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Microbial glycolipid antigen recognition by invariant natural killer T cells

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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.

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Understanding cytokine receptor signalling

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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.

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Mechanistic insights into the complement system

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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-

accelaration activity" or by binding protease factor I in "co-factor activity" to degrade C3b into inactive iC3b [7]. Non-protected labelled surface, on the other hand, trigger the terminal pathway of complement activation, which is started by cleavage of C5 (a homologue of C3) into C5b and association with MAC-protein C6 into C5b6. C5b6 in turn binds other MAC proteins C7, C8 [8] and multiple C9s causing MAC formation and cell lysis.

[1] B.J.C. Janssen *et al., Nature* **2005**, *437,* 505-511. [2] B.J.C. Janssen *et al., Nature* **2006,** *444***,** 213-216. [3] F.J. Milder *et al., Nature SMB* **2007***, 14,* 224-228. [4] B.J.C. Janssen *et al. EMBO J.* **2009**, *28*, 2469-2479. [5] F. Forneris *et al., Science* **2010***, 330*, 1816-1820. [6] S.H.M. Rooijakkers, J. Wu *et al., Nature Immunology* **2009***, 10,* 721-727. [7] J. Wu *et al., Nature Immunology* **2009,** *10,* 728-733. [8] M.A. Hadders *et al.*, *Science* **2007,** *317*,1552-1554.

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Structural basis of disease resistance in flax against flax rust

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Plant diseases are a major issue for economical important crops worldwide. Plant immunity is triggered by the recognition of a pathogen effector protein by a plant resistance (R) protein, leading to the activation of plant defences, which often culminate in a localized cell death response.

The R proteins can be divided into a few conserved families, while the effectors are diverse in both sequence and structure, and have roles in virulence. Recognition of effectors by R proteins, and the subsequent activation and downstream signaling events, are poorly understood at the molecular and structural level. We have used the interaction between flax, and the fungal pathogen, flax rust, as a model system to characterize this process. The flax-R proteins consist of a central nucleotide-binding (NB) domain, a C-terminal leucine-rich-repeat (LRR) domain, and an N-terminal Toll-interleukin-receptor like (TIR) domain. The LRR domains of flax R proteins are involved in direct interaction with corresponding flax-rust effectors [1,3], while the NB and TIR domains have roles in activation and signalling, respectively.

Here, we report the first crystal structure of a TIR domain from a plant R protein (L6) at 2.3 Å resolution [4]. The structure reveals important differences from the structures of mammalian and bacterial TIR domains. Analysis of the structure combined with site-directed mutagenesis suggests that TIR domain self-association is a requirement for immune signaling, and reveals distinct surface regions involved in self-association, signaling, and autoregulation.

We have also determined crystal structures of two different variants of the flax rust-effector protein AvrM. One of these variants AvrM-A, is recognized by the M resistance protein in flax, which results in activation of a necrotic immune response. The second variant avrM, is not detected by M and promotes disease. Both structures have a novel L-shaped helical fold, with two chains forming a dimer with an unusual non-globular shape. Comparison of the two structures provides insight into the structural basis of effector recognition by R proteins.

Our results bring us a step closer to understanding the molecular basis for the disease resistance process in plants, which is a prerequisite for the future engineering of novel resistance specificities into commercially important crops.

[1] *Dodds et al., Proc Natl Acad Sci USA* **2006,** *103*, 8888. [2] Catanzariti et al *Mol Plant Microbe Interact* **2010,** *23,* 49. [3] Wang et al *Plant Cell* **2007,** *19*, 2898. [4] Bernoux and Ve et al., *Cell Host and Microbe* **2011,** *9*, 200.

Keywords: immunobiology, crystallography, plants

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Structure of Complement C6 Suggests a Universal Model for Pore Formation by Cytolysins

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The Membrane Attack Complex (MAC) forms lytic pores in the outer membranes of Gram-negative bacteria. Pore formation begins with conversion of C5 to C5b, which promotes assembly of an initiation complex on the membrane surface comprising 4 structural elements: C6, C7, C8 α and C8 β, each of which contains a "MACPF" domain, a building block of the circular pore. This complex then recruits ~10 copies of a 5th MACPF domain protein, C9 forming membrane-spanning β-barrel. The transformation of MACPF domain from a pre-pore to a pore conformation leads to unwinding of two helical subdomains, CH1 (TMH1) and CH2 (TMH2), to form transmembrane β-hairpins. The structural and regulatory principals of initiation and propagation of assembly and membrane insertion are poorly understood. Each structural protein has "auxiliary" domains N- and C-terminal to the MACPF domain whose role has thus far been unclear. Here we describe the atomic resolution structure of full-length C6, the longest of the structural proteins, which shows how N- and C-terminal auxiliary domains cooperate to stabilize a "closed" conformation of the central β-sheet of the MACPF domain, while locking the membrane-inserting elements into their "pre-pore" state. On the basis of structural comparisons with C8 and perforin, we propose a model in which the "opening" of the β-sheet is key to initiating and propagating assembly. We show that only open conformations of the β-sheets are capable of forming a circular concatamer in which the edges of each β-sheet are shared with its neighbors, forming a single contiguous barrel. As the pore is constructed, the exposed edge of an open sheet provides a template that promotes opening of the next MACPF domain to join the ring, thus propagating assembly. Comparisons with the 3 structurally-related classes of cytolysins, (Perforin, *Photorhabdus luminescens* (PLU) and the cholesterol dependent cytolysin, PFO) suggest that they adopt a remarkably similar 3-dimensional organization and regulatory mechanism, despite differences in the tertiary folds of the auxiliary domains and their location in the primary sequence (N- versus C-terminal to the MACPF domain). This similarity includes a special domain at the "base" of C6 that may mediate initial membrane attachment and position it at an optimal height for membrane insertion, as well as the domain that both locks the β-sheet in its closed conformation and the membrane-inserting elements in their pre-pore conformation.

Keywords: complement, immunology