Journal of Korean Medical Science
Vol. 10, No. 6, 393-398 December 1995

Superantigen and Class II MHC Molecules

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INTRODUCTION

Superantigen(reviewed in Marrack and Kappler, 1990 ; Murray et al., 1995) is a class of immunostimulatory molecules that share the property of being able to stimulate polyclonal T cells expressing particular Vβ gene segments(White et al., 1989 ; Irwin et al., 1993). Members of the superantigen family include toxins from Staphylococcus aureus and other bacteria, as well as viral superantigens from mouse mammary tumor virus. The bacterial superantigens are associated with food poisoning and toxic shock syndrome, and the viral superantigen plays an important role in viral transmission. The toxicity of bacterial superantigens is mediated by their potent T-cell stimulating activities, leading to overproduction of lymphokines(Marrack et al., 1990 ; Miethke et al., 1992). The activation of large numbers of mature T cells is preceded by binding of superantigens to class II major histocompatibility complex(MHC) molecules (Fleischer and Schreznmeier, 1988 ; Carlson et al., 1989). Although the fraction of responding T cells by superantigens is orders of magnitude greater than that stimulated by conventional antigens, superantigens have similarities to conventional ones. For example, both types of antigens must be presented to a T-cell receptor(TCR) by a MHC molecule(Fleischer and Schreznmeier, 1988 ; Carlson et al., 1989), and both types form ternary complexes with the MHC and TCR that trigger T-cell proliferation and lymphokine production. But superantigens are different from conventional protein antigens. Superantigens do not require active cellular processing for presentation to the MHC molecule, they do not occupy the MHC peptide binding groove(Karp et al., 1990), and their presentation is not restricted by a particular class II MHC molecule(Mollick et al., 1989 ; White et al., 1989). The interaction of superantigens with TCR and the mechanism by which superantigens stimulate T-cells also differs from those of conventional peptide antigens(Choi et al., 1990).

The molecular structures of superantigen Staphylococcus enterotoxin B(SEB) and toxic shock syndrome toxin-1(TSST-1) have been determined by x-ray crystallography(Swaminathan et al., 1992 ; Prasad et al., 1993 ; Acharya et al., 1994). Although TSST-1 and SEB show only 16% sequence identity, their three-dimensional structures are similar. Both superantigens consist of two domains. The N-terminal domain is thought to bind the class II MHC molecule, while C-terminal domain contains the residues important for mitogenic activity of superantigen. The x-ray crystal structure of a complex between the superantigen SEB and the human class II MHC molecule HLA-DR1 was determined recently(Jardetzky et al., 1994). It revealed that SEB binds exclusively to the α-chain of DR1 off one edge of the peptide binding groove. One loop of SEB covers residues of DR1 recognized by the TCR during conventional antigen recognition, which suggests an unconventional model for the interaction between the TCR and MHC during superantigen activation. The three-dimensional structure of a bacterial superantigen, TSST-1, complexed with HLA-DR1 has also been determined by x-ray crystallography(Kim et al., 1994). Interestingly, the TSST-1 binding site on DR1 overlaps that of SEB, but the two binding modes are very different, although the tertiary structures of SEB and TSST-1 are similar.

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While SEB binds primarily off one edge of the DR1 peptide binding site, TSST-1 extends over almost one-half of the binding site contacting both the flanking MHC α-helices and the bound peptide. This may result from the sequence difference of SEB and TSST-1, and suggests that TCR would bind to TSST-1 : DR1 complex very differently than to DR1 : Peptide complex or SEB : DR1 complex. It also suggests that TSST-1 binding may be peptide dependent, though less so than TCR, binding providing a possible explanation for the inability of TSST-1 to block competitively SEB binding to all DR1 molecules on cells, even though the binding sites of TSST-1 and SEB on DR1 overlap almost completely(Thibodeau et al., 1994b).

RESULTS AND DISCUSSIONS

Overall structure of TSST-1 : HLA DR1 complex

The overall structure of TSST-1 : DR1 complex is shown in Fig. 1. DR1 appears as a dimer of αβ hetero-dimer, as has been observed in other DR1 crystal forms(Brown et al., 1993 ; Jardetzky et al., 1994 ; Stern et al., 1994). TSST-1 binds on the top surface of the α1 domain of the DR1 interacting primarily with amino acids from the first and third turns of the β-sheets and from the α-helix. Additionally, TSST-1 interacts with some residues from the β1 domain of DR1 and C-terminal region of the antigenic peptide bound on the DR1. As a consequence, this region of the peptide binding groove is buried by TSST-1 molecule. The N-terminal β-barrel domain of the TSST-1 is primarily involved in complex formation of the DR1 and TSST-1 molecules, as had been indicated by x-ray crystallographic analysis of TSST-1(Prasad et al., 1993 ; Acharya et al., 1994) and peptide studies mapping the TSST-1 : MHC interaction(Edwin et al., 1991 ; Soos et al., 1993). The C-terminal domain of the TSST-1 is oriented up and away from the DR1 molecule, interacting with other symmetry related TSST-1 molecule in the crystal. Mutation studies have suggested that this region could interact with TCR(Mollick et al., 1993).

Description of the interface between TSST-1 and HLA-DR1

TSST-1 : DR1 complex formation is primarily accomplished by interdigitation of two loops from DR1 molecule and two loops from TSST-1 molecule(contact region I in Fig. 1), by α/β type packing of α-helix from DR1 and β-strands from TSST-1(contact region II), and by interaciton between the antigenic peptide bound on DR1 and β-strands from the TSST-1(contact region III). Relatively extensive contacts occur at the interface between TSST-1 and DR1 molecule with the buried solvent accessible surface area of~1,000Å^2(Kim et al., 1994).

In contact region I, two loops from TSST-1(T26-T35 and T46-T54) and from DR1 molecule(A13-A21 and A34-A41) interdigitate and form a tight interface. The major driving force of this interaction is likely to be hydrogen bond formation and hydrophobic interaction. Side chains from Gln A18 and Lys T58 form a potential hydrogen bond, and Lys A39 and Ser T53 form a potential hydrogen bond. Lys A39 also forms potential hydrogen bonds with main chain carbonyl oxygen from Phe T47 and Ser T49. Side chains of Asp T27 and Tyr A13 are also located at this interface. The mutation of Lys A39 to Ser or Ala abolishes TSST-1 binding to HLA-DR7 (Panina-Dordignon et al., 1992 ; Thibodeau et al., 1994a). Met A36 interacts with Leu T30 forming a hydrophobic ridge with other hydrophobic residues, Tyr A13 and Ile A63. Mutagenesis study has indicated that Met A36 is important for TSST-1 binding-(Panina-Dordignon et al., 1992). Mutation of the Met A36 to Ile markedly decreased the ability of the DR7 molecule to bind and present TSST-1.

In contact region II, one α-helix from the α1 domain of DR1(A55-A75) and four β-strands from the N-terminal domain of the TSST-1 form a sort of α/β type folding. The N-terminal domain of the TSST-1 form a β-barrel where the concave surface of theses β-strands is distinguished by a prominant clustering of solvent-accessible hydrophobic residues(Acharya et al., 1994). Interestingly, the α-helix from the α1 domain of DR1 is alligned in parallel along the concave face of the β-strands. Leu A60 interaccts with Ile T46 and Pro T48, and Val A65 forms a hydrophobic core with Leu T44, Phe T83 and Ile T81. In addition to the hydrophobic interactions, electrostatic and hydrogen bonding interaction is also important for this region. Glu A71 forms a salt bridge with Arg T34, and the side chains from Lys A67 forms a potential hydrogen bond with the main chain carbonyl oxygen of Asp T27.

TSST-1 also interacts with some residues from the β1 domain of DR1 and the antigenic peptide bound on DR1 molecule. The interaction does not seem to

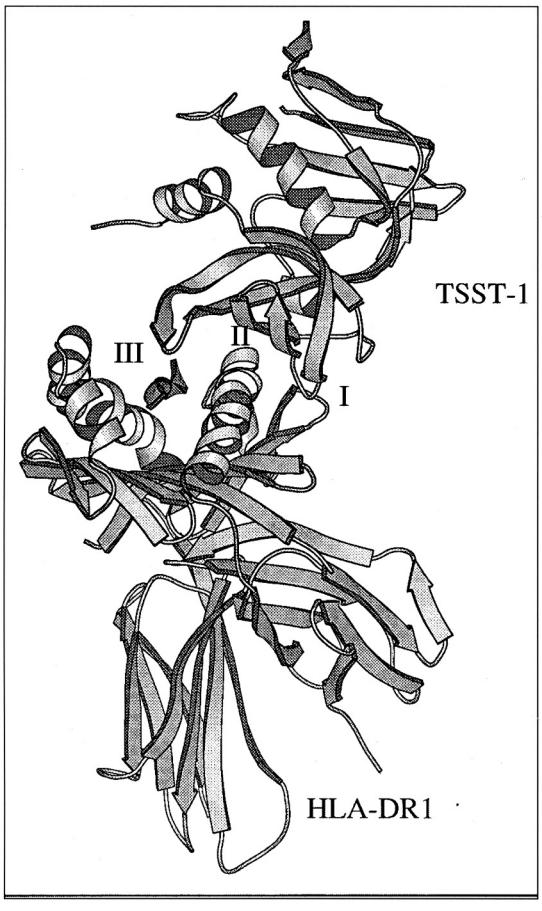

Fig. 1. A ribbon diagram of TSST-1 : HLA-DR1 complex. The TSST-1 molecule cover the entire top of the DR1 α1 domain and about half of the peptide binding groove. Although a continuous surface, the TSST-1 : DR1 interface can be usefully divided into three major contact regions : Contact region I, II and III designated by I, II and III, respectively in this figure.

be substantial, however, the β-strands from the TSST-1(T70-T83) and the C-terminal region of the peptide(P7-P13) are close together. Side chains from Gln T73 form potential hydrogen bonds with residues Tyr B60 and Gln B64, and side chains of Glu B66 and His T74 are also located around this interface. The location of the TSST-1 molecule above the antigenic peptide clearly suggests that the conventional MHC II : TCR interaction is hard to imagine in the presence of superantigen without any rearrangement of either DR1 or TSST-1 molecule. It also suggests the possibility that T cell activation by superantigen could be directed by peptide antigen.

SEB and HLA-DR1

A structural alignment of the SEB and TSST-1 shows that the major features of the SEB binding interface on HLA DR1 are not conserved in TSST-1(Swaminathan et al., 1992 ; Prasad et al., 1993 ; Acharya et al., 1994 ; Jardetzky et al., 1994). In particular, Phe 44 in SEB, which forms hydrophobic ridge with Tyr A13, Met A36, Leu A60 and Ile A63, is changed to Ser T29 in TSST-1 and the SEB residues Glu 67, Tyr 89 and Tyr 115 which interact with Lys A39 are changed to Ile T46, Thr T69 and Ile T98 in TSST-1(Jardetzky et al., 1994). However, mutation of Met A36 to Ile or Lys A39 to Ser, abolishes TSST-1 binding to HLA-DR7 and mutation of Lys A39 to Ala disrupts TSST-1 binding as well as SEB binding(Panina-Dordignon et al., 1992 ; Thibodeau et al., 1994a). These results have indicated that although SEB and TSST-1 are sensitive to mutations in the same region of the class II MHC molecule, the specific interactions may be substantially different. In fact, the structure of TSST-1 : DR1 complex is very different from that of SEB : DR1 complex(Kim et al., 1994), although two superantigens bind to similar regions of DR1 molecule. In TSST-1 : DR1 structure, TSST-1 binds more close to the α-helix of α1 domain and peptide binding groove of DR1 molecule. As a result, the antigenic peptide and the peptide binding groove of DR1 is almost covered by TSST-1 molecules and the overall geometry of TSST-1 : DR1 complex is significantly different from that of SEB : DR1 complex.

Contact region I in TSST-1 : DR1 complex seems to be common with that in SEB : DR1 complex although the details of the side chain interaction is substantially different. Hydrophobic residues Tyr A13, Met A36, Leu A60 and Ile A63 interact with the SEB Phe 44, Leu 45 and Phe 47 in SEB : DR1 structure-(Jardetzky et al., 1994), while these residues interact with Leu T30 in TSST-1 : DR1 structure(Kim et al., 1994). Lys A39 forms a completely buried salt bridge with Glu 67 of SEB, surrounded by hydrogen bonds from SEB Tyr 89 and Tyr 115, while the Lys A39 forms hydrogen bonds with the side chain of Ser T53 and the carbonyl oxygens from Phe T47 and Ser T49.

In SEB : DR1 structure, the disulfide loop residues-(SEB 94-97) contact the α1 domain α-helix of the DR1 molecule. This disulfide loop is absent in TSST-1 and the β-strands from the N-terminal β-barrel

domain directly interact with the α-helix of DR1 in TSST-1 : DR1 structure. Different binding modes of SEB and TSST-1 might primarilly result from the presence of the disulfide loop in SEB. The disulfide loop protrudes from the β4 and β5 strands and block the concave face of the N-terminal β-barrel which interacts with the α-helix of the α1 domain of the DR1 molecule in TSST-1 : DR1 structure. Also, SEB does not interact with the β1 domain of DR1 and the antigenic peptide bound on DR1, while TSST-1 interacts with these regions.

TCR binding site on Superantigen : MHC complex

Mutation studies have shown that some residues at the C-terminal domain of TSST-1 are important for mitogenic activity(Blanco et al., 1990 ; Bonventre et al., 1993 ; Murray et al., 1993). These residues include Tyr T115, Glu T132, His T135, Ile T140, His T141 and Tyr T144, and line a region between the two domains of the superantigen defining a potential TCR binding site that is located above and to one side of the MHC peptide binding groove(Kim et al., 1994). Direct binding studies with a soluble TCR β chain indicate that the TCR β chain may be sufficient to mediate recognition of a superantigen : MHC II complex of sufficient strength to bind cells(Gascoigne and Ames, 1991). Particularlly, the hypervariable region 4(HV4) of the Vβ domain of the TCR is important for superantigen interactions(Cazenave et al., 1990 ; Choi et al., 1990 ; White et al., 1993). However, recent results indicate that other components of the TCR including CDR1 and the α subunit of the TCR could also be involved in recognition of superantigen : MHC II complex(Patten et al., 1993 ; Bellio et al., 1994).

Since the structure of the TCR is believed to be similar to that of Fab fragment of immunoglobulin-(Chothia et al., 1988 ; Davis and Bjorkman, 1988) based on the amino acid sequence homology, it is tempting to build a structural model for the ternary complex between MHC, TSST-1 and TCR using the hypothetical model of the TCR and TSST-1 : MHC structure. The docking model(Fig. 2) generated by alligning the DR1 dimer two-fold axis and the pseudo two-fold axis that relates the α and β subunits of the TCR in parallel(normal to the hypothetical membrane), and by juxtaposition of the HV4 region of the TCR with the TSST-1 residues involved in TCR interactions suggests interesting implications for the formation of the ternary complex between MHC,

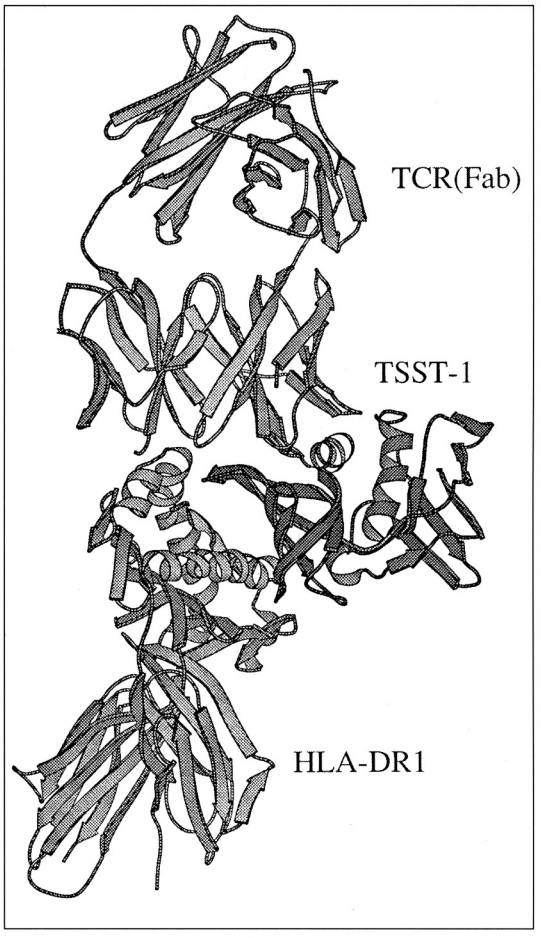

Fig. 2. A structural model for the complex between class II MHC molecule, superantigen and TCR. This model was generated by graphical superposition of the crystal structures of TSST-1 : DR1 complex and Fab fragment using a graphics program TOM/FRODO.

TSST-1 and TCR : (a) Not only HV4 but also other components of the TCR β-subunit(CDR1, CDR2 and CDR3) could interact with the TSST-1 molecule(both N-terminal and C-terminal domains) as had been implicated by mutagenesis studies of TCR and TSST-1 ; (b) The α subunit of the TCR could interact with the β subunit of class II MHC molecule. This interaction is likely to be analogous to that in the conventional MHC : TCR complex ; (c) Neither subunit of the TCR would interact with the α subunit of the DR1 and the antigenic peptide which are buried by the TSST-1 molecule. The β subunit of the TCR, which

interact with the α subunit of the DR1 and the antigenic peptide in the conventional MHC : TCR complex, would rather interact with the TSST-1 molecule. Superantigens may have been evolved to bind class II MHC molecules and to mimic the α subunit of the MHC molecules to stimulate the T-cells ; and (d) The proposed model for the DR1 : TSST-1 : TCR ternary complex is similar in macroscopic view to that of DR1 : SEB : TCR complex(Jardetzky et al., 1994) although specific interactions between DR1 : TSST-1 and DR1 : SEB are substantially different, and the interactions between TSST-1 : TCR and SEB : TCR could also be different. The mechanism of T-cell stimulation by superantigens may use general idea of interacting TCR β subunit with superantigens and TCR α subunit with the DR1 β subunit. In this sense, the interactions between TCR and MHC molecules may be different during superantigen stimulation from the interactions involved in antigenic peptide stimulation.

However, the very different mode of binding of TSST-1 to class II MHC molecules, relative to SEB, suggests that TCRs may be oriented very differently in complexes with various superantigens. Although the structures of SEB : DR1 and TSST-1 : DR1 both suggest that TCR could simultaneously contact superantigen and DR1 molecules(Jardetzky et al., 1994), the structure of TSST-1 : DR1 complex also suggests the possibility that TCRs might contact only TSST-1 and be blocked from contacting DR1. This could result from the fact that the TSST-1 already occupied more than half of the TCR recognition site on MHC peptide binding groove(Kim et al., 1994). It should be revealed by further mutagenesis study and x-ray crystallographic analysis.

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