**TEL AVIV UNIVERSITY**
**THE SACKLER SCHOOL OF MEDICINE**
**DEPARTMENT OF HUMAN MICROBIOLOGY**

# INTERACTIONS OF *KLEBSIELLA PNEUMONIAE* WITH HOST CELLS AND THEIR CONTRIBUTION TO PATHOGENESIS

**THESIS SUBMITTED FOR THE DEGREE OF „DOCTOR OF PHILOSOPHY“**
**BY**

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| | |
|---|---|
| Aqua bidest. | bidestilled water |
| $Arg^-$ | auxotrophic for arginine |
| $Arg^+$ | prototrophic for arginine |
| Arg | arginine |
| AS | ankylosing Spondylitis (Spondylitis ankylosans) |
| ATCC | American Type Culture Collection |
| BHI | brain heart infusion |
| CFU | colony forming unit |
| CL | chemiluminescence |
| CPM | counts per minute |
| CPS | capsular polysaccharides |
| CRD | carbohydrate recognition domaines |
| $D_2O$ | deuteriumoxide |
| GNP | glutaraldehyde natrium cacodylate buffer |
| EBSS | Earle's balanced salt solution |
| ELISA | enzyme linked immunosorbent assay |
| FCS | fetal calf serum |
| FU | fluorescence unit |
| HA | hemagglutination |
| HBSS | Hanks' balanced salt solution |
| HEPES | (N-[2-Hydroxyethyl]piperazine-N´-[2-ethanesulfonic acid]) |
| His | histidine |

| | |
|---|---|
| $His^-$ | auxotrophic for histidine |
| $His^+$ | prototrophic for histidine |
| HLA | human leukocyte antigen |
| IU | international unit |
| DMMV | Department of Medical Microbiology and Virology, University of Kiel, Germany |
| K-Antigen | capsular antigen |
| Kan | kanamycin |
| $Kan^r$ | kanamycin resistance/ resistant |
| KPTA | *Klebsiella pneumoniae* origin Tel Aviv |
| LPS | lipopolysaccharide |
| MIC | minimal inhibitory concentration |
| MR/K-HA | mannose resistant, *Klebsiella*-like hemagglutinin (agglutination) |
| MSHA | mannose sensitive hemagglutinin (agglutination) |
| NA | nutrient agar |
| Nal | nalidixic acid |
| $Nal^r$ | nalidixic acid resitant |
| NB | nutrient broth |
| NCTC | national collection of type cultures (Britain) |
| NHS | normal human serum |
| M9 | minimal medium |
| PAGE | polyacrylamide gel electrophoresis |
| PBS | phosphate buffered saline |

| | |
|---|---|
| PFGE | Pulsed-field gel electrophoresis |
| R-LPS | rough forms of lipopolysaccharide |
| PMNL | polymorphonuclear leukocytes |
| RPMI | Rosewell Park Memorial Institute |
| RrSP-D | SP-D dodecamers |
| RrSP-Dser15,20 | SP-D trimers |
| SD | standard deviation |
| SDS | sodium dodecyl sulfate |
| S-LPS | smooth forms of lipopolysaccharide |
| SP-A | surfactant protein A |
| SP-D | surfactant protein D |
| spp. | species (plural) |
| ssp. | Subspecies |
| Tris | 2-amino-2-(hydroxymethyl)-1,3-propandiol |
| RPM | rotation per minute |

**ABSTRACT**

*Klebsiellae* are opportunistic pathogens that cause severe infections, such as septicemia, pneumonia, urinary tract infections (UTI), and soft tissue infections, especially in hospitalized, immunocompromised patients with severe underlying diseases. *Klebsiellae* are among the most important nosocomial pathogens, being the causative agent in 5-7% of all hospital-acquired infections. They are also thought to induce ankylosing spondylitis (AS) in HLA-B27-positive individuals.

In *Klebsiella*, the main virulence factors are located on the surface of the bacterium. Two major classes of glycoconjugates have been identified on the surface of *Klebsiella*: capsular polysaccharides (CPS) and bacterial lipopolysaccharides (LPS) (Ørskov and Ørskov, 1984, Podschun and Ullmann, 1998). Based on the structural and immunological variability of the CPS and the O-antigen of LPS, *Klebsiellae* have been classified into 77 capsular and nine O-serotypes, which appear to have differential effects on the outcome of *Klebsiella* infections. There is evidence that the differences in their pathogenicity and epidemiological relevance are due to the ability of certain capsular and O-antigens to interact differentially with cellular and humoral components of the innate immune system. The innate immune system's cellular components include granulcytes, macrophages, and epithelial cells. The innate immune system's humoral components include serum components such as natural antibodies and proteins of the complement system and secretory tissue proteins, among which are the surfactant protein D and A, and the lung collectins.

In the present study, I focused my research on the interactions of *Klebsiella* with host cells (e.g. nonphagocytic and phagocytic cells) and the role of the CPS and LPS in such interactions. Specifically, I examined: (i) the interactions of capsulated and noncapsulated *Klebsiella* variants

and their capsule-switched derivative with intestinal and bladder epithelial cell lines, (ii) the interactions between noncapsulated variants and the surfactant protein D (SP-D), and (iii) the interactions of capsulated and noncapsulated variants as well as a capsule-switched derivative with polymorphonuclear leukocytes (PMNL). I used the virulent K2 parent strain, which is isolated at a high frequency from septicemia, and the non-virulent K21a, K26, K36 and K50 serotypes. The K36 capsular genes were exchanged with those of the K2 strain. In addition, 20 well-characterized clinical isolates of *Klebsiella* strains expressing the O1, O3, O4 or O5 serotypes were employed. The identity of the variants with the parent strains was assured by various serological and biochemical tests.

The adhesion of K21a, K26, K36, and K50-capsulated *Klebsiella* strains to ileocecal (HCT-8) and bladder (T24) epithelial cell lines was significantly weaker than that of their corresponding spontaneous noncapsulated variants K21a/3, K26/1, K36/3, K50/3**.** Internalization of the capsulated bacteria by both epithelial cell lines was also significantly reduced. Similarly, a capsule-switched derivative, K2(K36), which had a morphologically larger K36 capsule than the parent strains and produced more capsular material, was less able to invade the ileocecal epithelial cell line than the corresponding K2 parent strain. None of the capsulated strains possessed significant numbers of mannose-sensitive type 1 fimbriae (MSHA), whereas two of the noncapsulated variants, K21a/3 and K50/3, exhibited potent mannose-sensitive hemagglutinating activity. Although hemagglutinating activity attributable to mannose-resistant *Klebsiella* type 3 fimbriae (MR/K-HA) was weak in all strains, several capsulated parent strains exhibited lower titers than their corresponding noncapsulated variants. While the level of adhesion to the ileocecal cells did not differ from that to bladder cells, bacterial internalization by bladder cells was significantly lower than internalization by ileocecal cells, suggesting that bladder cells lack components required for the internalization of *Klebsiella.*

Studies on the interactions of the *Klebsiella pneumoniae* strains with SP-D revealed that only the noncapsulated phase variants can bind and agglutinate the collectin, consistent with previous findings. Although SP-D bound the core oligosaccharide domain of LPS isolated from all strains, I could show for the first time that, based on the structural variability of the O-antigens, the *Klebsiella* strains can be functionally classified into those which express SP-D-reactive and those which express SP-D-non-reactive O-antigens. This conclusion is based on experiments in which the LPS molecules of each isolate were electrophoretically separated into those containing the core region (R-LPS) only and those containing the core region linked to the O-antigens with various numbers of oligosaccharides repeating units (S-LPS). Blotting the gels with SP-D revealed that SP-D bound at relatively low concentrations to the core region of LPS obtained from all tested strains, whereas it bound to S-LPS molecules from only some strains. The repeating oligosaccharide units of the O-antigens of the SP-D-reactive strains are rich in mannose and have been identified serologically as O3 and O5 serotypes, whereas those of the non-reactive strains lack these oligosaccharide structures and have been classified as O1 and O4 serotypes. SP-D was more potent at agglutinating noncapsulated phase variants of the *Klebsiella*-reactive O-serotypes and, as a consequence, more effectively inhibited their adhesion to lung epithelial cells, an initial step in the colonization process necessary for lung infection. This anti-adhesion activity requires the multimerization of trimeric SP-D subunits. Analysis of the O-serotypes of clinical isolates revealed that *Klebsiella* expressing “non-reactive” LPS O-antigens were isolated at a significantly higher frequency from patients with *Klebsiella* pneumonia. Our findings suggest that SP-D plays an important role in the natural pulmonary clearance of opportunistic gram-negative bacteria and contributes to known serotypic differences in the pathogenicity of *Klebsiella*.

The K2 parent strain induced low levels of chemiluminescence (CL) in PMNL obtained from both healthy individuals and AS patients, whereas the K36 capsulated strain induced a potent CL response in PMNL from both groups. Furthermore, if the K2 capsule was switched to K36, or if the noncapsulated K2/3 variants were tested, it was found that the capsule switched to the derivative K2(K36) variant, or that the noncapsulated variant induced a significantly higher CL response than the K2 parent strain. These findings are consistent with the notion that the K2 capsule is anti-phagocytic by virtue of its inability to react with PMNL in the presence of serum. The noncapsulated variant K36/3 induced a CL response in PMNL of healthy individuals that was as potent as that induced by the capsulated parent strain. Interestingly, the CL response induced by the noncapsulated variant K36/3 was significantly lower in AS PMNL than in healthy PMNL. These results imply that PMNL from HLA-B27-positive AS patients are less susceptible to stimulation by the serum-treated noncapsulated K36/3 serotype. It is possible that the receptors for the C3b fragment on AS PMNL poorly recognize the C3b ligand on the surface of the K36/3 noncapsulated variant.

These findings suggest that the virulence of *Klebsiella* is determined by interactions between the components of the innate immune system, such as complement, SP-D, and mucosal epithelial cells on the one hand, and the molecular structures of the LPS and CPS on the other. These interactions produce differences in virulence that are expressed as differences in the epidemiological predominance of the various *Klebsiella* serotypes in infections such as pneumonia and septicemia.