Crystal Structure of Staphylococcal Enterotoxin I (SEI) in Complex with a Human Major Histocompatibility Complex Class II Molecule*

Marisa M. Fernández 1,2,†, Rongjin Guan 1,2,‡, Chittoor P. Swaminathan 3, Emilio L. Malchiodi 4,5, § and Roy A. Mariuzza 4,5, §

From the 1 Cátedra de Inmunología and Instituto de Estudios de la Inmunidad Humoral, Laboratorio de Inmunología Estructural, Consejo Nacional de Investigaciones Científicas y Técnicas, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Junín 956 4° P, 1113 Buenos Aires, Argentina and the 2 Center for Advanced Research in Biotechnology, W. M. Keck Laboratory for Structural Biology, University of Maryland Biotechnology Institute, Rockville, Maryland 20850

Superantigens are bacterial or viral proteins that elicit massive T cell activation through simultaneous binding to major histocompatibility complex (MHC) class II and T cell receptors. This activation results in uncontrolled release of inflammatory cytokines, causing toxic shock. A remarkable property of superantigens, which distinguishes them from T cell receptors, is their ability to interact with multiple MHC class II alleles independently of MHC-bound peptide. Previous crystallographic studies have shown that staphylococcal and streptococcal superantigens belonging to the zinc family bind to a high affinity site on the class II β-chain. However, the basis for promiscuous MHC recognition by zinc-dependent superantigens is not obvious, because the β-chain is polymorphic and the MHC-bound peptide forms part of the binding interface. To understand how zinc-dependent superantigens recognize MHC, we determined the crystal structure, at 2.0 Å resolution, of staphylococcal enterotoxin I bound to the human class II molecule HLA-DR1 bearing a peptide from influenza hemagglutinin. Interactions between the superantigen and DR1 β-chain are mediated by a zinc ion, and 22% of the buried surface of peptide-MHC is contributed by the peptide. Comparison of the staphylococcal enterotoxin I–peptide–DR1 structure with ones determined previously revealed that zinc-dependent superantigens achieve promiscuous binding to MHC by targeting conservatively substituted residues of the polymorphic β-chain. Additionally, these superantigens circumvent peptide specificity by engaging MHC-bound peptides at their conformationally conserved N-terminal regions while minimizing sequence-specific interactions with peptide residues to enhance cross-reactivity.

Superantigens (SAGs) are a class of disease-causing and immunostimulatory proteins of bacterial or viral origin with the ability to activate up to 20% of all T cells, as compared with the on order of only 0.001% of T cells for conventional peptide antigens (hence the term “superantigen”) (1, 2). SAGs activate T cells by simultaneously binding T cell receptors (TCRs) and MHC class II molecules, resulting in the massive release of inflammatory cytokines, such as interleukin-1, interleukin-2, tumor necrosis factor-α, and tumor necrosis factor-β (3). These host cytokines are believed to be responsible for the most severe consequences of SAG intoxication, including capillary leak, renal failure, acute respiratory distress, and death (3).

The best-characterized group of SAGs belongs to the pyrogenic toxin SAG family, which currently numbers 22 members produced by Staphylococcus aureus and Streptococcus pyogenes (3, 4). These toxins include staphylococcal enterotoxins A through M (SEA to SEM, except there is no F), staphylococcal toxic shock syndrome toxin-1 (TSST-1), streptococcal superantigen (SSA), streptococcal mitogenic exotoxin Z (SMEZ), and streptococcal pyrogenic exotoxins A (SPEA), SPEC, SPEG, SPEH, and SPEJ (5–8). These proteins are among the most potent pyrogens known and are capable of inducing toxic shock syndrome, an acute onset illness characterized by high fever and hypotension that can lead to multiple organ failure and lethal shock (3). Because of their extreme virulence and the ease with which they can be produced and disseminated, bacterial SAGs have been identified as category B agents of bioterrorism by the U. S. Centers for Disease Control and Prevention.

All known bacterial SAGs share a characteristic three-dimensional structure consisting of an N-terminal β-barrel domain and a C-terminal β-grasp domain (2). Despite this common architecture, the complexes formed between SAGs and MHC molecules are structurally diverse, considerably more so than SAG–TCR complexes (4) Thus, x-ray crystallographic and binding studies have shown that MHC class II molecules possess two independent binding sites for bacterial SAGs: 1) a low affinity site ($K_D \sim 10^{-5} \text{M}$).
on the conserved $\alpha$-chain; and 2) a zinc-dependent, high affinity site ($K_D \sim 10^{-7} \text{ m}$) on the polymorphic $\beta$-chain. Based on sequence analysis, SAGs may be divided into three groups according to how they bind MHC: 1) those that bind only the MHC $\alpha$-chain through the low affinity site (SEB, SEC1–3, SEG, TSST-1, SPEA, SSA); 2) those that bind only the MHC $\beta$-chain through the zinc-dependent, high affinity site (SEH, SEI, SEJ, SEK, SEL, SEM, SPEC, SPEG, SPEH, SPEJ, SMEZ); and 3) those that cross-link two MHC molecules by simultaneously binding the $\alpha$-chain of one MHC and the $\beta$-chain of another MHC through the low and high affinity sites, respectively (SEA, SEJ, SEE).

A unique feature of bacterial SAGs, which is a major contributor to their toxicity, is the ability of individual SAGs to bind different MHC class II molecules, largely irrespective of the sequence of the MHC-bound peptide. In this way, SAGs maximize TCR-MHC interactions and the resulting T cell activation. For SAGs such as SEB and SEC3, which bind to the low affinity site on the class II $\alpha$-chain, recognition of multiple MHC alleles by a single toxin is readily explained by crystal structures of these SAGs bound to HLA-DR1, a human class II molecule (10, 11). In these complexes, the N-terminal domain of SEB or SEC3 contacts the $\alpha_1$ domain of DR1, away from the peptide-binding groove. Because the DR $\alpha$-chain is nonpolymorphic and because the SAG does not contact the MHC-bound peptide, the result is promiscuous MHC recognition. By contrast, the structural basis for cross-recognition of MHC by zinc-dependent SAGs is much less apparent, despite the available structures of two such SAGs, SEH and SPEC, bound to HLA-DR1 and HLA-DR2a, respectively (12, 13). In these complexes, the C-terminal domain of the SAG contacts the $\beta_1$ domain of HLA-DR, which is polymorphic, as well as the MHC-bound peptide. Indeed, the peptide accounts for ~25% of the surface area of the MHC molecule buried by SEH or SPEC, similar to TCR-peptide-MHC complexes, in which the TCR exhibits exquisite specificity for both peptide and MHC (14).

To better understand MHC recognition by zinc-dependent SAGs, we determined the crystal structure of staphylococcal enterotoxin I (SEI) bound to HLA-DR1 bearing a peptide from influenza virus hemagglutinin (HA-(306–318)). SEI, which is encoded in the egc operon of S. aureus (15), is associated with both menstrual and nonmenstrual toxic shock syndrome, in addition to food poisoning (16) and various veterinary diseases (17). Comparison of the SEI-HA:HLA-DR1 structure with those of SEH and SPEC bound to MHC class II (12, 13) reveals how zinc-dependent SAGs circumvent peptide specificity and how they target key residues of the polymorphic class II $\beta$-chain to achieve promiscuous binding to peptide-MHC.

**EXPERIMENTAL PROCEDURES**

Expression and Purification of SEI—The sei gene was cloned and expressed in *Escherichia coli* as described previously (18). Briefly, sei from clinically isolated *S. aureus* strain Fc30 (AAX84810) was cloned into the BamHI/EcoRI cloning site of the bacterial expression vector pET32a.3C, which encodes thioredoxin and a 3C protease recognition sequence (19) directly upstream of the BamHI site. Six histidine-encoding triplets were grafted onto the 3′ terminus of the sei gene to facilitate purification of the thioredoxin-SEI fusion protein. For expression, transformed Origami (DE3) cells (Novagen) were grown at 30 °C in LB medium containing 100 μg/ml ampicillin and 30 μg/ml chloramphenicol. Bacteria were induced with 0.1 mM isopropyl-1-thiogalactopyranoside at an absorbance of 1.0 at 600 nm. After induction, the cultures were incubated for 4 h with constant shaking. SEI was expressed as a soluble cytoplasmic fusion protein (20). The protein was purified using a Ni$^{2+}$ chelate adsorbent (Qiagen), cleaved with 3C protease (kindly provided by Dr. R. Langley) and resubjected to Ni$^{2+}$ affinity chromatography to separate SEI from thioredoxin. SEI was further purified using a Mono S cation exchange column (Amersham Biosciences).

Expression and Purification of HLA-DR1—HLA-DR1 was produced by *in vitro* folding from bacterial inclusion bodies as described (21). Briefly, plasmids encoding the HLA-DR1 $\alpha$-chain (DRA*0101) and $\beta$ (DRB1*0101) chain were transformed separately into *E. coli* BL21(DE3) cells (Stratagene). Bacteria were grown at 37 °C to an absorbance of 0.6–0.7 at 600 nm, and 1 mM isopropyl-1-thiogalactopyranoside was added. Inclusion bodies were washed extensively and the subunits purified under denaturing and reducing conditions using an HQ50 anion exchange column (PerSeptive Biosystems). Yields of DR1 $\alpha$- and $\beta$-subunits were 16 and 20 mg/liter of culture medium, respectively. Purified subunits were diluted dropwise with constant stirring to a final concentration of 50 μg/ml into a folding solution of 20 mM Tris-HCl (pH 8.5), 25% (v/v) glycerol, 0.5 mM EDTA, 3 mM reduced glutathione, and 0.3 mM oxidized glutathione and kept at 4 °C for 2 days in the presence of 1 μM HA-(306–318) peptide (PKYVKQNTLKLAT). Recombinant HA+HLA-DR1 was purified from the folding mixture using a Mono Q anion exchange column (Amersham Biosciences) equilibrated with 20 mM Tris-HCl (pH 8.0) and developed with a linear NaCl gradient. The protein eluted as a single peak at 0.15 M NaCl.

Crystallization and Data Collection—HLA-DR1-HA and SEI were premixed in a 1:1 molar ratio, and the complex was purified with a Superdex S-200 column (Amersham Biosciences) prior to crystallization. Crystals were grown at room temperature in hanging drops by mixing 1 μl of protein solution at 10 mg/ml in 10 mM Hepes and 15 mM NaCl (pH 7.5) with 1 μl of reservoir solution containing 10% (v/v) dioxane, 1.2 mM (NH$_4$)$_2$SO$_4$, and 100 mM Hepes (pH 7.5). Large, but twinned, pyramidal crystals grew over a period of 2 weeks. Single crystals were obtained by microseeding at a protein concentration of 7 mg/ml.

For data collection, crystals were cryoprotected by brief soaking in mother liquor containing 15% (v/v) glycerol and flash-cooled in a nitrogen stream. X-ray diffraction data to 2.0 Å resolution were recorded in-house at 110 K using an R-axis IV$^{2+}$ image plate detector equipped with Osmic mirrors and mounted on a Rigaku rotating anode Cu-Kα x-ray generator. The data were processed and scaled with CrystalClear (22). Data collection statistics are summarized in Table 1.

Structure Determination and Refinement—The structure of the SEI-HA:HLA-DR1 complex was solved by molecular replacement using Phaser (23). The search models consisted of SEA (24) (Protein Data Bank accession code 1SXT) and HA-(306–318):HLA-DR1 (25) (1DLH). Only one SEI-HA:HLA-DR1 complex molecule
Crystal Structure of SEI Bound to MHC Class II

| TABLE 1 | Data collection and structure refinement statistics |
|------------------|------------------|
| Data collection  | Resolution range (Å) 40.0-2.0 |
|                  | Space group C2 |
|                  | Cell parameters (Å, °) |
|                  | a = 150.40, b = 99.93, c = 72.92 |
|                  | β = 92.0 |
|                  | Unique reflections* 72,148 (7089) |
|                  | Completeness (%)* 99.2 (98.0) |
|                  | Rmerge (%)* 7.5 (37.8) |
|                  | l(Tl) 9.7 (3.3) |
|                  | Redundancy 4.06 (3.9) |
| Refinement       | Resolution range (Å) 40.0-2.0 |
|                  | Rcryst (%) 21.3 |
|                  | Rsym (%) 25.2 |
|                  | No. of reflections used 69,190 |
|                  | No. of reflections in Rmerge set 2,949 |
|                  | No. of non-hydrogen protein atoms 4,859 |
|                  | No. of SO4 ions 4 |
|                  | No. of Zn ions 1 |
|                  | No. of Hepes molecules 1 |
|                  | No. of dioxane molecules 2 |
|                  | No. of water molecules 460 |
|                  | r.m.s. deviation bond lengths (Å)* 0.012 |
|                  | r.m.s. deviation bond angles (°)* 1.08 |
|                  | Average temperature factors (Å²) Protein main chain atoms 33.6 |
|                  | Protein side chain atoms 34.2 |
|                  | Waters 35.6 |
|                  | Temperature factor from Wilson plot (Å²) 29.4 |
|                  | Ramachandran plot statistics |
|                  | Generously allowed (%) 90.8 |
|                  | Additional allowed (%) 8.8 |
|                  | Fortunately allowed (%) 0.4 |

* Values in parentheses are statistics of the highest resolution shell (2.07-2.00 Å).

was found in the asymmetric unit, corresponding to a Matthews coefficient of 3.72 (solvent content ~67%). The molecular replacement solution was refined with Refmac 5.2 (26), and the model was adjusted manually with XtalView (27) based on σA-weighted Fo – Fc and 2Fo – Fc electron density maps. TLS parameters were refined as well as temperature (B) factors. The final model comprises residues 4–216 of SEI, 4–181 of DR1-β, and 306–318 of HA and 460 water molecules. The model also contains one zinc ion, four sulfate ions, two dioxane molecules, and one Hepes molecule, resulting in a final Rcryst of 21.3% and Rsym of 25.2% at 2.0 Å resolution. The quality of the model was examined with PROCHECK (28) and Molprobity (29). Refinement statistics are summarized in Table 1. Atomic coordinates and structure factors for the SEI·HA·HLA-DR1 complex have been deposited in the Protein Data Bank under accession code 2G9H.

RESULTS AND DISCUSSION

Overview of the Complex—The structure of SEI bound to HA-(306–318)·HLA-DR1 was determined by molecular replacement to 2.0 Å resolution (Table 1). The overall structure of the complex is shown in Fig. 1A. All residues in the interface between SAG and MHC molecules show unambiguous electron density. In addition, all SEI residues are well defined, including those forming the β4-β5 loop (see below). In the complex, all contacts made by SEI are to the DR1 β-chain and HA peptide; there are no interactions between the DR1 α-chain and the SAG. The β-grasp motif of the C-terminal portion of SEI contacts the α-helix of the β1 domain of HLA-DR1, as well as the N-terminal portion of HA-(306–318) (Fig. 1B).

The interaction between SEI and the DR1 β-chain is mediated in part by a zinc ion, for which identity was confirmed by atomic absorption spectroscopy (data not shown). The importance of Zn2+ to complex stabilization is supported by the finding that the addition of EDTA abolishes binding of SEI to HLA-DR1 in solution (18). However, the occupancy of Zn2+ in the crystal is surprisingly low. In the final refined model, its occupancy was set to 0.3, which gave a temperature factor of 30.7 Å², very close to the average main chain atom B-value of 33.6 Å² for the protein molecules (Table 1). The low zinc occupancy could be explained by traces of EDTA in the protein solution used for crystallization, because EDTA was present throughout the preparation of HLA-DR1 except at the final dialysis stage. Residual EDTA could have stripped Zn2+ from SEI, either before or after crystal formation. Although EDTA eliminates detectable binding of SEI to HLA-DR1 during gel filtration (18), it is likely that the SAG retains some affinity for MHC, even in the absence of Zn2+. Under the high protein concentrations in crystallization drops, low affinities often suffice for co-crystallization. Alternatively, even if each complex molecule in the crystal initially contained Zn2+, its partial removal by EDTA would not necessarily disrupt the SEI·HA·DR1 complex, because lattice contacts could compensate for reductions in affinity.

Conformation of the β4-β5 Loop and Implications for TCR Binding—In nearly all previously reported SAG structures, both free and bound to MHC or TCR ligands, little or no electron density could be observed for residues connecting β-strands 4 and 5 of the N-terminal domain (10, 24, 30–37), implying flexibility. In the SEI·HA·DR1 complex, by contrast, the β4-β5 loop of SEI (residues 67–75) is well ordered (Fig. 2A), even though it lacks the disulfide bond typical of other SAGs. Sequence alignments show that this loop is 1–6 residues shorter in SEI than in most SAGs (Fig. 3), which could explain why it adopts a defined conformation in the SEI·HA·DR1 complex (the structure of unbound SEI is unknown). Alternatively, lattice contacts with a neighboring complex molecule in the crystal (which include several hydrogen bonds between loop residues Cys73 and Ser75 and a symmetry-related DR1 molecule) may account for the ordering of the β4-β5 loop in the SEI·HA·DR1 structure.

The conformation and amino acid sequence of the β4-β5 loop may influence the Vβ binding specificity of SEI and other SAGs. Thus, the structure of SPEC in complex with human Vβ2.1 showed that the β4-β5 loop makes a substantial contribution to the binding interface (38). Like SPEC, the five other known zinc-dependent SAGs from Streptococcus (SPEG, SPEH, SPEI, SMEZ1, and SMEZ2) also stimulate T cells expressing Vβ2.1 (39). By contrast, no zinc-dependent SAG from Staphylococcus, including SEI, recognizes Vβ2.1. To help explain the lack of interaction between SEI and Vβ2.1, SEI was superposed onto SPEC in the SPEC-Vβ2.1 structure (38). In the docked complex (Fig. 2B), major steric clashes are evident between the β4-β5 loop of SEI and the first and
second complementarity-determining regions (CDR1 and CDR2) of Vβ2.1. These clashes arise from the very different orientations of the β4-β5 loop in SEI and SPEC, underscoring the importance of this loop in determining Vβ-binding specificity.

Structure of the Interface and Interactions with Zinc and Peptide-MHC—The complex of SEI with HA-DR1 buries 1163 Å² of surface area as calculated with Areaimol in the CCP4 program suite (40), of which 596 Å² is contributed by peptide-MHC and 567 Å² by the SAG. By comparison, the total buried surfaces in the SEH-HA-DR1 and SPEC-MBP-DR2a complexes are considerably greater: 1465 and 1628 Å², respectively (12, 13). For the SEI-HA-DR1 complex, 22% (132 Å²) of the buried surface of peptide-MHC is contributed by the MHC-bound peptide, compared with 28% (208 Å²) for the SEH-HA-DR1 complex and 34%
(265 Å²) for the SPEC-MBP-DR2a complex. No major rearrangements in the polypeptide backbone of HA-DR1 are associated with complex formation; the free (25) and SEI-bound HA-DR1 molecules superpose with a root-mean-square difference of 0.39 Å for 182 carbon atoms of the DR1 domains, including the HA peptide. However, several small adjustments in side chain position are evident. For example, in unbound HA-DR1, Gln70 of DR1 forms one hydrogen bond, via its side chain, to P4 Gln of HA. However, in the SEI-HA-DR1 complex, the Gln70 side chain is slightly shifted to form two hydrogen bonds with P4 Gln.

The site on HA-DR1 recognized by SEI is composed of: 1) the N-terminal portion of the α-helix of the DR1 domain, which contacts strands β7, β8, β12, β15, and the β8-β9 turn of SEI (Fig. 4A); 2) the turn between the β1 and β2 strands of the DR1 domain, which contacts the β12-β13 turn of SEI; and 3) the
N-terminal portion of the HA peptide, which contacts strands \(\beta 8\) and \(\beta 15\) of the SAG (Fig. 4B). This site overlaps those recognized by SEH and SPEC. However, unlike SEH, SEI makes no contacts with the DR1 \(\alpha 1\) domain.

The interaction of the DR1 \(\beta 1\) \(\alpha\)-helix with SEI is mediated by residues Glu\(^{307}\), Asp\(^{366}\), Thr\(^{77}\), Arg\(^{80}\), and His\(^{81}\), which form five hydrogen bonds and numerous van der Waals contacts with the SAG (Table 2). By contrast, the interaction of the \(\beta 1\)-\(\beta 2\) turn of DR1 is restricted to Thr\(^{211}\), which makes a single van der Waals contact with Asn\(^{177}\) of SEI (Table 2). A zinc ion is observed to bridge HLADR1 and SEI by tetrahedrally coordinating three ligands from the SAG (His\(^{307}\) from strand \(\beta 12\) and His\(^{207}\) and Asp\(^{309}\) from strand \(\beta 15\)) with one ligand from the MHC \(\beta 1\) \(\alpha\)-helix (His\(^{81}\)) (Fig. 4C). Histidines 169 and 207 of SEI bind through their N\(\delta 1\) and N\(e 2\) atoms, respectively, whereas His\(^{81}\) of HLADR1 binds through its N\(\delta 1\) atom (Fig. 4C). All of the metal ligand distances (1.8–2.3 \(\AA\)) and coordination angles (89–135\(^\circ\)) fall within the ranges reported for tetrahedrally coordinated zinc ions in proteins (41).

In the complex, SEI also contacts the N-terminal portion of the HA peptide from P\(^{1}\) to P\(^{2}\) (Fig. 4B), where P1 Tyr is the first anchor residue. Just two peptide residues, P\(^{1}\) Lys and P\(^{2}\) Val, account for all of the interactions with the SAG (Table 2) and are, respectively, 55 and 88% buried in the interface. These residues pack against Asn\(^{98}\) and Trp\(^{100}\) of the \(\beta 8\) strand of SAG to form a hydrophobic cluster in the main hydrophobic cluster (Fig. 4B). In addition, a side chain–side chain hydrogen bond links the N\(\zeta\) atom of P\(^{1}\) Lys to the O\(e 2\) atom of Glu\(^{111}\), the sole hydrogen bond between peptide and SAG in the complex.

Comparison with Other SAG-MHC Complexes—Comparison of the SEI-HA-DR1 structure with the two other available structures of zinc-dependent SAGs (SEH and SPEC) bound to MHC class II (12, 13) enabled us to understand how individual SAGs can interact with different HLADR1 molecules at the high affinity site, largely irrespective of the MHC-bound peptide. In the case of SAGs such as SEB (10) and SEC3 (42) that bind to the low affinity site on the class II \(\alpha\)-chain, recognition of multiple HLADR alleles by a single toxin is easily explained because the
Table 2

| Protein | HA/DR1 | SEH | HA/DR1 | SPEC | MBP/DR2a |
|---------|--------|-----|--------|------|----------|
| Asn171  | Thr218 | Gln305 | Asp164 | Cys18 | Arg209 |
| Thr107  | Gln305 | Asp164 | Glu169 | Cys18 | Arg209 |
| Pro183 , Ile287 , Asn301 | Thr115 | Glu119 | Asp164 , Asp92 | Cys18 | Arg209 |
| Asn171  | Thr218 | Gln305 | Asp164 | Cys18 | Arg209 |
| Thr107  | Gln305 | Asp164 | Glu169 | Cys18 | Arg209 |
| Pro183 , Ile287 , Asn301 | Thr115 | Glu119 | Asp164 , Asp92 | Cys18 | Arg209 |
| Lys408 | Val P-1 | Trp115 , Gln120 | Lys P-1 , Lys P3 | Phe191 | Asn P3 |
| Asp209 | Gln120 | Asp164 | Cys18 | Arg209 |
| Trp115 | Trp115 , Gln120 | Lys P-1 , Lys P3 | Phe191 | Asn P3 |
| Asn108 | Val P-1 | Trp115 , Gln120 | Lys P-1 , Lys P3 | Phe191 | Asn P3 |

**Zn**<sup>2+</sup> coordination

| Protein | HA/DR1 | SEH | HA/DR1 | SPEC | MBP/DR2a |
|---------|--------|-----|--------|------|----------|
| Asn171  | Thr218 | Gln305 | Asp164 | Cys18 | Arg209 |
| Thr107  | Gln305 | Asp164 | Glu169 | Cys18 | Arg209 |
| Pro183 , Ile287 , Asn301 | Thr115 | Glu119 | Asp164 , Asp92 | Cys18 | Arg209 |
| Asn171  | Thr218 | Gln305 | Asp164 | Cys18 | Arg209 |
| Thr107  | Gln305 | Asp164 | Glu169 | Cys18 | Arg209 |
| Pro183 , Ile287 , Asn301 | Thr115 | Glu119 | Asp164 , Asp92 | Cys18 | Arg209 |
| Lys408 | Val P-1 | Trp115 , Gln120 | Lys P-1 , Lys P3 | Phe191 | Asn P3 |
| Asp209 | Gln120 | Asp164 | Cys18 | Arg209 |
| Trp115 | Trp115 , Gln120 | Lys P-1 , Lys P3 | Phe191 | Asn P3 |
| Asn108 | Val P-1 | Trp115 , Gln120 | Lys P-1 , Lys P3 | Phe191 | Asn P3 |

**Hydrogen bonds**

| Protein | HA/DR1 | SEH | HA/DR1 | SPEC | MBP/DR2a |
|---------|--------|-----|--------|------|----------|
| Asn171  | Thr218 | Gln305 | Asp164 | Cys18 | Arg209 |
| Thr107  | Gln305 | Asp164 | Glu169 | Cys18 | Arg209 |
| Pro183 , Ile287 , Asn301 | Thr115 | Glu119 | Asp164 , Asp92 | Cys18 | Arg209 |
| Asn171  | Thr218 | Gln305 | Asp164 | Cys18 | Arg209 |
| Thr107  | Gln305 | Asp164 | Glu169 | Cys18 | Arg209 |
| Pro183 , Ile287 , Asn301 | Thr115 | Glu119 | Asp164 , Asp92 | Cys18 | Arg209 |
| Lys408 | Val P-1 | Trp115 , Gln120 | Lys P-1 , Lys P3 | Phe191 | Asn P3 |
| Asp209 | Gln120 | Asp164 | Cys18 | Arg209 |
| Trp115 | Trp115 , Gln120 | Lys P-1 , Lys P3 | Phe191 | Asn P3 |
| Asn108 | Val P-1 | Trp115 , Gln120 | Lys P-1 , Lys P3 | Phe191 | Asn P3 |

**van der Waals contacts**

| Protein | HA/DR1 | SEH | HA/DR1 | SPEC | MBP/DR2a |
|---------|--------|-----|--------|------|----------|
| Asn171  | Thr218 | Gln305 | Asp164 | Cys18 | Arg209 |
| Thr107  | Gln305 | Asp164 | Glu169 | Cys18 | Arg209 |
| Pro183 , Ile287 , Asn301 | Thr115 | Glu119 | Asp164 , Asp92 | Cys18 | Arg209 |
| Asn171  | Thr218 | Gln305 | Asp164 | Cys18 | Arg209 |
| Thr107  | Gln305 | Asp164 | Glu169 | Cys18 | Arg209 |
| Pro183 , Ile287 , Asn301 | Thr115 | Glu119 | Asp164 , Asp92 | Cys18 | Arg209 |
| Lys408 | Val P-1 | Trp115 , Gln120 | Lys P-1 , Lys P3 | Phe191 | Asn P3 |
| Asp209 | Gln120 | Asp164 | Cys18 | Arg209 |
| Trp115 | Trp115 , Gln120 | Lys P-1 , Lys P3 | Phe191 | Asn P3 |
| Asn108 | Val P-1 | Trp115 , Gln120 | Lys P-1 , Lys P3 | Phe191 | Asn P3 |

---

DR α-chain is nonpolymorphic and the SAG makes no contacts with the bound peptide. By contrast, zinc-dependent SAGs bind the class II β-chain, which is polymorphic, and also interact with peptide. To account for cross-reactivity, the SEI-HA/DR1, SEH-HA/DR1, and SPEC-MBP/DR2a complexes may be compared at three levels: 1) zinc-mediated interactions between MHC and SAG, 2) direct interactions between MHC and SAG, and 3) interactions between the MHC-bound peptide and SAG.

All three SAG residues that coordinate zinc in the SEI-HA/DR1 complex (His169, His209, and Asp209) (Fig. 4C) are structurally conserved in the SPEC-MBP/DR2a complex (Fig. 4C), although SEI His169 is from strand β12 of the SAG, whereas the corresponding SPEC residue (His167) is from strand β13 (Fig. 3). In the SEH-HA/DR1 complex (Fig. 4F), the equivalent residue is replaced by aspartate and is not coordinated to zinc (a water molecule possibly serves as a surrogate ligand). Indeed, SEI residues His207 and Asp209 are identical across the entire family of zinc-dependent SAGs, and His169 is present in 13 of 16 members, if one includes SPEC (Fig. 3). On the MHC side of the interface, His81β, which is highly conserved in class II molecules, provides the fourth zinc ligand, thereby linking SAG and MHC in all three complexes (Fig. 4, C, F, and I). Further connecting SEI and HLA-DR1 in the region of the zinc ion is a side chain–main chain hydrogen bond, Asn89-Nδ2-O Thr77β, which is also present in the SEHH-HA/DR1 and SPEC-MBP/DR2a complexes (Table 2). Notably, SEI Asn98, like zinc-coordinating residues His207 and Asp209, is strictly conserved in all zinc-dependent SAGs (Fig. 3).

In addition to Thr77β and His81β, zinc-dependent SAGs form key interactions with several other conserved, or conservatively substituted, residues of the polymorphic DR β-chain, in particular Glu168β and Asp187β (Table 2). By focusing recognition on this relatively conserved subset of β-chain residues, individual SAGs are able to bind multiple MHC class II alleles, hence avoiding the MHC restriction of TCRs and increasing their T cell stimulatory capacity. However, cross-reactivity is not absolute, as some zinc-dependent SAGs exhibit MHC specificity. For example, SPEC binds to both human HLA-DR and mouse I-E molecules, but not to I-A (43), possibly because I-A contains an insertion between residues 84β and 85β. On the other hand, T cell stimulation assays indicate that SEI can be presented by I-A, as well as I-E and HLA-DR (18).

A remarkable feature of the SEH-HA/DR1 complex, one that is shared by the SEHH-HA/DR1 and SPEC-MBP/DR2a complexes, is the substantial interaction between the toxin and MHC-bound peptide, such that peptide accounts for ~25% of the surface area of the MHC molecule buried in the complexes. This is similar to TCR-peptide-MHC complexes, where ~30% of the buried MHC class I or class II surface involves the antigenic peptide (14). However, although TCRs display exquisite peptide specificity, SAGs in general, including members of the zinc family, bind MHC regardless of the sequence of the associated peptide (1, 2), although some peptides may modulate the affinity of the interaction, at least in the case of TSST-1 (44). Very likely, SAGs have evolved peptide-independent binding to maximize MHC interactions and thus their biological potency. The SEI-HA/DR1, SEHH-HA/DR1, and SPEC-MBP/DR2a structures reveal how zinc-dependent SAGs circumvent peptide specificity, despite the obvious involvement of peptide in the binding interface.

In contrast to TCRs, which generally (14), although not always (45), focus on the central portion of the MHC-bound peptide at and around the P5 position, SEI, SHE, and SPEC all
recognize the N-terminal portion between positions P−3 and P3 (Table 2). In this regard, superposition of known peptide-MHC class II crystal structures has shown that the N-terminal segment (to residue P4) of peptides bound to class II molecules displays considerably less conformational variability than the central and C-terminal segments (from residue P5) (46), as may be seen by comparing the overall conformations of MHC-bound HA and MBP peptides, in which the sequences are unrelated (Fig. 4, E and H). This indicates that zinc-dependent SAGs recognize a more structurally conserved region on the surface of peptide-MHC than most TCRs, which would facilitate SAG binding to MHC molecules bearing diverse peptides. In support of this idea, we recently described an autoimmune TCR (3A6), which, unlike TCRs specific for microbial peptides, primarily recognizes the N-terminal portion of the MHC-bound peptide similar to SEI, SHE, and SPEC (47). As demonstrated using combinatorial peptide libraries (48), TCR 3A6 is much more promiscuous in terms of peptide recognition than TCRs targeting the more structurally variable central and C-terminal portions of bound peptides.

Besides engaging peptides at their more conserved N-terminal regions, zinc-dependent SAGs augment cross-reactivity by minimizing sequence-specific interactions with peptide residues. This may be achieved in various ways, as shown by the SEI-HA-DR1 and SEH-HA-DR1 complexes, in which two different SAGs bind an identical peptide-MHC ligand. In the SEI-HA-DR1 complex, SEI contacts only two HA residues, P−1 Lys and P2 Val, and makes only one hydrogen bond with peptide, to the amino group of P−1 Lys (Table 2). More extensive interactions are observed in the SEH-HA-DR1 complex, including two hydrogen bonds between SEH Gln130 and P3 Lys. However, as these bonds are to the backbone of the P3 residue, they are not expected to confer sequence specificity. The two hydrogen bonds are conserved in the SPEC-MBP-DR2a complex (but not the SEI-HA-DR1 complex), even though a completely different peptide is present.

Conclusions—Bacterial SAGs have evolved two distinct modes for interacting with MHC class II, involving either a low affinity site on the invariant α-chain or a high affinity site the variable β-chain. Our structural analysis of MHC recognition by zinc-dependent SAGs, which contact both the β-chain and MHC-bound peptide, demonstrates that these toxins achieve broad MHC cross-reactivity by binding conserved residues of the polymorphic β-chain while mostly avoiding specific interactions with side chains of the peptide. This represents a markedly different strategy for escaping peptide-MHC restriction from the one adopted by SAGs that bind to the nonpolymorphic α-chain away from the peptide-binding cleft. Nevertheless, both strategies produce similar end results: high-density binding of toxin molecules to the surface of antigen-presenting cells expressing diverse MHC alleles, which leads to highly efficient T-cell activation, massive release of pyrogenic cytokines, and toxic shock.

Because the biological potency of bacterial SAGs, which are potential agents of bioterrorism, derives from simultaneous engagement of TCR and MHC molecules, competitive blocking of the TCR- or MHC-binding sites on SAGs should, in principle, reduce toxicity. Indeed, we previously showed that an affinity-matured variant of a mouse TCR Vβ domain prevents T cell activation by SEC3 or SEB in vitro (9). However, this Vβ showed no neutralizing activity against any other SAGs tested, probably because of topological variability at the TCR-binding site. The present study suggests that a better strategy for developing broad-spectrum therapeutics for SAG-mediated diseases (i.e. a strategy that is capable of neutralizing a wide range of toxins) may be to target the MHC- rather than TCR-binding sites of SAGs. Because the low and high affinity sites for MHC are structurally conserved, as few as two SAG-binding reagents (proteins or small molecules), each directed to one of these sites, may serve as effective antagonists against multiple toxins. As all 22 known staphylococcal and streptococcal SAGs are capable of causing toxic shock, any credible program to develop therapeutics for SAG-induced disease must include a comprehensive strategy, such as targeting MHC-binding sites, for neutralizing as broad a spectrum of toxins as possible using a limited number of different antagonist molecules.

Acknowledgments—We thank L. J. Stern (University of Massachusetts) for the gift of HLA-DR1 expression plasmids, J. Fraser (University of Auckland) for the pET32a.3C vector, and S. Long (National Institute of Standards and Technology) for performing atomic absorption spectroscopy measurements.

REFERENCES

1. Scherer, M. T., Ignatowicz, L., Winslow, G. M., Kappler, J. W., and Marrack, P. (1993) Annu. Rev. Cell Biol. 9, 101–128
2. Li, H., Llera, A., Malchiodi, E. L., and Mariuzza, R. A. (1999) Annu. Rev. Immunol. 17, 435–466
3. McCormick, J. K., Yarwood, J. M., and Schlievert, P. M. (2001) Annu. Rev. Microbiol. 55, 77–104
4. Sundberg, E. J., Li, Y., and Mariuzza, R. A. (2002) Curr. Opin. Immunol. 14, 36–44
5. Bohach, G. A., Fast, D. J., Nelson, R. D., and Schlievert, P. M. (1990) Crit. Rev. Microbiol. 17, 251–272
6. Koth, M. (1998) Curr. Opin. Microbiol. 1, 56–65
7. Dinges, M. M., Orwin, P. M., and Schlievert, P. M. (2000) Clin. Microbiol. Rev. 13, 16–34
8. McCormick, J. K., and Schlievert, P. M. (2000) in Gram Positive Pathogens (Fischetti, V., Novick, R., Ferretti, J., Portnoy, D., and Rood, J., eds) pp. 43–52, American Society for Microbiology, Washington, D.C.
9. Kieke, M. C., Sundberg, E., Shusta, E. V., Mariuzza, R. A., Wittrup, K. D., and Kranz, D. M. (2001) J. Mol. Biol. 307, 1305–1315
10. Jardetzky, T. S., Brown, J. H., Gorga, J. C., Stern, L. J., Urban, R. G., Chi, Y. I., Stauffacher, C., Strominger, J. L., and Wiley D. C. (1994) Nature 368, 711–718
11. Sundberg, E. J., Anderssen, P. P., Schlievert, P. M., Karjalainen, K., and Mariuzza, R. A. (2003) Structure (Camb.) 11, 1151–1161
12. Petersson, K., Hakansson, M., Nilsson, H., Forsberg, G., Svensson, L., Lijas, A., and Walse, B. (2001) EMBO J. 20, 3306–3312
13. Li, Y., Li, H., Dimassi, N., McCormick, J. K., Martin, R., Schuck, P., Schlievert, P. M., and Mariuzza, R. A. (2001) Immunity 14, 93–104
14. Rudolph, M. G., Stanfield, R. L., and Wilson, I. A. (2006) Annu. Rev. Immunol. 24, 419–466
15. Jarraud, S., Peyrat, M. A., Lim, A., Tristan, A., Bes, M., Mougel, C., Etienne, J., Vandenesch, F., Bonneville, M., and Lina G. (2001) J. Immunol. 166, 669–677
16. Banks, M. C., Kamel, N. S., Zabriskie, J. B., Larone, D. H., Ursea, D., and Posnett, D. N. (2003) J. Infect. Dis. 187, 77–86
17. Omoe, K., Ishikawa, M., Shimoda, Y., Hu, D. L., Ueda, S., and Shinagawa, K. (2002) J. Clin. Microbiol. 40, 857–862

September 1, 2006•Volume 281•Number 35
Journal of Biological Chemistry
Crystal Structure of SEI Bound to MHC Class II

18. Fernández, M. M., De Marzi, M. C., Bergher, P., Burzyn, D., Langlely, R. J., Piazzon, I., Mariuzza, R. A., and Malchiodi, E. L. (2006) Mol. Immunol. 43, 927–938

19. Walker, P. A., Leong, L. E., Ng, P. W., Tan, S. H., Waller, S., Murphy, D., and Porter, A. G. (1994) Bio/Technology 12, 601–605

20. Langley, R., Wines, B., Willoughby, N., Basu, L., Proft, T., and Fraser, J. (2005) J. Immunol. 174, 2926–2933

21. Frayser, M., Sato, A. K., Xu, L., and Stern, L. J. (1999) Protein Expr. Purif. 15, 105–114

22. Pflugrath, J. W. (1999) Acta Crystallogr. Sect. D Biol. Crystallogr. 55, 1718–1725

23. McCoy, A. J., Grosse-Kunstleve, R. W., Storoni, L. C., and Read, R. J. (2005) Acta Crystallogr. Sect. D Biol. Crystallogr. 61, 458–464

24. Sundstrom, M., Hallen, D., Svensson, A., Dohlsten, M., and Abrahmsen, L. (1996) J. Biol. Chem. 271, 2212–2216

25. Stern, L., Brown, J., Jardetzky, T., Gorga, J., Urban, R., Strominger, J., and Wiley, D. (1994) Nature 368, 215–221

26. Murshudov, G. N., Vagin, A. A., and Dodson, E. J. (1999) Acta Crystallogr. Sect. D Biol. Crystallogr. 53, 240–255

27. McRee, D. E. (1999) J. Struct. Biol. 125, 156–165

28. Laskowski, R., Moss, D., and Thornton, J. (1993) J. Mol. Biol. 231, 1049–1067

29. Davis, I. W., Murray, L. W., Richardson, J. S., and Richardson, D. C. (2004) Nucleic Acids Res. 32, W615–W619

30. Schad, E. M., Zaitseva, I., Zaitsev, V. N., Dohlsten, M., Kalland, T., Schlievert, P. M., Ohlendorf, D. H., and Svensson, L. A. (1995) EMBO J. 14, 3292–3301

31. Fields, B. A., Malchiodi, E. L., Li, H., Ysern, X., Stauffacher, C. V., Schlievert, P., Karjalainen, K., and Mariuzza, R. A. (1996) Nature 384, 188–192

32. Sundberg, E., and Jardetzky, T. (1999) Nat. Struct. Biol. 6, 123–129

33. Li, H., Llera, A., Tsuchiya, D., Leder, L., Ysern, X., Schlievert, P. M., Karjalainen, P. M., and Mariuzza R. A. (1998) Immunity 9, 807–816

34. Roussel, A., Anderson, B. F., Baker, H. M., Fraser, J. D., and Baker, E. N. (1997) Nat. Struct. Biol. 4, 635–643

35. Papageorgiou, A. C., Tranter, H. S., and Acharya, K. R. (1998) J. Biol. Chem. 273, 61–79

36. Papageorgiou, A. C., Baker, M., McLeod, J., Goda, S., Manzotti, C., Sansom, D., Tranter, H., and Acharya, K. R. (2004) J. Biol. Chem. 279, 1297–1303

37. Cho, S., Swaminathan, C. P., Yang, J., Kerzic, M. C., Guan, R., Kieke, M. C., Kranz, D. M., Mariuzza, R. A., and Sundberg, E. J. (2005) Structure (Camb.) 13, 1775–1787

38. Sundberg, E. J., Li, H., Llera, A. S., McCormick, J. K., Tormo, I., Schlievert, P. M., Karjalainen, K., and Mariuzza, R. A. (2002) Structure (Lond.) 10, 687–699

39. Proft, T., and Fraser, J. D. (2003) Clin. Exp. Immunol. 133, 229–306

40. Collaborative Computational Project, Number 4 (CCP4) (1994) Acta Crystallogr. Sect. D Biol. Crystallogr. 50, 760–763

41. Alberts, I. L., Nadassy, K., and Wodak, S. J. (1998) Protein Sci. 7, 1700–1716

42. Zavala-Ruiz, Z., Sundberg, E. J., stone, J. D., DeOliveira, D. B., Chan, I. C., Svendsen, J., Mariuzza, R. A., and Stern, L. J. (2003) J. Biol. Chem. 278, 44904–44912

43. Li, P. L., Tiedemann, R. E., Moffat, S. L., and Fraser, J. D. (1997) J. Exp. Med. 186, 375–383

44. Kim, J., Urban, R. G., Strominger, J. L., and Wiley, D. C. (1994) Science 266, 1870–1874

45. Nicholson, M. J., Hahn, M., and Wucherpfennig, K. W. (2005) Immunity 23, 351–360

46. Ghosh, P., Amaya, M., Mellins, E., and Wiley, D. C. (1995) Nature 378, 457–462

47. Li, Y., Huang, Y., Lue, J., Quandt, J. A., Martin, R., and Mariuzza, R. A. (2005) EMBO J. 24, 2968–2979

48. Hemmer, B., Pinilla, C., Gran, B., Vergelli, M., Ling, N., Coulon, P., McFarland, H. F., Houghten, R., and Martin, R. (2000) J. Immunol. 164, 861–871