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STRUCTURE NOTE

Rhesus macaque: A tight homodimeric CD8αα

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INTRODUCTION

Simian immunodeficiency virus (SIV) infection of rhesus macaque (*macaca mulatta*) is widely used as an animal model for human immunodeficiency virus (HIV) infection[1–3] as well as other human diseases. It is known that the host cytotoxic T lymphocyte (CTL) responses provide powerful protection against HIV infection, and CTL-based immunization is currently believed to be the most promising approach toward vaccine development.[4]

As a coreceptor of T cell receptor (TCR) on the surface of CTLs, CD8 molecules stabilize the interaction of the TCR with major histocompatibility complex (MHC) by binding to the MHC class I (MHCI) molecule on the surface of antigen-presenting cells. In the absence of CD8 interaction, MHCI-restricted immune responses are hampered.[5] In addition, recent data indicate that CD8 has the ability to bind to a nonclassical MHC class I-like molecule, TL antigen, independently of TCR and CD3, expanding the function of CD8 further to an immunomodulator.[6–8] Moreover, soluble forms of CD8 can disrupt activation of some T cell clones with higher efficacy than anti-CD8 antibodies.[9,10]

In both human and mouse, the functions of CD8 involved in immune responses have been extensively studied.[11,12] The crystal structures of the human HLA-A*0201-CD8αα complex,[13,14] murine MHC H-2K^b-CD8αα complex,[15] TL antigen-CD8αα complex,[16] and the murine CD8αβ heterodimer[17] have been solved. For macaque monkeys, however, little is known on the structures of the CTL-related molecules (e.g., TCR, MHC, and CD8). Only the structure of MHC allele Mamu-A*01 has been recently solved in our laboratory.[18]

In this article, we present the crystal structure of rhesus macaque CD8αα (rCD8αα) homodimer and discuss the relatedness and uniqueness of rCD8αα structure with that of human/mouse CD8αα homodimer. Strikingly, with two Thr43 residues in C–C′ loop, rCD8αα shows a unique extra hydrogen bond in the homodimeric interface indicating a tighter homodimeric interaction.

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MATERIALS AND METHODS

Expression and purification of rCD8αα homodimer

Rhesus macaque CD8 (rCD8) alpha chain nucleotides covering amino acids 1–120 of the ectodomain were synthesized based on the sequence of Indian origin rhesus (GeneBank ID: 698329). Inclusion bodies of rCD8α were prepared, and rCD8αα homodimer was renatured and purified by using the protocols described earlier.[13,14]

Crystallization, data collection, and processing

All crystallization attempts were performed at 18°C by the hanging drop vapor diffusion method. Ideal rCD8αα crystals grew from a 1:1 mixture of the protein solution (10 mg/mL) with crystallization reagent of 0.05*M* potassium phosphate monobasic, 20% w/v polyethylene glycol 8000. Data were collected using a Rigaku MicroMax007 rotating-anode X-ray generator (Cu Kα; λ = 1.5418 Å) equipped with an R-AXIS VII++ image-plate detector. Data were processed and scaled using HKL2000.[19]

Structure solution, refinement, and analysis

Data were analyzed by molecular replacement[20] using Molrep in the CCP4 package,[21] taking human CD8αα as the search probe (PDB code: 1AKJ).[14] Final rounds of refinement resulted in a final Rcryst of 21.3% (R_{free} = 25.7%) for all data between 35.0 and 2.20 Å.

Buried surface areas were calculated using SURFACE[21] with a 1.4 Å probe radius. The PyMOL Molecular Graphics System (DeLano Scientific, http://www.pymol.org) was used to prepare figures. Geometry of the refined structure was validated according to Ramachandran plot criteria.[22] The data collection and refinement statistics of the structure are shown in Table I.

Accession number

Atomic coordinates of rhesus macaque CD8αα homodimer have been deposited in the Protein Data Bank (PDB, http://www.rcsb.org/pdb) under accession code: 2Q3A.

Table I
X-Ray Diffraction Data Processing and Refinement Statistics

Data collecting	
Space group	$P2_12_12_1$
Unit cell dimensions (*a*, *b*, *c*)	46.54, 56.26, 82.31
Unit cell dimensions (α, β, γ)	90.00, 90.00, 90.00
Resolution range (Å)	40.00–2.20 (2.32–2.20)[a]
Total number of reflections	138,752
Number of unique reflections	17,807
Number of molecule in the asymmetric unit	2
Average redundancy	6.60 (5.90)
Completeness (%)	99.6 (100.0)
R_{merge} (%)	9.6 (29.5)
I/σ	12.8 (5.7)
Refinement	
Resolution (Å)	35.85–2.20
R-factor (%)	21.3
R_{free}[b] (%)	25.7
RMS deviations from restraint target values:	
Bond lengths (Å)	0.006
Bond angles (°)	1.33
Ramachandran plot Quality:	
Residues in most favored regions	165 [84.2%]
Residues in additional allowed	27 [13.8%]
Residues in generously allowed	4 [2.0%]
Residues in disallowed regions	0 [0%]

[a]Values in parentheses are given for the highest resolution shell.
[b]R_{free} is calculated over reflections in a test set (5%) not included in atomic refinement.

RESULTS AND DISCUSSION

Overall structure of rhesus macaque CD8αα homodimer

The crystals contained two CD8αα molecules as a dimer in a hand-shaking mode per crystallographic asymmetric unit. Belonging to the V set of Ig folds,[14] the overall structure comparison of rCD8αα homodimer with the human counterpart is shown in Figure 1(A). Each CD8α molecule is primarily composed of β structure arranged into two antiparallel β sheets. Short regions of 3_{10} helix are found between the E and F strands, which are not commonly found in CD8β molecules.

All residues corresponding to the HLA-A2-CD8αα interface remain the same in MHC Mamu-A*01 and rCD8αα.[18] The interaction of HLA-CD8 is mainly based on charge complementarity and exhibits relatively low affinity (KD = 100–223 μ*M*) and rapid kinetics.[8,23] The molecular surfaces of Mamu-A*01 and rCD8αα show similar complementarities (data not shown), indicating their interaction.

Structural comparison of rhesus macaque with human or murine CD8αα homodimer

Superposition of the final structure of rCD8αα dimer shows closer resemblance to human CD8αα homodimer (1AKJ, in complex with HLA-A2)[14] than to murine CD8αα (mCD8αα) (1BQH, in complex with Kd)[15] [Fig. 1(A,B)]. Root mean square deviation (RMSD) of rCD8αα and hCD8αα dimer results in 0.831 E for all Cα atoms, which is much smaller than that of rCD8αα with mCD8 dimer of 1.634 E.

The comparison of r/h/m CD8αα reveals some differences in loop regions, especially in complementarity

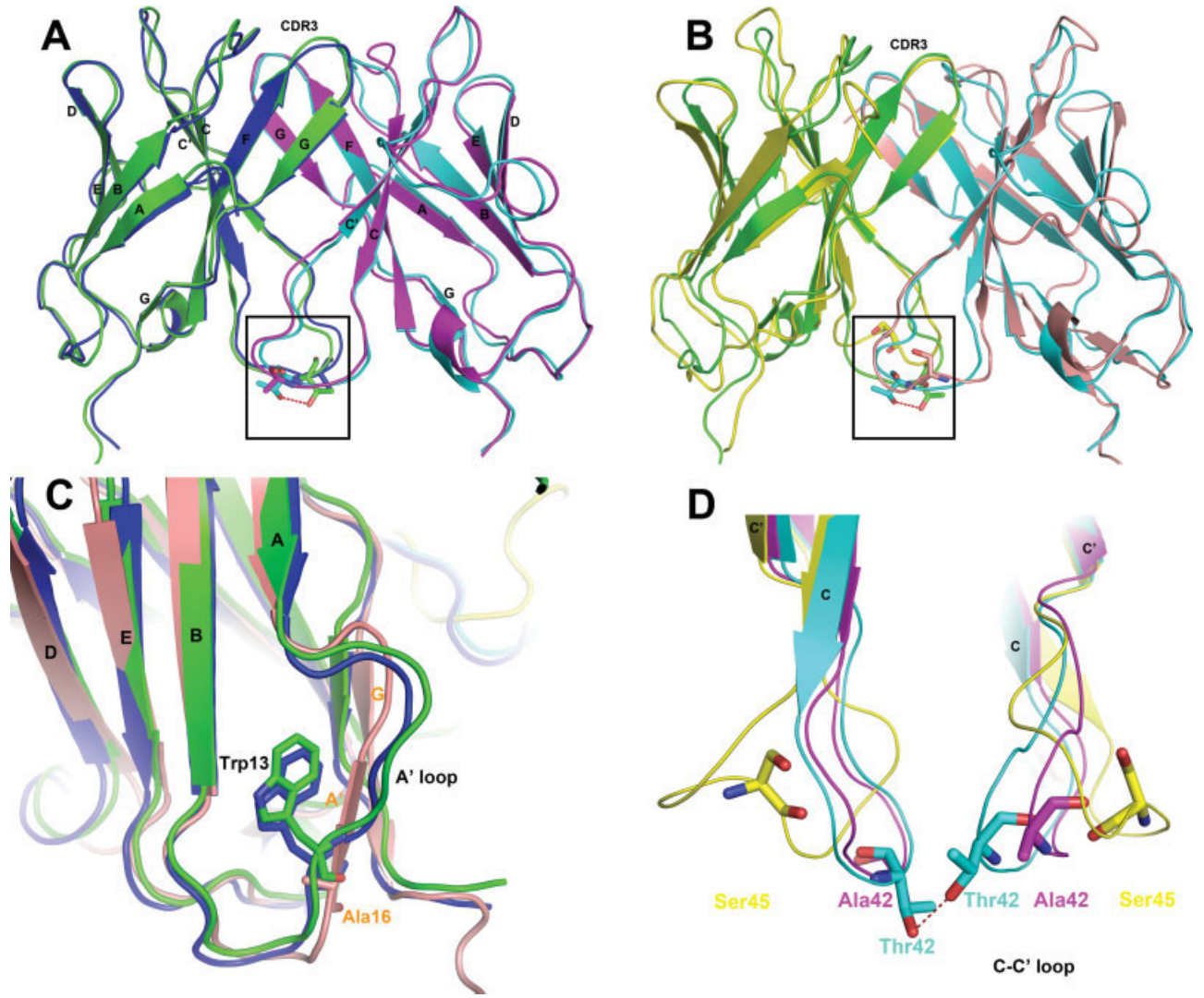

Figure 1

Crystal structure of rCD8αα homodimer and its superposition with human and mouse CD8αα structures. The ribbon diagram of each CD8αα was drawn and color-coded as: rCD8αα, green and cyan; hCD8αα, blue and magenta; mCD8αα, yellow and orange. (**A**) Superposition of rCD8αα and hCD8αα. The extra hydrogen bond region of rCD8αα was boxed. (**B**) Superposition of rCD8αα and mCD8αα. The extra hydrogen bond region of rCD8αα was boxed. (**C**) The transition of A'-β strand in mCD8αα and A' loop in both rCD8αα/hCD8αα as the residue changes (Ala16 to Trp13). (**D**) Thr43 residues in C-C' loop of rCD8αα homodimer (cyan) form extra hydrogen bonds. Hydrogen bond between two main-chains is shown as red dashed line.

determining regions (CDRs), which are involved in MHCIs binding. Interestingly, though CDR1 and CDR2 are structurally variable, CDR3 has almost the same conformation among r/h/m CD8αα molecules [Fig. 1(A,B)].

As one of the elements commonly found in many Ig domains, the first β strand of each domain is split into two shorter strands (A and A′).[20] rCD8αα homodimer can also be characterized by a *cis*-proline (Pro7) at the transition point at the A strand. However, at the place of the A′ strand, a loop is located in rhesus macaque as well as human CD8α molecules as the result of the big side chain of the residue Trp13, which is equivalent to Ala16 in mCD8α molecules [Fig. 1(C)].

Corresponding to Ala42 in hCD8α and Ser48 in mCD8α, Thr43 residues in rCD8α form an additional hydrogen bond to each other. This extra H-bond enhances the dimeric interaction and brings the C–C′ loop of the two molecules into a much closer position [Fig. 1(D)]. As a result, the interface size of the two rCD8α subunits (∼2274 Å^2, total buried solvent-accessible surface area) is significantly larger than that of human CD8α interface (∼2038 Å^2) indicating a tighter homodimeric interaction of CD8αα in rhesus macaque.

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