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MHC class I and class II reactivity mediated by a single T cell receptor

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αβ T cell receptors (TCRs) corecognize peptide antigens bound to self-major histocompatibility complex molecules (pMHC). pMHC-I and pMHC-II are differentially recognized by TCRs present on $CD8^+$ and $CD4^+$ T cells respectively. The TCR genes, however, originate from a shared repertoire with only minor differences in their usage. The factors governing the inherent restriction of TCRs for pMHC-I versus pMHC-II are the subject of intense debate as structural correlates of this differentiation have not been identified.

Recently, microbial-pMHC-I and self-pMHC-II cross-reactive $CD8^+$ T cell clones have been isolated from human peripheral blood mononuclear cells [1], [2] violating the general exclusivity of MHC-I and MHC-II restriction.

The focus of our study is a T cell clone (2G8) [1] expressing a single TCR specific for the peptide TRATKMQVI (TRAT), derived from human cytomegalovirus (CMV) and presented by the MHC-I allotype HLA-Cw*0602. 2G8 T cells cross-react strongly on the MHC-II allotypes HLA-DRB1*0401/0408 and less strongly on HLA-DRB1*0410, whereas DRB1*0402/03/04/06/07 are not recognized.

We have confirmed the dual MHC-I and MHC-II recognition by the 2G8 T cell clone using stably transduced T cell lines expressing the 2G8 TCR. We have also determined the crystal structure of the ternary complex 2G8 TCR- HLA-Cw*0602/TRAT/hβ_2m at 3.2Å resolution. The 2G8 TCR docked at approximately 67° across the long axis of the pMHC-I, a docking angle that falls within the range of TCR-pMHC docking angles determined to date. Unlike the majority of antimicrobial TCRs that are positioned roughly over the center and C-terminal end of the pMHC surface, a tilted binding mode and a shift towards the CMV-epitope N-terminus is adopted by the 2G8 TCR. A similar docking mode was previously observed for an antimicrobial TCR and ascribed to the need for self-tolerance reminiscent of MHC-II restricted autoimmune TCR complexes. Despite the markedly shifted N-terminal footprint of 2G8, the complementarity determining regions (CDR) of both, variable α- and β-chains contribute to the pMHC interaction and the affinity of the TCR for TRAT/HLA-Cw*0602 was typical for an microbial agonist peptide of ~4.2 μM as determined by surface plasmon resonance (SPR). In addition residues 65, 69 and 155 on the MHC molecule were TCR contact sites as reported for other TCRs and called the 'restriction triad'.

To determine the roles of the 2G8 TCR CDRs in the recognition of MHC-I/II, we mutated 23 residues contacting the pMHC-I to alanine.

Our findings demonstrate how TCRs recognize HLA-C and have the potential to delineate distinct mechanisms for recognition of pMHC-I versus pMHC-II.

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Structure of human C8 suggests how complement forms circular membrane pores

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C8 is one of five complement proteins that assemble on bacterial membranes to form the lethal, pore-like "membrane attack complex" (MAC) of complement. The MAC consists of one C5b, C6, C7, and C8, and 12-18 molecules of C9. Pore formation leads to loss of osmotic integrity and death of the cell under attack.

C8 is composed of three genetically distinct subunits, C8α, C8β and C8γ. The C6, C7, C8α, C8β and C9 proteins are homologous and evolved from an ancestral lysin, likely most similar to contemporary C9. Together they comprise the MAC family of proteins. All contain N- and C-terminal modules and a central 40-kDa MACPF domain that has a key role in forming the MAC pore. Here, we report the 2.5 Å resolution crystal structure of human C8 purified from blood. This is the first structure of a MAC family member and of a human MACPF-containing protein.

The structure shows the modules in C8α and C8β form domains which are located on the periphery of C8 and not likely to interact with the target membrane. The multiple disulfide bonds in the modules and carbohydrate moieties on the module's surface likely protect the MAC from the highly proteolytic environment at sites of inflammation. The carbohydrate attached to thrombospondin modules are C-linked mannosyltryptophan residues. This is an infrequent posttranslational modification and C8 is the first crystal structure of a protein with such residues. It appears that β-linkages fits better to electron density than α-mannosyls.

The C8γ subunit is a member of the lipocalin family of proteins that in general bind and transport small lipophilic molecules. In C8, however, the subunit has its putative ligand binding cavity empty and the entrance blocked by C8α.

C8α and C8β are related by a rotation of approximately 22º with only a small translational component (1.6 Å) along the rotation axis. Assuming this binding mode was conserved during the complement pathway evolution, one can speculate that the binding geometry between these two homologous subunits is similar to the arrangement of C9 molecules within the MAC pore. This leads to a model of the MAC that explains how C8-C9 and C9-C9 interactions could facilitate refolding and insertion of putative MACPF transmembrane β-hairpins to form a circular pore. The model proposed by us is a simple extension of the unique oligomeric nature of C8 into the circular MAC using evolutionary arguments. The model is in excellent agreement with independent electron microscopy estimates of the MAC dimensions [1]. Based on EM studies, two diametrically opposite molecular models have been proposed for pores formed by homologous lysins: bacterial cholesterol dependent cytolysins (CDC) [2] and perforin [3]. Our model supports the CDC model.

Recognition of "self" by human complement is inhibited through interaction with the ubiquitously distributed membrane-associated protein CD59, which binds to a defined segment of C8α. Our MAC model suggests that that this binding inhibits the recruitment of the first C9 molecule into MAC.

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