Pneumolysin is responsible for differential gene expression and modifications in the epigenetic landscape of primary monocyte derived macrophages
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Introduction: Pneumolysin (PLY), has shown to be a key virulence factor of *Streptococcus pneumoniae*, helping its transmission by manipulating host cells. While some aspects of the suppression of host immune responses are understood, its role in manipulating primary macrophages is still unknown. Targeting this manipulation, this study aims to understand the effects of PLY on the epigenetic landscape of macrophages and the associated transcriptional response.

Methods: Two *S. pneumoniae* serotype 2 strain D39 were used to infect human monocyte derived macrophages (MDMs), a PLY-deficient mutant and a reconstituted mutant which expresses PLY. After the assay Cytokines (TNF-α and IL-6) levels were measured by ELISA and RT-qPCR. Besides, differential gene expression was evaluated, and the abundance was used to determine active pathways. In addition, post translational modifications (PTMs) were analyzed by quantitative proteomics using mass spectrometry searching for different modifications. In the case of histones PTMs, nano-LC was used for the analysis.

Results: MDMs transcriptome changes with bacterial challenge. PLY deficient strains have the same intracellular viability as wild-type, allowing the comparison. PLY-dependent transcriptome changes are predominantly upregulated cell metabolism related genes, but also altered stress related genes. Importantly, tumor necrosis factors (TNF), heme oxygenase 1, NFkB and CD40 signaling networks were upregulated. Proteomics results showed that proteins involved in cell metabolism were upregulated in a PLY-dependent manner. Although tested, no PTM results are shown. Histone modifications were altered after bacterial challenge in a PLY-dependent manner. The mutation of PLY leads to an up-regulation of TNF-α, meaning that PLY decreases the inflammatory response, in a specific window of time. Treatments with histone deacetylase inhibitors lead to a reduction of TNF-α levels in PLY mutants, hinting to a putative mechanism for PLY action.

Conclusions: In summary, it is shown that pneumococci infection triggers epigenetic, transcriptional and translational responses, specially related with innate immune responses with PLY has a prominent role in modulating macrophage response to infection. PLY reduced TNF-α and IL-6 transcriptional responses, possibly through histone post-translational modifications. Finally, this report paves the way to novel therapeutic approaches, by modulating histone PTMs to improve infection host responses (**Figure 1**).

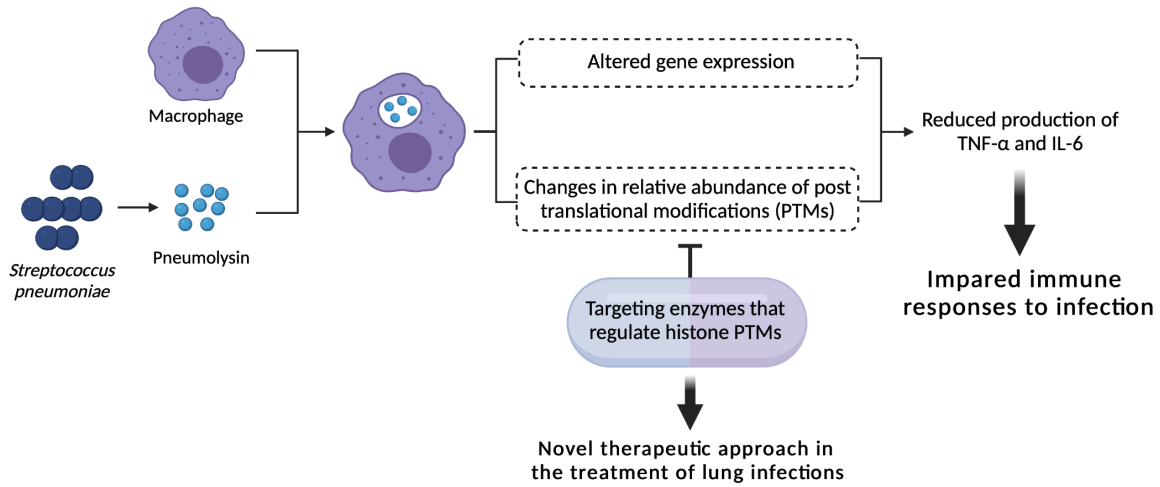

Figure 1 - During lung infection, *S. pneumoniae* releases the virulence factor PLY that will be recognized by macrophages. PLY alters gene expression in macrophages and promotes changes in the abundance of histone PTMs. Alteration of the epigenetic landscape has functional consequences to key immune responses impairing the infection resolution. Therefore, drugs targeting enzymes that regulate histone PTMs can be a novel therapeutic approach in the treatment of lung infections.