LytA is Involved in the Evasion of Complement-Mediated Immunity and Invasive Disease by a Pneumolysin-Independent Mechanism

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ABSTRACT:

Streptococcus pneumoniae is one of the major causes of invasive disease accounting for more deaths than any other vaccine-preventable bacterial infection. Recognition by the complement system is a vital event in the clearance of this important human pathogen. We show here that the main autolytic enzyme of the bacterium, the amidase *LytA*, avoid complement-mediated immunity by a complex mechanism of impaired activation and increased down-regulation. Moreover, we demonstrated that both *ply* and *lytA* mutants were attenuated in pneumonia and sepsis and the virulence of the double mutant was greatly impaired demonstrating that both proteins play an important role in the establishment of the pneumococcal invasive disease.

Keywords: Complement system, LytA, Pneumolysin down-regulators.

1. Introduction

The pneumococcal capsule is one of the most important virulence factors of *Streptococcus pneumoniae*, although there are several proteins that play an important role in pathogenesis. Among them, pneumolysin (Ply) is a member of the cholesterol-dependent cytolysin family that plays a significant role in virulence by binding to cholesterol containing membranes, forming ring-shaped pores that mediate cell death of the human host [1]. In addition, Ply interacts with different components of the host immune response such as TLR4 and complement-mediated immunity modifying the inflammatory response and the recognition of *S. pneumoniae* by the first complement component of the classical pathway C1q [2–4]. The contribution of LytA to virulence is thought to be mediated by its function in the release of Ply and inflammatory mediators such as teichoic acids and peptidoglycan from lysed bacterial cells. The complement system is one of the main host defence mechanisms against invading pathogens such as S. *pneumoniae*. Activation of the three complement cascades leads to the formation of the key component C3b that is essential for opsonisation of microorganisms, phagocytosis, and inflammation. The main goal of this study was to investigate the role of Ply and LytA in essential aspects of the pathogenesis process including host immune response evasion and pneumococcal dissemination.

2. Methods

We analyzed the interaction of LytA and Ply with the complement system by using a flow cytometry assay.

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3. Results

3.1. Pneumolysin and LytA cooperate in the evasion of complement-mediated recognition

C3b deposition was increased on the *ply* and *lytA* single mutants, whereas the double mutant had remarkably high levels of C3b bound compared to the wild-type or the single mutants. These results suggest that the combination of both LytA and *Ply* is highly effective in the inhibition of the complement activity. Interaction with the classical and alternative pathways was explored by using human sera depleted in C1q and factor B (Bf) respectively. Loss of C1q only increased the deposition of C3b on the surface of the *lytA* and *ply lytA* mutant strains. Mutants lacking Ply and LytA had higher levels of C3b than the wild-type strain when bacteria were incubated in Bf-depleted sera confirming that Ply avoids the activation of the classical pathway and LytA interferes with both cascades. Ply and LytA avoided classical pathway activation by reducing the deposition of the first component C1q. However, the interaction with CRP was increased in the *lytA* mutant background, but not in the *ply* mutant.

3.2. LytA recruits fluid phase down- regulators to avoid complement-mediated immunity

Binding to fluid-phase down-regulators of the complement system is a clever strategy used by different pathogens to escape complement mediated immunity. The interaction of Ply, LytA, and PspC with C4BP and Factor H was explored by flow cytometry. Our results showed that C4BP (but not factor H) binding was slightly decreased in the absence of Ply. Recruitment of both C4BP and factor H down-regulators was significantly reduced in the absence of PspC and LytA compared to the wild-type strain.

3.3. LytA and Ply enhance the establishment of pneumococcal pneumonia and sepsis

Lack of Ply or LytA was associated with a significant attenuation in virulence in a mouse sepsis model in comparison to the wild-type strain. The role of Ply and LytA in pneumococcal pneumonia was also investigated. The levels of *ply* or *lytA* single mutants recovered from BALF, lung and blood samples were significantly lower than those obtained with the parental strain, indicating that Ply and LytA are involved in the pathogenesis of pneumococcal pneumonia. In both models of infection, loss of both Ply and LytA had a pronounced attenuation in virulence at all the investigated locations demonstrating that the activity of both proteins is required for the full virulence of the bacteria.

4. Conclusion

Our study shows that LytA and Ply are very important virulence factors of *S. pneumoniae* that divert complement-mediated immunity allowing the bacterium an efficient dissemination to the systemic circulation.

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