MINIREVIEW

Superantigens: Structure and Relevance to Human Disease* (43996)

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Abstract. **Superantigens are a class of immunostimulatory molecules produced by bacteria and viruses. Their potent immune effects are due to their unique ability to bind to the major histocompatibility complex (MHC) outside the antigen-binding cleft and to stimulate T cells in a T-cell receptor (TCR) Vβ–specific manner. Structural studies have revealed the binding sites involved in the MHC/superantigen/TCR complex. The bacterial superantigens are responsible for a number of syndromes, including food poisoning and toxic shock syndrome, but their effects may be not only acute but also chronic and complex. Recent evidence suggests that superantigens may be relevant to the pathogenesis of autoimmune and immunodeficiency disorders. To illustrate the detrimental effects of superantigens on disease outcome, evidence demonstrating the modulation of experimental allergic encephalomyelitis, an animal model for multiple sclerosis, by superantigen, as well as the potential role of superantigens in HIV pathogenesis of AIDS, will be presented. The information presented may provide valuable insight into the role of superantigens in autoimmunity and HIV infection.** [P.S.E.B.M. 1996, Vol 212]

The prototype superantigens, staphylococcal enterotoxins (SEs), are responsible for staphylococcal food poisoning and toxic shock syndrome (1). Superantigens form complexes with major histocompatibility complex (MHC) class II molecules on antigen-presenting cells (APC) by binding to the outside of the antigen-binding cleft and polyclonally activate Vβ–specific T cells (2–5). As much as 20% of the T-cell repertoire can be activated by superantigen. This activation results in prodigious production of cytokines, which are responsible for all or part of the toxicity associated with superantigens (4, 6, 7).

Superantigens are not restricted to bacterial toxins, but may be associated with a number of viruses, including retroviruses, as well as with *Mycobacterium tuberculosis* (Table I). Though their clinical effects may be acute, they may also be chronic, protracted, and complex. Evidence suggests that superantigens are involved in autoimmune disorders (25, 26), and may play a role in the immunodeficiency associated with acquired immune deficiency syndrome (AIDS) (27).

Recent observations have brought a number of advances in the understanding of the structural interactions between MHC class II molecules, superantigens, and the T-cell receptor (TCR). In addition, superantigens have been implicated as environmental factors that play a role in the exacerbation of certain autoimmune and immunodeficiency disorders. We will focus on experimental allergic encephalomyelitis (EAE), a mouse model for multiple sclerosis (MS), to illustrate how superantigens can exacerbate EAE in a remitting/relapsing manner, similar to MS (10, 11). In addition we will discuss the possible role of superantigens in

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Table I. List of Bacterial and Viral Superantigens

	Protein	Superantigen-associated disease	References
Bacterial			
Prototype: Staphylococcal enterotoxins			
Organism			
Staphylococcus aureus	Enterotoxins	Food poisoning	1
		Toxic shock syndrome	1
		Kawasaki's disease (?)	8, 9
		Multiple sclerosis (?)	10, 11
Group A streptococci	Pyrogenic exotoxins	Psoriasis (?)	9, 12
		Rheumatic heart disease (?)	9
Mycoplasma arthitidis	T-cell mitogen	Arthritis (?)	13
Mycobacterium tuberculosis	Not identified	Tuberculosis	14
Yersinia pestis	Not identified	Reiter's syndrome	15
		Reactive arthritis	15
Clostridium perfringens	Exotoxin	Sudden infant death syndrome (?)	16, 17
Viral			
Prototype: Superantigen from mouse mammary tumor virus (MMTV)			
Virus		Protein	
Mouse mammary tumor virus (type B retrovirus)	ORF product	Mammary tumors	18
Mouse leukemia virus (type C retrovirus)	Gag protein	MAIDS	19
Human immunodeficiency virus (lentivirus)	Not identified	AIDS	20, 21, Torres BA *et al.*, submitted
Human spumaretrovirus (foamy virus)	bel 3 gene product	Grave's disease (?)	22
Rabies	Nucleocapsid protein	Rabies	23, 24
Epstein-Barr virus (human herpes virus)	Not identified	B cell lymphoma (?)	9
		Chronic fatigue syndrome (?)	9

the pathogenesis of human immunodeficiency viruses (HIV). Altered Vβ T-cell profiles in HIV-infected individuals have been shown, suggesting the presence of a superantigen (28–30). We have recently shown that the Nef protein of HIV is a T-cell mitogen with properties characteristic of a superantigen (21). Thus, the data and models presented should shed light on the role of superantigens in autoimmunity and viral diseases such as AIDS.

Structure of Staphylococcal Enterotoxins

Initial efforts toward understanding the structural basis for the biological activities of SEs involved secondary structure predictions using the amino acid sequence compared with structural information derived from techniques such as circular dichroism (CD). The SEs characteristically have low α-helical content together with a high content of β-structure, as indicated by CD analysis (31). The CD spectra of staphylococcal enterotoxin B (SEB) and the closely related SEC (65% sequence homology) are essentially identical (31, 32). The CD spectrum of SEA varies somewhat from that of SEB or SEC, although it reveals the same predominance of β-structure (32, 33). While SEE is similar to SEA in the high percentage of β-structure, SEA has more α-helical content than SEE (33). Toxic shock syndrome toxin-1 (TSST-1) possesses low α-helix and high β-structure content characteristic of the other SEs, but shares minimal sequence homology with them (34). Thus, despite differences in amino acid sequences and antigenic properties, all of the SEs, including TSST-1, exhibit a similar pattern of secondary structure (low α-helix, high β-sheet), which is compatible with their common biological functions.

The 3-dimensional structures of SEB and TSST-1 have recently been determined using x-ray crystallography. The crystal structure of SEB revealed a main chain fold containing two domains, the first of which is composed of residues 1–120 and contains five β-strands and three α-helices (35). The β-strands of the first domain form a cylindrical barrel forming a criss-cross pattern. The second domain is composed of residues 127–239 and contains seven β-strands and two α-helices. The β-strands 6 and 12 are parallel and form an infrequently observed left-handed crossover. A shallow cavity formed by the two domains is considered to be a TCR-binding site. The TSST-1 crystal structure revealed a single-chain, two-domain molecule (36, 37). The first domain is composed of residues

1–89 and contains five β-strands and one α-helix. The β-strands of the first domain form a continuous roll or β barrel. The second domain is composed of residues 90–194 and contains seven β-strands and one α-helix. The α-helix in the second domain appears to be cupped by the surrounding β-strands forming an elaborate β-grasp motif. The topology of SEB and TSST-1 is similar in that they are both composed of two domains and exhibit a high content of β-structure relative to α-helix. However, a number of differences in the SEB and TSST-1 structures exist. Among them are (i) that TSST-1 is unique from SEB in that it contains a β-grasp motif; (ii) that TSST-1 is extensively truncated in a number of loop regions present in SEB; (iii) that there is no structural equivalent of the N terminus of SEB in the TSST-1 structure; and (iv) that the TSST-1 structure contains fewer α-helices than the SEB structure. Thus, a general two-domain motif is apparent from the SEB and TSST-1 3-dimensional structures, but certain structural features important for SEB are not present in TSST-1, probably due to the lack of TSST-1 amino acid sequence homology with the other staphylococcal enterotoxins.

The 3-dimensional structure of SEB complexed to a human MHC class II molecule, HLA-DR1, revealed the DR1-α1 domain as a binding site for SEB and confirmed studies, which will be discussed below, that demonstrated the N terminus of SEB as a binding site for HLA-DR1 (38). No overt conformational changes were observed to occur in the MHC class II molecule or SEB upon formation of the complex. Interestingly, the MHC/SEB crystal structure suggests a model of the MHC/superantigen/TCR complex where MHC interaction with the TCR is distinct from interactions induced by peptide antigen presentation and may even block such peptide-mediated interaction.

The structural basis for SEs interaction with MHC class II molecules has been elucidated by several approaches, with the synthetic peptide approach providing particularly valuable results. Studies of SEA employing overlapping synthetic peptides of the entire molecule identified regions 1–45, 39–66, 62–86 and 121–149 as being able to compete with radiolabeled SEA for binding to HLA-DR (39–41). SEA (1–45) also inhibited SEA induction of T-cell proliferation and gamma interferon (IFN-γ) production (39). Interestingly, the SEA peptide 121–149 itself possesses superantigenic-like activity in that it stimulates T-cell proliferation, but has to be presented *via* interaction with the MHC class II molecule (Hobeika AC, Johnson HM, unpublished data). Furthermore, peptide 121–149 stimulates macrophages through a lymphocyte pathway to produce tumor necrosis factor–α and interleukin-1 (IL-2) (42). These observations suggest that the staphylococcal enterotoxin superantigens are more versatile in their interaction with cellular receptors then is indicated by x-ray crystallographic studies.

Sites on the MHC class II molecule important for interaction with SEA have also been identified. Overlapping peptides of the MHC class II β-chain of the H-2^b haplotype were examined for their ability to inhibit SEA binding to MHC class II and HLA-DR. The region encompassing amino acid residues 65–85, corresponding to the predicted α-helix along the antigen-binding cleft of the β-chain, preferentially inhibited SEA binding to MHC class II and HLA-DR and SEA stimulation of human and mouse T-cell proliferation (43). Likewise, a peptide encompassing amino acid residues 51–80 of the MHC class II H-2^b haplotype α-chain, corresponding to the predicted α-helix along the antigen binding cleft of the α-chain, inhibited SEA-induced human and mouse T-cell proliferation and bound directly to SEA in a solid phase binding assay (44). Subsequent site-directed mutagenesis studies have confirmed the results described above and have identified specific residues of the MHC class II β-chain, in particular histidine 81, as important for binding SEA (45).

With the same approach as above, the functional sites on the enterotoxins, TSST-1 and SEB, for binding to MHC class II molecules have been identified. Synthetic peptides of the TSST-1 molecule encompassing amino acid residues 39–68 and 155–194 competed with radiolabeled TSST-1 for binding to mouse A20 cells and human Raji cells (46). Like SEA, multiple and complex MHC class II binding sites have been identified on SEB. Studies were undertaken to compare the regions of SEB important for binding to L cells transfected with HLA-DR1 and Raji cells bearing HLA-DR3, DRw10, DQw1, and DQw2 (47). Synthetic peptides encompassing SEB amino acid residues 1–33, 31–64, and 179–212 inhibited binding of radiolabeled SEB to DR1 transfected L cells. In contrast, SEB peptides encompassing residues 1–33, 124–154, 150–183, and 179–212 inhibited binding of SEB to Raji cells. SEB peptide 124–154 also inhibited SEB induced human T cell proliferation. While SEs do not exhibit MHC restriction, they do exhibit haplotype preference as evidenced by the fact that HLA-DR haplotypes vary in their ability to bind and present some of the SEs (48). These results suggest that different regions of SEB may be involved in binding to various alleles of HLA-DR as well as DQ molecules expressed on Raji cells. The binding of different regions of SEB to MHC class II molecules would imply that the Vβ regions of the T cell receptor may not all recognize a given superantigen in the same way. Superantigen Vβ specificity may vary depending on the MHC class II molecule involved in presentation. Thus, it would appear that the contacts in the MHC/superantigen/TCR com-

plex are quite dynamic, depending on the nature of the binding of superantigen to MHC class II.

Superantigens in Autoimmunity

Superantigens in Neurologic Inflammatory Disease. MS is an inflammatory demyelinating autoimmune disease of the central nervous system manifested by paralysis that affects speech, motor functions, and vision. The symptomology of MS can often be observed to occur in a relapsing/remitting manner, consistent with presentation of clinical symptoms of MS followed by periods of remission. The relapsing/remitting nature of some autoimmune disorders has raised the question of how environmental factors affect patients with autoimmunity. Intuitively, superantigens are suspected of playing a role in causing or modulating some autoimmune disorders based on their ubiquitous presence in the environment and their potent polyclonal T cell stimulatory effects. Studies were conducted to determine if superantigens could modulate the course of EAE in PL/J mice (25), an autoimmune neuropathogenic disorder that is studied as a model for MS (49).

PL/J mice immunized with rat myelin basic protein (MBP) develop acute demyelination manifested clinically as tail or hind limb weakness and paralysis. Those mice that survive the acute episode of disease will usually resolve these clinical symptoms and do not develop spontaneous or MBP-induced relapses (50). It has been shown that, in the PL/J mouse, the predominant initiating T cells are an oligoclonal population bearing the Vβ8 TCR. Analysis of TCR gene rearrangements from MS patient brain plaques suggests that $V\beta5.2^+$ T cells may be critical in MS (51).

Two scenarios were examined in assessing superantigen modulation of EAE in PL/J mice. The first being possible protective effects of treatment with a Vβ8-specific superantigen prior to immunization with rat MBP in order to induce Vβ8 T-cell anergy and deletion, thus blocking EAE development. The second is to administer superantigen after sensitization of mice to MBP to determine if superantigen reactivates or exacerbates disease.

To study the potential protective effect of superantigen pretreatment, PL/J mice were treated with Vβ8-specific staphylococcal enterotoxin B (SEB). After 1 week, Vβ8 T cells of the treated mice failed to respond to SEB *in vitro* (52). Injection of these mice with SEB prior to immunization with rat MBP in Freund's complete adjuvant (CFA) and pertussis toxin, resulted in protection against EAE induction when compared with controls. Protection from EAE by superantigen appears to be dependent on the Vβ specificity of the superantigen.

Similar observations were made for MRL/lpr mice, a model for systemic lupus erythematosus in which anti-DNA autoantibody production and immune complexes correlates with the onset of disease at 3 months of age (53). As with EAE, Vβ8 T cells are involved in this disease. In mice given SEB prior to appearance of antibodies, the time of onset and severity of disease were significantly reduced. Administration of SEB after the appearance of anti-DNA autoantibodies did not affect the onset of the disease (unpublished observation). Thus, superantigen-induced anergy and/or deletion of a particular Vβ-specific T-cell subset before autoimmune events reach a critical stage can protect against induction of certain autoimmune disorders.

Superantigen exacerbation and/or induction of EAE in mice already sensitized to rat MBP was also examined. Accordingly, SEB exposure induces EAE reactivation after immunization with MBP and resolution of an initial episode (11) (Fig. 1). SEB induces multiple relapses (up to three) over a 4-month period, and induces disease in mice that are immunized but do

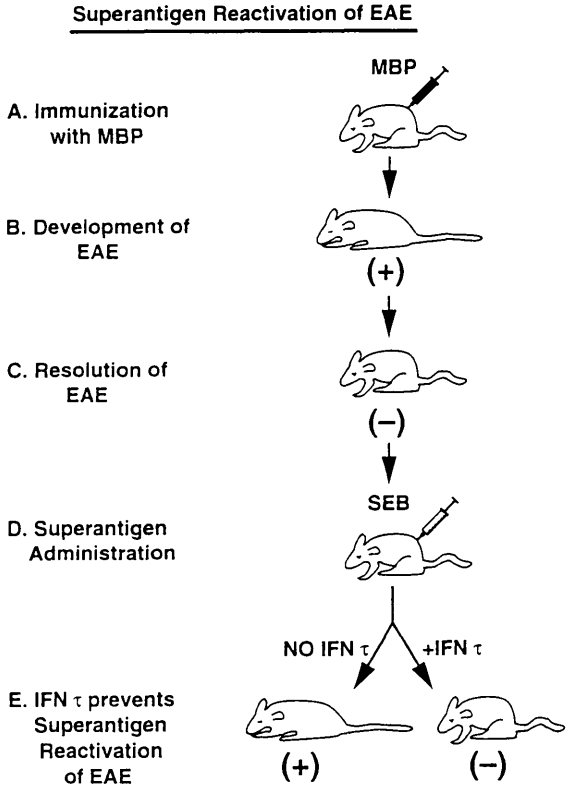

Figure 1. Reactivation of EAE by the staphylococcal enterotoxin superantigen SEB and prevention of superantigen reactivated EAE by IFN-τ. (A) PL/J mice employed in the reactivation study were initially immunized with MBP in Complete Freund's Adjuvant (CFA) and injected with Pertussis toxin on the day of immunization and 48 hr later. (B) Development of EAE occurred on or about Day 10–14. (C) Mice were allowed to recover from clinical symptoms for at least 2–4 weeks. (D) PL/J mice that had recovered from EAE were injected with SEB. (E) Mice that received no IFN-τ treatment developed superantigen-reactivated EAE on or about 7 days after exposure to superantigen. In contrast, mice which received IFN-τ 48 hr prior to SEB injection, on the day of SEB injection and 48 hr after SEB injection were protected from development of superantigen-reactivated EAE.

not develop an initial episode of disease. In the case of reactivation, SEB can stimulate but not anergize $V\beta8^+$ T cells. PL/J mice immunized with MBP in CFA or CFA alone were resistant to anergy induced by SEB unlike their naive counterparts (10). Thus, activated T cells appear to be refractory to induction of anergy by superantigens.

SEA, which is not Vβ8 specific, was able to reactivate disease in mice that had been immunized with whole MBP as effectively as SEB (10, 11). SEA did not induce statistically significant clinical disease in (PL/J × SJL)F1 mice immunized with an acetylated N-terminal MBP peptide instead of whole MBP (54). It has been previously shown that the amino terminus of MBP is encephalitogenic in PL/J and (PL/J × SJL)F1 mice (49).

The fact that EAE can be reactivated by SEA in mice immunized with MBP but not acetylated N-terminal peptide suggests that subdominant or cryptic EAE inducing epitopes are present in MBP. Further, these epitopes may activate Vβ-bearing T cells that can be stimulated by SEA. PL/J mice projected against development of EAE by SEB pretreatment developed an accelerated onset of paralysis with increased severity when administered SEA (11). These mice exhibited anergy and deletion of the Vβ subset, an expansion of the Vβ10 subset (a Vβ for which SEA is specific) and T-cell recognition of the MBP Ac-1-17 peptide. Thus, T cells bearing receptors other than Vβ8 can recognize the amino terminus of MBP and potentially contribute to disease. It is also possible that, while subdominant and/or cryptic epitopes may not sensitize T cells sufficiently to induce disease, these cells may cause disease when pushed over the threshold by superantigens such as SEA. Preliminary data suggest that T cells reactive to epitopes in the region 100–140 of MBP are present in SEA reactivated PL/J mice (Soos JM, Johnson HM, unpublished data). Thus, paralytic disease in PL/J mice after reactivation of disease by superantigen may not be restricted to Vβ8 T cells specific for the N terminus of MBP, but is more complex, reflecting the complexity of MS.

A type I interferon, IFN-β1b, has recently been approved by the FDA for treatment of MS in humans. Type I IFNs have been shown to block SE induction of IL-2, T-cell proliferation, and Vβ-specific T-cell expansions (55). The IFN pregnancy recognition hormone, IFN-τ, also blocks both the development and superantigen reactivation of paralysis in the EAE model (56) (Fig. 1). The observation that IFN-τ can block superantigen induced exacerbations of EAE may be a corollary to the reduction in disease exacerbations in MS patients undergoing treatment with IFN-β1b (57). Interestingly, treatment with IFN-τ does not result in toxic side effects like those observed during IFN-β1b treatment of MS. Studies are also in progress to block EAE exacerbation by SEs using neutralizing antibodies. Such therapies could assist in the amelioration of superantigen exacerbated autoimmune diseases through the presence of high-titer neutralizing antibodies.

Mechanism of IFN-τ Protection Against Superantigen-Induced EAE

It is possible that a number of immunosuppressive activities that IFN-τ can exert are responsible for the mechanism by which IFN-τ can prevent superantigen-induced EAE. IFN-τ reduced T cell proliferation in response to both the staphylococcal enterotoxin superantigens (55) and to specific antigen, in this case MBP (56). IFN-τ also reduced Vβ-specific T-cell expansions *in vivo* (56). IFN-τ, when injected concurrently with SEB, reduced the number of Vβ8 T cells when compared with the number of Vβ8 T cells present in mice injected with only SEB. In addition, SEB induced IL-2 (55) and TNF-α (56) production was also reduced in the presence of IFN-τ. Additional functions of IFN-τ are currently being examined to determine if they also contribute to the mechanism by which IFN-τ prevents superantigen-induced EAE. Early studies of the type I IFNs revealed their ability to induce suppressor cell function (58). Inhibition of anti-sheep red blood cell (SRBC) antibody production was demonstrated when normal mouse spleen cells were either co-cultured with type I IFN–treated spleen cells or treated with type I IFN–induced suppressor factor (the supernatant from cells that had been treated with Type I IFN for 24 h) in the presence of SRBCs. Preliminary data suggest that IFN-τ induces suppressor cell function which can reduce antigen-specific T-cell responses to MBP (Soos JM, Johnson HM, unpublished data). Additionally, IFN-τ effects on production of anti-MBP antibodies and expression of adhesion molecules and MHC class I and II molecules are currently being pursued.

Mouse Mammary Tumor Virus Superantigens

The first viral superantigen to be described is that encoded in an open reading frame (ORF) in the 3′ long terminal repeat of the type B retrovirus, mouse mammary tumor virus (MMTV) (59–62). A recent addition to the family of viral superantigens is the one encoded by the rabies virus (23, 24). Although MMTV superantigens were recognized in the 1990s, they were originally described by Festenstein in 1973 as minor lymphocyte-stimulating (mls) antigens (63). The ability of T cells from certain mouse strains to be stimulated by lymphocytes from MHC-identical strains was ascribed by Festenstein to the presence of mls antigens. More recently, mls antigens were found to be endogenous superantigens from germline-encoded MMTV provirus (64, 65).

Like their bacterial counterparts, MMTV superantigens are presented in the context of class II MHC antigens and stimulate T cells in a Vβ-specific fashion. MMTV superantigens show MHC preference, with more efficient presentation occurring in the context of I-E as opposed to I-A (66). Interestingly, this MHC preference corresponds with the greater infectivity of B cells bearing I-E (67).

Until recently, no direct evidence was available on the ability of MMTV superantigen to bind to class II MHC antigens, although strong evidence exists as to MHC requirement for activity (68). Through the use of synthetic peptides, a site was identified on the MMTV-1 superantigen that binds to mouse class II MHC antigens. Overlapping peptides corresponding to the predicted extracellular domain of the MMTV-1 (ORF) superantigen were tested for their relative abilities to compete with radiolabeled SEA for binding to the mouse B-cell line, A20 (69). Only one peptide, ORF(76–119), significantly reduced SEA binding to mouse A20 cells and consistently competed in a dose-response manner. Furthermore, in direct binding studies radiolabeled ORF(76–119) peptide specifically bound to A20 cells and was effectively inhibited by both unlabeled SEA and ORF(76–119) peptide. No binding of ORF(76–119) peptide was seen to a class II–negative cell line. Binding of ORF(76–119) peptide to A20 cells was blocked by antibodies to I-E and I-A, thereby indicating that binding was occurring to class II antigens. Further evidence of binding to MHC was obtained by radioimmunoassay, which showed that SEA and ORF(76–119) peptide competed with both radiolabeled SEA and ORF(76–119) peptide for binding to a peptide corresponding to the α-helical region (residues 60–90) of the mouse class II MHC antigen β chain. Thus, the data indicate that SEA and ORF(76–119) peptide bind to a similar region on the β-chain of the mouse class II MHC molecule.

Biochemical analyses suggest that MMTV superantigen is a Type II membrane protein with its Vβ specificity residing in the C terminus and with a hydrophobic transmembrane region near the N terminus (70). Although no direct evidence exists, the variability of the C-terminal residues of the ORF proteins of various MMTV strains seems to correlate with the differences in Vβ specificity, suggesting that this region binds TCR (71). In one study, truncations of the ORF gene were transfected into class I MHC–bearing cells and tested for superantigen activity. Complete loss of superantigen activity occurred when the MMTV ORF gene was N-terminally truncated to the third methionine (residue 122) and beyond (70). Further studies suggest that the superantigen encoded by MMTV-7 is synthesized as a precursor molecule, which is proteolytically cleaved and expressed as an 18.5K surface protein (72). Binding studies performed with 28K and 18K versions of the MMTV-7 superantigen showed that both molecules bind to human MHC class II antigens (73). Thus, studies by our laboratory indicate one site for binding to mouse class II MHC antigens, whereas studies performed by others suggest that two sites on MMTV superantigen are involved in binding to human MHC class II antigens.

It has been known for several years that infectivity by MMTV requires an intact immune system (reviewed in Ref. 18). MMTV transmission to offspring can occur *via* infectious virions in mother's milk or can occur vertically an endogenous provirus. Most mouse strains have been shown to contain one or more copies of endogenous MMTV, and many contain distinct MMTV strains (74). Upon passage into the gut of the host, virus enters the gut-associated immune tissue and infects B cells. B cells then express the MMTV-encoded superantigen, presumably in the context of class II antigens, causing Vβ-specific T-cell stimulation. Although no actual infection of T cells occurs (18), it is thought that T-cell stimulation indirectly enhances the further infection of B cells (67). Migration of infected cells to the mammary gland the main site of viral infection occurs, and infectious virions are produced and transmitted in milk.

Germline-encoded MMTV serves an important protective mechanism for the host. It is known that superantigens can cause anergy and/or deletion of Vβ-specific T cells (25). Similarly, expression of MMTV superantigen early in the ontogeny of the immune system induces the eventual deletion of Vβ-specific T cells that would otherwise be stimulated. In this manner, the host is protected against subsequent infection by MMTV strains that stimulate those Vβ-specific T-cell populations. This has been shown in mice transgenic for a MMTV superantigen that stimulates $V\beta14^+$ T cells (75). Transgenic mice showed partial to complete deletion of $V\beta14^+$ T cells, depending on the level of superantigen expression. Those mice in which $V\beta14^+$ T cells were deleted were subsequently protected from infection upon challenge with the same MMTV strain.

HIV Superantigen

A hallmark of infection with HIV is the alteration of CD4/CD8 T-cell ratios due to loss of $CD4^+$ T helper cells. Such losses ultimately result in the inability of an infected individual to mount effective immune responses against infections, thus resulting in death. Several possible mechanisms by which CD4 loss occurs have been postulated, including direct cytolysis by HIV (76), HIV-induced syncitia formation (77, 78) and/or cytolytic T-cell activity against infected CD4 cells (79). In addition to skewing of the CD4/CD8 ratios, other immunologic alterations seen in HIV-

infected individuals include polyclonal activation of B cells with increased immunoglobulin production, reduced antigen and mitogen responses, and increased natural killer cell activity (reviewed in Ref. 80).

Because of the known induction of T-cell anergy and/or deletion by superantigens, it has been speculated that the HIV genome encodes for a superantigen that may cause some of the immunopathologies observed with AIDS. Upon interaction with MHC class II antigens and TCR, Vβ-specific T-cell populations would be activated and expand, eventually leading to functional (anergy) or actual (deletion) loss of T cells. Several points of evidence suggest a role for a HIV-derived superantigen. Although initial studies suggested that AIDS patients had deletions in Vβ T-cell populations (81), such deletions could not be found by others (82). $V\beta12^+$ T cells were shown to support enhanced replication of HIV compared with other Vβ subsets and proliferated in response to cells from HIV-positive patients, suggesting the presence of a superantigen (82). Interestingly, one study found skewed Vβ T-cell profiles in monozygotic twins that were discordant for HIV infection, with perturbations in several Vβs (83). Thus, these studies hint at the presence of a superantigen encoded by HIV that may play a role in HIV pathogenesis by augmenting a population of T cells, resulting in a reservoir for virus replication, with eventual deletion of those T cells.

We have shown that Nef, a HIV regulatory protein, binds to MHC class II antigens at a site(s) involved in bacterial superantigen binding (21). Like MMTV superantigen, Nef protein is encoded in the 3′ long terminal repeat of the genome of lentiviruses such as HIV-1 and simian immunodeficiency virus (SIV). Nef induced significant levels of proliferation in human peripheral blood mononuclear cells (PBMC) from a wide sampling of donors (85%–90%), although these responses are lower than those induced by SEA (Torres BA *et al.*, submitted). Furthermore, Nef stimulation resulted in the production of the T helper cell cytokines, IL-2 and IFN-γ. Proliferation in response to Nef was observed in reconstituted cultures consisting of T cells and inactivated APCs, which is compelling evidence for Nef superantigen activity. Furthermore, Nef did not induce purified T cells to proliferate in the absence of APCs, which is consistent with Nef superantigen activity. No apparent Vβ expansions were detected using flow cytometry, possibly because of the low but significant level of proliferation. Therefore, we developed a simple amplification detection method using immobilized anti-Vβ antibodies (Tanabe T, *et al.*, submitted). PBMC from HIV-negative donors were pretreated for 24 h with Nef and then restimulated with immobilized anti-Vβ antibodies. Compared to untreated PBMC, significant expansion of Vβ2, Vβ5.2/5.3, and Vβ18 were detected in Nef-treated cultures (84). Nef-induced Vβ18 occurred with PBMC from all donors tested. Vβ2 and Vβ5.2/5.3 expansion occurred in >50% of donors. Other Vβ subsets were not found to be expanded. Thus, Nef has superantigen-like characteristics in that it binds to class II antigens, does not require processing by APC, and activates T cells to proliferate in a Vβ-specific manner and release cytokines such as IL-2 and IFN-γ.

The functional role that Nef plays in the lentiviral pathogenesis is uncertain, although its importance is reflected in vaccine studies in which a Nef-deleted SIV mutant did not cause disease and protected animals upon challenge with the pathogenic wild-type strain (85). The viral load in SIV mutant-infected animals was considerably lower than in animals challenged with wild-type SIV. Within this context, the ability of Nef to activate T cells is interesting in that active HIV replication requires T-cell activation. Furthermore, although resting or quiescent T cells can be infected, evidence suggests that HIV replication only occurs upon subsequent cellular activation.

Since T-cell activation occurs with exogenous Nef protein, we determined whether Nef stimulation of PBMC was sufficient to induce HIV-1 replication. Unstimulated and mitogen-stimulated PBMC were infected with HIV-1 and cultures were monitored for signs of viral replication. Supernatants from unstimulated PBMC showed only marginal reverse transcriptase (RT) activity. RT levels were high in cells stimulated with T cell mitogens, in particular SEA (Fig. 2). Interestingly, Nef-stimulated cells produced moderate to high levels of RT, indicating that these cells were capable of sustaining virus replication. Virus replication in Nef-stimulated cultures yielded infectious virus, rather than defective particles (84). Flow cytometric analysis showed that increased per-

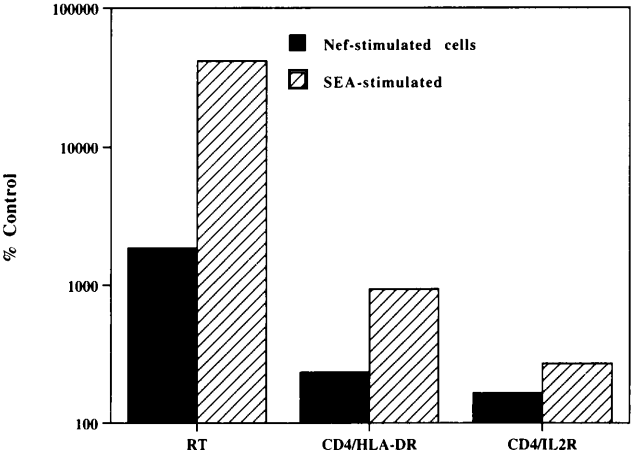

Figure 2. Nef-induced T-cell activation is sufficient for active HIV replication. Nef-stimulated PBMC were infected with HIV and cultures monitored for reverse transcriptase (RT) activity. T-cell activation induced by Nef was determined by flow cytometric analyses. PBMC were double-stained with anti-CD4 and antibodies to either HLA-DR or IL-2R.

centages of cells that stained for both CD4 and HLA-DR were present in Nef-stimulated cultures, as compared with unstimulated cultures (Fig. 2). Furthermore, increased percentages of cells that stained for both CD4 and IL-2R were observed in Nef-stimulated cultures. These results are consistent with our previous studies showing production of T helper cytokines upon stimulation with Nef. Thus, stimulation of PBMC with Nef protein induces T-cell activation and such activation is sufficient for HIV replication.

Nef could not be detected in culture supernatants of infected cells, suggesting that Nef may not be present in a soluble form in these cultures. Previously, we showed that paraformaldehyde-inactivated cells were capable of presenting Nef to T cells, indicating that Nef did not require processing (84). We investigated the possibility that HIV-infected cells could activate autologous PBMC. To achieve maximal infection, PBMC were stimulated with mitogen, infected with HIV, and inactivated with paraformaldehyde. Proliferation of fresh autologous PBMC cultured in the presence of infected cells was observed (Torres BA, Tanabe T, Yamamoto JK, Johnson HM, unpublished data). Furthermore, proliferative responses were significantly reduced by the addition of polyclonal anti-Nef antibodies. These data suggest that proliferation occurred as a result of Nef expressed on the surface of HIV-infected cells, thereby indicating that Nef may be a virally-encoded mitogen.

Nef has been shown to induce differentiation of human B cells to immunoglobulin secreting cells (86). Monoclonal antibodies to HLA-DR and adhesion molecules abrogated Nef-induced differentiation. B-cell stimulation required T cells and monocytes, the latter producing IL-6 upon Nef stimulation. Interestingly, staphylococcal superantigens have been shown to induce B-cell differentiation analogous to that described for Nef (87). Thus, Nef can stimulate both T and B cells in a manner similar to the staphylococcal enterotoxin superantigens.

This leads to the question of the activation events(s) that occurs during HIV-1 exposure and that ultimately lead to the establishment of infection. Our results suggest a model in which Nef acts as a HIV-encoded T cell mitogen (Fig. 3). Nef may interact with T cells either as an integral part of the membranes of infected T cells or in a soluble form released as the result of lysis of infected cells. Nef has been shown to be expressed on the membranes of infected T cells (88), which is consistent with this model. Nef expression may occur in the context of the T cell activation marker, HLA-DR, and the Nef/HLA-DR complex would stimulate uninfected T cells, as well as B cells. Such stimulation would allow for the expansion of a cellular reservoir for replication of the virus, eventually leading to T-cell anergy and/or apoptosis. B-cell activation and differentiation could ultimately lead to hypergammaglobulinemia. Thus, the HIV genome may encode for its own T-cell mitogen, which would induce the amplification of virus replication in the host, with ultimate deleterious effects on the immune system. As such, Nef could be classified as a virulence factor for HIV.

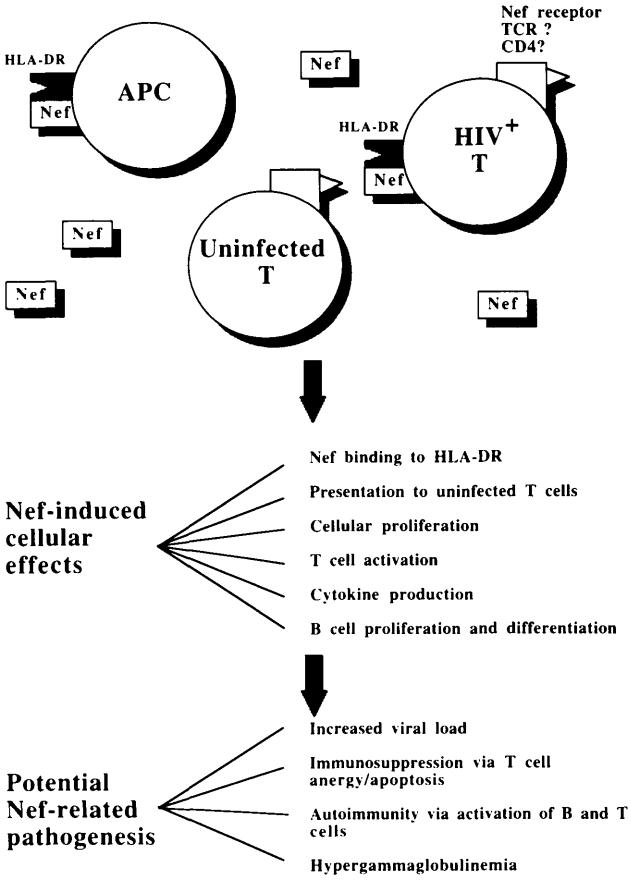

Figure 3. Model for the role of Nef in the pathogenesis of HIV. Activation of uninfected T cells may occur by interaction with Nef in either of two forms: as soluble Nef released from lysed cells, presented in the context of MHC class II antigens on APC, or as an integral part of the membranes of infected T cells, complexed to HLA-DR. Although the Nef receptor on T cells has not been elucidated, Vβ expansion data suggest that binding to uninfected T cells occurs *via* TCR. Activation of T cells results in proliferation and release of cytokines such as IFN-γ and IL-2, thereby creating a cellular reservoir for virus and increasing the viral load in the host. T-cell activation may result in depletion of T cells *via* virus production, anergy, and/or apoptosis. Differentiation of B cells, possibly mediated by T-cell cytokine release, results in hypergammaglobulinemia. Autoimmune-like sequelae may result from B cell differentiation into Ig-secreting cells, and activation of T cells.

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1. Bergdoll MS. The staphylococcal enterotoxins—an update. In: Jelaszewics J, Ed. The Staphylococci. New York: Gustav Fisher Verlag, pp247–266, 1985.

2. Mollick JA, Cook RG, Rich RR. Class II MHC molecules are specific receptors for staphylococcus enterotoxin A. Science **244**:817–820, 1989.
3. Peavy DL, Adler WH, Smith RT. Mitogenic effects of endotoxin and SEB on mouse spleen cells and human lymphocytes. J Immunol **105**:1453–1458, 1970.
4. Langford MP, Stanton GJ, Johnson HM. Biological effects of staphylococcal enterotoxin A on human peripheral lymphocytes. Infect Immun **22**:62–68, 1978.
5. Callahan JE, Herman A, Kappler J, Marrack P. Stimulation of B10.BR T cells with superantigenic staphylococcal enterotoxins. J Immunol **144**:2473–2479, 1990.
6. Carlsson R, Sjogren HO. Kinetics of IL 2 and IFNγ production, expression of IL 2 receptors, and cell proliferation in human mononuclear cells exposed to SEA. Cell Immunol **96**:175–183, 1985.
7. Johnson HM, Magazine HI. Potent mitogenic activity of staphylococcal enterotoxin A requires induction of interleukin 2. Int Arch Allergy Appl Immunol **87**:87–90, 1988.
8. Abe J, Kotzin BL, Jujo K, Melish ME, Glode MP, Kohsaka T, Leung DYM. Selective expansion of T cells expressing T cell receptor variable region Vβ2 and Vβ8 in Kawasaki disease. Proc Natl Acad Sci USA **89**:4066–4070, 1992.
9. Kotzin BL, Leung DYM, Kappler J, Marrack P. Superantigens and their potential role in human disease. Adv Immunol **54**:99–166, 1993.
10. Schiffenbauer J, Johnson HM, Butfiloski E, Wegrzyn L, Soos JM. Staphylococcal enterotoxins can re-activate experimental allergic encephalomyelitis. Proc Natl Acad Sci USA **90**:8543–8546, 1993.
11. Soos JM, Hobeika AC, Butfiloski E, Schiffenbauer J, Johnson HM. Accelerated induction of experimental allergic encephalomyelitis in PL/J mice by a non-Vβ8 specific superantigen. Proc Natl Acad Sci USA **92**:6082–6086, 1995.
12. Lewis HM, Baker BS, Bokth S, Powles AV, Garioch JJ, Valdimarsson H, Fry L. Restricted T cell receptor Vβ gene usage in the skin of patients with guttate and chronic plaque psoriasis. Br J Dermatol **129**:514–520, 1993.
13. Cole BC, Griffiths MM. Triggering and exacerbation of autoimmune arthritis by the *Mycoplasma arthriditis* superantigen MAM. Arth Rheum **36**:994–1002, 1993.
14. Ohmen JD, Barnes PF, Grisso CL, Bloom BR, Modlin RL. Evidence for a superantigen in human tuberculosis. Immunity **1**:35–43, 1994.
15. Uchiyama T, Miyoshi-Akiyama T, Kato H, Fujimaki W, Imanishi K, Yan X. Superantigenic properties of a novel mitogenic substance produced by Yersinia pseudotuberculosis isolated from patients manifesting acute and systemic symptoms. J Immunol **151**:4407–4413, 1993.
16. Lindsey JA, Johnson HM, Wallace FM, Soos JM. Can superantigens trigger sudden infant death? Med Hypoth **43**:81–85, 1994.
17. Murrell TG, Murrell WG, Lindsey JA. Sudden infant death syndrome (SIDS): Are common bacterial toxins responsible, and do they have a vaccine potential? Vaccine **12**:365–368, 1994.
18. Held W, Acha-Orbea H, MacDonald HR, Waanders GA. Superantigens and retroviral infection: Insights from mouse mammary tumor virus. Immunol Today **15**:184–190, 1994.
19. Hugin AW, Vacchio MS, Morse HC III. A virus-encoded "superantigen" in a retrovirus-induced immunodeficiency syndrome of mice. Science **252**:424–427, 1991.
20. Schnittman SM, Fauci AS. Human immunodeficiency virus and acquired immunodeficiency syndrome: An update. Adv Intern Med **39**:305–355, 1994.
21. Torres BA, Johnson HM. Identification of an HIV-1 Nef peptide that binds to HLA class II antigens. Biochem Biophys Res Commun **200**:1059–1065, 1994.
22. Wick G, Trieb K, Aguzzi A, Recheis H, Anderl H, Grubeck-Loebenstein B. Possible role of human foamy virus in Graves' disease. Intervirology **35**:101–107, 1993.
23. Lafon M, Lafage M, Martinez-Arends A, Ramirez R, Vuillier F, Charron D, Lotteau V, Scott-Algara D. Evidence for a viral superantigen in humans. Nature **358**:507–510, 1992.
24. Lafon M, Scott-Algara D, Marche PN, Cazenave PA, Jouvin-Marche E. Neonatal deletion and selective expansion of mouse T cells by exposure to rabies virus nucleocapsid superantigen. J Exp Med **180**:1207–1215, 1994.
25. Johnson HM, Russell JK, Pontzer CH. Role of superantigens in human disease. Sci Am **266**:92–101, 1992.
26. Friedman S, Posnett D, Tumang J, et al. A potential role for microbial superantigens in pathogenesis of systemic autoimmune disease. Arth Rheum **34**:468–480, 1991.
27. Soudeyns H, Rebai N, Pantaleo GP, Ciurli C, Sekaly RP, Fauci AS. The T cell receptor Vβ repertoire in HIV-1 infection and disease. Semin Immunol **5**:175–185, 1993.
28. Laurence J, Hodstev AS, Posnett DN. Superantigen implicated in dependence of HIV-1 replication in T cells on TCR Vβ expression. Nature **358**:255–258, 1992.
29. Dalgleish AG, Wilson S, Gompels M, Ludlam C, Gazzard B, Coates AM, Habeshaw J. T-cell receptor variable gene products and early HIV-1 infection. Lancet **339**:824–828, 1992.
30. Rebai N, Pantaleo G, Demarest J, et al. Analysis of the TCR Vβ repertoire in monozygotic twins discordant for HIV. Proc Natl Acad Sci USA **91**:1529–1533, 1994.
31. Singh BR, Evenson ML, Bergdoll MS. Structural analysis of staphylococcal enterotoxin B and C1 using circular dichroism and fluorescence spectroscopy. Biochemistry **27**:8735–8741, 1988.
32. Middelbrook JL, Spero L, Argos P. The secondary structure of staphylococcal enterotoxins A, B, and C. Biochem Biophys Acta **621**:233–240, 1980.
33. Singh BR, Betley MJ. Comparative structural analysis of staphylococcal enterotoxins A and E. J Biol Chem **264**:4404–4411, 1989.
34. Singh BR, Kokan-Moore NP, Bergdoll MS. Molecular topography of toxic shock syndrome toxin 1 as revealed by spectroscopic studies. Biochemistry **27**:8730–8735, 1988.
35. Swaminathan S, Furey W, Pletcher J, Sax M. Crystal structure of staphylococcal enterotoxin B, a superantigen. Nature **359**:801–806, 1992.
36. Prasad GS, Earhart CA, Murray DL, Novick RP, Schlievert PM, Ohlendorf DH. Structure of toxic shock syndrome toxin 1. Biochemistry **32**:13761–13766, 1993.
37. Acharya KR, Passalacqua EF, Jones EY, Harlos K, Stuart DI, Brehm RD, Tranter HS. Structural basis of superantigen action inferred from crystal structure of toxic shock syndrome toxin 1. Nature **367**:94–97, 1994.
38. Jardetzky TS, Brown JH, Gorga JC, Stern LJ, Urban RG, Chi Y, Stauffacher C, Strominger JL, Wiley DC. Three-dimensional structure of a human class II histocompatibility molecules complexed with superantigen. Nature **368**:711–718, 1994.
39. Pontzer CH, Russell JK, Johnson HM. Localization of an immune functional site on staphylococcal enterotoxin A using the synthetic peptide approach. J Immunol **143**:280–284, 1989.
40. Pontzer CH, Russell JK, Johnson HM. Site of non-restrictive binding of SEA to class II MHC antigens. Int Arch Allergy Appl Immunol **93**:107–112, 1991.
41. Griggs ND, Pontzer CH, Jarpe MA, Johnson HM. Mapping of multiple binding domains of the superantigen staphylococcal enterotoxin A for HLA. J Immunol **148**:2516–2521, 1992.
42. Pontzer CH, Griggs ND, Johnson HM. Agonist properties of a

microbial superantigen peptide. Biochem Biophys Res Commun **193**:1191–1197, 1993.
43. Russell JK, Pontzer CH, Johnson HM. The I-Aβb region (65–85) is a binding site for the superantigen, staphylococcal enterotoxin A. Biochem Biophys Res Commun **168**:696–701, 1990.
44. Russell JK, Pontzer CH, Johnson HM. Both α-helices along the major histocompatibility complex binding cleft are required for staphylococcal enterotoxin A function. Proc Natl Acad Sci USA **88**:7228–7232, 1991.
45. Herman A, Labrecque N, Thibodeau J, Marrack P, Kappler JW, Sekaly RP. Identification of the staphylococcal enterotoxin A superantigen binding site in the β1 domain of the human histocompatibility antigen HLA-DR. Proc Natl Acad Sci USA **88**:9954–9958, 1991.
46. Soos JM, Russell JK, Jarpe MA, Pontzer CH, Johnson HM. Identification of binding domains on the superantigen toxic shock syndrome toxin 1 for class II MHC molecules. Biochem Biophys Res Commun **191**:1211–1217, 1993.
47. Soos JM, Johnson HM. Multiple binding sites on the superantigen staphylococcal enterotoxin B impart versatility in binding to MHC class II molecules. Biochem Biophys Res Commun **201**:596–602, 1994.
48. Herman A, Croteau G, Sekaly RP, Kappler JW, Marrack P. HLA-DR alleles differ in their ability to present staphylococcal enterotoxins to T cells. J Exp Med **172**:709–717, 1990.
49. Zamvil SS, Steinman L. The T lymphocyte in experimental allergic encephalomyelitis. Annu Rev Immunol **8**:579–621, 1990.
50. Fritz RB, Chou C, McFarlin DE. Relapsing murine experimental allergic encephalomyelitis induced by myelin basic protein. J Immunol **130**:1024–1026, 1983.
51. Oksenberg J, Panzara M, Begovich A, et al. Selection for TCR Vβ-D β-J β gene rearrangements with specificity for a myelin basic protein peptide in brain lesions of multiple sclerosis. Nature **362**:68–70, 1993.
52. Soos JM, Schiffenbauer J, Johnson HM. Treatment of PL/J mice with the superantigen, staphylococcal enterotoxin B, prevents development of experimental allergic encephalomyelitis. J Neuroimmunol **44**:39–44, 1993.
53. Kim C, Siminovitch KA, Ochi A. Reduction of lupus nephritis in MRL/lpr mice by a bacterial superantigen treatment. J Exp Med **174**:1431–1437, 1991.
54. Brocke S, Gaur A, Piercy C, et al. Induction of relapsing paralysis in experimental autoimmune encephalomyelitis by bacterial superantigen. Nature **365**:642–644, 1993.
55. Soos JM, Johnson HM. Type I IFN inhibition of superantigen-induced stimulation: Implications for treatment of superantigen associated disease. J. IFN Cytokine Res **15**:39–45, 1995.
56. Soos JM, Subramaniam PS, Hobeika AC, Schiffenbauer J, Johnson HM. The interferon pregnancy recognition hormone, IFN-tau, blocks both development and superantigen reactivation of experimental allergic encephalomyelitis without associated toxicity. J Immunol **155**:2747–2753, 1995.
57. IFNβ Multiple Sclerosis Study Group. Interferonβ-1b is effective in relapsing-remitting multiple sclerosis. I. Clinical results of a multicenter, randomized, double-blind, placebo controlled trial. Neurology **43**:655, 1993.
58. Johnson HM, Blalock JE, Interferon immunosuppression: Mediation by a suppressor factor. Infect Immun **29**:301–305, 1980.
59. Marrack P, Kushnir E, Kappler J. A maternally inherited superantigen encoded by a mammary tumor virus. Nature **349**:524–526, 1991.
60. Frankel WN, Rudy C, Coffin JM, Huber BT. Linkage of Mls genes to endogenous mammary tumour viruses of inbred mice. Nature **349**:526–528, 1991.
61. Woodland DL, Happ MP, Gollob KJ, Palmer E. An endogenous retrovirus mediating deletion of αβ T cells? Nature **349**:529–530, 1991.
62. Dyson PJ, Knight AM, Fairchild S, Simpson E, Tomonari K. Genes encoding ligands for deletion of Vβ11 T cells cosegregate with mammary tumour virus genomes. Nature **349**:531–532, 1991.
63. Festenstein H. Immunogenetic and biological aspects of in vitro lymphocyte allotransformation (MLR) in the mouse. Transplant Rev **15**:62–68, 1973.
64. Choi Y, Kappler JW, Marrack P. A superantigen encoded in the open reading frame of the 3′ long terminal repeat of mouse mammary tumour virus. Nature **350**:203–207, 1992.
65. Acha-Orbea H, Shakhov AN, Scarpellino L, Kolb E, Muller V, Vessaz-Shaw A, Fuchs R, Blochinger K, Rollini P, Billotte J, Sarafidou M, MacDonald HR, Diggelmann H. Conal deletion of Vβ14-bearing T cells in mice transgenic for mammary tumour virus. Nature **350**:207–211, 1992.
66. MacDonald HR, Glasebrook AL, Schneider R, Lees RK, Pircher HP, Pedrazzini T, Kanagawa O, Nicholas J-F, Howe RC, Zinkernagel RM, Hentgartner H. T-cell reactivity and tolerance to Mls^a-encoded antigens. Immunol Rev **107**:89–108, 1989.
67. Held W, Waanders GA, MacDonald HR, Acha-Orbea H. MHC class II hierarchy of superantigen presentation predicts efficiency of infection with mouse mammary tumor virus. Int Immunol **6**:1403–1407, 1994.
68. Herman A, Kappler JW, Marrack P, Pullen A. Superantigens: Mechanism of T cell stimulation and role in immune responses. Annu Rev Immunol **9**:745–772, 1991.
69. Torres BA, Griggs ND, Johnson HM. Bacterial and retroviral superantigens share a common binding region on MHC class II antigens. Nature **364**:152–154, 1993.
70. Choi Y, Marrack P, Kappler JW. Structural analysis of a mouse mammary tumor virus superantigen. J Exp Med **175**:847–851, 1992.
71. Pullen AM, Choi Y, Kushnir E, Kappler JW, Marrack P. The open reading frame in the 3′ long terminal repeats of several mouse mammary tumor virus intergrants encode Vβ3 specific superantigens. J Exp Med **175**:41–47, 1992.
72. Winslow GM, Scherer MT, Kappler JW, Marrack P. Detection and biochemical characterization of the mouse mammary tumor virus 7 superantigen (Mls-1^a). Cell **71**:719–730, 1992.
73. Mottershead DG, Hsu P-N, Urban RG, Strominger JL, Huber BT. Direct binding of Mtv7 superantigen (Mls-1) to soluble MHC class II molecules. Immunity **2**:149–154, 1995.
74. Salmons B, Gunzburg WH. Current perspectives in the biology of mouse mammary tumor virus. Virus Res **8**:81–102, 1987.
75. Golovkina TV, Chervonsky A, Dudley JP, Ross SR. Transgenic mouse mammary tumor virus superantigen expression prevents viral infection. Cell **69**:637–645, 1992.
76. Lemaitre M, Guerard D, Henin Y, Montagnier L, Zerial A. Protective activity of tetracycline analogs against cytopathic effect of HIV in CEM cells. Res Virol **141**:5–13, 1990.
77. Lifson JD, Feinberg MB, Reyes GR, Rabin L, Banapour B, Chakrabati S, Moss B, Wong-Staal F, Steimer KS, Engelman EG. Induction of CD4-dependent cell fusion by the HTLV-III/LAV envelope glycoprotein. Nature **323**:725–728, 1986.
78. Sodroski J, Goh WC, Rosen C, Campbell K, Haseltine WA. Role of the HTLV-III/LAV envelope in syncytium formation and cytopathicity. Nature **322**:470–474, 1986.
79. Zinkernagel R, Hengartner H. T-cell mediated immunopathology versus direct cytolysis by virus: Implications for HIV and AIDS. Immunol Today **15**:262–268, 1994.
80. Edelman AS, Zolla-Pazner S. AIDS: A syndrome of immune dysregulation, dysfunction, and deficiency. FASEB J **3**:22–29, 1989.
81. Imberti L, Sottini A, Bettinardi A, Puoti M, Primi D. Selective

depletion in HIV infection of T cells that bear specific T cell receptor V beta sequences. Science **254:**860–862, 1991.
82. Laurence J, Hodstev AS, Posnett DN. Superantigen implicated in dependence of HIV-1 replication in T cells on TCR Vβ expression. Nature **358:**255–258, 1992.
83. Rebai N, Pantaleo G, Demarest JF, Ciurli C, Soudeyns H, Adelsberger JW, Vaccarezza M, Walker RE, Sekaly RP, Fauci AS. Analysis of the TCR Vβ repertoire in monozygotic twins discordant for HIV. Proc Natl Acad Sci USA **91:**1529–1533, 1994.
84. Torres BA, Tanabe T, Yamamoto JK, Johnson HM. HIV encodes for its own CD4 T cell superantigen. Proc Natl Acad Sci USA (in press).
85. Daniel MD, Kirchhoff F, Czajak SC, Sehgal PK, Desrosiers RC. Protective effects of a live attenuated SIV vaccine with a deletion in the *nef* gene. Science **258:**1938–1941, 1992.
86. Chirmule N, Oyaizu N, Saxinger C, Pahwa S. Nef protein of HIV-1 has B-cell stimulatory activity. AIDS **8:**733–739, 1994.
87. Stohl W, Elliott JE, Linsley PS. Human T cell–dependent B cell differentiation induced by staphylococcal superantigens. J Immunol **153:**117–127, 1994.
88. Fujii Y, Nishino Y, Nakaya T, Tokunaga K, Ikuta K. Expression of human immunodeficiency virus type I Nef antigen on the surface of acutely infected and persistently infected human T cells. Vaccine **11:**1240–1246, 1993.