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# A Review on Superantigens

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**Abstract:** ***Superantigens are microbial products which are immunostimulatory and disease causing. They stimulate a large fraction of T cells unlike conventional antigens. These superantigens include various staphylococcal enterotoxins, streptococcal toxic shock syndrome toxins, viral proteins etc. The superantigen family comprise of proteins that range from 22-29 kDa in size and highly resistant to proteases and heat denaturation. Superantigens posses an N- and C- terminal domain divided by a long, solvent accessible α-helix which spans the centre of the molecule and contains several hydrophobic residues in its solvent-exposed regions while the C-terminal domain adopts a β grasp motif. Various human diseases are caused by superantigens as food poisoning, toxic shock syndrome, Kawasaki disease and some autoimmune disorders. Different approaches have been developed for the effective protection and treatment of superantigens based diseases. The ability of superantigens to stimulate a large population of T-cells has been explored for cancer immunotherapy and treatment of infection and autoimmune diseases.***

**Keywords:** Superantigen (SAg), Staphylococcal Enterotoxins (SEs), T cell receptor (TCR), MHC class II molecules, Antigen Presenting Cell (APC), Immunomodulatory.

## 1. Introduction

The term "superantigen" was coined by Marrack and Kappler [1] to describe microbial proteins that activate large number of specific T-cells against conventional antigens. Superantigens (SAgs) are a class of immunostimulatory and disease-causing proteins of bacterial or viral origin with the ability to activate large fractions of T-cells (up to 5-20%), compared to only one in $10^5$ to $10^6$ T-cells during normal antigen presentation [2]. The first superantigens to be described were the minor-lymphocytes-stimulating (MLS) antigens in the mouse thymus. Endogenous MLS antigens in mice were originally described by Festenstein in 1973 and have subsequently been shown to be derived from mouse mammary tumor viruses. 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 enterotoxin A (SEA) for its potent enterotoxic properties. All superantigens defined so far are microbial products, implying a role for these molecules in the life cycle of the microbe. These super-antigens make use of the host immune system, most probably for facilitating their own propagation [3].

### 1.1 Bacterial Superantigens

The best-characterized group of SAgs belongs to the pyrogenic toxin SAg family, which includes staphylococcal enterotoxins (SEs), staphylococcal toxic shock syndrome toxin-1 (TSST-1), streptococcal superantigen (SSA), and streptococcal pyrogenic exotoxin A (SPE-A), SPE-C, SPE-G, SPE-H and SPE-J [4,5,6,7,8]. These bacterial SAgs are among the most potent pyrogens known and are capable of inducing a highly lethal toxic shock syndrome. Twelve SAgs have been identified in Group A Streptococci (GAS), predominantly but not exclusively produced by *Streptococcus 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. Superantigens 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, SPE-I, SPE-L and SPE-M (98% sequence identity) indicating another horizontal transfer from *S. pyogenes* to *S. equi* or *vice versa*. Other two SAgs have been identified from *S. dysgalactiae* called *Streptococcus dysgalactiae* Mitogen (SDM) and SPE-$G^{dys}$. SDM is most similar to SPE-M and SPE-$G^{dys}$ is most similar to SPE-G [9]. Bacterial superantigens that do not belong to the pyrogenic toxin family include the staphylococcal exfoliative toxins (ET) A and B [10], *Mycoplasma arthritidis* mitogen (MAM) [11] and *Yersinia pseudotuberculosis* mitogen (YPM) [12]. There are about 41 bacterial SAgs described in the literature and the number is growing steadily.

### 1.2 Viral Superantigens

Among SAg proteins of viral origin, only mouse mammary tumor virus (MMTV), a milk-transmitted B-type retrovirus, causing murine mammary carcinoma has been described in detail [13]. The T-cell response to MLS antigens is similar to the response to bacterial SAgs with expansion of unique TCR Vβ subsets. It has been demonstrated that mouse MLS endogenous SAgs are encoded by MMTV proviral DNA that has been integrated into germline, demonstrating a link between endogenous SAgs and infectious agents. Other reports have shown superantigenic activity by the rabies virus nucleocapsid protein and by two human tumor viruses, cytomegalovirus and Epstein-Barr virus. Recently, the envelope gene of an endogenous human retrovirus isolated from pancreatic islets was shown to encode an MHC class II- dependent SAg specific for Vβ7 [14]. A selective depletion of T-cells that express specific Vβ chains also occurs in the patients infected with human immunodeficiency virus (HIV), suggesting that superantigen exists [15].

## 2. Structure of Superantigen

The superantigen family comprises of proteins that range from 22-29 kDa in size. They are robust proteins, highly resistant to proteases and heat denaturation [1]. Amino acid sequence-based alignment of streptococcal and staphylococcal superantigens allows their classification into subfamilies: (a) SEA, SED, SEE, SEH, SEJ and SPE-H; (b) SEB, SEC1-3, SPE-A1-3, SSA and SEG; (c) SPE-C, SPE-J, SPE-G, SME-$Z_1$ and SME-$Z_2$; (d) SEI, SEK, SEL and SEQ; (e) SDM. However, SDM shows greatest homology to SPE-M and SPE-L from *Streptococcus pyogens* appearing to form a group independent from those of the classical superantigens [16]. TSST-1 has ~28% homology with other SEs (Staphylococcal enterotoxins) and cannot be grouped with any of these subfamilies; the SETs (staphylococcal enterotoxin-like toxins) appear to be its closest relations, although the overall sequence identity is low, at only 26%; and the SET proteins show no apparent superantigenicity [17]. Analysis of three-dimensional structure of superantigens reveals that archetype superantigen to be comprised of an N- and C- terminal domain divided by a long, solvent-accessible α-helix which spans the centre of the molecule. The N- terminal domain displays a characteristic OB (oligosaccharide/oligonucleotide-binding) fold and contains several hydrophobic residues in its solvent-exposed regions while the C-terminal domain adopts a β-grasp motif [18, 19]. A feature seen in many, but not all superantigens is a highly flexible disulphide loop, located in the N-terminal domain of SEs and SPE-A, but not in TSST-1 [20, 21] and SPE-C [22]. This flexible loop is implicated in the emetic properties of the SEs and their ability to bind certain TCR Vβ elements [23]. The additional feature of the superantigen family is the presence of one or more zinc-binding sites. Different and structurally non-equivalent zinc-binding sites have been identified in SEA, SED, SEH and SPE-C to which other superantigens have been found to have homologous zinc sites. The zinc ions have been shown to posses a role in the formation of: (a) stable homodimers (in SED and SEH) (b) a second high affinity MHC class II binding sites (in SEA, SEC, SED, SPE-A1 and SEH) and (c) in the thermostability of superantigens [24].

## 3. Bioactivity

### 3.1 Conventional Antigen Versus Superantigens

Superantigens differ from conventional peptide antigens in four major aspects: (i) They elicit a strong primary response, while *in vivo* priming and boosting are necessary to detect T-cell proliferation *in vitro* in response to normal antigen. (ii) The TCR Vβ chain is sufficient for recognition of a superantigen [25], in contrast to that of a conventional peptide antigen, which requires a very specific interaction with the third hypervariable region of the TCR **(Fig- 1)**. (iii) All superantigens discovered so far require presentation by MHC class II proteins, however, the T-cell response to superantigens is not class II restricted, thereby violating the golden rule of MHC restriction that governs all other antigen specific T-cell responses [26]. Superantigens are more efficiently presented by certain human HLA-DR alleles than by mouse MHC class II to both human and murine T-cells. (iv) Superantigens are presented in an unprocessed form, whereas normal antigens require breakdown into peptides, which are then loaded into the MHC-binding groove. The combination of these four features unequivocally defines a superantigen.

### 3.2 Interaction of superantigens with MHC class II binding sites

Superantigens bind directly to APCs on the outside of MHC class II molecules. Structural analyses have revealed the presence of two distinct superantigen-binding sites on MHC class II molecules [18, 19]. The generic site is located on the α-chain of the MHC class II molecule. A second, high-affinity (~100 times higher affinity than the generic site), zinc-dependent site is located on the β-chain. The binding mode of SEB and TSST-1 through the α-chain of DR1 involves the hydrophobic core at the N-terminal domain of the toxin molecule. Similar hydrophobic ridge regions form the generic site in other superantigens (except SPE-C, SPE-H and SME-$Z_2$) and are implicated in class II binding. Most superantigens except SEB, TSST-1 and SSA possess either one or two zinc-binding sites [19]. Binding is mediated by a bridging zinc ion, which tetrahedrally coordinates three ligands from SPE-C (His 167, His 201 and Asp 203) [27] and two ligands in the case of SEH (His 206, Asp 208) with one from the MHC class II β1 helix (His 81), respectively. SEH does not possess a generic MHC binding site and its interaction with MHC class II via the zinc site is the strongest known among the staphylococcal enterotoxins [28]. The high affinity C-terminal zinc binding site in SEA has a $K_d$ of 100 nM for DR1 recognition [29, 30], compared to its generic site (N-terminal domain) which has considerably lower affinity ($K_d$ of 10 μM) for class II binding. If the two binding sites co-exist, SEA shows a $K_d$ of 13 nM [31]. The existence of two distinct MHC class II binding sites may enable the formation of a trimeric SEA-MHC-SEA complex [32]. SEC and SPE-A do not possess the high-affinity zinc-binding site observed in SEA, instead a new N-terminal zinc-binding site with somewhat lower affinity (the estimated dissociation constant for the zinc ion in SEC2 is < 1 μM) is present [33, 34]. SED [35] and SPE-C [22] can form zinc-dependent homodimers (in SED) and zinc-independent homodimers (in SPE-C) and bind solely to the chain of MHC class II molecules by a zinc-mediated mechanism similar to that of SEA and could form either trimers or tetramers. SME-$Z_2$, SPE-G and SPE-H bind to MHC class II molecules in a zinc-dependent fashion [36]. The presence of multiple sites of interaction with MHC class II molecules affords these toxins a great deal of diversity, either through zinc-mediated interaction, via the generic site or a combination of both. This gives each superantigen a unique array of possible interactions through which it can elicit immune function [24].

### 3.3 Interaction of superantigens with TCR

The primary targets of SAgs are the $CD4^+$ T-cells, activation of which results in T helper type 1 (Th1) response [37]. Almost all SAgs interact exclusively with the Vβ region of the TCR resulting in the stimulation of up to 10% of resting T-cells [25]. The interaction of SAgs and TCR molecules share a common core of residues with specificity for particular Vβ elements. Thus, sequence differences within

their TCR-binding sites provide the basis for a characteristic Vβ repertoire for each superantigen [38]. The TCR-binding site has been shown to involve a shallow cavity between the two domains of the toxins. In SEB, this cavity is formed by the α2-helix, β2-β3 loop, β4-strand, β4-β5 loop, part of the β5-strand and α5-helix [39]. Main interactions are shown to be between the side chain atoms of the superantigen and complementarity determining regions 1 and 2 (CDR 1 and 2), and hypervariable region 4 (HV4) of the Vβ chain [19] of TCR. The change of one residue within the TCR-binding site is enough to alter Vβ specificity [40]. T21, S206 and N207 have been identified as the probable specificity defining residues in SEA [39]. The location of the TCR-binding site of TSST-1 differs from that of the other superantigens. It is situated in the C-terminal domain on the long α2-helix and between the β7-β8 and α2-β9 loops as part of the α1-helix [20, 21]. In another study done by Petersson and coworkers [41] showed stimulation of human T- cells by SEH through direct interaction with the TCR Vα domain (Vα 10), no TCR Vβ domain.

## 4. Superantigen-Induced Signal Transduction Pathways

### 4.1 The signal cascade

Signal transduction pathways are hierarchical cascades that originate at the cell membrane with the embedded receptors for the appropriate effector molecules, such as mitogens, growth factors, certain hormones, toxins and many other types of bioactive molecules. Transmission of signals from the cell membrane to the nucleus occurs principally by conversion of a series of proteins to their active state upon phosphorylation by kinases **(Fig- 2)**. A variety of stimuli have been shown to generate intracellular responses that converge on various kinase pathways [42]. There is no evidence that this pathway is directly linked to the TCR/MHC II receptors. SEA and SEB modulate IL-2 receptor (IL-2R) expression and signal transduction involving the Jak/STAT pathway [43]. Mice exposed to SEA or SEB also showed activation of the Jak/STAT pathway in their T-cells [44, 45]. Protein kinase C (PKC) shows widespread action in response to SE exposure and there is interdependence between PKC, phospholipases and eicosanoids (arachidonic acid metabolites).

### 4.2 Kinases involved with TCR/MHC II

After binding of Staphylococcal enterotoxins to TCR, Tyrosine phosphorylation of TCR subunit is an initial step of TCR stimulation along with recruitment and tyrosine phosphorylation of the protein tyrosine kinase ZAP-70 [46, 47]. Then activation of the Src (sarcoma) family protein tyrosine kinases, Lck and Fyn occurs and as a consequence, the phosphorylation of numerous substrates, including several TCR-CD3 subunits takes palce [48]. Cytoplasmic tail binds ZAP-70 which in turn is phosphorylated at tyrosine residue by SEA or SEB stimulation [49]. During the initial binding of SE to MHC II/ TCR, protein tyrosine kinase activity increases at TCR CD3 complex [47] and tyrosine phosphorylation of phospholipase C-γ (PLC-γ) occurs in T-cells and in antigen presenting cell [50]. The TCR complex is thought to activate PLC-γ through a specific transmembrane adapter protein, LAT (linker for activation of T-cells) [51]. The activated PLC-γ cleaves phosphatidylinositol 4, 5-bisphosphate to release two potent signalling molecules, inositol 1, 4, 5-triphosphate ($IP_3$), which increases calcium mobilization and diacylglycerol (DAG), a potent stimulator of protein kinase C (PKC). Protein kinase C is involved in numerous effects induced by SEs. Protein kinase C activation is essential for the regulation of SEB-induced cell death. When peripheral blood mononuclear cells are stimulated with TSST-1 or SEB, the major cell producers of TNF-α are T-cells. PI-3 kinase regulates TNF-α production in TSST-1 or SEB treated cells and inhibitors of PI-3 kinase block TNF-α production [52, 53]. Both Protein tyrosine kinase (PTK) and protein kinase C (PKC) play essential role in HLA class II molecule mediated signal transduction elicited by SEB, and PTK may precede PKC activation in signalling pathway [54]. PTK include increased expression of the IL-12/p40 gene in macrophages, which leads activation and nuclear translocation of nuclear factor κB (NF-κB).

## 5. Superantigens in Human Diseases

### 5.1 Food poisoning

A form of gastroenteritis known as staphylococcal food poisoning occurs upon ingestion of food colonized with toxin-producing strains of *S. aureus.* The symptoms, which include vomiting and diarrhoea, are generally short-term, lasting no longer than 1-2 days. Quantities of less than 1µg of toxin are sufficient to trigger vomiting in humans [55].

### 5.2 Toxic shock syndrome (TSS)

Toxic shock syndrome (TSS) is a rapid-onset of illness causing fever, hypotension, rash, vomiting, diarrhoea and eventually multiple organ failure. TSS toxin-1 (TSST-1) and SEs implicated in TSS have the ability to cross the mucosa. Cytokines such as interleukin-1 (IL-1) and tumor necrosis factor (TNF-α) have been shown to rise during the course of TSS [4, 7].

### 5.3 Streptococcal toxic shock syndrome (STSS)

Streptococcal toxic shock syndrome (STSS), caused by *S. pyogenes*, is the most severe form of invasive streptococcal disease with mortality rates of up to 50%. STSS is often associated with bacteraemia, myositis or necrotizing fasciitis. Streptococcal SAgs have been implicated in STSS [4, 9].

### 5.4 Acute rheumatic fever (ARF)

Acute rheumatic fever (ARF), a post-infection sequel, is the leading cause of preventable pediatric heart disease. It usually occurs in school age children and young adults after pharyngeal infection with *S. pyogenes*. Several novel streptococcal SAgs, for example, SPE-K, SPE-L or SPE-M have been identified from ARF-associated serotypes [56]. 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 [9].

### 5.5 Kawasaki disease (KD)

Kawasaki disease (KD) is an acute multi-system vasculitis of unknown etiology 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 with respect to clinical symptoms. A potential association between KD and the *Y. pseudotuberculosis* mitogenic (YPM) factor has also been reported [9].

### 5.6 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. SAgs break the tolerance or suppression of autoreactive T-cell clones and induce a state of autoimmunity [57]. The different autoimmune diseases are as follows:

#### 5.6.1 Multiple sclerosis (MS)

Multiple Sclerosis (MS) is an inflammatory demyelinating autoimmune disease of the central nervous system that causes paralysis, and affects speech, motor functions and vision [58].

#### 5.6.2 Rheumatoid arthritis (RA)

Superantigens have also been implicated in rheumatoid arthritis (RA). In this disease staphylococcal superantigens increase the cellular cytotoxic activity of T-cells, with synovial fibroblast being the targets of this cyotoxicity. Superantigens have been shown to reactivate bacterial wall-induced arthritis and collagen induced arthritis [58].

#### 5.6.3 Psoriasis

It is a cutaneous inflammatory disorder characterized by epidermal keratinocyte hyper proliferation in association with inflammatory infiltrates. In the disease of guttate and chronic plaque psoriasis, number of Vβ bearing T-cells increases in the dermis and epidermis. Skin lesion eruptions in guttate psoriasis have been linked with throat infections and increased antibody titers to streptococcal antigens [58].

#### 5.6.4 Diabetes

Insulin-dependent diabetes mellitus (IDDM) is an autoimmune disorder in which pancreatic β cells are destroyed. There is evidence that autoreactive $V\beta7^+$ cells are responsible for the destruction of pancreatic cells, suggesting activity of a superantigen [59].

#### 5.6.5 Human immunodeficiency virus (HIV)

Human immunodeficiency virus (HIV) causes a loss of $CD4^+$ T-cells over the course of the disease, resulting in the inability to effectively combat infections by other microbial agents. An HIV-encoded superantigen, Nef, has been shown to induce the differentiation of human B-cells to immunoglobulin-secreting cells, probably as a result of T-cell activation and release of cytokines that aid in B-cell activation and differentiation [58].

#### 5.6.6 Mouse mammary tumor virus (MMTV) and cancer

The prototype for viral superantigen is produced by mouse mammary tumor virus (MMTV), a type-B retrovirus that causes mammary tumors [60]. MMTV superantigens, previously identified as MLS antigens [61] have been implicated in the migration of infected immune cells to the mammary gland and in the subsequent efficient infection of mammary tissue [58].

## 6. Different Therapeutic Methods for Superantigens Based Diseases

By the knowledge of molecular mechanism by which SAgs stimulate the host immune system, a variety of therapeutic strategies have been developed. These methods offer effective protection against SAgs by blocking the initial stages of pathogenesis prior to activation of T-cell proliferation and cytokine release, thereby preventing the resultant cascade of downstream effect on the host immune system. These strategies fall under following categories:

### 6.1 Development of neutralizing antibodies against superantigens

Passive immunization through intravenous infusion of human IgG containing anti-SAg antibodies was successful in relieving the infections. Neutralizing Abs against SAgs have been identified that showed cross-reactivity between different SAgs and inhibit SAg-induced host effects in tissue culture [62, 63]. In order to elicit protective antibodies in animals, mice were immunized with attenuated variants or mutants of SEA and SEB, thus reducing the toxic effects and suppression of the immune system associated with injection of wild-type SAgs [64, 65]. These mutations were constructed by site-directed mutagenesis to alter critical residues that are required for binding to the MHC class II receptor, thus rendering the SAgs less toxic while maintaining antigenicity. The attenuated SAgs were highly immunogenic in mice, eliciting neutralizing antibodies that were effective in protecting 100% immunized animals against re-challenge with 10-30 $LD_{50}$ of intact SEA or SEB. Rhesus monkeys challenged with SEA or SEB through inhalation also produced a neutralizing antibody response. One-hundred percent of the monkeys successfully survived re-challenge with up to 30 $LD_{50}$ SAgs compared with non-immunized control primates.

### 6.2 Protection by superantigen derived peptides

Peptides derived from SAg protein sequences have been successfully used to map domains that are involved in TCR and MHC class II binding, stimulation of cytokine production and T-cell proliferation [66, 67]. These peptides interfere with SAgs binding to MHC class II and TCR besides eliciting antibodies that recognize and clear SAgs from the body. A common theme to this approach has been to use conserved peptide domains in the SAgs. The dodecapeptide, YNKKKATVQELD, a variant of wild-type SEB toxin residues 150-161, exhibited inhibition of cytokine mRNA expression in peripheral blood mononuclear cells (PBMCs) stimulated with SEB, with 18- and 10- fold reduction in IL-2 and IFN-γ mRNA, respectively, in the presence of 100-200-fold excess of the peptide [68, 69]. Visvanathan and coworkers [70] identified another dodecapeptide sequence CMYGGVTEHEGN that exhibited

antagonist properties against SAg-based host response, albeit with less efficacy. In this study, antibodies were raised in rabbits against a peptide consisting of two fused consensus sequences found in *Staphylococcus aureus* SAgs, **CMYGGVTEHEGN**VTVQELDYKIRKYLVDNKKLYG C. The anti-peptide serum was able to protect rabbits from SEB-induced lethal effects compared with rabbits that received preimmune normal rabbit serum [71].

### 6.3 Targetting toxin production in *S. aureus*

In this approach, SAg synthesis was inhibited to prevent subsequent toxin secretion by *S. aureus* into the host circulatory system. In *S. aureus*, SAg production is partially regulated by a quorom-sensing mechanism using RNAIII molecule encoded by the *agr* locus [72]. Expression of RNAIII is both positively regulated by the constitutively produced RNAIII activating protein (RAP) and negatively regulated by the RNAIII inhibiting peptide (RIP), a YSPXTNF hexapeptide, through phosphorylation of the TRAP protein (Target for RNA III activating protein) [73, 74, 75]. To block RAP activity, mice were immunized with purified RAP to allow the animals to produce antibodies to the RAP protein [76]. RIP was also shown to reduce the onset of other pathologies using different strains of *S. aureus* and host animals, including keratitis and osteomyelitis in rabbits, mastitis in cows and septic arthritis in mice [77]. Synthetic analogs of RIP have also proved to be efficient inhibitors of SAg production in *S. aureus* [78].

### 6.4 Development of MHC DRα1-TCRVβ chimeric proteins

Using the available structural data regarding toxin interactions with MHC class II and TCRs [79, 80, 81], chimeric proteins were developed as competitive inhibitors of SAg that prevent the effects of SAg binding to host cells [82]. In addition to cytokine release, incubation with the chimera also inhibited T-cell proliferation in SEB-stimulated PBMC [71].

### 6.5 Development of high affinity TCRs

Despite the potent effects of SAg binding to immune cells, the binding affinities between SAgs and their natural binding partners, the MHC class II and TCRs, have been measured to be quite low, of the order of $K_d \approx 1\text{-}100\mu M$ [83, 84, 85]. In an attempt to increase the binding affinities of the target receptors to SAgs and create potentially effective antagonists to T-cell activation, the yeast display system was employed to select the variants of TCR that exhibited a higher binding affinity [86, 87]. In this system, a mutagenized library of single chain TCR (Vβ-linker-Vα) was expressed as fusion proteins to the yeast cell surface mating protein Aga2. Upon induction of expression, the highest affinity mutant isolated in this manner, mL2.1/A52V, exhibited a ~1000-fold increase in binding affinity to SEC3, with $K_d \approx 7$ nM. Incubation of the mL2.1/A52V mutant with SEC3-stimulated $^{51}$Cr-labeled cytolytic T lymphocytes resulted in significant inhibition of $^{51}$Cr release from the T-cells compared with control TCR variants, indicating that the mL2.1/A52V mutant blocked SEC3-mediated T-cell lysis [71].

### 6.6 Blocking co-stimulatory signals to inhibit superantigen-induced responses

Apart from the activation of TCR, additional co-stimulatory signals are required for successful T-cell stimulation. A critical co-stimulatory interaction has been identified as the engagement of the CD28 receptor on the T-cell by the B7 ligand family members on the APC, resulting in the full T-cell response, including cytokine production, clonal expansion and prevention of anergy [88]. A second co-stimulatory receptor, CTLA4 or CD152, shares homology with CD28 and binds with higher affinity to B7, but negatively regulates this pathway by inhibiting cytokine production and cell cycle progression [89]. Prior to the elucidation of CTLA4 function, immunomodulatory reagents fusing the extracellular domain of the soluble CTLA4 receptor to the IgG1 heavy chain, termed CTLA4Ig, were observed to act as a competitive inhibitors of CD28/B7 co-stimulation [90]. The use of CTLA4Ig to block TSST-induced signaling resulted in inhibition of cell proliferation by up to 90% and rescue from lethality of 75% mice co-administered with TSST-1 and CTLA4Ig compared with control mice [91]. CTLA4Ig treatment exhibited a reduction in epidermal hyperplasia, decreased number of activated lesional T-cells and dendritic cells, and decreased expression of T-cell activation markers [92, 93].

### 6.7 Cytokine Therapy

Anti-inflammatory cytokine IL-10 plays an important role in inhibiting SAg-induced host effects and inducing tolerance to SAg in animal studies. Incubation of APC with exogenous IL-10 has been shown to significantly inhibit IFN-γ and IL-2 production in SEB-stimulated T-cells [94, 95]. In a more detailed study, incubation of IL-10 with TSST-stimulated PBMCs inhibited the production of TNF-α, IL-1, IL-6 and IFN-γ by 68, 93, 70 and 86%, respectively [96]. In whole animal studies, introduction of IL-10 has also protected mice from SEB-induced lethal shock in a dose-dependent manner [97]. Furthermore, IL-10 levels were shown to increase upon induction of immunological tolerance to SEA mucosal challenge in mice, whereas homozygous negative $IL10^{-/-}$ mice could not undergo this conversion to SEA tolerance, suggesting that IL-10 may play a role in preventing toxic shock [98]. Depending on the state of T-cell activation, IL-10 can mediate both stimulatory and inhibitory effects, inducing anergy in T-cells when incubated with allogeneic monocytes, while promoting growth of T-cells stimulated with anti-CD3 MAb [99, 100].

### 6.8 Small molecule inhibitors of superantigen-induced immune cell effects

Compounds that reduce circulating levels of TNF-α and other pro-inflammatory cytokines, such as pirfenidone [101], niacinamide [102], pentoxifylline [103], plant polyphenol, chlorogenic acid [104] dexamethasone [105] have been shown to prevent lethal shock caused by SAg exposure in mice.

## 7. Superantigens in Medical Therapy

A large number of researchers are concerned about the

therapeutic uses of SAgs. The potential applications of SAg derivatives include cancer immunotherapy and the treatment of infectious and autoimmune diseases. The recruitment of antigen-specific cytotoxic T lymphocytes (CTLs) is a major goal for the immunotherapy of malignant tumors. An attractive approach for immunotherapy is to use antibodies specific for tumor-associated antigens to target large numbers of T-cells to the tumor. Taking advantage of the ability of SAgs to activate large populations of T-cells, chemical conjugates of SEA and the colon carcinoma-reacting monoclonal antibodies (MAbs) C215 or C242 were shown to mediate T-cell-dependent destruction of colon carcinoma cells lacking MHC class II molecules [106]. In subsequent work, a recombinant fusion protein of SEA and the Fab region of the C215 MAb were found to efficiently target T-cells to lyse C215C MHC class II negative human colon carcinoma cells [107]. In other experiments, SEA bound to specific anti-carcinoma cell or anti-ganglioside GD2 MAbs displayed T-cell-mediated cytotoxicity toward MHC class II negative lymphatic leukemia cell lines or neuroblastoma cells, respectively [108, 109].

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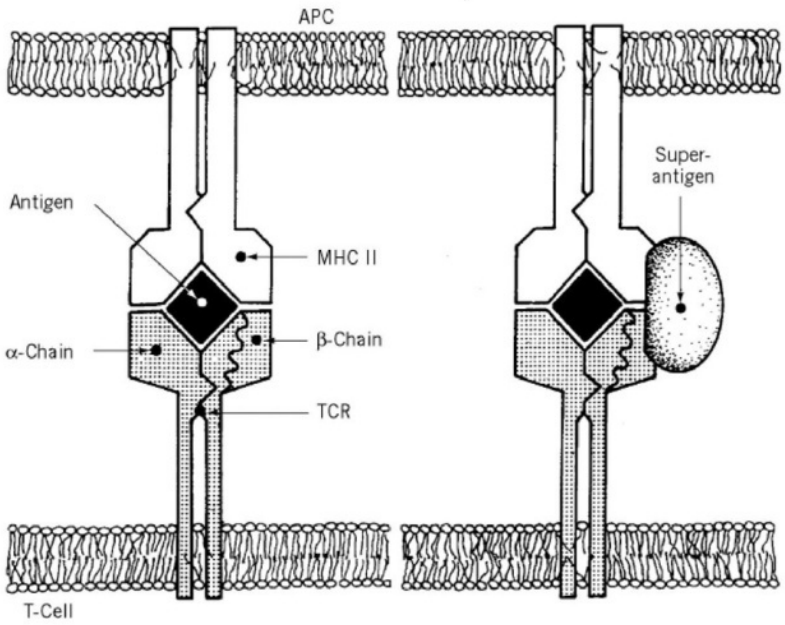


**Figure 1:** Interaction of conventional antigen and superantigen with MHC class II molecule and TCR.

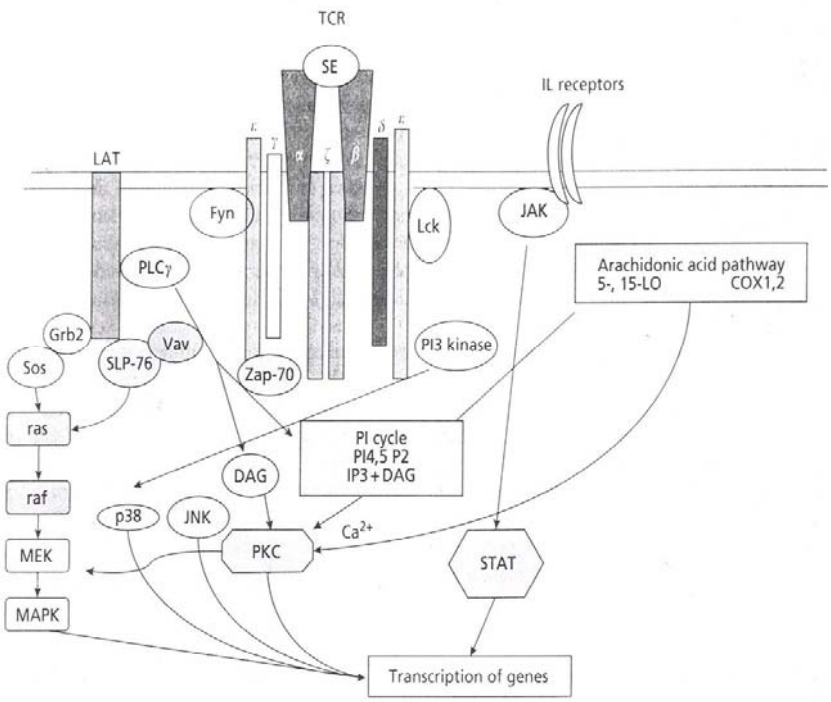


**Figure 2:** Diagrammatic representation of the interactions of various kinases and related signaling molecules involved in staphylococcal enterotoxin-induced signal transduction cascade.