The Staphylococcal Enterotoxins and Their Relatives

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Staphylococcal enterotoxins and a group of related proteins made by *Streptococci* cause food poisoning and shock in man and animals. These proteins share an ability to bind to human and mouse major histocompatibility complex proteins. The complex ligand so formed has specificity for a particular part of T cell receptors, V_β, and by engaging V_β can stimulate many T cells. It is likely that some or all of the pathological effects of these toxins are caused by their ability to activate quickly so many T cells. It is also possible that encounters with such toxins have caused mice, at least, to evolve mechanisms for varying their T cell V_β repertoires, such that they are less susceptible to attack by the toxins.

A COMPLEX BALANCE CONTROLS THE INTERACTION BETWEEN humans and the microorganisms that colonize us. Many of these organisms are normally benign, unable to damage their host, or held in check by intact barriers such as epithelial tissue or an active immune system. Such organisms may only rarely cause disease, infecting their hosts opportunistically when barriers are down or causing pathology when the organism produces a new, damaging product. Included in these products are a group of bacterial toxins that have long been recognized as pathogenic in man, but for which only recently have plausible mechanisms of toxicity been discovered.

The toxins in question comprise a large group of proteins produced by several different types of bacteria, including staphylococci, streptococci, and mycoplasma (*1–3*). These toxins are responsible for a number of diseases with various symptoms in man and animals [Table 1 (*1, 3–7*)]. A group of toxins produced by various strains of *Staphylococcus aureus*, for example, causes food poisoning in man, and is responsible for about a quarter of the outbreaks of this disease reported in the United States (*1, 4*). A distantly related protein, the toxic shock syndrome toxin TSST1, also produced by *S. aureus*, was isolated in the early 1980s and is responsible for the induction of tampon-related toxic shock (*5*). Streptococcal toxins with similar properties have also been described (*7*). A product of a mycoplasma, *Mycoplasma arthriditis*, is associated with shock-like symptoms in rats, mice, and rabbits (*3*). Although, unlike most of the other toxins listed in Table 1, the structure of the *M. arthriditis* toxin is not known, it is a small, basic protein, and probably shares its mode of action with the other toxins listed in Table 1, for reasons discussed below. Infection with several of the organisms listed as toxin sources in Table 1 can have autoimmune consequences. Streptococcal infection, for example, can lead to rheumatic fever and heart damage. *Mycoplasma arthritidis* infection was shown in the 1970s to cause arthritis in rats (*8*).

In spite of extensive work, until recently the toxicity of the proteins listed in Table 1 could not be satisfactorily explained; it is now thought that these diverse molecules may use similar mechanisms to cause pathology. The recent findings, however, that nearly all of the toxins bind to major histocompatibility complex (MHC) proteins and that these complexes of toxins and MHC proteins stimulate large numbers of T cells have led to some new ideas. It is these findings and ideas that are discussed in the rest of this article.

Structures of Enterotoxin-Related Proteins

The *S. aureus* enterotoxins are intermediately sized proteins. The sequences of some of these products were established by analyses of the proteins, in large part by M. Bergdoll and collaborators in the 1970s (*5, 9, 10*). Amino acid sequences for many of the toxins listed in Table 1 have also been deduced from the DNA encoding them (*9–17*). These sequences, aligned for greatest similarity, are shown in Fig. 1. Many of these toxins are closely related. Staphylococcal enterotoxin A (SEA) and SEE are more than 90% alike in amino acid sequence, for example, and are certainly descended from a common gene. Other toxins are less like SEA, but still share many stretches of sequences, such that it is not difficult to match the two

Table 1. Diseases caused by the staphylococcal enterotoxin-like toxins.

Toxin	Source	Sequelae	Reference
Staphylococcal enterotoxins (SE) A, B, C1, C2, C3, D, and E	*S. aureus*	Food poisoning, shock	(*1, 4*)
Toxic shock syndrome toxin (TSST1)	*S. aureus*	Toxic shock	(*5*)
Exfoliating toxins A and B	*S. aureus*	Scalded skin syndrome	(*6*)
Pyrogenic exotoxins A, B, and C	*S. pyogenes*	Fever, rash, shock	(*7*)
M. arthriditis mitogen	*M. arthriditis*	Shock	(*3*)

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sets of proteins up with respect to each other with similar sequences aligned.

Comparison of the sequences of the staphylococcal toxins indicates that they fall into two groups, SEA, E, and D are most similar to each other, as are SEB, C1, and C3. Work discussed below shows that although all the toxins shown in Fig. 1 have the same broad functions, they have distinguishable specificities. To some degree, these distinctions match their sequence differences, thus the members of a given group sometimes share specificities and differ in this from members of the other group. The two streptococcal toxins, SPE A and C, are about as similar to each of the staphylococcal toxin groups as they are to each other. So far, specificity studies of the type that distinguish the two staphylococcal toxin groups have not been done with SPE A and C, so at present these toxins cannot be formally assigned to either group.

The toxic shock syndrome–associated toxin can also be aligned with the toxin sequences shown in Fig. 1, albeit with some difficulty. As will become apparent below, this toxin has a different specificity pattern from those of either of the two groups of toxins. For the exfoliating toxins, however, the matter of relatedness is much less clear. These proteins are about the same size as SEA and SEB, and, as will be discussed below, they appear to have modes of action that are similar to these other staphylococcal-derived toxins. In spite of this, it is difficult to align the sequences of this last group with those of the staphylococcal enterotoxins. Several regions are loosely similar, which may indicate relatedness, but no alignments are striking enough to lead the scientist to predict that these proteins have common modes of action.

Overall, however, there are several stretches at which similarities are apparent throughout this group of toxins. The largest of these, located about two-thirds of the way through the proteins, is similar to a sequence found at the COOH-terminal end of human and mouse invariant chain [Fig. 2 (*17*)]. Invariant chain is a polypeptide associated with nascent MHC class II molecules (*18*). Class II molecules bind peptides and present them to T cells during immune responses (*19*). It is thought that the association of invariant chain with nascent class II prevents occupancy of the peptide binding site on class II molecules by peptides produced endogenously in the cell (*20, 21*). The separation of invariant chain from class II as the complex reaches the surface of the cell may promote association of class II with peptides derived from other sources, such as invading organisms. The similarity in a stretch of sequence between the bacterial toxins and the invariant chain may not be coincidental; many of these toxins bind to class II molecules themselves (see below). Perhaps the shared sequence indicates some or all of the invariant chain and toxin binding sites for class II.

Toxins Bind to MHC Class II Molecules

Soon after the isolation of these toxins, the search began for their target ligands in animals. Some reports suggested binding of various toxins to intestinal or mast cells, and various candidates for ligands were described (*22, 23*). Convincing biochemical characterization of the toxin ligands waited until an explosion of literature on the subject in the last few years. Many of the toxins discussed in this article have significant binding affinities for a collection of proteins that are intimately involved with immune responses and stimulation of T cells, the MHC class II molecules (*24–26*). Fraser, for example, has shown that SEA has a K_d for human class II of about $3.2 \times 10^{-7} M$ (*23*), as have Fischer and co-workers (*24*). Scholl and co-workers have estimated dissociation constants of about 10^{-7} and $10^{-6} M$ for TSST1 and SEB binding, respectively, to human class II (*24*). The work of Hermann and co-workers, in contrast, indicates that the exfoliating toxins bind only weakly or not at all to class II (*26*). SEA and SEB probably bind to the same site on class II, because they cross-compete for binding (*24*). In contrast, experiments with SEB and TSST1 have suggested that these two toxins have different binding sites on class II molecules (*24*). This second finding is rather surprising, as SEB and TSST1 are similar in amino acid sequence and the two toxins have related specificities for T cells in that both bind T cells through V_β (see below). One might have predicted that different MHC-toxin complexes with the common property of engaging T cell receptor V_βs might have required common structures themselves. This may not always be the case.

Little is known about the conformations of the toxin–class II complexes. The structure of class II molecules was deduced from the solution of the structure of MHC class I molecules (*27*) and is thought to consist of two immunoglobulin-like domains, located close to the cell membrane, which support a structure constructed from the NH_2-terminal regions of both polypeptides of the protein and comprise an extended β pleated sheet supporting two α helices, separated by a cleft. It is believed that peptides derived from foreign material, or from proteolysis of self proteins, normally lie in this groove, and that it is this complex of MHC and peptide that stimulates T cells bearing αβ receptors (*28*).

All the evidence to date indicates that the bacterial toxins do not normally bind to MHC molecules by occupying this groove, and therefore they do not behave like conventional peptide, MHC-binding antigens. Dellabona and co-workers, for example, have shown that the association of SEB with mouse class II molecules

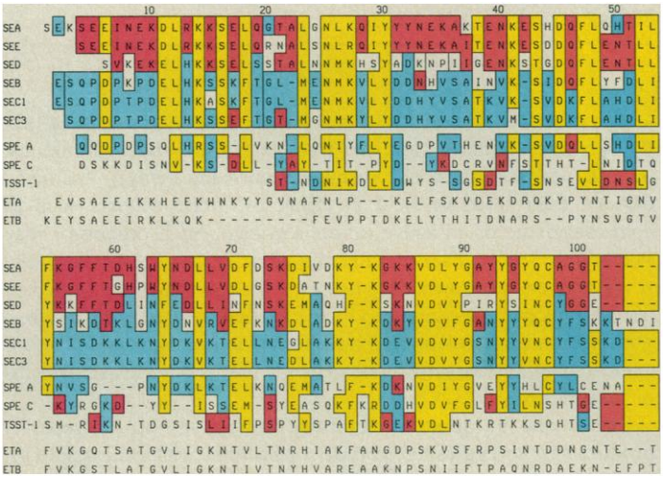


Fig. 1. Comparison of the primary sequences of the staphylococcal enterotoxins and their relatives. The complete primary amino acid sequences of the staphylococcal enterotoxins and related proteins are shown aligned, with the exception of the sequences of the exfoliating toxins, which are shown aligned with each other, but not with the remaining toxins. The exfoliating toxin sequences are shown here for completeness, and because these toxins have properties related to those of the others (see below). Toxins shown are as follows: SEA to SEE, *Staphylococcus aureus* enterotoxins A to E; SPE A and C, *Streptococcus pyogenes* toxins A and C; TSST1, *S. aureus* toxic shock–associated toxin; ETA and ETB, *S. aureus* exfoliating toxins A and B. Data are from (*9–17*). Residues that are identical or that have changed to an amino acid with similar properties among at least two of the following: SEA, SEE, and SED, are highlighted in pink. Residues that are identical or that have changed to an amino acid with similar properties among at least two of the following: SEB, SEC1, and SEC3, are highlighted in blue. Residues that are identical, or that have changed to an amino acid with similar properties among at least two of SEA, SEE, and SED and at least two of SEB, SEC1, and SEC2, are highlighted in yellow. Single letter abbreviations for the amino acid residues are: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

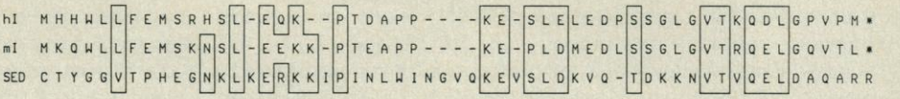
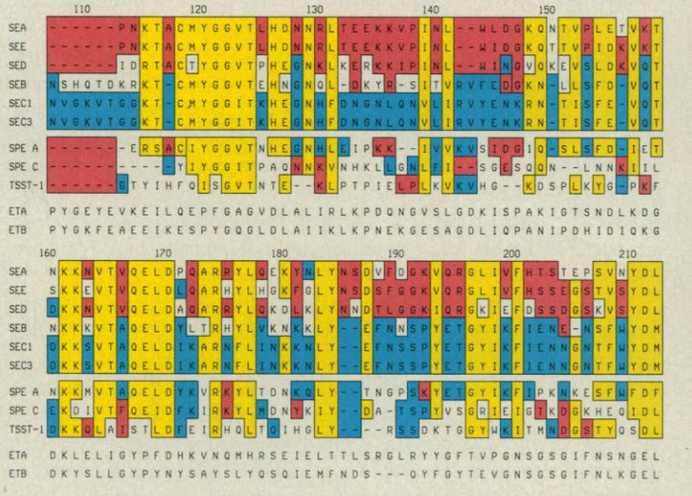


Fig. 2. The staphylococcal enterotoxins have a region that is similar to a region in invariant chain. A stretch of amino acids from residues 118 to 175 of SED is shown aligned with sequences 121 to 169 of mouse (mI) and 168 to 216 of human (hI) invariant chain. Data are from (*12, 18*).



does not inhibit presentation of an authentic antigenic peptide (derived from hen egg lysozyme) to T cells (*29*). Toxin binding to MHC also appears to be sensitive to limited proteolysis, because toxins treated in such a way and then added to class II bearing cells do not stimulate T cells, unlike their untreated counterparts (*24*). Occupancy of the antigen binding groove of MHC molecules by conventional antigenic peptides is, of course, unaffected by denaturation and usually requires limited proteolysis (*28*), so in these experiments the toxins are not behaving like conventional antigens.

If the toxins do not bind in the groove of class II molecules, where do they associate? Some clues may be deduced from the differential association of the toxins with different class II molecules. In humans, three different types of class II proteins have been described, now called DR, DP, and DQ. In mouse two are known, I-A and I-E. Affinities for these molecules of the toxins have only been measured properly for SEA, SEB, and TSST1, which, as mentioned above, have good affinities for some human class II proteins (*24*). There is evidence, however, that not all class II isotypes have equally high affinities for the toxins. Scholl and co-workers could not detect binding of labelled SEB or TSST1 to DP, although the two toxins seemed to bind equally well to a variety of DR and DQ alleles (*30*). Hermann and co-workers also found in coprecipitation experiments that the staphylococcal toxins did not bind to DP, although they did bind well to DR proteins. Unlike Scholl and co-workers (*30*), these authors found that, apart from SEC2 and SEC3, the toxins did not bind to DQ, a contradiction that needs to be resolved.

Toxin–class II complexes have also been tested for their ability to stimulate target T cells. This assay is less direct than measurements of binding of labeled toxin because it depends both on the binding of toxin to class II, and on the binding of toxin–class II complexes to T cell receptors. From such experiments Herman and co-workers deduced that most of the staphylococcal toxins bind preferentially to the DR class II protein, somewhat less well to DQ, and not at all to DP (*31*). They also found evidence that the different DR alleles have different affinities for a few of the toxins, most notably SEE.

Measurements of this types therefore indicate that the toxins bind well to DR, less well to DQ, and perhaps not at all to DP. Parallel results have been obtained in mouse, where complexes of toxins plus I-E (the murine equivalent of DR) stimulate T cells more efficiently than complexes of toxins with I-A [the mouse DQ analog (*32*)]. There is some evidence for weak haplotype specificity. For example, toxins bound to I-A^k stimulate T cells less well than toxins bound to I-A^d or I-A^b (*33*), although, as discussed above, the limitations of the type of measurement done so far do not allow us to distinguish between poor binding of the toxin to I-A^k, or poor stimulation of T cells by the ligand so formed. Finally, direct measurements of binding affinities and of T cell reactivity suggest that the *S. aureus* toxins bind more efficiently to human class II proteins than to mouse (*31, 34*). Since *S. aureus* is indigenous to humans and a different species of *Staphylococcus, xylosus*, is found on mice (*35*), the differential binding affinities may be the consequence of evolutionary divergence of the bacteria with their hosts.

Can anything be learned from these direct and indirect measurements of binding of the toxins to different class II molecules? A comparison of the amino acid sequences of class II molecules that do or do not bind the toxins with high affinity (*36*) suggests that the differences may not lie in the base of the peptide binding groove of the molecules, nor in the α helical barrels which line the groove. This latter conclusion also was suggested by the work of Dellabona and co-workers who have shown that point mutations in amino acids pointing upwards (towards the T cell receptor?) in one of these barrels affects presentation of peptides, but not presentation of toxins to T cells by class II (*29*). A more likely location for toxin binding to MHC may be at the sides of the class II molecules where two "wings," the ends of the β pleated strands, extend to either side of the proteins. Comparisons of binding and nonbinding sequences (loosely defined as discussed above) suggest a sequence on the β chain side of these wings may be important (Fig. 3). Janeway and co-workers and Fleischer have suggested models of this type on theoretical grounds in the past (*32, 37*).

Toxins Stimulate T Cells Through V_β

One of the earliest known properties of this group of bacterial toxins was their ability to stimulate many T cells, both in humans and mice (*38*). The toxins were not acting as mitogens such as concanavalin A. That is, the toxins did not activate in vitro as large a proportion of T cells as the stimulatory plant lectins and some T cell clones could not be stimulated by particular toxins (*39, 40*). This stimulation was dependent upon the presence of class II bearing cells in the cultures, a condition that was difficult to avoid in studies on human T cells, since activated human T cells are class II^+, but easier to prove for mouse T cells, which are class II^-.

Recent work has uncovered the reasons for the massive but somewhat limited T cell stimulation by such toxins. T cell receptors for antigenic peptides bound to MHC proteins are made up of five clonally variable components, V_α, J_α, V_β, D_β, and J_β, as well as N region amino acids, contributed by nongermline-encoded bases inserted at the junctional points between V_α and J_α, V_β and D_β, and D_β and J_β (*41*). Recognition of most conventional antigenic peptides bound to MHC proteins involves contributions from all the variable components of the T cell receptor (*42*). In contrast, the toxins discussed in this article appear to stimulate T cells almost exclusively via the V_β region of the T cell receptor expressed by the T cell (*32, 40, 43, 44*). In mice, for example, SEB stimulates almost all T cells bearing V_β3, 7, 8.1, 8.2, 8.3, and 17 (*32, 40, 44*). The

toxin responsible for toxic shock, TSST1, on the other hand, stimulates murine T cells bearing $V_\beta 3$, 10, 15, and 17 (*44*). In humans, similar findings apply. Staphylococcal EB stimulates T cells bearing $V_\beta 3$, TSST1 stimulates cells bearing $V_\beta 2$ (*45*). A complete comparison of the V_β specificities of the toxins for human and mouse V_βs is shown in Table 2. This V_β specificity is almost independent of the rest of the structure of the T cell receptor. Staphylococcal EA, for example, stimulates almost all T cells bearing mouse $V_\beta 3$, and the structure of the rest of the receptor on such cells is presumably heterogeneous. This property is reminiscent of other so-called superantigens that are synthesized endogenously by mice themselves and that have been named Mls antigens (*46, 47*). The relationships, structural and otherwise, between superantigens that are bacterial toxins and superantigens that are made by mice are discussed below.

The patterns of V_β specificity of the different toxins correspond loosely with their groupings by sequence similarity. SEA, SED, and SEE, for example, all stimulate mouse T cells bearing $V_\beta 11$, and SEE and SED both stimulate human T cells bearing $V_\beta 5$. SEB and the SECs, in contrast, stimulate mouse T cells bearing members of the $V_\beta 8$ family, and human T cells positive for $V_\beta 12$. There are exceptions, however. Staphylococcal ED, by sequence similarity a member of the SEA family, stimulates T cells bearing the $V_\beta 8$s, unlike SEA and SEE. Exfoliating toxin and TSST1, which are not particularly related by sequence, have similar specificities for V_β, both in mouse and humans. The data suggest that the V_βs have, for one reason or another, conserved their ability to bind certain toxin–class II complexes through the millions of years that separate mouse and humans. The mouse $V_\beta 8$s, for example are most like $V_\beta 12$ in man, and T cells bearing these V_βs share their spectra of toxin reactivity. The implications of this finding will be discussed again below.

Recent experiments have suggested that the bacterial toxins, and other superantigens, do not bind to T cell receptors at those regions thought, by modeling onto antibody structures, to be involved in binding to conventional antigenic peptides plus MHC. On the contrary, the superantigens appear to engage V_β on an exposed face of V_β, a region that is predicted to be a β pleated sheet and exposed on the side of the T cell receptor (*48*). Such a finding is in good agreement with a model for toxin binding to class II and V_β that has recently been proposed by Janeway (*32*), and a similar model is shown in Fig. 3. The model predicts that the toxins act as clamps, engaging the sides of class II and V_β, and bringing into close proximity the surfaces of the T cell receptor and MHC that would contact each other during T cell recognition of conventional antigens bound in the groove of MHC. The model predicts that binding energies contributed usually by contact of receptor residues with the antigenic peptide are substituted by binding of the toxin to V_β. Most of the elements of this proposal are probably correct, although their proper confirmation must await the x-ray crystallographic solution of the complex. Some other factors must be taken into account, however. It is very difficult to stimulate T cells with the toxins in the complete absence of class II, even if the toxins are extensively cross-linked, for example, by binding plastic. This suggests either that binding to MHC induces a conformational change in the toxin molecule, uncovering a V_β binding site, or that a considerable portion of the binding energy of T cell receptors for the toxin–class II complex is contributed by binding of the T cell receptor to class II. Secondly, there is an element of class II specificity about the interaction of the T cell receptor with this complex ligand. When individual T cells are examined, some fail to respond, even though they bear the appropriate V_β, to some toxin–class II complexes (*40*).

The toxins have detectable affinities for class II. Yet neither class II nor the toxins separately have detectable affinities for the T cell receptors in question, although the combinations of toxins and class II proteins do. This indicates that the affinity of the T cell receptor for either ligand alone is low, and that contact of T cell receptor amino acids with both toxin and class II is needed to generate good binding. The final binding complex therefore seems to be constructed similarly to that of T cell receptors, MHC proteins, and conventional antigenic peptides. For conventional antigens, the antigen has detectable affinity for MHC, but neither antigen nor MHC alone can bind the T cell receptor (*20*). Only after the complex peptide-MHC ligand has formed can it functionally engage the T cell receptor.

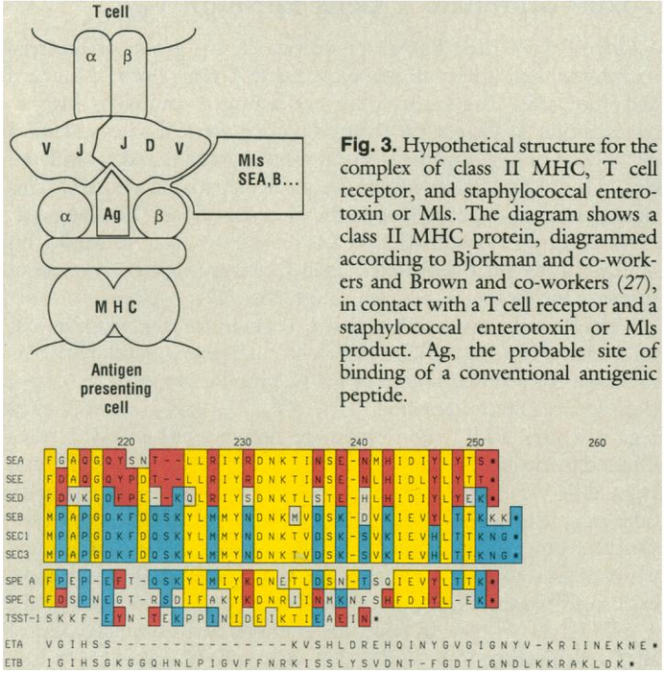


Fig. 3. Hypothetical structure for the complex of class II MHC, T cell receptor, and staphylococcal enterotoxin or Mls. The diagram shows a class II MHC protein, diagrammed according to Bjorkman and co-workers and Brown and co-workers (*27*), in contact with a T cell receptor and a staphylococcal enterotoxin or Mls product. Ag, the probable site of binding of a conventional antigenic peptide.

Mice Express Endogenous Equivalents of the Toxins

Some years ago Festenstein discovered an unexpected phenomenon in mice (*46*). T cells from some mice responded well to spleen cells from some other animals, even though both responder and stimulator were identical at the MHC. It had been known that T cells respond powerfully to MHC incompatible stimulator cells, but good stimulation in response to other antigens that are different between two mice does not usually occur. The antigens discovered by Festenstein were therefore given a special name, minor lymphocyte stimulating antigens (Mls), to distinguish them from other non-MHC differences between one mouse and another. At the time the phenomenon was discovered, it was thought that the Mls antigens mapped to a single genetic locus, on mouse chromosome 1, but it is now known that there are many Mls-like products produced by mice, controlled by nonlinked loci. Not all of these products differ from one strain of mouse to another. For example, Mls-1 has two alleles, a and b. Mls-1^a is expressed by mice such as CBA/J and stimulates T cells from MHC identical, Mls-1^b animals such as CBA/Ca. In contrast, another Mls-like molecule, which is produced by B cells and binds to I-E class II molecules, is produced by all strains of mice so far examined (*49*).

The last few years have witnessed a revolution in our understand-

ing of the phenomena of Mls. It is now apparent that these products stimulate T cells bearing particular V_βs. Mls-1^a, the product discovered by Festenstein, for example, in combination with mouse class II molecules, stimulates nearly all T cells bearing mouse V_β6, 7, 8.1, and 9 (*47, 50–52*). A list of the Mls-like products known so far, and the V_βs they engage, is shown in Table 3 (*47, 50–55*). Mls products have not yet been found in humans, although they may exist. Many properties of the Mls products are similar to those of the bacterial toxins discussed above. They stimulate T cells bearing particular V_βs almost regardless of the rest of the structure of the receptor on the T cell. This activity of Mls depends upon simultaneous expression by the presenting cell of class II proteins. Some class II products, most notably I-E molecules, present Mls products or bacterial toxins better than others (*34*). Many of the V_βs involved in recognition of Mls products are the same as those involved in presentation of the bacterial toxins. V_β3, for example, is a frequent participant. Finally, Mls appears to engage V_β at about the same site, on an exposed face of the polypeptide, as toxins (*48*).

The similarities between the properties of the bacterial toxins and mouse Mls products might lead one to suggest a structural similarity. Nothing is known about the structure of the Mls molecules. Some circumstantial evidence, however, suggests that they are not endogenous mouse analogs of the bacterial toxins. Were they to be class II–binding, 220 amino acid proteins they would probably have been detected in the many published analyses of class II structure. In addition, antibodies to them would probably have been produced. Antibodies can readily be made against the bacterial toxins. Alternate suggestions for the structures of Mls products have therefore been made. For example, they may be CD4-like accessory molecules (*56*) or may be a more fundamental membrane component than a protein, a lipid for example (*57*). None of these suggestions can be dismissed, and so far the only data that indicate a structure are the findings that the Mls products associate with class II and stimulate T cells via V_β, that is, they act as superantigens, and Mls products can be transferred from one cell to another.

Whatever the structure of the Mls products may be, there are consequences for the mouse that expresses them. They cause the deletion, in the thymus, of all prospective T cells bearing V_βs with which they can interact. Mice expressing Mls-1^a, for example, contain very few T cells bearing V_β6, 7, 8.1, or 9 (*47, 50–52*), and hence are deprived of about 20% of their total potential T cell repertoire. In spite of this, mice containing numerous Mls-like products do not seem to be particularly susceptible to disease. In fact, an examination of Mls expression in wild mice showed that such products were rampant in animals trapped in Florida. A wild mouse could hardly be identified that had not deleted some part of its T cell repertoire because of the expression of such products (*58*).

Why Are Bacterial Toxins Pathogenic?

The staphylococcal toxins were identified because they cause human diseases such as food poisoning, shock, and scalded skin syndrome. In mice the toxins also produce rapid weight loss and even death. What property of the toxins causes such symptoms, and is it related to the T cell stimulating activities of the proteins? Some members of this group of toxins are not very like the others in amino acid sequence, and yet all can bind MHC class II molecules and stimulate T cells, and all can cause disease. It is therefore likely that these properties are related to one another. The toxins could cause disease through class II binding or T cell stimulation in several ways. Engagement of class II molecules by the toxins, on macrophages or mast cells, could stimulate these cells and cause release of soluble mediators beneficial to the host in small quantities, but damaging in large. Indeed, production of macrophage-derived mediators such as interleukin-1 and tumor necrosis factor has been demonstrated after toxin stimulation of human cells (*59*). Both of these lymphokines are known to be pathogenic at high levels, causing fever, weight loss, and osmotic imbalances that can lead to death.

Alternatively, these toxins could stimulate a high proportion of T cells in mouse or man to divide and produce lymphokines. Staphylococcal enterotoxin B, for example, by engaging mouse T cells bearing V_β3, 7, 8.1, 8.2, and 8.3, stimulates 30 to 40% of all T cells in some mice. Toxic SST1, which engages human T cells bearing V_β2, stimulates about 10% of all $\alpha\beta^+$ T cells in humans. This is a very high proportion of cells, much higher than would be engaged by any conventional antigen such as a flu virus, for example. It is possible, therefore, that the symptoms of these toxins are caused by massive T cell stimulation and consequent release of T cell–derived lymphokines such as interleukin-2 or tumor necrosis factor (*60*).

This second explanation seems to account for the toxicity of these materials in mice. Mice which lack T cells, but contain functional, class II–bearing macrophages, are not affected by SEB, unlike their littermates that have T cells. Recently we constructed a collection of mice that contained normal numbers of T cells, but because of V_β gene deletions and endogenous Mls expression, lacked T cells bearing the V_βs with which SEB interacts. These animals were likewise unaffected by injection of doses of SEB that would cause considerable weight loss or even death in other mice (*51*). It is more difficult to determine how the toxins cause disease in man. The ability of these proteins, especially TSST1, to cause toxic shock is most likely related to their ability to stimulate T cells. During an episode of toxic shock caused by TSST1, the percentage of peripheral T cells in many human patients bearing the V_β with which this toxin interacts, V_β2, rises from about 9% in normal individuals to as much as 50% (*61*). Also, the symptoms of toxic shock are similar to

Table 2. V_β specificities of the staphylococcal enterotoxin-like toxins. Data are from (*32, 34, 40, 43–45*). V_β evaluation for man is incomplete because all human V_βs have probably not yet been described and the V_β specificity of some of the toxins in man has only been evaluated with the limited number of available antibodies to human V_βs and not with quantitative polymerase chain reactions (*45*).

Toxin	V_β specificity	
	Human	Mouse
SEA	?	1, 3, 10, 11, 17
SEE	5.1, 6.1–3, 8, 18	11, 15, 17
SED	5, 12, ?	3, 7, 8.1–3, 11, 17
SEB	3, 12, 14, 15, 17, 20	3, 7, 8.1–3, 17
SEC1	12, ?	3, 8.2, 8.3, 11, 17
SEC2	12, 13.1, 13.2, 14, 15, 17, 20	3, 8.2, 10, 17
SEC3	5, 12, ?	3, 7, 8.1, 8.2
TSST1	2	3, 15, 17
ExFT	2	3, 10, 11, 15, 17
MAM	?	6, 8.1, 8.2, 8.3

Table 3. Mls-like products identified in mouse.

Locus	V_β specificity	MHC association	Reference
Mls-1^a	6, 7, 8.1, 9	Class II (except q)	(*47, 50, 51*)
Mls-2^a	3	Class II (except q)	(*52*)
Mls-3^a	3	Class II (except q)	(*52*)
?	5	I-E	(*53*)
?	7	I-E	(*54*)
?	11	I-E	(*53*)
?	17	I-E	(*55*)

those observed in patients who for other reasons had massive T cell stimulation, such as those treated with antibodies to CD3. Interleukin-2 and tumor necrosis factor, two of the expected products of stimulated T cells, cause shock-like symptoms when given to patients in high concentrations (*62*).

The symptoms induced by other toxins in this group, particularly those associated with food poisoning, are less easily explained. Perhaps toxin binding to class II on mast cells or other cells lining the gut causes the release of mediators that concentrate locally and induce diarrhea and vomiting. Alternatively, perhaps the toxins stimulate T cells located in the gut, and it is the local production of lymphokines by these cells that causes the symptoms. Alternatively, and less likely, these toxins may have another group of receptors in addition to that of class II and T cell V_βs that mediate food poisoning in humans.

Some of these toxins may cause autoimmune diseases, either in humans or in experimental animals. Streptococcal infections in some individuals may lead to many different autoimmune manifestations including rheumatic fever and rash. The toxin produced by *Mycoplasma arthriditis* causes arthritis in rats and mice. Again it is possible that these sequelae are related wholly or in part to the ability of streptococcal or mycobacterial toxins to stimulate T cells. For example, some individuals may contain a few potentially autoreactive cells that have escaped the processes of tolerance. Normally such cells may be at concentrations too low to permit reactivity and autoattack. The bacterial toxins may by chance stimulate such cells through V_β and thereby raise the numbers or activity of such cells above a threshold level; the cells are then at concentrations high enough to damage the host.

Bacterial-Host Relations

Clearly the bacteria must derive some advantage from the production of toxins of this type. Several different organisms produce proteins with the properties described in this article, although the proteins are not closely related to each other in sequence. These toxins are immunosuppressive (*51, 63*), causing reductions in both T and B cell responses; perhaps the advantage to the bacteria is that the toxins reduce host resistance to invasion. Alternatively the toxins may induce local inflammation, in the gut or elsewhere, thereby increasing blood and nutrient supply and benefiting the bacterium. The same toxins cause disease, bind class II, and stimulate T cells through V_β in mammals as diverse as humans and mice. The last two properties are maintained even though the class II molecules are not very similar in amino acid sequence between the two species, and V_βs are in some cases even less alike. For example, as mentioned above, TSST1 stimulates human T cells bearing $V_\beta 2$. The major V_β target for this toxin in mice is $V_\beta 15$. $V_\beta 15$ is the mouse V_β that is closest in sequence to human $V_\beta 2$; nevertheless, the two regions are only 45% identical. In spite of this, over the course of 70 million years on divergent evolution, these two V_βs have retained their ability to engage the complex of TSST1 and class II.

Why have the mammals been unable to mutate either class II so that it does not bind these toxins or alter their V_βs so that they do not bind the toxin–class II complexes? Retention of binding to class II may be related to the sequence homology between some of the toxins and invariant chain. If transport to the cell surface in combination with invariant chain is essential to the proper function of class II, then the binding site for toxins on class II may have been retained evolutionarily because of a requirement to bind invariant chain.

It is more difficult to understand why T cell receptor V_βs have retained the ability to bind complexes of toxin and class II. One possibility is that, as for class II binding, the toxins are capitalizing on some essential structural feature of the V_βs, a feature that cannot be altered without loss of function. In support of this hypothesis we have recently noticed that an important phase in T cell development, positive selection, is controlled in part by interaction with V_β (*64*). The appearance of functional T cells in animals is dependent upon the still mysterious process of positive selection; unless the cell successfully undergoes this test it will die in the thymus and never emerge to be useful to its host in the periphery. If positive selection sometimes, or perhaps always, involves interaction of an endogenous toxin–like product with V_β on the developing thymocyte then it may be that loss of function leads to loss of ability to be positively selected.

In spite of these observations, at least one mammalian species, that of *Mus musculus domesticus*, has in fact taken steps that eliminate at least some toxin-reactive T cells. Two surveys of wild mice have shown that deletions of V_β genes are common in the genomes of wild mice (*58, 65*). We, for example, have recently surveyed about 40 wild mice trapped in Florida. Of these about one-third were homozygous for a large deletion at V_β, a deletion that removed many of the V_βs known to be reactive to staphylococcal toxins. Mls-like elements, moreover, caused the elimination in many of these mice of additional V_βs. We do not know for certain what pressures select in mice for large gaps in their T cell repertoires. Since the pressures have caused variations in V_β repertoire, either by gene deletion or T cell elimination, it is likely that they themselves act on V_β. Thus it is possible that production of toxins like those made by *Staphylococcus aureus* has caused wild mice to limit V_β expression in different mice, by any means at their disposal.

In summary, studies of the staphylococcal enterotoxins and related proteins have provided scientists with rich and unexpected vision of the complex relationships between bacteria and their hosts, and have also yielded some insight into what might have been expected to be a totally unrelated subject, namely the T cell repertoire.

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