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*J Immunol* 2002; 169:2907-2914; doi: 10.4049/jimmunol.169.6.2907
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Superantigens are microbial proteins that induce massive activation, proliferation, and cytokine production by CD4+ T cells via specific Vβ elements on the TCR. In this study we examine superantigen enhancement of Ag-specific CD4+ T cell activity for humoral B cell responses to T-dependent Ags BSA and HIV gp120 envelope, type I T-independent Ag LPS, and type II T-independent Ag pneumococcal polysaccharides. Injection of BSA followed by a combination of superantigens staphylococcal enterotoxin A and staphylococcal enterotoxin B (SEB) 7 days later enhanced the anti-BSA Ab response in mice ~4-fold compared with mice given BSA alone. The anti-gp120 response was enhanced ~3-fold by superantigens. The type II T-independent Ag pneumococcal polysaccharide response was enhanced ~2.3-fold by superantigens, whereas no effect was observed on the response to the type I T-independent Ag LPS. The superantigen effect was completely blocked by the CD4+ T cell inhibitory cytokine IL-10. SEB-stimulated human CD4+ T cells were examined to determine the role of the mitogen-activated protein (MAP) kinase signal transduction pathway in superantigen activation of T cells. Inhibitors of the mitogen pathway of MAP kinase blocked SEB-induced proliferation and IFN-γ production, while an inhibitor of the p38 stress pathway had no effect. Consistent with this, SEB activated extracellular signal-regulated kinase/MAP kinase as well as MAP kinase-interacting kinase, a kinase that phosphorolylates eIF4E, which is an important component of the eukaryotic protein synthesis initiation complex. Both kinases were inhibited by IL-10. Thus, superantigens enhance humoral immunity via Ag-specific CD4+ T cells involving the stress-independent pathway of MAP kinase. The Journal of Immunology, 2002, 169: 2907–2914.

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 (reviewed in Ref. 1). Acute effects include food poisoning and toxic shock syndrome. Long-term effects include autoimmune diseases and immunodeficiency (1). These effects have generally been considered “bad” and “ugly.” However, if the superantigen effects could be harnessed and exploited, then superantigens can have “good” effects for the host, such as enhancement of desirable Ag-specific immune responses.

Upon stimulation by superantigens, naive T cells respond and quickly become anergized and/or deleted (2–5). In contrast, T cells that are actively undergoing activation by specific Ag at the time of superantigen stimulation do not become anergized (6, 7). This is an important characteristic of superantigens that can potentially be exploited when attempting to enhance specific Ag responses. Superantigens can cause anergy and/or deletion of potentially competing naive T cells bearing the same Vβ element(s) as primed T cells of desired Ag specificity. In other words, primed T cells of a desired Ag specificity would become further and more potently expanded by superantigens, while naive T cells of the same Vβ specificity would become anergized. Thus, there would be less “competition” for cytokines and the desired specific immune response would be amplified.

We first tested this hypothesis in a mouse model for melanoma (8). B16F10 melanoma is a tumor derived from C57BL/6 mice that has been found to be poorly immunogenic and highly aggressive. We showed that vaccination of mice with a combination of staphylococcal enterotoxin A (SEA), staphylococcal enterotoxin B (SEB), and inactivated B16F10 cells led to significant and specific protection against subsequent challenge with viable B16F10 cells (at least 25-fold greater than a lethal dose) (8). Seventy-five percent of mice surviving >170 days remained tumor free after rechallenge with a lethal dose of B16F10, evidence of the development of strong immunologic memory. Additional studies showed increased numbers of CD4+ and CD8+ T cells, CTL activity, and IFN-γ production. Furthermore, failure to produce protection in either CD4− or CD8− T cell knockout mice is evidence that both CD4+ and CD8+ T cells probably played an essential role in induction of protective immunity. These results showed that superantigen administration subsequent to vaccination with inactivated tumor cells resulted in protective antitumor immunity.

In the present study we address the question of whether CD4+ T cell activation by superantigens extends to the subpopulation that provides helper signals for B cell activation and production of Abs to soluble Ags. If superantigens can significantly enhance B cell production of Abs in an Ag-specific manner, then this would suggest that the Th2 subpopulation of CD4+ T cells can also be amplified in an Ag-specific manner. Superantigens would thus be

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Received for publication December 26, 2001. Accepted for publication July 18, 2002.

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3 Abbreviations used in this paper: SEA, staphylococcal enterotoxin A; SEB, staphylococcal enterotoxin B; MAP, mitogen-activated protein; ERK, extracellular signal-regulated kinase; MBP, myelin basic protein; MNK, MAP kinase-interacting kinase; EAE, experimental allergic encephalomyelitis; MEK, MAP/ERK kinase.
potent activators of both the cellular and humoral arms of the immune system in an Ag-specific manner. This would suggest that superantigens could function as potent novel adjuvants to cellular and humoral immunity against cancer and infectious diseases.

Materials and Methods

Mouse studies

Six- to 8-wk-old female C57BL/6 mice (The Jackson Laboratory, Bar Harbor, ME) were used in these studies. Mice were bled before injection with Ag. The Ags used included BSA, LPS, pneumococcal polysaccharides (Pneumovax 23; Merck, Whitehouse Station, NJ), and the HIV-1 p24 envelope protein, gp120 (Advanced Biotechnologies, Columbia, MD). Fifty micrograms of each Ag were injected i.p. into mice, with the exception of gp120, which was administered i.p. at 5 μg per mouse. One week later, mice were injected i.p. with a combination (25 μg each) of SEA and SEB. Highly purified SEA and SEB were purchased from Toxin Technology (Sarasota, FL). Mice were bled once a week until the completion of each experiment.

Detection of specific Abs in mouse sera

Sera from mice were tested for specific Abs using a standard ELISA protocol. Fifty microliters of Ag (25 ng/well) in binding buffer (0.1 M carbonate/bicarbonate, pH 9.6) were placed in wells of 96-well plates and allowed to adhere overnight at room temperature. Plates were washed in wash buffer (150 mM NaCl, 0.05% Tween 20) and four reactive sites on the plastic were blocked for 2 h with 200 μl/well blocking buffer (PBS (pH 7.2) containing 5% nonfat instant milk). After washing plates, sera were diluted and 50 μl were placed in the wells for 1.5 h. Plates were again washed and alkaline phosphatase-conjugated anti-mouse IgG whole molecule or anti-mouse IgM (50 μl; Sigma-Aldrich, St. Louis, MO) was added to wells. After 45 min, plates were washed and 200 μl of substrate (1 mg/ml p-nitrophenyl phosphate in binding buffer) was added to plates. Color was allowed to develop for 30–60 min, after which 50 μl of stop solution (2 M H2SO4) was added. Absorbance was read at 405 nm using a Model 450 Bio-Rad Microplate Reader (Bio-Rad, Hercules, CA).

Isolation of PBMCs and cell culture

Human cells were collected from the whole blood of normal healthy volunteers or from leukocyte source packs (Civitan Regional Blood Center, Gainesville, FL). PBMCs were isolated by Histopaque (Sigma-Aldrich) density gradient centrifugation and viability was determined to be >95% by trypan blue exclusion. CD4+ T cells were isolated from PBMCs using the RosetteSep Human CD4+ T cell enrichment mixture (STEMCELL Technologies, Vancouver, Canada) as per the manufacturer’s instructions. Cell purity was assessed by flow cytometry. Cells were maintained in RPMI 1640 supplemented with 10% (v/v) heat-inactivated FBS, 200 μM penicillin, and 200 μg streptomycin in a 5% CO2 atmosphere at 37°C and used immediately.

Proliferation assay

The proliferative response of purified CD4+ T cells to SEB was performed by measuring the incorporation of [3H]thymidine into DNA. Specifically, purified human CD4+ T cells were added to 96-well plates at a concentration of 2.5 × 10^5 cells/ml, which was the optimal number of cells necessary to obtain substantial proliferation, as determined empirically. In the case of the specific mitogen-activated protein (MAP) kinase inhibitors PD98059 and SB202190 (Calbiochem, La Jolla, CA), the cells were pre-treated with various concentrations of the inhibitors for 1 h before stimulation with SEB. SEB at a concentration of 3 ng/ml was then added and the cultures were incubated in a final volume of 150 μl/well. After 90 h, 1 μCi of [3H]thymidine (Amersham, Arlington Heights, IL) was added per well and the plates were incubated for 6–8 h before harvest. [3H]Thymidine incorporation was measured as cpm on a liquid scintillation counter (BD Biosciences, San Jose, CA). All experiments were performed in quadruplicate.

Immunoblotting

Cells were lysed at 4°C for 20 min in ice-cold lysis buffer consisting of 50 mM/L Tris-HCl (pH 7.5), 250 mM/L NaCl, 1% (v/v) Triton X-100, 2 mM/L EDTA, 2 mM/L EGTA, 50 mM/L NaF, 20 mM/L β-glycerophosphate, 2 mM/L Na3VO4, 10 μg/ml aprotinin, 10 μg/ml leupeptin, 10 μg/ml benzamidine, and 1 mM/L PMSF (9). Samples were centrifuged at 13,000 rpm for 10 min and protein concentrations of the supernatants were determined using bicinechonic acid protein assay (Pierce, Rockford, IL). Equal amounts of protein from cell lysates (30–70 μg/lane) were subjected to SDS gel electrophoresis. After Western transfer, membranes were probed with the Abs indicated and developed using an ECL detection kit (Amersham). Densitometric analysis of radiographic film using IA-1000 Digital Analysis Software (Alpha Innotech, San Leandro, CA) was used to determine fold increase or decrease between band intensities based on total pixel value.

Extracellular signal-regulated kinase (ERK) assay

CD4+ T cells were isolated as described previously and incubated in medium without FBS for 1–2 h to decrease basal levels of activity before use (9). Cells (1 × 10⁶) were added to 1.5-ml microtube flasks and incubated with or without IL-10 at 100 U/ml for 30 min at 37°C. These cells were then stimulated as described for the times indicated and centrifuged at 2000 rpm for 10 s. Supernatants were removed and the cells were quick-frozen in liquid nitrogen before storage at −70°C until use. Cells were lysed and centrifuged as described above. Protein concentrations were determined and samples were normalized for protein content as described above. Abs to ERK-1 and ERK-2 (1 μg each; Santa Cruz Biotechnology, Santa Cruz, CA) were added and the samples were immunoprecipitated overnight at 4°C. Protein A-Sepharose slurry (40 μl; Sigma-Aldrich) was added and samples were incubated for 2 h on a rotator at 4°C before centrifugation at 2000 rpm for 10 s. The immunoprecipitates were then washed twice with cold lysis buffer and twice with cold kinase buffer, consisting of 20 mM MOPS (pH 7.2), 2 mM EGTA, 20 mM MgCl2, and 1 mM DTT, before being resuspended in 30 μl of kinase buffer. Upon addition of 0.5 μg of ATP, 10 mCi of [γ-32P]ATP (Amersham) and 6 μg of myelin basic protein (MBP), samples were incubated for 20 min at 35°C. The samples were then eluted in SDS-PAGE sample buffer, separated by SDS-PAGE, and visualized by autoradiography.

ELISA for human IFN-γ

Human CD4+ culture supernatants were tested for IFN-γ using the CytoScreen Immunoassay kit from BioSource International (Camarillo, CA). The kit was used as per the manufacturer’s instructions.

Deconvolution microscopy for MAP kinase-interacting kinase (MNK) cellular localization

Human CD4+ T cells were isolated as described above and incubated in medium without FBS for 1–2 h before use. CD4+ T cells were treated with 100 U/ml IL-10 for 30 min before stimulation with 3 ng/ml SEB for 15 min. Cells were cytoton centrifuged onto microscope slides and immediately fixed in methanol (∼20°C) as previously described (10). Cells were then permeabilized using 0.5% Triton X-100 in TBS (100 mM Tris-HCl and 0.9% NaCl) for 10 min. Slides were washed with TBS and nonspecific sites were blocked using blocking buffer (5% nonfat instant dry milk in TBS). Cells were then immunofluorescently stained with anti-MNK Abs in block buffer. After washing, nuclei of cells were stained with a solution of DAPI according to the manufacturer’s recommendations (Molecular Probes, Eugene, OR). After washing, cells were mounted in Prolong antifade solution (Molecular Probes), covered with a coverslip, and sealed with varnish (10).

The images were obtained using an Olympus 1 × 70 deconvolution microscope (Olympus, Melville, NY) under a ×60 oil immersion objective and an auxiliary ×1.5 magnification as previously described. These images were further deconvolved using the Delta Vision convolution algorithm (Applied Precision, Issaquah, WA).

Results

Superantigens such as the staphylococcal enterotoxins are potent stimulators of CD4+ T cells in terms of proliferation and cytokine production (1). Therefore, we first determined the ability of SEA and SEB to enhance the Ab response of mice primed with the T-dependent Ag BSA as well as a type I T-independent (LPS) and a type II T-independent (pneumococcal polysaccharides) Ag (type I and II T-dependent Ags are reviewed in Refs. 11 and 12). The rationale behind the approach was 2-fold. First, we have previously shown that superantigens can exacerbate the autoimmune disorder experimental allergic encephalomyelitis (EAE) in mice primed to MBP (7). Second, mice primed to weak tumor-specific Ags of melanoma cells were prophylactically protected against lethal melanoma with protective immunological memory of >170 days (8). Mice were first injected with BSA, followed 1 wk later by a combination of SEA and SEB at a dose that was previously shown to enhance specific cytolytic immune responses against tumors.
Mice were bled weekly from the start of the experiment, and sera were tested by ELISA for the presence of anti-BSA Abs. Significantly higher levels of anti-BSA Abs were produced in mice given superantigens as compared with mice given BSA alone, 4-fold at a 1/100 dilution (Fig. 1). Interestingly, Ab production to type II T-independent Ags such as pneumococcal polysaccharides was also enhanced by superantigen administration (~2.3-fold at 1/100 dilution), whereas anti-LPS (type I T-independent) IgM Ab titers were not affected (Fig. 1, B and C), suggesting that Th cells provide help to B cells responding to type II, but not type I, T-independent Ags. Thus, SEA and SEB superantigens significantly enhanced the Ab response to T-dependent and type II T-independent Ags.

The titrations of Fig. 1 of sera from Ag plus superantigen-treated mice were approximately linear with a correlation coefficient of ~99% for an arithmetic OD up to 1.7 plotted against the reciprocal of antisera dilution. Approximately 3-fold difference in OD for BSA plus superantigen was observed for a 10-fold difference in relative Ab concentrations. Thus, a doubling of OD conservatively indicates a doubling of Ab concentration in the test sample.

The anti-BSA Ab response was further characterized as a function of time. Abs to BSA were initially detected at low levels on day 7 in mice given BSA alone (Fig. 2). Day 7 was the day that superantigens were administered (after bleeding of mice). At day 14, significant differences in anti-BSA Abs between mice that received BSA alone vs BSA plus SEA/SEB were observed. Mice administered superantigens produced 3- to 4-fold higher levels of Abs than did mice given BSA only. Anti-BSA Ab production peaked by days 21–28 in both groups of mice. BSA plus superantigen mice maintained relatively high levels of Ab as late as 60 days, while Ab levels in BSA alone mouse sera were virtually undetectable. These results indicate that superantigen administration resulted in prolonged Ab production to BSA as well as higher levels in vivo.

The surface envelope protein of HIV, gp120, is a candidate protein for an effective vaccine against HIV. Abs against certain regions of gp120 can neutralize the virus by blocking entry into cells. Therefore, we determined the adjuvant effect of superantigens on Ab production to gp120. Mice were injected with gp120, followed by superantigens 1 wk later. As was the case for BSA, significantly higher and prolonged Ab production was observed in sera from mice administered superantigens as compared with mice injected...
with gp120 alone. At a 1/1000 dilution, sera from both groups of mice produced Abs at similarly high levels but of shorter duration for gp120 alone (Fig. 3A). Differences can clearly be seen at a 1/3000 dilution, where sera from mice given gp120 alone had significantly lower levels of Abs (Fig. 3B).

To control for Ag specificity of immunization with BSA followed 7 days later by administration of SEA/SEB, we compared the IgG Ab response to BSA to that of a control Ag, gp120. Mice were injected with BSA alone, BSA followed by SEA/SEB, SEA/SEB alone, or PBS alone. As can be seen in the ELISA measurements of Fig. 4A, BSA alone induced an Ab response that was enhanced >2-fold by SEA/SEB at a 1/100 dilution of sera. There was no Ab response to gp120 in the same sera as evidenced by the low, similar ELISA profiles for BSA alone, BSA followed by SEA/SEB, SEA/SEB alone, or PBS alone. Thus, the enhancement effect of SEA/SEB was specific for the primary Ag BSA.

We next looked at IgM Ab specific for BSA in the same sera. At 14 days following BSA injection there was no evidence of specific IgM Ab to BSA as per Fig. 4B, where the BSA response was compared with that of PBS. Importantly, a comparison of BSA with BSA followed by SEA/SEB showed the same profile. Thus, SEA/SEB did not enhance the IgM response to BSA under the same conditions under which it enhanced the IgG response.

A comparison of the total IgG levels of the above sera from mice treated with BSA alone, BSA followed by SEA/SEB, SEA/SEB alone, or PBS did not show any significant differences (Fig. 4C). Thus, an enhancement effect of SEA/SEB on the total IgG levels was not observed. Furthermore, the enhancement of the BSA response by SEA/SEB could not be attributed to simple non-specific enhancement of total IgG. We conclude that the SEA/SEB enhancement of the IgG Ab response to BSA is Ag specific.

We were interested in determining whether the superantigen effect could be abrogated by the cytokine IL-10. We previously
showed that superantigens activate T cells by decreasing the cellular level of the tumor suppressor gene product, p27, and that IL-10 reversed the superantigen effect by restoring the basal level of p27 (9). One group of mice received three injections of IL-10: one on the day before immunization, one on the day of immunization with BSA, and one on the day after immunization. One week after immunization, the mice received an injection of superantigens. Mice were bled regularly throughout the term of the experiment and sera were tested for anti-BSA Abs. As shown previously, superantigen administration resulted in rapid induction and high titers of specific Abs as compared with mice that received BSA alone (Fig. 5). However, IL-10 significantly suppressed superantigen-amplified Ab production. It also inhibited Ab production to a lesser degree in mice that did not receive superantigens. Furthermore, IL-10 suppressed superantigen-induced mouse splenocyte proliferation in vitro (M. G. Mjuberg and H. M. Johnson, unpublished data). Thus, the immunoenhancing effects of superantigens are down-regulated by IL-10. This may be a significant observation for up-regulation or down-regulation of immune responses, as the situation may require.

We next addressed the question of the mechanism of superantigen direct effects on CD4+ T cells and the modulation of these effects by IL-10 at the level of signal transduction. We have previously shown that the superantigen SEB can directly activate purified human CD4+ T cells and that IL-10 can block this activation by inhibition of down-regulation of the tumor suppressor gene, p27Kip1 (9). SEB was first examined for activation of subpathways of the MAP kinase pathway of human CD4+ T cells using specific inhibitors. Purified CD4+ T cells were treated with SEB in the presence or absence of the MAP/ERK kinase (MEK) inhibitor, PD98059, and the p38 inhibitor, SB202190. As seen in Fig. 6A, the MEK inhibitor, PD98059, significantly inhibited SEB-induced T cell proliferation in a dose-dependent manner, while the p38 inhibitor had no significant effect on SEB-induced proliferation. The inhibition included blockade of IFN-γ production and was observed over several days (Fig. 6B). MEK is part of the mitogen activation portion of the MAP kinase pathway, while p38 is part of the stress-induced portion of MAP kinase (13). Con A has been shown to activate the p38 pathway of MAP kinase (14) and, consistent with this, the p38 inhibitor SB202190 selectively blocked Con A-induced production of IFN-γ (Fig. 6C). The results show that SEB stimulates signal transduction via the mitogen component and not the stress component of MAP kinase. This is in contrast to stimulation of CD4+ T cells with the mitogen Con A, where p38 is required (14).

**FIGURE 5.** IL-10 abrogates the enhancement of the humoral immune response to BSA by superantigens. Mice were administered IL-10 (100 U/dose) 1 day before immunization with BSA (50 μg), the day of immunization, and 1 day after immunization. One week later, mice were given a dose of SEA/SEB as described in Fig. 1. Sera from mice given superantigens alone did not have detectable levels of anti-BSA Abs. No anti-BSA Abs were detected in preimmunization sera. Data are from sera obtained on day 21. Data are representative of three experiments, each performed in triplicate. Significance, as determined by Student’s t test, for BSA plus SEA/SEB vs BSA, SEA/SEB, and IL-10 was p < 0.001.

**FIGURE 6.** SEB-induced proliferation and IFN-γ production by human CD4+ T cells is inhibited by PD98059, an inhibitor of MEK, but not by an inhibitor (SB202190) of p38 kinase. CD4+ T cells were purified from human PBMCs and cultured in the presence of SEB and inhibitors. A, Proliferation of CD4+ T cells was measured by [3H]thymidine incorporation. Data are presented as cpm ± SD. B, Production of IFN-γ by CD4+ T cells was measured in culture supernatants taken at various time points using a commercial ELISA kit. Data are presented as picograms of IFN-γ per milliliter. Data from both sets of experiments are representative of three experiments, each performed in triplicate. C, IFN-γ production by Con A-activated CD4+ T cells is inhibited by SB202190, an inhibitor of p38 kinase, but not by an inhibitor of MEK (PD98059). IFN-γ was measured by ELISA in 48-h culture supernatants of CD4+ T cells stimulated with either 2 μg/ml Con A or 3 ng/ml SEB in the absence and presence of various concentrations of inhibitors. Data are presented as picograms of IFN-γ per milliliter. Data from both sets of experiments are representative of three experiments, each performed in triplicate. Significance, as determined by Student’s t test, for Con A plus 10 μM SB202190 vs Con A alone was p < 0.006.
Having established that SEB uses the MAP kinase pathway in activating CD4+ T cells with specific inhibitors, we next examined the inhibitory effect of IL-10 on SEB-induced phosphorylation of the MAP kinase pathway protein, ERK. IL-10 has previously been shown to affect the activation state of MAP kinases in CD40-stimulated monocytes (15), but the signaling cascades in monocytes differ from those of CD4+ T cells. Primary human CD4+ T cells, depleted of monocytes (9), were serum-starved to reduce background MAP kinase activity, treated with 100 U/ml IL-10 for 30 min, and then stimulated with 3 ng/ml SEB for 15 min. The cells were then lysed and the MAP kinase proteins ERK-1 and ERK-2 were immunoprecipitated from cell lysates and assayed for ERK activity. As seen in Fig. 7, IL-10 significantly inhibited the ability of MAP kinase ERK to phosphorylate the substrate MBP, as compared with cells stimulated with SEB in the absence of IL-10.

Recently, a protein called MNK was identified that acts as a substrate for MAP kinases and is important in the initiation of protein synthesis (16, 17). When phosphorylated by MAP kinase, MNK then phosphorylates eIF4E, an important component of the eukaryotic protein synthesis initiation complex (17). Once phosphorylated, eIF4E is able to bind the initiation factor complex, forming a stable eIF4E unit necessary for initiation of protein synthesis (18). Because MNK has been shown to be a substrate for ERK and is also involved in regulating eIF4E, we wanted to determine whether the effects of IL-10 extended to the interaction between MNK and eIF4E. It has been shown that MNK colocalizes with eIF4E, forming a perinuclear ring in cells stimulated with phorbol ester (19). Using deconvolution microscopy, we show in Fig. 8 that MNK exhibited perinuclear localization in human CD4+ cells stimulated with SEB, as compared with untreated cells. We also show that IL-10 treatment inhibited this perinuclear localization of MNK. This effect on MNK perinuclear localization is probably the result of IL-10 inhibition of ERK activity. Thus, we show here for the first time that IL-10 interferes with formation of the stable protein synthesis complex between MNK and eIF4E via inhibition of ERK activation of MNK. These findings provide a mechanism for IL-10 inhibition of protein synthesis in superantigen-activated CD4+ T cells.

**Discussion**

We originally showed that the staphylococcal superantigens were T cell mitogens (20) and that they were in fact the most potent T cell mitogens known (21). Superantigens are probably the most common cause of food poisoning, and in this context we showed that they could exacerbate autoimmune disorders such as EAE (7). This raised the question of possible induction and exacerbation of autoimmune disorders such as multiple sclerosis by superantigens (22).

Recently, we addressed the question of whether the potent T cell activation properties of superantigens could be harnessed to enhance desirable immune functions such as tumor immunity. Accordingly, we immunized C57BL/6 mice with irradiated B16F10 melanoma cells followed by treatment with a combination of SEA and SEB (8). Challenge with at least a 25-fold lethal dose of B16F10 cells resulted in protection against death. Mice that received only irradiated cells or only superantigen were not protected. Importantly, 75% of the protected mice survived a second lethal challenge of B16F10 cells administered >170 days postvaccination. Protection involved both CD4+ and CD8+ T cells, as per specific knockout mice, and was accompanied by induction of the Th1 cytokine IFN-γ. The CD8+ T cell requirement was consistent with specific induction of cytotoxic T cells. These findings are indicative of potent augmentation of specific T cell immunity by superantigens. Thus, superantigens augmented vaccination and persistent immunological memory against lethal doses of melanoma involving the tumor’s weak tumor-specific Ag(s) (8).

In the present study we examined the immunoenhancing effects of superantigens on the humoral arm of the immune response. Immunization of mice with the prototype T-dependent Ag BSA and with HIV envelope protein gp120 followed by SEA/SEB resulted in significantly increased relative Ab levels over a longer period of time when compared with Ag alone. Superantigens similarly enhanced the specific Ab response to the type II T-independent Ag pneumococcal polysaccharide. T cells have previously been shown to enhance the Ab response to type II T-independent Ags (11). The results presented here, combined with those of our previous findings on superantigen enhancement of tumor-specific immunity to mouse melanoma (8), are evidence that superantigens such as SEA and SEB can significantly boost Ag-specific immune responses of both Th1 and Th2 CD4+ T cells. Preliminary gene microarray studies suggest that both Th1 and Th2 cytokines such as IFN-γ, IL-2, IL-4, and TGF-β can be induced in culture at the same time under superantigen treatment.

There is an inherent characteristic of superantigen effects on naive vs Ag-primed T cells that is a plus for their immunoenhancing properties. Naïve T cells initially undergo cell division when treated with superantigens, followed shortly by anergy and/or deletion. Ag-primed T cells also expand when treated with superantigen but, in contrast, do not undergo the anergy/deletion characteristic of naïve T cells (2–4, 6, 7). Thus, the Vβ-specific polyclonal expansion associated with superantigens is tilted toward primed Ag-specific T cells.

The mitogen arm of the MAP kinase signal transduction pathway appears to be required for superantigen activation of CD4+ T
cells. Inhibitors of the mitogen arm of the MAP kinase pathway blocked superantigen activation of CD4⁺ T cells as well as induction of IFN-γ. Consistent with this, superantigens induced ERK and MNK activities, the latter being required for initiation of protein synthesis (17). An inhibitor of the p38 stress kinase arm of MAP kinase had no effect on superantigen activity. This finding sets staphylococcal superantigens strongly apart from T cell mitogens such as Con A, where the p38 branch of MAP kinase has been shown to be required for T cell activation (14).

IL-10 treatment of mice blocked the immunoenhancement effects of superantigens. We have previously shown that type I IFN induction of IL-10 is partially responsible for IFN protection against EAE and superantigen exacerbation of EAE (23, 24). Thus, a Th2 cytokine can inhibit a known Th2 cell function, enhancement of Ab production by superantigens. Consistent with this, IL-10 blocked the mitogen arm of the MAP kinase activation by superantigens, providing support for this pathway as a mediator of the superantigen immunoenhancing effects. The findings presented here raise an interesting question. Because IL-10 can block the enhancement of Ab production by superantigens, is production of IL-10 by Ag-primed Th2 cells relatively suppressed compared with cytokines such as IL-4 when the cells are treated with superantigens? Using a combination of microarray technology and real-time PCR, we are currently assessing superantigen-induced cytokine production in T cell subsets. Thus, future studies will test the ability of superantigens to induce expression of IL-10 in naive vs Ag-primed Th2 cells.

Our demonstration that superantigens can significantly enhance the humoral arm of the immune response, coupled with our previous demonstration of strong enhancement of both cellular immunity and memory in the mouse melanoma model (8), is of particular interest in the context of the use of manipulated dendritic cells to enhance immunity against diseases such as cancer (reviewed in Ref. 25). The question arises as to whether the relatively simple administration of superantigen in conjunction with specific Ag raises the level of immune response comparable to that of the

![FIGURE 8.](image_url)
more tedious and complex approach of isolation, treatment, and administration of dendritic cell subsets. Future studies by our laboratory and that of others should address this issue.

Others have reported superantigen effects on specific immune responses. In one study rabies virus superantigen showed adjuvant affects when injected at the same time as Ag (26), while in another study SEB did not boost the immune response to an ongoing influenza infection (27). We administered SEB 7 days after Ag to avoid anergy and death and we did not use an infection system, so it is difficult to directly compare our findings with the influenza infections.

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