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Structure of the Superantigen Staphylococcal Enterotoxin B in Complex with TCR and Peptide–MHC Demonstrates Absence of TCR–Peptide Contacts

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Superantigens are immune-stimulatory toxins produced by Staphylococcus aureus, which are able to interact with host immune receptors to induce a massive release of cytokines, causing toxic shock syndrome and possibly death. In this article, we present the x-ray structure of staphylococcal enterotoxin B (SEB) in complex with its receptors, the TCR and MHC class II, forming a ternary complex. The structure, in combination with functional analyses, clearly shows how SEB adopts a wedge-like position when binding to the β-chain of TCR, allowing for an interaction between the α-chain of TCR and MHC. Furthermore, the binding mode also circumvents contact between TCR and the peptide presented by MHC, which enables SEB to initiate a peptide-independent activation of T cells. The Journal of Immunology, 2014, 193: 1998–2004.

Staphylococcal enterotoxin B (SEB) is a toxin produced by Staphylococcus aureus. The bacteria produce a repertoire of staphylococcal enterotoxins collectively called superantigens (SAgs) because of their inherent property to activate large amounts of T cells (1). SEB was one of the first SAgs to be discovered and was shown to cause vomiting and diarrhea after ingestion (2). More than 20 years later, it was demonstrated that SAg toxicity was caused by cross-linking of the MHC class II and the TCR (3). In contrast with conventional Ags, which are presented as processed peptides by MHC, SAgs bind as unprocessed molecules directly to the MHC molecule and are recognized by one of the variable domains of TCR (TCR α variable [TRAV] and TCR β variable [TRBV]), more precisely by a subset of the four variable loops (CDR1-3 and HV4) (4, 5). This cross-linking induces an overactive immune response and leads to production of cytokines, which may result in diseases (6).

The different SAgs can be classified into five distinct evolutionary groups (I-V) (7). Although these toxins have a conserved tertiary structure, each group engages its immune receptors in structurally and functionally diverse ways. SEB is classified into group II, characterized by a low-affinity binding to the side of MHC class II in a peptide-independent manner (8). It is most likely important for the SAg to circumvent contact between the peptide and the TCR to trigger a large immune response. Nevertheless, binding to the side of MHC does not seem to prevent TCR–peptide interaction. Computer models of TCR–SAg–MHC complexes comprising class II SAgs, generated from the previously determined dimer structures (SEB-MHC and SEB/SEC3-TCR), suggest that TCR would not be able to make contacts with the presented peptide (8–10). So far, only two complete TCR–SAg–MHC complexes are available. One describes how staphylococcal enterotoxin H (SEH) binds to MHC and TCR (11). SEH is an atypical SAg because it activates and binds T cells through the TRAV domain (5, 12). Moreover, SEH binds on top of the peptide–MHC complex and contacts the peptide backbone (13). Hence it is clear that SEH prevents interactions between TCR and the peptide by steric hindrance (11). The other available TCR–SAg–MHC structure is of the Mycoplasma arthritidis mitogen, which is structurally unrelated to the staphylococcal enterotoxins and streptococcal pyrogenic exotoxins, but still binds TCR and MHC class II (14). Thus, the TCR–M. arthritidis mitogen–MHC complex is a clear example of another evolutionary route to the same outcome: a large activation of T cells by protection of the bound peptide against the hypervariable loops of TCR.

We have determined the structure of the complex between a human TCR, human MHC class II, and the SAg SEB. This SAg binds to the side of the MHC α-chain and to the TRBV domain of TCR. The structure demonstrates that the SAg forces the TCR to angle ~25° away from the MHC surface, as compared with a regular MHC–TCR complex. This enables the ternary complex to form without any significant contacts between the TCR and the peptide. In addition, there is a third interface between the TRAV domain and the MHC β-chain formed upon SEB binding, which stabilizes the formation of the ternary complex.

Materials and Methods

Protein expression and purification

Escherichia coli harboring a vector with the SEB gene (15) was used for protein expression. Cultivation for 24 h was carried out at 29°C and 200 rpm in 1 l SB media pH 7.0 containing 17 mM KH₂PO₄, 72 mM K₂HPO₄,
and 25 mg/l kanamycin. The periplasmic content was released and purified according to a previous protocol (11), except cationic exchange in 20 mM HEPES pH 7.0 with a 0–200 mM NaCl gradient.

TCRs were produced according to previously published protocols (16, 17), with changes as follows: *E. coli* BL21 (DE3) Star (Invitrogen) was used for expression, and refolding was carried out in 100 mM Tris-HCl pH 8.0, 5 M urea, 400 mM L-arginine, 0.83 mg/l cysteamine hydrochloride, and 0.73 mg/l cystamine dihydrochloride. Targeted mutagenesis to generate a Lys55Ala substitution in TRAV22 was carried out using a Quik-Change mutagenesis kit (Qiagen). Primers were generated using the oligo-mutator script (courtesy of Associate Prof. Martin Schmidt). In total, three different TCRs were purified: TRAV22/TRBV7-9, TRAV22/TRBV19, and K55A-TRA V22/TRBV19.

MHC class II, HLA-DR1 (α-chain DR*0101 and β-chain DRB1*0101) was produced as described previously (11, 18), with minor exceptions. Changes are as follows: *E. coli* BL21 (DE3) Star cells (Invitrogen) were used, cells were centrifuged and resuspended in fresh 2xYT media before IPTG induction, and refolding was carried out by dilution to a final concentration of 0.24 µM of each chain in 2 l refolding buffer.

All proteins were subjected to size exclusion chromatography in TBS buffer for crystallization or HBS supplemented with 0.005 or 0.01% Tween 20 for surface plasmon resonance (SPR).

### SPR experiments

SPR experiments were carried out using a BIAcore 2000 (GE Healthcare) at 25°C and a flow rate of 40 µl/min. SEB or TCRs were diluted in 10 mM sodium acetate pH 5.0 or 4.5, respectively, and coupled to a CM5 chip, using an amine coupling kit (GE Healthcare), to 350 resonance units (RU) for SEB and 200 RU for the TCR (TRA V22/TRBV19). A 2-fold dilution series, 0.6–160 m, of TCRs (TRAV22/TRBV19 and TRAV22/TRBV7-9) or SEB in HBS running buffer supplemented with Tween 20 was injected, and responses against a blank flow cell were recorded. Each sample was injected three times, and the mean value is shown. Figures 1A–D show the response for a mixture of SEB with wild type (wt) or mutant TCRs, with concentrations of 0.8–50 µM was injected. Response differences between the flow cell containing MHC and a blank flow cell were measured, and a running buffer injection was subtracted from all response curves. The experiment was repeated three times with the same result.

**Figure 1.** SPR experiments of SEB-TCR. (A) Responses for TRAV22/TRBV19 samples injected over immobilized SEB. Concentrations shown are 0.6–160 µM, as well as an injection of TRAV22/TRBV7-9, as negative control. (B) The response values for TRAV22/TRBV19 plotted against concentration. Each sample was injected three times, and the mean value is shown. (C) Responses for SEB samples injected over immobilized TRAV22/TRBV19. Concentrations shown are 0.6–160 µM for SEB. (D) The response values for SEB plotted against concentration. Each sample was injected three times, and the mean value is shown.

### Data processing and structure determination

Before crystallization, SEB, MHC class II (HLA-DR1), and TCR (TRAV22/TRBV19) were mixed in a 2:1:2 ratio with a total protein concentration of 6.5 mg/ml. Crystals were grown in 8% polyethylene glycol 20000, 0.1 M sodium citrate pH 5.5, and 0.09 M MgCl2 with the hanging drop method, and formed in a couple of weeks. For cryoprotection, reservoir solution containing 20% (v/v) glycerol was used. Diffraction data were collected at beamline ID23-1 at the European Synchrotron Radiation Facility at λ 1.07227 Å and 100 K on a single crystal.

### crystallization and X-ray diffraction experiments

X-ray diffraction images were indexed and integrated using XDS (19), and intensities were scaled and merged using Scala in the CCP4 suite (20), with 5% of the reflections chosen as a subset for cross-validation using calculation of free R values. Molecular replacement was carried out in Phaser (21), using the TRAC, TRBC, and TRBV domains from 1OGA (22) and the TRAV domain from 2IAL (23) as a model of TCR, and 1SEB (8) as a model of MHC.
was used for SEB and MHC models. Missing or differing amino acids were built or substituted manually in Coot (24) and refined with Refmac5 (25). Final model-building and structural refinements were made in Coot (24) and autoBUSTER (26). The final model comprised two TCR-SEB-MHC complexes, which included residues 3–203 in TCRα (excluding 151–152, 165–168, and 179–181 in complex I [CI] and 166–168 and 203 in complex II [CII]), 4–243 in TCRβ (excluding 4 in CII), 3–180 in MHCα (excluding 3 in CI and 243 in CII), 2–190 in MHCβ (excluding 106–110 in CI), 1–13 in the peptide, and 2–237 in SEB (excluding 101–108 and 237 in CI and 100–108 in CII). A composite omit map, with 5% of the structure omitted, was calculated in the CNS (27). Ramachandran statistics were calculated, with 89.4% of all residues within favored regions, 10.0% in allowed, 0.3% in generously allowed, and 0.3% in disallowed regions. All figures depicting structures were generated using PyMOL version 1.3 (Schrodinger).

Results

SEB binds TCR in the micromolar range

To determine the binding capacity of SEB to TCR, we performed SPR experiments, using TCRs with either variable domains TRAV22/TRBV19 or TRAV22/TRBV7-9. These domains are all isolated from HLA-A2–specific TCRs, reactive against a telomerase peptide (sequence ILAKFLHWL) for TRAV22, an influenza matrix peptide (sequence GILGFVFTL) for TRBV19, and a survivin peptide (sequence ELTLGEFLKL) for TRBV7-9. SEB was coupled to the SPR chip, and TRAV22/TRBV19 and TRAV22/TRBV7-9 were injected. The TCR-bearing variable domain TRBV19 clearly binds to SEB, whereas the one bearing TRBV7-9 failed to bind (Fig. 1A). In addition, the TRAV22/TRBV19 TCR was coupled to the surface and SEB was injected (Fig. 1C). The $K_D$ was determined to $\sim 4 \times 10^{-3}$ to $9 \times 10^{-2}$ M (Fig. 1B, 1D), which is in the same range as earlier reported SAg–TCR interactions (12, 28).

Overall structure of the TCR–SEB–MHC complex

The ternary complex of SEB, together with the extracellular domains of human TCR (TRAV22/TRBV19) and MHC class II (HLA-DR1) with bound HA-peptide, was crystallized and the structure was determined to 2.9 Å (Fig. 2A and Table I). The complex crystallized with two TCR–SEB–MHC complexes in the asymmetric unit, referred to as CI and CII. Residues are designated a, b, s, $\alpha$, $\beta$, and p, for TRA V, TRBV, SEB, MHCa, MHCb, and peptide, respectively. The two ternary complexes within the asymmetric unit are similar, with RMSD values for the $\alpha$-atoms of 1.2, 0.98, 0.52, 0.33, 0.47, and 0.17 Å for TCRα, TCRβ, SEB, MHCα, MHCβ, and the peptide, respectively. All proteins, SEB, TCR, and MHC, are globular proteins and exhibit the conventional folds as previously described (29–31). The TCR chains are kept together by an introduced disulphide bridge between Cys160α and Cys171β (17).

In the complex, TCR is placed on top of MHC, with SEB binding to the side of MHCα and to the TRBV domain of TCR, in a wedge-like fashion (Fig. 2A). Because of the positioning of the toxin, the TCR is angled $\sim 25^\circ$ away from the MHC surface, preventing the TRBV domain to contact either the MHC or the peptide. However, the TRAV domain is in proximity to the MHC $\beta$-domain, and there are intermolecular contacts between them. Thus, three protein–protein interfaces are present: SEB-TRBV, SEB-MHCa, and TRAV-MHCb, which are described separately (Fig. 2).

Interactions between SEB and TRBV19

SEB engages TRBV through its TCR-binding cleft, a shallow groove located between its N-terminal $\beta$-barrel and the $\alpha_2$-helix.

FIGURE 2. Three-dimensional structure of the TCR–SEB–MHC complex. (A) Ribbon representation of the overall structure, with TCR in purple and blue (TCRα and TCRβ, respectively), SEB in orange, MHC in green, and the peptide in black. (B–D) Close-up views of the three interfaces, with hydrogen bonds marked as dotted lines and a 2Fo-Fc electron density map shown around selected residues. The SEB–TCR interface with electron density for residues 52–59 in TRBV (B), the SEB–MHC interface, with electron density around residues 43–47 in SEB (C), and the TCR–MHC interface with electron density around residues 52–55 in TRAV is shown (D).
The buried surface areas between TRBV and SEB are 1464 and 1428 Å² for CI and CII, respectively, and SEB contacts mainly the CDR2 (39%), HV4 loops (19%), and the FR3 sheet (35%). There are slight differences in the SEB–TRBV interface between CI and CII, but the majority of the hydrogen bonds and van der Waals contacts are conserved. In total, there are 14 residues from SEB and 17 from TCR (16 in CII) creating the interface (Supplemental Table I). Among these contacts, there are six hydrogen bonds, five common to both CI and CII (Fig. 2B and Table II). Notably, the side-chain oxygen and nitrogen of Asn23 are used to form two hydrogen bonds to the backbone of Phe57. This asparagine is highly conserved in a range of other SAgs and is generally used to engage TCRs (11). Moreover, there are several van der Waals contacts that could be important for complex formation, for example, Val26 that contacts the CDR2 loop, and Tyr90 and Tyr91 that create a hydrophobic patch packing against residues 52–56 in the CDR2 loop of TRBV (Fig. 2B). These tyrosines are also well conserved among related SAgs (11).

Interface between SEB and MHC class II

In line with the earlier published structure of the SEB–MHC complex, SEB binds to the side of MHC without interactions to the peptide (8). The buried surface areas between SEB and MHC are 1476 (in CI) and 1517 Å² (in CII). In total, there are 11 hydrogen bonds between SEB and MHC in each complex, whereof 7 are conserved (Fig. 2C and Table II). In addition, SEB has a hydrophobic region consisting of nonpolar residues (Phe44, Leu45, and Phe47), which fit into a hydrophobic pocket in MHC (Fig. 2C). In all, there are 17 residues from SEB that form van der Waals contacts with MHC in CI and 15 residues in CII (Supplemental Table I).

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and CII, respectively. There is one hydrogen bond between TRAV and MHC in CI (Lys<sup>55a</sup>-Asp<sup>66b</sup>) and three in CII (Lys<sup>55a</sup>-Asp<sup>66b</sup> and two between Thr<sup>54a</sup> and Glu<sup>69b</sup>; Table II). Moreover, there are several van der Waals contacts from the CDR2 loop (Ser<sup>52a</sup>, Glu<sup>53b</sup>, Thr<sup>54a</sup>, Lys<sup>55a</sup>) and the HV4 loop (Ala<sup>66a</sup>, Thr<sup>67a</sup>) of TRAV to helix one in MHCβ (Fig. 2D and Supplemental Table I). Lys<sup>55a</sup> was substituted to alanine and SPR measurements were performed, to investigate the role of the TRAV22–MHCβ interface. MHC was immobilized on the SPR chip and SEB alone was injected, followed by dilution series of TCRs containing either TRAV22 or K55A-TRAV22 (both with TRBV19), with and without SEB. Neither wt TCR nor K55A-TCR bind to MHC without SEB present, whereas an increase in response is observed for TRAV22/TRBV19 mixed with SEB, as compared with SEB alone (Fig. 3). The K55A-TCR is, however, not able to increase the response when injected along with SEB (Fig. 3). Hence the presence of a TRAV–MHCβ interface in the crystal structure (Fig. 4), in combination with these SPR results, highlights the importance of the TRAV domain for ternary complex formation.

**Discussion**

Conventional T cell activation depends on the MHC molecule and the peptide presented (32), where the peptide is known to be the hotspot for TCR binding. This results in a very specific immune response, where \(<0.001\%\) of all T cells become activated. The outcome is entirely different when a SAg is responsible for T cell activation, because it initiates up to 20% of all T cells. The mechanism of SAg action is illustrated by the structure presented in this article, which demonstrates how the SAg displaces the TCR loops away from the peptide. In addition, an interface between the TRAV domain and the MHC β-chain is formed upon SAg binding, which has not previously been studied with x-ray crystallography.

Previously, it has been suggested that MHCβ and the TCR α-chain are able to influence T cell activation for the SEB-like SAgs, although these domains are not in direct contact with the SAg (33). In the structure presented in this article, we observe a third interface between the MHCβ and TRAV, which consists of residues from the CDR2α and the HV4α loops. To elucidate the role of the TRAV domain, we substituted Lys<sup>55a</sup> in CDR2α, which forms a hydrogen bond to MHCβ, to an alanine. This leads to a significantly decreased SPR response to immobilized MHC when K55A-TRAV22 is coinjected with SEB, compared with wt-TRAV22 (Fig. 3). Consequently, this suggests that the interface between TCR and MHC observed in the x-ray structure occurs in solution and that it contributes to the binding affinity of the complete complex. Interestingly, this lysine is rather well conserved among the human TRAV domains. Most likely, both the sequence and the length of the CDR2α loop are of importance for putative complex formation, because a longer or shorter loop would most likely influence the position of the lysine. Furthermore, other features in the TRAV-MHCβ interface have been shown to affect the formation of the ternary complex. For instance, it has been demonstrated that Glu<sup>69b</sup> in a murine MHC affects T cell activation by SEB (34). This glutamic acid contacts TRAV22 in the TCR–SEB–MHC structure presented in this article (Fig. 2D). Moreover, the CDR2α loop has been shown to be of importance for ternary complex formation for SEB-like SAgs. For example, a substitution of Ser<sup>51a</sup> to proline in a murine TCR has been shown to reduce the additive effect on complex formation upon SEC3 binding (33). Interestingly, residue Ser<sup>51a</sup> corresponds approximately to Ser<sup>52a</sup> in our structure, which contributes to the TRAV–MHCβ interface. However, there are also differences in the SAgs complexes, such as in the TCR–SEC3–MHC complex. We observe contacts between both CDR2α and HV4α to MHC, whereas only contacts between CDR2α and MHC were predicted in the previous computer model of the TCR–SEC3–MHC complex (35). Hence this interface is likely to be present in all SAgs belonging to group II, but may differ slightly between different SAgs. Moreover, this interface may also depend on how the α- and β-chains of TCR are organized with respect to each other, which can differ between each αβ pair.

With this structure available, a comparison of how two different TCRs, TRBV19 and the murine TRBV13-2 (10), recognizing the same SAgs, can be performed. Li and coworkers (10) suggest that SEB engages TRBV domains depending on the conformation of the FRββ domain and CDRβ loop, which is conserved between mTRBV13-2 and TRBV19. This clearly has a major impact on

![FIGURE 3.](http://www.jimmunol.org) SPR measurements of the TCR–SEB–MHC complex. Two-fold dilution series, \(0.8–50\mu M\), of SEB, wt-TRAV22/TRBV19, K55A-TRAV22/TRBV19, as well as two series of SEB mixed either with an equimolar ratio wt-TCR or K55A-TCR, were injected over immobilized MHC, and the responses are shown against the concentration. Wild type (wt) TCR injected together with SEB yields a considerably higher response compared with K55A-TCR along with SEB.

![FIGURE 4.](http://www.jimmunol.org) Comparison of the ternary TCR–SAg–MHC complexes with SEB and SEH. All proteins are shown in ribbon representation. SEB (left panel) is shown in orange and SEH (right panel) in yellow, the TCR α-chain in purple, the TCR β-chain in blue, and MHC class II in green.
specificity, and because of the relative rarity of this loop conformation, the TRBV specificity of SEB remains rather narrow. In addition, it explains the inability of SEB to bind TRBV7-9, which has a different loop conformation (Fig. 1A). Still, it is not possible to explain the lack of activation of T cells bearing TRBV6 (36), which all have an identical loop conformation as TRBV19. Our structure implies that in addition to the conformation of this loop, certain amino acids are of importance for the TRBV specificity. A common feature of the TRBV6 family is Gly55b within CDR2β (TRBV19 numbering), which is also present in mTRBV13-2. The lack of a side chain in mTRBV13-2 at this position disables interactions with Val26s in SEB, leading to a two to three times lower affinity (~140 μM) for mTRBV13-2 than for TRBV19 (37). Our structure suggests that in the presence of a larger amino acid instead of Gly55b, for example, an asparagine as in TRBV19, an interaction can be formed with Val26s, which could lead to increased shape complementarity and higher affinity. Furthermore, in the ternary complex, there is one additional hydrogen bond between Arg110s and Glu74, possibly to keep the HV4 interaction, as well as the conservation of the lysines in the HV4 loop, away from MHC. This highlights the remarkable variability in the HLA-DR1 complexed with an influenza virus peptide. Nat. Commun. 1: 119.


