So many ways of getting in the way: diversity in the molecular architecture of superantigen-dependent T-cell signaling complexes

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Superantigens (SAGs) elicit massive T-cell proliferation through simultaneous interaction with MHC and TCR molecules. SAGs have been implicated in toxic shock syndrome and food poisoning, and they may also play a pathogenic role in autoimmune diseases. The best-characterized group of SAGs are the pyrogenic bacterial SAGs, which utilize a high degree of genetic variation on a common structural scaffold to achieve a wide range of MHC-binding and T-cell-stimulating effects while assisting pathogen evasion of the adaptive immune response. Several new structures of SAG–MHC and SAG–TCR complexes have significantly increased understanding of the molecular bases for high-affinity peptide/MHC binding by SAGs and for TCR Vβ domain specificity of SAGs. Using the currently available SAG–MHC and SAG–TCR complex structures, models of various trimolecular MHC–SAG–TCR complexes may be constructed that reveal wide diversity in the architecture of SAG-dependent T-cell signaling complexes, which nevertheless may result in similar signaling outcomes.

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Abbreviations

CDR2	complementarity-determining region 2
FR2	framework region 2
HA	hemagglutinin
HV4	hypervariable region 4
hVβ2.1	human Vβ2.1
MBP	myelin basic protein
mVβ8.2	mouse Vβ8.2
SAG	superantigen

Introduction

Superantigens (SAGs) are immunostimulatory and disease-associated proteins of bacterial or viral origin that, contrary to processed antigenic peptides, bind to MHC molecules outside of the peptide-binding groove and interact with TCR Vβ domains [1,2]. This results in the stimulation of a large fraction (up to 5%–20%) of the T-cell population, compared with only 1 in 10^5–10^6 T cells during normal antigen presentation, in which the MHC-bound peptide contacts the hypervariable loops of the TCR. As SAG/TCR interactions are limited to TCR Vβ domains, SAGs are classically thought to stimulate T-cell subsets independently of T cell CD4 or CD8 surface expression. Expansion of T-cell subsets *in vitro* by bacterial SAGs seems to bear this out, although *in vivo* studies suggest that they might require $CD4^+$ T cells [3]. Viral SAGs from the mouse mammary tumor virus appear to stimulate $CD4^+$-T-cell-mediated help while eliciting no $CD8^+$-T-cell response [4]. SAGs have been implicated in the pathogenesis of a number of human diseases, including toxic shock syndrome, food poisoning [5,6] and several autoimmune disorders [7], such as Crohn's disease [8•] and type I diabetes [9••]. Their virulence is thought to be a result of the stimulation of extraordinarily large numbers of T cells and the subsequent release of massive amounts of pyrogenic and inflammatory cytokines such as IL-1, IL-2, TNFα and TNFβ.

Recent identification and characterization of novel SAGs, of both bacterial and viral origin, and new crystal structures of bacterial SAGs in complex with TCR and MHC molecules (with bound antigenic peptides) have greatly advanced our understanding of diversity in the molecular architecture of MHC–SAG–TCR ternary complexes and the pathogenesis of SAG-mediated disease. Most of what we know about SAG structure and function derives from studies with bacterial SAGs and, thus, we will focus predominantly on them in this review.

A varied arsenal for immunomodulation

Twenty-one pyrogenic SAG family members produced by certain strains of the bacteria *Staphylococcus aureus* and *Streptococcus pyogenes* have so far been characterized genetically. The SAGs can be divided into three genetic subfamilies (containing the SAGs SpeC, SpeJ, SpeG and SMEZ; SEC1, SEC2, SEC3, SEB, SSA, SpeA and SEG; and SEA, SEE, SED, SEH, SEI, SEJ, SEK and SEL) and two individual branches (containing SpeH and TSST-1) that translate loosely to functional subfamilies according to biochemical and structural characterization. All of these SAGs are two-domain proteins of similar size with remarkable structural conservation considering their low level of sequence identity. The carboxy-terminal domain has high structural similarity to the immunoglobulin-binding motifs of streptococcal proteins G [10] and L [11], whereas the amino-terminal domain is structurally homologous to the oligosaccharide/oligonucleotide-binding (OB) fold shared by the B subunits of AB_5 heat-labile enterotoxins, cholera toxin, pertussis toxin and verotoxin [12]. The OB fold has been proposed to represent an ancient fold-motif that contains a stable, fold-related binding face that can accommodate high sequence and function variability [13]. The structural similarities between SAGs and other bacterial proteins suggest strongly that the former have evolved through the recombination of these two smaller structural motifs [14].

Homologous recombination events, as opposed to random point-mutations, are also the primary reason for SAG

genetic polymorphism as revealed by the identification of 24 alleles of the *smez* gene [15,16,17••], in which 30 out of 31 polymorphic positions have only two different amino acids. Additionally, in the case of *smez*, the recombination event appears to have occurred between two copies of the gene originating from different *S. pyogenes* strains; further, less significant, polymorphism has been attributed to individual point-mutations occurring independently in different strains [17••].

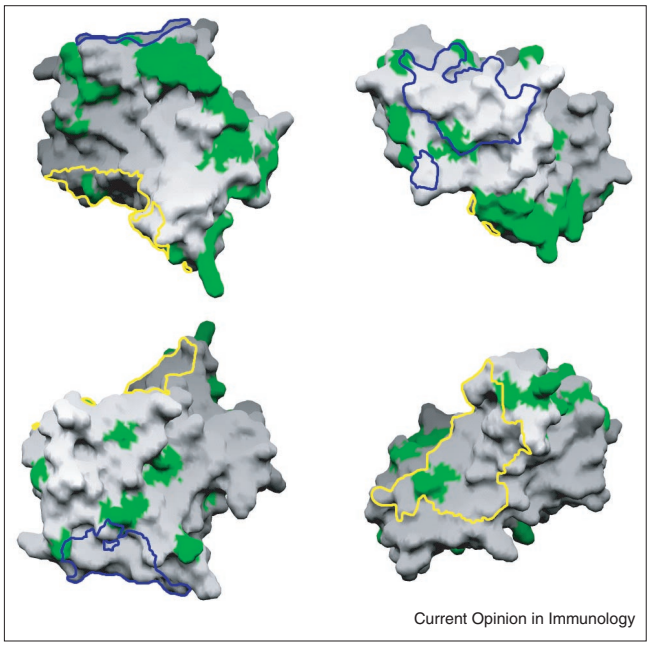
Interestingly, the extreme allelic variation of *smez* was found to not affect either TCR Vβ domain specificity or mitogenicity, but instead gave rise to significant antigenic differences [17••]. This can be seen most clearly in Figure 1, in which the polymorphic positions of *smez* have been mapped to the molecular surface of the atomic structure of one of its allelic products, SMEZ-*2* [18•], along with its putative MHC- and TCR-binding sites, as determined by homology modeling with the analogous SpeC complexes ([19••]; EJ Sundberg, RA Mariuzza, unpublished data). Indeed, the vast majority of allelic variation is observed outside of the TCR- and MHC-binding interfaces, including a highly clustered region of polymorphism, encompassing residues 91–100, positioned well away from the functional surfaces of the *smez* gene product. In this way, structural variation of the SMEZ molecular surface is likely to limit the ability of the adaptive immune response to interfere with its superantigenic function, particularly via antibody-mediated neutralization.

Genetic variability of the common structural scaffold of the conserved two-domain fold of SAGs provides not only for a degree of immunological evasion but also for remarkable adaptability in MHC- and TCR-binding by distinct SAGs. To date, the family of pyrogenic SAGs have been observed to use three unique interfaces to interact with MHC class II molecules, two of which are overlapping and found on the SAG amino-terminal domain and a third that is on the carboxy-terminal domain. SAG/TCR interactions are mediated through the cleft formed between the amino- and carboxy-terminal SAG domains, allowing for specific TCR contacts by residues from both of these domains. By altering a minimal number of residues on this common three-dimensional fold, bacterial strains are thereby able to produce a multitude of weapons for cross-linking MHC and TCR molecules. Indeed, the various strains of both *S. aureus* and *S. pyogenes* collectively produce collections of SAGs capable of providing them essentially the full range of SAG-dependent T-cell stimulation.

Superantigen interactions with MHC class II molecules and bound peptides

HLA-DR molecules, with which most binding analysis has been performed, possess two independent binding sites for bacterial SAGs: a low-affinity site (K_D~10^{-5} M) on the conserved α chain and a zinc-dependent, high-affinity site (K_D~10^{-7} M) on the polymorphic β chain [2]. Not all MHC molecules necessarily share these sites, and even

Figure 1

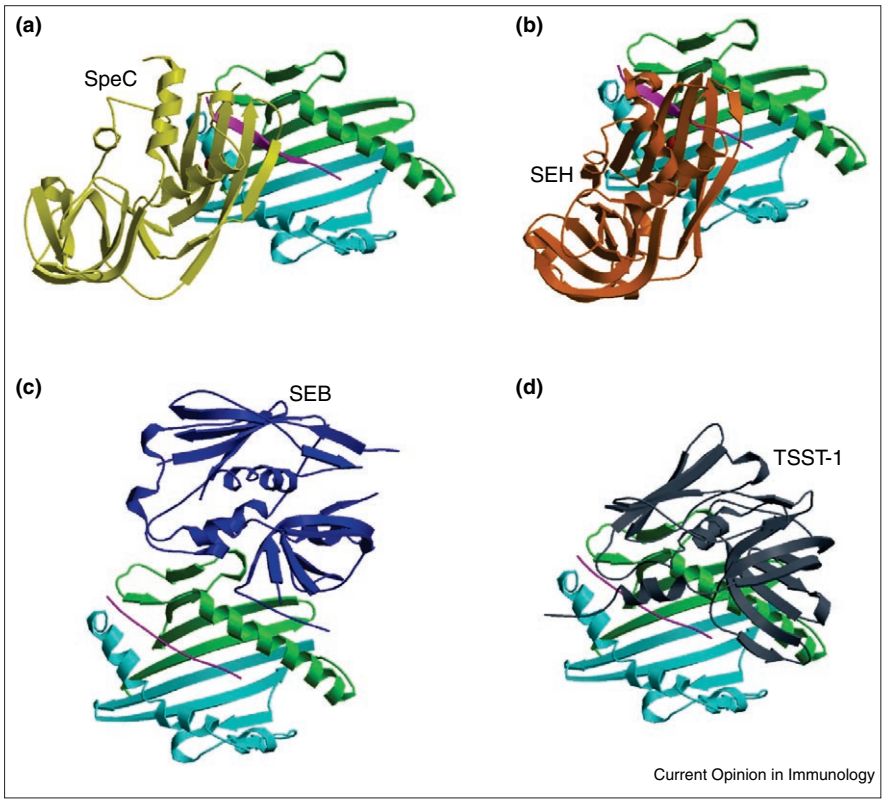


Allelic variation in SMEZ provides an elusive antigenic target while maintaining superantigen function. Four views of the SMEZ-2 molecular surface in which each panel shows the molecule rotated 90° about the vertical axis, going from top left to top right, then bottom left and bottom right. Residues subject to allelic variation amongst 24 SMEZ alleles [17••] are colored green. The binding sites of TCR and MHC class II molecules – as determined by homology modeling to the SpeC–hVβ2.1 (EJ Sundberg, RA Mariuzza, unpublished data) and SpeC–HLA-DR2a [19••] complex structures – are outlined in yellow and blue, respectively.

the low-affinity sites of HLA-DR and -DQ diverge sufficiently in structure to distinguish between SAGs [20]. Certain SAGs bind exclusively to the low-affinity site (e.g. SEB and TSST-1), whereas others bind to both the low- and high-affinity sites (e.g. SEA), or to only the high-affinity one (e.g. SpeC and SEH) of HLA-DR. Until recently, only the low-affinity interaction had been defined by X-ray crystallography [21,22]. The crystal structures of SpeC bound to HLA-DR2a [19••], and of SEH in complex with HLA-DR1 [23••], have now revealed the basis for SAG binding to the high-affinity, zinc-dependent site on MHC class II in atomic detail.

In both the HLA-DR2a–SpeC and HLA-DR1–SEH complexes, the carboxy-terminal domain of the SAG contacts the α helix of the β1 domain of the class II molecule, as well as the amino-terminal portion of the bound peptide (Figure 2a,b). There are no direct contacts with the HLA-DR α1 domain. The interaction is mediated, in part, by a bridging zinc ion that tetrahedrally coordinates ligands from SpeC or SEH with His81β from the MHC β1 helix. This mode of MHC engagement is completely distinct from those employed by SEB [21] (Figure 2c) and TSST-1 [22] (Figure 2d), both of which bind the α1 domain,

Figure 2



Comparison of docking modes of bacterial SAGs on MHC class II. **(a)** View of the HLA-DR2a–SpeC complex looking down into the peptide-binding groove of the class II molecule [19••]. The amino terminus of the peptide (pink) is at the upper left. Colors are as follows: DR2a α1 domain (green); DR2a β1 domain (light blue); and SpeC (yellow). **(b)** The complex between SEH (brown) and HLA-DR1 [23••]. **(c)** The complex between SEB (dark blue) and HLA-DR1 [21]. **(d)** The complex between TSST-1 (gray) and HLA-DR1 [22].

without the participation of metal ions. In addition, residues of SEB and TSST-1 in contact with MHC class II derive from the amino-terminal rather than carboxy-terminal domain of the SAG, as in the case of SpeC or SEH.

A surprising feature of the HLA-DR2a–SpeC and HLA-DR1–SEH complexes is the extensive interaction of the peptide bound to HLA-DR with the SAG, such that the peptide accounts for approximately one-third of the surface area of the MHC molecule buried in the complex (Figure 2a,b). This is comparable to the contribution of the antigenic peptide in TCR–MHC complexes [24,25], strongly implying that peptide plays an important role in binding SpeC and SEH.

Crystal structures have been determined for SpeC in complex with HLA-DR2a bearing two different self-peptides from myelin basic protein (MBP): $MBP_{89–101}$ and $MBP_{151–160}$ ([19••]; Y Li, RA Mariuzza, unpublished data) (Figure 3a,b). The interaction of SEH with a hemagglutinin peptide ($HA_{306–318}$) bound to class II in the HLA-DR1–SEH complex [23••] is illustrated in Figure 3c. In all three cases, the SAG contacts the amino-terminal portion of the peptide, from P–3 to P3 of $MBP_{89–101}$, P–2 to P3 of $MBP_{151–160}$ and P–1 to P3 of $HA_{306–318}$, where P1 is the first anchor residue. TCRs, by contrast, bind antigenic peptides more centrally, at and around the P5 position [24,25].

Importantly, the amino-terminal segments (to residue P4) of peptides bound to class II molecules display less conformational variability than their carboxy-terminal segments [26,27]. This suggests that SpeC and SEH bind a more structurally conserved region of the antigen-recognition surface of peptide–MHC complexes than do TCRs. Moreover, these SAGs make several conserved interactions with bound peptide. In particular, Gln113 of SpeC and the corresponding Gln120 of SEH form hydrogen bonds to the backbone of the P3 residue of all three peptides shown in Figure 3. Certain other interactions involving peptide side-chains are unique to the particular peptide, indicating that a broad range of affinities for SpeC or SEH binding to peptide–MHC complexes on antigen-presenting cells might be expected.

Whereas there is currently no information on the degree to which the binding of SpeC or SEH to HLA-DR is peptide-dependent, several studies have provided evidence that the presentation of some bacterial SAGs by MHC class II is strongly influenced by peptide sequence, thereby modulating SAG activity [28–31]. Although this may seem inconsistent with the idea that SAGs have evolved to maximize TCR/MHC interactions, an intriguing possibility is that the ability of certain SAGs to discriminate between different peptide–MHC complexes actually enhances their potency [30,32]. According to this hypothesis, the peptide dependence of SAGs like SEA, TSST-1, SpeC and

SEH serves as a mechanism for promoting high-affinity, but low-density, binding to MHC class II. By mimicking the low antigen densities normally encountered by T cells on antigen-presenting cells, these SAGs may avoid excessive T-cell signaling related to high densities that could result if they were able to bind equally well to all class II molecules on the APC surface.

Indeed, the capacity of high ligand densities to induce T-cell apoptosis through supra-optimal signaling is well documented, both for peptide–MHC and SAGs [33]. The threshold for SAG density resulting in T-cell death has been estimated recently for SEA [34•]. Occupation of total cell-surface MHC class II by SEA at levels below 0.3% resulted in a normal sequence of early T-cell activation events, whereas higher levels resulted in apoptosis. The restriction of SAG presentation density through peptide-dependent binding, therefore, may ensure appropriate activation at the level of the individual T cell, whereas the high frequency of responding T cells, as dictated by the Vβ specificity of the SAG, could determine the overall strength of the response.

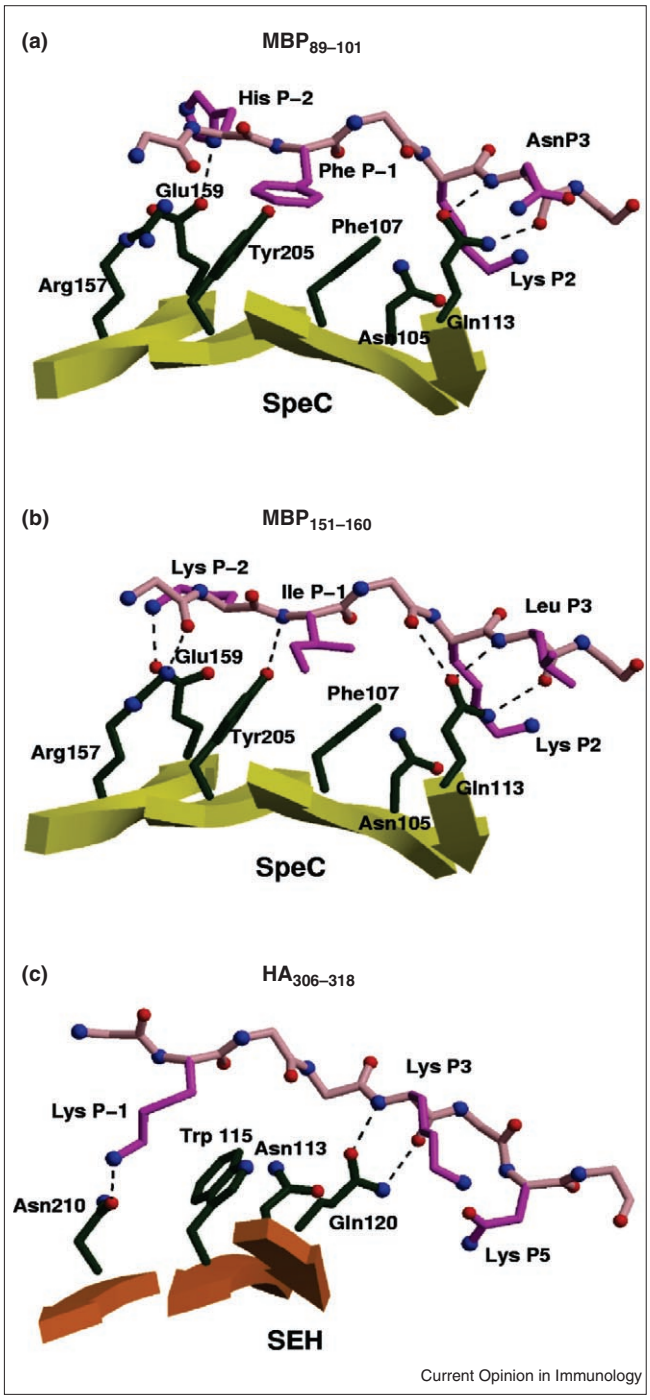
Understanding superantigen specificity for T cells at the structural level

Until just recently, structural studies of SAG/TCR-β-chain interactions had been limited to a single TCR β chain molecule, mouse Vβ8.2 (mVβ8.2), in complex with two different SAGs: SEC3 [35] or SEB [36]. These studies revealed a single binding mode for SAG/TCR interactions. Both SEC3 and SEB bind to mVβ8.2 predominantly through contacts with residues from complementarity-determining region 2 (CDR2), framework region 2 (FR2) and, to a lesser extent, hypervariable region 4 (HV4) and FR3.

Furthermore, all of the hydrogen bonds in these complexes involve only main-chain atoms of mVβ8.2 and, thus, it has been proposed that their binding mechanism is one of simple conformational dependence [35,36]. We define conformationally dependent binding as interactions that rely predominantly on the protein-backbone atomic structure of the Vβ domain, largely independent of amino acid sequence and, consequently, independent of side-chain structure.

Structural variation in Vβ domains is mainly restricted to its three CDR loops, extending from a common structural foundation, the framework regions. Even the first two CDR loops are not excessively variable. A recent analysis of Vβ gene segments and known atomic structures [37] estimates that 90% of these gene segments have CDR1 and CDR2 loops of any of three known canonical structures each. CDR3 loops of TCR β chains are much more variable in sequence and structure because of recombination of Vβ/Dβ/Jβ gene segments. Recently, we have determined the structures of two additional SAG–TCR-β-chain complexes — SpeA bound to the same mVβ8.2, and SpeC in association with human Vβ2.1 (hVβ2.1) (EJ Sundberg, RA Mariuzza, unpublished data) — allowing for a far more comprehensive analysis of SAG/TCR interactions and revealing a much broader variability in binding requirements.

Figure 3



Interaction of MHC-class-II-bound peptides with bacterial SAGs. **(a)** Interactions between SpeC and the MBP peptide (pink) in the HLA-DR2a–$MBP_{89–101}$–SpeC complex [19••]. Peptide residues in contact with the SAG are labeled. SAG residues that contact the peptide are shown as stick structures below the peptide stick structures. Hydrogen bonds are represented as broken black lines. **(b)** Interactions between SpeC and the MBP peptide in the HLA-DR2a–$MBP_{151–160}$–SpeC complex (Y Li, RA Mariuzza, unpublished data). **(c)** Interactions between SEH and the HA peptide in the HLA-DR1–$HA_{306–318}$–SEH complex [20].

As SpeA is highly structurally homologous to SEB, its complex with mVβ8.2 is, unsurprisingly, quite similar to that of SEB with the same TCR β chain. There are, however, two distinguishing structural features of the SpeA–mVβ8.2 complex.

First, because of differences in the sequences and structures of the disulfide loops in these two SAGs, the CDR1 loop of mVβ8.2 is involved in direct intermolecular contacts with SpeA. The terminal carboxyl atom of a glutamic acid side-chain in the SpeA disulphide loop forms a hydrogen bond with the side-chain nitrogen of an asparagine residue of the mVβ8.2 CDR1 loop. In SEB, a threonine residue is positionally equivalent to this SpeA glutamic acid residue but, because of the difference in side-chain length, it is unable to form a productive intermolecular contact with the asparagine residue in the mVβ8.2 CDR1 loop or any other residue nearby.

Second, in stark contrast to the formation of hydrogen bonds exclusively with main-chain atoms in the SEB–mVβ8.2 complex, there exist many hydrogen bonds formed directly between side-chain atoms of both the SpeA and mVβ8.2 molecules. Thus, SpeA seems to require some Vβ sequence specificity that restricts its reactivity beyond the simple conformation dependence required by SEB. The increased number of intermolecular interactions in the SpeA–mVβ8.2 versus the SEB–mVβ8.2 complex may also explain the difference in affinity of these two SAGs for the common mVβ8.2 target, with which SpeA forms an approximately 20-fold tighter complex than SEB [38].

The interaction between SpeC and hVβ2.1 has revealed an altogether different SAG–TCR-β-chain binding mode. This interaction includes a significantly larger buried-surface area than the SEB–mVβ8.2 and SpeA–mVβ8.2 complexes, more similar in size to peptide–MHC–TCR complexes. The network of intermolecular contacts is much more extensive than in the other structurally defined SAG–TCR-β-chain complexes and incorporates contacts with all three of the hVβ2.1 CDR loops as well as HV4, FR2 and FR3. These include numerous specific electrostatic interactions between side-chain atoms of SpeC and hVβ2.1 throughout the interface.

SpeC binds TCR β chains in a highly specific manner, primarily activating human T cells bearing Vβ2.1 [39]. Whereas the β-stranded core of the hVβ2.1 domain is very similar to that of mVβ8.2 and other Vβ domains, the binding surface of hVβ2.1 presented to SpeC is unique. Several features of hVβ2.1 contribute clearly to its specificity for SpeC, including two single amino acid insertions, one each in CDR1 and CDR2, and an extended CDR3 loop. The CDR1 insertion results in a semi-rigid body movement of this loop by nearly 2 Å away from its position in other Vβ domains and towards the SpeC molecule, resulting in numerous hydrogen bonds and van der Waals interactions. The CDR2 insertion results in a non-canonical CDR2 loop conformation in which the inserted residue is positioned optimally for the most productive set of interactions found in the interface with SpeC.

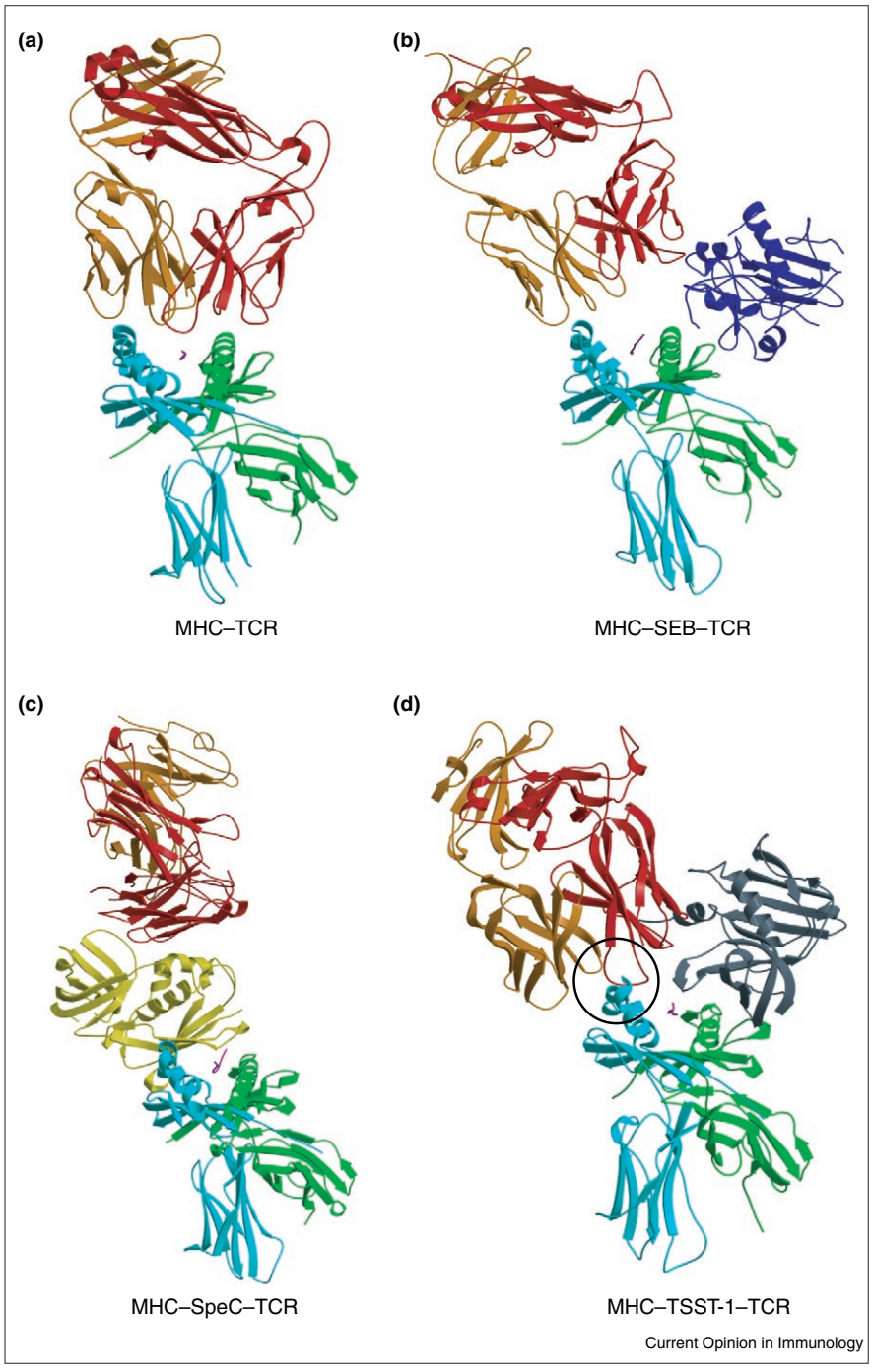
Although SpeC interacts with both hVβ2.1 and hVβ4.1, stimulation of human T cells bearing Vβ2.1 is 15-fold greater than in T cells presenting Vβ4.1 domains [39], whose sequence includes the CDR2 insertion, but lacks that in the CDR1 loop. Other Vβ domains that have the CDR1 insertion but lack the one in CDR2, such as hVβ20.1, are not recognized by SpeC. Thus, of these two rare insertions, the CDR2 insertion may confer SAG specificity, whereas the CDR1 insertion may be responsible for increased affinity. The extended conformation of the hVβ2.1 CDR3 loop results in several specific molecular contacts with SpeC. As no structure of this TCR β chain exists in its uncomplexed form, it is impossible to determine whether this is simply a serendipitous interaction due to the flexibility of the CDR3 loop. Flexibility of TCR CDR3 loops is not uncommon and has been proposed to be important in TCR recognition of peptide–MHC complexes [40]. It may be that protein plasticity in the Vβ domain is also important in SAG recognition. CDR3 residues have been reported to influence T-cell reactivity toward other microbial SAGs [41,42].

With this more diverse range of SAG–TCR interactions now characterized on the structural level, we can begin to observe a new paradigm for the selectivity of T-cell activation by SAGs, depending on varying levels of Vβ domain specificity. One group of highly promiscuous T-cell activators, including SEB and SEC3, binds TCR β chains in the simple conformation-dependent manner described above and interacts with only a single CDR loop, CDR2. Moderately promiscuous T-cell activators, including SpeA, can be grouped together on the basis of their dependence on numerous specific interactions mediated by direct side-chain/side-chain contacts overlayed onto the conformation dependence of the first group, and the additional involvement of the CDR1 loop. A third group of highly selective T-cell activators, including SpeC, binds those TCR Vβ domains that have the highest degree of structural dissimilarity, including non-canonical CDR-loop conformations and residue insertions, as well as usage of all three CDR loops, incorporating the highly variable CDR3 loop. TSST-1, which activates only human T cells bearing Vβ2.1 domains, may be the most extreme example in this third selectivity group. Surprisingly, this type of Vβ-domain specificity grouping is not wholly coincident with groupings according to primary or tertiary structure alignments, or to MHC binding characteristics.

Putting it all together: MHC–SAG–TCR ternary-complex models

Although no MHC–SAG–TCR ternary complex structures have been elucidated, one can build models for three such supramolecular complexes according to the variable MHC-binding characterististics of SEB, SpeC and TSST-1 (Figure 4).

Figure 4



Comparison of MHC-class-II-dependent T-cell signaling complexes. **(a)** The MHC–peptide–TCR complex [37]. **(b)** A model of the MHC–SEB–TCR ternary complex produced by superposition of the common elements of the HLA-DR1–SEB complex [21], the 14.3.d TCR-β-chain–SEB complex [36] and the 2C αβ TCR [49]. **(c)** A model of the MHC–SpeC–TCR ternary complex model produced by the superposition of the HLA-DR2a–SpeC complex [19••], the hVβ2.1-SpeC complex (EJ Sundberg, RA Mariuzza, unpublished data) and the 2C αβ TCR [49]. **(d)** A model of the MHC–TSST-1–TCR ternary complex produced by superposition of the common elements of the HLA-DR1–TSST-1 complex [22], a model of the hVβ2.1–TSST-1 complex (produced by superimposing the amino-terminal domain of TSST-1 onto that of SpeC in the hVβ2.1–SpeC complex [EJ Sundberg, RA Mariuzza, unpublished data]) and the 2C αβ TCR [49]. The circle encompasses portions of the MHC β chain and of the CDR3 loops of the TCR α and β chains which may interact in the MHC–TSST-1–TCR ternary complex. In all panels, the colors are as follows: MHC α chain (green); MHC β chain (light blue); antigenic peptide (pink); TCR α chain (orange); TCR β chain (red); SEB (dark blue); SpeC (yellow); and TSST-1 (gray).

A model of the MHC–SEB–TCR ternary complex (Figure 4b; [36]) shows that SEB acts as a wedge, essentially rotating the TCR about a contact point between the MHC β subunit α-helix and the TCR α chain CDR2 loop, relative to the MHC–TCR complex (Figure 4a; [43•]). This effectively prevents the peptide and all of the CDR loops, except Vα CDR2, from making specific contacts in the signaling complex. The interaction between residues from the MHC β chain and the Vα CDR2 loop has been confirmed by biochemical and mutational studies [44].

A model of the MHC–SpeC–TCR ternary signaling complex is shown in Figure 4c. Unlike the MHC/TCR cross-linking mechanism of SEB, SpeC acts as a bridge between the MHC and TCR molecules. This results in a displacement of the TCR away from its position in the MHC–TCR complex (Figure 4a) and a complete disruption of any possible MHC/TCR contacts. The TCR molecule in the MHC–SpeC–TCR model is rotated, relative to the MHC–TCR complex, about the horizontal axis by a similar amount as in the MHC–SEB–TCR model, but

in the opposite direction, and about the vertical axis by approximately 90°.

Figure 4d shows a model of the MHC–TSST-1–TCR ternary signaling complex. Similar to the SEB-dependent T-cell signaling complex, TSST-1 acts as a wedge between the MHC and TCR molecules, although the relative angular disruption from the MHC–TCR complex (Figure 4a) is less severe and the entire TCR molecule is shifted towards the MHC β chain. This model provides the possibility of a number of intermolecular contacts between the CDR3 loops of both the TCR α and β chains and the α-helix of the MHC β subunit, as highlighted by the circle in Figure 4d. These potential molecular interactions have yet to be confirmed biochemically.

Even though TCR is engaged very differently in MHC–TCR, MHC–SEB/SEC–TCR, MHC–SpeC–TCR and MHC–TSST-1–TCR complexes, it is remarkable that the end result — highly efficient T-cell activation — is very similar. This strongly implies that the specific geometry of TCR ligation may be less critical than other factors, such as the affinity and kinetics of the binding reaction, in triggering T cells. Indeed, we have previously shown that the half-life of the MHC–SEC3–TCR complex (~8 s [44]) falls within the range measured for specific MHC–peptide–TCR complexes (1 s–60 s [45]). In this case, maximum stabilization is achieved through direct MHC/TCR interactions that compensate for the weak individual affinities of SEC3 for MHC and TCR and increase the overall half-life of the ternary signaling complex.

Although we have not measured the half-lives of the MHC–SpeC–TCR or MHC–TSST-1–TCR complexes, we expect that they too will be comparable to those of TCR–peptide–MHC complexes. Since direct MHC/TCR interactions are precluded in the MHC–SpeC–TCR complex, sufficient stabilization must be achieved through the interplay of SAG/TCR and SAG/MHC interactions alone. This suggests that the far higher affinity of the SpeC/MHC interaction relative to that of SEC3/MHC interaction (K_Ds $\approx 4 \times 10^{-8}$ M and 3×10^{-4} M, respectively [19••,44]), may circumvent the need for MHC/TCR contacts in the MHC–SpeC–TCR complex. Some combination of factors similar to those that govern the MHC–SEC3–TCR and MHC–SpeC–TCR ternary complexes may be involved in stabilization of the MHC–TSST-1–TCR signaling complex.

Conclusions: on the superantigen horizon

With a grasp on the molecular mechanisms by which the pyrogenic family of exotoxins stimulate T cells, it is expected that much focus will now be directed towards the characterization of other SAGs. These include *Mycoplasma arthritidis* mitogen (MAM) and mouse retroviral Mtv (mammary tumor virus) SAGs [2]; these SAGs are structurally dissimilar to those discussed above, which were identified some time ago but have resisted X-ray crystallographic analysis. Several novel SAGs have also been identified that further underscore the diversity of this class of proteins. Of particular interest is a human endogenous retroviral SAG, designated IDDMK$_{1,2}$*22*, that may contribute to type I diabetes by activating autoreactive T cells [9••,46••]. A group of staphylococcal-exotoxin-like genes, *set1–set5*, have been identified whose gene products stimulate human peripheral-blood mononuclear cells and have some sequence homology with SpeC and TSST-1 [47•]. It is likely that the structures of these proteins will reveal further variations on the same theme used by the pyrogenic exotoxins.

A novel SAG, I2, that is produced by normal bacterial colonists of mouse and human ileum and is associated with the inflammatory bowel disease Crohn's disease, has been identified recently [8•]. I2 lacks sequence homology to other bacterial or viral SAGs but stimulates a subset of T cells that overlaps with those stimulated by MAM.

The genome of *S. aureus* now has been sequenced fully [48•]. This has revealed 70 newly identified potential virulence factors, several of which are likely to possess superantigenic properties. Structural characterization of these virulence factors will undoubtedly reveal greater diversity in the structural basis for SAG-dependent T-cell activation.

Why there exists such structural variablity in MHC–SAG–TCR ternary complexes is not presently clear. It may be that the formation of different cross-linking architectures results in distinct physiological outcomes. Although the general characteristics of T-cell stimulation by different SAGs appear broadly similar, small, but significant, differences in cytokine production, and the immune signals that follow, may be specific to individual SAGs. Because MHC binding, TCR selectivity and genetic classification of SAGs do not necessarily overlap, it is difficult to identify clear evolutionary pathways that have led to particular SAG-dependent signaling complexes. Some of the diversity in molecular architectures could be a result of convergent evolution as various strains of bacteria may have followed different paths to produce similarly functioning toxins.

As with other virulence factors, it is not altogether clear why certain bacteria produce SAGs. It may be that hyperstimulation of a key aspect of the immune response, T-cell proliferation, tilts the physiological balance in some way to favor bacterial survival. Now that such a diverse set of molecular architectures for SAG-dependent T-cell signaling complexes has been structurally defined, future studies can begin to investigate why such diversity exists, how it is advantageous for SAG-producing bacteria and what role it plays in disease pathogenesis.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

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