Université de la Méditerrannée, INSERM, U631, CNRS UMR6102, F-13288 Marseille, France.
E-mail: jean-baptiste.reiser@ibs.fr

Despite their tremendous potential diversity, T cell responses directed against defined major histocompatibility complex presented peptides (pMHC) are sometimes dominated by clones bearing highly related or identical «public» T cell receptors (TCR) in unrelated individuals. Such a selection of high avidity public T cell clones is observed along chronic human cytomegalovirus (HCMV) infections and favors an efficient immune response [1]. Understanding the principles that guide TCR repertoire diversity is essential to control the efficacy of the cellular adaptive immune response.
Through structural, biophysical and functional analyses of human TCRs recognizing a major HLA-A*0201-restricted antigen from HCMV, $pp65_{495-503}$, we show that a public TCR (RA14) selected from a diverse repertoire after chronic stimulations, interacts with the full array of available peptide residues and specifically focuses on three of them. Our data fully support the preferred selection of specific sets of V(D) J gene segments for the recognition of this HCMV antigen. Furthermore, most of RA14 TCR contacting amino acids are conserved by lower affinity TCRs, suggesting a shared TCR-pMHC docking mode and an antigen-driven selection of the best-fitted TCR.
Our present study thus highlights the structural characteristics that could explain the immunodominance of the RA14 TCR in response to $pp65_{495-503}$-HLA-A2 in an immunodepressed context associated with HCMV reactivation [2]. The mechanism contrasts from that observed for the immunodominant MP_{58-66}-HLA-A2 and FLR-HLA-B8 pMHC since the quasi-unique TCRs used for their recognition essentially interact with a unique feature on the pMHC surface [3,4]. Instead, the emergence of an optimal public solution out of an oligoclonal antigen specific response after repeated HCMV stimulations is based on a TCR with a very favorable structural complementarity with the entire peptide and focused interactions with three peptide hotspot. As these high avidity TCRs highly contribute to protective immunity, our findings should help in the development of effective vaccines against HCMV infection, which remains a key health issue in patients undergoing bone marrow transplantation.

[1] Trautmann, L. et al. *J Immunol*, **2005**, 175(9), 6123-6132. [2] Gras, S et al *J. Immunol*, **2009**, To be published. [3] Stewart-Jones, G. B. et al. *Nature immunology*, **2003**, 4(7), 657-663. [4] Kjer-Nielsen, L. et al. *Immunity*, **2003**, 18(1), 53-64

Keywords : structures of T cell receptor; HLA; virus host interactions

FA1-MS05-P04

The SARS-unique Domain of SARS-CoV Contains Two Macrodomains that Bind G-quadruplexes.

Jinzhi Tan[a], Clemens Vonrhein[b], Oliver S. Smart[b], Gerard Bricogne[b], Michela Bollati[a], Yuri Kusov[a], Guido Hansen[a], Jeroen R. Mesters[a], Christian L. Schmidt[a], Rolf Hilgenfeld[a,c]. [a]*Institute of Biochemistry,Center for Structural and Cell Biology in Medicine, University of Lübeck, Germany.* [b]*Global Phasing Ltd., Cambridge, UK.* [c]*Laboratory for Structural Biology of Infection and Inflammation, c/o DESY, Hamburg, Germany.*
E-mail: jztan@biochem.uni-luebeck.de

The SARS coronavirus (SARS-CoV) is much more pathogenic for humans than any other coronavirus. Therefore, protein domains encoded by the SARS-CoV genome that are absent in other coronaviruses are of particular interest, because they may be responsible for the extraordinary virulence. The most prominent such domain has been identified by bioinformatics as part of non-structural protein 3 (Nsp3) of the virus and appropriately named the "SARS-unique domain" (SUD) [1]. Through the efforts of several laboratories around the world, the structures of a number of non-structural proteins of the SARS coronavirus replicase/transcriptase complex have been determined. However, the SUD has escaped all attempts at crystallizing it because of its instability.
After construction of many fragments, we have managed to obtain the core of the SUD in a crystalline form, and determined its X-ray structures at 2.2 and 2.8 Å resolution, respectively. This revealed that SUD_{core} contains two copies of the so-called macrodomain. Furthermore, we have shown that each of these, as well as the entire SUD_{core} and full-length SUD, specifically bind to G-quadruplexes, both in the DNA and RNA form [2,3]. G-quadruplexes occur in the 3'-nontranslated regions of mRNAs coding for host cell proteins involved in apoptosis or signal transduction [4]. By mutational studies, we could show that replacement of pairs of lysine residues on the C-terminal subdomain of SUD_{core} abolished binding of G-quadruplexes completely.
There is also a link to poly(ADP-ribose) polymerase (PARPs), as the structure of the SUD subdomains show some distant similarity to the catalytic domain of these enzymes, and other macrodomains have been shown to bind ADP-ribose (whereas SUD does not). Further, PARP-1 also binds G-quadruplexes [5] and PARP domains exist in the antiviral protein ZAP, which is active against viruses containing a macrodomain (e.g. alphaviruses and coronaviruses). We speculate that SARS-CoV could inactivate ZAP by competing with it for G-quadruplex regions in RNA, suggesting a way for the SARS virus to fight the innate immune system of the host cell.

[1] Snijder E.J., Bredenbeek P.J., Dobbe J.C., Thiel V., Ziebuhr J., et al. *J. Mol. Biol.*, **2003**, 331, 991. [2] Tan J., Kusov Y., Mutschall D., Tech S., Nagarajan K., Hilgenfeld R., Schmidt C.L. *Biochem. Biophys. Res. Commun.* **2007**, 364, 877. [3] Tan J., Vonrhein C., Smart O.S., Bricogne G., Bollati M., Kusov Y., Hansen G., Mesters J.R., Schmidt C.L., Hilgenfeld R. *PLoS Path.*, **2009**, in press. [4] Han J.W., Flemington C., Houghton A.B., Gu Z., Zambetti GP, Lutz R.J., Zhu L., Chittenden T. *Proc. Natl. Acad. Sci. USA,* **2001**, 98, 11318. [5] Soldatenkov V.A., Vetcher A.A., Duka T., Ladame S. *ACS Chem Biol.*, **2008**, 3, 214.

Keywords: SARS coronavirus; SARS-unique domain; G-quadruplex