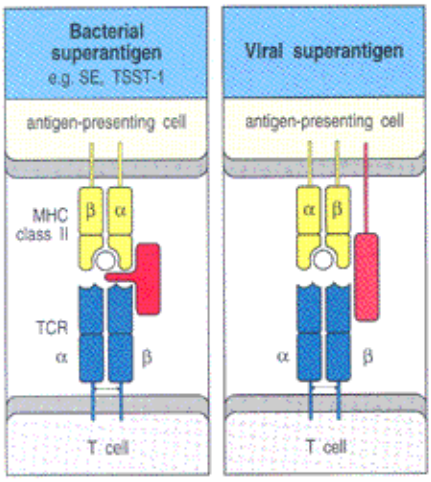
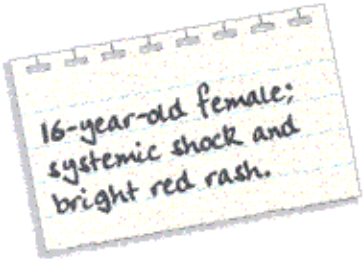


Fig. 7.1 Superantigens bind directly to T-cell receptors and MHC molecules. Superantigens interact with MHC class II molecules and T-cell receptors in a way that is quite distinct from the way that normal peptide antigens bind. Superantigens bind independently to MHC class II molecules and to T-cell receptors, binding to the V_β domain of the T-cell receptor, away from the peptide-binding site. The T-cell receptor α chain is not directly involved in binding superantigen.

The case of Claire Bourbon: life-threatening shock from a superantigen.

Claire Bourbon was a healthy 16-year-old with a history of mild asthma and allergic rhinitis who suddenly became ill with a fever, general muscle aches, and dizziness. She felt nauseous and vomited. Claire's temperature rose to 39.8°C and her mother rushed her to the family physician. En route she briefly lost consciousness, and a red rash developed over her arms and spread rapidly to most of the body.

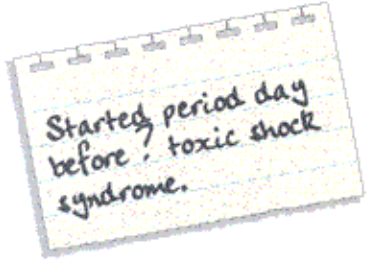
Upon arrival at the physician's she appeared quite ill and was immediately referred to the Emergency Department. She was alert but listless and her general condition gave cause for concern. On examination, her temperature was 37.8°C, and heart rate and respiration rate were markedly elevated, at 140 beats per minute and 24 breaths per minute respectively. Blood pressure was depressed—98/67 lying supine, 83/49 when seated, and 67/25 when standing—and showed evidence of significant volume depletion.



A bright red rash of flat and raised lesions was apparent on her trunk and extremities, but there were no petechiae (small subcutaneous hemorrhages) and no signs of localized infection.

Questioning revealed that Claire had not taken alcohol or drugs and had not been exposed to other ill individuals. Her last menstrual period had been 6 weeks before, and she had developed vaginal bleeding on the day previous to the onset of her illness. She had not used a tampon overnight, but had inserted one that morning, before she became ill.

Given her critical status, extensive laboratory tests were carried out. Her white blood cell count was raised, at 21,000 cells μl^{-1}, with a predominance of neutrophils and band forms (immature neutrophils), indicating increased mobilization of neutrophils from the bone marrow. Serum electrolyte levels were within normal limits. The blood coagulation time was slightly prolonged and serum transaminase levels were raised; both of these signs are consistent with abnormal liver function.



Cerebrospinal fluid (CSF) was normal and showed no evidence of infection. Cultures of blood, urine, CSF, and vaginal fluid were made and Claire was given a cephalosporin antibiotic (ceftriaxone) along with 2 liters of fluid intravenously. Her blood pressure improved and she was immediately admitted to the intensive care unit, where she developed petechiae. She was treated with intravenous fluids, two anti-staphylococcal antibiotics (oxacillin and clindamycin) as well as a cephalosporin (cefotaxime) with gradual improvement in her overall condition.

Her blood, urine, and CSF cultures remained sterile, but her vaginal culture was positive for abundant *S. aureus*. She was subsequently transferred to the regular in-patient ward and treated for 7 days with anti-staphylococcal antibiotics. The rash gradually faded.

Toxic shock syndrome.

Claire suffered from staphylococcal toxic shock syndrome (TSS), a striking example of the dramatic physiologic alterations caused by superantigens. TSS is a serious disease characterized by rapid onset of fever, a rash, organ failure, and shock. The majority of cases occur in menstruating women, typically in their teenage years, but cases do occur in all age groups. TSS is typically associated with a localized *S. aureus* infection (for example, subcutaneous abscesses, osteomyelitis, and infected wounds), staphylococcal food poisoning, or local colonization, as occurred in the vagina in this case. When kept in the vagina for a long time (>12 hours) tampons soaked with menstrual fluids can enhance the growth of the bacteria which are the source of superantigens. It is unlikely that the tampon played a part in Claire's disease as it was inserted less than 6 hours before the onset of symptoms. As well as TSST-1, toxigenic strains of *S. aureus* can produce other enterotoxins (such as enterotoxin B) that act as superantigens, with similar clinical consequences. In addition, microorganisms other than *S. aureus* secrete superantigens that can cause disease (Fig. 7.2).

Disease	Superantigen	TCR V_β
Definite role for superantigen		
Toxic shock syndrome	TSST-1	$V_\beta2$
Staphylococcal food poisoning	SEA	$V_\beta3$, $V_\beta11$
	SEB	$V_\beta3$, $V_\beta12$, $V_\beta14$, $V_\beta15$, $V_\beta17$,$V_\beta20$
	SEC	$V_\beta5$, $V_\beta12$, $V_\beta13.1$–2, $V_\beta14$, $V_\beta15$, $V_\beta17$,$V_\beta20$
	SED	$V_\beta5$, $V_\beta12$
	SEE	$V_\beta5.1$, $V_\beta6.1$–3, $V_\beta8$, $V_\beta18$
Streptococcal toxic shock syndrome	SPE-A	$V_\beta8$, $V_\beta12$, $V_\beta14$
Scarlet fever	SPE-B	$V_\beta2$, $V_\beta8$
Mycoplasma arthritidis (rodent)	MAM	$V_\beta17$
Clostridium perfringens	Enterotoxin	$V_\beta6.9$, $V_\beta22$
Suspected role for superantigen		
HIV	CMV	$V_\beta12$
Type I diabetes mellitus	MMTV-like	$V_\beta7$
Rabies virus	Nucleocapsid	$V_\beta8$
Toxoplasmosis	?	$V_\beta5$
Mycobacterium tuberculosis	?	$V_\beta8$
Yersinia enterocolitica	?	$V_\beta3$, $V_\beta6$, $V_\beta11$
Kawasaki disease	?	$V_\beta2$, $V_\beta8$

Fig. 7.2 Superantigens, V_β usage and disease.

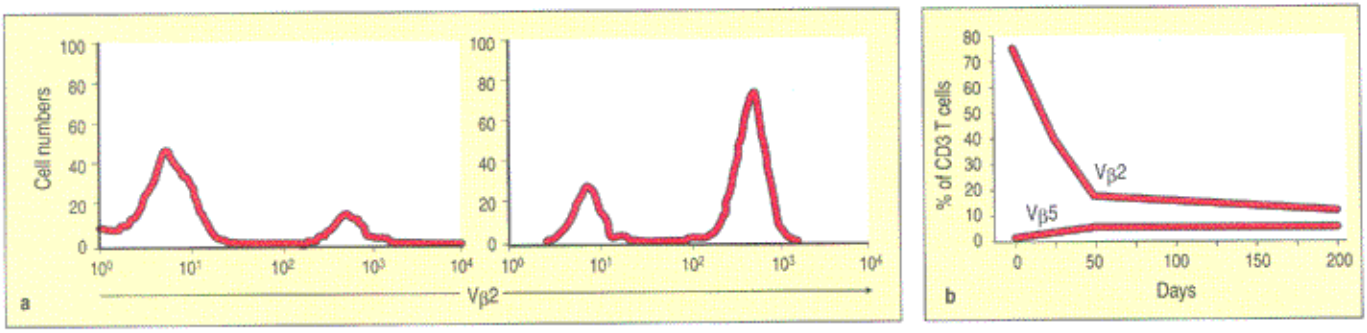


Fig. 7.3 Expansion in numbers of $V_\beta 2$ T cells in toxic shock syndrome (TSS). Panel a, FACS analysis. Peripheral blood T cells from a normal control (left) and a patient with acute TSS (right). Cells are stained with anti-$V_\beta 2$ monoclonal antibody with a fluorescein tag. There is an increased percentage of $V_\beta 2$ T cells in the patient. The horizontal axis represents the mean fluorescence intensity. Panel b, time course of persistence of high numbers and return to normal of $V_\beta 2$ T cells in a patient with TSS.

Consistent with the $V_\beta 2$ specificity of TSST-1, examination of the circulating lymphocytes from patients in the acute phase of TSS typically reveals a much higher proportion of circulating $V_\beta 2$ T cells compared with cells using other V_β segments. As the illness resolves there is a gradual return to near normal proportions. The expansion in the numbers of $V_\beta 2$ cells can be measured by examining the surface expression of T-cell receptors containing a $V_\beta 2$ region using immunofluorescence (Fig. 7.3) or by semiquantitative measurement of mRNA transcripts encoding $V_\beta 2$ T-cell receptor chains using reverse transcription and the polymerase chain reaction (RT-PCR) (Fig. 7.4).

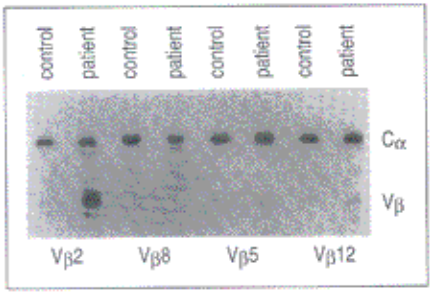


Fig. 7.4 RT-PCR analysis of T-cell receptor V_β mRNA. Autoradiograms of T-cell receptor chain transcripts amplified by reverse transcription followed by polymerase chain reaction (RT-PCR). T cells from a patient with toxic shock syndrome and a control individual were stimulated with anti-CD3 antibody and IL-2 before the extraction of RNA and generation of cDNA. Each reaction contained specific oligonucleotide primers to expand the particular V_β gene segment indicated (170–220 base pairs), as well as a C_α gene segment (approx. 600 bp) as a control to ascertain that equivalent amounts of mRNA were used. Photograph courtesy of Y. Choy.

Although all the T cells activated by a given superantigen share a common V_β region, they will differ in their specificity for conventional peptide antigens. Sequencing of the T-cell receptor from superantigen-activated T cells reveals a different use of D and J gene segments by the β chains and a wide diversity of α chains. These receptors will encompass a wide variety of antigen specificities. In contrast to superantigen, conventional antigen induces the clonotypic expansion of T cells. All the T cells in any given clone will have identical D and J gene segments in their β chains and identical α chains. Because the pool of V_β-restricted T cells activated by superantigen may contain autoreactive T cells, it has been postulated that superantigens may trigger autoimmune disease.

Many of the manifestations of TSS are the result of massive and unregulated cytokine production triggered by the activation of immune system cells. TSST-1 is more effective than bacterial lipopolysaccharide in inducing the synthesis and secretion of interleukin (IL)-1 and tumor necrosis factor (TNF)-α by monocytes. TSST-1 is also a potent T-cell mitogen for those T cells whose receptors it engages; it also induces them to produce large amounts of cytokines, including IL-2 and interferon (IFN)-γ.

IL-1 and TNF-α are critical in the induction of the acute-phase response and induce fever and the production of IL-6. IL-1 and TNF-α also activate vascular endothelium and, together with IL-2, increase vascular permeability with the subsequent leakage of fluid from the intravascular space into the perivasculature. It is these effects of the massive overproduction of TNF-α that result in the toxic shock: edema and intravascular volume depletion leading to hypotension and shock with multiple organ failure.

Susceptibility to TSS seems to correlate with a poor antibody response to TSST-1. While the majority of healthy individuals have protective antibody titers to TSST-1, more than 80% of patients with TSS lack anti-TSST-1 antibodies during the acute illness, and most fail to develop anti-TSST-1 antibodies following convalescence. Possible explanations include an inability on the patient's part to mount an antibody response against TSST-1 and staphylococcal enterotoxins, or a specific inhibition of such a response by the toxins.

Discussion and questions.

1 How do you determine whether a protein behaves as a superantigen?

Superantigens, but not conventional antigens, can activate naive T cells. Superantigens will thus induce the proliferation of lymphocytes from newborns and from the thymus, as previous exposure to the antigen and expansion of the number of antigen-reactive cells is not required. Superantigens do not require processing by accessory cells and are thus able to induce the proliferation of purified T cells in the presence of paraformaldehyde-treated monocytes, which lack the capacity to process antigen. Direct binding of a labeled protein to cells positive for MHC class II, or its co-precipitation with MHC class II molecules, confirms it as a superantigen.

2 Explain the rapid progression of clinical symptoms following introduction of superantigen compared with the delay in apparent responses to conventional antigen.

During the evolution of an adaptive immune response to conventional antigen, a cascade of events must occur over a relatively long period of time. The antigen has to be internalized, processed, and presented as peptide:MHC complexes by antigen-presenting cells. The complexes are recognized only by those T cells bearing a T-cell receptor specific for the antigen-derived peptides—a fraction of a percent (<0.1%) of the entire T-cell pool. These few antigen-specific T cells must then proliferate and bystander cells be recruited before an effective response can be mounted. In contrast, superantigen-induced immune activation is independent of antigen processing, thus bypassing the first step, and immediately activates a sizeable fraction of T cells. A very small number of superantigen molecules is sufficient to activate a T cell with the appropriate V_β region in its receptor (<10 molecules per T cell). Activation results in a massive secretion of T-cell cytokines, which include IL-2, IFN-γ, TNF-α, and TNF-β. In addition, superantigens can directly activate monocytes and dendritic cells by cross-linking their surface MHC class II molecules. Cross-linking is effected by superantigens bound to T-cell receptor β chains and/or because a number of superantigens, including TSST-1, have two distinct binding sites for MHC class II molecules. Cross-linking of MHC class II molecules causes a rapid and massive release of cytokines such as IL-1, TNF-α, IL-6, IL-8, and IL-12. This is associated with the upregulation of B7 co-stimulatory molecules on these cells, which, together with cytokine action, further amplifies T-cell activation by superantigen. Thus, minute amounts of superantigen are sufficient to rapidly activate a large number of T cells and monocytes/macrophages, resulting in an amplificatory loop and in a massive outpouring of cytokines, which leads to the rapid appearance of clinical symptoms.

3 What are the potential mechanisms of liver injury in TSS?

Liver injury may occur as a result of decreased organ perfusion during hypotension. However, immunologic mechanisms may also contribute to injury. Hepatocytes express Fas, a cell-surface molecule crucial for the induction of apoptosis (programmed cell death). T-cell activation by superantigens and the massive release of cytokines results in the upregulation of the natural ligand for Fas—FasL—on the surface of circulating lymphocytes. Cross-linking of Fas on hepatocytes by FasL on circulating lymphocytes results in the triggering of apoptosis in hepatocytes. In addition, circulating cytokines such as TNF-α are also capable of triggering cell death and can result in liver injury.

4 Is Claire susceptible to another bout of TSS?

Protection against toxic shock is conferred by antibodies against the superantigen, which neutralize it before it can cause disease. In order to stimulate an antibody response, the superantigen must be recognized, internalized, and processed by superantigen-specific B cells which then present the antigenic peptides to antigen-specific T cells. These are activated to become helper T cells that can in turn stimulate the production of superantigen-specific antibodies on reexposure to the superantigen. Antibodies to other antigens that cross-react with the superantigen may also confer protection.

In humans, there is evidence that during and following TSST-associated illness, $V_\beta 2$ T cells become anergic and thus cannot provide help to superantigen-specific B cells. Patients with TSS therefore fail to develop TSST-1-specific antibody. So Claire is, unfortunately, likely to be at risk of another episode of TSS. Hopefully, she will eventually develop anti-TSST-1 antibodies.