Chapter 25

Molecular recognition of diverse ligands by T-Cell receptors

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Summary

T-cell receptors (TCRs) are structurally related to antibodies, and also interact with a diverse set of ligands. TCRs recognize foreign peptide antigens displayed by major histocompatibility complex (MHC) molecules and foreign lipid-based antigens presented by CD1. These interactions initiate an immune response through T-cell activation. These critical surveillance and response initiation functions of the adaptive immune system are not perfect, though, as TCR interactions with self antigens can lead to autoimmune disease. Mutated peptides can also be recognized specifically by TCRs, and may be important in tumor immunity. TCRs are also bound specifically by a family of bacterial toxins called superantigens, which over-stimulate the immune system to cause numerous human diseases.

Key words: T-cell receptor, Peptide antigen, Lipid antigen, Superantigen, X-ray crystallography.

1. Introduction

T-cell receptors (TCRs) are structural cousins to antibodies, and also interact with a diverse set of ligands. Their primary function is to recognize antigens displayed by specialized molecules on the cell surface, binding to foreign peptide antigens in the context of major histocompatibility complex (MHC) and to foreign lipid-based antigens presented by CD1, thereby initiating an immune response through T-cell activation. In this way, TCRs serve a critical surveillance and response initiation function in the adaptive immune system. These recognition events are not perfect, however, as TCR interactions with self antigens lead to autoimmune disease. Mutated peptides or altered-self epitopes

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can also be recognized specifically by TCRs, and may serve an important immunosurveillance role in tumor immunity. TCRs are also bound specifically by a family of bacterial toxins called superantigens, which over-stimulate the immune system to cause numerous human diseases.

2. Anatomy of the T-Cell Receptor

TCRs are composed of multiple immunoglobulin (Ig) domains and are structurally equivalent to a single Fab fragment of an antibody (**Fig. 1**). Just like the variable domains of antibodies (V_H and V_L), those of TCRs (Vα and Vβ) contain complementarity-determining regions (CDRs) that connect the framework of β-strands in the Ig domain. These CDR loops lie in close spatial proximity on the surface of the molecule and together form a contiguous hypervariable

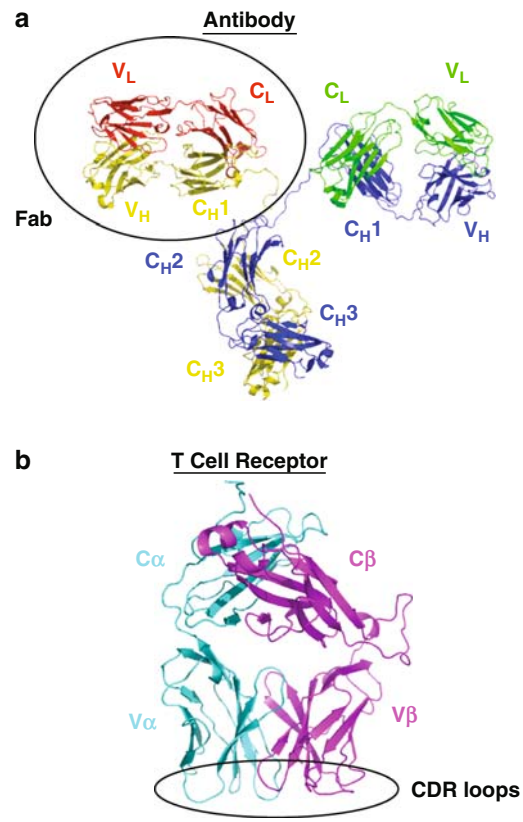

Fig. 1. Structural similarities between antibodies and T-cell receptors. (**a**) An intact antibody molecule in which the Fab fragment is highlighted by the *oval*. (**b**) A T-cell receptor. The CDR loops are highlighted by the *oval* (*see Color Plates*).

surface that is able to recognize specifically a nearly limitless array of antigenic epitopes. One major difference between antibodies and TCRs is that the former undergo an affinity maturation process via somatic hypermutation after the initial encounter with the antigen, while the latter are genetically static once they have undergone recombination and selection in the developing thymus. This reflects their distinct antigen-binding affinity requirements, approximately nm for antibodies, but only in the μm range for TCRs.

3. TCR Recognition of Peptide–MHC Complexes

T-cell receptors (TCRs) are an integral part of the adaptive immune system that has evolved to distinguish nonself pathogens from self tissues. Whereas, T-cell recognition of foreign peptides is essential for immune defense against invading microorganisms, recognition of self-peptides is thought to cause autoimmune disease, and T-cell epitopes involving altered-self peptides resulting from mutations accumulated during aging or disease are often associated with immunity to cancer *(1, 2)*. In terms of T-cell recognition, the boundaries separating foreign, self, and altered-self epitopes are somewhat blurred. Here, the structural characteristics of TCR recognition of these three broad classes of peptide–major histocompatibility complexes (pMHCs) are discussed.

3.1. Recognition of Foreign Peptide Antigens by TCRs

The earliest structural studies of TCR/pMHC complexes targeted TCRs specific for microbial and other foreign epitopes, or displaying alloreactivity *(3)*. These studies demonstrated remarkable similarities in the overall topology of TCR binding to pMHC, irrespective of MHC class I or class II restriction. In general, the TCR is positioned diagonally across the compound surface created by the peptide and the MHC α-helices that flank the peptide-binding groove, although some class I-restricted TCRs adopt a more orthogonal binding mode *(4)*. The orientation angle, defined as the angle between the line formed by the peptide direction and a line between the centers of mass of the Vα and Vβ domains, for all reported foreign pMHC class I- or class II-restricted TCRs is 45–80° *(3)*. The diagonal orientation is exemplified by the structure of human TCR HA1.7 bound to an influenza virus hemagglutinin (HA) peptide and HLA-DR1 (**Fig. 2a**) *(5)*. The most structurally diverse CDR loops, CDR3α and CDR3β, are generally located over the central peptide residue at position P5, and form a pocket that accommodates the P5 side chain. This docking mode maximizes interactions between the CDR3 loops and the MHC-bound peptide. This concentration of CDR3 loop contacts with foreign antigen is reminiscent of

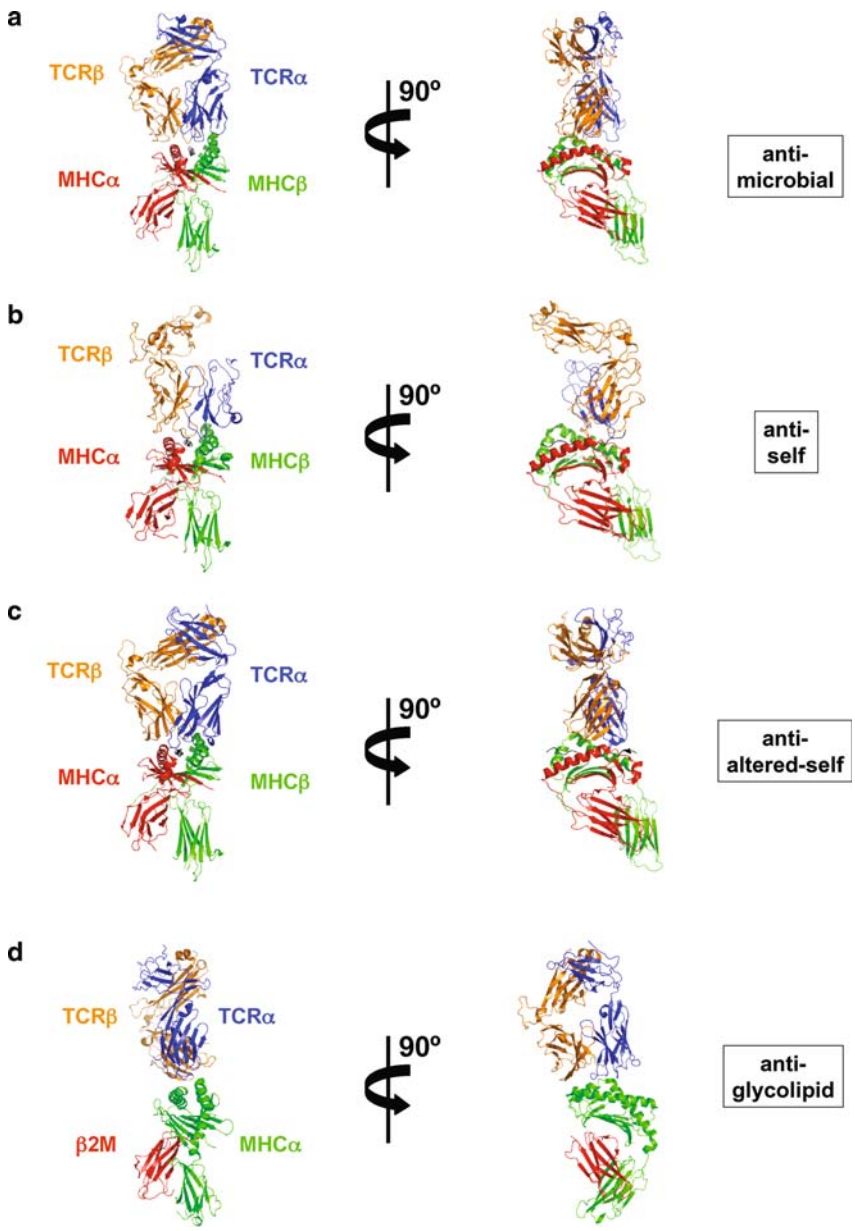

Fig. 2. TCR recognition of peptidic and lipid-based antigens displayed by MHC and CD1 molecules. (**a**) A representative anti-microbial complex, the structure of TCR HA1.7 bound to an influenza hemagglutinin peptide displayed by the MHC class II molecule HLA-DR1. (**b**) A representative anti-self complex, the structure of TCR Ob.1A12 bound to the $MBP_{85–99}$ peptide displayed by the MHC class II molecule HLA-DR2b. (**c**) A representative anti–altered-self complex, the structure of TCR E8 bound to the mutant TPI peptide displayed by the MHC class II molecule HLA-DR1. (**d**) A representative anti-glycolipid complex, the structure of NKT TCR bound to the α-GalCer glycolipid displayed by CD1d (*see Color Plates*).

antibody–antigen interactions. The central diagonal orientation commonly observed for TCRs recognizing microbial epitopes represents an optimal binding mode for maximizing interactions between TCR and the MHC-bound peptide, resulting in relatively high affinity for pMHC (K_D ~ 1–100 μM) *(6)*.

3.2. Recognition of Self Peptide Antigens by TCRs

The overall similarities among the initial structures of TCRs bound to MHC class I and II that display foreign peptide antigens created the expectation that all TCRs bind pMHC complexes similarly, and that pMHC recognition by autoreactive TCRs would be qualitatively indistinguishable from these. Several recent structures of autoimmune TCR/pMHC complexes, though, have revealed that autoimmune TCRs engage pMHC with distinct unconventional binding topologies compared with TCRs specific for foreign antigens.

In one such autoimmune complex between the TCR Ob.1A12 and a peptide composed of residues 85–99 of myelin basic protein (MBP) presented by the MHC class II molecule HLA-DR2b *(7)*, the TCR is not centered over pMHC and only contacts the N-terminal portion of the self peptide (**Fig. 2b**). Furthermore, this TCR exhibits a counter-clockwise rotation relative to pMHC compared with anti-foreign TCRs, resulting in a highly asymmetrical interaction with MHC and an orientation angle of 110°. In another autoimmune complex between human TCR 3A6 and MBP 89–101 presented by HLA-DR2a *(8)*, the orientation angle of TCR to peptide/MHC is within the range of anti-foreign TCR/pMHC complexes, although the CDR footprint of this TCR on pMHC is shifted markedly towards the N-terminus of the bound peptide, and towards the MHC β1 α-helix, compared with the CDR footprint on representative foreign pMHC complexes. A third autoimmune TCR/pMHC complex is unusual in that the N-terminal one-third of the binding groove is empty *(9)*, and as a consequence, this TCR recognizes only six peptide residues and uses only two CDRs to engage the peptide.

It is possible that autoimmune TCRs are intrinsically more cross-reactive than anti-foreign TCRs, which would increase the probability of self pMHC recognition, and the pathogenic potential of T cells expressing such TCRs would be enhanced, thus resulting in autoimmunity. Besides their sub-optimal binding topologies, these autoimmune TCR/pMHC complexes are commonly characterized by a scarcity of intermolecular hydrogen binding interactions. Additionally, these interactions exhibit much weaker affinities than do anti-foreign TCR/pMHC complexes and/or exceedingly short half-lives.

3.3. Recognition of Altered-Self Peptide Antigens by TCR

Recently, the crystal structure of a human tumor-specific TCR bound to a melanoma peptide epitope derived from the enzyme triosephosphate isomerase (TPI), in which a single-site mutation has occurred, and an MHC class II molecule has been determined *(10)*. This complex reveals a number of features intermediate between those of anti-foreign and autoimmune TCR-pMHC class II complexes that may reflect the hybrid nature of altered-self (**Fig. 2c**). These include a shift of the TCR toward the N-terminus of the bound peptide relative to anti-foreign TCRs, though not

as extreme as for autoimmune TCRs, while maintaining the generally diagonal binding orientation of anti-foreign TCRs. As a consequence of this shift, the CDR3 loops of the TCR are positioned directly over the mutated residue of the altered-self peptide epitope. This focus on the N-terminal half of self-peptides, which may be prevalent among both anti-self and anti-altered-self TCRs that have escaped negative selection during thymic development, implies that the N-terminal site is intrinsically less favorable for TCR binding than the central site typically utilized by TCRs recognizing foreign epitopes *(3, 5, 11)*. As with autoimmune TCRs, the TCR/altered-self pMHC complex exhibits very low affinity, although the altered-self mutation at the TCR-contacting position of the peptide epitope results in a modest increase in binding strength. The TCR in this complex is tilted toward the DR β-chain, with which it makes many more contacts (~80% of total contacts) than it does with the MHC α-chain *(10)*, a feature that also generally distinguishes autoimmune from anti-microbial TRC/pMHC complexes.

4. TCR Recognition of Glycolipid Antigens Displayed by CD1 Molecules

CD1 molecules comprise a large cluster of nonpolymorphic MHC class I-like molecules that present lipid-based antigens, such as glycolipids, to TCRs. The antigen-binding clefts of CD1s are characterized by vastly larger and more hydrophobic pockets than those formed in MHC molecules that accommodate the lipid moieties of these antigens. This allows the hydrophilic portions of the antigen, such as sugars, to protrude from the top surface of the CD1 molecule where they can be easily encountered by TCRs. The lipids, sugars, and peptides synthesized by microbes are significantly different from those made by vertebrates, thereby providing a basis for antigenicity.

4.1. Structural Characteristics of TCR/Glycolipid–CD1 Complexes

Certain T-cells, known as invariant natural killer T (NKT) cells, in part because of their expression of a semi-invariant TCR (NKT TCR), in which the TCR α chain is invariant while the β chain is restricted, are known to recognize the glycolipid α-galactosylceramide (α-GalCer) displayed by CD1d. A recent structure of the NKT TCR/α-GalCer/CD1d complex *(12)* provides the first view of how a TCR recognizes nonpeptidic antigens (**Fig. 2d**). In this structure, NKT TCR is positioned over the end of the CD1d antigen-binding cleft, at the F' pocket, analogous to the C-terminus of antigenic peptides displayed by MHC molecules. Furthermore, NKT TCR binds essentially parallel to the antigen-binding cleft, in stark contrast to the vast array of diagonal to orthogonal binding modes of

TCR/pMHC complexes. These two distinctive features of the NKT TCR/α-GalCer/CD1d complex results in the sugar moiety of the antigen being contacted by only the TCR Vα domain and not at all by the Vβ domain. These TCR-antigen contacts are roughly split between the CDR1α and CDR3α loops, the latter of which form contacts as well with both α-helices of the CD1d molecule. The TCR Vβ domain, conversely, accounts for less than 30% of all intermolecular contacts in the complex and only forms contacts with the CD1d molecule confined to the extreme C-terminal end of the α1 α-helix. By comparing the structure of the complex with that of the unbound NKT TCR *(13, 14)*, it is evident that the NKT TCR/α-GalCer/CD1d complex exhibits the hallmarks of a lock-and-key interaction, with no significant conformational changes induced upon complex formation. Accordingly, binding of this complex has been shown to be insensitive to changes in temperature *(15)*. This is quite unlike TCR/pMHC complexes, for which protein plasticity is a common feature.

TCR recognition of glycolipid antigen presented by CD1 seems to blur the line between innate and adaptive immunity. Like mechanisms of innate immunity, CD1 molecules exhibit a high degree of cross-species recognition. Also, the exclusive invariant domain usage for antigen recognition, at least in the case of the NKT TCR/α-GalCer/CD1d complex is, by definition, innate-like. However, the recognition of distinct CD1d/antigen complexes by various NKT cells, provides an adaptive component to immunity to lipid-based antigens.

5. TCR Recognition of Superantigens

Bacterial superantigens (SAGs) comprise a large family of disease-associated proteins that are produced predominantly by *Staphylococcus aureus* and *Streptococcus pyogenes (16)*, as well as by a number of other bacteria and viruses. SAGs function by simultaneously interacting with class II MHC and TCR molecules on antigen presenting cells and T lymphocytes, respectively *(17)*. Contrary to the peptidic and lipid-based antigens discussed above, SAGs bind to MHC molecules outside of their peptide-binding grooves and interact predominantly with only the Vβ domains of TCRs, resulting in the stimulation of up to 20% of the entire T-cell population. In this way, SAGs initiate a systemic release of inflammatory cytokines that results in various immune-mediated diseases including a condition known as toxic shock syndrome (TSS) that can ultimately lead to multi-organ failure and death. SAGs have also been implicated in the pathogeneses of arthritis, asthma and inflammatory bowel syndrome, and are classified as bioterror reagents.

5.1. Superantigen-TCR Specificity and Cross-Reactivity

With some 30 SAGs from *S. aureus* and *S. pyogenes* and more than 50 TCR Vβ domains encoded by the human genome, SAG–TCR interactions constitute a complex molecular recognition problem, in which some SAGs are strictly specific for a single Vβ domain, while others bind much more promiscuously to a multitude of Vβ domain targets. The recently expanded database of SAG–TCR Vβ domain crystal structures allows the construction of a paradigm for how SAGs confer specificity and cross-reactivity in TCR recognition.

The least specific SAGs (including staphylococcal enterotoxins B (SEB) and C (SEC3)) depend primarily on a common conformation adopted by the CDR2 loop and the fourth hypervariable (HV4) loop in many Vβ domains *(18, 19)*. In these complexes (**Fig. 3a**), hydrogen bonds are made only to Vβ main chain atoms, such that numerous combinations of amino acid

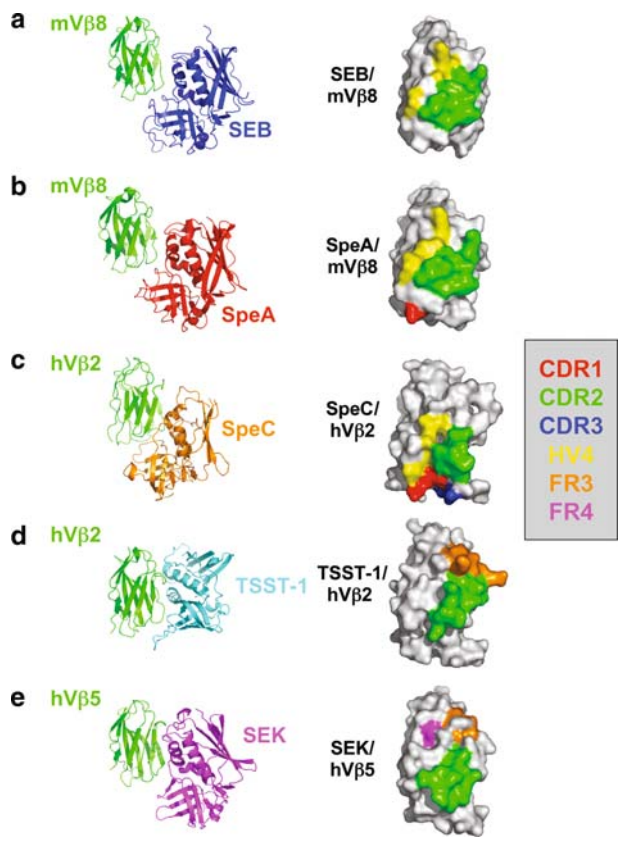

Fig. 3. Superantigen engagement of the T-cell receptor Vβ domain. *Left*, structures of the (**a**) SEB/mVβ8, (**b**) SpeA/mVβ8, (**c**) SpeC/hVβ2, (**d**) TSST-1/hVβ2, and (**e**) SEK/hVβ5 complexes. The Vβ domains are aligned to one another to highlight the distinct orientations by which these SAGs engage their TCR ligands. *Right*, TCR Vβ domain molecular surface buried by various SAGs. Hypervariable and framework region surface residues buried in the interface formed by TSST-1, SEB, SpeC, and SEK are color-coded as follows: CDR1 (*red*); CDR2 (*green*); CDR3 (*blue*); HV4 (*yellow*); FR3 (*orange*); and FR4 (*magenta*). The Vβ domains in the *right* are rotated counter-clockwise approximately 90? about the vertical axis of the page relative to their positions in the *left* (*see Color Plates*).

sequences in CDR2 and HV4 can satisfy the binding requirements for these SAGs, as long as they do not change the lengths of these hypervariable loops nor disrupt the common structural conformation adopted.

As TCR specificity increases (e.g., for streptococcal pyrogenic exotoxin A (SpeA)), the number of hypervariable loops with which the SAG interacts increases beyond CDR2 and HV4 to include CDR1 (**Fig. 3b**). Additionally, the interface becomes increasingly populated by hydrogen bonds formed directly between side chain atoms from both SAG and TCR *(20)*.

As TCR Vβ domain binding partners become restricted even further (e.g., for SpeC), the engagement of the entire repertoire of TCR hypervariable elements is observed (**Fig. 3c**). The CDR loops with which the SAG interacts also have incorporated non-canonical residue insertions that alter both their length and conformation to provide highly unique binding sites *(20)*.

SAG–TCR specificity is thus accomplished with increased side chain-to-side chain hydrogen bond interactions, an expanded set of hypervariable elements engaged, and an accumulation of non-canonical CDR loop structures, which is effectively exhausted at this point. In order to exhibit even greater specificity than SpeC, toxic shock syndrome toxin-1 (TSST-1) appears to target a structural element, a loop in the framework region (FR3), that adopts a common conformation in all but a few Vβ domains, at the expense of interacting with each of the hypervariable structures (**Fig. 3d**). The fine specificity of TSST-1 for TCR Vβ domains is enhanced by requiring a particular residue at a particular position in FR3 in order to bind and efficiently activate T cells.

This targeting of rarely variable regions, at the expense of canonical hypervariable regions, in Vβ domains as a means for TCR specificity may constitute a general mechanism for enhancing SAG-TCR specificity, as the structural analysis of SEK in complex with one of its Vβ ligands, hVβ5.1, shows similar characteristics *(21)* (**Fig. 3e**). SEK appears to derive its specificity, at least in part, through interactions with relatively uncommon residues in both FR3 and FR4, with which a single residue in SEK forms side chain-to-side chain hydrogen bonds *(21)*.

The distinct orientations with which each of these SAGs engage the TCR Vβ domain result in unique patterns of hypervariable and framework region surfaces that are buried (**Fig. 3e**, right panel). Binding to the TCR Vβ CDR2 loop is a requirement for all bacterial SAGs, and the proportion of the SAG-TCR interface that is contributed by the CDR2 loop is invariably the greatest in any SAG-TCR complex, relative to any other single hypervariable or framework region. Involvement of Vβ domain regions beyond the CDR2 loop, however, plays a significant role in the TCR Vβ domain specificity and cross-reactivity of a SAG *(22–24)*. SEK and TSST-1 engage one or more framework

region apical loops, at the expense of contacting the hypervariable elements. SEK buries significant molecular surface belonging to both the FR3 and FR4, while TSST-1 contacts only residues from FR3. The lower relative positions of SEB and SpeC on the Vβ domain result in their engagement of hypervariable elements at the expense of binding the apical loops of the framework regions. SEB buries molecular surface belonging to HV4, while SpeC contacts residues from CDR1, CDR3, and HV4.

5.2. Superantigen-Mediated T-cell Signaling Complexes

There exist three known binding modes for SAGs to interact with pMHC complexes. These binding modes are exemplified by the following SAGs: TSST-1, which binds predominantly to the MHC α subunit at a site that overlaps with that of SEB but also extends over the surface of the peptide to make contacts with the β subunit *(25)*; SEB, which binds MHC exclusively to its α subunit with no contacts made with the antigenic peptide *(26)*; and SpeC, which binds the MHC β subunit through coordination of a zinc ion and makes numerous contacts with the displayed peptide *(21, 27, 28)*. Crystal structures of TSST-1 *(29)*, SEB *(18)*, and SpeC *(20)* in complex with their TCR β chain ligands have allowed the construction of models of those MHC/SAG/TCR ternary complexes that are necessary for efficient T-cell activation that are distinct from pMHC-TCR complexes.

TSST-1 bridges the pMHC and TCR molecules such that two protein–protein interfaces, SAG/MHC and SAG/TCR, are formed (**Fig. 4a**). No direct MHC–TCR contacts are made. The relative orientation of the TCR and pMHC is such that a plane that passes through both the TCR α and β chains and one that is aligned with the MHC-displayed peptide are approximately perpendicular to one another.

In the SEB-dependent T-cell signaling complex (**Fig. 4b**), SEB acts as a wedge between the pMHC and TCR molecules, effectively rotating the TCR about a contact point between the MHC β subunit and the TCR α chain. This removes the antigenic peptide from any possible contacts with the TCR. The relative orientation of pMHC and TCR is otherwise akin to that observed in the TSST-1–mediated T-cell signaling complex model. In this supramolecular complex there exist three protein–protein interfaces: SEB/MHC, SEB/TCR and MHC/TCR. The presence of the direct MHC/TCR interaction (as indicated by the arrow in **Fig. 4b**) has been verified biochemically *(30)*.

SpeC, in contrast to SEB but similar to TSST-1, bridges the MHC and TCR molecules (**Fig. 4c**). There exists no direct interaction between MHC and TCR, and thus only two distinct protein–protein interfaces (i.e., SAG/MHC and SAG/TCR) comprise this complex. However, the TCR and pMHC are oriented such that planes passing through the TCR α and β chains and the antigenic peptide are approximately parallel to one another.

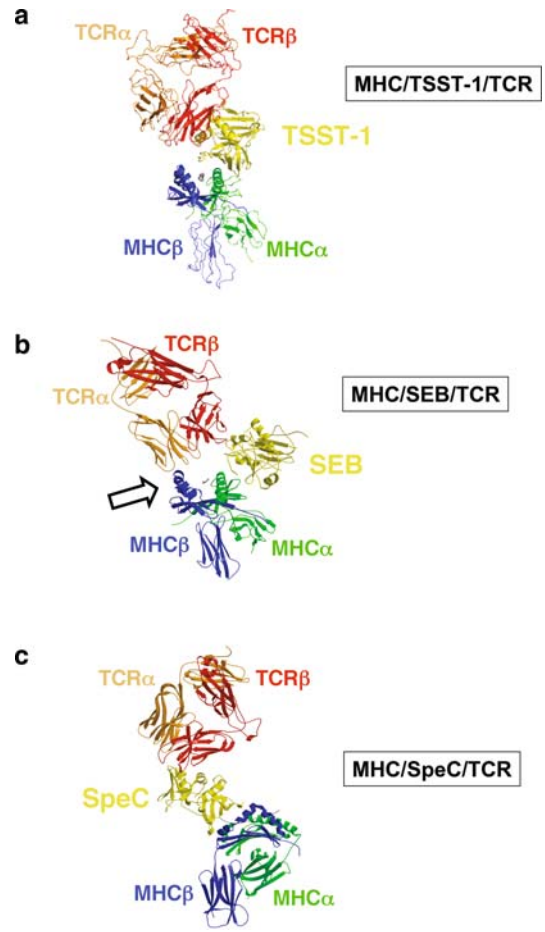

Fig. 4. MHC/SAG/TCR ternary signaling complexes mediated by (**a**) TSST-1, (**b**) SEB, and (**c**) SpeC. Colors are as follows: MHC α subunit, *green*; MHC β subunit, *blue*; antigenic peptide, *gray*; TCR α chain, *orange*; TCR β chain, *red*; SAGs, *yellow*. For clarity, the MHC/SAG/TCR complexes mediated by SpeC (**c**) is rotated approximately 90? clockwise about the vertical axis of the page relative to those mediated by TSST-1 (**a**) and SEB (**b**) (*see Color Plates*).

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