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REVIEW

Bacterial superantigens

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INTRODUCTION

Superantigens (SAgs) are the most powerful T cell mitogens ever discovered. Concentrations of less than 0·1 pg/ml of a bacterial superantigen are sufficient to stimulate the T lymphocytes in an uncontrolled manner resulting in fever, shock and death [1–3]. SAgs bind, as intact molecules to the class II major histocompatibility complex (MHC) antigens expressed on professional antigen presenting cells (APCs) outside the peptide-binding groove then sequentially bind the T cell receptor (TcR) via the variable region of the TcR β-chain [3–6]. Every SAg binds a subset of TcR Vβ domains and as the number of different Vβ regions in the human T cell repertoire is restricted to approximately 50, comprising about 24 major types of Vβ elements, a substantial number of T cells are activated by SAgs. This can be as high as 20% compared with only 1 in 10^5–10^6 naive T cells that are responsive to conventional peptide antigen. This results in massive systemic release of pro-inflammatory cytokines, such as tumour necrosis factor-alpha (TNF-α) and interleukin-beta (IL-1β), and T cell mediators, such as IL-2, which can lead to fever and shock [3,5,7,8].

Over the last 4 years the number of known bacterial SAgs has increased sharply, due mainly to various microbial genome sequencing projects [9–14]. There are now 41 bacterial SAgs described in the literature (Table 1) and the number is growing steadily. In addition, a new family of SAg-related proteins has been identified in *Staphylococcus aureus* that show sequence and structural homology to the 'classical' SAgs, but appear to have a quite different role. This review provides a summary of the field to date with some of the more recent discoveries that shed light on the how superantigens are able to trigger such strong T cell responses and the diseases related to SAg intoxication.

MECHANISM OF ACTION OF SAgs

SAgs are characterized by their ability to bind both MHC class II molecules and T cell receptors [5,6]. This occurs in a sequential fashion. The sole purpose of SAgs appears to be to bring these two critical molecules together in order to activate as many T cells as possible. The net result is the release of a large and sudden bolus of cytokines which causes the acute condition toxic shock [3,5,7,8] The histocompatibility class II molecule, despite its polymorphism, is the principal cell receptor for all SAgs but the affinity for MHC class II varies depending on the class II molecule and the SAg [15–17]. All SAgs examined so far display higher affinities towards human MHC class II molecules than mouse class II, which explains partly why SAgs are several orders of magnitude more potent on human T cells than mouse T cells. A variety of binding modes exist to both MHC class II and TcR (described in more detail below) which indicates the lengths that the bacteria have gone to target these two critical molecules of the adaptive immune response.

THE BACTERIAL SUPERANTIGENS

The prototype SAgs from S. aureus *and* Streptococcus pyogenes The first bacterial SAg was isolated in the late 1960s by Bergdoll and coworkers as a secreted toxin of *S. aureus* and was named staphylococcal enterotoxins A (SEA) for its potent enterotoxic properties. The staphylococcal enterotoxins (SE) are the causative agent in staphylococcal food poisoning and induce vomiting and diarrhoea within 1–2 h following ingestion. The mitogenic activity of SEs was discovered many years later, but the term 'superantigen' was not coined until 1989 when Marrack and coworkers found that the mitogenic activity was a result of a massive expansion of T cells that all shared the same T cell receptor Vβ chain domains [2]. Today, 18 different SEs have been described in the literature (Table 1) and all are potent T cell mitogens with half maximum stimulation values as low as 0·1 pg/ml. The SAg family also includes the *S. aureus* toxic shock syndrome toxin (TSST) which is the causative agent in toxic shock syndrome [18].

Twelve SAgs have been identified in Group A Streptococci (GAS), predominantly but not exclusively produced by *S. pyogenes*. These are the streptococcal pyrogenic exotoxins (SPEs) A, C, G-M, the streptococcal superantigen (SSA) and the streptococcal mitogenic exotoxin (SMEZ) 1 and 2. Many new SAgs have been identified by screening the completed *S. pyogenes* genomes; serotypes M1 (Oklahoma University, USA), M3 and M18 (Rocky Mountain Laboratories, NIH, USA) with conserved sequence motifs [10–13]. The sudden explosion of new superantigen

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Table 1. Functional properties of superantigens and their associated diseases

SAg	MW (kDa)	Organism	Crystal structure	Zinc binding	MHC II binding α/β chain	Human TcR Vβ specificity	P_{50} (h) (pg/ml)	Disease
SEA	27·1	*S. aureus*	+	+	+/+	1·1, 5·3, 6·3, 6·4, 6·9, 7·3, 7·4, 9·1, 23·1	0·1	FP
SEB	28·4	*S. aureus*	+	–	+/–	1·1, 3·2, 6·4, 15·1	0·8	FP
SEC1	27·5	*S. aureus*	–	–	+/–	3·2, 6·4, 6·9, 12, 15·1	0·2	FP
SEC2	27·6	*S. aureus*	+	–	+/–	12, 13, 14, 15, 17, 20	0·2	FP
SEC3	27·6	*S. aureus*	+	–	+/–	5·1, 12	0·2	FP
SED	26·9	*S. aureus*	+	+	+/+	1·1, 5·3, 6·9, 7·4, 8·1, 12·1		FP
SEE	26·8	*S. aureus*	–	+	+/+	5·1, 6·3, 6·4, 6·9, 8·1	0·2	FP
SEG	27·0	*S. aureus*	–	?	?	3, 12, 13·1, 13·2, 14, 15		FP
SEH	25·2	*S. aureus*	+	+	–/+	?		TSS
SEI	24·9	*S. aureus*	–	?	?	1·1, 5·1, 5·3, 23		FP
SEJ	28·5	*S. aureus*	–	?	?	?		?
SEK	25·3	*S. aureus*	–	?	?	5·1, 5·2, 6·7		?
SEL	24·7	*S. aureus*	–	?	?	?		?
SEM	24·8	*S. aureus*	–	?	?	?		?
SEN	26·1	*S. aureus*	–	?	?	?		?
SEO	26·7	*S. aureus*	–	?	?	?		?
SEP	26·4	*S. aureus*	–	?	?	?		?
SEQ	26·0	*S. aureus*	–	?	?	2·1, 5·1, 21·3		?
TSST	21·9	*S. aureus*	+	–	+/–	2·1	0·2	TSS
SPE-A	26·0	*S. pyogenes*	+	–	+/–	2·1, 12·2, 14·1, 15·1		SF
SPE-C	24·4	*S. pyogenes*	+	+	–/+	2·1, 3·2, 12·5, 15·1	0·1	STSS, KD?
SPE-G	24·6	*S. pyogenes*	–	+	?/+	2·1, 4·1, 6·9, 9·1, 12·3	2	?
SPE-H	23·6	*S. pyogenes*	+	+	–/+	2·1, 7·3, 9·1, 23·1	50	?
SPE-I	26·0	*S. pyogenes*	–	+	?/+	6·9, 9·1, 18·1, 22	0·1	?
SPE-J	24·6	*S. pyogenes*	–	+	–/+	2·1	0·1	?
SPE-L/K	27·4	*S. pyogenes*	–	+	?/+	1·1, 5·1, 23·1	1	ARF?
SPE-M	26·2	*S. pyogenes*	–	+	?/+	1·1, 5·1, 23·1	10	ARF?
SPE-M*	25·3	*S. pyogenes*	–	+	?	1·1, 5·1, 23·1		ARF?
SSA	26·9	*S. pyogenes*	–	–	?	1·1, 3, 15		?
SMEZ1	24·3	*S. pyogenes*	–	+	?/+	2·1, 4·1, 7·3, 8·1	0·08	STSS
SMEZ2	24·1	*S. pyogenes*	+	+	?/+	4·1, 8·1	0·02	STSS
SePE-H	23·6	*S. equi*	–	+	?	?		ES?
SePE-I	25·7	*S. equi*	–	+	?	?		ES?
SePE-L	27·4	*S. equi*	–	+	?	?		ES?
SePE-M	26·2	*S. equi*	–	+	?	?		ES?
SPE-A7	25·9	*S. dysgalactiae*	–	?	?	?		?
SPE-G^{dys}	24·4	*S. dysgalactiae*	–	?	?	?		?
SDM	25·0	*S. dysgalactiae*	–	?	?	1·1, 23		?
YPM-A	14·5	*Y. pseudotuberculosis*	–	?	?	3, 9, 13·1, 13·2		KD?
YPM-B	14·6	*Y. pseudotuberculosis*	–	?	?	3, 9, 13·1, 13·2		KD?
MAM	25·2	*M. arthritidis*	–	+	?	6, 8		Arthritis?
K18	?	HERV-K	–	?	?	7,13·1		IDDM?

sequences has resulted in confusing nomenclature where some sequences have been given two different names. For example, SPE-K identified in a serotype M3 strain from the United States is identical to SPE-L found in an M3 strain from Japan and an M89 strain from New Zealand [10,19,20]. The superantigen gene *spe-l* found in an M18 strain from the United States is identical to a gene named *spe-m* found in New Zealand [13,20]. For the purposes of this review, *spe-m** is the gene described in the United States as *spe-m*.

The mitogenic potency varies between both the streptococcal SAgs and the staphylococcal SAgs. The least potent of all superantigens so far examined is SPE-H, which produces a 50% maximal response (P_{50}) in human PBL of 50 pg/ml, while SMEZ-2 is the most potent SAg known thus far with a P_{50} of 0·08 pg/ml. This is equivalent to 8×10^{-14} gm/ml or 21 000 molecules/ml [12].

By structural comparison, the staphylococcal and streptococcal superantigens build a large protein family (Fig. 1), indicating that they have all evolved from a single primordial superantigen. Primary amino acid sequence homologies vary greatly from as low as 15·5% sequence identity, e.g. between SEB and SEK to over 90% (SEA *versus* SEE). Nevertheless, all SAgs possess a characteristic PROSITE amino acid sequence signature K-X(2)-[LIVF]-X(4)-[LIVF]-D-X(3)-R-X(2)-L-X(5)-[LIV]-Y (PS00278). So far, 11 superantigens have been crystallised and all show remarkable similarities in their overall structure despite very different primary amino acid sequences (see below).

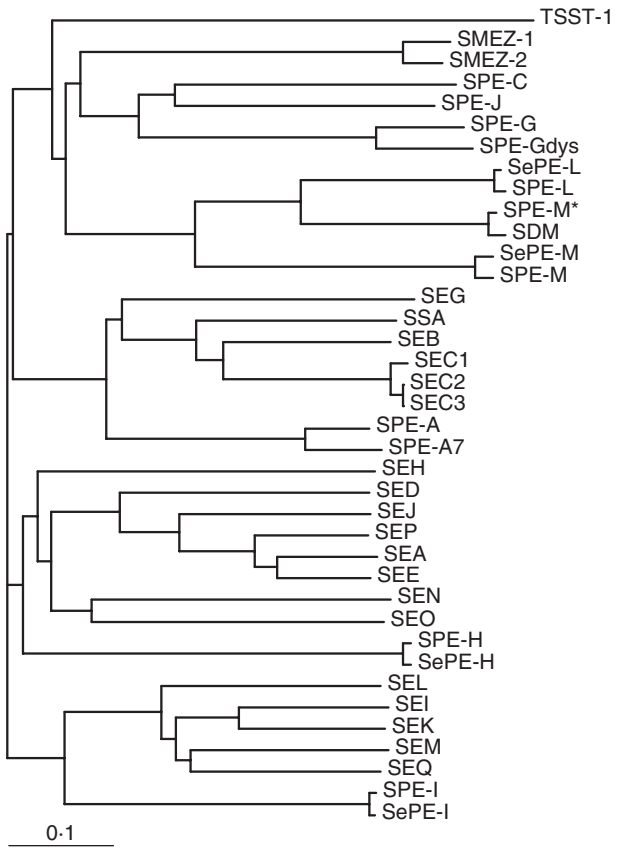

Fig. 1. An alignment of all streptococcal and staphylococcal superantigens based on amino acid sequence. Note that TSST-1 is a clear outlier of this group and that several clusters are formed based loosely on whether they are streptococcal or staphylococcal superantigens.

The streptococcal SAgs SPE-A, SPE-H, SPE-I and SSA are related more closely to the staphylococcal SAgs than to any other streptococcal SAg (Fig. 1) and the genes for these toxins are all located on mobile elements, so it is likely that this SAg subgroup in *S. pyogenes* arose through the horizontal transfer from *S. aureus* rather than evolving from existing streptococcal superantigen genes.

For many years the streptococcal proteins SPE-B and SPE-F were considered to be SAgs but have since been shown to be due to contamination from the potent SAg SMEZ-2. SPE-B is a cysteine protease and SPE-F (also known as mitogenic factor or MF) is in fact streptococcal DNase.

The non-GAS superantigens

SAgs have also been found in two different group C streptococci. The *Streptococcus equi* pyrogenic exotoxins (SePE) H, I, L and M are homologous to their *S. pyogenes* counterparts SPE-H, I, L and M (>98% sequence identity) indicating another horizontal transfer from *S. pyogenes* to *S. equi* or vice versa [20,21]. Another two SAgs have been identified from *S. dysgalactiae* called SDM [22] and SPE-G^{dys} [23]. SDM is most similar to SPE-M* and SPE-G^{dys} is most similar to SPE-G. Amino acid exchanges are outside the MHC class II and TcR binding sites suggesting that the GAS toxins and the non-GAS toxins are orthologues with identical functions. SPE-I and SPE-H are both located on the defective prophage SF370·2, SPE-L is located on the active prophage ΦNIH1·1 and SPE-M* was found on $\Phi_{speL/speM}$ [11,13,19]. This suggests that horizontal gene transfer between GAS and non-GAS occurred more recently than between GAS and *S. aureus*.

SAgs in other bacteria

SAgs have also been isolated from the Gram-negative bacteria *Yersinia pseudotuberculosis* and *Mycoplasma arthritidis*. The *Y. pseudotuberculosis* mitogens (YPM) A and B are 21 kDa proteins, which both target human TcR Vβ3, 9, 13·1 and 13·2 regions [24]. The *M. arthritidis* mitogen (MAM) is a 25-kDa protein that targets T cells bearing the Vβ6 and Vβ8 TcR [25]. These Vβ profiles differ from any profile of the 'classical' SAgs (Table 1). MAM and YPM-A/B are unrelated by amino acid sequence to the 'classical' SAgs and also lack the SAg family signature sequence. The protein structures of these SAgs have yet to be solved, so their mode of action remains a mystery. However, functional studies have shown that MAM binds preferentially to murine I-E or its human equivalent HLA-DR. The DR4, DR7 and DR12 subtypes present MAM most efficiently [26]. A study published in 1998 on the interaction between MAM and TcR indicated that MAM might contact not only the germ-line encoded TcRVβ region, but also the hypervariable CDR3 region [27]. This has also been shown to be the case for SPE-C (see below).

THE MHC CLASS II BINDING SITE

The crystal structure of seven staphylococcal SAgs (SEA, SEB, SEC2, SEC3, SED, SEH and TSST) and four streptococcal SAgs (SPE-A, SPE-C, SPE-H and SMEZ-2) have been solved and revealed a common core-fold based on two globular domains: a smaller N-terminal pseudo β-barrel domain, which is most similar to the classical oligosaccharide/oligonucleotide-binding fold (OB-fold) found in many bacterial proteins that bind oligomeric molecules and a larger C-terminal β-grasp domain. The two domains are separated by a long solvent-accessible α-helix that extends down the centre of the molecule [28–35].

Several co-crystal structures of SAgs bound to MHC-II have also been solved (Fig. 2). The first was the crystal structure of SEB bound to HLA-DR1 followed closely by a structure of TSST-HLA-DR1 [36,37]. Both SEB and TSST possess an exposed hydrophobic loop region in the smaller N-terminal domain to bind to a hydrophobic groove located in the distal region of the invariant α1 domain of HLA-DR. However, the structures are not identical. SEB binds out to the side of MHC-II away from the peptide binding groove while TSST sits over the top of the groove and makes considerable contacts with peptide residues. As a consequence, TSST prevents any contact between MHC-II and the TcR while SEB relies on continued contacts between MHC and TcR to strengthen the interaction.

The affinity of SAgs towards MHC class II varies considerably. SAgs that utilize the generic HLA-DR α-chain (such as SEB and TSST) bind with relatively low affinity (K_D~10^{-5} M). Other SAgs, such as SEA and SEE, have in addition to the generic low affinity α-chain binding site a high-affinity zinc-mediated binding site (K_D~10^{-7} M) for the polymorphic HLA-DR β-chain. The zinc cation forms a tetrameric coordination complex with three residues from the C-terminal SAg domain and with a conserved histidine residue (H81) from the HLA-DR β-chain. This group of SAgs can bind to both sites of the molecule, cross-linking MHC-

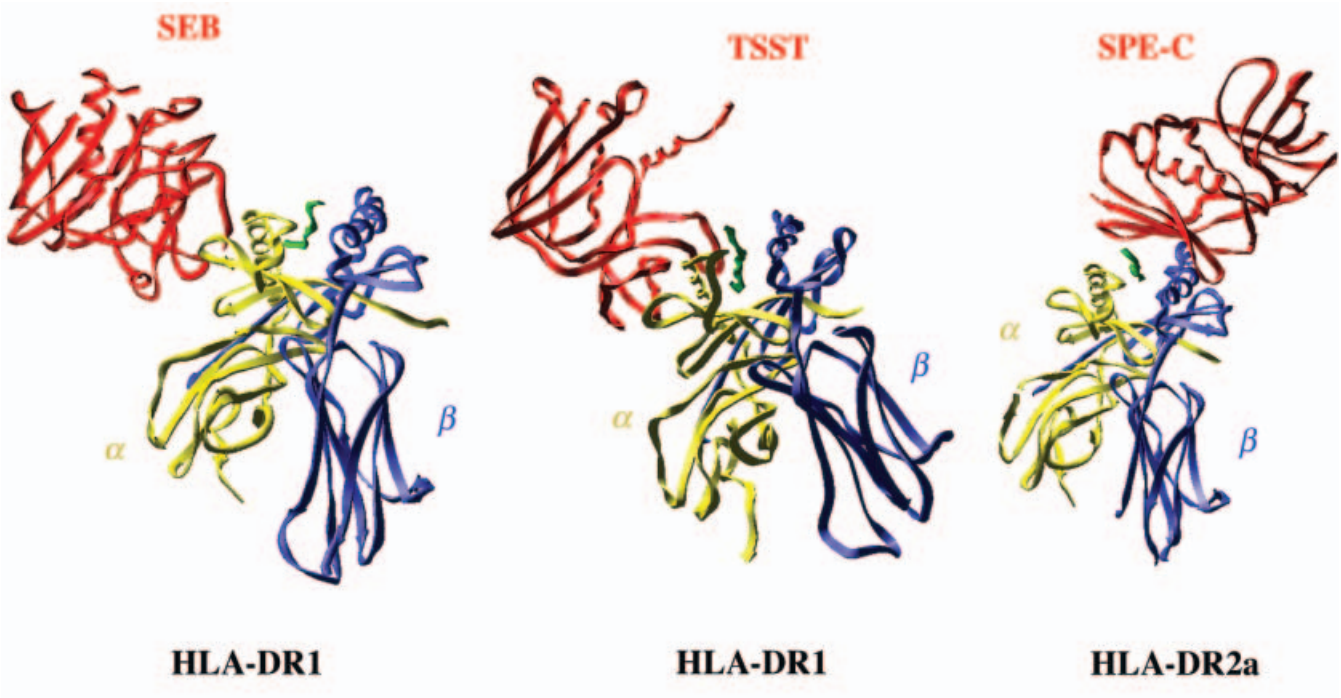

Fig. 2. A comparison of the co-crystal structures of three superantigens bound to MHC class II. The first is SEB which binds to the invariant α-chain of HLA-DR out to one side [36]. The TcR interacts with both SEB and MHC class II residues. The second shows TSST-1 bound to the same chain but is positioned further over the peptide groove and interacts with peptides [37]. The third structure is SPE-C bound to the polymorphic β-chain of HLA-DR2 via a zinc atom. It sits clearly over the top of the peptide groove and prevents any interaction between the TcR and the MHC molecules [42,43].

II molecules on the surface of APC and resulting in increased secretion of IL-1 and TNF-α [34,38–41]

Yet another subgroup of the bacterial SAgs, such as SEH, SPE-C, SPE-H and SMEZ, lack the generic low-affinity α-chain site and only bind the polymorphic HLA-DR β-chain. The recently solved crystal structures of SPE-C bound to HLA-DR2a and of SEH:HLA-DR1 show that the C-terminal domain of the SAgs contacts the α-helix of the MHC-II β1-domain, as well as the N-terminal part of the bound peptide [42,43]. Peptide-restricted binding to MHC-II has also been shown in biochemical studies for other SAgs and raises the possibility that certain bound peptides could enhance the potency of the SAg by promoting high-affinity binding [44,45].

Yet another mechanism of MHC-II binding is seen with SED and SPE-C. Both SAgs are capable of forming zinc-mediated homodimers that can cross-link MHC molecules on the cell surface of APCs [32,46]. The SPE-C crystal structure revealed that the dimer structure is formed through an interface where the low-affinity N-terminal binding-site is normally located [32].

THE TCR BINDING SITE

Less is known about the way in which SAgs bind TcR. In 1996, Mariuzza and colleagues succeeded in crystallizing the SAg SEC3 with a soluble form of the murine Vβ8·1 TcR β-chain [47]. In this complex, SEC3 makes multiple contacts with residues from the complementarity determinining region 2 (CDR2), the third framework region (FR3) and the 4th hypervariable loop (HV4) of the TcR β-chain. Furthermore, all the hydrogen bonds involve only main-chain atoms of the TcR β-chain, so that side-chain amino acid variation is less likely to affect the affinity of binding. In an attempt to correlate binding affinity to the potency to activate T cells, Leder *et al*. performed mutational analysis of the TcR binding site in SEC3. Most interestingly, their results showed a proportional relationship between binding affinity and potency, which is in sharp contrast to conventional peptides recognition, where even the smallest reduction in affinity results in complete loss of T cell stimulation [48]. In 1998, the crystal structure of SEB bound to murine Vβ8·1 TcR was solved and showed little difference from the SEC3-Vβ8·1 TcR complex [49]. However, in 2002 two additional complexes were determined – that of SPE-A bound to the same Vβ8·1 TcR and the streptococcal superantigen SPE-C bound to human Vβ2·1. These showed striking differences in SAg/TcR interaction. The SPE-A binds Vβ8·1 complex via many hydrogen bond-mediated contacts between the side-chain atoms of both molecules, suggesting that Vβ sequence specificity is required to restrict SPE-A reactivity, whereas SEC3/TcR interactions solely depend on conformation. The SPE-C/hVβ2·1 complex revealed a completely different binding mode involving not only residues in the CDR2, but also the CDR1 and the hypervariable CDR3 regions, including numerous specific electrostatic interactions between side-chain atoms of SPE-C and hVβ2·1 [50].

One question that has yet to be answered adequately is why there so many different binding modes for molecules intent on doing the same thing – that is bringing MHC and TcR together. One possible answer is that the different modes of MHC-II and TcR binding are a result of different immunological responses that might be related to different TcR β-chain subsets of T cells. This functional targeting of SAgs might also explain why a single bacterium often secretes more than one SAg.

SETS – NOVEL STAPHYLOCOCCAL VIRULENCE FACTORS IN SUPERANTIGEN DISGUISE?

In 2000, a novel gene cluster in the newly completed staphylococcal genome was identified that harboured five related genes with the characteristic PROSITE SAg family signature (PS00278). The corresponding gene products were named staphylococcal enterotoxin-like toxins (SET) 1–5 [51]. The SETs most closely resemble the TSST amino acid sequence, which prior to their discovery, had occupied a single isolated branch of the SAg family tree (Fig. 1). The addition of the SETs to the SAg family tree generated a new clade that now includes TSST. The SETs are all secreted from *S. aureus* and most individuals produce strong anti-SET antibody titres [52]. One SET (SET3) has been crystallized and the structure determined revealing a typical SAg fold, with the N-terminal pseudo β-barrel domain and the C-terminal β-grasp motif, separated by a long solvent-accessible α-helix [52]. A structural comparison of SET3 to TSST is provided in Fig. 3.

However, so far none of the SETs have exhibited any of the functional hallmarks of all SAgs, such as MHC class II binding or T cell stimulation. Thus, while they are structurally related, they appear to have very different functions.

The determination of the complete genome sequence of *S. aureus* strains Mu50, MW2 and N315 revealed additional *set* genes, all clustered on pathogenicity islands. *Set* 6–15 were found within SaPln2 on strain N315 and within the homologous SaPlm2 on strain Mu50 [14], while *set* 16–26 were found within vSaα on strain MW2 [9]. The function of SETs is still unknown, but their location within pathogenicity islands suggests a role as virulence factors and it is most likely they will have some role in defence against host immune functions of both the adaptive and innate immune responses.

VIRAL SUPERANTIGENS

Mouse mammary tumour virus (MMTV)

MMTV, a milk-transmitted B-type retrovirus, causes murine mammary carcinomas. The MMTV SAgs were discovered first by Felstenstein in 1974 and were referred to as minor lymphocyte stimulating (Mls) antigens. The T cell response to Mls antigens is similar to the response to bacterial SAgs with expansion of unique TcR Vβ subsets [53]. The SAg gene was identified later within the 3′ long-terminal repeat (LTR) of the MMTV genome and did not show any homology to the bacterial SAg genes [54]. The gene product is a 45-kDa type II transmembrane protein with a 10–14 amino acid polymorphic region at the C-terminus, which is responsible for the TcR Vβ specificity. Infectious MMTV is present in mammary tissue and breast milk of only a few mouse strains. The SAg molecule is an essential component of the virus life cycle, providing efficient viral replication in newly infected gut B cells by recruiting Vβ mediated T cell 'help' and promoting B cell proliferation. The endogenous SAg is inherited in Mendelian fashion and causes T cell deletion as a result of self-tolerance induction in the thymus. As a result, the transmission of an infectious virus carrying the identical SAg will be hampered by the lack of responder T cells, thereby protecting the mouse from MMTV infection.

Endogenous SAgs in humans

For many years, MMTV was the only virus that was known for certain to express a SAg. In 1996, Sutkowski *et al*. observed that Epstein–Barr virus (EBV) infected human B cell lines induced into the lytic cycle with a B cell mitogen that selectively stimulate Vβ13 bearing T cells suggesting the existence of a EBV encoded SAg [55]. More recently, the same group showed that the previously described EBV-related SAg activity is in fact encoded by alleles of the human endogenous retrovirus (HERV)-K18 env gene, which is transcriptionally activated by EBV [56]. HERV-K18 is located on chromosome 1 within the first intron of CD48, which possesses an upstream EBV-inducible enhancer. Furthermore, expression of HERV-K18 is strongly induced by IFN-α [57] Three alleles of HERV-K18 env were identified (K18·1–3) and all of them had mitogenic activity towards Vβ7 and Vβ13·1 T cells. HERV-K18·1 is identical to the previously identified insulin-dependent diabetes mellitus associated retrovirus IDDMK$_{1,2}$22 [58]. The authors propose that endogenous SAgs might facilitate the transmission of the EBV virus, similar to MMTV in mice and could contribute to viral pathogenesis, e.g. the extensive T cell infiltrates in EBV-associated tumours.

SUPERANTIGENS IN HUMAN DISEASE

Food poisoning

The staphylococcal superantigens SEA-SEE and SEG-SEI are potent gastrointestinal toxins responsible for staphylococcal food

Fig. 3. A structural comparison between the superantigen TSST-1 and the non-superantigenic secreted toxin SET3 [52]. SET3 shares a two-domain structure with TSST-1 with a larger C-terminal domain of the β-grasp motif and smaller N-terminal β-barrel domain. Two main α-helices are both shared and the larger α-helix contains the highly conserved superantigen PROSITE motif K-X(2)-[LIVF]-X(4)-[LIVF]-D-X(3)-R-X(2)-L-X(5)-[LIV]-Y (PS00278).

poisoning. Quantities of less than 1 μg of toxin are sufficient to trigger vomiting in humans. This enterotoxin function appears to be distinct from the SAg activity but this remains controversial. A highly flexible disulphide-loop within the N-terminal domain has been implicated with the emetic properties, but the exact mechanism that leads to the disease or a specific receptor molecule have not yet been identified [59].

Toxic shock syndrome (TSS)

Classical toxic shock syndrome (TSS) caused by *S. aureus* can be considered as a capillary leak syndrome and includes symptoms, such as hypotension, rash, desquamation, fever and major organ involvement [60]. Like endotoxic shock, TSS is mediated through TNF-α. TSST is regarded as the primary causative agent for menstrual TSS, which is associated with the use of certain tampons, particularly those of high absorbency that promotes the growth of *S. aureus*. In contrast to other staphylococcal SAgs, TSST has the ability to cross the mucosa. TSST and other staphylococcal SAgs have been associated with non-menstrual TSS, which can occur in any patient population [1,61]. This is supported by the observation that these toxins induce TSS-like symptoms in animal models in the rabbit and in rodents [18].

Streptococcal toxic shock syndrome (STSS)

STSS, caused by *S. pyogenes*, is the most severe form of invasive streptococcal disease, with mortality rates of up to 50%. The clinical symptoms are very similar to those in TSS, but STSS is often associated with bacteraemia, myositis or necrotizing fasciitis [62,63]. Streptococcal SAgs have been implicated in STSS and supporting evidence includes the following. The *spe-a* and *spe-c* genes were found at higher frequencies in isolates from STSS patients compared to control groups [64,65], lack of protective anti-SAg antibodies was found to be associated with an increased risk for STSS [66,67] and circulating SAgs were found in several patients suffering from STSS [68].

Acute rheumatic fever (ARF)

ARF, a post-infection sequelae, is the leading cause of preventable paediatric heart disease. It usually occurs in school-age children and young adults after pharyngeal infection with *S. pyogenes*. ARF is a cross-reactive immune response to the host's cardiac tissue and it has been proposed that the reactive T cells might be driven by SAgs. Recently, several novel streptococcal SAgs have been identified from ARF-associated serotypes. The genes for SPE-K/L were found in high frequencies on serotypes M3 (USA and Japan) [10,19] and on M89 (New Zealand) [20], while SPE-M and SPE-M* were found in M18 (USA) [13]. Smoot *et al*. showed that antibodies against SPE-M and SPE-M* were more common in convalescent sera from ARF patients compared to patients with pharyngitis [69]. Interestingly, a common target of the SAgs SPE-K/L, SPE-M and SPE-M* are T cells bearing the TcRs with Vβ1·1.

Kawasaki disease (KD)

KD is an acute multi-system vasculitis of unknown aetiology that affects mainly young children and is now recognized as the leading cause of acquired heart disease in children in the developed world.

KD is associated with marked activation of T cells and monocytes and there is a remarkable similarity among KD, TSS, STSS and scarlet fever in the clinical symptoms. Intravenous immunoglobulin therapy is highly effective when given early, suggesting that the causative agent is a toxin. Several investigators reported the selective expansion of T cells bearing the Vβ2·1 TcR, which points towards a SAg involvement in the disease [70,71]. A potential association between KD and the Y. *pseudotuberculosis* mitogenic factor (YPM) has also been reported [72].

Autoimmune diseases

It has been proposed that SAgs might contribute to the pathogenesis of autoimmune disease by activating T cells that are specific for self antigens. Although there is no direct evidence of SAg involvement, it has been suggested that SAgs could, under the right conditions, break the tolerance or suppression of autoreactive T cell clones and induce a state of autoimmunity. Evidence for this hypothesis came from an animal model of multiple sclerosis: experimental autoimmune encephalomyelitis (EAE), where it was shown that administration of SEB to mice recovering from EAE triggered direct stimulation of the Vβ3 positive autoreactive MBP peptide specific T cells resulting in a rapid relapse of the disease [73,74].

Conrad and colleagues found a biased TcR usage in T cells from IDMM patients towards Vβ7 suggesting the activity of a SAg [58]. They showed that the mitogenic activity was encoded by a gene residing on an endogenous retrovirus, named IDDMK$_{1,2}$22 and that viral expression occurred only in IDMM patients. It was shown later by the same group that IDDMK$_{1,2}$22 is identical to one allele of the EBV-inducible HERV-K18 carrying the Vβ7-specific SAg K18·3 [56] (see above).

Superantigens and streptococcal disease susceptibility

One of the most intriguing questions is why do some patients develop severe diseases after GAS infections, while others show only minor symptoms, such as pharyngitis? Several investigators have shown an association between severe streptococcal disease and *spe*-a or *spe*-c genotype of the disease causing GAS isolate [64,65]. However, most experiments were carried out before the majority of streptococcal SAgs were discovered and more recent genotyping has not confirmed those preliminary results. Moreover, the genes for some potent SAgs, such as SMEZ and SPE-J, are present in virtually all GAS isolates.

A lack of protective neutralizing antibodies against individual SAgs has also been proposed as a possible risk factor for the development of severe SAg-mediated disease [66,67]. Unfortunately, to date, interpretations of experiments aimed at examining neutralizing responses in patient sera have been limited by the number of known SAgs available. Now that most of the SAgs have been identified from the various completed staphylococcal and streptococcal genomes, a larger cohort of disease sera should be examined to test this theory.

HLA alleles differ in their ability to present different SAgs and recent structural analysis revealed that the bound peptide also plays a role in modulating SAg binding to the MHC molecule (see above), implying an MHC linkage with susceptibility to SAg toxicity [15–17,50]. Recently, Kotb and colleagues showed that indeed the immunogenetics of the host strongly influence the outcome of invasive streptococcal infection. Specific human HLA haplotypes conferred strong protection from severe systemic disease caused by invasive streptococcal infection, whereas other haplotypes actually increased the risk of severe disease [75]. This was the first clearly identifiable link between MHC class II polymorphism and the activity of individual SAgs. It now remains to

match the MHC susceptible alleles with individual SAgs expressed by the invading bacteria.

Superantigens have received a great deal of attention since the discovery of their mechanisms in 1989. Since then, a wealth of knowledge about their structure and molecular mechanisms has been presented, yet little information has been forthcoming on their direct role in diseases, other than the obvious food poisoning and toxic shock. Nevertheless, there has been considerable speculation that they are involved in other immune-related diseases. They are a remarkably family of molecules, refined to subvert the adaptive immune response ruthlessly by targeting the two most important antigen recognition molecules the TcR and MHC class II. They are clearly designed as a defence against a hostile immune system: of this much we are certain. What is not certain is exactly how this random stimulation of many T cells results in protection for the microbe. Both *S. aureus* and *S. pyogenes* are commensal organisms in humans, so the fact that they have the potential to activate the immune response in such a dramatic fashion means that their expression must be tightly controlled and that the immune system must deal with their continuous presence. Perhaps this continual subliminal T cell activation is of some benefit to the host as well.

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