Immunogenicity of a 24-Valent *Klebsiella* Capsular Polysaccharide Vaccine and an Eight-Valent *Pseudomonas* O-Polysaccharide Conjugate Vaccine Administered to Victims of Acute Trauma

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We measured the antibody response in 10 victims of acute blunt trauma and penetrating trauma who were immunized against *Klebsiella pneumoniae* and *Pseudomonas* species within 72 hours of injury. The two vaccines, which were previously shown to be safe and immunogenic in uninjured humans, were a 24-valent *K. pneumoniae* capsular polysaccharide vaccine and an eight-valent *Pseudomonas* O-polysaccharide-toxin A conjugate vaccine. The patients were between 18 and 44 years of age, had Injury Severity Scores that ranged between 9 and 34, and did not have chronic infections or malignancies. On days 14 and 28 after immunization, all patients had a response of greater than fourfold to at least six of the nine *Pseudomonas* vaccine antigens. Half of the patients responded to eight of the nine antigens. Nine patients responded to at least 18 of 24 *Klebsiella* antigens, and seven patients responded to 22 of the 24 antigens. No important side effects were attributed to the vaccines. The results of this preliminary study indicate that active immunization against potential pathogens is possible in victims of acute trauma.

The victim of serious trauma is predisposed to infection with gram-negative bacteria. Infections with these organisms increase the likelihood of multiple organ dysfunction and death. The more severe the trauma, the greater the risk for infection and organ dysfunction [1]. Multiple immunologic alterations have been described in persons who have been injured, and these alterations may predispose to the development of infections [1]. These immunologic alterations have not been well controlled with use of current therapies. If active immunization raises the titers of protective antibodies to specific pathogens and results in protection against infection with those pathogens, immunization of injured civilians or potentially injured soldiers would be valuable. We evaluated two vaccines that have proved safe and immunogenic in >2,000 volunteers [2].

Methods

Patients who were selected had been admitted to the R. A. Cowley Shock Trauma Center (Baltimore) and were considered evaluable if they were <45 years of age; had sustained acute blunt or penetrating trauma with an Injury Severity Score (ISS) [3] of ≥9 within the previous 48 hours; had no known chronic infections, malignancies, or autoimmune diseases; and provided informed consent. Blood samples were obtained to determine preimmunization antibody concentrations. Following phlebotomy, 1,200 μg of the polyvalent *Klebsiella* capsular polysaccharide vaccine (Klebvax) was injected deeply into the right deltoid muscle, and 200 μg of *Pseudomas* O-polysaccharide-toxin A (Nosovac) conjugate vaccine was injected into the left deltoid muscle. The vaccines, which were manufactured and supplied by the Swiss Serum and Vaccine Institute in Berne, Switzerland, and are described in detail by Edelman et al. [4] had been previously shown to be safe and effective [2, 4]. The vaccines were lyophilized and reconstituted prior to use.

Following the injection, patients were observed for 1 hour for any acute adverse reactions, and the vaccination sites were examined over a 48-hour period for local reaction. Phlebotomy was performed ~14 days and 28 days after vaccination to measure antibody response to the vaccine components. All blood samples were frozen at −70°C until they were assayed. Serum IgG antibody concentrations were measured for each of the 33 vaccine antigens (24 *Klebsiella* antigens and nine *Pseudomonas* antigens [8 *Pseudomonas* and toxin A]) by a previously described ELISA [4].

Results

Thirteen patients gave informed consent for the study; one patient later declined participation, and two did not return for postimmunization phlebotomy. The ages of the patients ranged from 18 to 43 years, 70% were male, and half had sustained blunt trauma. None of the patients had undergone splenectomy.

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Informed consent was obtained from the patients or their parents or guardians, and the guidelines for human experimentation of the U. S. Department of Health and Human Services and the University of Maryland were followed in the conduct of this study.

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Table 1. Geometric mean IgG antibody responses among 10 patients who received eight-valent *Pseudomonas* O-polysaccharide-toxin A conjugate vaccine.

Antigen	Preimmunization antibody concentrations (μg/mL ± SD)*	Antibody concentrations on day 14 after immunization (μg/mL ± SD)*	Antibody concentrations on day 28 after immunization (μg/mL ± SD)*	Percentage of patients with at least a fourfold rise in antibody concentrations after immunization
IT-1	11.7 ± 2.2	28.3 ± 2.2	187.2 ± 2.7	100
IT-2	48.8 ± 1.7	267.1 ± 2.0	247.4 ± 2.1	70
IT-3	7.24 ± 1.9	43.6 ± 1.8	36.4 ± 2.1	70
IT-4	14.1 ± 1.5	141.2 ± 1.6	103.9 ± 1.8	100
IT-5	7.8 ± 1.7	87.5 ± 2.2	62.1 ± 2.5	80
IT-7	5.6 ± 2.1	38.3 ± 2.3	43.2 ± 2.3	90
Habs3	18.9 ± 2.0	110.8 ± 2.7	112.2 ± 2.8	60
Habs4	18.1 ± 1.8	299.7 ± 2.3	228.5 ± 2.6	100
Toxin A	1.36 ± 1.5	10.1 ± 3.5	9.85 ± 3.0	90

* Only nine samples were obtained on days 14 and 28.

ISS scores ranged from 9 to 34 (mean score, 18). Seven of 10 patients were enrolled within 48 hours of injury. Three patients received the vaccines between 52 and 62 hours after injury.

None of the patients enrolled in the study died. One patient failed to return for phlebotomy on day 14; therefore, nine postimmunization specimens were collected around day 14 (range of collection times, days 11–17). One patient failed to return for phlebotomy on day 28; therefore, nine specimens were collected around day 28 (range of collection times, days 24–40). The geometric mean antibody concentration (GMAC) in serum before immunization and at day 14 and day 28 following immunization was determined for 24 components of the *Klebsiella* vaccine and nine components (eight polysaccharide antigens and toxin A) of the *Pseudomonas* vaccine.

Antibody response. All patients responded with a greater than fourfold rise in GMAC to six or more of the nine antigens in the *Pseudomonas* vaccine (table 1). Half of the patients responded to eight of the nine antigens. All patients responded to IT-1, IT-3, and H4. In only two cases, a response to an individual antigen that was not present on day 14 was present on day 28.

All patients but one responded to at least 18 of the 24 antigens in the *Klebsiella* capsular polysaccharide with a greater than fourfold rise in GMAC (table 2). Seven of the 10 patients responded to 22 of the 24 antigens. In only three cases, a response to an individual antigen that was not present on day 14 was present on day 28. One antigen, K35, was poorly immunogenic (only 5 of 10 patients responded). Antibody response to this antigen has also been weak in uninjured humans [4].

Tetanus toxoid. Five of the ten patients received tetanus toxoid within 24 hours of admission. All patients confirmed to have received the tetanus vaccine had greater than fourfold increases in serum IgG antibody levels. A sixth patient, who had no record of having previously received tetanus toxoid, had a greater than 20-fold increase in IgG antibody to tetanus. This magnitude of response is consistent with that of the five other tetanus vaccine recipients. This patient may have received tetanus toxoid that had not been documented in the medical record. The other four patients who did not receive tetanus toxoid had no significant increase in antibody titer.

Adverse reactions. None of the patients had acute systemic reactions to the vaccines. Three patients developed low-grade fevers (temperatures ranged from 99.2°F to 101°F) within 48 hours of vaccination. Only one patient had a local reaction to the *Pseudomonas* vaccine, with some mild tenderness at the injection site 24 hours after injection. There were no vaccine-related abnormalities of liver enzyme levels.

Discussion

During the 1980s, *Pseudomonas aeruginosa* accounted for 10% of infections and *Klebsiella pneumoniae* accounted for 9% of infections at our trauma center [1]. In this preliminary study, we have demonstrated that patients who have sustained moderate injuries (mean ISS score, 18) can be actively immunized with a polyvalent *Klebsiella* capsular polysaccharide vaccine and a *Pseudomonas* O-polysaccharide-toxin A conjugate vaccine. All patients immunized with the *Pseudomonas* conjugate vaccine had a greater than fourfold elevation in titers of antibodies to a minimum of six of the nine antigens. Ninety percent of the patients vaccinated with the *Klebsiella* polysaccharide vaccine had a greater than fourfold rise in antibody concentrations to at least 18 of the 24 antigens. It was not apparent that the immunologic responses of these patients were diminished by the type of injury or the severity of injury or by the receipt of homologous blood transfusions prior to immunization (three patients had received ⩾2 units of blood).

We measured the responses on days 14 and 28, as was done in previous studies with volunteers [4]. An antibody response was manifest by day 14 in almost all cases. However, patients who have been injured are at risk for developing gram-negative

Table 2. Geometric mean IgG antibody responses among 10 patients who received 24-valent *Klebsiella* capsular polysaccharide vaccine.

Antigen	Preimmunization antibody concentrations (μg/mL ± SD)*	Antibody concentrations on day 14 after immunization (μg/mL ± SD)*	Antibody concentrations on day 28 after immunization (μg/mL ± SD)*	Percentage of patients with at least a fourfold rise in antibody concentrations with immunization
K2	2.3 ± 2.1	60.3 ± 4.6	31.6 ± 4.6	80
K3	1.5 ± 1.4	10.0 ± 2.9	7.2 ± 2.9	70
K5	4.3 ± 1.8	67.6 ± 3.7	40.7 ± 5.4	80
K9	1.6 ± 1.4	19.5 ± 3.1	12.3 ± 2.4	80
K10	1.3 ± 1.4	24.0 ± 2.3	26.9 ± 3.5	100
K15	2.6 ± 2.5	57.5 ± 2.5	40.7 ± 2.8	100
K16	1.2 ± 1.5	28.2 ± 4.6	22.9 ± 2.2	90
K17	3.2 ± 2.3	27.5 ± 3.4	20.4 ± 2.7	100
K18	1.9 ± 2.5	31.6 ± 3.5	30.8 ± 2.8	100
K21	2.45 ± 3.0	19.1 ± 2.2	12.3 ± 2.1	90
K22	2.7 ± 3.0	23.4 ± 2.1	16.6 ± 2.5	80
K25	2.9 ± 2.3	40.7 ± 2.9	26.3 ± 3.5	90
K28	4.9 ± 2.0	77.6 ± 3.7	46.8 ± 4.3	80
K30	1.6 ± 2.2	4.27 ± 2.9	15.5 ± 3.2	90
K35	4.4 ± 1.9	22.4 ± 3.5	19.1 ± 3.5	50
K43	2.8 ± 2.4	36.3 ± 4.3	12.6 ± 3.2	70
K52	1.9 ± 2.1	17.4 ± 3.1	23.4 ± 2.7	90
K53	4.1 ± 2.8	8.5 ± 4.0	46.8 ± 4.4	90
K55	2.1 ± 1.6	45.7 ± 4.1	28.8 ± 4.4	90
K60	2.2 ± 3.0	4.90 ± 4.1	25.7 ± 4.8	90
K61	1.8 ± 2.1	46.9 ± 2.9	47.9 ± 2.9	90
K62	2.3 ± 1.7	29.5 ± 3.6	21.4 ± 3.8	90
K63	1.1 ± 1.2	29.5 ± 3.6	21.4 ± 3.8	90
K64	2.9 ± 2.8	25.7 ± 4.3	24.0 ± 3.9	80

* Only nine samples were obtained on days 14 and 28.

infections early following the injury; hence, the rapidity of antibody response after immunization may be crucial.

In animal models, antibodies to the capsular polysaccharide of *Klebsiella* have provided significant protection against death due to infection with *Klebsiella* species. Likewise, antibodies to products of *P. aeruginosa* have been protective in mice [2]. To determine if this mode of immune enhancement is effective in preventing infections due to *Klebsiella* species and *P. aeruginosa* in trauma victims, it must now be determined whether an adequate antibody response occurs in more seriously injured patients and whether it occurs earlier after immunization. In subsequent studies, both IgG and IgM antibody concentrations should be measured, and the possible immunosuppressive effect of homologous blood transfusions should be considered. If active immunization is effective in quickly generating levels of antibodies that are protective, its safety and economy make it an attractive prophylactic technique.

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