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International Journal of Food Microbiology 61 (2000) 1–10

INTERNATIONAL JOURNAL OF
Food Microbiology

www.elsevier.nl/locate/ijfoodmicro

Review

Staphylococcal enterotoxins

Naomi Balaban[a], Avraham Rasooly[b,c,*]

[a]*Department of Medical Pathology, University of California, Davis, CA 95616, USA*
[b]*Center of Veterinary Medicine US Food and Drug Administration, Washington, DC, USA*
[c]*Center of Food Safety and Applied Nutrition US Food and Drug Administration, Washington, DC, USA*

Received 6 May 2000; received in revised form 12 May 2000; accepted 5 June 2000

Abstract

Staphylococcus aureus is a major human pathogen that produces a wide array of toxins, thus causing various types of disease symptoms. Staphylococcal enterotoxins (SEs), a family of nine major serological types of heat stable enterotoxins, are a leading cause of gastroenteritis resulting from consumption of contaminated food. In addition, SEs are powerful superantigens that stimulate non-specific T-cell proliferation. SEs share close phylogenetic relationships, with similar structures and activities. Here we review the structure and function of each known enterotoxin.

Keywords: *Staphylococcus aureus*; Enterotoxin; Human pathogen

1. Staphylococcal enterotoxins

Staphylococcal enterotoxins (SEs) belong to a large family of staphylococcal and streptococcal pyrogenic exotoxins (PT), sharing common phylogenetic relationships, structure, function, and sequence homology. These toxins cause toxic shock-like syndromes and have been implicated in food poisoning and several allergic and autoimmune diseases. Included within this group are the staphylococcal enterotoxins, two forms of toxic shock syndrome toxin (TSST), and a group of streptococci pyrogenic exotoxins.

SEs are a family of major serological types of heat stable enterotoxins (SEA through SEE and SEG through SEJ). SEs function both as potent gastrointestinal toxins as well as superantigens that stimulate non-specific T-cell proliferation. Although these are two separate functions localized on separate domains of the proteins, there is a high correlation between these activities and in most cases a loss of superantigen activity (because of a genetic mutation) results

*Corresponding author. Tel.: +1-202-205-4192; fax: +1-202-401-7740.

E-mail address: axr@vm.cfsan.fda.gov (A. Rasooly).

0168-1605/00/$ – see front matter
PII: S0168-1605(00)00377-9

in loss of enterotoxic activity as well (Harris et al., 1993).

2. Staphylococcal food-borne diseases

Food borne diseases have a major public health impact. It is estimated that in the US, each year food borne illnesses affect 6 to 80 million people, causing up to 9000 deaths, and cost about 5 billion US dollars (Altekruse et al., 1997; Buzby and Roberts, 1997). Staphylococcal food borne diseases resulting from consumption of food contaminated with SEs are the second most common cause of reported food-borne illnesses (Archer and Young, 1988; Bean et al., 1990; Bunning et al., 1997; Garthright et al., 1988; Levine et al., 1991; MacDonald and Griffin, 1986), where 10% of the affected individuals visited or were admitted to hospitals. Staphylococcal food poisoning is characterized by a short incubation period (2 to 6 h) after ingestion of preformed toxins, followed by nausea, vomiting, abdominal pain, and diarrhea. Vomiting usually begins first, with diarrhea following soon after. SEA is the most common enterotoxin recovered from food-poisoning outbreaks in the US (77.8% of all outbreaks) followed by SED (37.5%) and SEB (10%) (Casman, 1965). The intoxication is not lethal and the elderly are more susceptible to morbidity and mortality from food borne-induced gastroenteritis than younger individuals. In very rare cases, acute staphylococcal enterotoxicosis can cause death due to complications.

The amount of enterotoxin necessary to cause intoxication is very small. The emetic dose in a monkey assay is ~5–20 μg/animal (Bergdoll, 1967). In an outbreak of gastroenteritis in the US due to chocolate milk containing staphylococcal enterotoxin A (SEA) (Evenson et al., 1988), the mean amount of SEA in the 400-ml container was only 0.5 ng/ml and a total dose of ~200 ng. Enterotoxins are detectable with a low inoculum of 10^3/g (Meyrand et al., 1998). Some staphylococcal strains produce detectable amounts of enterotoxins after 24 h incubation at 30°C (Pereira et al., 1991). In *S. aureus* cultures grown in enriched medium as well as in mushrooms contaminated with *S. aureus* and incubated at 37°C, SEA is routinely detected after ~2 h (Rasooly and Rasooly, 1998).

In a study of food poisoning in England, the most prevalent contaminated foods (75%) were meat (ham), poultry (chicken) or their products. Other contaminated food products included fish and shellfish (7%) and milk products (8%). Most contamination took place in the home followed by restaurants and food stores (Wieneke et al., 1993).

SEs are resistant to inactivation by gastrointestinal proteases such as pepsin as well as by heat. Heat stability is one of the most important properties of SEs in terms of food safety (Denny et al., 1971; Hernandez et al., 1993; Hilker et al., 1968; Hoover et al., 1983; Humber et al., 1975; Lee et al., 1977). Recent work showed that in mushrooms, even after 28 min at 121°C (or 15 min at 127°C) SEA retains some biological activity, as measured by the kitten animal assay (Anderson et al., 1996). Other studies showed loss of activity after 11 min at 121°C (using kitten assay) or 8 min at 121°C (using monkey assay) (Denny et al., 1966). Heat stability seems to be dependent on the media the toxin is in, the pH, salt concentration and other environmental factors related to the level of toxin denaturation. The central and the C-terminal domains of SEs were shown to cause emesis while the removal of the 59 N-terminal residues region (tested in SEC1) retains its ability to cause emesis in monkeys (Spero et al., 1976).

3. Enterotoxins' superantigen activity

In the cellular immune response, the primary event is recognition of the antigen by the membrane-bound T cell antigen receptor (TCR) (Fields et al., 1996; Jardetzky et al., 1994; Seth et al., 1994; Swaminathan et al., 1992). The TCR is a glycosylated polymorphic heterodimer, composed either of α and β or of γ and δ chains. The antigen is presented to TCR in the form of processed peptides bound to molecules of the major histocompatibility complex (MHC), class I and II, which are membrane-bound proteins displayed on the surface of antigen-presenting cells (APC) (Fink et al., 1986). This type of recognition is the key to the high specificity of the immune response, where only few T-cells can recognize a specific antigen. Superantigens, on the other hand, can interact with many T cells in a nonspecific manner, and are the most potent activators of T lymphocytes. They require only recognition of specific TCR Vβ for interaction

and cross-link the T-cell receptor and antigen-presenting cells of MHC class II, causing activation (Choi et al., 1989, 1990; Marrack et al., 1990; Johnson et al., 1991; Kappler et al., 1994) (Fig. 1).

The receptors for SE on antigen-presenting cells are MHC class II molecules. Recent studies have shown that a complex of SE and MHC class II molecules is required for binding to the variable region of the T cell antigen receptor beta-chain (Fields et al., 1996; Hayball et al., 1994). SE mitogenic activity is dependent on induction of interleukins (Gerwien et al., 1998; Huang and Koller, 1998; Lavkan et al., 1998; Mason et al., 1998), that may be intimately involved in the mechanism of SE toxicity.

Although their mode of action as superantigens is well understood, little is known about how SEs enter the body via the intestine and cause the symptoms of food poisoning. Using an in vitro culture system to study the capacity of class II MHC-negative human intestinal epithelial cells (Hamad et al., 1997) to transcytose several staphylococcal toxins, it was found that epithelial cells are capable of dose-dependent, facilitated transcytosis of SEB and TSST-1, but not SEA. In vivo mice studies showed that ingested SEB appears in the blood more efficiently than SEA, suggesting that these toxins can cross the epithelium in an immunologically intact form.

4. Phylogenetic relationships of SEs

The PT family is composed of SEs, toxic shock syndrome toxin (TSST-1), and streptococcal pyrogenic exotoxins (SPE). Phylogenetic relationships for members of the PT family were estimated by examining nucleotide sequences of the genes encoding SPE type A, SPE type C, SEA, SEB, SEC, SEC1, SEC2, SEC3, SED, and SEE (Van den Bussche et al., 1993). A total of 611 mutational events within the PT genes were studied (Van den Bussche et al., 1993), and sequence divergence was partitioned in a hierarchical fashion into two large groups (Fig. 2). One group consists of the staphylococcal toxins SEA, SEE, and SED, being closely related to the streptococcal toxin SPEC (clade I)

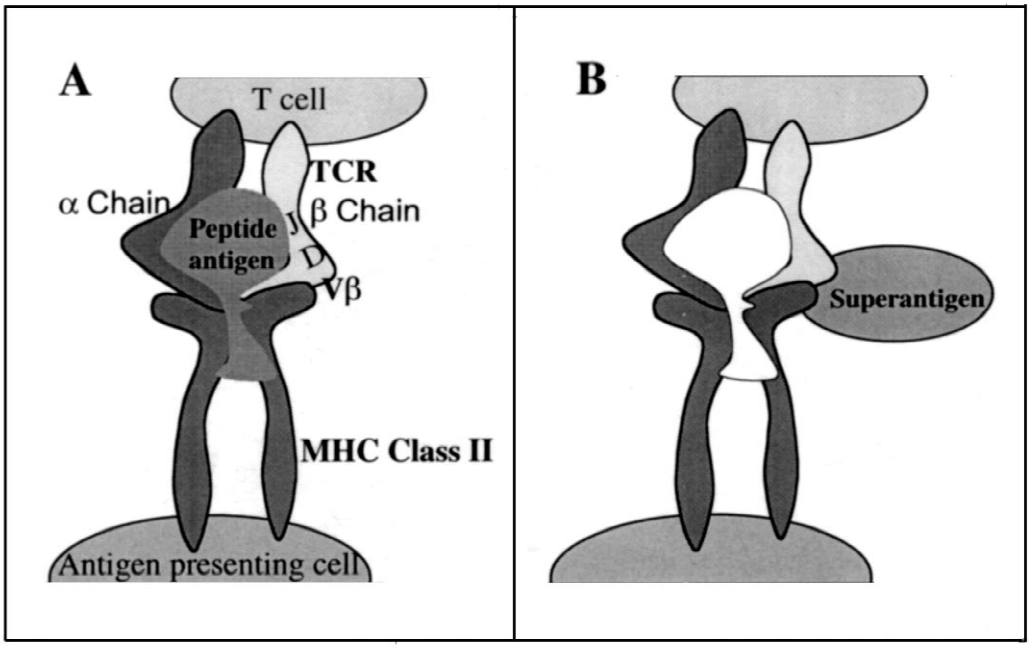

Fig. 1. Model for the structure of the complex MHC class II and T-cell receptor. (A) Conventional antigen. (B) Superantigen. The model shows the processed antigen peptide (pink) presented by MHC class II (blue) which attracts specific T-cell bearing antigen specific T-cell receptor (TCR, green) variable (Vβ) chain. In contrast, superantigens (orange) bind directly to the outside of the MHC molecule and cross-link it to Vβ chain, which initiates non-specific activation of the cell.

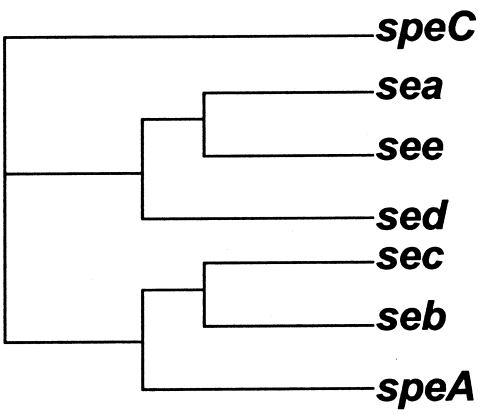

Fig. 2. Phylogenetic relationships for members of the PT family. Simplified diagram showing the phylogenetic relationships for members of the PT family adapted from Van den Bussche et al. (1993). The predicted amino acid sequences were used in this analysis.

(Van den Bussche et al., 1993), with an overall amino acid homology of 51–81%. The other clade depicts the close relationships of the staphylococcal toxins SEC and SEB with the streptococcal toxin SPEA (clade II) (Van den Bussche et al., 1993), with an overall protein sequence homology of 42–67%. We analyzed SEs that have been characterized more recently. SEJ appears closely related to the toxins in clade I (52–66% homology) (Fig. 3). SEI and SEH are more distantly related to the SEs, although they are somewhat closer to clade II toxins (31–38% sequence identity) than to clade I SEs. In general, the amino acid homology between members of the two clades is 22–33%. Degree of homology between SEs was determined using CLUSTAL W (Thompson et al., 1994) and is shown in Fig. 3.

	SEC1	SEG	SEA	SEE	SEJ	SED	SEH	SEI
SEB	67	46	31	30	30	33	24	23
SEC1		42	25	27	22	27	25	22
SEG			24	25	24	22	26	24
SEA				81	66	51	37	33
SEE					64	54	38	31
SEJ						52	36	35
SED							38	31
SEH								25

Fig. 3. Comparison of % amino acid sequence homology among SEs. Red, clade I. Blue, clade II. Black, % homology between clade I and clade II.

5. Sequence analysis of SEs

We, and others, performed alignment analysis of SEs using multiple sequence alignment with hierarchical clustering (Corpet, 1988) (Fig. 4). Sequences include the mapped SE regions related to their interactions with the MHC class II and TCR, which were deduced from crystallographic data (Swaminathan et al., 1992; Soos and Johnson, 1994; Papageorgiou et al., 1995, 1998; Schad et al., 1995; Ulrich et al., 1995; Fields et al., 1996; Sundstrom et al., 1996a,b) and from mutational analysis (Irwin et al., 1992; Kappler et al., 1992; Abrahmsen et al., 1995; Harris and Betley, 1995; Hayball et al., 1994, 1995; Hudson et al., 1995; Briggs et al., 1997; Rosendahl et al., 1997). Of note is the fact that the amino acid numbers in Fig. 4 are from the alignment analysis and not from the original studies.

All SEs, from both clades, share several conserved regions, and have a similar two-domain topology with a β-barrel (up to amino acid (aa) ~126) motif and a β-grasp motif (from aa ~127) separated by a shallow cavity (Schad et al., 1995; Swaminathan et al., 1992). The junction between the two domains (aa 119–129) appears to be highly conserved among all SEs. Mutational analyses of SEB and SEA suggest that the TCR binding site is located in this shallow cavity (Antonsson et al., 1997; Garcia et al., 1998; Swaminathan et al., 1992). The region spanning aa 118–175 also shares homology to a region of the COOH-terminal end of the mouse and human invariant chain, a polypeptide associated with nascent MHC class II molecule which seems to prevent binding of peptides to the binding site on MHC class II molecule (Marrack and Kappler, 1990). This similarity may enable SEs to bind to the MHC class II, thereby inducing non-specific binding and facilitating superantigen activity.

In addition, mutational analysis has identified three N-terminal regions involved in SEB MHC class II V-β interaction (Kappler et al., 1992). Mutations in aa 13–27 affect both MHC class II binding as well as T cell interaction. Similarly, mutations in residues 45–58 of SEB reduce the toxin's ability to bind to MHC class II. Finally, mutations in the conserved tyrosine 66 affect the interaction of SEA with V-β 7 and 8.1.

SEA, SEC2, SED, SEE, SEH and SEJ (but not SEB) are dependent upon Zn^{2+} for high affinity

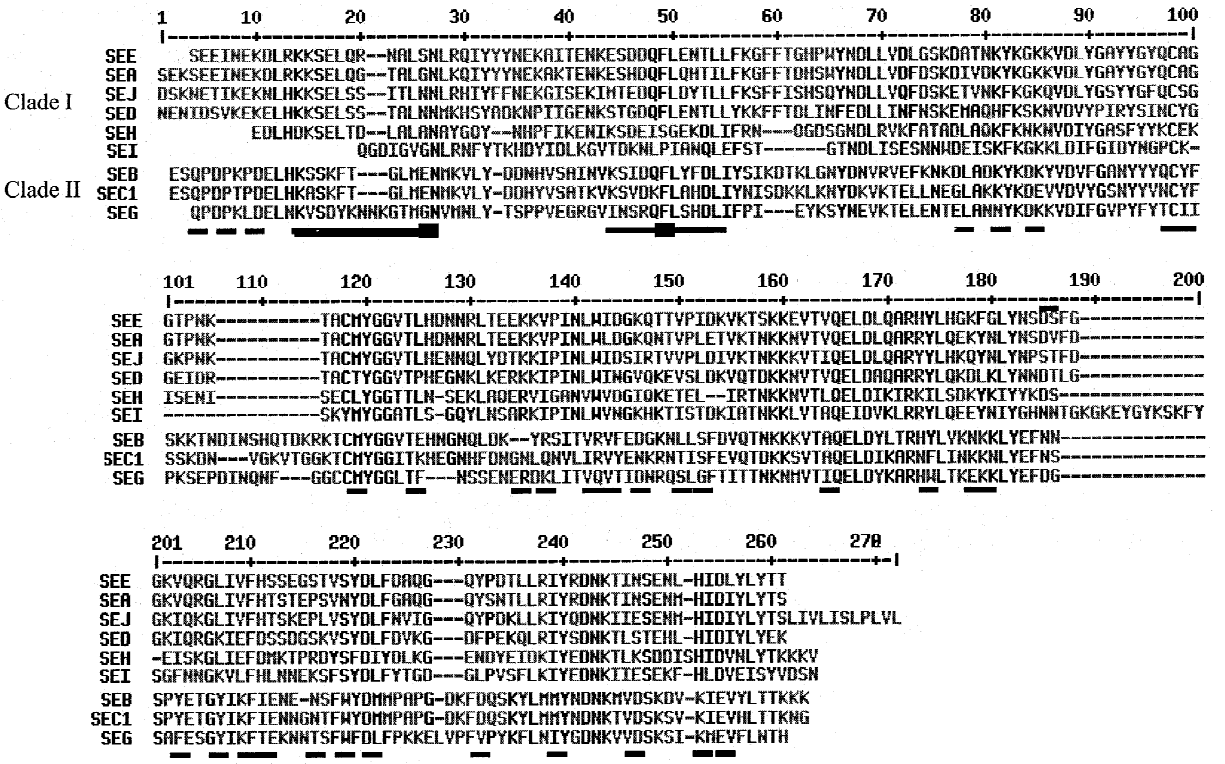

Fig. 4. Comparison of amino acid sequences of SEs. The protein sequences, aligned using MultAlin, are divided into clade I and clade II. Residues that are identical in at least eight of the toxins are shown in red, while those that are identical in at least half of the SEs are shown in blue. Residues that are on the surface of the toxins and that are involved in the interaction between MHC class II and TCR are underlined: MHC class II (red line); TCR (green line); Zn-binding region (blue line). Amino acid numbers are from the alignment analysis and not from the original studies.

interactions with MHC class II molecules (Abrahmsen et al., 1995; Schad et al., 1995, 1997; Sundstrom et al., 1996a,b; Al-Daccak et al., 1998), all containing a conserved Zn-binding site which forms a molecular bridge with a residue (H81) in β chain of MHC class II.

5.1. Staphylococcus enterotoxin A (SEA)

SEA is the most common toxin implicated in staphylococcal food poisoning. (Holmberg and Blake, 1984). The gene for SEA (*entA*) is carried by a temperate bacteriophage (Betley and Mekalanos, 1985; Borst and Betley, 1994). Hybridization analysis of DNA from *entA*-converting phage suggests that this phage integrated into the bacterial chromosome by circularization and reciprocal crossover, and that the *entA* gene is located near the phage attachment site. The *sea* gene is composed of 771 base pairs and encodes an enterotoxin A precursor of 257 amino acid residues (Huang et al., 1987). A 24-residue N-terminal hydrophobic leader sequence is apparently processed, yielding the mature form of SEA (M_r 27,100 dalton) (Betley and Mekalanos, 1988). There are three SEA isoforms with three different isoelectric points, suggesting variation in processing or post translation modification. As mentioned above, the mature SEA is a monomeric two-domain protein composed of a 13-barrel and a 13-grasp motif (Schad et al., 1995), the same general structure found in other enterotoxins. SEA has a Zn^{2+} coordination site involved in MHC class II binding.

SEA is expressed from the mid-exponential phase of growth, but is not regulated by the accessory gene regulator *agr* (Tremaine et al., 1993), unlike *seb*, *sec*,

and *sed*, which require a functional *agr* for maximal expression.

5.2. Staphylococcus enterotoxin B (SEB)

The coding region of the (*entB*) gene (Johns and Khan, 1988) contains ~900 nucleotides. The SEB precursor protein contains 267 amino acids (M_r 31,400 dalton) and includes an N-terminal signal peptide of 27 amino acids. The *entB* gene is chromosomal in clinical isolates of *S. aureus* from food-poisoning cases (Shafer and Iandolo, 1978). However, in other bacterial strains, the gene is carried by a 750 kb plasmid (Shalita et al., 1977).

The T-cell receptor binding site encompasses a shallow cavity formed by both domains and the MHC class II molecule binds to an adjacent site (Kappler et al., 1992; Papageorgiou et al., 1998; Soos and Johnson, 1994). Analysis of the TCR-binding sites of SEB (Fields et al., 1996; Hayball et al., 1994), SEA and SEC2 shows significant differences, which may account for the ability of each superantigen to bind specific V-β.

5.3. Staphylococcal enterotoxin type C (SEC)

SECs are a group of highly conserved proteins with significant immunological cross-reactivity (Bergdoll et al., 1965). The three antigenically distinct SEC subtypes are SEC1, SEC2, and SEC3. The *entC3* gene contains 801 bp and encodes a precursor protein of 267 amino acids (Hovde et al., 1990) containing a 27-residue signal peptide (Bohach and Schlievert, 1989; Hovde et al., 1990). The *entC3* gene is closely related to the gene for staphylococcal enterotoxin type C1, with 98% nucleotide sequence identity (Couch and Betley, 1989). SEC3 differs from enterotoxins C2 and C1 by four and nine amino acids, respectively.

Sequence similarities between *entC3*, *entC1*, and *entB* genes suggest that an ancestral *entC1*-like gene was formed by recombination between the *entC3* and *entB* genes (Couch and Betley, 1989). The *entC2* structural gene contains an 801-bp open reading frame encoding a 267-amino-acid precursor. Mature SEC2, produced by removal of the signal peptide, contains 239 amino acids. The C-terminal residues of the three toxins are identical, except for one conserved amino acid substitution in enterotoxin C3 (Hovde et al., 1990). The divergent N-termini of Type C enterotoxins determine subtype-specific antigenic epitopes, while the conserved C-terminal regions determine biological properties and cross-reactive antigenic epitopes shared with other pyrogenic toxins.

S. aureus isolated from different animal species produce a unique host-specific SEC (Marr et al., 1993), suggesting that toxin heterogeneity is due to selection for modified SEC sequences that facilitate the survival of *S. aureus* isolates in their respective hosts.

5.4. Staphylococcal enterotoxin D (SED)

SED (Chang and Bergdoll, 1979) is the second most common serotype associated with food poisoning. The gene encoding SED is *entD*, which is located on a 27.6-kilobase penicillinase plasmid designated pIB485 (Bayles and Iandolo, 1989). The *entD* gene product contains 258 amino acids, including a 30-amino-acid signal peptide (Bayles and Iandolo, 1989). The 228-amino-acid mature polypeptide shows a high degree of sequence similarity to other staphylococcal enterotoxins. The SED superantigen is dependent upon Zn^{2+} for high affinity interactions with MHC class II molecules and thus SED was co-crystallized with Zn^{2+} (Sundstrom et al., 1996a). The three-dimensional structure of SED is similar to structures of other bacterial superantigens, although SED has the unique capability of forming dimers in the presence of Zn^{2+}. The high affinity Zn^{2+} binding site used in dimer formation is located on the surface of the beta-sheet in the C-terminal domain. Two bound metal ions are coordinated by residues from both molecules in the dimer interface and thus contribute directly to formation of the dimer. A second Zn^{2+} binding site is located on the surface of the protein close to the domain interface of the molecule. The ability of SED to form a Zn^{2+}-dependent homodimer seems to facilitate novel and biologically relevant multimeric interactions with MHC class II molecules.

5.5. Staphylococcal enterotoxin type E (SEE)

The gene for SEE (*entE*) encodes a 29-kDa protein that is apparently processed to a mature extracellular form with a molecular mass of 26 kDa

(Couch et al., 1988). DNA sequence identity indicate that SEE, SED and SEA are closely related (Van den Bussche et al., 1993). SEE shares high sequence homology (81%) with SEA (Fig. 3).

5.6. Staphylococcal enterotoxin type G (SEG)

The *entG* gene encodes a 258-amino acid precursor protein that is cleaved to form a toxin with 233 amino acids (Munson et al., 1998). SEG is most similar to SpeA, SEB, SEC, and SSA (Munson et al., 1998).

5.7. Staphylococcal enterotoxin type H (SEH)

SEH is a recently discovered enterotoxin with a molecular mass of 27,300 dalton (Su and Wong, 1995). The NH_2-terminal amino acid sequence of the enterotoxin is unique and immunodiffusion assays do not detect cross-reactivity between SEH and previously identified enterotoxins. As discussed above, the homology SEH shares with clade I is 36–38% (Fig. 3), suggesting that SEH is a more distant enterotoxin.

5.8. Staphylococcal enterotoxin type I (SEI)

The *entI* gene encodes a precursor protein of 242 amino acids (Munson et al., 1998). The signal sequence of pre-SEI is cleaved to form a toxin containing 218 amino acids. SEI have the lowest homology to other SEs although it shares more homology to clade I than to clade II.

5.9. Staphylococcal enterotoxin type J (SEJ)

Characterization of the enterotoxin D-encoding plasmid revealed the presence of an open reading frame which encodes a previously unidentified enterotoxin, designated staphylococcal enterotoxin J (SEJ) (Zhang et al., 1998). The enterotoxin D and J open reading frames are transcribed in opposite directions and are separated by an 895 nucleotide intergenic region which contains a perfect inverted repeat, with each arm of the repeat having a length of 21 nucleotides. The predicted 269-amino acid SEJ protein has substantial sequence similarity to SEA, SEE and SED (64–66%). PCR amplification suggests that the *entJ* determinant may be present on all SED-encoding plasmids.

6. Conclusion

SEs function both as potent gastrointestinal toxins as well as superantigens that stimulate non-specific T-cell proliferation. Structurally, these two functions are located in two separate domains, targeting different host tissues. An open question remains whether these separate functions are related. This question may also be related to how the toxins enter the body via the intestine. It was found that epithelial cells are capable of a dose-dependent, facilitated transcytosis of SEs (tested on SEB and TSST-1) and that ingested SEB for example appears in the blood more readily than SEA (Hamad et al., 1997). A working hypothesis is that the enterogenic activity may facilitate transcytosis, thus enabling the toxin to interact with T cells in the bloodstream, leading to superantigenic activity. More studies are needed to answer the questions regarding the activities of SEs both as enterogenic and as superantigens.

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