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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Keywords: structural_genomics, crystallography, macrophage

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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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MD-1 is a 162 amino-acid glycoprotein that associates with a B-cell-specific RP105 protein on the cell surface and has a low sequence identity of 16% to MD-2 that associates with Toll-like receptor 4 and recognizes endotoxic lipopolysaccharide. MD-1 and RP105 are supposed to mediate lipopolysaccharide recognition, however, little is known about their structures and functions. To obtain structural insights into the RP105 and MD-1 system, we have determined the crystal structure of mouse MD-1.

Mouse MD-1 was expressed in yeast *Pichia pastoris*, and was purified to homogeneity. Crystallization was performed with the sitting-drop vapor-diffusion method. Two crystal forms, hexagonal and tetragonal, were obtained. The crystal structure of the hexagonal form was initially determined at 2.4 Å resolution with the single isomorphous replacement method, and then the structure was further refined to 1.65 Å resolution against the tetragonal form.

MD-1 is folded into a single domain consisting of two antiparallel β-sheets in the β-cup fold; one sheet consists of three strands, and the other of six strands. A deep hydrophobic cavity of 1,915 $Å^3$ is formed between these sheets as is MD-2. No charged residues are located on the cavity entrance. Continuous electron-densities attributable to bound phosphatidylcholine were observed in the cavity. It is likely that phosphatidylcholine is an endogenous ligand of MD-1 and plays a role in retaining the MD-1 structure since the bound phosphatidylcholine from *P. pastoris* is copurified with MD-1. Together with the binding assay with tetra-acylated lipid IVa, MD-1 is shown to be a lipid-binding coreceptor.

Keywords: receptor, immune, lipid

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Structure and mechanisms of SIGN-R1, pneumococcal and sialylated protein receptor on macrophages

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The intricate system of serum complement proteins provides resistance to infection. SIGN-R1 is a transmembrane receptor found in Spleen macrophages, providing resistance against blood-borne S. Pneumoniae, and that can also recognize C. albicans, HIV and M. tuberculosis. SIGN-R1 extracellular moiety comprises a neck region formed by six repeats and a carboxyl-terminal calcium-type carbohydrate recognition domain (CRD). Pneumococcal recognition by SIGN-R1 activates the classical complement pathway independent immunoglobulins, as well as promotes phagocytes processes [1]. Furthermore SIGN-R1 has been probed to be required in anti-inflammatory activity of sialylated Fc fragments [2]. In order to better understand these processes we have performed the structural determination of SIGN-R1 CRD by X-Ray crystallography. The three-dimensional structures of the SIGN-R1 CRD in complex with a pneumococcal polysaccharide analogue (dextran sulphate) and sialic acid have been obtained at 2.5Å and 2.6Å of resolution respectively. SIGN-R1 CRD structure displays the typical long-form C-type lectin-like domains (CTLDs) fold [3] with two Ca2+ sites mediating carbohydrate binding. Binding site is altered by the unusual orientation of the long loop region, moreover two additional secondary structures elements are present: a 3_{10} helix and a small β-sheet arising from the extended β-sheet 2. Unexpectedly, crystal structure reveals five sulfate binding sites not been observed previously. In addition, docking experiments with models of pneumococcal CSP (CPS14) and *Staphylococcus aureus* "poliribitol phosphate" ligands have been done. We found that sialylated-glyco-proteins could bind to the classical lectin polysaccharide recognition zone while CPS seems to have a new secondary recognition region. This two different recognition sites will allow SIGN-R1 to bind both glyco-proteins and CPS simultaneously. Structural description and carbohydrate recognition are in deep described in the poster.

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Structural basis of carbohydrate recognition by calreticulin

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The calnexin cycle is a process by which glycosylated proteins are subjected to folding cycles in the endoplasmic reticulum (ER) lumen via binding to the membrane protein calnexin (CNX) or to its soluble homolog calreticulin (CRT). Defects in the calnexin cycle and ER protein folding in general are important for a number of diseases and conditions, ranging from cystic fibrosis to malfunctions of the ER stress response due to aging, genetic mutations, or environmental factors. Components of the calnexin cycle additionally play key roles in the assembly of major histocompatibility complex (MHC) class I molecules where CRT associates with protein disulfide isomerase ERp57, tapasin, the heavy chain/β2-microglobulin heterodimer and the TAP peptide transporter to form the peptide-loading complex. The previous crystal structure of CNX revealed two main structural components, a globular lectin domain and an extended arm-like domain, called the P-domain [1]. CNX/CRT specifically recognize monoglucosylated $Glc_1Man_9GlcNAc_2$ glycans, but the structural determinants underlying this specificity were unknown.

Here, we determined a 1.95 Å crystal structure of the CRT lectin domain in complex with the tetrasaccharide fragment from the glucosylated arm of the $Glc_1Man_9GlcNAc_2$ glycan [2]. The lectin domain shows a jelly roll fold similar to leguminous lectins and largely consists of a beta-sandwich formed by two curved beta-sheets. It also contains a single high-affinity calcium-binding site that plays an important role in stabilizing the protein but does not participate in carbohydrate recognition. The tetrasaccharide binds to a long channel on CRT formed by a concave beta-sheet. All four sugar moieties are engaged in the protein binding via an extensive network of hydrogen bonds and hydrophobic contacts. The structure explains the requirement for glucose at the non-reducing end of carbohydrate; the oxygen O2 of glucose perfectly fits to a pocket formed by CRT side chains while forming direct hydrogen bonds with carbonyl of Gly124 and the side chain of Lys111. The structure also explains a requirement for the Cys105-Cys137 disulfide bond in CRT/CNX for efficient carbohydrate binding. The Cys105-Cys137 disulfide bond is involved in intimate contacts with the third and fourth sugar moieties of the Glc_1Man_3 tetrasaccharide. Finally, the structure rationalizes previous mutagenesis