Progress in Immunization against *Klebsiella* Infections

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Nosocomial infections with *Klebsiella* spp. are a leading cause of morbidity and mortality. The ability of *Klebsiella* spp. readily to colonize hospitalized patients, complications in treatment of infections due to R-factor-acquired antibiotic resistance, and the high mortality rate in certain patient populations, point to the need for immunoprophylactic/immunotherapeutic agents for disease control. The potential for vaccination against *Klebsiella* spp. is discussed in light of recent developments concerning the pathogenesis of *Klebsiella* infections as relates to the identification of protective antigens as possible vaccine candidates.

The ability of *Klebsiella* to cause serious disease in man has been recognized since its isolation from the lungs of pneumonia patients by Friélander in 1882. Until the introduction of wide-spectrum antibiotics, infections with *Klebsiella* were only sporadically reported. An increase in the frequency of *Klebsiella* infections was first noted in hospitalized patients in the 1950s (1, 2). Currently, the overwhelming majority of *Klebsiella* infections are nosocomial in origin and account for approximately 7.5% of hospital-acquired infections in the United States (3). Of particular significance is the occurrence of *Klebsiella* epidemics in neonatal wards (4, 5). Even under non-epidemic conditions, *Klebsiella* infections in pediatric patients are a major concern. In one study (6), *Klebsiella* was the third most common pathogen isolated and was responsible for a wide variety of pediatric disease syndromes, including meningitis, sepsis, and enterocolitis. *Klebsiella* is most frequently isolated from the urine and respiratory tract (3, 7, 8). However, several recent studies have shown it to be a leading cause of bacteremia (2, 3, 8) and wound infections (3). The frequent isolation of *Klebsiella* from burn wounds has also been noted (9).

Currently, there are no means available for the immunological control of *Klebsiella* infections. The need for such immunoprophylactic/immunotherapeutic agents is underscored by the extremely high mortality rates listed in Table 1. In several studies the fatality rate approached, or exceeded, 50%. Additional studies have shown that in burn patients, *Klebsiella* spp. was second only to *Pseudomonas aeruginosa* as the source of fatal infections (12, 13). In yet another report, *Klebsiella* pneumonia and bacteremia were the leading causes of death associated with nosocomial infections (14). Overall, antibiotic therapy for treatment of *Klebsiella* pneumonia and bacteremia has been found to be moderately effective (8). The recent occurrence of *Klebsiella* strains harboring R-factors coding for multiple antibiotic resistance can greatly complicate chemotherapy (15, 16).

**Virulence of Klebsiella**

Several *Klebsiella* somatic antigens (including pili and capsular polysaccharide) have been implicated as important virulence factors. The majority of *Klebsiella* isolates are piliated. Pili have been shown to mediate attachment to urinary tract epithelial cells in experimental infections (17, 18). Such adherence allows for colonization to occur leading to subsequent disease.
The vast majority of clinical Klebsiella isolates possess a well-defined capsule, although size may vary. Two distinct classes of extracellular capsular material have been described: complex acidic polysaccharides which confer serotype specificity (K antigens) (19, 20) and non-type-specific neutral polysaccharides (21). Encapsulation appears to be essential for virulence, at least in experimental infections (22). Capsule size has been shown to greatly influence virulence. Domenico et al. (23) has described two colonial variants derived from the same strain of Klebsiella pneumoniae, KP1-0, which possesses a large capsule, and KP1-T, which has a substantially smaller capsule. KP1-T was found to be far less virulent for mice and displayed a greatly reduced potential for causing lobar pneumonia in rats (23). Similarly, KP1-0 was found far more virulent for burn-traumatized mice than KP1-T (24). Several mechanisms by which encapsulation may act to increase virulence have been proposed, and include: 1) antiphagocytic activity (23), 2) interference with the mounting of a protective immune response (25, 26), and 3) release of capsular material from the bacterium acting to adsorb protective antibody (23). The last hypothesis is supported by the findings of Pollack (27) that antigenemia (capsular material) in patients suffering from Klebsiella infections correlated with a poorer prognosis.

Several studies have linked virulence to a particular capsular serotype. Mizuta et al. (28) investigating the virulence of 82 Klebsiella O1 group strains (0-antigen determined by lipopolysaccharide) found that only strains which possessed K1 or K2 capsular antigens were highly virulent for mice. However, not all O1 : K1 and O1 : K2 strains were virulent. The predominance of serotypes K2 and K21 among clinical isolates that led Caswell and Talsania (29) to suggest that these serotypes may have "unrecognized advantages" over other serotypes relating to virulence. A similar hypothesis has been proposed by Riser and Noone (7) relating to capsular type and site of isolation. However, increased virulence may not be due solely to capsular type, but may depend on a variety of other traits (antibiotic resistance, piliation, etc.), which together may provide these strains with certain advantages in the hospital environment (10).

Immunization against Experimental Klebsiella Infections

Immunization of mice with either killed whole-cell or ribosomal vaccines provides good serotype-specific protection (30). Antisera elicited against ribosomal preparations were also protective, indicating protection was mediated by humoral antibody. Further studies have confirmed that the protective antigen present in several immunizing preparations (ribosomal vaccines and crude cell-surface extracts) was capsular polysaccharide (31, 32). Antibody to LPS appeared to play no role in protection. Interestingly, immunization with purified capsular material resulted in an insignificant decrease in mortality. This finding was attributed to the poor immunogenicity of the purified capsular antigen.

Parenteral or intranasal immunization of mice with a killed whole-cell vaccine was effective at preventing fatal pulmonary infection following intranasal challenge (33). Protection was attributed to the stimulation of a local immune response (IgG and IgA) to capsular antigen, which functioned to opsonize and clear the bacterial challenge. Subsequent studies demonstrated that antibody to a surface antigen, other than capsular polysaccharide or LPS, was protective (34). This antigen was present on several strains of Klebsiella and initial results suggest it may be an outer membrane protein. Antisera to a highly purified, chemically/physically defined polysaccharide used to passively immunize mice afforded significant protection against fatal Klebsiella pneumoniae burn wound sepsis (unpublished observation of author). Of critical importance was the ability of anticapsular polysaccharide to prevent bacteremia. In further unpublished studies capsular polysaccharides were found to be highly immunogenic in mice, conferring high levels of serotype-specific protection.

Development of Vaccines for Human Use

Existing data from animal studies would indicate that capsular polysaccharide is a viable vaccine candidate. While other bacterial capsular polysaccharides (meningococcal and pneumococcal) have been shown to be clinically effective, no