of MHC-restricted and non-autoreactive TCRs, the specific response to an antigen is characterized by the expression of a limited TCR repertoire with preferred gene segment usage. We focused our studies on the T cell response against the human beta-herpesvirus cytomegalovirus (HCMV) that infects 60 to 90% of the population and can cause life-threatening diseases in immunocompromised patients. In most individuals sharing the widespread MHC allele HLA-A*0201 (A2), HCMV-specific T cells target the same epitope NLV, derived from the viral protein pp65. While the NLV-specific TCR repertoire is heterogeneous in healthy donors, a dramatic diversity reduction and a high affinity TCR selection occur in chronic inflammation and immunodepression. To provide new insights into the structural basis of antigen recognition by T cells and generation of protective immune response against a major infectious agent in human, we have determined the crystal structure of the ternary complex between NLV, A2 and a TCR expressed by a predominant clone (RA14) derived from a rheumatoid arthritis patient. These data, with binding and activation experiments, provide evidences of constrains for preferred TCR gene usage in this viral peptide recognition. Contrasting with known structures of public TCRs, a novel antigen read-out is observed with the recognition of three peptide and one A2 specific hot spots.

Keywords: structures of T-cell receptor complexes, HLA, X-ray crystallography of proteins

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Crystal structure of a pattern recognition protein required for fungal detection in *Drosophila*

Yumiko Mishima[1], Franck Coste[1], Nadege Hervouet[1], Vanessa Bovezeau[1], Christine Kellenberger[1], Jessica Quintin[2], Dominique Ferrandon[2], Alain Roussel[1]
[1]CNRS-CBM, Rue Charles Sadron, Orleans, 45071, France, [2]IBMC-CNRS, Strasbourg, France, E-mail：mishima@cnrs-orleans.fr

In *Drosophila*, the synthesis of antimicrobial peptides in response to microbial infections is under the control of the Toll and Imd signaling pathways. The Toll signaling pathway responds mainly to gram-positive bacterial and fungal infection while the Imd pathway mediates the response to gram-negative bacteria. Microbial recognition upstream of Toll involves peptidoglycan recognition proteins (PGRPs) and glucan binding proteins (GNBP). The sensing of gram-positive bacteria is mediated by PGRP-SA and GNBP1 that cooperate to detect the presence of lysine-type peptidoglycan in the host. Recently it has been shown that the pattern recognition receptor GNBP3 is required for the detection of fungal cell wall components. We have solved the crystal structure of the N-terminal domain of GNBP3 at 1.8 Å by single wavelength anomalous dispersion (SAD) method using a Sm derivative. This structure together with the functional studies contributes to improve understanding of the molecular mechanisms underlying innate immune system in *Drosophila*.

Keywords: innate immunity, pattern recognition, molecular recognition

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Structure-function correlations in vertebrate defensins

Jacek Lubkowski[1], Cyril Barinka[1], Wuyuan Lu[2]
[1]National Cancer Institute, Macromolecular Crystallography Laboratory, 1050 Boyles Streeet, Bldg. 539, Frederick, MD, 21702, USA, [2]Institute of Human Virology, University of Maryland School of Medicine, 725 West Lombard Street, Baltimore, MD, 21201, USA, E-mail：jacek@ncifcrf.gov

Defensins are small (3-5 kD), cysteine-rich cationic proteins, mostly recognized for their antimicrobial properties. The last several years of research have also revealed the ability of defensins to interact specifically with a range of cellular receptors, including chemokine receptors. Using X-ray crystallography, functional assays, and other biophysical methods, we attempted to correlate the biological activities of defensins with their structural characteristics. We were able to demonstrate that the N-terminal fragment of human beta-defensin is critical for productive signaling through the chemokine receptor CCR6. Specifically, we can explain why human beta-defensins 1 through 3 activate CCR6, whereas beta-defensin 4 does not. We also demonstrated that the human receptor CCR6 can be activated by non-human defensins, if the molecules contain the required structural determinants. Our data suggest that for most (possibly for all) biological activities of defensins that belong to both alpha and beta subfamilies, specific oligomerization is irrelevant. Furthermore, we have established specific roles for highly-conserved residues in human alpha-defensins. We explained the functional/structural roles of a salt bridge (Arg-Glu) or of the presence of a Gly residue, both nearly invariant in alpha-defensins. Although all alpha-defensins studied to date appear to form conserved dimers in solution, we have found the first case of a native alpha-defensin in which mutual arrangement of monomers is strikingly different from all examples reported previously. Our conclusions are supported by nearly 30 high-resolution crystal structures, accompanied by thorough biological studies in vitro, and, for selected proteins, additional studies in solution.

Keywords: defensins, chemotaxis, antimicrobial

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Molecular recognition of the natural killer cell receptors 2B4 and Ly49 with their respect ligands

Lu Deng, Alejandro Velikovsky, Sangwoo Cho, Roy A Mariuzza
University of Maryland Biotechnology Institute, Center for Advanced Research in Biotechnology, 9600 Gudelsky Drive, Rockville, Maryland, 20850, USA, E-mail：dengl@umbi.umd.edu

Natural killer (NK) cells eliminate virally infected and tumor cells. Among the receptors regulating NK cell function we are interested in 2B4, a member of the signaling lymphocyte activation molecule (SLAM) family that binds CD48; and the Ly49 family, regulating NK cell function by sensing major histocompatibility complex class I (MHC-I) molecules on target cells. 2B4 is the only heterophilic receptor of the SLAM family. The complex structure between the N-terminal domains of mouse 2B4 and CD48 displayed an association mode related to, yet distinct from, that of the NK-T-B-antigen dimer, suggesting a model that permits intermixing of SLAM receptors with MHC-specific receptors in the NK cell immune synapse. The crystal structures of Ly49C, Ly49G and Ly49C－H-2Kb complex, combined with mutational analysis of Ly49A, permitted a structure-based classification of Ly49s that we used to dissect the binding site into three distinct regions, each having different roles in MHC recognition. One region, located at the center of the binding site, has a similar structure across the Ly49 family and mediates conserved interactions with MHC-I that contribute most to binding. However, the preference of individual Ly49s for particular MHC-I molecules is governed by two regions that flank the central

region, and are structurally more variable. One of the flanking regions divides Ly49s into those that recognize both H-2D and H-2K versus only H-2D ligands, whereas the other discriminates among H-2D or H-2K alleles. The modular design of Ly49 binding sites provides a framework for predicting the MHC-binding specificity of Ly49s that have not been characterized experimentally.

Keywords: natural killer cell, crystal structure, immune system

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Structure of the subdominant TCR in complex with HLA-B8FLRGRAYGL

Stephanie Gras[1], Lars Kjer-Nielsen[2], Craig S Clements[1], Scott R Burrows[3], James McCluskey[2], Jamie Rossjohn[1]
[1]Monash University, Biochemistry and Molecular Biology, Monash University, Clayton campus, Calyton 3800, Vic, Clayton, Victoria, 3800, Australia, [2]Department of Microbiology & Immunology, University of Melbourne, Parkville, Victoria 3010, Australia, [3]Cellular Immunology Laboratory, Queensland Institute of Medical Research, Brisbane 4029, Australia, E-mail：stephanie.gras@med.monash.edu.au

The cytotoxic T cell response towards viruses is directed towards class I Major Histocompatibility Complex (MHC-I) molecules complexed to peptide antigens (pMHC-I). These pMHC-I complexes are expressed on the surface of infected cells and are recognized by clonally distributed ab T cell receptors (TCR) on CD8+ T-cells. Appropriately armed and activated CD8+ T-cells can eliminate infected cells and prevent viral replication. The CD8+ T-cell response towards many viruses can be extremely focused with viral eradication occurring through the recognition of only one or two immunodominant epitopes. Epstein Barr virus (EBV) is a ubiquitous human pathogen with around 90% of the population persistently infected. EBV infection, although typically asymptomatic in immuno-competent individuals, is the causative agent of infections mononucleosis and has been linked to the development of cancers. Viral infection and persistence is achieved through a balance of lytic and latent infections controlled by a series of lytic or latent proteins respectively. The immunodominant HLA-B8 restricted epitope, FLRGRAYGL (FLR), is from EBNA 3A (latent protein). We have previously studied the biased TCR usage of the B8+ individuals infected by EBV, and solved the structure of the public TCR named LC13 in complex with B8-FLR. Interestingly the public LC13 TCR displays alloreactivity towards HLA-B44. Accordingly, in HLA B8+/B44+ individuals, the CTL responses towards FLR express different TCRs that exhibit altered specificity. We present recent findings in this area that allows us to compare how two different TCRs can interact with the same pMHC-I. These results are important, as well as EBV is the best know and most widely studied herpes virus due to its clinical and oncogenic importance.

Keywords: T cell receptor, HLA, Epstein Barr virus

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Crossreactive T cells spotlight the germline rules for TCR interactions with MHC molecules

Shaodong Dai, Philippa Marrack, John Kappler
HHMI/Natioanl Jewish Medical and Research Center, Immunology, 1400 Jackson St, Denver, Colorado, 80247, USA, E-mail：dais@njc.org

To test whether highly crossreactive alpha/beta T cell receptors (TCRs) produced during limited negative selection best illustrate evolutionarily conserved interactions between TCR and major histocompatibility complex (MHC) molecules, we solved the structures of three TCRs bound to the same MHC II peptide (IAb-3K). The TCRs had similar affinities for IAb-3K but varied from noncrossreactive to extremely crossreactive with other peptides and MHCs. Crossreactivity correlated with a shrinking, increasingly hydrophobic TCR-ligand interface, involving fewer TCR amino acids. A few CDR1 and CDR2 amino acids dominated the most crossreactive TCR interface with MHC, including Vbeta8 48Y and 54E and Valpha4 29Y, arranged to impose the familiar diagonal orientation of TCR on MHC. These interactions contribute to MHC binding by other TCRs using related V regions, but not usually so dominantly. These data show that crossreactive TCRs can spotlight the evolutionarily conserved features of TCR-MHC interactions and that these interactions impose the diagonal docking of TCRs on MHC.

Keywords: T cell receptors, MHC complexes, immune system

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Ligand binding to pentraxins

Halina Mikolajek[1], Patrizia Mangione[2], Stephen P Wood[3], Mark B Pepys[4]
[1]Univeristy of Southampton, Biological Sciences, Biomedical Sciences Building, Bassett Crescent East, Southampton, Hampshire, SO16 7PX, UK, [2]Centre for Amyloidosis and Acute Phase Proteins, Department of Medicine, University College London, UK, [3]Centre for Amyloidosis and Acute Phase Proteins, Department of Medicine, University College London, UK, [4]Centre for Amyloidosis and Acute Phase Proteins, Department of Medicine, University College London, UK, E-mail：hm1002@soton.ac.uk

The human pentraxin proteins, serum amyloid P component (SAP) and C-reactive protein (CRP) have emerged as potentially important targets in the treatment of amyloidosis and cardiovascular diseases respectively, although their normal physiological functions are unclear. Structurally highly conserved homologous proteins are present in common experimental animals such as the rat, mouse, rabbit and hamster but there are major differences from the human pentraxins in their normal behaviour as acute phase proteins, fine ligand specificity and capacity to activate the complement system. SAP binds to amyloid fibrils of all types and may contribute to their formation, stabilisation and persistence. Since important biological functions of proteins are often conserved among species, the structural differences between the rat and human pentraxins were investigated. Here we report the X-ray crystal structure of rat SAP in complex with phosphocholine (PC) to 2.2 Å resolution. The structure reveals the pentraxin fold and a bound PC in a pocket on each subunit indicating that rat SAP is also a PC-binding protein. This pentameric structure displayed subtle differences in the electrostatic properties. It remains to be determined whether this has an effect on avid binding of SAP to deoxyribonucleic acid (DNA), a functional property of human SAP still poorly understood.

Keywords: pentraxin, serum amyloid P component, phosphocholine