Superantigens - Fancy Pathogens, or Passing Fancy?

David R. Karp, M.D., Ph.D.
Internal Medicine Grand Rounds,
Parkland Hospital
University of Texas Southwestern Medical Center

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Introduction

Five years ago, the term "superantigen" did not exist in the immunologist's vocabulary. Now, these proteins are known to be part of the pathogenesis of a variety of infectious diseases. The characteristic evidence of superantigen stimulation of the immune system has led to the speculation that they are involved in diseases for which the pathogenesis is unclear. These include diseases of a presumed infectious origin, such as Kawasaki disease, as well as autoimmune disorders such as rheumatoid arthritis and systemic lupus erythematosus.

In certain cases the knowledge of the superantigen is considerable. For example, the bacterial toxins from *Staphylococcus aureus* are well studied. The structure of these proteins, including x-ray crystallographic data, is known. Their genetics have been characterized, and their effects on the immune system at molecular, cellular, and organism level have been detailed. Other superantigens, such as a protein derived from *Mycoplasma arthriditis*, or the retroviral superantigens in mice, are just now being characterized at the molecular level. Finally, there are superantigens that exist only as suggested answers to puzzling experimental or clinical observations. This group of proteins may yet be the most intriguing as they offer clues to a number of disease states that have remained unsolved.

Definition

No "official" definition of a superantigen exists. An operational definition stems from a comparison to features of nominal peptide antigen presentation (reviewed below). All currently known superantigens are proteins of microbial (bacterial or viral) origin. They are capable of polyclonal T cell activation that depends on the expression of certain oligoclonal determinants on the T cell antigen receptor. Their optimal action requires the presence of cells bearing class II molecules of

the major histocompatibility complex (MHC). Lastly, they function as intact proteins, not as proteolytic fragments.

Review of Antigen Presentation

The unique way that superantigens stimulate T cells can be compared to the way that a more typical antigen is recognized in a protective immune response. The past ten years have brought a revolution of understanding to the field of immunology. It is now clear that the role of polymorphic products of the major histocompatibility complex (MHC) is to bind a wide array of small peptides and present them to antigen-specific T cells. Through complementary mechanisms, different MHC molecules sample a sea of peptides produced from both intra- and extracellular proteins. These peptides bind to a specialized structural motif in the MHC molecules and are displayed to T cells. Polymorphic residues within the T cell receptor (TcR) then can interact with both the amino acid side chains of the peptide and the MHC. If the overall affinity of the interaction is high enough, a signal will be delivered to the T cell causing it to proliferate, secrete cytokines, or become cytotoxic to target cells.

MHC Molecules

Within the MHC are genes for several types, or classes, of molecules. Both class I and class II MHC molecules are responsible for the presentation of peptide antigens, whereas only class II molecules participate in superantigen function. The molecular biology of both classes of MHC molecules is similar, and their three-dimensional structures are nearly identical. However, there are differences in both structure and cell biology between class I and class II MHC molecules that belie strategic differences in their function in host defense.

Class I

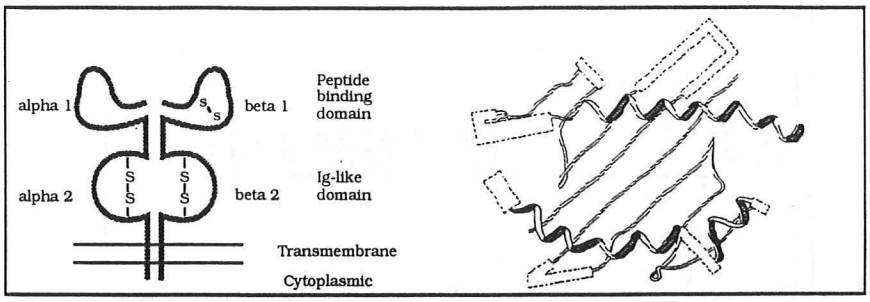
Class I MHC molecules include classical transplantation antigens such as HLA-A, -B, and -C in humans, as well as structurally similar molecules with less well-defined function such as HLA-E and -G. These molecules consist of a polymorphic heavy chain of ~40 kilodaltons that associates with β2 microglobulin, a non-polymorphic 12 kilodalton protein that is not encoded within the MHC. They are expressed on all nucleated cells. Within the endoplasmic reticulum, newly synthesized class I heavy chains, β2 microglobulin, and short (8 or 9 amino acid) peptides fold to form a single trimolecular unit (1, 2). In this complex, the presence of peptide is absolutely necessary. Mutant cell lines that fail to produce peptides or transport them into the ER fail to generate properly folded class I molecules (3). These molecules are recognized as conformationally wrong and are not exported to the cell surface. In this way, the class I molecules that reach the cell surface are all effectively loaded with peptide. During a viral infection, the majority of the peptides that reach the ER will be of viral origin. This explains why T cells that are specific for viral proteins are almost always restricted by the class I haplotype (alleles) of the antigen presenting cell (4).

Class II

Class II molecules are normally found only on certain myeloid cells, including B lymphocytes, monocyte/macrophages, and dendritic cells. However, their expression on other cells such as synoviocytes or fibroblasts can be induced by gamma interferon, expanding the number of possible types of antigen-presenting cells during an active immune response. In contrast to class I molecules, class II MHC proteins (HLA-DR, -DQ and -DP in humans) are primarily involved in the binding of peptides produced from extracellular antigens (5). Class II molecules consist of two non-covalently linked polypeptide chains, termed α (~35 kilodaltons) and β (~28 kilodaltons). Both of these polypeptides are encoded within the MHC. Following biosynthesis, the α and β chains associate with a third protein, the invariant chain. As the name implies, invariant chain is not polymorphic and is not encoded within the MHC.

The invariant chain has at least two functions. First, its association with the αβ heterodimer prevents the binding of peptides to class II in the ER (6, 7). Second, its sequence has signals for the intracellular movement and localization of class II (8, 9). The trimolecular complex is transported to a vesicle of the endosomal/lysosomal pathway. This compartment is has low pH and contains both acid proteases and extracellular antigens such as foreign proteins, opsonized or phagocytosed bacteria, parasites and neutralized viruses. In this environment, the complex antigens are denatured and degraded to peptides. In addition, the invariant chain is clipped into smaller fragments and dissociates from the α and β chains of class II. This allows the binding of antigenic peptides that are, in general, longer (13-25 amino acids) than those that bind to class I (10). The peptide-loaded class II molecules then transit to the cell surface. As in the case with class I there is evidence that class II molecule that fail to bind peptides at this time will not fold in a conformation that is transported to the cell surface (11).

Despite these differences on function, the overall structure of class I and class II molecules is remarkably similar (12). They each have four large (~90 amino acid) extracellular domains, a transmembrane domain, and short intra-cytoplasmic tails. In the case of class I, one of extracellular domains is comprised of β2 microglobulin. In both class I and class II molecules, the two membrane proximal domains (α3 of class I; α2 and β2 of class II) have primary, secondary, and tertiary structures typical of immunoglobulin domains. The membrane distal domains (α1 and α2 in class I; α1 and β1 in class II) form a structure suited to the binding of peptides. X-ray crystallographic studies of both class I and class II molecules have shown that these regions consist of a "floor" composed of β pleated sheet region that is overlaid on two sides by stretches of α helix (13, 14). These two helices form a "groove" at the most membrane distal part of the MHC molecule. Antigenic peptides have been demonstrated to bind in an extended conformation within this groove .



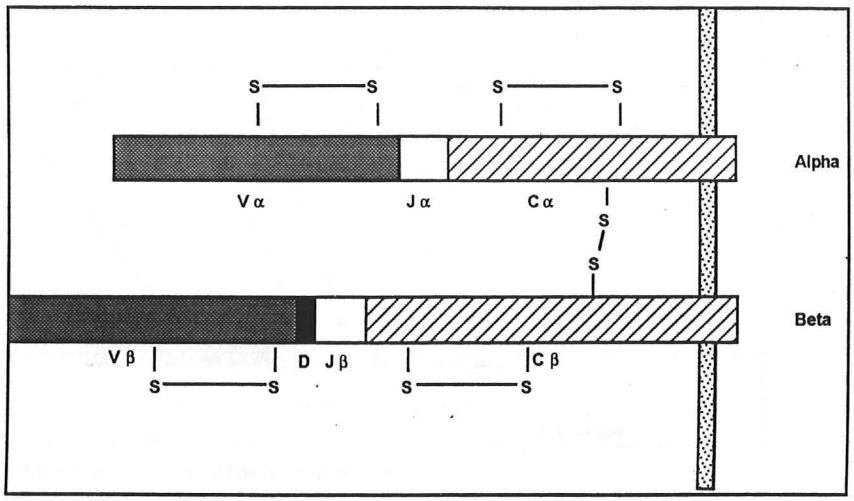
MHC Class II Structure

Peptide binding site of class II (ref. 14)

T Cell Receptor

Structure

The T cell antigen receptor (TcR) is the molecule that recognizes the antigenic peptide/MHC complex. The use of monoclonal antibodies and recombinant DNA techniques have provided information on both the structure and genetics of the TcR. Overall, the amino acid sequence of TcR is homologous to immunoglobulin. TcR is a heterodimer with each chain having constant and variable regions. Unlike immunoglobulin, the two chains of TcR are of approximately the same molecular mass, and are both transmembrane glycoproteins. Two forms of TcR have been described, $\alpha\beta$ and $\gamma\delta$. The latter is expressed only on a minority of peripheral T cells and some tissue lymphocytes, and its physiological role is unclear. Each of the four polypeptides is coded by a separate genetic locus, and only one form is expressed in a given T cell. Only the $\alpha\beta$ TcR is consistently associated with a response to superantigens.

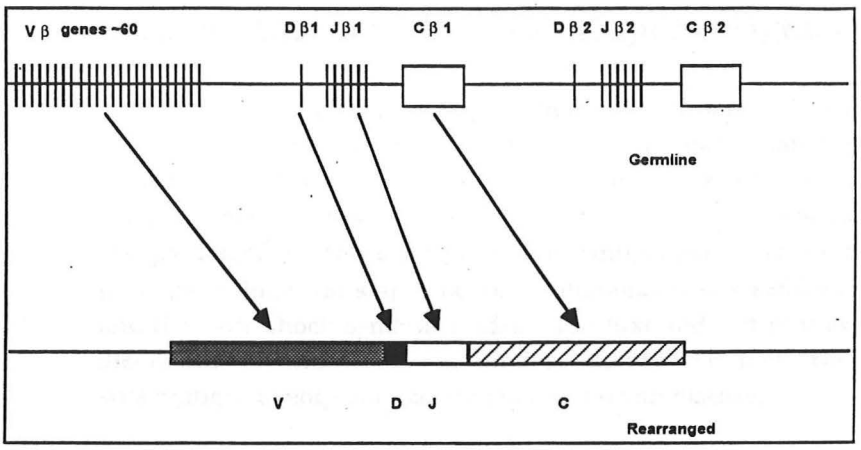


Structure of the T Cell Antigen Receptor

Genetics

Like immunoglobulin, the variable and constant regions of TcR chains are coded by separate genes. In humans there is a single Cα gene and two Cβ genes. The variable region genes are grouped into families according to sequence similarity. There are approximately 13 human Vα families each with from 3 to 20 members. The total number of Vα genes is 100. Located between the Vα genes and Cα gene are approximately 50 junctional, or J region, genes. During T cell maturation the germline gene is rearranged to form a functional Vα-Jα-Cα unit. Similarly, there are at least 22 human Vβ gene families each with 1 to 9 members giving a total of 57-60 Vβ genes. There are 12 Jβ regions and, in addition 2 diversity (D) regions not found in the α chain gene. The mature β chain gene is composed of Vβ-Dβ-Jβ-Cβ.

When all the possible combinations of variable region gene segments are calculated, the number of possible αβ TcR's is very large. In addition, the joining of each segment is not perfect, and individual



Generation of Diversity in the T Cell Receptor Beta Chain

nucleotides not in the germline can be added. The final estimate of the number of T cell receptors in a single individual is greater than 10^{15} (15). Each receptor is potentially most reactive with a single combination of MHC and hypothetical peptide. There is redundancy and cross-reactivity so that the ultimate precursor frequency of T cells specific for a given peptide antigen presented by a particular MHC class I or class II molecule is on the order of $1/10^5$.

The most polymorphic residues of the TcR α and β chains cluster into discrete areas termed complementarity determining regions (CDR's). Three CDRs (CDR1, CDR2, and CDR3) are made up by the combination of germline sequences and nucleotide addition/deletion during recombination. By analogy to the three-dimensional structure of immunoglobulin, these CDRs align in a plane at the membrane distal portion of the mature TcR. CDR1 and 2 appear to interact primarily with MHC residues, while CDR3 polymorphisms affect the ability to recognize antigenic peptides. A fourth polymorphic domain, CDR4, appears on the "side" of the TcR β chain. The polymorphism in this region is determined mainly by the Vβ family-specific sequences. It is these residues that have been implicated in the binding of bacterial and viral superantigens (16, 17).

Medical Effects of Known Superantigens

The best understood superantigens are a panel of exoproteins produced by *Staphylococcus aureus*. Their biochemistry, genetics, microbiology, and pathogenicity have been extensively studied. The effect of these proteins on cells of the immune system is profound, and is the paradigm for the concept of superantigenicity. These bacterial proteins include the staphylococcal enterotoxins, the exfoliative toxin, and the toxic shock syndrome toxin. The first and last of these will be discussed to illustrate our understanding (or lack thereof) of superantigen biology and participation in human disease.

Staphylococcal Enterotoxins

The staphylococcal enterotoxins (SE) are a series of structurally related proteins isolated from strains of *S. aureus* that cause gastroenteritis (reviewed in 18). The original serotyping nomenclature (SEA, SEB, SEC_{1-3}, SED, and SEE) is used to describe the different molecules. They all have a molecular mass of 25-30,000. They are structurally and functionally resistant to heat and proteolysis. There is a high degree of primary sequence homology among the different toxins, particularly within regions that have been predicted to be essential for their function. When given parenterally, the toxins produce fever and lethal shock in experimental animals (19). The LD_{50} for rabbits is 50 μg/kg. Enteral administration to primates causes a prompt (ca. 30 minute) emetic response. In tissue culture, the toxins are potent T cell mitogens (20). This observation led to the elucidation of superantigen action.

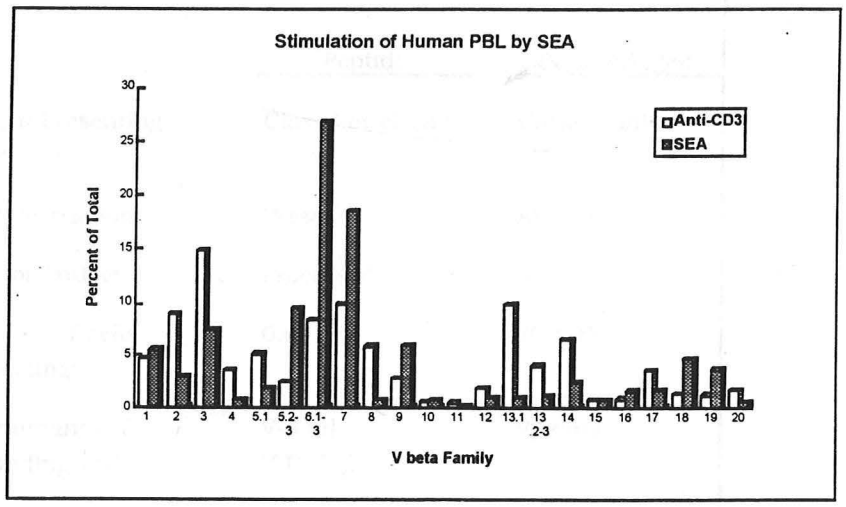
Unlike conventional mitogens such as concanavalin A, the staphylococcal toxins are active at sub-nanomolar concentrations. They stimulate the production of IL-2 and gamma interferon from T cells (21, 22), and IL-1 and tumor necrosis factor from monocytes (23, 24). The ability of the toxins to stimulate T cells requires the presence of class II-bearing cells in the culture (25). Unlike the response to conventional

antigen, this response does not require that the MHC haplotypes of the T cell and APC be the same (MHC restriction). Formaldehyde fixation of the antigen presenting cells, which abrogates their ability to process protein antigens such as ovalbumin, does not alter their ability to present superantigens. Fragmentation of the toxins results in loss of their activity (26). In contrast, proteolytic digests of nominal protein antigens are still active. Finally, direct binding of the staphylococcal enterotoxins to cells that express class II, but not class II-negative cells, has been demonstrated (26, 27, 28, 29). This binding could be demonstrated on class II molecules of different isotypes and alleles, suggesting that a conserved region of the molecule was responsible for binding.

The most striking feature of staphylococcal toxin-induced T cell stimulation is the nature of the T cell receptors expressed by the responding cells. As many as 20 - 50% of all the T cells in a culture are capable of responding to a given toxin (30, 31). As noted above, only 1 in 100,000 T cells is capable of responding to a typical peptide antigen. The high proportion of cells responding to superantigens suggested that a structure less polymorphic than CDR1-3 is involved. Flow cytometry of toxin-simulated T cells stained with monoclonal antibodies to different Vβ domains demonstrates preferential expansion. Each toxin stimulates a discrete set of cells with different Vβ domains.

When staphylococcal toxins are administered to neonatal animals, there is an alteration in the adult T cell repertoire (30). There is specific loss of cortical thymocytes bearing the Vβ domains that are stimulated *in vitro*. Presumably, the presence of a strong activating signal at this stage of T cell development leads to negative selection of these T cells. Therefore, exposure to a superantigen at this age could affect the presentation of disease later in life by the elimination of either protective or regulatory T cell clones. While large alterations of the T cell repertoire due to endogenous superantigens have been described in mice (see below), there is no evidence that this occurs in humans. Nevertheless, it provides an attractive hypothesis to explain individual variation in susceptibility to disease.

The analysis of T cell receptor repertoire has been simplified by using the polymerase chain reaction (PCR) to quantify the expression of different Vβ domains. This technique is necessary to analyze all Vβ-expressing T cells, as there are some Vβ's for which no monoclonal antibody is available. Typically, RNA is isolated from T cells stimulated by a putative superantigen, and converted to cDNA. Aliquots of the cDNA are exponentially amplified by PCR using a primer that is specific for the Vβ region and the constant region. The amplification must be calibrated to be certain that the product accurately reflects the proportion of the mRNA in the starting material. The PCR products are applied to a membrane, and probed with a radioactive oligonucleotide common to all TcR β chain DNA. The bound radioactivity can be quantified and relative proportions of the different Vβ usage calculated.



Stimulation of T cells Expressing Different Vβ Families by SEA

For example, SEA preferentially stimulates several families of T cells from peripheral blood. Vβ5.2-3, Vβ6, Vβ7, Vβ9, Vβ18, and Vβ19 were enriched by co-culture of lymphocytes with this toxin. The control is stimulation with anti-CD3, which should activate all cells.

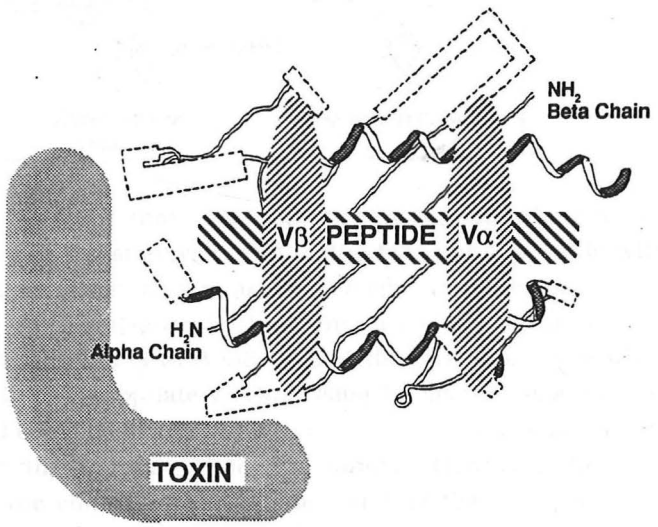
This technique is simple to perform, although there are several pitfalls to its interpretation. First, the choice of cells is critical. Many laboratories treat the T cells with IL-2 or anti-CD3 *in vitro* to have more mRNA to start with. This assumes that all cells will proliferate equally. Seldom is this tested experimentally. Second, the amplification must be performed in a manner that ensures that all Vβs are amplified with similar efficiencies. Third, this methodology should not become the "tail that wagged the dog". There is the tendency to apply the method and assume that any Vβ bias represents a superantigen effect. In the absence of an isolated molecule, this is circumstantial evidence at best and all other explanations (including technical artifact) should be considered.

Comparison of Peptide and Superantigen Presentation

	Peptide	Superantigen
Antigen Presenting Cells:	Class I or class II	Class II only
MHC Restriction:	Present	Absent
Form of Antigen:	Processed	Intact
Percent of T cells responding:	0.001%	20-50%
Determinants of responding TcR:	V-J (α) V-D-J (β)	Vβ only
Effect of *in vivo* administration:	Immunity (if given in proper form)	Deletion or anergy of responders

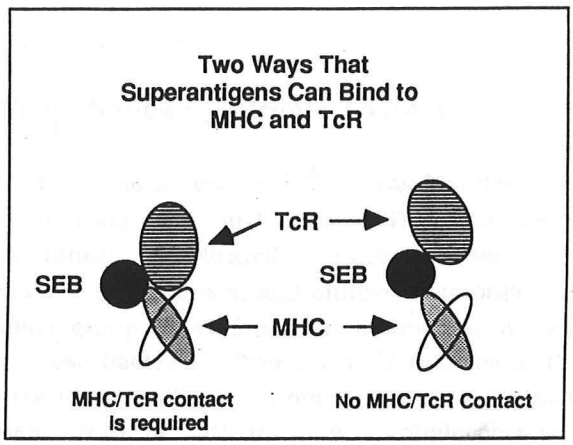
The molecular basis of staphylococcal toxin action is currently the subject of great interest. Cross competition experiments have

demonstrated separate, perhaps overlapping, binding sites for the toxins on class II molecules (28, 32). They are known to bind outside the antigen binding groove (33, 34). Two of the toxins, SEA and SEE, appear to contain a zinc atom (35). A major part of their binding to class II involves the coordination of this zinc between several histidines on the toxin and a critical histidine residue on class II (36). This histidine is highly conserved both evolutionarily and among different isotypes and alleles of class II. Therefore, these staphylococcal toxins have taken advantage of a motif that is probably essential for class II structure or function. The binding site(s) of other enterotoxins on class II is less well characterized.



The crystal structure of SEB has been solved (37). It has two major domains, each of which has residues that were shown to be important for class II binding by mutagenesis experiments. A shallow cavity formed by both domains contains the region suspected of interacting with TcR β chain. This raises the possibility that SEB acts by binding simultaneously to a complex of MHC and TcR. The alternative is that the enterotoxins are bifunctional crosslinking agents, without any requirement for TcR/MHC interaction. There are allelic differences in the

action of the staphylococcal enterotoxins on cloned T cells. This supports a role for TcR/MHC binding. However, this implies an innate affinity of TcR for MHC, regardless of antigen specificity. This has not been proven.



A major question that has not been solved is whether the superantigenicity of the staphylococcal toxins has anything to do with their clinical effect. Experimental animal models have demonstrated a requirement for T cell activation in the cachexia and shock that follows parenteral administration of SEB (38). This effect was only seen when inbred mice had the appropriate Vβ-expressing T cells to respond to the enterotoxin, and could be blocked by cyclosporin A. In humans, the role of the enterotoxins in toxic shock is minor. However, they are responsible for the clinical effects of one-fourth of the food poisoning cases in this country. The incidence of staphylococcal intoxication is higher in parts of the world where refrigeration of meat- or dairy-containing foodstuffs is a problem. The effect of the enterotoxins on the GI tract appears to be local, as denervated animals still have an emetic response. There is some evidence that activation of tissue mast cells within the GI tract leads to histamine release and symptoms of gastroenteritis. Intradermal injection of SEB in non-immune animals leads to rapid mast cell degranulation and an immediate type hypersensitivity reaction (39). This response could be blocked by H1 and

H2 receptor blockers as well as calcium channel blockers. These same agents were effective in blocking the emetic response after gastric challenge with SEB. Based on studies with chemically modified SEB, the portion of the molecule that is responsible for mast cell activation is distinct from the predicted TcR or MHC binding sites, and is conserved among the enterotoxins.

Toxic Shock Syndrome Toxin-1

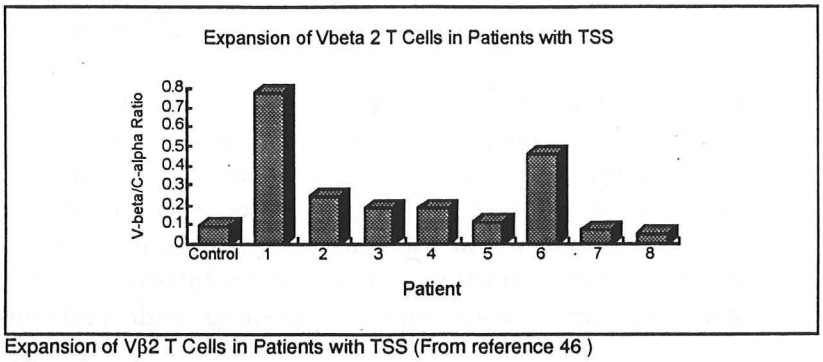
Toxic shock syndrome (TSS) was first described as an independent entity by Todd, et al., in 1978 (40). They had observed 7 children with a febrile illness, complicated by a desquamating rash, particularly of the palms and soles, shock, and multi-system organ failure. Staphylococci of phage group I were found in all patients. A novel toxin was isolated from these bacteria. Over the next two years, there were increasing reports of a similar syndrome, occurring primarily in menstruating women. 98% of patients grew staphylococci from vaginal cultures (versus 7% of controls). There was increased risk with tampon use. This epidemic peaked in mid-1980 with almost 140 cases per month reported to the CDC. 7.8% of reported cases were fatal. Since that time, the annual incidence has diminished to a constant level (224 cases reported in 1992) consisting of both menstrual and non-menstrual cases. The latter occur post-partum, in surgical wound infections, and soft-tissue infections. Diagnosis is based on the presence of fever, rash (diffuse erythroderma early; desquamation in 1-2 weeks), hypotension, and dysfunction of multiple organ systems in the absence of positive cultures for other infectious agents. Treatment is supportive, although antibiotics may prevent relapses.

The toxic shock syndrome toxin-1 (TSST-1) is produced by over 90% of staphylococcal strains isolated from patients with TSS (41). It can be isolated at concentrations of 3-50 ng/ml from blood, urine vaginal fluid, and abscesses. Similar to the enterotoxins, this protein has a molecular mass of ~22,000, and is easily purified from culture filtrates. The primary sequence of TSST-1 is quite different from the staphylococcal enterotoxins or exfoliative toxin (42). Experimental

animal models of TSS have been developed, primarily in rabbits. Fever, hypotension, mucocutaneous inflammation, dyspnea, liver dysfunction, and oliguria have all been demonstrated by either slow continuous subcutaneous infusion, or parenteral injection of TSST-1 (43). The LD_{50} in rabbits is between 20 and 70 μg/kg. In addition, TSST-1 has been shown to potentiate the lethal effects of endotoxin administration (44). However, it is not clear whether endotoxin is necessary for the development of TSS.

The effect of TSST-1 *in vitro* led to its description as a superantigen. In bulk cultures of peripheral blood mononuclear cells, it is a potent T cell mitogen and strong inducer of IL-1, tumor necrosis factor, and gamma interferon synthesis. There is also suppression of immunoglobulin synthesis by B cells that is characteristic of superantigen stimulation in mixed lymphocyte culture (45). TSST-1 binds directly to human and mouse class II molecules. Differences in the strength of binding to particular isotypes of class II have allowed mapping of the region that interact with the toxin. The binding site for TSST-1 appears to be distinct from that for other toxins, particularly SEB.

In vitro, TSST-1 stimulates T cells bearing the Vβ2 domain of the TcR almost exclusively (31). Normally, cells with this receptor make up a small fraction of peripheral T cells. Analyses of the T cell repertoire of patients with TSS have been performed. In one report, peripheral blood was obtained from eight patients with TSS (46). In four of the cases, TSS was menstruation-related. RNA was isolated from T cells that were stimulated by IL-2 and anti-CD3 antibodies. Semi-quantitative PCR was used to determine the relative expression of Vβ2-, Vβ5-, Vβ8-, and Vβ12-bearing cells.



Expansion of Vβ2 T Cells in Patients with TSS (From reference 46)

Five of the eight patients had elevation in Vβ2 T cells above the normal range during the acute phase of their illness. No difference was seen for the other Vβ families. 50% of circulating $CD4^+$ T cells bore Vβ2, as determining by monoclonal antibodies specific for Vβ2. $CD8^+$ cells were enriched for this Vβ as well. The level of Vβ2 T cells slowly returned to normal over 200 days. This expansion of Vβ2 cells is direct evidence of a superantigen effect in TSS. It documents a level of T cell activation not seen in the response to conventional antigens. The persistence of these cells is unlike the long-term depletion of responding cells seen in animals injected with staphylococcal toxins. This suggests that species differences in reaction to superantigens may be responsible for predominance of certain clinical effects.

Despite the *in vivo* evidence that TSST-1 is stimulating T cells as a superantigen, the exact pathogenesis of organ dysfunction in TSS is subject to debate. Certainly the release of large amounts of cytokines (particularly IL-1 and TNF) could cause the fever and constitutional symptoms. Hypotension could be the result of endothelial damage subsequent to tissue activation of T cells, a direct cardiodepressive effect, or the enhanced sensitivity to endotoxin. Hypotheses for the organ failure in TSS invoke mediators such as kinins, complement proteins, leukotrienes, and others, which would be secondary to the widespread T cell activation.

Streptococcal Toxins

Several of the proteins of *Streptococcus pyogenes* exhibit superantigen activity *in vitro*. This effect has been implicated in clinical syndromes caused by this bacteria. However, epidemiological studies and animal models are not as impressive as the case with the staphylococcal enterotoxins. A resurgence of rheumatic fever and fulminant streptococcal infections that arose in the late 1980's, suggests that a knowledge of these agents may be important in the management of severe infections.

Streptococcal Shock Syndrome

In 1987 - 89, a series of reports appeared describing a toxic shock-like syndrome in patients infected with highly virulent strains of *S. pyogenes* . Stevens, et al., published the most complete collection of information about this syndrome (47). They described 20 patients with fulminant Group A streptococcal infection. Most of these patients had soft tissue or intra-abdominal infections. All but one had septic shock, and the majority developed ARDS and acute renal failure. Despite antibiotics and intensive cardiovascular support, there was a 30% mortality. Bacteriological studies were performed on ten clinical isolates. There was a predominance of serotypes M-1 and M-3. Eight of the ten isolates also produced streptococcal pyrogenic exotoxin A (SPEA). This toxin was not found in bacteria isolated between 1976 and 1986, prior to the emergence of newly virulent strains. Strains of streptococcus isolated from scarlet fever patients prior to 1940 all produced SPEA. This suggests a possible relationship between scarlet fever and the toxic shock-like syndrome.

The related toxins streptococcal pyrogenic toxin -A (SPEA) and -B (SPEB) have been studied as superantigens (48). The purified toxins stimulated human peripheral blood T cells in an MHC class II-dependent, but not restricted manner. Semi-quantitative PCR was used to determine the Vβ repertoire of the responding T cells. SPEA preferentially stimulated Vβ8, Vβ12, and Vβ14. SPEB stimulated Vβ2

and Vβ8. There is little information on the clinical importance of these toxins.

Mollick, et al. isolated a novel toxin from a specimen of one of Stevens' original patients (49). This toxin is a 30 kD protein that exhibits class II-dependent T cell mitogen activity. The investigators termed this molecule SrSA (**Str**eptococcal **S**uper**a**ntigen). Interestingly, its amino acid sequence is highly homologous to the staphylococcal toxin SEB, rather than any of the known streptococcal toxins, including SPEA. The T cell receptor Vβ specificity of SrSA is distinct from SEB or SPEA. 10 of 12 bacterial isolates from patients with toxic shock-like syndrome produced this toxin. In addition, 11 of 12 isolates from patients with recurrent pharyngitis without systemic symptoms also produced this toxin.

Acute Rheumatic Fever

The third streptococcal protein that has superantigen properties is the M protein. This surface protein is the major virulence factor of streptococci. For many years the association of certain serotypes of *S. pyogenes* and acute rheumatic fever (ARF) suggested a direct role for the M protein in the pathogenesis of the disease. An attractive hypothesis has been that antigenic epitopes of M protein stimulate cross-reacting antibodies to cardiac tissue. In fact, M proteins from bacterial strains associated with ARF (e.g., M5 and M6) but not those from non-rheumatogenic strains (e.g., M24) share antibody epitopes with human cardiac tissue. Rabbit antisera raised against streptococci cross-react with human heart tissue. Patients with acute rheumatic fever with carditis and chorea often have antibodies in their sera that bind to sarcolemmal membranes of the heart and/or the caudate or sub-thalamic nuclei in the brain. However, no firm causal relationship between these antibodies and tissue destruction has been shown. Their presence may be only a reflection of the polyclonal gammopathy that accompanies the disease, and not a specific immune or auto-immune response.

In tissue culture, rheumatogenic M6 protein was shown to elicit non-MHC restricted cytotoxic T lymphocytes which could kill cultured human myocardial cells. In contrast, non-rheumatogenic M24 protein caused the expansion of cells that were not cytotoxic and suppressed the response to M6 (50). The proliferative effect of M protein was dependent on the presence of antigen presenting cells that express class II molecules. As expected for a superantigenic response, there was no requirement that the MHC haplotype of the stimulator and responder cells be the same. Direct binding of M protein to cells expressing class II was demonstrated (51). Finally, preferential expansion of Vβ2-, Vβ4-, and Vβ8-bearing T cells was seen in peripheral blood lymphocyte populations stimulated by M5 (52).

Although the superantigenic effects of the streptococcal exotoxins and M protein can be documented *in vitro*, there is only coincidental evidence that they are responsible for disease. The similarity of the clinical features found in the staphylococcal and streptococcal shock syndromes makes it appealing to invoke a common mechanism, namely superantigen intoxication. Further studies with patients with fulminant streptococcal infections will be needed to assess the potential alteration in T cell function and repertoire. Likewise, the role of M protein superantigenicity in ARF is only speculative. It is not evident whether the M protein is cleaved from the surface of the bacteria, enters the bloodstream, then somehow causes localized inflammatory responses. Alternatively, polyclonal T cell activation by M protein as a superantigen may be a byproduct of the immune response to a pharyngeal infection. This may lead to a break in T cell tolerance to auto-antigens in susceptible individuals (determined by HLA type, expression of T cell receptor Vα domains, etc.). As in the case with streptococcal shock, a careful analysis of T cell receptor expression in patients with ARF would be strong evidence of an *in vivo* superantigen effect. Efforts could then be focused to interfere with this mechanism of T cell stimulation in the hope that the systemic or autoimmune effects could be regulated without sacrificing protective immunity.

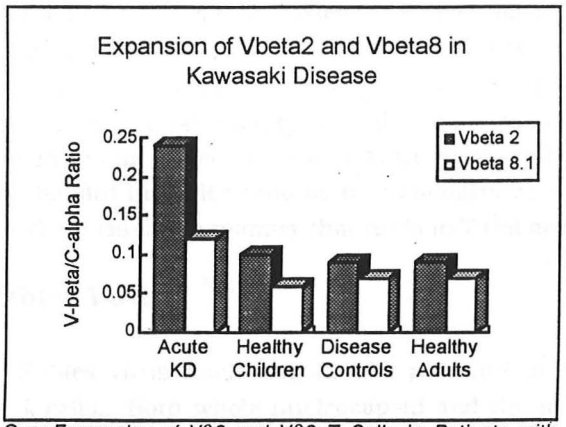
Diseases Potentially Involving Superantigens

Kawasaki Disease

The Vβ-specific expansion of T cells in response to stimulation by a superantigen is so striking that this observation alone is suggestive evidence for the involvement of one of these proteins in a particular disease process. One such example is Kawasaki disease (KD). This disease resembles toxic shock syndrome in some respects, although it has a very different epidemiologic pattern. It is primarily a disease of infants and children, with an increased prevalence in Japanese populations. It is characterized by nonexudative conjunctivitis, inflammatory mucocutaneous erythema, and cervical lymphadenopathy. Coronary vasculitis develops in 15-25% of cases. An infectious etiology for KD has been suspected, although no agent has been identified. Patients with KD show marked polyclonal T cell activation as well activation of monocyte/macrophages. This finding is consistent with superantigen stimulation. Further evidence for a superantigen in KD has been provided by the finding of restricted T cell Vβ usage in a group of patients from Japan and Hawaii (53). Fourteen patients with acute KD were analyzed just prior to treatment with intravenous immunoglobulin; eleven were re-tested 40 days later.

In most of the patients with acute KD, there was a selective expansion of T cells bearing Vβ2 and/or Vβ8.1. The level of the T cells was >2 standard deviations greater than the mean of normal populations. No other Vβ family was present at a level significantly different that normals. With convalescence, there was a return of the Vβ2 and Vβ8.1 cells to the normal range. KD is generally not seen in young infants who still have maternal antibody protection, nor in older children who are likely to have protective immunity to common pathogens. The authors of this study noted that a minority of patients with acute KD had specific antibody to streptococcal erthyrogenic toxin B (SPEB, see above). The T cells stimulated by this toxin are the same as

those expanded in the KD patients. This raises the possibility that KD is caused by streptococcal toxins or a functionally similar superantigen.



Over-Expression of Vβ2 and Vβ8 T Cells in Patients with Kawasaki Disease

Mouse Mammary Tumor Virus

The genomes of all mice carry 6-10 copies of integrated retroviruses. Most of these are defective viruses, however a few produce infectious particles. Many of them will cause mammary tumors, with virus appearing in the milk. Within the 3' long terminal repeat (LTR) of these mammary tumor viruses (mtv) is a large open reading frame coding for a ~320 amino acid protein (54). This protein is a potent superantigen that is expressed primarily in activated B cells (55). Sequence variation among MTV's confers selectivity for different sets of Vβ-bearing T cells (56). The mtv superantigen causes a mixed lymphocyte reaction between strains of mice that have identical MHC haplotypes. Because the superantigens are present from birth, the responding T cells in a mouse expressing that particular mtv are deleted during thymic ontogeny. Thus, for each mtv, there are strains of mice that can respond to the superantigen, and strains that express the superantigen, but cannot respond to it.

The B cells in the gut are the first cells to be infected by the milk-borne viruses. Superantigen expression here results in T cell activation and cytokine secretion that causes the B cell replication that is necessary for viral integration. The loss of T cells expressing several Vβ families does not seem to affect the functional immune repertoire of the mouse. Thus far, no similar system of endogenous superantigens has been described in other species. However, the study of the mtv superantigen will be helpful in understanding how endogenous proteins can interact with MHC class II in a manner that leads to T cell activation.

Rabies Virus

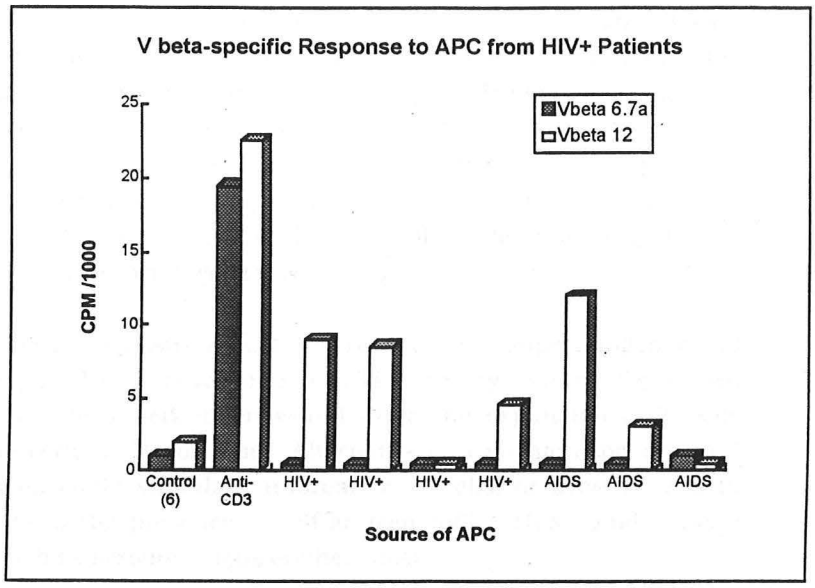
Rabies virus nucleocapsid is a powerful activator of peripheral blood T cells. Both whole nucleocapsid and the major constituent, N-protein cause proliferation when presented by either live or fixed antigen presenting cells (57). The proliferating population is enriched in Vβ8-bearing cells. Direct binding of nucleocapsid or purified N-protein to MHC class II molecules can be demonstrated by flow cytometry and Western blotting. N-protein appears to fulfill the "criteria" for being a superantigen. While there is no evidence that this superantigenic stimulation seen *in vitro* is related to the disease caused by rabies virus, there are implications for development of potent vaccines. This internal virus protein may have an adjuvant-like effect, leading to a more vigorous production of neutralizing antibodies against the viral envelope. However, if peripheral anergy follows a superantigen stimulation as in the case with the staphylococcal enterotoxins, then protective immunity could be compromised.

HIV

There are two reports that suggest a superantigen-like effect seen in patients infected with human immunodeficiency virus. Imberti, et al., noted a decrease in the expression of Vβ-14, -15, -16, -17, -18, -19, and -20 in AIDS patients compared to controls (58). The degree of abnormality of the T cell repertoire correlated with the stage of disease. Patients with CDC stage II had nearly normal expression of Vβ domains;

patients in stage III ($CD4^+$ lymphocytes $<200/mm^3$ with no opportunistic infections) had the most restricted pattern of Vβ expression. The authors conclude that this is consistent with the effect seen when superantigens are given to experimental animals. They hypothesize that there is an initial stimulation of certain T cells. This proliferation is necessary for retroviral integration. This is followed by deletion of those cells originally stimulated by the superantigen, whether they are infected, or not. This hypothesis is difficult to reconcile with the known kinetics of T cell lymphopenia in AIDS. Unfortunately, no other group has reproduced this data.

A second group has investigated the nature of T cells that are most susceptible to HIV infection (59). They established polyclonal T cell lines, expressing different Vβ domains, from normal donors and tested them for the ability to support HIV replication *in vitro*. Consistently, the T cells expressing Vβ12 had the highest level of $p24^{gag}$. This replication was confined to the $CD4^+$ fraction, and required the presence of antigen presenting cells during the initiation of the culture. Mitogen-stimulated T cells expressing other Vβ domains were able to support HIV replication to the same degree as un-stimulated Vβ12. These authors did not see any depletion of a particular Vβ family. In contrast, 2 of 4 patients showed a marked increase of Vβ12 bearing cells in the population that also stained with anti-gp120 antibody. Finally, the T cell-depleted PBL from $HIV\text{-}1^+$ patients or controls were used as stimulators for Vβ-enriched T cell lines (from uninfected donors). 5 of 7 HIV^+ patient samples caused proliferation of the Vβ12, but not the Vβ6.7a cell line. Normal PBL did not stimulate either line.



No candidate superantigen molecule has been isolated from HIV. There is no open reading frame in the LTR like mouse mammary tumor virus. The retrovirus that causes an AIDS-like disease in mice (MAIDS) expresses a gag polypeptide with superantigen effects. Whether this is the protein from human retroviruses that is responsible for the Vβ12 T cell activation is not known. If a superantigen is promoting HIV infectivity, its action could be the source of therapeutic intervention.

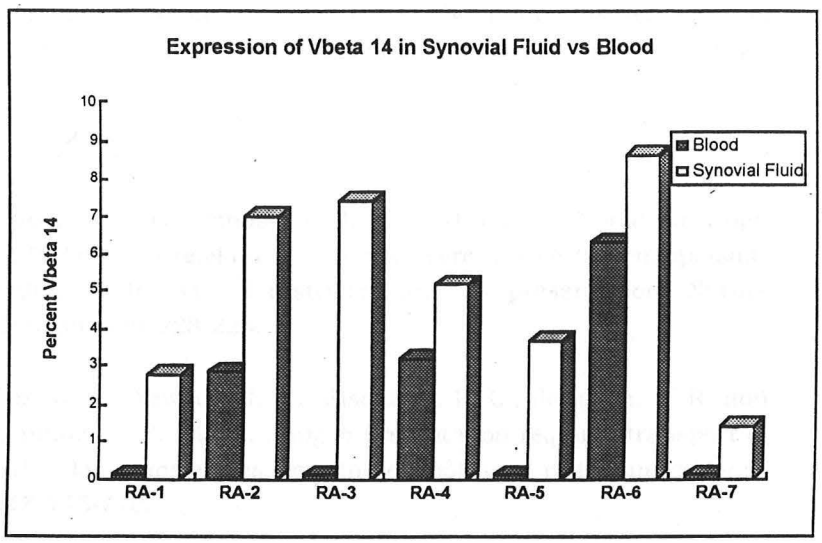
Autoimmune Disorders

There has been much speculation, a few suggestive reports, and continuing investigation into the question of whether superantigens play a role in autoimmunity. Their effect on lymphocyte activation could explain the immune hyper-responsiveness characteristic of many rheumatologic disorders. The implication of a microbial product in these disorders has been suggested since the 1890's. A rodent pathogen, *Mycoplasma arthriditis*, causes a chronic, destructive synovitis that is similar to rheumatoid arthritis in humans (60). This organism produces a superantigenic protein that stimulates human T cells (61). Other

evidence for involvement of infectious agents in autoimmune disease includes the finding that certain T cells cloned from the synovial fluid of patients with rheumatoid arthritis react to *Mycobacterium tuberculosis* or mycobacterial heat shock proteins (62, 63, 64). In addition, mRNA for human T lymphotropic virus-I (HTLV-I) was isolated from synovial cells of patients with tropical spastic paraparesis who exhibit an inflammatory arthritis (65). These cells also stimulate T cell proliferation, suggesting a possible role for retroviral gene products in the synovitis.

In theory, a tissue-specific expression of a superantigen could cause the polyclonal activation of T cells in the synovium. Continued exposure to the superantigen would drive the expansion of T cells expressing certain Vβ domains. Alternatively, systemic exposure to a superantigen could stimulate autoreactive T cells, or drive B cells to differentiate in the presence of self-antigen (66). This could explain diseases such as systemic lupus erythematosus.

A number of studies have appeared to address this question in rheumatoid arthritis (RA). In general, there is no agreement as to whether there is a Vβ bias, or which Vβ's are predominant. Two studies found evidence for selective Vβ expansion in patients with RA. Paliard, et al., found that T cells bearing Vβ14 were enriched in the synovial fluid of patients with severe RA, when compared to the level of Vβ14 cells in the peripheral blood (67). 6 of 9 patients had no Vβ14 cells in the periphery. This was distinct from patients with other forms of arthritis or normal controls. $V\beta14^+$ T cells in the synovial fluid demonstrated restricted sequence heterogeneity. The interpretation of these data is that an unknown superantigen activated Vβ14 T cells, causing their ultimate depletion in the periphery. Some of these Vβ14 cells might react to synovial autoantigens, home to the joint, and initiate an inflammatory response.



A second paper demonstrated expansion of Vβ3 and 17, in addition to Vβ14 T cells from the synovium of RA patients (68). A region of sequence similarity among these T cell receptors was found that corresponded to the CDR4 domain shown to control bacterial and retroviral superantigen binding. It is important to note that both of these studies used IL-2 activated cells from patients with severe disease. There was no control for the percentage of $CD4^+$ or $CD8^+$ cells isolated, or for MHC haplotype, all of which may affect the distribution of Vβ families. Finally, these findings have not been corroborated by other groups studying RA using identical techniques.

Conclusion

Superantigens are currently the focus of over 50 laboratories worldwide. Many others are using them as reagents to probe the effects of polyclonal T cell activation. They play a central role in the pathogenesis of several infectious diseases. The true involvement of these substances in diseases such as rheumatic fever, Kawasaki disease, and sarcoidosis is being evaluated. These agents may hold clues to the

cause(s) of autoimmunity. Finally, the study of superantigen function may lead to promising development of novel immunotherapeutic agents.

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