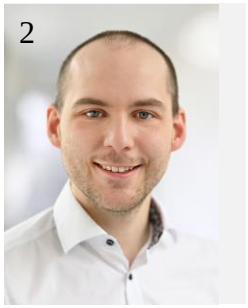
ImmunoTools *FlowISiAM* Award 2024



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Functional role and diagnostic potential of activated monocytes for pneumococcus-induced sepsis

Background: *Streptococcus (S.) pneumoniae* infection is the leading cause of community-acquired pneumonia (CAP). One of the key virulence factors of *S. pneumoniae* is pneumolysin (Ply), a cholesterol-dependent cytolysin and part of a family of toxins expressed in Gram-positive bacteria (1). Notably, when investigating pathogen prevalence in sepsis patients requiring intensive care, *S. pneumoniae* emerges as a leading pathogen associated with high mortality (2). Ply is released by *S. pneumonia* as a soluble monomer. These monomers subsequently undergo oligomerization and a radical, irreversible conformational change from helical to sheet, which is crucial for their transformation into membrane-insertable channels (3). *S. pneumoniae* produce and release Ply after reaching a high density in an autolytic process. Ply monomers then bind to surrounding membranes of host cells and polymerize to lytic pores. Due to the burst-like Ply release, infiltrating immune cells and parenchymal cells in the lung get lysed instantaneously, reducing the host's immune response, generating a nutrient-rich environment, and allowing the bacteria to break through barriers and spread (4). The significant lytic effects on its environment and immunosuppressive features make Ply a key bacterial virulence factor, rendering the invasiveness and severity of invasive pneumococcal diseases (IPD). The host recognizes Ply through pattern recognition and purinergic receptors (5-7). Additionally, Ply triggers the inflammasome (8). Those mechanisms are crucial for the host to initiate inflammatory and cellular host responses to pneumococci. Binding of Ply to cholesterol is independent from pore formation, and excess of cholesterol is able to scavenge Ply monomers, leading to cell detoxification (9). Indeed, Ply enhances early cholesterol synthesis as a potential cell-inherent mechanism of detoxification (10).

Aim: We hypothesize that Ply monomers and Ply-cholesterol complexes may appear early in infected CAP and IPD patients even before the proposed autolytic burst of the bacteria, and

that most of them are phagocytized by activated monocytes. In that case, the *FlowISiAM* technology may be used for early patient stratification. A separate project funded by the German Research Foundation (Project Nr. 550453157) is currently addressing the use of synthetic steroids for potential therapeutic intervention. The final goal would be to identify CAP and IPD patients infected with disseminating pneumococci expressing Ply that would benefit from a potential therapeutic intervention with synthetic steroids in order to prevent lytic effects early on. In addition, systemic presence of Ply may be a trigger for sepsis. A second goal would therefore be to identify a potential threshold of Ply-positive monocytes as a risk factor for sepsis. The identification of a systemic infection in CAP and IPD patients can be challenging, and the *FlowISiAM* technology could be a valuable diagnostic tool for sepsis stratification.

Methods: In a first set of experiments we will test the ability of activated monocytes in blood derived from healthy donors to phagocytize exogenously added Ply using the *FlowISiAM* technology with commercially available antibodies against Ply. In a second step we will titrate the concentration of Ply to determine the sensitivity of the applied *FlowISiAM* technology. We will then expand Ply spiking experiments with *S. pneumoniae* infection of blood cultures derived from healthy donors to determine the required bacteria titer and incubation time for early PLY detection in activated monocytes by the *FlowISiAM* technology. In a final step, we will design and apply a clinical study with blood samples derived from CAP and IPD patients to evaluate the *FlowISiAM* technology as a potential diagnostic tool for patient stratification.

Cooperation partner: Experiments will be conducted in the Department of Anesthesiology and Intensive Care Medicine of the University Hospital Jena under direction of Prof. Dr. Markus Gräler and Jun.-Prof. Dr. Adrian Press. ImmunoTools will provide technical support and provide reagents to conduct the proposed *FlowISiAM* technology. INVIGATE will support the selection of qualified antibodies against Ply and will provide further assistance for the optional development of custom antibodies in case of positive results.

References:

1. Cole J, Angyal A, Emes RD, Mitchell TJ, Dickman MJ, Dockrell DH. Pneumolysin Is Responsible for Differential Gene Expression and Modifications in the Epigenetic Landscape of Primary Monocyte Derived Macrophages. Front Immunol. 2021;12:573266.
2. Cilloniz C, Torres A, Niederman MS. Management of pneumonia in critically ill patients. BMJ. 2021;375:e065871.
3. Faraj BHA, Collard L, Cliffe R, Blount LA, Lonnen R, Wallis R, et al. Formation of pre-pore complexes of pneumolysin is accompanied by a decrease in short-range order of lipid molecules throughout vesicle bilayers. Sci Rep. 2020;10(1):4585.
4. Pereira JM, Xu S, Leong JM, Sousa S. The Yin and Yang of Pneumolysin During Pneumococcal Infection. Front Immunol. 2022;13:878244.

5. Malley R, Henneke P, Morse SC, Cieslewicz MJ, Lipsitch M, Thompson CM, et al. Recognition of pneumolysin by Toll-like receptor 4 confers resistance to pneumococcal infection. Proc Natl Acad Sci U S A. 2003;100(4):1966-71.
6. Olotu C, Lehmensiek F, Koch B, Kiefmann M, Riegel AK, Hammerschmidt S, et al. Streptococcus pneumoniae inhibits purinergic signaling and promotes purinergic receptor P2Y(2) internalization in alveolar epithelial cells. J Biol Chem. 2019;294(34):12795-806.
7. Srivastava A, Henneke P, Visintin A, Morse SC, Martin V, Watkins C, et al. The apoptotic response to pneumolysin is Toll-like receptor 4 dependent and protects against pneumococcal disease. Infect Immun. 2005;73(10):6479-87.
8. McNeela EA, Burke A, Neill DR, Baxter C, Fernandes VE, Ferreira D, et al. Pneumolysin activates the NLRP3 inflammasome and promotes proinflammatory cytokines independently of TLR4. PLoS Pathog. 2010;6(11):e1001191.
9. Kammann T, Hoff J, Yildirim I, Shkodra B, Muller T, Weber C, et al. Intracellularly Released Cholesterol from Polymer-Based Delivery Systems Alters Cellular Responses to Pneumolysin and Promotes Cell Survival. Metabolites. 2021;11(12).
10. Weber M, Lambeck S, Ding N, Henken S, Kohl M, Deigner HP, et al. Hepatic induction of cholesterol biosynthesis reflects a remote adaptive response to pneumococcal pneumonia. FASEB J. 2012;26(6):2424-36.

ImmunoTools *FlowISiAM* AWARD for

Markus H. Gräler and **Adrian T. Press** includes

antibodies for *FlowISiAM*, know how transfer and protocol, support regarding selection of specific antibodies against specific biomarkers from INVIGATE, expert assistance in evaluating the results obtained, and integration into the ImmunoTools *FlowISiAM* network.