MS.50.1

Acta Cryst. (2011) A**67,** C118

Microbial glycolipid antigen recognition by invariant natural killer T cells

Dirk M. Zajonc,[a] Yali Li,[a] Enrico Girardi,[a] Esther Dawen Yu,[a] Jing Wang,[a] Gavin F. Painter,[b] Petr Illarionov,[c] Yuki Kinjo,[d] Mitchell Kronenberg,[e] *[a]Division of Cell Biology, La Jolla Institute for Allergy and Immunology, La Jolla, CA 92037 (USA). [b]Carbohydrate Chemistry Team, Industrial Research Limited, Lower Hutt (New Zealand). [c]School of Biosciences, University of Birmingham, Edgbaston (UK) [d]Department of Chemotherapy and Mycoses, National Institute for Infectious Diseases, Tokyo (Japan). [e]Division of Developmental Immunology, La Jolla Institute for Allergy and Immunology, La Jolla, CA 92037 (USA).* E-mail: dzajonc@liai.org

Invariant Natural Killer T (iNKT) cells are an evolutionary conserved T cell population characterized by features of both the innate and adaptive immune response. Studies have shown that iNKT cells are required for protective responses to pathogens such as *Borrelia burgorferi* and *Streptococcus pneumoniae*, and that these cells recognize bacterial diacylglycerol antigens presented by CD1d, a non-classical antigen presenting molecule.

Here we report the first crystal structures of the iNKT cell TCR bound to various natural, microbial glycolipids presented by CD1d [1], [2]. Binding of the TCR induced complementarity determining region 3 (CDR3) dependent structural changes in the F' roof of CD1d; these changes resemble those occurring in the absence of TCR engagement when the highly potent synthetic antigen alpha-galactosylceramide (alpha-GalCer) binds CD1d [3]. Furthermore, TCR binding caused a marked repositioning of the sugar headgroups into an orientation that closely resembles alpha-GalCer. The TCR-dependent re-orientation of the sugar moieties, together with the induced CD1d fit, help explain the weaker potency of the microbial antigens compared to alpha-GalCer.

Our studies have established that the TCR of iNKT cells binds with a conserved footprint onto CD1d, regardless of the bound glycolipid antigen, and that for microbial antigens this unique binding mode requires TCR-initiated conformational changes. Therefore, not only do our studies illuminate the mechanism of glycolipid recognition for antigens from important pathogens, but also they have important implications for the development of immunomodulatory compounds that act on iNKT cells.

[1] Y. Li, E. Girardi, J. Wang, E.D. Yu, G.F. Painter, M. Kronenberg, D.M. Zajonc. *J Exp Med* **2010,** *207*, 2383-2393. [2] E. Girardi, E.D. Yu, Y. Li, N. Tarumoto, B. Pei, J. Wang, P. Illarionov, Y. Kinjo, M. Kronenberg, D.M. Zajonc. *submitted* **2011.** [3] D.G. Pellicci, O. Patel, L. Kjer-Nielsen, S.S. Pang, L.C. Sullivan, K. Kyparissoudis, A.G. Brooks, H.H. Reid, S. Gras, I.S. Lucet, R. Koh, M.J. Smyth, T. Mallevaey, J.L. Matsuda, L. Gapin, J. McCluskey, D.I. Godfrey, J. Rossjohn. *Immunity* **2009,** 31,47-59.

Keywords: glycolipid antigen, CD1d, TCR recognition

MS.50.2

Acta Cryst. (2011) A**67,** C118

Understanding cytokine receptor signalling

Michael W. Parker,[a] Sophie E. Broughton,[a] Guido Hansen,[a] Jack King-Scott,[a] Timothy R. Hercus,[b] Angel F. Lopez,[b] *[a]St. Vincent's Institute of Medical Research, Fitzroy, Melbourne, Victoria (Australia). [b]Division of Human Immunology, Centre for Cancer Biology, Adelaide, South Australia (Australia).* E-mail: mparker@svi.edu.au

The granulocyte-macrophage colony stimulating factor (GM-CSF), interleukin-3 (IL-3) and IL-5 family of cytokines regulates the survival, proliferation, differentiation and functional activation of hematopoietic cells [1], [2]. These same cytokines have also been implicated in multiple pathologies resulting from the excessive or aberrant expression of the cytokine or their receptors, in conditions such as arthritis, asthma, autoimmunity and leukaemia. The receptors for these cytokines are expressed on the surface of hematopoietic cells and comprise a cytokine-specific alpha subunit and a beta subunit that is common to all three receptors. The alpha subunit binds cytokine with low affinity forming a complex that is able to recruit the beta subunit, converting the binding to a high affinity state.

We recently determined the structure of a GM-CSF:receptor ternary complex, representing the first structure of an "activated" receptor of this family of cytokines [3]. Inspection of the structure revealed novel insights into the mechanism of receptor activation whereby the receptor likely signals via higher order networks. This model of signalling provides a unifying molecular explanation for the diverse functional properties of related cytokine:receptor systems. We have now determined the structure of the binary complex of cytokine bound to alpha chain which highlights the importance of the N-terminal domain of the alpha chain in the assembly of the GM-CSF receptor into a signalling competent state. In more recent studies we have shown that IL-3 assembles into a similar ternary complex to that of the GM-CSF receptor suggesting a general paradigm for the whole family.

[1] T.R. Hercus, D. Thomas, M.A. Guthridge, P.G. Ekert, J. King-Scott, M.W. Parker, A.L. Lopez, *Blood* **2009**, *114*, 1289-1298. [2] A.F. Lopez, T.R. Hercus, P. Ekert, D.R. Littler, M. Guthridge, D. Thomas, H.S. Ramshaw, F. Stomski, M. Perugini, R. D'Andrea, M. Grimbaldeston, M.W. Parker, *IUBMB Life* **2010**, *62*, 509-518. [3] G. Hansen, T.R. Hercus, B.J. McClure, F.C. Stomski, M. Dottore, J. Powell, H. Ramshaw, J.M. Woodcock, Y. Xu, M. Guthridge, W.J. McKinstry, A.F. Lopez, M.W. Parker, *Cell* **2008**, *134*, 496-507.

Keywords: cytokine, immunology, cell signalling

MS.50.3

Acta Cryst. (2011) A**67,** C118-C119

Mechanistic insights into the complement system

Piet Gros, Federico Forneris, Michael Hadders, Jin Wu, *Crystal and Structural Chemistry, Bijvoet Center, Utrecht University, Utrecht (The Netherlands).* E-mail: p.gros@uu.nl

The complement system is an integral part of the innate immune defense in mammals that enables the host to lyse and clear invading pathogens and altered host cells from blood and interstitial fluids, while protecting healthy host cells and tissue. Complement is formed by ~30 large multi-domain plasma proteins and cell-surface receptors. Through structural studies we have revealed the molecular mechanisms responsible for the central amplification steps, the host protection by complement regulators and the initial event in formation of the membrane-attack complex (MAC).

Structures of the central complement component C3 (1,641 res.) and its activated form C3b revealed an intricate domain arrangement and marked conformational changes that lead to covalent attachment of C3b to target cells labeling these cells for immune clearance [1,2]. Amplification of the labeling is performed by labile protease complexes, called C3 convertases, which are formed on the target cell surfaces by an interplay of complex formation and proteolysis [3,4,5]. The resulting active convertase (with half-life time of ~90 s) consists of C3b in complex with fragment Bb of factor B, where C3b likely provides a major binding site for the substrate C3 though C3b:C3 dimerization [6]. Complement regulators, like factor H, bind to C3b through a long extended interface and disrupt the C3b-Bb complex yielding "decay-