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<tbody>
<tr>
<td>All patients with systemic GNB infections</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>16/126</td>
<td>23/136</td>
<td>1.3</td>
<td>NS</td>
</tr>
<tr>
<td>Shock</td>
<td>6/126</td>
<td>15/136</td>
<td>2.3</td>
<td>0.049</td>
</tr>
<tr>
<td>Lethal shock</td>
<td>2/126</td>
<td>9/136</td>
<td>4.2</td>
<td>0.033</td>
</tr>
</tbody>
</table>

| Abdominal surgery patients with systemic GNB infections |         |         |     |     |
| Total                | 8/71    | 15/83   | 1.6 | NS  |
| Shock                | 2/71    | 13/83   | 5.6 | 0.006|
| Lethal shock         | 1/71    | 9/83    | 7.7 | 0.017|

Treatment given on admission to ICU, then every 5 days and at onset of sepsis.

Was failure to confirm clinical benefit from passive administration of anti-core endotoxin antibodies in subsequent studies (Calandra et al, 1988; Cometta et al; J5 Study Group) due to 

*flawed concept or faulty implementation?*
Anti-core glycolipid (CGL) antibodies associated with improved outcome from sepsis


- Decrease in anti-CGL ab during septic episode predicted poor outcome (Pollack, et al, 1983)

- Maintenance of “adequate levels” of anti-CGL antibodies led to decrease in circulating LPS levels and increased survival (Schedel et al, 1991)
Need adequate levels of anti-endotoxin antibodies

- Swiss-Dutch study showed no protection from infusion of "hyperimmune" J5 IVIG; however, anti-J5 antibody level increased only 2-fold before plasma fractionation (Calandra et al, 1988)

- No benefit of anti-J5 LPS antibody for meningococcal sepsis, but when measured at 6 hr after infusion, no increase in anti-J5 ab over baseline (?consumption)(J5 Study Group);

- Pooled plasma from blood donors with naturally high levels of antibody to CGL (Re595) unable to prevent sepsis, but when measured at 2 days, ab level <50% that obtained at 2 hr post infusion (Cometta et al)(?consumption).

THUS, in these studies, unlikely that adequate levels of anti-endotoxin antibodies were present.

Conclude that insufficient data to prove or disprove hypothesis that anti-endotoxin core antibodies will improve outcome from sepsis.
Detoxified J5 LPS Vaccine for the Prevention and Treatment of Sepsis

- Lipopolysaccharide (LPS) from the J5 mutant of *E. coli* O111 purified and detoxified by removal of ester-linked fatty acids from the lipid A portion of molecule.

- Group B meningococcal outer membrane protein (OMP) prepared from group B *N. meningitidis* and complexed non-covalently with the detoxified J5 LPS.
Active immunization with dLPS/OMP vaccine with or without CpG protects against polymicrobial sepsis (cecal ligation/puncture model)
Specific consumption of anti-J5 IgG during experimental sepsis

<table>
<thead>
<tr>
<th></th>
<th>dLPS-J5/OMP</th>
<th>dLPS-J5/OMP+CPG</th>
<th>CPG Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fold Decrease</td>
<td>-3</td>
<td>-2</td>
<td>-1</td>
</tr>
<tr>
<td>Decrease in Antibody</td>
<td>OMP IgG</td>
<td>Total IgG</td>
<td>J5 IgG</td>
</tr>
</tbody>
</table>

*P<.05  **P<.005
Phase I Study of J5dLPS/OMP Vaccine

Antibody Levels

Table 2. Anti-J5 LPS ELISA Titers of Sera From Volunteers in the Phase I Trial

<table>
<thead>
<tr>
<th>Group</th>
<th>IgG</th>
<th>IgA</th>
<th>IgM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Fold Rise</td>
</tr>
<tr>
<td>5µg</td>
<td>1.7a</td>
<td>+0.28</td>
<td>3.6</td>
</tr>
<tr>
<td></td>
<td>+0.71</td>
<td></td>
<td>+0.14</td>
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<td>10µg</td>
<td>2.8</td>
<td>+0.50</td>
<td>5.8</td>
</tr>
<tr>
<td></td>
<td>+1.9</td>
<td></td>
<td>+0.6</td>
</tr>
<tr>
<td>25µg</td>
<td>2.1</td>
<td>+0.18</td>
<td>4.9</td>
</tr>
<tr>
<td></td>
<td>+0.6</td>
<td></td>
<td>+0.3</td>
</tr>
</tbody>
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Phase 1 study of vaccine administered in conjunction with CpG to begin November, 2011
Survivors of Severe Sepsis

- Have increased risk of death for up to 5 years after septic episode (JAMA 1997;277:1058).


- Develop cognitive and functional disability (JAMA 2010;304:1787)


This suggests that “Surviving Sepsis” is not good enough. Need to prevent sepsis or to prevent progression to severe sepsis.
Proposed Strategies for an Effective Anti-Endotoxin Vaccine

1. Active immunization of individuals at higher risk of sepsis
   - fire fighters
   - police
   - military
   - patients undergoing complicated elective surgery
   - trauma and burn patients
   - soldiers/ICU patients infected with *Acinetobacter*

2. Passive treatment of septic patients

3. Active/passive therapy
   - Supplement consumption of actively induced antibody during septic episode.
Active vs Passive Immunization

- Passive immunization:
  - Is reactive, but
  - Would bypass possible lack of responsiveness of target population;
  - Hyperimmune IVIGs are expensive and
  - Require increased regulatory burden.

- If hurdles to active immunization could be overcome, this approach:
  - Could be more cost-effective, and
  - Unlike passive immunotherapy, may reduce incidence and/or morbidity of infection.
Hurdles to development of vaccines for nosocomial infections

- Unlike “universal” vaccines, vaccines for nosocomial infections (e.g. sepsis, MRSA, C. difficile) would be administered to a subset of patients at risk.

- Target populations and their immune responsiveness not clearly defined;

- While immunization schedules for universal vaccines well-established, strategies for nosocomial vaccines need to be developed;

- Role for adjuvants.
Proposed strategies

• Target hospitalized patients who are more likely to be hospitalized in the future (similar to strategy advocated for pneumococcal immunization) (JAMA 1990;264:1117 & MMWR 1997;46:919).

• Followup immunizations as outpatients.
Malnutrition, Gut Permeability and Endotoxemia

Gut mucosal cell damage in meningococcal sepsis in children: Relation with clinical outcome*

Joep P. M. Derikx, MD; Else M. Bijker, MSc; Gijs D. Vos, MD, PhD; Annemarie A. van Bijnen, BSc; Erik Heineman, MD, PhD; Wim A. Buurman, PhD; Dick A. van Waardenburg, MD

Malnutrition, Gut Permeability Results from Endotoxemia in Severe

Community and International Nutrition

For Commentary on this article see: J. Nutr. 133: 1237, 2003.

Growth Faltering in Rural Gambian Infants Is Associated with Impaired Small Intestinal Barrier Function, Leading to Endotoxemia and Systemic Inflammation

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Anti-endotoxin “nutriceutical”

Goal: To develop a bovine colostrum-based product enriched in anti-endotoxin antibodies to break the cycle of increased gut permeability and endotoxin-mediated pathology during malnutrition, HIV infection and sepsis (e.g. secondary to trauma or burns).

Plan: To immunize cows with J5dLPS/OMP vaccine, harvest colostrum and process into an orally-administered product which can be formulated additional health-promoting supplements.
Conclusions

• Anti-LPS antibodies merit a “second look” in the prevention/therapy of sepsis.

• Both O-specific and core-glycolipid antibody approaches feasible.

• Given the likelihood of antibody consumption, particularly during fulminant sepsis, antibody levels must be monitored.

• Active immunization strategies for anti-endotoxin vaccines and other “nosocomial vaccines”, must be developed.