Do Streptococcus pneumoniae and Respiratory Syncytial Virus synergise to promote invasive disease?

Khandaker Shadial, Laura Jacques1, Abbie-Jasmine Harrison2, Marie Yang1, Aras Radiolu1
1 Department of Clinical Infection, Microbiology and Immunology, 2Department of Infection Biology
Institute of Infection and Global Health, University of Liverpool, UK

Introduction

Streptococcus pneumoniae (Sp) and Respiratory Syncytial Virus (RSV) are two major pathogens commonly coexisting in respiratory secretions of patients presenting with acute respiratory infections. Though there is increasing evidence of a synergistic interplay between these two pathogens, the exact mechanisms remain obscure.

The aim of our study was to decipher how confection with RSV alters pneumococcal growth dynamics and host immune response and how this impact on the colonisation and invasive properties of Sp.

Experimental approaches

An integrative experimental approach encompassing murine models, in vitro cell culture systems and quantitative mass spectrometry technique was exploited to investigate pneumococcal growth dynamics and its interaction with host during RSV confection.

Results I. Pneumococcal density and dissemination during RSV confection in mice previously colonised with Sp

- Heightened RSV-induced weight loss with delayed recovery in Sp-RSV coinfected animals.
- Increased bacterial density and decreased clearance from the nasopharynx.
- Dissemination of pneumococci to the lungs, but not in the blood (blood data not shown here).
- Lower recruitment of macrophages and neutrophils but increased influx of regulatory T cells (Tregs) indicate coordinated effort to maintain Sp carriage in the nasopharynx.

Results II. Pneumococcal in vitro growth dynamics and epithelial barrier function during coinfection with RSV

- The presence of RSV promotes the growth of Sp in a dose-dependent manner, as depicted by the gradual shortening of the lag phase of the growth curve (Figure 3A).
- The integrity of a Detroit 562 epithelial monolayer remains unaffected upon Sp-RSV confection. In fact, measurements showed a higher transepithelial electrical resistance (TEER) in Sp-RSV coinfected cells, and lower Sp migration towards the basal compartment during dual infection.

Our results show that a number of keratin proteins of the intermediate filament family were upregulated upon Sp-RSV coinfection. These proteins were described as regulators of innate immunity and moderate cytoskeletal organisation during endocytosis — this may have contributed to the trans-cellular migration of Sp. Of particular interest, Unconventional myosin-VI (MYO6) protein was also upregulated. It acts as a cell-junction protein and appears to be involved in the very early stage of clathrin-mediated endocytosis in polystyrene epithelial cells, a phenomenon which was described in Sp endocytosis through epithelial barrier.

Several pneumococcal key proteins were upregulated during confection. KEGG pathway analysis determined that proteins involved in fatty acid metabolism and biosynthesis (cysK, fabF,fabG1,fabH1,fabF) were highly enriched. For instance, superoxide dismutase (soDA), histone-like DNA binding protein (HbpA), Thrombospondin (TTRX) proteins were upregulated which play a critical role against oxidative stress, affecting both the survival and the virulence of Sp. HbpA proposed to have a role in the pathogenesis of streptococcus-induced tissue inflammation by forming a soluble complex with lipotechoic acid. Among downregulated bacterial proteins, pneumolysin (pyA), and manganese ABC transporter substrate-binding protein (psaA) is noteworthy. Downregulated byA may indicate less autolysis and thus more survival of Sp at the infection site.

Conclusion

- Confection with RSV augments pneumococcal growth and density both in vivo and in vitro. Persistence of pneumococci in the nasopharynx may result from dysfunctional immune response characterised by the decrease recruitment of macrophage and/or neutrophil in our murine model. Increased accumulation of Sp may also play a role for maintenance of colonisation.
- Co-infection with Sp-RSV did not affect the epithelial integrity of a human nasopharyngeal cell monolayer in vitro, but instead, resulted in increased epithelial resistance. Enrichment of the cell-junction proteins during confection may explain these observations, but needs further clarification.
- Several pneumococcal proteins were upregulated in our proteomic analysis which are involved in bacterial metabolism as well as viability during oxidative stress, thus may impacting on bacterial survival which evident in our result in the form of increased density at infection site.

Bibliography