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CUTTING EDGE

Cutting Edge: Evidence of Direct TCR α-Chain Interaction with Superantigen[1]

Nick Pumphrey, Annelise Vuidepot,* Bent Jakobsen,* Göran Forsberg,† Björn Walse,‡ and Karin Lindkvist-Petersson[2§]*

Superantigens are known to activate a large number of T cells. The SAg is presented by MHC class II on the APC and its classical feature is that it recognizes the variable region of the β-chain of the TCR. In this article, we report, by direct binding studies, that staphylococcal enterotoxin (SE) H (SEH), a bacterial SAg secreted by* Staphylococcus aureus*, instead recognizes the variable α-chain (TRAV27) of TCR. Furthermore, we show that different SAgs (e.g., SEH and SEA) can simultaneously bind to one TCR by binding the α-chain and the β-chain, respectively. Theoretical three-dimensional models of the penta complexes are presented. Hence, these findings open up a new dimension of the biology of the staphylococcal enterotoxins. *The Journal of Immunology,* **2007, 179: 2700–2704.**

Superantigens (SAgs)[3] are a class of immunostimulatory and disease-causing proteins of viral or bacterial origin. They can activate large fractions (5–20%) of the T cell population and stimulate them to proliferate and secrete cytokines. These properties are attributed to their unique ability to be presented by MHC class II on APCs as unprocessed SAgs and recognized by the TCR on T cells. The staphylococcal enterotoxins (SEs) are bacterial SAgs secreted by certain strains of *Staphylococcus aureus* (1). During the recent sequencing of the staphylococcal genome new SAgs have been identified and, to date, the SE family is comprised of SEA, SEB, SEC, SED, SEE, SEG, SEH, SEI, SEJ, SEK, SEL, SEM, SEO, SEP, and SEQ (2). SEs bind as unprocessed SAgs to MHC class II by using two distinct binding sites. Site-directed mutagenesis and structural studies have revealed that SEA, SEB, SEC, SED, and SEE bind to the α-chain of MHC class II with low affinity by using their N-terminal domain (3–5). SEH and SEI have been structurally verified to bind, with high affinity via a zinc-dependent interaction in their C-terminal domains, to the β-chain of MHC class II (6–8). SEA, SED, and SEE also require binding to the β-chain of MHC class II via an "SEH-like" site in their C-terminal domains (3, 9, 10). When SAgs are presented by MHC class II they are recognized by specific subsets of T cells. A classical feature of SAgs is that they interact with the variable region of the TCR β-chain, with each SE family member displaying its own unique Vβ profile (11). However, the characterization of SEH has to some extent generated a paradigm shift in the field of SAg biology, because SEH is able to induce specific activation of human T cells dependent on the variable region of the TCR α-chain (TRAV) (12). Before this study, some observations identified skewed TRAV repertoires of T cells after SE stimulation, although this was explained by a contact between MHC and TCR, and recently a crystal structure of the *Mycoplasma arthritidis* mitogen (MAM) in complex with MHC and TCR showed that MAM contacts both the α-domain and the β-domain of TCR (13, 14). SEH was shown to induce a specific expansion of cells expressing TCRVα10 (TRAV27 in ImMunoGeneTics nomenclature) and therefore possesses unique biological properties (12). In this study, we have confirmed that SEH does not only induce a specific expansion of TRAV27-expressing cells, it also interacts directly with TCRs containing TRAV27 with an affinity in the low micromolar range.

Materials and Methods

Cloning, protein expression, and purification

Recombinant SEA and SEH were expressed as secreted products in *Escherichia coli* K12 strain UL635. The expression vector contained a gene coding for one of the different SAgs, the constitutive *S. aureus* protein A promoter, a synthetic signal peptide and a kanamycin resistance gene (3). The cultivations were conducted in bioreactors and the SAgs were recovered as previously described (15). All of the purified SAgs were >95% pure according to Coomassie-stained SDS-PAGE.

Soluble TCRs were produced as disulfide-linked αβ heterodimers as previously described (16, 17). In the TCR-biotin constructs the TCR β-chain was C-terminally extended to encode the optimized biotinylation sequence GSGGGLNDIFEAQKIEWH. Tagged TCR was biotinylated with BirA biotin ligase (Avidity) according to the manufacturer's instruction.

BIAcore analysis

Binding studies were conducted by surface plasmon resonance detection using a BIAcore 3000 instrument. Before immobilization, ligands were diluted in 10

*Avidex Limited, Abingdon, Oxon, United Kingdom; †Active Biotech Research AB, Lund, Sweden; ‡SARomics AB, Lund, Sweden; and §Department of Cell and Molecular Biology, Göteborg University, Göteborg, Sweden.

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[2] Address correspondence and reprint requests to Dr. Karin Lindkvist-Petersson, Department of Cell and Molecular Biology, Göteborg University, Box 462, S-405 30 Göteborg, Sweden. E-mail address: karin.lindkvist@gmm.gu.se

[3] Abbreviations used in this paper: SAg, superantigen; RU, resonance unit; SE, staphylococcal enterotoxin; SPE-A, streptococcal pyrogenic exotoxin type A; TRAV, variable region of TCR α-chain; TRBV, variable region of TCR β-chain.

mM sodium acetate (pH 5) to 20–100 μg/μl. Coupling to the dextran matrix of the BIAcore CM5 sensor chip was performed using standard amine-coupling chemistry. Experiments were conducted at 25°C in HBS (10 mM HEPES, 3.4 mM EDTA, 150 mM NaCl, and 0.005% Tween 20 (pH 7.4)). Proteins were dialyzed against HBS before BIAcore analysis. A nonimmobilized surface was used as reference surface in all experiments, as well as a nonbinding TCR or SAg.

The equilibrium analyses were performed using 1-min injection at flow rate at 20 μl/min at 25°C. SAgs were immobilized at ~300 resonance units (RU), TCRs were immobilized at ~1000 RU, and the experiments were conducted as described above. Concentrations ranging from 20–0.04 μM of SAg or TCR were used as analytes. In the experiment investigating the cobinding of SEH and SEA to TCRs, constant concentration at a near saturation level of SEA (10 μM) was used and SEH was titrated as described above. The data for the blank reference flow cell was subtracted and the binding levels at equilibrium were recorded. Affinities of TCRs for SEH were calculated from these levels using nonlinear curve fitting within the program GraphPad Prism.

Three-dimensional models

The molecular model of the TRAV27-SEH interaction was constructed by superimposing common elements in the crystal structures of the SEH-MHC complex (Protein Data Bank code 1HXY) (8) and TCR (TRAV27/TRBV19) (Protein Data Bank code 1OGT) (18) with the crystal structure of the streptococcal pyrogenic exotoxin type A (SPE-A)-TCR complex (Protein Data Bank code 1L0Y) (19). SEH was superimposed onto SPE-A and the TRAV27 chain was superimposed onto the variable region of the TCR β-chain (TRBV). The TRAV27 chain was then manually adjusted to avoid steric clashes and to create better interactions. The final SEH-TRAV27 complex was subjected a short energy minimization consisting of 1000 steps involving only the residues in the interface. The minimization was performed with the MacroModel module from the Schrödinger computational chemistry suite of programs using a dielectric constant of 1 and the OPLS2005 force field.

Results

SEH binds to the variable α-chain of TCR

T cells expressing TRAV27 are expanded after SEH stimulation (12). To confirm that the expansion of TRAV27-expressing cells is caused by an interaction between SEH and TRAV27, direct binding measurements were made by using surface plasmon resonance. Three different TCRs were coupled to the BIAcore chip as ligands (TRAV27/TRBV19, TRAV27/TRBV7.9, and TRAV22/TRBV7.9) and SEH and SEA were investigated as analytes. An interaction between SEH and the two TCRs containing TRAV27 is observed, whereas no interaction is seen between SEH and the TCR containing TRAV22 (Fig. 1, *D–F*). Because the same TCRVβ is present in both TRAV22/TRBV7.9 and TRAV27/TRBV7.9, the lack of interaction between TRAV22/TRBV7.9 and SEH therefore infers that SEH binds to the α-chain. In addition, we have earlier seen that SEH does not induce any specific expansion of T cells expressing certain TRBVs, either in FACS analyses or in RT-PCR analyses (12). SEA was used as control analyte to verify the functionality of TRAV22/TRBV7.9 because it is known to activate and bind to T cells expressing TCRs containing TRBV7.9. In Fig. 1, *A* and *C*, it is seen that SEA binds to TRAV27/TRBV7.9 as well as to TRAV22/TRBV7.9. Taken together, these results show that SEH binds to the α-chain of TCR.

A specific TCR can bind two different SAgs simultaneously

Following the above observations, we investigated whether one TCR heterodimer can bind to two different SAgs at the same time, one with its Vα-chain and one with its Vβ-chain. The TCR containing TRAV27/TRBV7.9 was used to detect simultaneous binding to both SEA and SEH. As controls, TRAV27/TRBV19 and TRAV22/TRBV7.9 were used. First, the concentration of SEA to achieve near saturation of the TRBV7.9 was determined to be 10 μM (see Fig. 4*C*). The experiments were then performed such that SEA was kept at a constant concentration of 10 μM while SEH was titrated from 20 to 0.15 μM (Fig. 2, *A–C*). The results show a clear titration of SEH binding over TRAV27/TRBV19 where SEA does not bind (Fig. 2*A*), which is similar to what happens when SEA is not included in the titration (Fig. 1*E*). As expected, there is no titration of SEH binding on TRAV22/TRBV7.9, although it is interesting to note that SEA binding is not significantly affected by the presence of SEH (Fig. 2*B*). On TRAV27/TRBV7.9 we find that, in addition to SEA binding, the titration of SEH results in a similar titration of binding as that to TRAV27/TRBV19 (Fig. 2*C*). When the level of SEA binding is subtracted from TRAV27/TRBV7.9 and the affinities of the SEH to TRAV27 are calculated, we find that the affinity of the SEH for TRAV27 is not significantly affected by the presence of SEA (Fig. 2*D*). Hence, we find that SEH and SEA can simultaneously bind to their respective α- and β-chains in an independent manner.

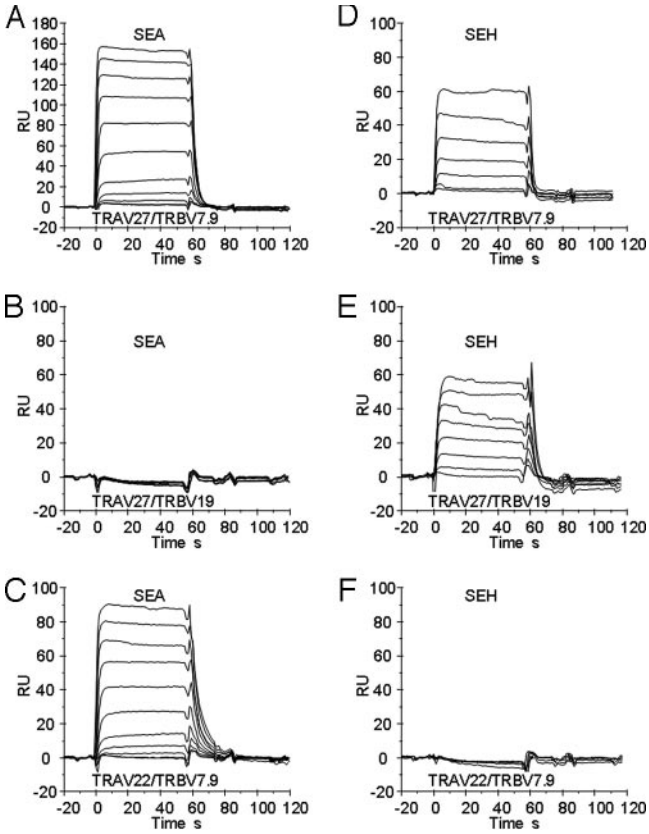

FIGURE 1. BIAcore analyses of SEH and SEA binding to various TCRs. Shown are the injection of titrations of SEA (*A*, *B*, and *C*) and SEH (*D*, *E*, and *F*) over immobilized TCRs, TRAV27/TRBV7.9 (*A* and *D*), TRAV27/TRBV19 (*B* and *E*), and TRAV22/TRBV7.9 (C and *F*). Responses from a blank flow cell were subtracted.

Three-dimensional model of the SEA-SEH-TCR-MHC complex

The crystal structure of a TCR containing TRAV27 is known (18). This structure was used to construct a structural model of the ternary complex between SEH, TCR, and MHC (Fig. 3*A*). The resulting model, after subsequent manual adjustment and energy minimization, produced a complex where the TRAV27 chain binds in a slightly tilted conformation compared with the Vβ-chain in the SPE-A-TCR structure. This is because the CDR2 loop and framework region 3 in TRAV27 have a different conformation compared with the Vβ-chain due to strand

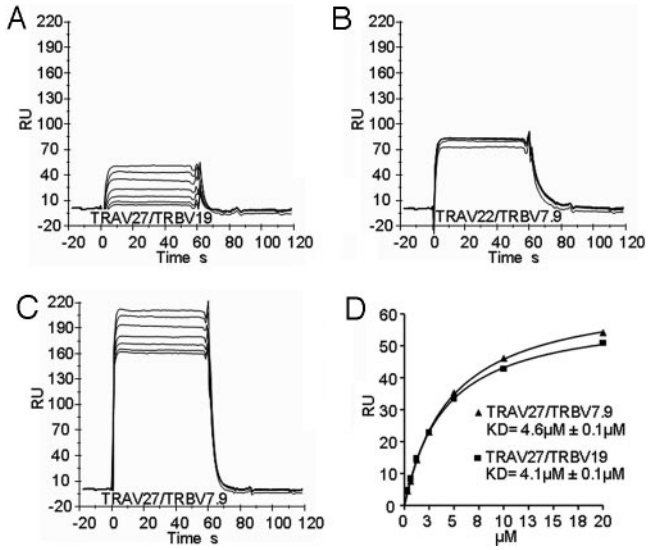

FIGURE 2. Titration of SEH with constant concentration of SEA. *A–C*, SEA-SEH binding to TRAV27/TRBV19 (*A*), where only SEH binds, TRAV22/TRBV7.9 (*B*), where only SEA binds, and TRAV27/TRBV7.9 (*C*), where both SEA and SEH bind. *D*, Plotting the response at 40 s with SEH concentration shows that the affinity of SEH for TRAV27/TRBV19 is similar to its affinity for TRAV27/TRBV7.9 once the 156 RU contribution of SEA binding to TRAV27/TRBV7.9 had been subtracted.

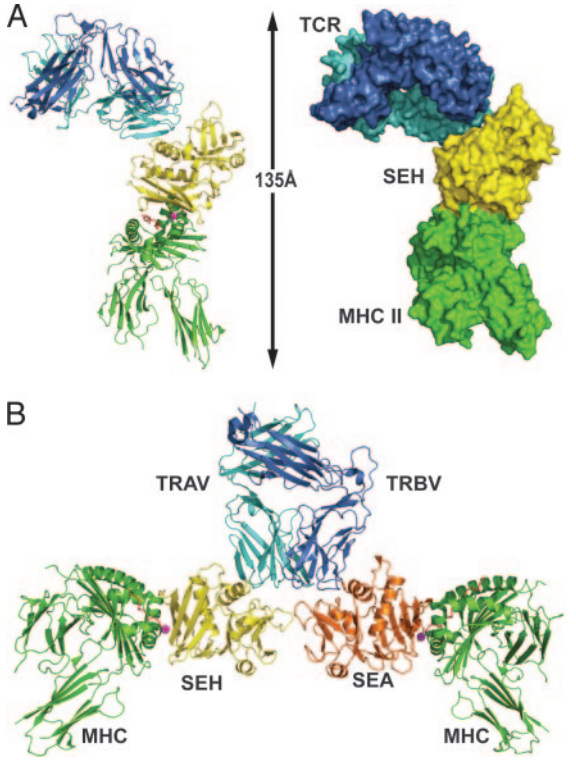

FIGURE 3. Ribbon and surface representations of three-dimensional model complexes. *A*, Ribbon and surface representations of a model of the ternary complex. The model contains a TCR (TRAV27/TRBV19, cyan and blue) that binds SEH (yellow) and MHC (green). *B*, Ribbon representation of a structural model of TCR (TRAV27/TRBV19, cyan and blue) binding SEH (yellow), SEA (orange), and MHC (green), simultaneously.

swapping. In Vα-chains like TRAV27 the anti-parallel β sheet that creates CDR2 is formed between the c″ β-strand and the following d β-strand and not between the c″ β-strand and the preceding c′ β-strand as in the majority of Vβ-chains (20). In addition, the different orientation between the Vα and Cα domains compared with the Vβ and Cβ domains of TCR contributes to the tilted conformation. SEH interacts with TRAV27 using similar regions as those of SPE-A in the model. The most important is that several residues in helix 2 of SEH interacts with CDR2 on TRAV27 and that residues in the α4-β9 loop interacts with residues on the c″ β-strand of TRAV27. The distance between the MHC and TCR termini is ~135 Å in the model of the ternary complex, which is in agreement with distances in normal MHC-TCR complexes (130 Å) (21).

In Fig. 3*B*, a model of the penta complex (TCR-SEA-SEH-2MHC) is presented. It was constructed by additional superposition of the TCR-SEH-MHC structure onto a second SEH-MHC complex where SEH had been exchanged with SEA. According to the presented model, both SEA and SEH can bind to the same TCR simultaneously without any steric hindrance and, hence, suggests that such a formation can happen on the cell surface as well.

Affinity measurements of the SEH-SEA-TCR complexes

As an alternative confirmation of the binding, the affinity between SEH and TRAV27 was determined. SEH was immobilized to ~300 RU and TRAV27/TRBV19 was injected at various concentrations (20–0.04 μM) (Fig. 4*D*). In addition, the affinity between TRAV22/TRBV7.9 and SEA was determined, and this TCR contact also served as negative control for the SEH measurements (Fig. 4, *B* and *E*). The response was measured where the equilibrium had been reached (40 s into the injection) and the data were analyzed using GraphPad Prism. SEH binds to TRAV27 with an affinity of ~4 μM (Fig. 4*F*), whereas SEA binds to TRBV7.9 with an affinity of ~2 μM (Fig. 4*C*).

Discussion

The established paradigm has been that SAgs bind a specific repertoire of TCR Vβ-chains and hence mediate T cell activation dependent on the T cell Vβ expression. In this study, we demonstrate that one SAg (SEH) is capable of binding to the α-chain (TRAV27) of a TCR (Fig. 1). SEH activation of human T cells does not induce any obvious Vβ-specific expansion of the cells according to FACS analyses or RT-PCR (12). In addition, from a biological point of view SEH appears to belong to a different class of SAgs with no reactivity in the mouse (15) and a unique preference for a subset of human T cells. In this study, we investigated the interaction between SEH and TRAV27 and detected a direct binding between the α-chain of TCR and SEH. In addition, it was also shown that SEH and SEA are able to bind two different chains of the TCR simultaneously, in agreement with previous results showing that SEA and SEH do not compete for binding to the same type of TCR even though they both activate the cells expressing it (12). We also show that a penta complex between TCR, SEH, SEA, and 2MHC (TCR-SEH-SEA-2MHC) can possibly form (Fig. 3*B*). However, SEA may not bind to both the high and the low affinity site on MHC at the same time as SEH binds their common TCR. Nevertheless, this lack of interaction is not expected to affect function, because it has been shown that SEA is still active when the low affinity site for MHC had been deleted

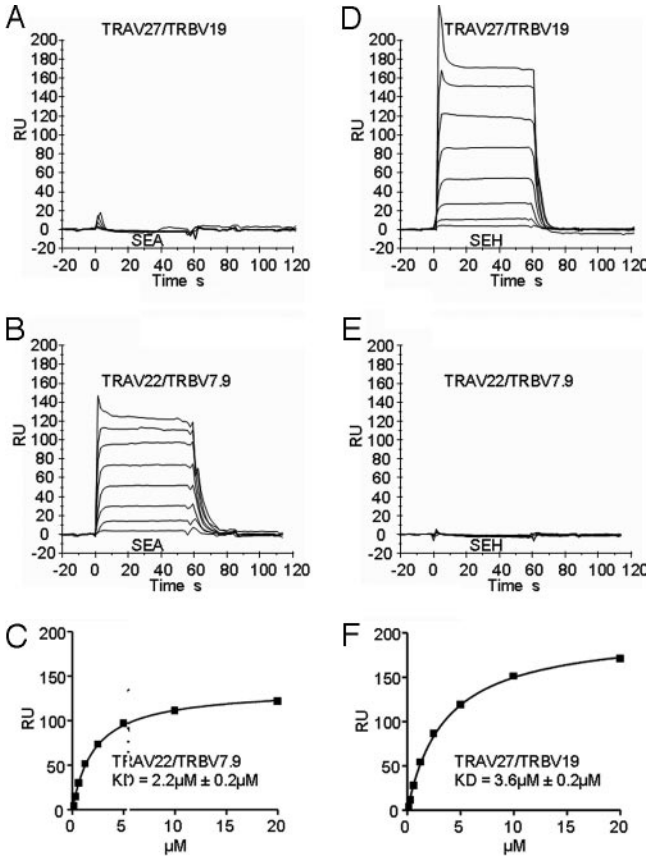

FIGURE 4. Affinity measurements of SEH binding to TCR. Injection of titrations of TCRs TRAV27/TRBV19 (*A* and *D*) and TRAV22/TRBV7.9 (*B* and *E*) over immobilized SAgs SEA (*A* and *B*) and SEH (*D* and *E*). Responses from a blank flow cell were subtracted. There was no binding seen to another negative control, SED (data not shown). *C* and *F*, Determination of the equilibrium K_D values using the measurements of the responses from the sensograms at 40 s (*B* and *D*, respectively).

(22). In addition, earlier studies have revealed a complementary effect of SAgs that seems to play an important role for the bacteria producing them. In particular, SEH coexpresses frequently with SEA and SEB (23). Hence, these bacteria may have evolved to produce SAgs that can activate a very broad repertoire of T cells and, in addition, be presented by MHC class II in different ways (24). Therefore, bacteria harboring the genes for such complementary SAgs are expected to be better suited to survive in a broad repertoire of hosts.

The affinity between SEH and TRAV27 was measured using equilibrium calculations and found to be ~4 μM. This is comparable to the affinities of other SAgs interacting with Vβ-chains, estimated to range from $K_D \approx 10^{-4}$ to 10^{-6} M (25). Hence, the affinity suggests that the SEH-TCR interaction is similar to other SAg-TRBV interactions. However, it has been reported that the putative TCR binding site in SEH differs from other SAgs by sequence and structural dissimilarities (26). From our suggested structural model we cannot explain why SEH specifically binds TRAV27, but important differences in the sequence of the interacting regions might explain why SEH behaves differently compared with other SAgs. For example, SEH has a glycine residue (Gly^{19}) on helix 4 where other SAgs have bulky aromatic residues interacting with the Vβ-chain. Similarly, SEH has a glycine residue (Gly^{50}) on the β2-β3 loop where other SAgs in most cases have a tyrosine residue. This loop also has a different conformation in SEH compared with other SAgs. However, further studies are needed to establish whether these hypotheses are correct.

The recent discovery that SAgs activate T cells by using the Vα-chain has several implications for the biology of SAgs, underlining the importance of continuing studies in this field. One important consequence is that investigating whether a patient has a skewed Vβ repertoire (27), which is a common way to determine whether a disease is caused by SAg or not, may be misleading. Because our results show that Vβ interaction is not the exclusive means of T cell activation by SAgs, it could be possible that such a disease may not be properly detected. In addition, this study defines the importance of the divergence needed for a broad T cell activation and also the value of having different SAgs present in the bacteria to start a superantigenic response. Hence, these results are of importance for the field of SAg biology and have consequences for the development of diagnostics as well as for the identification of therapeutic methods to treat SAg-mediated diseases.

Disclosures

The authors have no financial conflict of interest.

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