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Purification and Vaccine Potential of *Klebsiella* Capsular Polysaccharides

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Capsular polysaccharide (CPS) from 18 *Klebsiella* strains of different capsular types was isolated and characterized. Purified CPSs were composed primarily of carbohydrate with trace quantities of protein, nucleic acids, and lipopolysaccharide. All CPSs were of a high molecular weight, possessing a K_d of 0.01 to 0.11 as determined by gel filtration over Sepharose CL-4B. Low levels of lipopolysaccharide present in all preparations were responsible for the highly pyrogenic nature of one-half of the CPS preparations. Treatment of capsular material with dilute NaOH in 95% ethanol markedly reduced the pyrogenicity of all preparations and had a negligible effect on their molecular weight. The immunogenicity of the various native CPSs for mice varied considerably from serotype to serotype, but all evoked an anticapsular immunoglobulin G response. Five of 18 NaOH-treated polysaccharides were significantly ($P < 0.05$) less immunogenic than their native counterparts. Human immunoglobulin G prepared from volunteers immunized with either native or NaOH-treated KP1-0 capsular polysaccharide was equally effective at preventing experimental fatal *Klebsiella pneumoniae* burn wound sepsis in mice.

Infections due to *Klebsiella* spp. in compromised patient populations are a well-recognized problem (1, 24, 33). In the 5-year period from 1976 to 1980, *Klebsiella* spp. were found to be the second most frequently isolated gram-negative bacteria in cases of primary bacteremia (3). *Klebsiella* bacteremia and pneumonia carry an attendant mortality rate which can approach, or exceed, 50% (1, 15, 23, 33). Also, *Klebsiella* spp. can often be the cause of urinary tract, wound, soft-tissue, and respiratory tract infections (3, 9).

Recently, we have described the isolation, purification, and characterization of capsular polysaccharide (CPS) derived from *Klebsiella pneumoniae* KP1-0 (6, 6a). Anti-CPS antibody administered passively (6) or elicited in response to immunization with purified antigen (6a) was found to provide a high degree of protection against fatal *K. pneumoniae* KP1-0 burn wound sepsis. Furthermore, this capsular antigen was found to be safe and immunogenic in human volunteers (8). However, the above studies were limited to only a single capsular serotype.

While there have been numerous investigations on the biochemistry of *Klebsiella* CPS relating to monosaccharide composition and primary structure (18, 25), little is known regarding the various biological and chemical parameters relevant to their use as vaccine components (4). In the present study, we have isolated and purified 18 serotypes of *Klebsiella* CPSs. These serotypes were selected due to their prevalence among *Klebsiella* bacteremic isolates and account for approximately 70% of such strains (21, 24; P. M. Mortimer and S. J. Cryz, Jr., submitted for publication). The various antigen preparations were analyzed regarding purity, molecular weight, pyrogenicity, and immunogenicity in mice to determine their potential as human vaccine candidates.

MATERIALS AND METHODS

Bacterial strains and growth conditions. *Klebsiella* strains of known capsular and lipopolysaccharide (LPS) serotype (28) were kindly provided by I. Ørskov, Statens Seruminstitut, Copenhagen, Denmark. Cultures were stored at −20°C in a 10% skim milk solution. HYEM medium (containing 2% Hycase-SF [Humko-Sheffield, Memphis, Tenn.], 0.3% yeast extract [Difco Laboratories, Detroit, Mich.], and 2% maltose) was used for routine cultivation. Cultures (500 ml of medium in a 2-liter Erlenmeyer flask) were grown at 37°C with shaking (100 rpm) for 16 h. *K. pneumoniae* KP1-0 was the gift of D. Straus, Texas Tech Health Science Center, Lubbock.

Purification of CPS. CPS was purified by a slight modification of a previously published technique (13). Bacteria were removed from cultures by centrifugation at 10,000 × *g* for 30 min. The supernatant was then passed through a 0.45-μm filter to remove the remaining cells. Cetavlon (*N*-cetyl-*N*,*N*,*N*-trimethylammonium bromide; E. Merck AG, Darmstadt, Federal Republic of Germany), as a 10% (wt/vol) stock, was added to a final concentration of 0.5% (wt/vol). After stirring for 30 min at room temperature, the precipitate was collected by centrifugation. The material was dissolved in 1 M $CaCl_2$ (100 to 250 ml) and ethanol was added to equal 25% (vol/vol). After stirring for 30 to 60 min at room temperature, the precipitated nucleic acids were removed by centrifugation. The CPS was precipitated from the resulting supernatant by increasing the ethanol concentration to 80% (vol/vol). The CPS was dissolved in distilled water (50 to 100 ml) and extracted at least three times with an equal volume of chloroform-butanol (5:1). The pooled water phases were extensively dialyzed against distilled water at 4°C and then centrifuged at 100,000 × *g* for a minimum of 16 h to remove the majority of LPS. CPS was precipitated from the supernatant by ethanol precipitation (80%, vol/vol), collected by centrifugation, dissolved in distilled water, and lyophilized.

Purification of LPS. LPS was purified from cell wall fragments of stationary-phase cultures of *K. pneumoniae* KP1-0 as previously described (7).

Molecular weight determination. The CPS preparations were sized by gel filtration over a Sepharose CL-4B column (92 by 1.5 cm; Pharmacia Fine Chemicals, Uppsala, Sweden). The column was equilibrated in phosphate-buffered saline (PBS; pH 7.4) and calibrated, using blue dextran 2000 (Pharmacia Fine Chemicals) to define the void volume (V_0)

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and [^{14}C]sodium acetate (New England Nuclear, Zurich, Switzerland) to define the total bed volume (V_s). The distribution constant (K_d) was determined according to the method of Wong et al. (32). CPS (5 mg in 3 ml) was applied to the column, and fractions were monitored for total carbohydrate by the phenol-sulfuric acid method (11).

Quantitative analysis. Protein was measured by the method of Lowry et al. (19), using bovine serum albumin as a standard. Nucleic acids were quantitated by determining the A_{260} of a 1-mg/ml CPS solution (in water) in a 1-cm cuvette. The conversion formula used was that 50 μg of nucleic acids per ml yields an A_{260} of 1 in a 1-cm cuvette (32). Residual moisture was determined by use of a type 26-321A moisture analyzer (Dupont Instruments, Monrovia, Calif.). A known amount of CPS (approximately 10 mg) was placed onto a nickel weighing boat and inserted into the oven (105°C) for 20 min in the presence of dry N_2 (conditions shown to result in a maximal water loss from CPSs). The amount of water driven out of the sample was quantitated by an electrolysis cell and digitally displayed. To correct for atmospheric moisture, a blank of just an empty weighing boat was used. The corrected water loss value was divided by the initial weight of the CPS to yield percent residual moisture. 2-Keto-3-deoxyoctonate was measured by the thiobarbituric acid method (29). Total carbohydrate was determined by the phenol-sulfuric acid method, using dextran 500 (Pharmacia Fine Chemicals) as a standard (11).

Detoxification of LPS. LPS present in CPS preparations was detoxified via deacylation by the method of Seid and Sadoff (31). CPS (5 mg) was suspended in 2 ml of a 0.1 N NaOH solution in 95% ethanol for 30 min at 37°C. The solution was neutralized by the addition of 2 N acetic acid. The liquid was then removed with a Pasteur pipette. The CPS was dissolved in distilled water, extensively dialyzed against distilled water, and lyophilized.

Pyrogenicity assays. The pyrogenicity of CPS was evaluated in 1.8- to 2.2-kg New Zealand white rabbits. CPS was dissolved in physiological saline. Rabbits (a minimum of two per capsular antigen) each received 1 ml of CPS solution per kg of body weight intravenously. Temperature response was measured at 0.5-h intervals for 4 h. An increase of 0.3°C at any one time point was considered to be a pyrogenic response.

ELISA. Enzyme-linked immunosorbent assays (ELISA) for the detection of anti-CPS immunoglobulin G (IgG) were performed as previously described (6). An ELISA for detection of anti-LPS antibody was performed in a similar manner, using microtiter plates coated with 200 μl of a 20-μg/ml solution of LPS in PBS, pH 7.4, per well. Each plate contained eight control wells which were reacted with all reagents except serum.

Immunization of mice. Female NMRI mice (18 to 20 g) were used. Groups of 10 mice were immunized intramuscularly with 1 μg of CPS in 100 μl of PBS on days 0 and 14. Blood was taken from each mouse on day 28, and the serum was collected and stored at −20°C.

Antisera. Healthy adult volunteers were immunized subcutaneously with 25 or 50 μg of native or NaOH-treated *K. pneumoniae* KP1-0 CPS (8). Sera were obtained at the time of vaccination and 4 weeks postvaccination from seven volunteers per group and pooled. IgG was isolated from the crude sera by published methods (26). IgG ELISA titers were determined as previously described (6, 6a). The sera were diluted with sterile physiological saline so as to give equal titers for each preparation. The IgG titers were 98 and 85 for serum pools derived from volunteers immunized with native KP1-0 CPS or NaOH-treated KP1-0 CPS, respectively.

TABLE 1. Composition of *Klebsiella* CPSs

Capsular serotype	Component (% dry wt)[a]				
	Carbohydrate	Protein	Nucleic acid	KDO[b]	Residual moisture
2	77.6	1.03	1.32	0.04	9.5
3	73.7	0.73	0.88	0.10	8.5
7	86.8	0.91	0.51	0.10	9.9
9	78.1	2.05	1.24	0.05	10.6
10	78.9	1.11	0.70	0.16	13.6
20	57.4	0.28	0.21	0.06	8.0
21	70.7	1.57	0.97	0.06	9.5
24	79.8	0.16	0.36	0.05	9.0
25	76.2	0.5	0.69	0.02	7.4
30	70.1	2.44	0.55	0.16	11.5
35	79.0	1.0	2.45	0.12	8.6
43	71.8	1.33	0.54	0.11	12.3
47	69.4	0.44	0.44	0.15	9.9
54	62.9	1.74	1.33	0.10	9.4
55	63.2	0.71	0.36	0.04	9.2
56	74.3	2.50	1.10	0.18	9.3
60	86.3	1.20	1.40	0.20	7.1
61	69.2	1.17	0.61	0.13	6.7
Mean	73.6	1.15	0.86	0.101	9.44

[a] See text for analytical methods.
[b] KDO, 2-Keto-3-deoxyoctonate.

Protection studies. Mice (female NMRI weighing 18 to 20 g) each received human IgG (5.6 mg of protein per mouse) or human albumin (16.5 mg per mouse; Albuman, Swiss Serum and Vaccine Institute, Berne, Switzerland) intravenously in 0.3 ml of saline. Approximately 24 h later mice were burned and challenged with approximately 70 CFU of *K. pneumoniae* KP1-0 as previously described (5, 6). Mortality was recorded for 10 days postchallenge. For active vaccination studies mice were immunized on days 0 and 7 with 10 μg of KP1-0 CPS or pronase-treated KP1-0 CPS in 100 μl intramuscularly. KP1-0 CPS was treated with pronase in the following manner. To 500 μg of CPS in 1 ml of PBS, pronase (Boehringer GmbH, Mannheim, Federal Republic of Germany) was added to equal 50 μg/ml. The solution was incubated for 24 h at 37°C.

Statistical analysis. For challenge studies, significance was determined by the chi-square method. For antibody response studies, Student's *t* test was used.

RESULTS

CPSs were isolated from 18 strains of *Klebsiella* spp. which differed in serotype. Capsular material could readily be purified from all strains by using a scheme which entailed (i) coprecipitation from culture supernatants with a quaternary ammonium detergent (Cetavlon), (ii) removal of nucleic acids by ethanol precipitation, (iii) extraction with organic solvents to remove lipids and protein, and (iv) removal of LPS by ultracentrifugation. Yields of capsular antigens varied from 70 to 750 mg/liter for the various strains.

CPS preparations were composed primarily of carbohydrate (Table 1). Residual protein levels were in the range of 0.5 to 2.5%, with an average value of 1.15%. The nucleic acid content was comparably low, with all but one preparation (K35) containing ≤2% and an average value of 0.86%. Trace quantities of 2-keto-3-deoxyoctonate were detected in all preparations, indicating the presence of LPS. The above

TABLE 2. Pyrogenic potential of native and NaOH-treated *Klebsiella* CPSs

Capsular serotype	MPD (μg of antigen/ml per kg of rabbit body wt)[a]		Fold reduction in pyrogenicity
	Native	NaOH treated	
2	<0.1[b]	>10[c]	>100
3	0.5	>10	20
7	<0.1	>10	>100
9	2	>50	>25
10	<0.2	>10	>50
20	0.5	>10	>20
21	0.5	>10	>20
24	10	50	5
25	10	>50	>5
30	1	>50	>50
35	0.5	>10	>20
43	<0.1	5	>50
47	<0.1	>10	>100
54	<0.1	>10	>100
55	1	>50	>50
56	<0.1	>10	>100
60	<0.1	>10	>100
61	<0.1	>10	>100

[a] A pyrogenic response was termed an increase of ≥0.3°C.
[b] Lowest dose tested.
[c] Highest dose tested.

results demonstrate that CPSs could be obtained in good yields from all strains of *Klebsiella* spp. tested and could subsequently be purified to a high degree.

The pyrogenic potential of bacterial CPS for rabbits has previously been shown to be a fairly accurate indicator regarding the acceptability of such antigens in humans (16, 32). One-half (9 of 18) of the native preparations were found to be highly pyrogenic, with a minimal pyrogenic dose (MPD) of ≤0.2 μg/ml per kg (Table 2). Four preparations (K3, K20, K21, and K35) were termed moderately pyrogenic (MPD = 0.5 μg/ml per kg), while five (K9, K24, K25, K30, and K55) were comparatively nonpyrogenic (MPD ≥ 2 μg/ml per kg).

We have previously noted that treatment of K1 CPS in a solution of 95% ethanol containing 0.1 N NaOH markedly reduced its pyrogenicity by detoxifying the contaminating LPS via deacylation of the lipid A moiety (8). Therefore, the various capsular antigens were treated in a similar manner, and their MPDs were determined (Table 2). NaOH-treated capsular material was in all cases found to be less pyrogenic than the corresponding native material. For 11 of 18 capsular preparations which were initially highly or moderately pyrogenic, this treatment reduced the pyrogenicity by more than 50-fold. A less dramatic effect was observed when the native CPS was comparatively nonpyrogenic (serotypes 24 and 25).

The immunogenicity of bacterial CPSs is accepted to be dependent upon their molecular weight (14, 32). The molecular size of the various capsular antigens was determined by gel filtration over Sepharose CL-4B before (native) and after NaOH treatment (Table 3). NaOH treatment had a negligible effect on the elution profiles observed. All polysaccharides eluted at or near the void volume (K_d = 0.01 to 0.16), indicating an average molecular weight of $>10^6$. Furthermore, >97% of the total carbohydrate present in all preparations eluted with a K_d of ≤0.5. An example of the results obtained is shown in Fig. 1 (native K7 CPS).

We elected to study the IgG response to vaccination with *Klebsiella* CPSs since this class of anti-CPS antibody has been shown to afford good protection against experimental *Klebsiella* infections (6). The immunogenicity of native and NaOH-treated CPSs was evaluated in mice. The IgG antibody response following immunization with native capsular antigens was found to vary substantially among the serotypes (Table 4). Polysaccharides derived from capsular types 7, 10, 20, 24, 56, and 60 were highly immunogenic (mean IgG ELISA titers, ≥150). Material derived from types 43 and 61 was weakly immunogenic (mean IgG ELISA titers, ≤20). In most cases, NaOH treatment was found to have little, if any, effect on the immunogenicity. However, the antibody response to five preparations (K10, K30, K54, K55, and K61) was found to be significantly ($P \leq 0.05$) decreased after NaOH treatment.

The protective capacity of human IgG antibody elicited in response to immunization with native CPS or NaOH-treated CPS was evaluated in a murine burn wound sepsis model (Table 5). Passively transferred anti-native CPS or anti-NaOH-treated CPS conferred a comparable, and significant ($P < 0.01$), degree of protection against infection with the homologous strain of *K. pneumoniae*.

To document that the protection observed above was not due to antibody elicited to the protein or LPS contaminants present in the KP1-0 CPS vaccine, the following experiments were performed. Groups of mice (15 in each group) were immunized with PBS, KP1-0 CPS, or KP1-0 CPS treated with pronase and subsequently burned and challenged with *K. pneumoniae* KP1-0. The mortality rates for these three groups were 87, 26, and 20%, respectively. There was no significant difference in the protective capacity of native or pronase-treated KP1-0 CPS, with both preparations providing significant protection ($P < 0.01$) against fatal infection, indicating that the protein present in CPS is not responsible for eliciting a protective immune response. To determine if a protective immune response was elicited against residual LPS in CPS, groups of mice received human albumin, preimmune human IgG, or IgG prepared from volunteers immunized with NaOH-treated KP1-0 CPS (postimmune IgG) (Table 6). The transfer of preimmune IgG, which

TABLE 3. Molecular weight of *Klebsiella* CPSs before and after NaOH treatment

Capsular serotype	K_d		$K_d \leq 0.5$	
	Native	NaOH treated	Native	NaOH treated
2	0.04	0.03	99.4	99.2
3	0.04	0.05	98	98
7	0.01	0.08	100	100
9	0.03	0.07	98.7	97.8
10	0.08	0.11	98.5	98.7
20	0.11	0.11	100	99.2
21	0.10	0.05	98.3	99.2
24	0.05	0.05	99.8	99.3
25	0.02	0.08	100	99.2
30	0.05	0.02	99	100
35	0.02	0.01	99.8	97.9
43	0.02	0.01	97.8	100
47	0.06	0.06	97.9	98.9
54	0.02	0.01	99	100
55	0.10	0.06	99.7	99.2
56	0.06	0.16	100	98
60	0.07	0.01	100	98.1
61	0.06	0.05	99.9	98.1

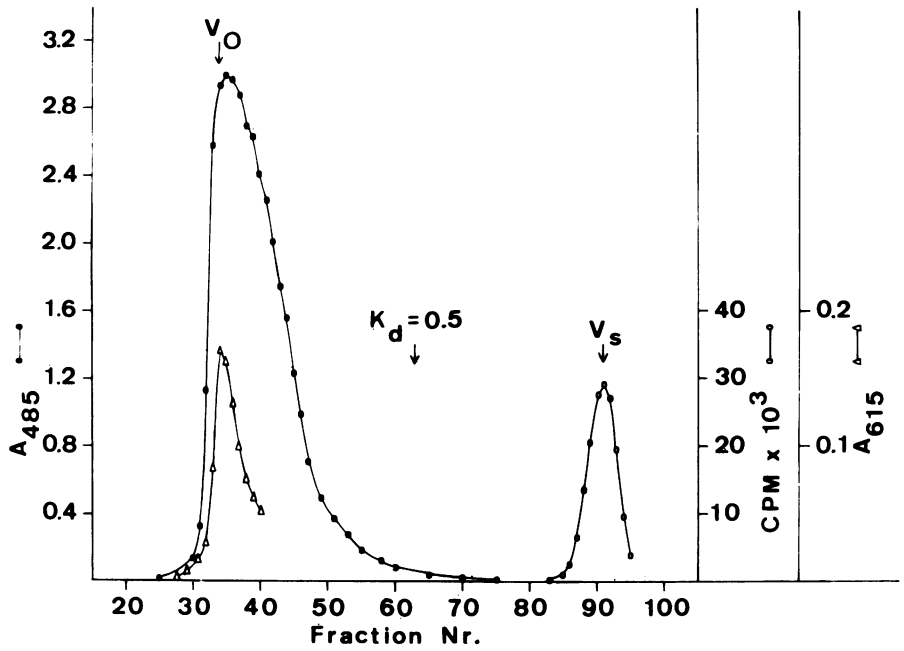

FIG. 1. Gel filtration of native K7 CPS over: Sepharose CL-4B (●; measured at 485 nm; phenol-sulfuric acid method); blue dextran 2000 (△; measured at 615 nm); [^{14}C]sodium acetate (○; quantitated by determining the counts per minute of a 200-μl fraction). V_0 = Void volume based upon the elution profile of blue dextran 2000. V_s = Total bed volume based upon the elution profile of [^{14}C]sodium acetate.

contained no detectable anti-CPS antibody but possessed anti-KP1-0 LPS antibody, was not found to be protective ($P > 0.05$). In contrast, postimmune IgG enriched in anti-CPS antibody but not in anti-LPS antibody afforded significant protection against mortality when compared with both the human albumin-treated and the preimmune IgG-treated groups ($P < 0.01$). The results demonstrate that, while immunization of humans with NaOH-treated KP1-0 CPS evokes a good anti-CPS antibody response, it does not engender an anti-LPS response. Furthermore, protection against fatal experimental infection correlates only with the transfer of anti-CPS antibody.

TABLE 4. IgG antibody response following immunization with native and NaOH-treated *Klebsiella* polysaccharides[a]

Capsular serotype	Mean IgG ELISA titer (range)[b]		P
	Native	NaOH treated	
2	72 (44–80)	107 (<10–720)	NS[c]
3	45 (<10–90)	39 (<10–60)	NS
7	362 (270–1,000)	192 (90–280)	NS
9	78 (44–160)	63 (33–77)	NS
10	519 (200–1,500)	30 (<10–94)	<0.01
20	225 (140–360)	214 (130–460)	NS
21	49 (<10–110)	32 (<10–125)	NS
24	169 (100–240)	307 (135–1,250)	NS
25	68 (48–160)	68 (63–97)	NS
30	431 (190–800)	135 (78–200)	<0.01
35	54 (<10–190)	32 (10–130)	NS
43	9.9 (<10–55)	8 (<10–80)	NS
47	47 (<10–180)	14 (<10–46)	NS
54	67 (50–100)	20 (<10–65)	<0.05
55	55 (17–80)	8 (<10–32)	<0.01
56	163 (90–1,000)	128 (45–530)	NS
60	155 (30–290)	136 (80–330)	NS
61	19 (<10–55)	2 (<10–24)	<0.05

[a] Each mouse received 1 μg of antigen intramuscularly on days 0 and 14. Blood was collected on day 28.
[b] Groups of 10 mice were used.
[c] NS, Not significant.

DISCUSSION

Infections caused by *Klebsiella* spp. constitute a major threat to immunocompromised patients (1, 23, 24, 33). Antibiotic therapy appears to have only a moderate impact on the mortality rate for *Klebsiella* bacteremia and pneumonia (15, 23). The recent emergence of multiply resistant strains greatly complicates antibiotic regimens (20, 22). At present, no immunoprophylactic or immunotherapeutic agents exist for the control of *Klebsiella* infections.

Several studies have described the critical role played by CPS during the course of experimental *Klebsiella* infections (5, 10). In addition, the protective capacity of type-specific CPS has been well documented in model infections (2, 6, 6a). However, Fournier et al. (12) reported that, while ribosomal vaccines provided serotype-specific protection against *Klebsiella* infections in mice, immunization with purified CPS was ineffective. It should be noted that neither the immune response to vaccination with the various antigens nor the

TABLE 5. Protection against fatal *K. pneumoniae* KP1-0 sepsis by passive transfer of anti-KP1-0 CPS

Antiserum[a]	Mortality (no. dead/total)	P
Human albumin	14/15	
Anti-native KP1-0 CPS	2/15	<0.01
Anti-NaOH-treated KP1-0 CPS	3/15	<0.01

[a] Mice each received 0.3 ml of antiserum or human albumin intravenously 24 h prior to challenge with 70 CFU of *K. pneumoniae* KP1-0.

TABLE 6. Protection against fatal *K. pneumoniae* KP1-0 infection by passive transfer of preimmune and postimmune human IgG[a]

IgG transferred[b]	IgG ELISA titer		Mortality (no. dead/ total)	P
	KP1-0 CPS	KP1-0 LPS		
Human albumin	ND[c]	ND	14/15	
Preimmune IgG	<2	61.7	12/15	>0.5[d]
Postimmune IgG[e]	128	45.8	2/15	<0.01[f]

[a] Preimmune IgG was obtained from the sera of human volunteers prior to immunization with NaOH-treated KP1-0 CPS. Postimmune IgG was obtained from the sera of volunteers 28 days after immunization.
[b] Mice each received 0.3 ml of human albumin or human IgG intravenously 24 h prior to challenge with 64 CFU of *K. pneumoniae* KP1-0.
[c] ND, Not determined.
[d] Compared with human albumin-treated group.
[e] Following vaccination with NaOH-treated KP1-0 CPS.
[f] Compared with both human albumin-treated group and preimmune IgG-treated group.

molecular weight of the CPS preparation was determined, therefore making it difficult to explain these findings in light of the present observations.

Although 77 *Klebsiella* capsular serotypes have been identified (27), their distribution among blood isolates is somewhat limited. The 18 serotypes currently investigated would encompass roughly 70% of *Klebsiella* bacteremic strains (21, 24; Mortimer and Cryz, in preparation). These findings, together with the fact that *Klebsiella* serotype 1 CPS has been found to be safe and immunogenic in humans, indicate that capsular antigens should be considered as prime vaccine candidates against *Klebsiella* infections. We have therefore studied the feasibility of such an approach by analyzing the chemical, biological, and immunological characteristics of these 18 capsular antigens relevant to vaccine development.

CPSs could be readily isolated and purified from all serotypes of *Klebsiella* strains studied. Although purity, in regard to contaminating protein, nucleic acid, and LPS, varied somewhat among the different serotypes, it was comparable to the standards currently set forth for capsular polysaccharide-based vaccines against *Neisseria meningitidis* and *Streptococcus pneumoniae* (30, 32).

All *Klebsiella* capsular preparations contained varying amounts of LPS, which, for the most part, correlated closely with their pyrogenicity. The majority of native capsular antigens were considered to be too pyrogenic for eventual use as components of a multivalent vaccine for human use. Detoxification of contaminating LPS by deacylation in the presence of dilute NaOH was found to be an effective method for lowering the pyrogenicity of *Klebsiella* capsular preparations. After NaOH treatment, ≥5 μg of antigen per kg of body weight was needed to evoke a fever response (≥0.3%) in rabbits. This represents a dose 100-fold greater than that currently allowed for the *N. meningitidis* capsular vaccine (32).

Of equal importance was the fact that *Klebsiella* CPSs were resistant to degradation by NaOH. Therefore, the high-molecular-weight characteristic of these capsular antigens necessary for their immunogenicity was preserved. This was evidenced by the fact that NaOH treatment had little effect on the IgG response mounted by mice to immunization with the majority of CPSs. Although NaOH treatment did significantly diminish the immunogenicity of five capsular serotypes, in no case was the immune response completely abrogated.

IgG antibody directed against either native or NaOH-treated serotype 1 CPS was equally effective at preventing fatal experimental burn wound sepsis. Antibody directed against LPS or protein antigens did not appear to contribute significantly to protection. Although protection studies were limited to a single serotype (due to the fact that serotypes of *Klebsiella* spp., other than 1 and 2, are nonvirulent for burn-traumatized mice at a reasonable challenge dose [$\leq 10^4$ bacteria]), the results obtained suggest that the protective antigenic determinants expressed by *Klebsiella* CPSs may be resistant to destruction or alteration by NaOH.

The present study demonstrates that high-molecular-weight capsular antigens can be isolated and purified from numerous clinically relevant serotypes of *Klebsiella* spp. Furthermore, the use of NaOH treatment to reduce the pyrogenic potential of purified capsular material may prove to be a useful technique for producing a safe and immunogenic *Klebsiella* polysaccharide vaccine.

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