INDUCTION OF APOPTOSIS AND PYROPTOSIS IN MACROPHAGES BY THE POLYSACCHARIDE CAPSULE OF ACINETOBACTER BAUMANNII

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Acinetobacter baumannii is a Gram-negative opportunistic bacterium that causes difficult-to-treat nosocomial infections. Due to its multidrug resistance and persistence in clinical environments, *A. baumannii* is classified by the World Health Organization as a critical priority pathogen [1]. One of the key virulence factors of *A. baumannii* is the polysaccharide capsule, which enables evasion of host immune defences [2], [3].

Macrophages are among the first innate immune effectors and play an important role in controlling bacterial spread. These cells are involved in phagocytosis, antigen presentation, and regulation of inflammation [4]. During infection, macrophages can undergo different inflammatory and non-inflammatory forms of cell death. The balance between these pathways influences the outcome of the immune response [5]. This study aimed to investigate how the polysaccharide capsule of *A. baumannii* modulates apoptotic (non-inflammatory) and pyroptotic (inflammatory) cell death pathways in macrophages.

We used a capsulated clinical *A. baumannii* strain and its non-capsulated mutants. *In vitro* infections were performed using murine J774A.1 and human THP-1 macrophages. Viability of macrophages was assessed by fluorescence microscopy, while apoptosis and pyroptosis markers were analysed by Western blot. Apoptosis was evaluated by detecting activation of the effector caspase-3 (Casp-3), while pyroptosis was assessed by detecting formation of the N-terminal gasdermin D fragment (GSDMD-N) and secretion of the pro-inflammatory cytokine interleukin-1β (IL-1β).

The non-capsulated Δ*galU* mutant resulted in increased viability of J774A.1 and THP-1 macrophages and induced weaker activation of Casp-3 compared to the capsulated strain. To assess whether this effect is strain-dependent, Casp-3 activation was also analysed using another capsulated clinical isolate and its non-capsulated Δ*wza* mutant. The study revealed that the Δ*wza* non-capsulated mutant likewise induced significantly weaker Casp-3 activation. In contrast, loss of the polysaccharide capsule did not significantly affect GSDMD-N formation and IL-1β secretion in J774A.1 and THP-1 cells.

Our findings show that the loss of *A. baumannii* polysaccharide capsule significantly increased macrophage viability and reduced activation of the effector apoptotic caspase-3 in both cell lines. However, the loss of bacterial capsule did not affect pyroptotic responses. These results indicate that the polysaccharide capsule of *A. baumannii* induces apoptotic rather than pyroptotic signalling in J774A.1 and THP-1 macrophages during infection.

Acknowledgements

This project has received funding from the Research Council of Lithuania (LMTLT), agreement No S-MIP-23-109.

[1] S. Corcione et al., "Risk factors for mortality in Acinetobacter baumannii bloodstream infections and development of a predictive mortality model," Journal of Global Antimicrobial Resistance, vol. 38, pp. 317–326, Jul. 2024, doi: 10.1016/j.jgar.2024.06.010.

[2] J. Skerniškytė, R. Krasauskas, C. Péchoux, S. Kulakauskas, J. Armalytė, and E. Sužiedėlienė, "Surface-Related Features and Virulence Among Acinetobacter baumannii Clinical Isolates Belonging to International Clones I and II," Frontiers in Microbiology, vol. 9, p. 3116, Jan. 2019, doi: 10.3389/fmicb.2018.03116.

[3] R. Krasauskas, J. Skerniškytė, J. Martinkus, J. Armalytė, and E. Sužiedėlienė, "Capsule Protects Acinetobacter baumannii From Inter-Bacterial Competition Mediated by CdiA Toxin," Frontiers in Microbiology, vol. 11, p. 1493, Jul. 2020, doi: 10.3389/fmicb.2020.01493.

[4] J. Brancewicz, N. Wójcik, Z. Sarnowska, J. Robak, and M. Król, "The multifaceted role of macrophages in biology and diseases," International Journal of Molecular Sciences, vol. 26, no. 5, p. 2107, Feb. 2025, doi: 10.3390/ijms26052107.

[5] M. Kist and D. Vucic, "Cell death pathways: intricate connections and disease implications," The EMBO Journal, vol. 40, no. 5, p. e106700, Jan. 2021, doi: 10.15252/embj.2020106700.