MINIREVIEW

Superantigens: The Good, the Bad, and the Ugly

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Increasing evidence suggests that superantigens play a role in immune-mediated diseases. Superantigens are potent activators of CD4+ T cells, causing rapid and massive proliferation of cells and cytokine production. This characteristic of superantigens can be exploited in diseases where strong immunologic responses are required, such as in the B16F10 animal model of melanoma. Superantigen administration is able to significantly enhance ineffective anti-tumor immune responses, resulting in potent and long-lived protective anti-tumor immunity. However, superantigens are more well-known for the role they play in diseases. Studies using an animal model for neurologic demyelinating diseases such as multiple sclerosis show that superantigens can induce severe relapses and activate autoreactive T cells not involved in the initial bout of disease. This may also involve epitope spreading of disease. Superantigens have also been implicated in acute diseases such as food poisoning and TSS, and in chronic diseases such as psoriasis and rheumatoid arthritis. Viral superantigens are also involved in the disease process, including superantigens derived from human immunodeficiency virus and mouse mammary tumor virus. Finally, immunotherapies that ameliorate the role played by superantigens in disease are discussed.

[Exp Biol Med Vol. 226(3):164–176, 2001]

Key words: superantigens; autoimmunity; cancer; prophylactic vaccination

Superantigens are microbial proteins that are potent activators of CD4+ T cells. As such, superantigens can have profound effects on the immune system, both acute and long-term (1, 2). Acute effects include food poisoning and toxic shock syndrome. Long-term effects include autoimmune diseases and immunodeficiency (Fig. 1). These effects have generally been considered "bad" and "ugly". However, if the burst of T-cell activation that occurs with superantigens could be harnessed and exploited, then superantigens can have "good" effects for the host, such as enhancement of desirable immune responses.

Superantigens are produced by bacteria or viruses and can activate large numbers of CD4+ T cells (1, 2). T-cell activation of this magnitude results in prodigious production of cytokines, which may be partly responsible for the acute toxic effects of superantigens. *Staphylococcus aureus* enterotoxins (A, B, C, D, E, and toxic shock syndrome toxin) are the prototypic superantigens, having been the first to be characterized extensively as to T-cell activation. A number of pathogenic bacteria and viruses have been shown to produce superantigenic proteins (Table I), which have been implicated in a wide array of human disorders, including acute diseases such as food poisoning and toxic shock syndrome (3), and in chronic diseases such as atopic allergy (8), Kawasaki's disease (6, 7), and periodontal disease (13). Further, superantigens can cause deregulation of immune responses, resulting in autoimmune disease (such as multiple sclerosis) or immunodeficiency (such as that associated with HIV). Superantigens are thought to act as virulence factors by subverting normal immune responses and causing delays in the establishment of pathogen-specific immunity.

Superantigens differ from conventional antigens in several ways (Fig. 2). Conventional antigens are taken up or are endogenously produced by antigen-presenting cells and are processed into discrete peptides, which are then presented to antigen-specific T cells in the antigen-binding groove of either MHC class I or class II molecules on the surface of the antigen-presenting cell. Superantigens, on the other hand, function as intact molecules and bind directly to MHC class II molecules on the surface of antigen-presenting cells

This work is supported by National Institutes of Health Grants R37 AI25904 and CA38587 to H.M.J. This article is Florida Agriculture Experiment Station Journal Series number R-07972.

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0037-9727/01/2263-0164$15.00

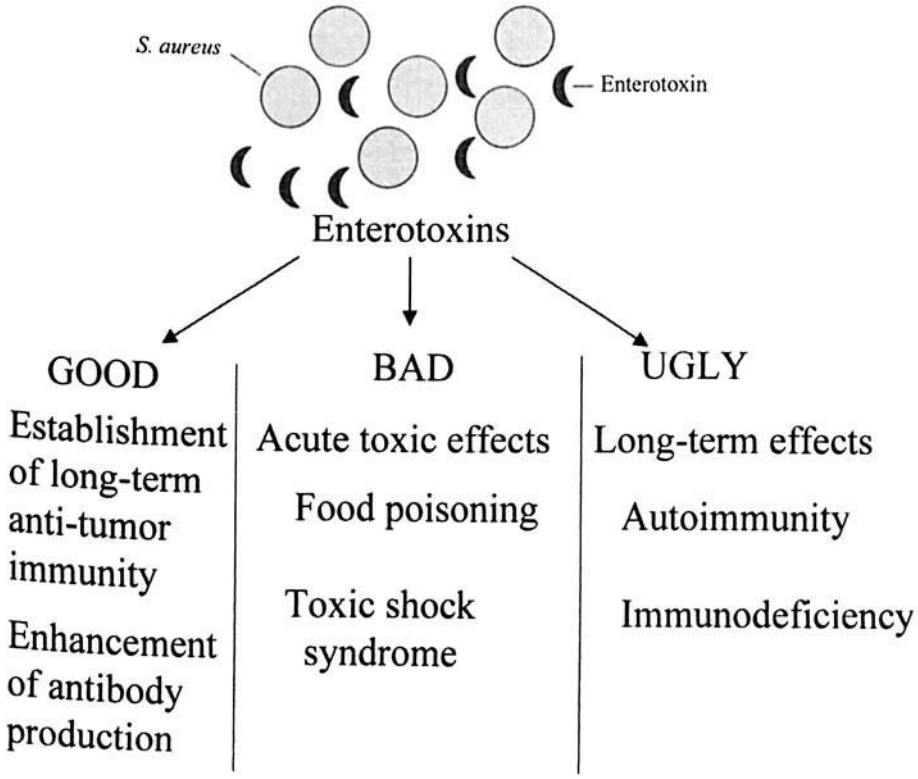

Figure 1. "Good", "bad", and "ugly" effects of the staphylococcal enterotoxin superantigens on the host. "Good" effects involve establishment of strong beneficial immune responses, such as humoral responses or responses against tumors. Superantigens can cause acute disease ("bad" effect) or lay the groundwork for chronic diseases ("ugly" effect).

Table I. Human Diseases Associated With Bacterial and Viral Superantigens

Organism	Superantigen	Disease	References
Bacterial			
Staphylococcus aureus	Enterotoxins[a]	Food poisoning	3
		Toxic shock syndrome	3
		Multiple sclerosis	4, 5
		Kawasaki's disease	6, 7
		Atopic allergy	8
Group A strptococci	Pyrogenic exotoxins	Psoriasis	7, 9
		Rheumatic heart disease	7
Mycoplasma arthriditis	T-cell mitogen	Arthritis	10
Mycobacterium tuberculosis	Not identified	Tuberculosis	11
Yersinia	Not identified	Reiter's syndrome	12
Prevotella intermedia	Not identified	Periodontal disease	13
Viral			
MMTV	vSAg gene[a]	Mammary tumors	14
Mouse leukemia virus	Gag protein	MAIDS	15
$IDDMK_{12}22$	pPOL-ENV-U3	Insulin-dependent diabetes mellitus	16, 17
HIV	Nef	AIDS	18
Rabies virus	Nucleocapsid protein	Rabies	19, 20
Epstein-Barr virus	Not identified	B-cell lymphoma	21

[a] Indicates prototype for either bacterial or viral superantigens.

in the absence of processing (22). Recently, superantigens have been shown to bind to class I MHC antigens (23), although these findings need to be confirmed. Superantigens can be presented to T cells by many types of immunologic class II-bearing cells, including monocytes/macrophages, B cells, and natural killer cells (24), and binding to class II occurs at a site outside the antigen-binding groove (25, 26). This complex of superantigen/MHC class II interacts directly with the variable region of the beta chain (Vβ) of the T-cell receptor (TCR) on T cells, thereby causing T-cell activation (27).

As many as 60 different Vβ elements of human TCRs have been identified to date. The subsets of Vβ-bearing T cells that are activated by one superantigen may differ from those activated by another superantigen. For example, toxic shock syndrome toxin-1 (TSST-1) from *S. aureus* interacts with human T cells bearing Vβ2, whereas staphylococcal enterotoxin B (SEB) activates human T cells expressing

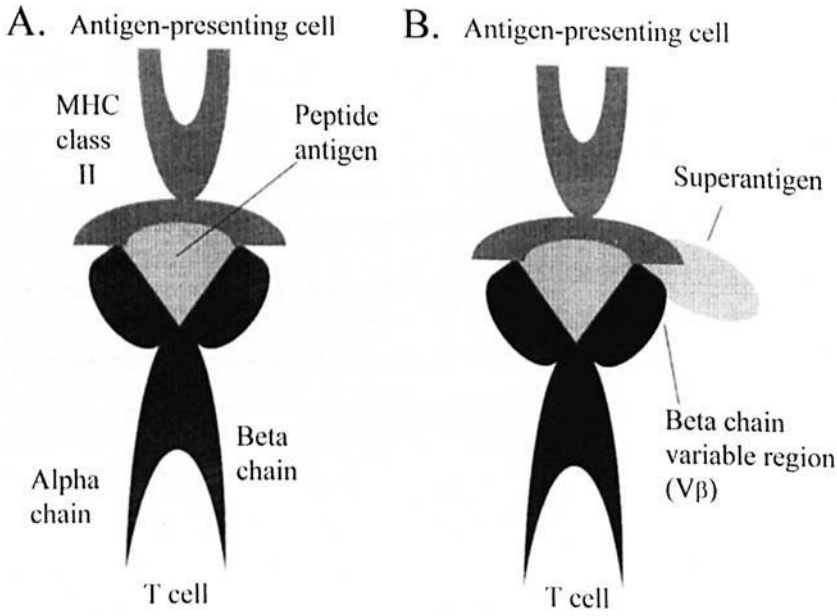

Figure 2. Differences between superantigens and conventional antigens. (A) Conventional antigens are processed by antigen-presenting cells into discrete peptides. Peptide antigens are then expressed on the surface of the cell in the peptide-binding groove of MHC class I or class II antigens. T-cell receptors (TCR) on T cells recognize the specific antigen in the context of MHC class II, thereby activating T cells. (B) Superantigens are not processed by antigen-presenting cells. Rather, they bind directly to MHC class II antigens at a site distant from the peptide-binding groove. This complex of superantigen:MHC class II interacts directly with the variable region of the β-chain (Vβ) of the TCR on T cells, thereby causing T-cell activation of specific subsets of T cells.

Vβ3, Vβ12, Vβ14, Vβ15, Vβ17, and Vβ20 (28). Thus, superantigens induce expansion of unique subsets of Vβ T cells independent of antigen specificity and can activate as many as 20% of cells in a given T-cell population.

T-cell stimulation by superantigens causes proliferation and the prodigious production of cytokines, primarily from $CD4^+$ cells (29–32). The predominant cytokines produced and released during superantigen activation are interleukin-2 (IL-2) and gamma interferon (IFNγ), both of which are intimately involved in the cascade of cytokines produced during immune responses. The levels of cytokines produced are higher than those normally achieved during conventional antigen-induced T-cell activation, presumably due to potency of superantigens in polyclonal activation of large numbers of cells.

The "Good"

Upon stimulation by superantigens, naïve T cells respond and then quickly become anergized and/or deleted (33–35). On the other hand, T cells that are actively undergoing activation by specific antigen at the time of superantigen stimulation do not become anergized (33, 35). This is an important characteristic of superantigens that can be exploited when attempting to enhance specific antigen responses. Superantigens can cause anergy and/or deletion of potentially competing naïve T cells bearing the same Vβ element(s) as primed T cells of a desired antigen specificity. In other words, primed T cells of the desired antigen specificity will be further and more potently expanded by superantigens while naïve T cells of the same Vβ specificity will become anergized. Thus there would be less "competition" for cytokines and the desired specific immune response will be amplified.

Superantigens have been shown to exacerbate both humoral and cellular autoimmune responses in an autoimmune animal model, suggesting that immune responses are enhanced by superantigens. It seemed to us that this negative property of superantigens could be exploited in cases where immune responses are needed to be rapidly and potently enhanced, such as in the case of cancer.

To this end, we began studies on the effects of superantigens on cancer using a mouse melanoma model, in which C57Bl/6 mice are challenged with live syngeneic B16F10 melanoma cells. Tumor cells given in this manner usually cause death of C57Bl/6 mice approximately 14 days after challenge. Mice were vaccinated with inactivated tumor cells, followed several days later with an injection of superantigens (Fig. 3). Mice were then challenged with live tumor cells. Sixty to one hundred percent of the mice given both vaccine and superantigen survived 136 days after tumor challenge whereas untreated mice or mice given vaccine only survived for only 14 and 17 days following tumor administration, respectively (Kominsky *et al.*, submitted). Not only did vaccine/superantigen mice survive for 136 days, they showed no signs of having tumors. The surviving mice were given another dose of live melanoma tumor cells and 80% of the mice were alive and tumor-free 50 days after the tumor rechallenge, indicating that immunologic anti-tumor memory was established in these animals.

The question arose as to the possibility of protecting mice with established melanoma tumors. To this end, mice were challenged with live melanoma cells, followed several days later with an injection of superantigens (Fig. 4). Untreated mice died by Day 13, whereas SEA/SEB mice survived twice as long (100% survival on Day 28). One superantigen-treated mouse has survived as long as 50 days and is still free of tumors. Thus, it is feasible to treat

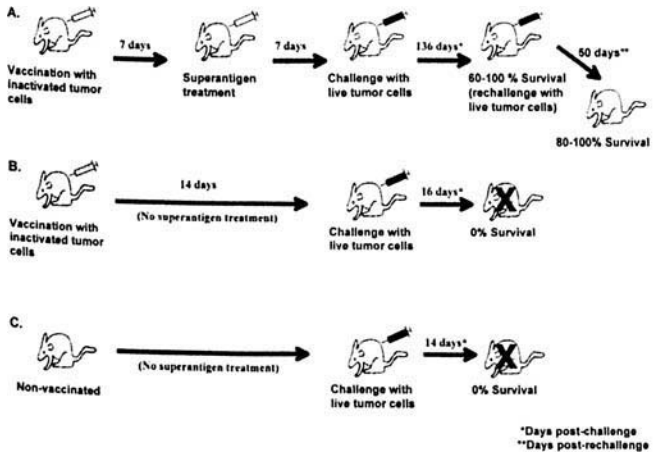

Figure 3. Combinational therapy of superantigens and vaccine can "cure" and/or significantly prolong the survival of mice challenged with live tumor cells. (A) Mice were treated with inactivated tumor cells, followed by treatment with SEA/SEB seven days later. Tumor challenge occurred seven days after superantigen treatment. Sixty to 100% of mice survived 136 days after challenge. These mice were again challenged, and 80–100% have survived greater than 50 days. (B) Mice were vaccinated with inactivated tumor cells and challenged 14 days later. All mice died by 16 days postchallenge. (C) Untreated mice were challenged with live tumor cells. All mice died by 14 days postchallenge.

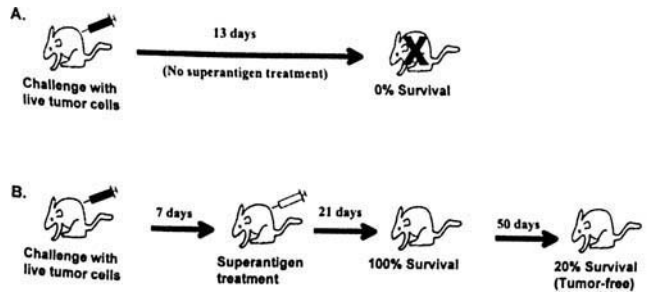

Figure 4. Superantigen treatment prolongs the survival of mice with established tumors. (A) One hundred percent of mice that were challenged with live tumor cells died by 13 days postchallenge. (B) Mice were challenged with live tumor cells and given superantigen 7 days later. One hundred percent of mice survived at least 21 days. Fifty days later, 20% of the mice were still alive with no signs of tumors.

tumor-bearing animals with superantigens and prolong survival, and in some instances, cure animals.

There may be several mechanisms at play in the protection of mice against melanoma by superantigens. One key mechanism is tumoricidal activity. It is possible that initial anti-tumor immunity is modulated by natural killer (NK) cell activity, as indicated by higher numbers of NK cells in vaccinated mice shortly after superantigen administration (data not shown). $CD8^+$ T-cell numbers also increased in these animals, and cytotoxic T lymphocyte (CTL) activity was highest in vaccinated/superantigen-treated animals. Thus, one mechanism of protection in these animals is immune cell-mediated tumoricidal activity involving initially NK cells and then CTL.

One of the hallmarks of superantigen activation of T cells is the production of cytokines, one of which is gamma interferon (IFNγ). In addition to important immunomodulatory activities, such as the enhancement of CTL, NK, and macrophage tumoricidal activity, IFNγ is known to have direct anti-proliferative properties (35–38). Thus, we investigated the effects of IFNγ on the growth rate of B16F10 melanoma cells *in vitro*. IFNγ had significant inhibitory effects on B16F10 melanoma cell growth, as much as 75% inhibition at a concentration of 10 U/ml (data not shown). As mentioned earlier, it is also possible that IFNγ enhances the tumoricidal activity of cells such as CTL, NK, and macrophages, thereby allowing for efficient removal of melanoma cells in superantigen/vaccination-treated mice. However, another possible mechanism for protection from melanoma by superantigen treatment may be the production of cytokines such as IFNγ, which may act directly on the tumor cells to inhibit their growth. Superantigens are known to activate $CD4^+$ T helper type 1 (T_H1) cells or inflammatory T cells, which are involved in cellular immune responses such as aiding in the generation of cytokines such as IFNγ and activation of cytotoxic $CD8^+$ T cells. The question arose as to the superantigen effects on $CD4^+$ T_H2 cells, which act as helper cells for antibody production by B cells *in vivo*. Thus, in addition to determining the effects of superantigens on cellular immune responses to tumor cells, studies were performed on the effects of superantigens on humoral immune responses to soluble antigens. The antigens used were a T-dependent antigen (bovine serum albumin; BSA) and T-independent antigens of either type I (such as lipopolysaccharide; LPS) or type II (pneumococcal polysaccharides). C57Bl/6 mice were injected with antigen alone, antigen followed by superantigen seven days later, or superantigens on Day 7. Antibody levels were determined by ELISA on serum from Day 14. Superantigens enhanced the T-dependent BSA antibody response by approximately 2-fold (Fig. 5). Interestingly, antibody production in response to type II T-independent antigens (pneumococcal polysaccharides) were also increased, suggesting that $CD4^+$ T cells play a role in enhancing humoral immune responses to type II T-independent antigens. The antibody response to type I T-independent antigen was not enhanced by superantigen. This is consistent with previous studies that showed that $CD4^+$ T cells enhance antibody responses to type II, but not type I, T-independent antigens (33, 39). Studies are underway to determine if increased $CD4^+$ T-cell activity of the T_H2 type played a role in superantigen enhancement of the antibody response to a soluble protein. These studies have potential importance for enhancing the antibody response against proteins as well as type II carbohydrate antigens in humoral responses to tumors.

Variations on a theme of staphylococcal enterotoxin (SE) transfection of tumor cells have been investigated in animal tumor models with good success (40, 41). In one study, B16 melanoma cells were transfected with the gene for SEA and irradiated for use as a vaccine (40). The melanoma cells were found to secrete SEA (approximately 9 ng/ml), even after irradiation with 3,500 rad. SEA-secreting melanoma cells, administered to mice either prior to or after

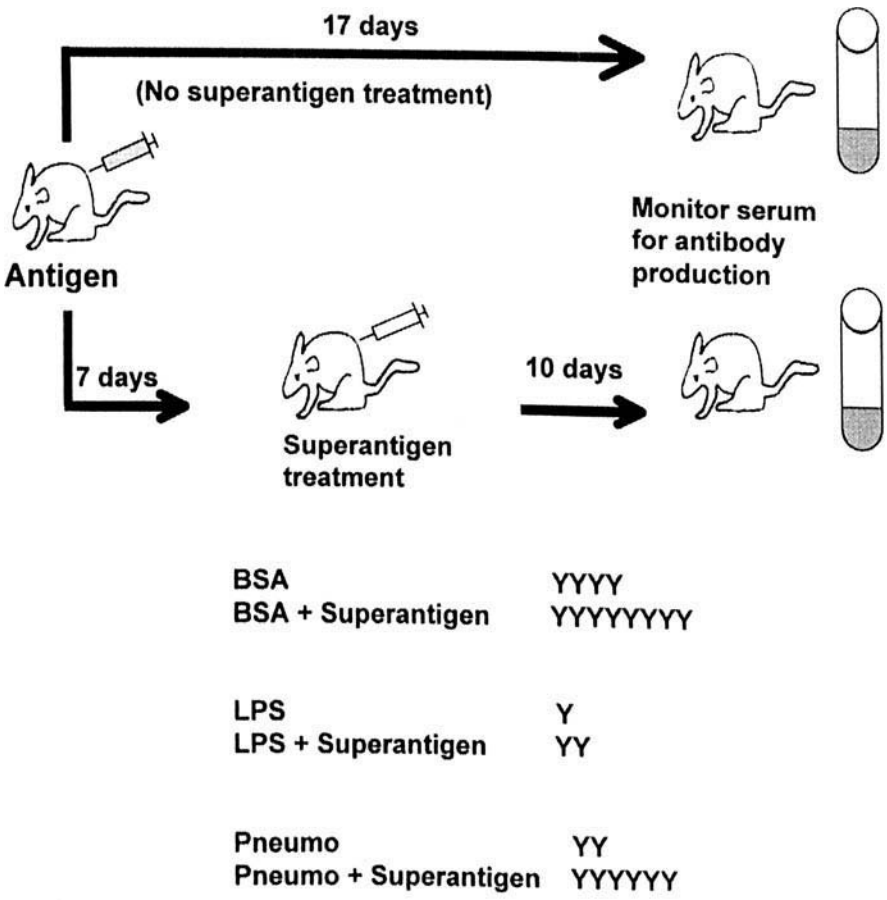

Figure 5. Effect of superantigens on the humoral response to T-cell-dependent and T-cell-independent antigens. Mice were immunized against either BSA, a T-cell-dependent antigen, or type I (LPS) or type II (pneumococcal polysaccharides) T-cell-independent antigens. Mice were either treated with superantigens or not given further treatment. Mice were tested for antigen-specific antibodies *via* ELISA. Two- and 3-fold increases in antibodies against BSA and pneumococcal polysaccharides, respectively, were observed. Little or no effect was noted in the antibody titers against LPS.

challenge with the live parent cell line, caused reduction in the size of primary tumors (40).

In another study, direct *in vivo* transfection with plasmid DNA containing sequences for SEB and GM-CSF was performed intratumorally on dogs having established malignant melanoma tumors (41). After transfection *in vitro*, SEB was expressed in 1–10% of tumor cells, and the SEB concentration (both intracellular and secreted) was estimated to be 1–10 fg/ml, which is within the range of responsiveness for human and canine cells (41). The primary tumors of stage III dogs were excised surgically and treated with intratumoral transfection with SEB/GM-CSF. Four of nine dogs survived to at least 90 weeks as compared to 17 weeks for dogs treated only with surgical excision.

Using a non-genetic approach, TSST-1 was passively anchored to P815 tumor cells by fusion with a hydrophobic transmembrane sequence (42). TSST-bound tumor cells were then used to vaccinate mice prior to administration of live tumor challenge. Smaller tumors were found in mice that received TSST-bound tumor cells, as compared to controls (42).

Another strategy for using superantigens as immunotherapeutics for cancer involves the fusion of tumor-specific antibodies to superantigens as a means of enhancing cytolytic T-cell activity *via* induction of proinflammatory cytokines (43). Antibodies specific for an immunodominant antigen in human melanoma cells, high-molecular-weight melanoma-associated antigen (HMM-MAA), were generated in cynomolgus monkeys that were immunized against human melanoma cells. These antibodies were fused to SEA and tested in severe-combined immunodeficient (SCID) mice for therapeutic effects against human melanoma challenge. Significant reduction in both the weight and number of tumors was observed in mice treated with the antibody–superantigen fusion protein (43). Similarly, it has been shown that SEA fused to antibodies directed against colon carcinoma antigens is effective in mediating superantigen–antibody-directed cellular cytotoxicity against human carcinoma cells in an MHC class II-independent manner (44). Thus, other approaches to treatment of tumors using superantigens are feasible and need to be more fully explored.

The "Bad" and the "Ugly"

Superantigens have been implicated in acute human diseases such as food poisoning and toxic shock syndrome. These acute diseases can be considered to be the 'bad" effects of superantigens. Both food poisoning and toxic shock syndrome are caused primarily by members of the family of SE superantigens, and will be discussed in more detail below. A possible ramification of rampant T-cell activation by superantigens is the proliferation of autoreactive T cells. Superantigen-producing pathogens are ubiquitous. Thus, superantigen-producing pathogens may

play a role in the establishment and/or exacerbation of autoimmune disorders (an "ugly" side effect) such as multiple sclerosis, rheumatoid arthritis, psoriasis, and diabetes.

Acute Effects: Food Poisoning and Toxic Shock Syndrome. A form of gastroenteritis known as staphylococcal food poisoning occurs upon ingestion of food colonized with toxin-producing strains of *S. aureus*. The symptoms, which include vomiting and diarrhea, are generally short-term, lasting no longer than 1–2 days. *S. aureus* is the most common cause of food poisoning in the United States (1, 2). Most of the enterotoxins (with the exception of TSST-1) produced by *S. aureus* can cause the emetic response seen in food poisoning, although SEA is usually the culprit.

An important question concerns the identity of the target cells for the emetic effects of the SE, since these effects may be independent of T-cell activation. Experimental results suggest that SEB stimulates mast cells to release leukotrienes, which are thought to be responsible for the emetic response of monkeys (45). Corroborative evidence supporting this mechanism of SEB action was the ability of a leukotriene LTD4/LTE4 receptor inhibitor (LY 171833) to block emesis (45). In another study, the emetic response correlated with the production of prostaglandin E2 and leukotriene B4 (46). The role of mast cells in SE-induced emesis has been speculative to date due to lack of evidence of SE receptor on mast cells. Since it has been established that class II major histocompatibility complex antigens serve as the receptors on nonlymphoid cells for SE, it is important to show that mast cells display such receptors. Recently, human cord blood mast cells have been shown to express the MHC class II antigens, HLA-DR and HLA-DQ (47, 48). Thus, SE may exert their biological effects through activation of T cells as well as other cells, such as mast cells. Finally, another mechanism by which SE may exert their biological effects involves SE-dependent, T cell-mediated cytotoxicity (49). MHC class II-expressing, SE-presenting cells are selectively and rapidly eliminated following their presentation of SE to T cells, presumably by means of lymphokines released by the activated T cells. This mechanism may represent a bacterial strategy to avoid immune recognition.

Toxic shock syndrome (TSS) was first described as a disease in young children with *S. aureus* infections (50). TSS became more extensively characterized as the result of an epidemic that occurred in the early 1980s involving young women using tampons during menstruation. A spectrum of symptoms are manifested in TSS, including an early rash (with desquamation occurring later), fever, and severe hypotension, the latter possibly leading to fatal shock. Several organ systems are affected during TSS, such as skin, kidneys (decreased renal function), liver (elevation in liver enzymes), and gastrointestinal tract (vomiting/diarrhea) (51). It was determined that the tampon-associated disease was caused by intravaginal colonization by strains of *S. aureus* that produce TSST-1 (52–55). Presumably, illness worsens and becomes more severe as the bacteria continue to grow and elaborate TSST-1. Cytokines such as interleukin 1 (IL-1) and tumor necrosis factor (TNF) have been shown to be elevated during the course of TSS, and thus have been implicated in the shock aspect of the disease (56–58). Little or no immunity against TSST-1 occurs, and even after several bouts of TSS, few patients seroconverted (52).

Chronic Effects: Superantigens in Autoimmunity. *Neurologic inflammatory disease.* Multiple sclerosis (MS) is an inflammatory demyelinating autoimmune disease of the central nervous system that causes paralysis, and affects speech, motor functions, and vision. The symptoms of MS can often be observed to occur in a relapsing/remitting manner. This form of MS consists of presentation with clinical symptoms of MS followed by periods of remission. How relapses and exacerbations occur and what causes the reactivation of autoimmune disease has been a topic of much speculation. It has been suggested that environmental influences may contribute to or even be responsible for exacerbations of autoimmune disease (59). Such influences from one's environment potentially include exposure to infectious agents as well as factors possessing immunostimulatory activity. As indicated previously, microbial superantigens are ubiquitous in our environment.

Experimental allergic encephalomyelitis (EAE) is an animal model that is useful for the study of the inflammatory demyelinating disease, MS (60). In the EAE model of neurologic inflammatory disease, components of the myelin sheath including myelin basic protein (MBP), proteolipid protein (PLP) and myelin oligodendrocyte protein, serve as central nervous system (CNS) antigens for induction of autoimmunity. Upon immunization with MBP, PL/J mice develop clinically observable tail and limb paralysis due to lymphocytic infiltration into the CNS accompanied by acute demyelination.

Exacerbation of disease manifested as a clinical relapse of EAE was first demonstrated by the administration of a microbial superantigen. In the PL/J strain, acute episodes of EAE usually resolve and clinical relapses do not occur (61). After resolution of all clinical signs of EAE induced by immunization with MBP, administration of either of the staphylococcal enterotoxin (SE) superantigens, SEB or SEA, caused reactivation of disease (5, 62). These results were confirmed by studies of SEB in mice (63) and SED in rats (64). Studies by our group revealed several interesting features of superantigen-induced EAE. In addition to reactivation of a single episode of disease, SEB also induced clinical disease in mice immunized with MBP but which never developed clinical signs of EAE (62). In this case, superantigens were able to initiate development of disease in immunized but asymptomatic animals bearing autoreactive T cells. Multiple injections of SEB also resulted in relapses of EAE over a three-month period, suggesting that

after superantigen activation these auto-reactive T cells were resistant to anergy and deletion.

EAE can also be prevented by administration of SEB prior to immunization with MBP (65, 66). Anergy and/or deletion of the $V\beta8^+$ T-cell subset that is responsible for the initial induction of EAE appears to be the mechanism for this protection. Naïve T cells appear to be susceptible to superantigen-induced anergy and/or deletion while activated T cells are not susceptible. Targeting of a specific Vβ T-cell population does not, however, provide absolute protection from development of EAE. When mice protected from development of EAE by SEB pretreatment are exposed to SEA (which has a different Vβ T-cell specificity from SEB), induction of EAE does occur (5). This SEA-induced EAE is characterized by severe paralysis and accelerated onset of clinical symptoms. Similarly, superantigens from *S. pyogenes* have been implicated in activation of auto-reactive cells in a case of acute disseminated encephalomyelitis (67). Thus, the effects of microbial superantigens introduce a profound complexity to autoimmune disease models such as EAE, akin to the complexity of the pathogenesis observed in MS (see Fig. 6).

It has been demonstrated that an event during the course of disease in the EAE model known as epitope spreading occurs (68, 69). At later time points after immunization of mice with a specific autoantigen, T-cell proliferative responses to other previously cryptic epitopes can be detected. Epitope spreading includes both intramolecular (spreading of T-cell responsiveness to other epitopes within the same autoantigen) and intermolecular (spreading of T-cell responsiveness to other separate autoantigens) spreading. It has been suggested that induction of epitope spreading may be linked to disease relapse and development of chronic disease.

We hypothesized that superantigen reactivation of EAE may result in the spreading of T-cell specificities for other epitopes of MBP. PL/J mice that had resolved an initial episode of EAE were treated with SEA and developed a second episode of paralysis. At the onset of symptoms, mice were sacrificed and splenocytes were stimulated *in vitro* with a panel of MBP peptides. EAE reactivation by SEA resulted in the spreading of T-cell specificities from the immunodominant epitope Ac1-17 to residues 100–120 of MBP (Soos *et al.*, manuscript in preparation). While intramolecular spreading did occur, spreading to other antigens did not occur as evidenced by the lack of response to a proteolipid protein (PLP) peptide and heat shock protein 60. To further characterize the epitope MBP 100–120, PL/J mice were immunized with MBP 100–120. No initial development of disease was observed. However, administration of SEA two weeks after MBP 100-120 immunization resulted in the onset of paralysis. In addition to a proliferative response to MBP 100-120, these mice also exhibited a proliferative response to the flanking MBP peptides 81–100 and 120–140. Thus, SEA is able to induce intramolecular epitope spreading in PL/J mice after reactivation of EAE.

These results suggest that superantigen involvement may increase the complexity of disease in the EAE model. In strains of mice that are able to develop a chronic form of EAE, it has been demonstrated that spreading of T-cell responses from a dominant autoantigen epitope to other subdominant or cryptic epitopes can occur (68, 69). Likewise, reactivation of EAE by superantigen can lead to the spreading of T-cell responses to other autoantigen epitopes in the

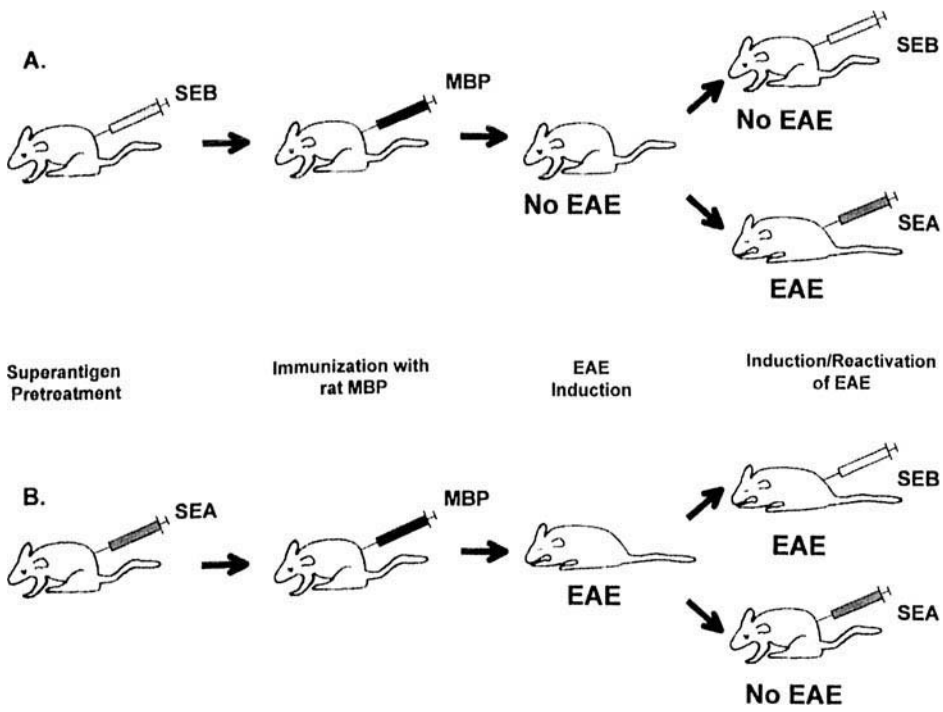

Figure 6. Modulation of EAE by superantigens with different Vβ specificities. The predicted outcome of the hypothesis proposed is denoted by "EAE" (induction of disease) and "No EAE" (absence of disease). The predicted outcome was confirmed by studies on the development of EAE after administration of either SEA or SEB. (A) In the first group, SEB pretreatment prevented development of EAE following injection of MBP and, while mice administered a second dose of SEB were refractory to development of disease, mice administered SEA exhibited accelerated onset of EAE. (B) In the second group, SEA pretreatment did not prevent EAE. After resolution of clinical symptoms, administration of a second dose of SEA did not reactivate disease. SEB administration, however, did reactivate EAE in the SEA-pretreated mice.

PL/J strain, which normally only develops an acute episode of disease after immunization for induction of EAE (61). Thus, a contributing mechanism for development of clinical disease in the EAE model by superantigen administration may be spreading of T-cell responses to other subdominant but pathogenic autoantigen epitopes.

Rheumatoid arthritis. Superantigens have also been implicated in rheumatoid arthritis, a chronic autoimmune disease. Superantigen effects are suggested by studies on peripheral and synovial $V\beta14^+$ T cells from rheumatoid arthritis patients versus controls (70), and SED-induced B-cell production of rheumatoid factor, autoantibodies that are reactive with immunoglobulins (71). Further, recent studies suggest that the staphylococcal superantigens increase the cellular cytotoxic activity of T cells, with synovial fibroblasts being the targets of this cytotoxicity (72).

Animal studies also suggest that superantigens may be involved in rheumatoid arthritis. Superantigens have been shown to reactivate bacterial wall-induced arthritis (73) and collagen-induced arthritis (10). In these models, reactivation was induced by immunization with autoantigen prior to superantigen exposure. Mice immunized with a cell wall preparation of *S. pyogenes* were administered TSST-1, resulting in rapid reactivation characterized by multiple episodes of inflammation lasting as long as six weeks (73). In collagen-induced arthritis, mice having undergone and resolved an episode of arthritis were subsequently challenged with *Mycoplasma arthriditis* mitogen (MAM). These mice showed reactivation of disease in 5–10 days (10). In another collagen-induced arthritis study, treatment with SEB prior to induction of disease by collagen administration resulted in significant protection against arthritis (74). Further, administration of SEB after immunization with collagen caused increased severity of arthritis (75). Infection with a superantigen-producing microorganism may also lead to autoimmunity after the initial infection has resolved. Examples include scarlet fever caused by *Streptococcus*, which can lead to rheumatic heart disease (7, 76), and *Yersinia enterocolitica* infection, leading to reactive arthritis and Reiter's syndrome (77). These findings are similar to those of the role of superantigens in EAE.

Psoriasis. Psoriasis is a cutaneous inflammatory disorder characterized by epidermal keratinocyte hyperproliferation in association with inflammatory infiltrates (78). Increases in the numbers of Vβ2 and Vβ5.1 T cells have been seen in the dermis and epidermis of patients with guttate and chronic plaque psoriasis, as compared to T-cell populations in peripheral blood (79). Skin lesion eruptions in guttate psoriasis have been linked with throat infections and increased antibody titers to streptococcal antigens (80). T cells specific for group A streptococcal antigens have been isolated from psoriatic lesions. The group A streptococci produce multiple superantigens, including streptococcal pyrogenic exotoxins, SPE-A, -B, and -C. Significantly, Vβ2 T cells are stimulated by SPE-A and SPE-C while Vβ15 T cells are stimulated by SPE-C (81). Such results appear to further substantiate the hypothesis that superantigens are involved in the etiology and/or exacerbation of psoriasis.

Diabetes. Insulin-dependent diabetes mellitus (IDDM) is an autoimmune disorder in which pancreatic β cells are destroyed. There is evidence that auto-reactive $V\beta7^+$ T cells are responsible for the destruction of pancreatic cells (16), suggesting that the disease may involve a superantigen. An endogenous human retrovirus has recently been isolated from IDDM patients that induces the proliferation of $V\beta7^+$, the same subset of T cells thought to be involved in destruction of the pancreas (17). Thus, initial evidence suggests that a virally encoded superantigen may modulate autoimmune disease.

Chronic effects: immunodeficiency. Following the intense activation by superantigens, Vβ-specific T cells may become anergic or even be deleted (34, 35), possibly resulting in a state of immunodeficiency in an individual. One mechanism by which anergy may be induced in T cells by superantigens is Vβ-specific internalization of TCR (82).

Human immunodeficiency virus (HIV) causes a loss of $CD4^+$ T cells over the course of the disease, resulting in the inability to effectively combat infections by other microbial agents. Other immunologic perturbations 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 (83).

It has been speculated that the immunologic perturbations observed during the course of infection may be due to an HIV-encoded superantigen. Several pieces of evidence implicate the involvement of an HIV superantigen (84). Initial studies suggested that HIV-infected patients had deletions in the Vβ repertoire (85), although later studies disagreed with this finding (86). Of all the Vβ T-cell subsets tested, $V\beta12^+$ cells were shown to proliferate in response to HIV-infected cells and were able to support enhanced HIV replication and proliferation in response to HIV-infected cells (86). Another study in which the T-cell subsets of monozygotic twins discordant for HIV infection were analyzed showed perturbations in several Vβ subsets (87).

An HIV-encoded superantigen has been identified (18). Nef, a regulatory protein expressed early in the infection of $CD4^+$ T cells, was shown to induce Vβ-specific T-cell proliferation in the absence of processing (18, 88). T-cell proliferation required the presence of antigen-presenting cells. Cytokine production, in particular IL-2 and IFNγ, was induced by Nef. More importantly, Nef-stimulated T cells were capable of supporting HIV replication. Antibodies to two regions of Nef, the carboxyl terminus and an internal site, blocked Nef-induced proliferation and the ability of cells to support virus replication. Further, HIV-infected cells caused proliferation and activation of autologous T cells that were then capable of supporting HIV replication. Anti-Nef antibodies blocked both of these events. Thus, the data suggest that Nef may be involved in the establishment of HIV infection by causing the expansion

of T-cell subsets that may act as cellular reservoirs for viral replication.

Nef has been shown to induce the differentiation of human B cells to immunoglobulin-secreting cells, probably as a result of T-cell activation and release of cytokines that aid in B-cell activation and differentiation (89). Antibodies to MHC class II antigens abrogated differentiation. B-cell differentiation required the presence of T cells and monocytes (89). Interestingly, superantigens such as the staphylococcal enterotoxins have also been shown to cause B-cell differentiation (90). These data show that Nef superantigen can result in both T- and B-cell activation in a manner reminiscent of the staphylococcal superantigens.

A model on the hypothetical role of Nef in the pathogenesis of HIV is presented in Fig. 7. Nef may be released in a soluble form as the result of lysis of infected cells and presented by antigen-presenting cells and/or be expressed on the surface of infected T cells. Interaction of T cells with Nef in either of these ways activates T cells to proliferate and produce cytokines such as IFNγ and IL-2. An outcome of Nef stimulation is the establishment of a cellular reservoir of activated $CD4^+$ T cells for virus production, with eventual depletion of T cells *via* virus replication, anergy and/or apoptosis. B-cell differentiation could be seen as the result of $CD4^+$ cells providing help and producing cytokines that aid in B-cell maturation. Nef activation may also explain, in part, the increased spontaneous immunoglobulin levels seen throughout the course of HIV infection (83). Thus, the outcome of Nef-induced immune activation may include increased viral yield with T-cell anergy/apoptosis and polyclonal B-cell activation, the latter resulting in hypergammaglobulinemia and possible autoimmune-like sequelae.

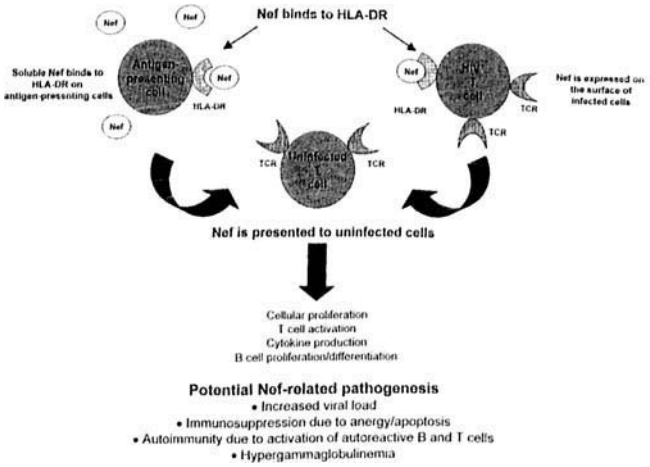

Figure 7. Model for the role of Nef in the pathogenesis of HIV. Soluble Nef released by lysed infected cells binds to HLA-DR on antigen-presenting cells, or is an integral component of the cell membranes of infected T cells. Nef is then presented to uninfected T cells, causing proliferation and activation of T cells with concomitant cytokine production. Such proliferation results in a cellular reservoir for virus replication. Differentiation of B cells may possibly be mediated by release of T-cell cytokines.

MMTV and Cancer. The prototype for viral superantigens is produced by MMTV, a type-B retrovirus that causes mammary tumors (91–94). Although MMTV superantigens were recognized in the 1990s, they were originally described in 1973 as minor lymphocyte-stimulating (mls) antigens (95). These antigens were identified by their ability to stimulate lymphocytes from MHC-identical mice. The mls antigens were determined to be the products of endogenous superantigens from germline-encoded MMTV provirus (96, 97).

Initial studies on the infectivity of MMTV indicated that an intact immune system was required for infection (14). Although MMTV ultimately infects mammary gland tissue, MMTV is ingested and initially infects B cells and T cells in the mucosal-associated lymphoid tissue. Both B cells and T cells produce infectious virions (98). It has been speculated that MMTV superantigen is required for amplification of virus replication by causing Vβ-specific T-cell expansion, which enhances the further infection of immune cells (14).

MMTV superantigen has been implicated in the migration of infected immune cells to the mammary gland and in the subsequent efficient infection of mammary tissue (99). Low or undetectable levels of virus were found in the mammary tissue of transgenic mice expressing endogenous MMTV superantigen (and thereby lacking superantigen-reactive T cells) when high virus doses were introduced directly into the mammary gland. These data indicate that immune cells are essential for the infection of mammary tissue, which is the site for transmission of virus *via* milk to suckling pups. Further, fewer of these transgenic mice had incidence of mammary tumors as compared to non-transgenic mice, probably as a consequence of significantly lower virus levels in these animals (99). Thus, MMTV superantigen acts as a virulence factor, not only in the establishment of infection in immune cells and mammary tissue but also in the tumorigenesis of the virus.

Immune-Based Therapies That Ameliorate "Bad" and "Ugly" Superantigen Effects. *Type I interferons (IFNs).* In spite of their undesirable side effects, IFNs, in particular the type I IFNs, are well established as useful drugs and their application is likely to expand as research continues. IFNβ was approved in 1993 by the FDA for the treatment of the relapsing/remitting form of MS and is currently being used in this capacity (100). Despite its positive effects in ameliorating the symptomology of MS, IFNβ has undesirable side effects, including bone marrow suppression and weight loss (101, 102).

A unique type I IFN, IFN tau (IFNτ), has been used in the EAE animal model (103–107). IFNτ was initially identified as a pregnancy recognition hormone in sheep (108). Cloning of its cDNA and comparison with other genes showed strong homology with IFNα (109). This led to the characterization of the antiviral activity of ovine IFNτ, which resulted in the demonstration of some very interesting biological properties (110, 111). Ovine IFNτ possesses

antiviral activity similar to IFNα across several species, including humans (110). However, unlike IFNα and IFNβ, IFNτ lacks toxicity for cells at high concentrations (110, 112), and does not induce weight loss or bone marrow suppression in animal models (103, 104, 106). This is a very important observation for the use of type I IFNs as therapeutics, particularly for treatment of MS.

IFNτ, administered both intraperitoneally (ip) and orally, was shown to induce remission in SJL/J mice that had ongoing chronic active EAE disease and protected mice against secondary relapses (104, 106). Treatment with IFNτ reversed lymphocyte infiltration and microglial activation in the CNS. Lower anti-MBP antibody levels were found in IFNβ-treated mice than in untreated mice in both the acute and chronic forms of EAE. MBP induced the proliferation of B cells in EAE mice, but activation was blocked by either *in vivo* or *in vitro* treatment with IFNτ. Further, IFNτ inhibited MBP activation of T cells from EAE mice. Thus, IFNτ inhibited both cellular and humoral immunity in EAE, possibly explaining the effectiveness of type I IFNs in the treatment of MS.

IFNτ was shown to prevent EAE by the induction of suppressor cells and suppressor factors (105). Specifically, the protective effects of IFNτ are mediated, at least in part, by $CD4^+$ Th2 suppressor cells and by the induction of suppressor factors consisting of interleukin-10 (IL-10) and transforming growth factor β (TGFβ) by these cells (104, 105). IL-10 and TGFβ were found to act synergistically to inhibit the proliferation of auto-reactive T cells from EAE mice in response to autoantigen. Further, administration of IFNτ to mice having either the chronic or relapsing/remitting forms of EAE resulted in IL-10 production *in vivo*.

Structure studies have shown that the N-terminus of IFNτ is involved in its lack of toxicity (113, 114). Having identified this region, a "humanized" chimeric has recently been constructed consisting of human IFNα and the N-terminus of IFNτ. This chimeric IFN possesses potent biological activity in tissue culture but, like ovine IFNτ, lacks the toxicity associated with IFNα (107). This chimeric has been constructed because a human IFNτ with the properties of ovine IFNτ has not been identified to date. A previous report of a human IFNτ has not held up or been confirmed (115). The chimeric IFN is currently being tested in animal models.

Interleukin 10 (IL-10). Studies have shown that one mechanism of protection of mice against antigen induction of EAE and superantigen reactivation of relapses is the induction of IL-10 by type I IFNs in treated mice (104, 105). IL-10 is a Th2 cytokine that suppresses the activity of $CD4^+$ Th1 cells (116). Antibodies to IL-10 block the protective effects of IL-10 against EAE. Focusing on cell cycle events, we have determined the effects of IL-10 on the entry of quiescent $CD4^+$ T cells into the cell cycle upon stimulation with the staphylococcal superantigen, SEB (119). IL-10 blocked cells at the G_0/G_1 phase of the cell cycle. IL-10 treatment prevented the down-regulation of $p27^{KIP1}$, an inhibitor protein that controls progression out of the G_0 phase of the cell cycle. IL-10 also prevented the up-regulation of the G_1 cyclins D2 and D3, proteins necessary for entry and progression through the G_1 phase of the cell cycle. Associated with the inhibition of the cell cycle, IL-10 suppressed SEB induction of IL-2 (117). Addition of exogenous IL-2 to IL-10-treated cells significantly reversed the antiproliferative effects of IL-10. Moreover, IL-10 effects on the early G_1 proteins $p27^{KIP1}$ and cyclin D2 were similarly reversed by exogenous IL-2. Although this reversal by IL-2 was pronounced, it was not complete, suggesting that IL-10 may have some effects not directly related to the suppression of IL-2 production.

Cell separation experiments suggest that IL-10 can affect purified $CD4^+$ T cells directly, providing functional evidence for the presence of IL-10 receptors on these cells. Further, IL-10 inhibited expression of IL-2 transcription regulators c-fos and c-jun, which also inhibit other cell functions. The studies show that the mechanism of IL-10 regulation of quiescent $CD4^+$ T-cell activation is mainly by blocking induction of IL-2 that is central to down-regulation of $p27^{KIP1}$ and up-regulation of D cyclins in T-cell activation and entry into the cell cycle (117).

The mitogen-activated protein (MAP) kinase pathway involving the kinase cascade Ras→Raf→Mek→Erk→Elk-1 is important for gene activation in T cells (118). IL-10 blocks the phosphorylation of Raf and Erk, thus inhibiting signal transduction *via* the MAP kinase pathway of T-cell activation (Perrin *et al.*, manuscript in preparation). Blockage of the MAP kinase pathway is one possible mechanism by which IL-10 blocks IL-2 induction by SEB and blocks the direct effects of SEB on T-cell activation.

Conclusions

Superantigens are produced by microbial organisms that are ubiquitous in the environment. Superantigen effects can be acute or chronic, the latter involving autoimmunity or immunodeficiency. These effects are deleterious, although immune-based therapies exist to help overcome superantigen effects. Further, the powerful effect of superantigens on the immune response can be exploited to help establish long-term anamnestic immune responses to cancer.

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