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Structural investigation of T cell Tolerance.
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During selection of the T cell repertoire, the immune system navigates the subtle distinction between self-restriction and self-tolerance, yet how this is achieved is unclear. We studied how self-tolerance toward a trans-HLA (human leukocyte antigen) allotype shapes T cell receptor (TCR) recognition of an Epstein-Barr virus (EBV) determinant (FLRGRAYGL named as FLR).
The X-ray crystallography structure of the immunodominant LC13 TCR in complex with HLA-B8-FLR showed a very C terminally focuses docking of this TCR [1]. The LC13 TCR is self-reactive with HLA-B44, so the HLA-B8+/HLA-B44+ individuals use another T cell repertoire to recognize the HLA-B8-FLR complex. In order to understand how this new T cell repertoire lacks to self-react with HLA-B44, we were interested to study the atomic level of the interaction between one TCR of this new repertoire and the HLA-B8 in complex with the EBV antigen, FLR.
We have solved by X-ray crystallography the structure of the CF34 TCR, from HLA-B8+/HLA-B44+ individuals, in complex with the HLA-B8-FLR at 2.80Å resolution [2].
This structure shows that the CF34 TCR docked at the N terminus of HLA-B8-FLR, whereas the alloreactive LC13 TCR docked at the C terminus of HLA-B8-FLR.
The CF34 TCR docking onto the peptide-MHC coincided with a polymorphic region between HLA-B8 and HLA-B44 allowing CF34 to "see" the difference between the two MHCs. To further test this hypothesis we have performed an extensive mutagenesis study on the HLA-B8 molecule in order to map the energetic footprint of those two TCRs and we have also add to this study another TCR, named RL42 from HLA-B8/B44+, for which we solved the structure in complex with HLA-B8-FLR. All together those unpublished data shed some light on the T cell Tolerance mechanism.

[1] Kjer-Nielsen L et al., Immunity, 2003. [2] Gras S et al., Immunity, 2009.

Keywords: immune system, TCRpMHC, Epstein Barr-virus

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Structure-based Discovery of Antivirals Targeting the Proteases of RNA Viruses.
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Current methods in structure-based discovery of antiviral lead compounds include *de-novo* design, virtual screening, fragment screening, and structure-guided medicinal chemistry, all of which require a detailed knowledge of the structure and function of the viral target proteins. We focus on the 3C and 3C-like proteases of enteroviruses and coronaviruses, resp., as targets for drug discovery [1-9]. The combined application of X-ray crystallography, STD-NMR spectroscopy, structure-guided medicinal chemistry, and viral replicons in our laboratories proved essential to carry several antivirals through preclinical development. Some of the compounds that are in animal tests at this time exhibit very broad activities against a wide range of positive-strand ssRNA viruses and might be of use in case of future zoonotic transmissions of RNA viruses to humans. The structural studies also allow us to predict the emergence of resistance mutations that would undoubtedly arise during wide-spread application of the new antivirals and suggest ways to cope with them by modifying the inhibitors.

[1] Steuber H., Hilgenfeld R. *Curr. Top. Med. Chem.,* 2010, 23, 323. [2] Chen S. et al. *Protein & Cell,* 2010, 1, 59. [3] Verschueren K.H.G. et al., *Chem. Biol.,* 2008*, 15*, 597. [4] Al-Gharabli S.I. et al., ChemBioChem, 2006, 7, 1048. [5] Tan J. et al., *J. Mol. Biol*. 2005, *354*, 25. [6] Anand K. et al. *Science,* 2003, *300*, 1763. [7] Yang H. et al., *Proc. Natl. Acad. Sci. USA,* 2003, *100*, 13190. [8] Mesters JR et al. *Curr. Opin. Struct. Biol.,* 2006, *16*, 776. [9] Schmidt M.F. et al., Angew. Chem. Int. Ed. Engl., *2008*, 47**, 3275.**

Keywords: Antiviral Drug Design, Viral Proteases, Fragment Screening

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Incorporation of the basal pilin FctB into the pilus of *Streptococcus pyogenes.*
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Many pathogenic and commensal bacteria express pili, long hair-like protein polymers that enable adhesion to host tissues. The pili of Gram-positive bacteria are unique as they are assembled by specialised transpeptidases (sortases) through covalent linkages. In the common human pathogen *Streptococcus pyogenes*, the major pilin FctA forms the polymeric pilus fibre, while the minor pilin Cpa is the adhesin found at the pilus tip. The pilus is anchored to the streptococcal cell wall through the basal pilin FctB. Here, we present the crystal structure of FctB at 1.9 Å resolution. It reveals an immunoglobulin (Ig)-like N-terminal domain with an extended proline-rich tail. Surprisingly, the Ig-like domain is structurally homologous to the N-terminal domain of the major pilin FctA. This led to the identification of a conserved lysine residue as the putative site of covalent linkage to the pilus assembly, which we confirmed by mass spectrometry analysis of complete *S. pyogenes* pili. The C-terminal tail

forms a polyproline-II-like helix that seems to be a common feature of many Gram-positive cell-wall anchored virulence factors, and particularly of basal pilins. Together, we identified structural characteristics of pilins that direct their incorporation into the pilus polymer.

Keywords: bacterial adhesion, pilus assembly, *Streptoccocus pyogenes*

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Identification, characterisation and exploitation of novel Gram-negative drug targets. Gunter Schneider, Tatyana Sandalova, Jolanta Kopec, Jason Schmidberger, Robert Schnell. *Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden.*
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Increases in the rates of bacterial infection and resistance to available antibiotics present an alarming health-problem worldwide. The situation is particularly serious with respect to Gram-negative bacteria and the identification and structural characterisation of novel, genetically validated drug targets for these bacteria would represent a major advance in biomedical science. The EC-funded AEROPATH project aims to contribute to early stage drug discovery by advancing, at the molecular level, fundamental and important aspects of Gram-negative bacteria using the important pathogen model *Pseudomonas aeruginosa*. Secondly the consortium wishes to exploit these discoveries and develop inhibitors as hit and lead compounds for further antibiotic development. The consortium consists of the University of Dundee, University of St. Andrews, Lionex GmbH, MFD Diagnostics GmbH, and Karolinska Institutet. Target validation is based on gene knock-out studies focusing on individual genes flagged as essential and an infection model in the mouse. Hits will be identified by virtual screening based on three-dimensional structures, HTS screens at the Scottish Hit Discovery Facility, and fragment-based screening using a combination of thermofluor/Stargazer technologies, NMR and X-ray methods. Enzyme inhibition assays, x-ray crystallography and Isothermal Calorimetry are used to characterize the binding mode and the kinetic parameters of the identified ligands. The progress on the 43 targets assigned to the consortium partner KI will be presented.

Keywords: infectious diseases, macromolecular crystallography, lead compounds