Commentary

Superantigens: Just Like Peptides Only Different

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The exotoxins produced by *Staphylococcus aureus* and *Streptococcus pyogenes* are prototype molecules for the larger family of superantigens (SAGs). This family now includes many structurally unrelated molecules of disparate origins reflecting a wide evolutionary convergence towards the common goal of subverting T cell antigen recognition. SAGs bind simultaneously to MHC class II molecules and TCRs, bringing them together in such a way as to induce profound T cell activation. How SAGs, and in particular the staphylococcal enterotoxins do this, has been the subject of intense interest over the last nine years. It's the subject of an elegant paper from Leder et al. at the Center for Advanced Research at the University of Maryland published in this issue of the Journal of Experimental Medicine (1). This paper examines the thermodynamics of staphylococcal enterotoxin (SE) B and C3 binding to TCR and ultimately raises questions about how similar peptides and SAGs are in their engagement and triggering of T cells.

Much is now known about the fine structure of the staphylococcal and streptococcal exotoxins. They are small, single chain proteins constructed from two globular domains. In all toxins except streptococcal pyrogenic exotoxin C (SPEC), the variable NH_2-terminal domain contains a generic binding site for the invariant α1 domain of MHC class II. In a subset of toxins including SEA, SED, SEE, and SPEC, an additional zinc-dependent site is located in the larger more conserved COOH-terminal domain that binds to the highly polymorphic β1 domain of MHC class II. The TCR binding site is located in a shallow groove between the two toxin domains (except in toxic shock syndrome toxin [TSST] and probably also SPEC). In the paper by Leder et al. (1) the energetic contribution of individual residues within the TCR binding site of SEC3 and SEB to a soluble form of mVβ8.2 TCR have been determined using a combination of sedimentation equilibrium and real-time bio-sensing techniques. A previous three-dimensional crystal structure of SEC3/Vβ8.2 is used as a guide to mutate all those residues that make contact with Vβ8.2 (2). The authors generate a thermodynamic map of the TCR site of SEC3 that shows that overall binding energy is shared fairly evenly among all residues but there are five that are clustered in the center of the binding site that make significantly more energy contributions than the others. Two residues, an asparagine at position 23 and a tyrosine at position 90, are conserved in all toxins, whereas another, a glutamine at position 210, varies between toxins and appears to be important in determining Vβ specificity. Using these mutants to stimulate Vβ8.2 transgenic T cells, the authors reveal a simple almost linear relationship between ligand affinity and biological potency. This might seem like a rather obvious association, but the relationship between T cell ligand affinity and the ensuing biological response is anything but obvious.

Do SAGs Fit Current Models of T Cell Triggering? The currently held model of T cell recognition is that the TCR discriminates exquisite differences in peptide–MHC (pepMHC) complexes on the basis of small quantitative differences in affinity (3). All pepMHC–TCR interactions measured thus far (barring one [reference 4]) have been found to exhibit low affinities and short half-lives. This fits well with a mechanism of sequential engagement of multiple TCR molecules by a single pepMHC complex elegantly revealed by Antonio Lanzavecchia and colleagues. They have shown that at limiting surface concentration, a single pepMHC complex triggers as many as 200 TCR complexes (5). Their serial triggering model predicts that not only must the interaction between pepMHC and TCR be brief, but also that increasing binding affinities might inhibit serial triggering and thus reduce efficiency. As an added level of complexity, pepMHC complexes with affinities that fall just below a threshold required for T cell activation are antagonists, inducing T cell anergy rather than activation. Rabinowitz et al. (6) have defined this difference in biological outcome as determined by the ratio of complete (positive) to negative (incomplete) signals via the TCR.

In real terms, affinity differences towards TCR of as little as 10-fold appear to be sufficient to induce antagonism. For instance, in a well studied Ova/K^b 42.12 TCR model, the affinity difference between the agonist Ova/K^b complex (6.5 μM) and two antagonist peptides, E1/K^b and R4/K^b (22 and 57 μM respectively), are only 3-fold and 9-fold respectively (7). In comparison, the binding affinities for the SEC3 mutant/mVβ8.2 interactions ranged from 3.5 μM to 240 μM, an affinity difference of >60-fold. Moreover, even the weakest binding SEC3 mutant SEC3 Y90A required a mere 45-fold more toxin to stimulate at wild-type levels. This represents only a small difference in potency considering that SAG responses are usually measured over five to six orders of magnitude. No antagonist response was seen even from the weakest binding SEC3 mutant.

A further anomaly of the data presented is the finding that SEB is 10-fold more potent than SEC3 to Vβ8.2-bearing T cells but showed much weaker binding to soluble Vβ8.2. The authors explain this contradiction by introducing the role of MHC class II in the cooperative stabilizing of SEB/TCR, interactions and indeed there is firm evi-

J. Exp. Med. © The Rockefeller University Press • 0022-1007/98/03/819/03 $2.00
Volume 187, Number 6, March 16, 1998 819–821
http://www.jem.org

dence to suggest this does occur. Seth et al. (8) showed that although SEB has only very weak individual affinities towards either HLA-DR1 and a hVβ3 TCR alone, SEB mixed with HLA-DR1 and TCR together produced a much more stable trimer with an apparent affinity far greater than the sum of the two components. It is important to mention that the solution affinities of the SEC3 mutants towards TCR were not calculated in the presence of MHC class II so we do not know yet whether MHC class II also stabilized the SEC3–TCR interaction. Therefore it is possible that the real ligand affinities of SEC3–MHC complexes to TCR are much higher.

So what can we deduce from these results about the response to SEC3 and its similarities to peptide antigens? First, the affinity and short half-life of the SEC3–mV β8.2 interaction (K_d = 3.5 μM and 5 s, respectively) in the absence of class II is very similar to those measured for several pepMHC–TCR solution complexes. Therefore we cannot attribute the extreme potency of SEC3 simply to tighter binding to TCR. In fact, an attempt by the authors to increase the affinity of SEB by engineering its site to look like that of SEC3 was only partially successful. Although the authors managed to increase the SEB–TCR affinity 10-fold with a single V26Y mutation, this achieved only a modest 4-fold increase in biological response. This result is interesting because it hints that SAGs can't be made to bind more strongly to TCR than they already do, and is consistent with the concept of "optimal affinity" proposed for the serial triggering model. We will have to wait and see whether other SAGs bind to TCRs with similar affinities in order to confirm this.

Where T cells appear to differ markedly in their response to SEB and SEC3 in comparison to peptides is in their apparent tolerance of significant differences in ligand affinity. For a pepMHC ligand where even the slightest difference in affinity results in an altered outcome, a 68-fold decrease should in theory abrogate the response altogether. This clearly does not happen even for the weakest binding SEC3 mutant and points to a fundamental difference between SAGs and peptide ligation of TCR that must reflect the different ways in which these two antigenic forms are presented. Unlike peptides, which are for all intents and purposes bound irreversibly to MHC class II, bacterial SAGs are in equilibrium between free and bound states. With affinities towards both MHC class II and TCR in the micromolar range, the vast majority of SEB or SEC3 molecules will be unbound at the physiological relevant picofemtomolar range. How then do they trigger T cells at concentrations many orders of magnitude less than their dissociation constants? In the case of SEB, any transitional dimer complex that forms with either TCR or with MHC class II molecules appears to be stabilized by an additional TCR–MHC interaction before the TCR is triggered. Serial triggering would require SEB to be bound more securely to MHC class II and only transiently engage TCR. On the basis of its measured affinities for both molecules, this does not seem possible.

Different Modes of SAG Binding to MHC Class II Reflect Alternative Mechanisms for Activation. SEB binds weakly to MHC class II α-chain and relies on continued engagement between TCR and MHC class II to stabilize an activation complex, whereas SEA has achieved stable binding to the APC surface in another way. It has developed a second zinc-dependent interaction with the polymorphic MHC class II β chain that is 100-fold stronger than its SEB-like α-chain interaction (9–11). This enables one SEA molecule to cooperatively bind and stabilize a second weak α-chain SEA interaction resulting initially in MHCII–$(SEA)_2$ trimers (12). Indeed, this second high affinity binding is so important to SEA that mutations in the zinc site completely destroy SEA activity even though SEA can still bind to the α chain and presumably ligate TCRs in the same way as SEB. Stabilizing SEA in this fashion presumably alleviates the reliance on MHCII–TCR compatibility required by SEB and expands the repertoire of T cells activated by SEA. Interestingly, in humans, SEB really only stimulates Vβ3 bearing cells en masse while SEA stimulates Vβ1, 5.3, 6.3, 6.4, 6.9, 7.3, 7.4, 9.1, and 23.1 (11). It also means that SEA achieves much greater stability on the surface of the APC, perhaps allowing it to act more like a pepMHCII complex in sequentially triggering multiple TCRs.

The second class II binding site of SEA also introduces another possible way for SAG-mediated T-cell activation, namely promotion of MHC cross-linking on the surface of APCs. SEA is a potent activator of APCs promoting cell adhesion and aggregation (13, 14). It is not yet known whether MHC class II cross-linking also enhances T cell signaling directly, but it might be possible that MHC aggregation promotes areas of high local ligand concentration, which in turn increases the avidity for TCR and induces local TCR clustering.

There are at least two more SAGs that are also able to cross-link class II, both using different mechanisms to SEA. As shown from its crystal structure, SED has the capability to form zinc-dependent homodimers by coordinate binding of two Zn^{2+} ions between the COOH-terminal domain (homologous to the high affinity zinc site of SEA) of two SED molecules. Binding to MHC class II is proposed to occur via the SED NH_2-terminal domain to the class II α chain in a similar fashion to SEB. This could potentially result in MHC cross-linking in an MHCIIα-SED-SED-MHCIIα mode (15). The recent crystal structure of SPEC indicates that the generic class II α chain binding site in the NH_2-terminal domain has been lost in favor of an SPEC dimer interface (16). SPEC instead binds only to the β chain by a zinc-mediated mechanism and thereby might cross-link class II in a MHCIIβ-SPEC-SPEC-MHCIIβ mode. This has been supported by the finding that SPEC readily dimerizes in solution and also cross-links MHC class II to induce homotypic cell adhesion (16). In contrast to the cross-linking mechanism of the bivalent SEA molecule, dimeric SAGs like SED and SPEC might well be able to promote TCR dimerization, due to the optimal location of two TCR binding sites in the dimer structure.

The fact that SAGs have coevolved at least three separate mechanisms to bind and coalesce MHC class II on the surface of APCs suggests that surface aggregation plays an important function in T cell triggering. However, it doesn't explain why toxins with only one MHC class II binding site like SEB, SEC1-3, and TSST are still as potent as those toxins that have two. We can only assume that every SAG has been optimized to stimulate efficiently under the limitations of its own modus operandi. The development of additional modes of MHC class II binding reflects a need by bacteria to stimulate more T cells by accommodating a greater range of individual SAG–TCR interactions.

In conclusion, while SAGs and pepMHC complexes bind to TCRs with similar affinities, the tolerance to a 60-fold decrease in SEC3 affinity for TCR and the apparent absence of any antagonist responses indicates that T cell activation by SAGs is optimal over a much broader range of affinities. Perhaps this reflects the importance of avidity rather than intrinsic affinity in SAG activation. For peptide activation, the window of affinity for agonist/antagonist responses, set during thymic development, is very narrow indeed and provides for both positive and negative TCR signals. The serial triggering model proposes a mechanism for peptide-mediated TcR signaling that is unlikely to require TcR dimerization or cross-linking and perhaps relies on its very absence to discriminate subtle differences in pepMHC affinities. On the other hand, SAGs have coevolved several elaborate mechanisms that promote MHC class II coalescence. This could promote TcR signaling through clustering and/or TCR dimerization.

The murky picture we have of SAG-induced T cell activation is not helped by the remarkable variations seen in SAG binding. A clearer picture is sure to emerge as more and more SAG–TCR interactions are examined using similar quantitative approaches to those used by Leder et al. (1). However, the most fundamental question still remains; why do bacteria produce SAGs in the first place?

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Received for publication 21 January 1998.

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