Modulation of Contact Sensitivity Responses by Bacterial Superantigen

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Superantigens are potent modulators of the immune system, especially T cells. Therefore, we determined the influence of superantigens on the T-cell–mediated immune response, contact sensitivity. We chose the combination of staphylococcal enterotoxin B (SEB) as superantigen and 2,4-dinitrofluorbenzene (DNFB) as the contact sensitizer, because in BALB/c mice SEB reacts almost exclusively with Vβ8$^+$ T cells, and these cells are capable of transferring contact sensitivity to DNFB from sensitized donors to naive syngeneic recipients. Pretreatment with a single intradermal injection of 50 ng SEB 24 h before DNFB exposure at the same site on the lower abdomen enhanced the induction of contact sensitivity: its intradermal injection permitted sensitization with non-sensitizing concentrations of DNFB as assessed by ear swelling responses after challenge with DNFB. In contrast, pretreatment with repeated intradermal injections of 50 ng SEB every other day over at least 1 week inhibited the induction of contact sensitivity following sensitization. The enhancing effect of SEB may be explained by the creation of a proinflammatory milieu in the skin after a single intradermal injection of the bacterial toxin, whereas the inhibitory effect may be due to tolerization of Vβ8$^+$ T cells. The data indicate that products of skin-colonizing bacteria that can serve as superantigens are able to augment or inhibit the developement of contact sensitivity. ***Key words: contact sensitivity/superantigen/staphylococcal enterotoxin B/2,4-dinitrofluorbenzene. J Invest Dermatol 105:220–224, 1995***

Contact allergic responses as a manifestation of delayed-type hypersensitivity are mediated by T cells [1,2]. During the induction phase, antigen/hapten-specific T cells are induced by appropriate presentation of the hapten in the context of major histocompatibility complex (MHC) class II molecules. Inappropriate presentation, however, leads to anergy and tolerance due to the lack or presence of soluble or membrane-bound factors [3,4]. Stimulation of T cells can also occur in the presence of superantigens that bind to the Vβ-region of the αβ T-cell receptor [5,6] and to MHC-class II molecules outside the regular antigen binding groove [7,8]. This interaction can lead to activation, cytokine production, and proliferation or to anergy and deletion of the responsive Vβ T-cell subsets [9–17]. In view of its broad effects on T-cell responses, we evaluated the effect of a superantigen on the development of contact sensitivity. Because of potential clinical significance, we chose a bacterial superantigen derived from bacteria that colonize the skin in various disease conditions, as such superantigenic products may very well influence contact dermatitis reactions in a clinical setting [18,19].

Staphylococcal enterotoxins (SE) have been shown by other investigators to suppress delayed-type hypersensitivity responses as well as other immune reactions *in vivo* [20–23], but true contact sensitivity was not investigated in these studies. Inhibition of contact sensitivity to 2,4-dinitrofluorbenzene (DNFB) has been described in only one investigation using C57BL/6 mice utilizing SEA as a superantigen [24], but no analysis of Vβ T-cell populations was included in this study.

In the present study we chose the combination of DNFB and SEB, as contact sensitivity to DNFB is a well-characterized model, and Vβ8$^+$ T cells are capable of transferring contact sensitivity to this hapten in BALB/c mice. In BALB/c mice Vβ8$^+$ T cells represent a large T-cell population and are the primary responders to SEB [7].

MATERIALS AND METHODS

Experimental Animals Female BALB/c mice (Jackson Laboratories, Bar Harbor, ME) of about 10 weeks of age and maintained under approved institutional guidelines were used in all experiments. These mice lack Vβ3- and Vβ17-positive T cells. Therefore Vβ7 (small fraction) and Vβ8 (major proportion) serve as the SEB-reactive T cells [7].

Contact Sensitization Unless otherwise indicated the lower abdomen was shaved with an electric clipper and painted on two consecutive days with 30 μl of DNFB (Sigma, St. Louis, MO) dissolved in oil/acetone (mixture of 1:4 parts) at a concentration of 0.5%. Control animals were painted with the solvent oil/acetone alone.

Testing for Contact Sensitivity After measurement of the baseline thickness of both ears, 30 μl of DNFB 0.2% dissolved in oil/acetone (mixture of 1:4 parts), was applied onto the dorsum of both ears, and ear thickness was measured with a modified micrometer 24 h later. The ear swelling response was calculated by subtracting the thickness prior to testing

Manuscript received December 8, 1994; revised manuscript received April 12, 1995; accepted for publication April 18, 1995.

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Abbreviations: CS, contact sensitivity; LN, lymph node(s); SALT, skin-associated lymphoid tissue; SE, staphylococcal enterotoxin.

0022-202X/95/$09.50 • SSDI0022-202X(95)00229-E •

from the thickness observed 24 h after testing. Control testing was performed with the solvent (oil/acetone) alone.

Pretreatment Fifty nanograms of staphylococcal enterotoxin B (SEB, Toxin Technology, Sarasota, FL) or alternatively (where indicated) 1 ng of SEA (Toxin Technology), which is about 100 times more potent a superantigen than SEB, was injected into the skin of the abdomen either once or repeatedly as described in *Results*. Control animals were injected only with the solvent PBS.

Transfer The cell preparations were suspended in one ml of PBS and injected into the tail vein of naive syngeneic recipients with a tuberculin syringe and a 26-gauge needle.

Cell Preparation In all experiments, local draining lymph node (LN) cells were used and prepared by removing the inguinal LN under sterile conditions and dispersing them mechanically through stainless steel mesh screens to provide single cell suspensions.

Separation of Vβ8$^+$ T cells Tissue culture flasks were directly coated with hybridoma culture supernatants of monoclonal Vβ8-antibody (F23.1, kindly provided by Drs. J. Kappler and P. Marrack, Denver, CO) at 4°C overnight. Afterwards the antibody supernatant was removed and the flasks were washed with tissue culture medium. Suspensions from the lymph nodes were then added and incubated at 37°C in 5% CO_2. After 2 h the flasks were gently washed with prewarmed medium (37°C) to remove unbound cells, and tissue culture medium containing interleukin 2 (IL-2, obtained from the Biological Response Modifier Program, NC) at a concentration of 50 U/ml was added, and retained cells were incubated at 37°C in 5% CO_2. After 3 d the cells were harvested, extensively washed, and transferred into recipients. An aliquot was checked for purity by flow cytometry (>98% Vβ8$^+$).

Flow Cytometry For cell staining, cells were placed in 96-well microtiter plates and washed with staining buffer (PBS supplemented with 2% heat-inactivated fetal bovine serum (FBS) and 0.1% sodium azide). 20 μl of 1:10 diluted mouse serum was added for blocking of nonspecific binding sites followed by 20 μl of biotinylated Vβ-antibody (kindly provided by Drs. J. Kappler and P. Marrrack) diluted in staining buffer. The incubation was carried out on ice for 30 min. After washing, 20 μl of diluted PE-streptavidin (Tago, Burlingame, CA) was added to the wells. For CD3 staining 20 μl of directly FITC-labeled antibody (kindly provided by Drs. J. Kappler and P. Marrack) diluted in staining buffer was added. This second incubation was carried out for 30 min on ice in the dark. After washing, cell pellets were resuspended in staining buffer and examined with an EPICS analyzer (Coulter Electronics, Hialeah, FL).

Tissue Culture Culture medium consisted of RPMI 1640 (Gibco, Grand Island, NY) with L-glutamine (2 mM), penicillin (100 U/ml), streptomycin (100 μg/ml), 2-mercaptoethanol (5mM), HEPES buffer (15 mM), Na-pyruvate (1mM) and FBS (10% Hyclone, Logan, UT). Conditions were 37°C and 5% CO_2.

Proliferation Assay LN cells were incubated in triplicate wells for 72 h in flat-bottom culture plates containing 200,000 cells per well. SEB was added at a final concentration of 1 μg/ml and Con A at a final concentration of 5 μg/ml. Proliferation was assessed by incorporation of [^{3}H]thymidine added at a concentration of 1 μCi (6.7 Ci/mM, ICN, Irvine, CA) to each well 6 h before harvesting.

Interferon-γ Assay IFN-γ was measured in culture supernatants with an ELISA-Kit (Genzyme, Cambridge, MA) according to the manufacturer's instructions. Cell-free supernatants were collected from 48-h cultures of single-cell suspensions from inguinal lymph nodes of differently pretreated animals incubated with medium alone or with SEB at a concentration of 1 μg/ml.

Statistics All results are depicted as mean ± SD. Student two-tailed unpaired t test was used to determine the level of difference (p) between two groups.

RESULTS

Determination of Sensitizing and Non-Sensitizing Protocols for DNFB Exposure Protocols that lead to the induction of contact sensitivity to DNFB are well established [4]. As expected, painting of the shaved abdomen with 30 μl of 0.5% DNFB in oil/acetone on two consecutive days led to the induction of contact sensitivity as assessed by positive ear swelling responses measured 24 h after application of 20 μl of 0.2% DNFB on the ears. Control animals that had been painted with oil/acetone only did not exhibit significant ear swelling responses after challenge with 0.2% DNFB on their ears (data not shown).

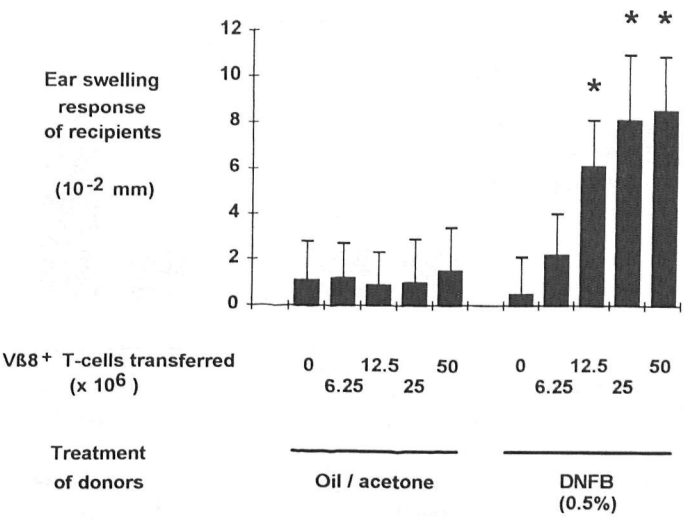

Figure 1. Transfer of contact sensitivity to DNFB by Vβ8$^+$ T cells. One group of donor BALB/c mice was sensitized to DNFB by painting of the abdominal skin with DNFB 0.5% in oil/acetone on two occasions (over 2 d). A control group was exposed to the solvent alone. After three additional days regional draining LN cells were prepared. Vβ8$^+$ T cells were purified by panning and expanded with IL-2 for 3 d. Cells were washed, purity was checked by flow cytometry (>98% Vβ8$^+$ T cells), and cells were transferred intravenously into naive syngeneic recipients. Recipients were challenged after one additional day with DNFB 0.2% to the ears and ear swelling responses were recorded 24 h later. The results are given as mean ± SD of two independent experiments with three animals in each group of recipients. *Significantly increased ear swelling responses ($p < 0.05$) compared to all groups of recipients of cells from non-sensitized donors.

Painting of the abdomen on two consecutive days with 0.2% DNFB followed by ear challenge with 0.2% DNFB also failed to induce ear swelling responses, indicating that contact sensitization did not take place in these mice (data not shown).

Vβ8$^+$ T cells Mediate Contact Sensitivity to DNFB To investigate whether Vβ8$^+$ T cells are able to mediate contact sensitivity to DNFB, donor mice were sensitized to DNFB by painting the shaved abdominal skin with 30 μl of DNFB 0.5% in oil/acetone on two consecutive days. After three days the mice were sacrificed and cells from the regional draining (inguinal) LN were isolated. Vβ8$^+$ T cells were positively selected by direct panning and expanded with interleukin-2 for three days. Before transfer into naive syngeneic recipients, cells were washed extensively and purity was confirmed by flow cytometry (>98% Vβ8$^+$). On the day following transfer, recipients were tested for contact sensitivity by ear challenge with DNFB 0.2% in oil/acetone and measurements of ear swelling response 24 h after challenge. Vβ8$^+$ T cells from DNFB-sensitized donors were able to transfer contact sensitivity to DNFB in a dose-dependent manner. At least 12.5×10^6 Vβ8$^+$ cells from DNFB-sensitized donors were needed to induce significant ($p < 0.05$) increases in ear swelling responses after challenge with DNFB compared to control recipients (see below) **(Fig 1)**. Following transfer of 25×10^6 Vβ8$^+$ T cells from DNFB-sensitized mice, increases in ear swelling responses after challenge with DNFB reached a plateau **(Fig 1)**. Transfer of T cells depleted of Vβ8$^+$ cells from DNFB-sensitized donors were 5–10 times less efficient than Vβ8$^+$ T cells (data not shown). Control recipients that were challenged in the same way and that had received Vβ8$^+$ T cells purified and expanded in the same way from control donors treated with oil/acetone alone did not exhibit any significant ear swelling responses after challenge with DNFB **(Fig 1)**.

Pretreatment With a Single Intradermal Injection of a Small Amount of SEB Enhances Induction of Contact Sensitivity to DNFB A single intradermal injection of SEB has previously been shown to induce a strong inflammatory response in

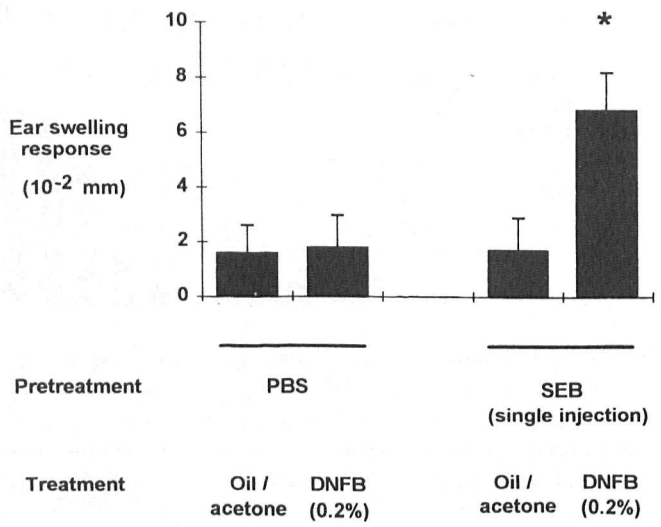

Figure 2. Augmentation of induction of contact sensitivity to DNFB following a single intracutaneous injection of SEB. One group of mice was injected with SEB (50 ng intradermally) in the lower abdomen, and a control group was injected with PBS alone. Twenty-four hours later, half of the mice were exposed to a low concentration of DNFB (0.2% in oil/acetone) and the other half to the solvent oil/acetone at the same sites where SEB had been injected before. After 3 additional days all mice were challenged with DNFB 0.2% to the ears, and ear swelling responses were recorded after 24 h. The results are given as mean ± SD of three independent experiments with three mice in each subgroup. *Significantly increased ear swelling response ($p < 0.05$) compared to all other subgroups.

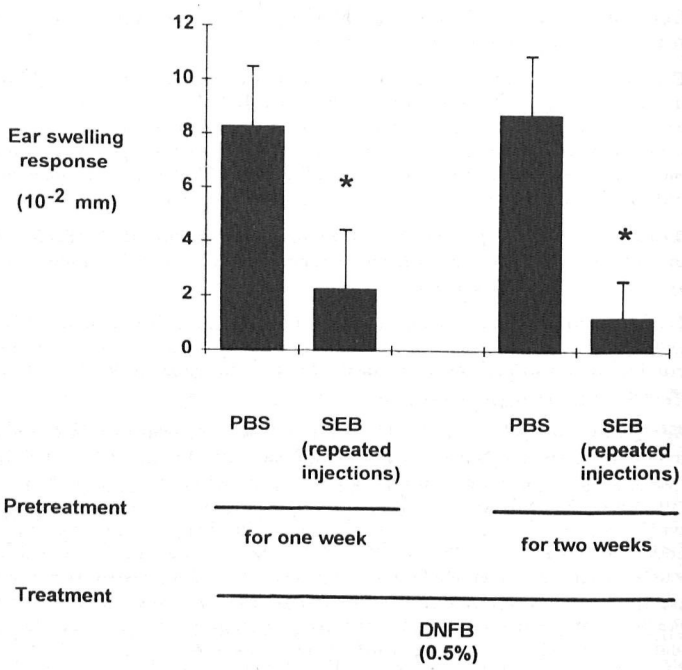

Figure 3. Inhibition of contact sensitivity to DNFB by repeated intradermal injections of SEB. Groups of mice were injected intradermally with SEB (50 ng in PBS) or PBS on the lower abdomen every other day for one week, and the other group was injected similarly for two weeks. All mice were then painted with DNFB (0.5% in oil/acetone) on two consecutive days on the abdomen at the site of the injections. Following an additional 3 d, all mice were challenged with DNFB 0.2% to the ears, and the ear swelling responses were recorded after 24 h. The results are given as mean ± SD of three independent experiments with three mice in each subgroup. *Significantly lower ear swelling responses compared to the PBS pretreated subgroups ($p < 0.05$).

the skin at the injected site.‡ To investigate the consequences of this proinflammatory effect of SEB in the skin on the induction of contact sensitivity to DNFB, we injected 50 ng of SEB intradermally into the shaved abdomen. On the following two days the skin was painted with DNFB 0.2% in oil/acetone at the same site. After three additional days the mice were tested for contact sensitivity to DNFB by ear challenge with DNFB 0.2% in oil/acetone and measurement of ear swelling responses 24 h later.

Mice pretreated by a single SEB-injection were sensitized by these low concentrations of DNFB, as they exhibited significant ($p < 0.05$) ear swelling responses after challenge with DNFB compared to control animals that had been pretreated with SEB, treated with oil/acetone alone, and challenged with DNFB in the same way **(Fig 2)**. Mice pretreated with PBS only were not sensitized to DNFB by painting with 0.2% DNFB, as they did not exhibit increased ear swelling responses after challenge with DNFB **(Fig 2)**. This indicates that although sensitization with DNFB 0.2% is not able to induce contact sensitivity to DNFB on its own, pretreatment with SEB converts a nonsensitizing dose to a sensitizing one. This augmenting effect was not long lasting, as it was not observed when mice were painted with DNFB one week after SEB pretreatment.

Pretreatment With Repeated Intradermal Injections of Small Amounts of SEB Inhibits Induction of Contact Sensitivity to DNFB As SEB can downregulate and induce deletion of $V\beta8^+$ T cells under certain conditions [9–15], we investigated the effects of repeated SEB treatment on the induction of contact sensitivity to DNFB. For this purpose mice were injected with 50 ng SEB into the shaved skin of the abdomen every other day over a period of 1 to 2 weeks. Mice in each group were then exposed to 30 μl of DNFB 0.5% in oil/acetone at the same site on two consecutive days. After an additional 3 d, contact sensitivity to DNFB was tested by ear challenge with DNFB 0.2% in oil/acetone and with measurement of ear swelling 24 h later. Animals pretreated with SEB injections for 1 week (or to a greater extent, if pretreated for 2 weeks) exhibited significantly ($p < 0.05$) decreased ear swelling responses after challenge with DNFB compared to PBS-pretreated control animals **(Fig 3)**. Animals that were pretreated with an equipotent dose of a superantigen that does not affect $V\beta8^+$ T cells (i.e., 1 ng of SEA [7] instead of 50 ng of SEB) but were otherwise treated in the same way as SEB pretreated mice were sensitized normally to DNFB (data not shown). These data indicate that repeated injections of SEB do not enhance but rather inhibit the development of contact sensitivity to DNFB. If mice were sensitized to DNFB after a period of two months following SEB injections, they exhibited normal ear swelling responses (data not shown), indicating that the inhibitory effect was not permanent.

Repeated Injections of SEB Do Not Lead to Deletion But to Functional Down-Regulation of $V\beta8^+$ T cells in the Skin Associated Lymphoid Tissue (SALT) To investigate the underlying mechanism responsible for the inhibitory effects of chronic SEB exposure on the induction of contact sensitivity to DNFB, we analyzed the main cell target of SEB in BALB/c mice, $V\beta8^+$ T cells. $V\beta14^+$ T cells, which do not react with SEB [7], were monitored in parallel. One week after repeated SEB exposure, the frequency of $V\beta8^+$ T cells in local draining (inguinal) LN were only slightly diminished (about 10%) but were inhibited in their proliferative response to SEB by more than 60% **(Table I)** compared to $V\beta8^+$ T cells of PBS-treated control animals. After 2 weeks of SEB injections, $V\beta8^+$ T cells were decreased to a greater extent (about 30%) and were inhibited even more in their proliferative response to SEB (about 85%, **Table I**). In contrast, $V\beta14^+$

‡Saloga J, Renz H, Leung DYM, Gelfand EW: Superantigens derived from skin colonizing staphylococci profoundly influence immunological reactions of the skin (abstr). *J Invest Dermatol* 101:406, 1993.

Table I. Decreased Frequency of Vβ8$^+$ T-Cells and Proliferative Responses to SEB of Inguinal LN Cells *In Vitro* After Repeated Cutaneous Exposure to SEB *In Vivo*[a]

	Pretreatment *In Vivo*			
	1 Week		2 Weeks	
	PBS	SEB	PBS	SEB
Vβ expression (as % of T cells)				
Vβ8$^+$	32.0 ± 1.5	27.2 ± 1.1[b]	33.2 ± 1.9	24.0 ± 1.2[b]
Vβ14$^+$	11.9 ± 1.2	12.0 ± 1.3	11.6 ± 1.1	12.1 ± 1.3
Proliferative response *in vitro* (cpm)				
Medium	1,168 ± 231	1,714 ± 217	1,109 ± 195	1,320 ± 248
SEB (1 μg/ml)	31,012 ± 3,622	11,398 ± 2,159[b]	29,639 ± 3,516	4,270 ± 717[b]
Con A (5 μg/ml)	80,900 ± 9,628	72,244 ± 9,261	74,889 ± 8,116	65,361 ± 7,852

[a] Mice were injected intradermally every other day for 1 or 2 weeks with SEB (50 ng in PBS) or PBS. Regional LN cells were prepared and analyzed by flow cytometry as described in *Materials and Methods*. The frequency of Vβ-expressing T cells is shown as the mean ± SD of five independent experiments, expressed as % of T cells (CD3$^+$). Cells from the same preparation were also analyzed for their proliferative response to SEB or Con A in 3 d cultures as described in *Materials and Methods*. The data (cpm) are shown as the mean ± SD of five independent experiments.

[b] Indicates results where the data of SEB-pretreated animals differ from PBS-pretreated animals significantly ($p < 0.05$).

T cells were not diminished and the proliferative response to Con A was only slightly reduced **(Table I)**, indicating the Vβ-specificity of the inhibitory effect of SEB in this model.

Modulation of IFN-γ Production by SEB In addition to the moderate decrease in frequency of Vβ8$^+$ T cells in the inguinal LN and the marked inhibition of their proliferative response to SEB after 2 weeks of SEB injections, we observed a marked inhibition of IFN-γ production in response to SEB *in vitro* **(Table II)**. Interestingly, IFN-γ production in response to SEB *in vitro* was enhanced when regional draining LN cells were collected 24 h after a single injection of 50 ng of SEB. Stimulation with DNFB itself or its water soluble counterpart $DNBSO_3$ *in vitro* did not yield consistent results.

The inhibition of cell proliferation and IFN-γ production by Vβ8$^+$ T cells in response to SEB *in vitro* may explain the decrease in development of contact sensitivity to DNFB after repeated SEB injections. In contrast, interferon-γ production and development of contact sensitivity were enhanced following a single injection of SEB.

DISCUSSION

In this study we demonstrate that treatment with a low concentration of the superantigen SEB enhances sensitivity to DNFB; in contrast, repeated injections over 1-2 weeks inhibits this process. Enhancement is most likely secondary to the development of local inflammation (including infiltration of the skin by T cells and expansion of Vβ8$^+$ T cells in the skin associated lymphoid tissues

Table II. Modulation of IFN-γ Production of Inguinal LN Cells in Response to SEB *In Vitro* After Cutaneous Exposure to SEB *In Vivo*[a]

	Pretreatment *In Vivo*		
Stimulation *In Vitro* (48 h)	Controls Untreated (pg/ml)	SEB Single Injection (pg/ml)	SEB Repeated Injections (pg/ml)
Medium	<500	<500	<500
SEB (1 μg/ml)	2,965 ± 728	10,374 ± 3,511[b]	<500[b]

[a] One group of mice was treated with repeated intradermal injections of SEB (50 ng) every other day over a period of two weeks, and received the last injection 24 h before sacrificing (repeated injections). A second group was injected once with SEB (50 ng) 24 h before sacrificing (single injection). The third group was untreated. Inguinal LN cells were prepared and stimulated *in vitro* as indicated. After 48 h, cell-free supernatants were harvested and IFN-γ was measured as described in *Materials and Methods*. The results are given as mean ± SD of two independent experiments.

[b] Indicates results where the data of SEB-pretreated animals differ from untreated animals significantly ($p < 0.05$).

that are involved in contact sensitivity to DNFB). A flare-up of reactions at sites where contact dermatitis has been previously induced have also been described after systemic application of higher amounts of superantigen [24]. One additional augmenting effect may be due to the increase in IFN-γ production in regional LN cells in response to SEB. The capacity of superantigens including SEB for induction of IFN-γ and other pro-inflammatory cytokines that play a role in contact sensitivity has been described in different experimental settings by other investigators [25,26].

The inhibitory effects of chronic SEB pretreatment, on the other hand, may be explained by the downregulation of Vβ8$^+$ T cells. This outcome would be expected, because this subset of T cells has been shown by us to be a major T-cell population that mediates contact sensitivity to DNFB in cell transfer experiments. The decreased proliferative response to SEB *in vitro* may point to the induction of anergy in DNFB-reactive Vβ8$^+$ T cells in the absence of Vβ8$^+$ T-cell deletion. The capacity to produce cytokines that are important for contact allergy was also reduced, as is demonstrated here for IFN-γ. Inhibition of SEB-induced IFN-γ production *in vitro* after SEB exposure *in vivo* has also been reported by other investigators [14]. Any differences between individual studies may be attributed to differences in the route of administration of the superantigen and the quantities injected. The inhibitory effect of SEB on the T cells was Vβ-specific, as Vβ8$^+$ T cells (SEB reactive) were decreased in frequency and response, whereas Vβ14$^+$ T cells were not significantly affected. Moreover, the proliferative response to SEB was inhibited, whereas the response to Con A remained intact. Additionally, migration of Vβ8$^+$ T cells to sites of contact allergen challenge may be inhibited by downregulation of important adhesion molecules after SEB treatment [27]. The lack of inhibition of contact sensitivity to DNFB after pretreatment with repeated injections of SEA does not exclude the possibility that there may be DNFB-specific T cells among SEA-reactive Vβ 1, (3), 10, 11, 12 and (17)-positive T cells [7,24]. It demonstrates, rather, that downregulation of these Vβ T-cell populations is not sufficient to inhibit contact sensitivity to DNFB, as it leaves Vβ8$^+$ T-cells, a major T-cell population able to mediate contact sensitivity to DNFB, unaffected.

In addition to binding to Vβ elements of the T-cell receptor, superantigens also target MHC class II cells. The binding of superantigens to MHC class II molecules on antigen-presenting cells leads to signaling events and secretion of cytokines and may affect their accessory cell function as well [28–30]. Altered general accessory cell function appears to be excluded as an explanation for the inhibition of induction of contact sensitivity to DNFB by chronic SEB exposure, because this function remained intact in LN and spleen cells, in view of the normal response to Con A. Whether another specialized function of these cells (or a subpopulation of

such cells e.g., epidermal Langerhans' cells) is inhibited remains to be determined.

Dermal mast cells are also affected by exposure of the skin to the superantigen SEB. In contrast to monkeys, where immediate type skin reactions have been reported to be elicited by superantigens [31] mast cell degranulation in mice occurs only after a few hours, pointing to a rather indirect effect of SEB on these cells.§ Nevertheless, mast cell degranulation may be an additional factor explaining the modulatory effect of superantigens on the development of contact sensivity, expecially the enhancing effect of a single injection of SEB [32,33].

The inhibition of different immunologic phenomena *in vivo* by superantigens, including modulation of delayed type hypersensitivity responses [20–23], has been described by other investigators. To the best of our knowledge, only one report exists concerning the modulation of contact sensitivity by superantigens. Kawaguchi-Nagata and coworkers [24] described the inhibition of contact sensitivity to DNFB by SEA in C57BL/6 mice. No analysis of Vβ T-cells mediating contact sensitivity to DNFB in these mice or investigation of changes in number or function of Vβ T-cell subsets was reported in this study.

In comparison to other studies exploring the effects of superantigens on Vβ T-cell subsets *in vivo* [11–13,34,35], deletion of V$\beta 8^+$ T cells after SEB exposure was rather limited in our experiments. This is likely due to the local inoculation of small amounts of the superantigen, which may closely resemble the physiologic situation with SEB-producing *staphylococci* on the skin [18,19]. Nevertheless, even these small amounts of superantigen applied locally had a noticeable effect on the skin itself, on the associated lymphoid tissue of the skin, and on skin-derived immune responses. In a clinical setting, superantigens of skin-colonizing bacteria may thus modulate the course of allergic contact dermatitis. Chronic exposure of the skin to staphylococcal enterotoxins as it occurs in atopic dermatitis [18,19] may contribute to the decrease in contact allergic responses reported to accompany this skin disorder [36,37].

This work was supported by DFG grants Sa 483 and Kn 120 and NIH grants HL 36577, AR 41256 and HL 37260.

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