CD4$^+$ T cell recognition of pneumolysin, a pore-forming cytolysin derived from *Streptococcus pneumoniae* presented by a common HLA allotype

Lisa Ciacchi[1], Martijn D. B. van de Garde[2], Jan Petersen[1], Carine Farenc[1], Martien C. M. Poelen[2], Hugh H. Reid[1], Kristin Ladell[3], David A. Price[3], Cecile A. C. M. van Els[2] & Jamie Rossjohn[1]

[1]Monash Biomedicine Discovery Institute, Monash University, Clayton, Australia,
[2]Centre for Infectious Disease Control, National Institute for Public Health and the Environment, Bilthoven, The Netherlands,
[3]Cardiff University School of Medicine, Cardiff, United Kingdom

lisa.ciacchi@monash.edu

Infection with the pathobiont *Streptococcus pneumoniae* (pneumococcus) can cause life-threatening invasive pneumococcal diseases (IPD), including pneumonia, sepsis, and meningitis [1-3]. With the emergence of new pneumococcal strains, there is an urgent need for vaccines that elicit broader population coverage against conserved pneumococcal antigens, irrespective of capsular serotype. Pneumolysin (Ply) is a key pneumococcal virulence factor belonging to a family of cholesterol-dependent cytolysins (CDCs) that disrupts host cell defence mechanisms and immune cell function. This cytotoxin is expressed by virtually all pneumococcal strains and pneumococcal carriage and infection induce natural immunity to Ply. A detoxified form of this protein has therefore been tested as a potential serotype-independent vaccine candidate to protect against IPD [4-6].

In this study, we identified a highly immunogenic human CD4$^+$ T cell epitope in pneumolysin, widely presented by a common HLA allotype. The nature of the Ply-specific T cell receptor (TCR) repertoire was evaluated in healthy HLA-typed individuals. HLA-Ply-specific tetramer$^+$ CD4$^+$ TCRs were cloned, expressed, and purified. The ternary structures of three TCRs, including examples of near-public (B1) and private sequences (B5 and 5F), were solved in complex with HLA-Ply.

All of these TCRs formed stabilizing contacts with solvent-exposed residues in the central region of the peptide via their hypervariable CDR3 loops. The immunodominance of this epitope can therefore be explained by the preferential selection of TCRs capable of this ubiquitous mode of recognition.

[1] Adams, W., Bhowmick, R., Bou Ghanem, E. N., Wade, K., Shchepetov, M., Weiser, J. N., McCormick, B. A., Tweten, R. K. & Leong, J. M. (2020). *J. Immunol.* **204**, 101.

[2] Backhaus, E., Berg, S., Andersson, R., Ockborn, G., Malmström, P., Dahl, M., Nasic, S. & Trollfors, B. (2016). *BMC Infect. Dis.* **16**, 367.

[3] Kaur, R., Surendran, N., Ochs, M. & Pichichero, M. (2014). *Infect. Immun.* **82**, 5069.

[4] Salha, D., Szeto, J., Myers, L., Claus, C., Sheung, A., Tang, M., Ljutic, B., Hanwell, D., Ogilvie, K., Ming, M., Messham, B., van den Dobbelsteen, G., Hopfer, R., Ochs, M. M. & Gallichan, S. (2012). *Infect. Immun.* **80**, 2212.

[5] van de Garde, M. D. B., van Westen, E., Poelen, M. C. M., Rots, N. Y. & van Els, A. C. M. (2019). *Infect. Immun.* **87**, e00098.

[6] Rossjohn, J., Gilbert, R. J. C., Crane, D., Morgan, P. J., Mitchell, T. J., Rowe, A. J., Andrew, P. W., Paton, J. C., Tweten, R. K. & Parker, M. W. (1998). *J. Mol. Biol.* **284**, 1223.

Keywords: TCR repertoire; cholesterol-dependent cytolysins; CD4+ T cell epitope; pneumolysin

We wish to acknowledge Josien Lanfermeijer from the RIVM/IIV for technical support, Frans Reubsaet from RIVM/IDS for providing clinical bacterial isolates, Sanofi-Pasteur for providing the peptide, and the NIH Tetramer Core Facility. We thank the staff at the Monash Macromolecular Crystallisation Facility and the Australian Synchrotron (beamlines MX1 and MX2) for assistance with crystallisation and data collection, respectively.