MINIREVIEW

Staphylococcal Enterotoxin Superantigens (43321A)

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Abstract. **Staphylococcal enterotoxins (SE) are a family of structurally related proteins that are produced by *Staphylococcus aureus*. They play a role in the pathogenesis of food poisoning and are the most potent activators of T lymphocytes known. The receptors for SE on antigen-presenting cells are major histocompatibility complex class II molecules. Recent studies have shown that a complex of SE and major histocompatibility complex class II molecules is required for binding to the variable region of the T cell antigen receptor β-chain. SE mitogenic activity is dependent on induction of interleukin 2, which may be intimately involved in the mechanism of SE toxicity. The minor lymphocyte-stimulating "endogenous" self-superantigen has recently been shown to be a retroviral gene product, so that this too is apparently a microbial superantigen. An understanding of the mechanism of action of these microbial superantigens has implications for normal and pathological immune functions.** [P.S.E.B.M. 1991, Vol 198]

Staphylococcal enterotoxins (SE) are among the most common causes of food poisoning. These structurally related proteins are produced by the bacterium *Staphylococcus aureus* (1). The fact that SE are mitogenic for lymphocytes has provided insight into the mechanism of their toxic effects and has led to their use as a tool for studying lymphocyte function. The SE have recently been called microbial superantigens because of their ability to stimulate subsets of T cells based on their T cell antigen receptor β-chain variable region (Vβ) usage (2, 3). The major histocompatibility complex (MHC) class II molecules and T cell antigen receptors (TcR) serve as the receptors for these toxins, and thus the mechanism by which SE regulate immune function is of particular interest. We will examine here the structure of the SE, their mechanism of action and biological effects, and their interactions with MHC class II and TcR molecules.

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Structure

SE are single chain molecules with molecular weights of approximately 24–30 kDa (Table 1). They are charged (pI from 7.0 to 8.6), acid and heat stable, and rich in serine, threonine, and aspartic acid residues. With the exception of toxic shock syndrome toxin-one (TSST-1), SE display a centrally located disulfide loop. Some studies have shown that enzymatic cleavage of staphylococcal enterotoxin A, B, or C (SEA, SEB, or SEC) in the region of the disulfide bridge does not affect biological activity (4, 5). In contrast, one recent study suggested that while monocytes can be induced to produce interleukin 1 and tumor necrosis factor-α by reduced and alkylated SE, T cell activation requires an intact disulfide loop (6).

SE are classified serologically into five groups, A through E, with SEC further divided into three subtypes on the basis of minor epitopes (1). Another enterotoxin, originally designated staphylococcal enterotoxin F, was renamed TSST-1 (7). In contrast to serological distinctions, the SE also display some antigenic cross-reactivity (8, 9). Not surprisingly, elucidation of SE amino acid sequences revealed appreciable homology (10). SEA is related in amino acid sequence to staphylococcal enterotoxins E and D, while SEB has greater homology with

Table I. Characteristics of Staphylococcal Enterotoxins

Toxin	Mol mass (kDa)	pI	Disulfide Bond	Homology to SEA[a]	CD structure		Murine Vβ Usage[b]
					α-helix (%)	% β-sheet (%)	
SEA	27.8	7.3	yes	120	11	36	1, 3, 10, 11, 17
SEB	28.3	8.6	yes	27.4	10	55	7, 8.1, 8.2, 8.3, 11, 17
SEC_1	26.0	8.6	yes	29.6	15	38	3, 7, 8.2, 8.3, 11, 17
SED	27.3	7.4	yes	ND[c]	ND	ND	3, 7, 8.1, 8.2, 8.3, 11, 17
SEE	29.6	7.0	yes	ND	ND	ND	11, 15, 17
TSST-1	24.0	7.2	no	5.2	6	51	3, 15, 17

[a] Homology is expressed as a *z*-value based on optimized homology scores, where $z > 3$ indicates possible homology, $z > 6$ indicates probable homology, and $z > 10$ indicates significant homology (see Ref. 10).
[b] For Vβ usage, see Ref. 57.
[c] ND, not determined.

SEC. Two highly conserved regions have been identified in the sequences of the SE (10, 11). One region in SEA spans Cys 106 to residue 119, while the other extends from residue 147 to residue 163. Conserved sequences may correspond to regions that are potentially important for function, and the carboxy terminus has been implicated in the gastrointestinal properties of SEC (12).

The degree of sequence homology among the SE ranges from 30% to 86%, yet even the less homologous molecules have similar biological functions (10). Initial efforts toward understanding the structural basis for such common biological activities have involved secondary structure predictions using the amino acid sequence compared with structural information derived from techniques such as circular dichroism (CD) and tryptophan quenching. The SE characteristically have low α-helical content together with a high content of β-structure, as indicated by CD analysis (Table 1; 13). The CD spectra of SEB and the closely related SEC (65% sequence homology) are essentially identical (13, 14). The CD spectrum of the SEA molecule varies somewhat from that of the SEB or SEC molecules, although it reveals the same predominance of β-sheet (14, 15). While staphylococcal enterotoxin E is similar to SEA in the high percentage of β-sheet, SEA has more α-helix than staphylococcal enterotoxin E (15). TSST-1 has the low α-helix and high β-sheet content characteristic of other SE, but shares minimal sequence homology with them (16). Thus, the functional similarity of TSST-1 with other enterotoxins may depend on an active site maintained at the level of secondary and tertiary structures. Since mitogenic plant lectins also have high β-sheet content, it has been speculated that particularly high β-structure may enhance the attachment of proteins to cell surface receptors (17), which may facilitate binding of the SE to both MHC and the TcR. Thus, despite differences in amino acid sequences and antigenic properties, all of the SE, including TSST-1, exhibit a similar pattern of secondary structure (low α-helix, high β-sheet), which is compatible with their common biological functions.

Biological Activity

The ability of SE to stimulate lymphocyte proliferation and lymphokine production at concentrations as low as 10^{-13} to 10^{-16} *M* makes them among the most potent T cell activators known (18–21). SEA-stimulated human peripheral lymphocyte cultures have been used to show that the SE induce mitogenesis indirectly via induction of interleukin 2 (22). SE can also modulate the humoral immune response, and this too appears to be due to activation of T cells. Antibody production by mouse splenic B cells is potently inhibited by SEA and SEB in an *in vitro* system in which the toxins and the antigen are simultaneously added to cultures (19). The correlation of inhibition of antibody production with γ-interferon induction in these cultures suggested that γ-interferon, a product of T cells, may modulate such suppressive effects. The addition of enterotoxins to cultures that have been stimulated with antigen for 2 days results in enhancement, rather than suppression, of the antibody response (19). Thus, suppression or enhancement of the immune response by SE depended upon the time of T cell stimulation relative to antigen stimulation. In *in vivo* experiments, suppression of antibody production and induction of weight loss in mice by SEB was also dependent on the presence of functional T cells (23). Furthermore, a rapid immunological response from rats parenterally exposed to SEA involved monocytes and small and medium lymphocytes only a few hours after administration of SEA (24).

There is also evidence that SE possess some properties that are dissociable from their T cell-mediated effects. Mapping functional sites on SEC_1 has been carried out by limited tryptic hydrolysis of the molecule (12). An N-terminal 6.5-kDa fragment that possessed mitogenic activity was not emetic, whereas a C-terminal 22-kDa fragment that was emetic, was not mitogenic. While it was concluded that mitogenic and emetic

properties originated from different sites on the molecule, the trypsin treatment itself significantly reduced both activities relative to the native SEC_1. Contrary to these results, a tryptic fragment of SEC with the N-terminal 59 amino acids deleted retained its mitogenicity (25). When taken together, results from two laboratories using carboxymethylated toxin suggested a dissociation of the emetic and mitogenic activities of SEB. While carboxymethylated toxin induced mitogenesis (26), carboxymethylated SEB was not emetic (27). Of interest, carboxymethylated SEB competitively inhibited the emetic effects of native SEB in monkeys (27).

An important question concerns the identity of the target cells for the emetic effects of SEB and other enterotoxins, since these effects may be independent of T cell activation. Experimental results suggest that SEB stimulates mast cells to release leukotrienes, which are thought to be responsible for the emetic response of monkeys (28). Corroborative evidence supporting this mechanism of SEB action was the ability of a leukotriene LTD_4/LTE_4 receptor inhibitor (LY171883) to block emesis. At present, direct binding and activation of isolated mast cells by enterotoxins needs to be investigated. Since it has been established that class II major histocompatibility complex antigens (see below) serve as the receptors on nonlymphoid cells for the SE, it is important to show that mast cells display such receptors. Thus, SE may exert their biological effects through activation of T cells as well as other cells, such as mast cells. Finally, another mechanism by which SE exert their biological effects involves SE-dependent, T cell-mediated cytotoxicity (29). MHC class II-expressing, SE-presenting cells are selectively and rapidly eliminated following their presentation of SE to T cells, presumably by means of lymphokines released by the activated T cell. This mechanism may represent a bacterial strategy to avoid immune recognition.

The Major Histocompatibility Complex Receptor

Accessory cells are required for SEA-induced T lymphocyte responses in a manner analogous to the accessory cell requirement for presentation of protein antigens to lymphocytes (30, 31). MHC class II molecules (Ia) on the antigen-presenting cells are the toxin receptors (2, 32–34). Unlike normal protein antigens, presentation of SE is not MHC-restricted and processing is not required (30, 31). Human cells can present enterotoxin to mouse T cells as effectively as mouse antigen-presenting cells; however, presentation varies among different MHC class II haplotypes (35). In the mouse, some SE bind preferentially to the I-E isotype, while others bind both I-A and I-E (2). In humans, the enterotoxins typically bind to human histocompatibility leukocyte D locus antigens DR and DQ, but not to DP (36). Enterotoxins bind to antigen-presenting cells with an affinity (K_d) between 10^{-6} and 10^{-7} M (30, 34, 37). SEA binds with higher affinity than does SEB (38), and enterotoxins bind human MHC molecules with higher affinity than they bind mouse MHC molecules.

While several approaches have been used to identify the sites of interaction between MHC class II molecules and SE, the use of synthetic peptides in competitive binding and functional assays has been particularly valuable. Overlapping peptides corresponding to regions 30–60, 50–70, 65–85, and 80–100 of the MHC class II antigen β-chain on mouse (H-2^b) accessory cells were synthesized and tested for their ability to inhibit SEA binding to a mouse B cell lymphoma antigen-presenting cell line (A20) and to human Burkitt's lymphoma line, Raji (39). The MHC class II β-chain peptide of the A isotype, b haplotype molecule I-A_β^b(65–85), corresponding to the predicted α-helix along the hypothetical antigen-binding cleft, preferentially inhibited SEA binding to the cells (Fig. 1; 40). Consistent with these results, I-A_β^b(65–85) also directly and specifically bound both the intact SEA molecule and its Ia binding site, represented by peptide SEA(1–45) (see below). Functionally, I-A_β^b(65–85) inhibited SEA stimulation of human and mouse T cell proliferation (41). Other overlapping peptides encompassing region (70–80), I-A_β^b(60–80), I-A_β^b(70–90), and I-A_β^b(60–90) also inhibited responsiveness to SEA in a similar fashion, suggesting that this α-helical region may be important for SEA function.

Using the synthetic peptide approach, the N-terminal part of the SEA molecule was identified as a site that interacts with the Ia molecule (42, 43). Specifically SEA(1–45) and, most recently, SEA(39–66) have been found to compete with radiolabeled SEA for interaction with HLA-DR (Nathan D. Griggs, personal communication). SEA(1–45) also inhibited SEA induction of T cell proliferation and γ-interferon production. In addition, the β-chain α-helical peptide I-A_β^b(65–85) was bound by SEA(1–45), suggesting that SEA interacts with the α-helix of the Ia β-chain via an N-terminal domain of the SEA molecule. The importance of the amino-terminal region of SEA, SEB, and SEC in T cell activation has been observed in earlier studies in which enzymatic hydrolysis was used to dissociate the emetic and mitogenic activities of the molecules (5, 12). A small amino-terminal peptide retained mitogenic activity (12). This is in contrast to a report that SEC retained mitogenic activity in the absence of the first 59 amino acid residues (25). Furthermore, a central 14-kDa fragment of TSST-1 had mitogenic activity (44, 45). Thus, different regions of the SE sequence may have biological relevance.

The structural basis for SE interaction with class II molecules has been further elucidated by a comparison of SEA and TSST-1 binding (46). Both toxins bound similarly to murine A20 cells, and inhibition of binding

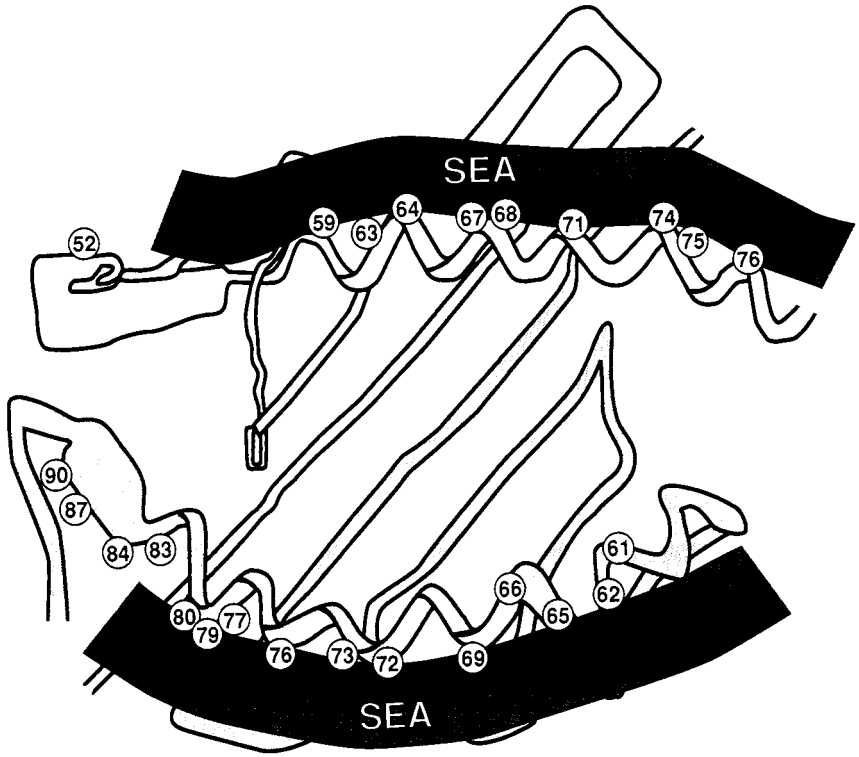

Figure 1. Proposed model for the interaction of SEA with a class II MHC molecule. The class II MHC antigen-binding cleft (40) is formed by the noncovalent association of a β-chain (lightly shaded) and an α-chain (open). SEA region(1–45) binds the α-helical β-chain. Another region of SEA binds the α-helix of the MHC α-chain. While the α-chain is not necessary for SEA binding, the β-chain is both necessary and sufficient for SEA binding. However, since neither MHC α-helix is sufficient for function, both are involved in mediating SEA effects. SEA is thought to bind the α-helices of the MHC β- and α-chains outside of the antigen-binding cleft, suggesting that some combination of these conserved amino acid residues predicted to face away from this groove (circled numbers) are involved in the interaction. It is not known whether the different molecular sites of SEA which bind each MHC molecule are on one or more than one SEA molecule.

was observed only with the homologous toxin, suggesting two distinct sites. In contrast, on human Raji cells, SEA was a better inhibitor of TSST-1 binding than was TSST-1 itself, which suggests that TSST-1 interacts with Raji cells at an SEA-binding site. The peptides SEA(1–45) and I-A_β^b(65–85) were capable of blocking SEA binding to both A20 and Raji cells, but inhibition was greater on A20 cells. Neither peptide blocked TSST-1 binding to either cell line. The data are compatible with a model in which SEA has a binding site on A20 cells involving SEA(1–45) and I-A_β^b(65–85) that is distinct from that which binds TSST-1, while at least two binding sites are present on Raji cells. One site involves predominantly the residue 1–45 region on SEA and the 65–85 region of the MHC β-chain, while the other site involves both a different region on the SEA molecule and a different site on the class II MHC molecule to which it binds. This latter site also binds TSST-1.

Evidence that the Ia α-chain is also involved in the binding of SE includes the results of one study wherein chimeric α- and β-chains of DR and DP expressed at the surface of transfected cells were used to show that the amino-terminal (α1 domain) region of DR was essential for high-affinity binding of TSST-1 (47). Using another approach, α-chain mutations with single alanine substitutions along the α-helix proposed to form one side of the antigen-binding groove produced negligible effects on SE presentation, although drastic effects on peptide presentation were observed (48). These studies both concluded that SE interact with MHC class II molecules outside of the antigen-binding groove.

Studies have also been carried out with synthetic peptides corresponding to various regions of the α-chain of mouse MHC class II molecules (41). Like the β-chain α-helical peptides, peptides corresponding to the α-chain α-helix inhibited SEA activation of T cells and directly bound SEA. Unlike the β-chain α-helical peptides, however, the α-chain α-helical peptides did not block binding of SEA to MHC class II molecules. It appears that the α-helix of the α-chain is a co-binder of SEA, but that it cannot block binding of SEA to the α-helix of the β-chain (Fig. 1).

Thus, the data generated using class II MHC α- and β-chain synthetic peptides suggest that both α-helices are important for SEA-induced function, although the β-chain α-helix is sufficient for SEA binding to cells. Taken together, the data are consistent with a model of class II molecule × SEA interaction wherein both α- and β-chain α-helices bind SEA to form a complex that is subsequently recognized by the TcR.

As mentioned above, SEA binding probably occurs outside of the antigen-binding groove (47, 48). While one molecule of SEA may bind at the same time to both α- and β-chains of an MHC molecule, it is also possible that more than one SEA molecule binds one MHC molecule.

The T Cell Antigen Receptors

Recently, it was shown that the TcR for antigenic peptides is also the receptor for SE (2, 3, 49). Furthermore, the ability of a given enterotoxin type to activate a particular T cell was dependent on the Vβ region of the TcR (2, 3). This property is the basis for coining the name "superantigens" for the SE (3). Vβ specificity is a quantitative effect rather than a qualitative one, since T cell clones of the same Vβ specificity vary in their response to microbial superantigens (50). Evidence for interaction of SE with TcR has been based primarily on studies with antibodies to the TcR.

Direct interaction of the microbial superantigen and MHC with the TcR has been demonstrated recently using a cell-binding assay that substantially increases the avidity of the interaction (51). This increased avidity may mimic *in vivo* T cell activation in which only about 0.5% of TcR occupancy was reported to be required for activity. For the binding studies, a truncated, secreted form of the β-chain of the TcR was employed that does not require interaction with the α-chain or accessory molecules such as CD3 for secretion (52). This isolated β-chain was sufficient to bind SEA complexed to cell surface class II MHC. While β-chain binding could be observed independently, the α-chain of the TcR may be necessary for elicitation of function (53). Activation of $\gamma\delta$-bearing T cells by SEA was dependent on the presence of the Vγ9 variable region (54), which argues against exclusive binding by the β-chain.

Contact residues involved in binding SE to the TcR Vβ are being elucidated. Studies have been carried out on SEC_2 induction of interleukin 2 in cells bearing chimerics of human Vβ 13.2 and Vβ 13.1 (55). Vβ 13.2 cells respond to SEC_2, whereas Vβ 13.1 cells do not. Cells that contained residues 67–77 from the Vβ 13.2 element that were introduced into the corresponding region of the Vβ 13.1 element responded to SEC_2. A synthetic peptide corresponding to amino acids 58–78 of the murine, SEA-responsive Vβ3 was able to block T cell activation by SEA (56). The potential binding region 58–78 is thought to lie on the side of the TcR molecule away from the MHC conventional antigen-binding site.

SE and the Self-Superantigen Minor Lymphocyte-Stimulating Antigen

There is a functional similarity between superantigens such as the SE and putative products of the mouse minor lymphocyte-stimulating antigen (Mls) locus (2, 57). Mls antigens were discovered when it was shown that T cells from some strains of mice could be stimulated by spleen cells from other mice, even though the mice were identical with respect to MHC (58). These antigens were called minor lymphocyte-stimulating antigens. Until recently, nothing was known about the function or structure of the Mls antigen. Although initially thought to be a single locus, it is now known that there are at least two Mls unlinked loci, each with a stimulator Mls^a and a responder Mls^b allele (2). The functional response of T cells to the uncharacterized Mls antigens is essentially the same as it is to the relatively well-characterized superantigens. However, the function of self-superantigens, such as Mls, is not known. One group has proposed that self-superantigens evolved in mice to eliminate those Vβ-bearing T cells that respond to microbial superantigens such as the SE (57). This would protect the mice against the negative effect of overstimulation of the immune system. Another group considers the self-superantigens (and by association, also the microbial superantigens) to be coligands with specific antigen, and that, in this capacity, they help the immune response to specific antigens (59).

Recently, genetic data have shown a linkage between the endogenous provirus murine mammary tumor virus and the Mls antigen (60). This finding has been confirmed and strengthened by several other reports (61–64), even though the structure of the murine mammary tumor virus that possesses the superantigen properties has not been identified. The findings suggest that Mls and possibly other "endogenous" superantigens are really virus-derived microbial superantigens. It would seem then that the "function" of superantigens remains to be determined. Certainly, the nature of their receptors in the immune system and the power with which they activate it provide the impetus to determine the role and importance of superantigens in the immune response to specific antigens, both self and foreign.

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