retained in the class II MHC peptide binding pocket until it is replaced by an antigen in the endosome. CLIP serves to stabilize the MHC heterodimer and also prevents binding of endogenous peptides. CLIP-to-antigen exchange, mediated by HLA-DM, is a key determinant of subsequent immunological events. HLA-CLIP stability is thought to be an important factor for triggering autoimmunity [1,2]. We have analyzed the non-covalent interaction between HLA-DQ2.5 and CLIP in order to determine the molecular basis for the unusually long half-life of the HLA-DQ2.5-CLIP complex. This is the first HLA-DQ-CLIP structure to be reported.

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Keywords: HLA-DQ2.5, autoimmunity, CLIP

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Structure, function and evolution of the serum pentraxins

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Wide-ranging studies on the serum pentraxins C-reactive protein (CRP) and serum amyloid P component (SAP) are aimed at the investigation of the structural, functional and evolutionary relationships, and of the humoral and cellular recognition properties, of the pentraxins from species ranging from invertebrate to man. Recognition properties of the pentraxins, homologues of which have been found in mammals, fish, amphibians, and invertebrates, include cell wall phospholipids and fungal and bacterial polysaccharides. In the absence of highly developed adaptive immunity, a diverse array of humoral components, such as the pentraxins, provides an essential and effective strategy for recognising and destroying disease-causing pathogens. In humans CRP, when bound to a suitable physiological ligand, binds C1q and efficiently activates classical complement.

The structures of pentraxins from man [1,2] and *Limulus polyphemus* [3] reveal variable aggregation of the conserved protomer fold. Unusually LimSAP displays two distinct molecular aggregations for the same molecule, a doubly stacked octamer and a doubly stacked heptamer. Although sequence homology with human SAP is relatively low, structural homology is high. This is due in part to a “topological” equivalence of side chain position. Upon binding phosphoethanolamine, LimSAP binds a third calcium in each subunit, with all three calcium ions contributing to the binding and orientation of the ligand. New structural studies of mammalian, fish and horseshoe crab pentraxins provide further unique insights into not only the evolutionary conservation of an important functional role in immunity, but also into the diversity of molecular aggregation built from a phylogenetically conserved protomer fold.

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Keywords: pentraxin, innate immunity, protein evolution

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Structural basis of recognition of pathogen-associated molecular patterns by pgrp-s

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Peptidoglycan recognition proteins (PGRPs) are involved in the recognition of pathogen-associated molecular patterns (PAMPs). The well known PAMPs include lipopolysaccharide (LPS) from Gram-negative bacteria and lipoteichoic acid (LTA) from Gram-positive bacteria. PGRP-short (PGRP-S) has been isolated from the mammary secretions of camel (CPGRP-S). It binds LPS and LTA with affinities of 1.6×10^{-9} M and 2.4×10^{-8} M respectively. The crystal structures of CPGRP-S complexes with LPS and LTA revealed that both compounds were held tightly inside the CPGRP-S tetrameric complex consisting of molecules A, B, C and D. The binding cleft is formed at the interface of molecules C and D which is extendable to the interface of A and C. The interface of molecules A and B is tightly packed while that between B and D forms an open channel. The hydrophilic moieties of these compounds occupy a common region while hydrophobic chains interact with distinct regions in the binding site. The flow cytometry studies showed that both LPS- and LTA-induced expressions of pro-inflammatory cytokines, TNF-α and IL-6 were inhibited by CPGRP-S. The results of animal studies using mice models indicated that both LPS- and LTA-induced mortality rates decreased drastically when CPGRP-S was administered. The recognition of both kinds of PAMPs from Gram-negative and Gram-positive bacteria, their high binding affinities to CPGRP-S, the significant decrease in the productions of LPS- and LTA-induced TNF-α and IL-6 on introduction of CPGRP-S and the drastic reductions in mortality rate in mice models by CPGRP-S suggest that CPGRP-S may be exploited as a common antibiotic agent for the welfare of mankind. This is particularly significant as there is an alarming rise in the incidence of bacterial resistance to known antibiotics. This also brings the amino acid sequence of CPGRP-S in focus particularly the presence of residues, Pro96 and Pro151 at one of the interfaces and the absence of three N-terminal residues and Cys8 as compared to human PGRP-S. So far in the family of PGRP-S, such a homotetrameric complex has been observed only for CPGRP-S.

Keywords: PGRP, PAMPs, LPS

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Structural studies of macrophage proteins using UQSG pipeline

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Macrophages are cells differentiated from circulating blood monocytes that represent the first line of defense against pathogen invasion. Macrophages are widely distributed throughout the body and are particularly abundant at the route of pathogen entry. They play a critical role in immune defense by initiating, promoting, preventing, suppressing or terminating immune responses.

We established a high-throughput pipeline at the University of Queensland to investigate the structures and functions of novel macrophage proteins [1]. My project began with the selection of 12

novel, biologically interesting and crystallization-feasible targets that were then designed into 96 different constructs. Processing of the 96 constructs was performed in parallel using simple automated applications of ligation-independent cloning, small-scale bacterial expression and purification, and solubility assessment. After processing the 96 constructs of 12 targets, I found that 16 constructs of three targets (25%) yielded soluble protein. From the three soluble targets, I have spent most time on two of these protein.

The first protein is a CARD domain containing protein that interacts with Bcl10. The primary function of Bcl10 is to interact with CARD proteins through CARD-CARD interactions to regulate its activity [2]. The crystal structure of this CARD containing protein solved at 1.5 Å resolution revealed six anti-parallel α-helices, showing that this protein is indeed similar to other CARD proteins with known structures. Approaches to determine the interaction between these two CARD domain containing proteins are currently being applied.

The second protein I worked on is a DUF59 domain containing protein with no function characterized yet. However, it has been reported that a family member is part of the MMXD protein complex involved in chromosome segregation [3]. I solved two crystal structures of this DUF59 domain protein to 1.8 Å resolution revealing, unusually, two different types of domain swapped-dimer. Functional characterization of this DUF59 domain containing protein, and of its domain swapping, is currently being investigated.

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Deciphering the mechanisms responsible for promiscuity in primary humoral response

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The antigenic repertoire is infinite. In order to generate an effective immune response, every antigen has to be specifically recognized in the primary encounter so that appropriate immune response could be mounted. In terms of the physico-chemical principles of antigen-antibody recognition, charge and shape complementarity is the key feature of antigenic discrimination and therefore, the number of antibodies required to neutralize the infinite population of antigens, ought to be unlimited. The fact that the germline antibody repertoire is finite (being limited by the number of temporal and spatial B cells and fixed recombinatorial potential of VDJ gene segments) implies that the germline antibodies could be broadly polyspecific in order to fulfill the physiological requirements of the primary immune response. While emerging data appear to invoke a paradigm shift on how Ag recognition is actually achieved in a primary humoral response, the structural mechanisms for promiscuous binding capabilities of germline antibodies have not been yet clearly illustrated.

Towards understanding the mechanistic basis for multispecificity in primary humoral response, structure and binding modes of a germline mAb BBE6.12H3 with multiple independent antigens were examined at atomic resolution. Our study demonstrates conformational flexibility of BBE6.12H3 paratope both in antigen-bound and antigen-free states. CDRH3 could undergo conformational rearrangements to adapt to independent and structurally different peptides. Six crystal structures of BBE6.12H3 illustrate diversity of antigen recognition repertoire and provide structural evidence for correlation of paratope flexibility with the multispecificity of germ line antibody. Moreover, comparative analysis of interacting residues in these complexes suggested that antigen combining site may be predesigned to be polyspecific. It is proposed that of primary antibody repertoire involves large, yet, finite germ line antibody clones, each capable of adopting discrete conformations which, in turn, show diverse binding modes.

Keywords: humoral immunity, immune recognition, paratope flexibility

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Crystal structure of CEL-IV, isolated from a sea cucumber, cucumaria echinata

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CEL-IV is a C-type lectin isolated from a sea cucumber, Cucumaria echinata. This lectin is composed of four identical C-type carbohydrate-recognition domains (CRDs). X-ray crystallographic analysis of CEL-IV revealed that its tetrameric structure was stabilized by multiple interchain disulfide bonds among the subunits [1]. Although CEL-IV has the EPN motif in its carbohydrate-binding sites, which is known to be characteristic of mannose binding C-type CRDs, it showed preferential binding of galactose and N-acetylgalactosamine. Structural analyses of CEL-IV-melibiose and CEL-IV-raffinose complexes revealed that their galactose residues were recognized in an inverted orientation compared with mannose binding C-type CRDs containing the EPN motif, by the aid of a stacking interaction with the side chain of Trp-79. Changes in the environment of Trp-79 induced by binding to galactose were detected by changes in the intrinsic fluorescence and UV absorption spectra of WT CEL-IV and its site-directed mutants. The binding specificity of CEL-IV toward complex oligosaccharides was analyzed by frontal affinity chromatography using various pyridylamino sugars, and the results indicate preferential binding to oligosaccharides containing Gal-beta-1–3/4(Fuc-alpha-1–3/4)GlcNAc structures. These findings suggest that the specificity for oligosaccharides may be largely affected by interactions with amino acid residues in the binding site other than those determining the monosaccharide specificity.

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Structural analyses of mouse MD-1 protein complexed with endogenous phospholipid

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