Oral Administration of the Bacterial Superantigen Staphylococcal Enterotoxin B Induces Activation and Cytokine Production by T Cells in Murine Gut-Associated Lymphoid Tissue

Gerburg M. Spiekermann and Cathryn Nagler-Anderson

*J Immunol* 1998; 161:5825-5831;
http://www.jimmunol.org/content/161/11/5825

---

**References**

This article cites 33 articles, 17 of which you can access for free at:
http://www.jimmunol.org/content/161/11/5825.full#ref-list-1

**Subscription**

Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

**Permissions**

Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**

Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Oral Administration of the Bacterial Superantigen Staphylococcal Enterotoxin B Induces Activation and Cytokine Production by T Cells in Murine Gut-Associated Lymphoid Tissue

Gerburg M. Spiekermann and Cathryn Nagler-Anderson

The toxicity of the staphylococcal enterotoxins (SEs) has been linked to the activation of large numbers of T cells in the peripheral lymphoid tissues. Because the primary manifestations of foodborne enterotoxic poisoning are associated with the gastrointestinal tract, we have compared the responses of T cells in the gut-associated lymphoid tissue and in the periphery to intragastric (i.g.) and i.p. administration of SEB. Intraexperimental SEB results in an early expansion of peripheral Vβ1+ T cells and Th1 cytokine secretion followed by deletion at 7–10 days. We found that i.g. SEB rapidly (within 4 h) leads to the expansion and activation of Vβ1+ T cells in the Peyer’s patch and mesenteric lymph nodes. Analysis of cytokine mRNA in purified Vβ1+ T cells by competitive RT-PCR showed that, 4 h after i.g. SEB, the induction of mRNA for IL-2 and IFN-γ is about 10-fold greater in mucosal than in peripheral lymphoid tissue. Our results show that activated mucosal T cells expand and up-regulate cytokine mRNA in response to luminal exposure to SEB, suggesting a role for the gut-associated lymphoid tissue in the gastrointestinal manifestations of enterotoxic poisoning. The Journal of Immunology, 1998, 161: 5825–5831.

The staphylococcal enterotoxins (SEs), including SEB, act as superantigens by stimulating all T cells bearing reactive Vβ-chains (1). In contrast to conventional Ags, superantigens do not require processing by the APC. They bind to MHC class II molecules outside of the peptide binding groove and interact with the variable region of the β-chain of the TCR. Although, in general, recognition of conventional Ags in the context of class II requires CD4 and in the context of class I requires CD8, superantigens such as SEB induce both CD4 and CD8 T cell proliferation and effector function (2, 3).

The toxic syndromes and foodborne illness (4, 5) associated with the SEs have been linked to the high levels of cytokine secretion induced by the simultaneous stimulation of large populations of T cells (6). It has been assumed that these toxic effects of SEs are due to their dissemination, via the blood, to T cells in the peripheral lymphoid tissues. The primary manifestations of foodborne enterotoxic poisoning, i.e., vomiting and diarrhea, are associated with the gastrointestinal tract and usually occur within 24 h after the ingestion of the toxin. If the SEs exert their toxic effects through the secretion of cytokines, the activation of large numbers of potentially responsive T cells present in the gut-associated lymphoid tissue (GALT) in the organized lymphoid tissues in the mesenteric lymph node (MLN) and Peyer’s patch (PP), as well as in the intestinal epithelium and lamina propria, might be expected to play an important role. Yet, to our knowledge, the responses of T cells in the GALT to either systemic or i.g. administration of SEB in vivo have not previously been examined.

Systemic administration of SEB by i.p. or i.v. injection results in an early (2–3 day) expansion of the responsive Vβ1+ T cells, which are subsequently deleted at 7–10 days (7–9). We have found that intragastric (i.g.) administration of SEB rapidly (within 4 h) leads to the expansion and activation of both CD4+ and CD8+ Vβ1+ T cells in the PP and MLN. Analysis of cytokine mRNA in purified Vβ1+ T cells by competitive RT-PCR showed that, 4 h after i.g. administration of SEB, the induction of mRNA for IL-2 and IFN-γ was about 10-fold greater in mucosal than in peripheral lymphoid tissue. SE elicited secretion of IFN-γ and TNF from lymphocytes has been shown to lead to epithelial transport and barrier abnormalities (10). Taken together, our results suggest that the rapid expansion of mucosal T cells, and concomitant cytokine release, induced by the presence of SEs in the intestinal lumen could contribute directly to the acute intestinal symptoms at the site of SEB uptake, i.e., the diarrhea and vomiting that are the hallmark of food poisoning.

Materials and Methods

Mice

Female BALB/cBy J mice (6–12 wk of age) were purchased from The Jackson Laboratory (Bar Harbor, ME) and maintained under specific viral pathogen-free conditions at an American Association for the Accreditation of Laboratory Animal Care accredited facility at Massachusetts General Hospital.

SEB treatment and flow cytometric analysis

Mice were anesthetized and fed i.g. with 50 μg of staphylococcal enterotoxin B (SEB; Sigma, St. Louis, MO) mixed with 400 μg of soybean
trypsin inhibitor (STI, Sigma) in 500 μl of PBS using a ball-tipped feeding needle. Controls were fed PBS and STI alone. Another group of mice received 50 μg of SEB dissolved in 500 μl of PBS and administered i.p. Single-cell suspensions were prepared by pressing the tissues through a 70-μM nylon cell strainer (Falcon, Lincoln Park, NJ). Spleen cell suspensions were depleted of RBCs by lysis with Tris-buffered ammonium chloride. Single-cell suspensions were then incubated with the following, fluorescently conjugated, activation marker-specific Abs: CD69 (phycoerythrin (PE)-labeled H1.2F3, hamster Ig, Pharmingen, San Diego, CA); CD25 (IL-2R α-chain, PE-labeled PC61 5.3, rat IgG1, Caltag, San Francisco, CA); and L-selectin/CD62L (PE-labeled MEL14, rat IgG2a, Pharmingen). PE-labeled hamster Ig, rat IgG1, and rat IgG2a (all purchased from Pharmingen) were used as isotype controls. The T cell subpopulations of interest were identified using FITC-labeled Abs directed against CD4 (RM4-5) and CD8 (F23.1, Pharmingen). In preliminary experiments, an Ab against Vβ9 (MR10-2) was used as an additional specificity control. Detection of the the biotinylated Abs with streptavidin-conjugated PerCP (Becton Dickinson, San Jose, CA) enabled us to analyze these samples by three-color flow cytometric analysis on a Becton Dickinson FACScan with CellQuest software. Dead cells and debris were excluded from analysis by gates set on forward and side angle light scatter. For each stained aliquot of cells, 50,000–200,000 events were acquired, gating on all cells with the forward and side scatter properties of lymphocytes. 6000–10000 events were analyzed for activation marker expression in the gated CD4 + or CD8 + Vβ8 + populations.

T cell purification

In some experiments, Vβ8 + T cells were purified from the single-cell suspensions obtained from spleen, MLN, and PP using the VarioMACS system (Miltenyi Biotec, Auburn, CA) following the manufacturer’s protocol. Cells were incubated with biotinylated anti-Vβ8,1,2,3 (F23.1, Pharmingen), for 25 min at 4°C, washed, and incubated with streptavidin-conjugated magnetic microbeads (Miltenyi Biotec) for an additional 25 min at 4°C. Streptavidin-conjugated PE (PharMingen) was also added as a tracer to assess the purity of the resulting cell preparations by flow cytometric analysis. The cells were then washed again and passed through a MACS V5 separator column. The purity of the magnetically sorted Vβ8 + T cells was >95% for cells derived from the spleen and MLN and equivalent for cell populations prepared from PBS- or SEB-treated mice. Multiple experiments (for a total of 30–40 mice) were required for the preparation of an enriched population of Vβ8 + cells from the PP of PBS- and SEB-treated mice. The lymphocytes in the various preparations ranged in purity from 50–90% Vβ8 + and were pooled together (estimated final purity, 75–90%) for the preparation of RNA for analysis by RT-PCR.

Semiquantitative and competitive RT-PCR

Total RNA was extracted from whole tissue preparations or from purified Vβ8 + T cells isolated from the spleen, MLN, and PP of SEB-treated mice and PBS controls using TRizol (Life Technologies, Gaithersburg, MD) according to the manufacturer’s instructions. In a 60-μl total reaction mixture, 10 μg of total RNA was reverse transcribed using 0.5 μM random hexamer primers, 0.67 mM dNTP, 50 μM of uracil leukemia virus reverse transcriptase, and 20 μl of RNase inhibitor (all purchased from Perkin-Elmer, Foster City, CA). The cDNA was then amplified by multiple PCR reactions with cytokine-specific primers for IL-2 (5'-TCTACTTCAAGCTTCACAG, 5'-GAGTCAAACTCAGAAAGCTG, 5'-C TTCATGAAAGCTTCATTTTGC), IL-4 (5'-CATCGGCTGAACGTGTTTG, 5'-GCTGTTGAGTTGACTCG), and IL-10 (5'-CCGTTTTACCTGGTAGAAGTGATG, 5'-TCTGTA GGTCCCGGACCGACGACTCA) derived from published sequences (11). TNF-α cDNA was amplified using an RT-PCR Amplimer Set from Clontech (Palo Alto, CA). PCR reactions were performed in a programmable Thermal Cycler (MJ Research, Watertown, MA) for 35 cycles. Pre- laboratory experiments established that amplification for 35 cycles was adequate for cell populations prepared from PBS- or SEB-treated animals. The PCR reaction mixtures contained 5 μl of buffer A (100 mM Tris-HCl, pH 8.3, 500 mM KCl, 15 mM MgCl2, 0.01% w/v gelatin; Fisher Scientific, Pittsburgh, PA), 1 μl of dNTP (10 mM; Perkin-Elmer), and 0.25 μl of Taq polymerase (Fisher Scientific). Each cycle was 95°C, 30 s; 58°C, 60 s; 75°C, 60 s; and a final extension of 72°C, 5 min. In each experiment the initial PCR reaction was performed using 8 μl of cDNA and HPRT-specific primers. The PCR products were separated by electrophoresis on a 1.5% agarose gel and visualized by EthBr staining and UV light. The amount of cDNA to be added to each cytokine-specific reaction mixture was then adjusted so that each experimental sample would contain equivalent amounts of DNA for the housekeeping gene. After HPRT normalization, each experimental sample was amplified using cytokine-specific primers. Twenty microinots of the total (5 μl) cytokine-specific PCR product from each reaction was separated by electrophoresis in a 1.5% agarose gel and visualized by EthBr staining and UV light. For competitive PCR for IL-2 and IFN-γ, 10-fold or 3-fold dilutions (in the pg/ml range) of the polyclonimer cytokine plasmid PQRS (Ref. 11; generously provided by Dr. Steven Reiner, University of Chicago, IL) were added to a constant, HPRT-normalized amount of experimental cDNA for the PCR reaction with each cytokine-specific primer. Competitive PCR for TNF-α was performed using serial 10-fold and 3-fold dilutions of TNF-α PCR product (MIMIC Clontech). PCR products were separated on agarose gels. The point at which the competitor and experimental bands are of equivalent intensity is designated as the concentration of experimental mRNA for the cytokine-specific gene.

Statistical analysis

Statistical differences in percentages of CD4 + and CD8 + Vβ8 + T cells for groups of mice given SEB i.p. or i.v. in comparison with PBS-treated controls were determined using a two-tailed Student’s t test with StatView software (Abacus Concepts, Berkeley, CA). A p value < 0.05 was considered significant.

Results

Intragastric administration of SEB leads to an early expansion of responsive Vβ8 + T cells in the PP and MLN but not in the spleen

Systemic administration of SEB by i.p. or i.v. injection results in a characteristic and well-documented early (2–3 day) expansion of the responsive Vβ8 + T cells in peripheral blood and spleen followed by deletion at 7–10 days (7–9). This is sometimes accompanied by an early loss of SEB-responsive cells within the first 24 h (12). Fig. 1 shows that i.p. injection of SEB results in a significant loss of responsive CD4 + Vβ8 + T cells in the spleen at 12 h followed by a marked expansion by 48 h, as expected. CD8 + Vβ8 + T cells in the spleen are expanded at 48 h as well. The expansion of CD4 + and CD8 + Vβ8 + T cells noted in the spleen at 48 h after systemic administration of SEB also occurs in the MLN, albeit to a lesser extent. The response of T cells in the PP to i.p. SEB differs from that in the spleen and MLN; CD4 + Vβ8 + T cells are expanded by 48 h, whereas CD8 + Vβ8 + T cells are markedly reduced at 12 h and have only returned to baseline levels by 48 h. In contrast, after i.g. administration of the same dose of SEB, no expansion of Vβ8 + T cells is seen in the spleen within the first 48 h (other laboratories have also shown that peripheral (spleen and blood) Vβ8 + T cells are deleted 7–10 days after i.g. administration of SEB without an accompanying early expansion at these sites (13, 14)). When we examined Vβ8 + T cells in the PP, however, we found that both CD4 + and CD8 + Vβ8 + T cells are significantly (p < 0.05) expanded by 12 h after i.g. administered SEB. CD4 + Vβ8 + T cells return to normal levels by 48 h, while CD8 + Vβ8 + T cells remain elevated. In the MLN, only CD4 + Vβ8 + T cells are significantly expanded.

Mucosal T cells are activated during the first 48 h after i.g. administration of SEB

This pattern of responsiveness suggested that i.g. administration of SEB resulted in the activation of mucosal T cells. Accordingly, we examined the induction of well-known early markers of cellular activation on both CD4 + Vβ8 + and CD8 + Vβ8 + T cells from the spleen, MLN, and PP during the first 48 h after oral i.p. injection of SEB. The first phenotypic change evidenced by Vβ8 + T cells after systemic administration of SEB in vivo is a marked down-regulation of L-selectin expression on T cells in the draining lymph nodes, which peaks at 4 h after SEB injection and returns to normal levels within 48 h (15). Fig. 2 shows that systemic, i.p.
administration of SEB leads to down-regulation of L-selectin on CD4\(^+\) T cells in the spleen as well as on mucosal CD4\(^+\) T cells in the MLN and PP. The subsequent up-regulation of L-selectin on these cells is delayed compared with that noted for SEB-responsive T cells in the spleen during the first 48 h. The magnitude and kinetics of induction of the early activation marker CD69 and the IL-2R \(\alpha\)-chain (CD25) are similar on CD4\(^+\), V\(\beta\)\(^8\)\(^+\) T cells in all three tissues. The response of CD8\(^+\), V\(\beta\)\(^8\)\(^+\) T cells paralleled that of CD4\(^+\), V\(\beta\)\(^8\)\(^+\) T cells during the first 48 h after i.p. administered SEB (data not shown). It is interesting to note that some of the V\(\beta\)\(^8\)-CD4\(^+\) and CD8\(^+\) T cells in the PP express a constitutively activated phenotype, since \(\sim 20\%\) are L-selectin\(^{low}\) and CD69\(^{high}\) before SEB treatment (Fig. 2 and Ref. 16).

A very different activation phenotype is seen after oral administration of SEB. During the first 48 h, most of the activated CD4\(^+\), V\(\beta\)\(^8\)\(^+\) T cells are detected in the PP, although a significant population of CD69\(^{high}\) cells can be detected in the MLN and a small population is detectable in the spleen. However, L-selectin is only partially down-regulated on CD4\(^+\) T cells in the PP and is not down-regulated in the MLN or spleen. The IL-2R \(\alpha\)-chain is not up-regulated on CD4\(^+\), V\(\beta\)\(^8\)\(^+\) T cells after oral administration of SEB in any of the three tissues examined. A somewhat different response pattern emerged when CD8\(^+\), V\(\beta\)\(^8\)\(^+\) T cells were examined (data not shown). Although CD69 was highly up-regulated on T cells in the PP, L-selectin was only partially down-regulated. There was no evidence for activation of CD8\(^+\), V\(\beta\)\(^8\)\(^+\) T cells in the MLN or spleen in the first 48 h.

When we examined the percentages of V\(\beta\)\(^8\)-CD4\(^+\) and CD8\(^+\) T cells in both mucosal and peripheral tissues 10 days after i.p. or i.g. administration of SEB, we found that i.p. injection of SEB resulted in a marked depletion of CD4\(^+\), V\(\beta\)\(^8\)\(^+\) T cells in all sites examined, as reported (7–9). However, i.g. administration of two different doses (50 and 200 \(\mu\)g) of SEB resulted in little depletion of CD4\(^+\) or CD8\(^+\), V\(\beta\)\(^8\)\(^+\) T cells in the spleen; significant depletion in the PBL was detected only at the higher (200 \(\mu\)g) dose, in agreement with a previous report (Ref. 14; data not shown). CD4\(^+\), V\(\beta\)\(^8\)\(^+\) T cells were, however, significantly depleted in both of the mucosal sites examined after i.g. SE, although the depletion was less marked than that seen after i.p. administration of SEB (data not shown).

mRNA for Th1 cytokines is rapidly up-regulated in mucosal lymphoid tissue after i.g. administration of SEB

We next analyzed the induction of mRNA for both Th1 and Th2 cytokines after i.g. administration of SEB, using semi-quantitative RT-PCR. Fig. 3 shows that IL-2, IFN-\(\gamma\), and TNF-\(\alpha\) mRNA are rapidly induced in the PP and MLN within 1.5 h. mRNA for each of these Th1 cytokines declines in the PP by 12 h, but can still be seen in the MLN. When we looked at cytokine induction outside of the GALT, we found that a small amount of IL-2 mRNA is present in the spleen at 4 h; IFN-\(\gamma\) and TNF-\(\alpha\) mRNA are not detectable in the spleen at any time point. mRNA from MLN and spleen cells prepared 4 h after i.p. administration of SEB is also included as a positive control for induction of cytokine mRNA. No clear induction of Th2 cytokine mRNA was noted after i.g. administration of SEB, although both IL-4 and IL-10 were detectable in the spleen and MLN after SEB was given i.p.. Although Th1 cytokine responses are typically associated with the response to the SEs, other work has shown that, after i.v. administration of SEB, both Th2 (IL-4 and IL-10) and Th1 (IFN-\(\gamma\) and IL-2) cytokines can be detected in the serum within 90 min (17).

We speculated that the amount of mRNA induction in the MLN appeared greater than that at other sites because this tissue contains mostly T cells, while B cells predominate in the spleen and PP. To quantify the induction of cytokine mRNA in each of these sites after i.g. administration of SEB, we purified V\(\beta\)\(^8\)\(^+\) T cells from the spleen and MLN and performed competitive RT-PCR for IL-2, IFN-\(\gamma\), and TNF-\(\alpha\) using 10-fold dilutions of competitor. We chose to examine the 4-h time point because this was the peak for IL-2 and IFN-\(\gamma\) mRNA induction in all of the tissues examined (see Fig. 3). Fig. 4A shows that, in the MLN, 4 h after i.g. administration of SEB, mRNA for IL-2 is increased by \(\sim\)100-fold and IFN-\(\gamma\) is increased 20-fold. In the spleen (Fig. 4B), mRNA induction for each of these cytokines is \(\sim\)10-fold lower than in the MLN. mRNA for IL-2 is increased 10-fold, while IFN-\(\gamma\) is increased only 5-fold. We then examined mRNA for both Th1 and
FIGURE 2. Mucosal, but not peripheral, Vβ8+ T cells are activated during the first 48 h after i.g. administration of SEB. Mice were fed (closed circles) or injected (open circles) with 50 μg of SEB as described in the legend to Fig. 1. Controls were fed PBS (open squares). Lymphocytes from the spleen, MLN, or PP were harvested at the indicated time points and stained for three-color flow cytometric analysis. Activation marker expression was analyzed on the gated Vβ8+CD4+ subpopulation. The results are expressed as the mean value for each group ± SEM. The results shown are representative of two independent experiments.

Discussion
The SEs act as superantigens for T cells bearing particular Vβ segments and are thus capable of activating large numbers of T cells. Even before its role as a superantigen was appreciated it was documented that orogastric administration of SEA in rats led to a rapid and fractionated PP and MLN in Fig. 3 is derived from non-T cell sources. In contrast to the induction seen after i.p. administration of SEB (Fig. 3), no induction of mRNA for the Th2 cytokines IL-4 or IL-10 was detectable in the PP.

FIGURE 3. mRNA for Th1 cytokines is rapidly up-regulated in mucosal lymphoid tissue after i.g. administration of SEB. SEB was administered as described for Figs. 1 and 2. RNA was prepared from single-cell suspensions of PP, MLN, and spleen at the indicated time points and subjected to semiquantitative RT-PCR with primers specific for both Th1 (IL-2, IFN-γ, TNF-α) and Th2 (IL-4, IL-10) cytokines.
Leukocytic infiltration in the stomach and duodenum (18). The "leukotactic" properties attributed to exposure to SEA in the intestinal lumen were confirmed and extended by another study in which the addition of SEB to explants of fetal human intestine in organ culture resulted in a marked expansion of intestinal T cells and accompanying cytokine release (19). However, before the present study, the response of T cells in the GALT upon luminal exposure to SEs in vivo had not been further explored. Indeed, because the signals are small and difficult to detect, few studies have examined Ag-specific responses of T cells in the GALT without in vitro restimulation. In this report, we use three-color flow cytometric analysis of populations of V:\beta\_8\^\(\text{+}\) T cells and a sensitive and quantitative competitive RT-PCR technique to show that T cells in the GALT, isolated directly ex vivo, are rapidly activated and stimulated to produce cytokines after i.g. administration of SEB.

Recent work has shown that SEB can bind to a class II-negative human intestinal epithelial cell line in vitro and can cross the epithelium in an immunologically intact form (14). Our data extend these results by showing that i.g. administration of SEB can also activate mucosal T cells. As in mice, SE-mediated shock in man is accompanied by T cell expansion and cytokine release (20, 21). Mice, however, are more resistant to the toxic effects of the SEs than humans, but at higher doses, they exhibit symptoms associated with T cell cytokine release such as weight loss and thymic involution (6, 22). The differences in susceptibility between mice and humans may be related to the magnitude of the response induced, since SEB binds with higher affinity to human than murine MHC class II (1). Indeed, mice treated with d-galactosamine to impair liver metabolism succumb to a T cell-mediated lethal shock after administration of small (2–20 \(\mu\)g) doses of SEB (22, 23). Our results in mice might therefore be extrapolated to propose a model for the role of T cells in the GALT in human enterotoxic poisoning. Toxins such as SEB may gain access to T cells in the PP by binding to specialized epithelial cells called M cells, which transport Ags from the intestinal lumen into the PP (24) and, at least

**FIGURE 4.** The induction of Th1 cytokine mRNA in V:\beta\_8\^\(\text{+}\) T cells decreases with increasing distance from the intestinal lumen 4 h after i.g. administration of SEB. BALB/c mice were fed PBS or 50 \(\mu\)g of SEB + STI. Four hours later, V:\beta\_8\^\(\text{+}\) T cells were purified from the spleen or MLN. RNA was extracted and reverse transcribed, and levels of the Th1 cytokines IL-2 and IFN-\(\gamma\) were quantitated by competitive RT-PCR through the addition of serial 10-fold dilutions of the PQRS poly-competitor cytokine construct to a constant HPRT-normalized amount of experimental cDNA as described in Materials and Methods. No competitor was added to the far right lane on each gel. PQRS competitor was added to a final concentration ranging from 0.0018 to 180 pg/ml (read from right to left side of each gel). The results shown are representative of two independent experiments. Arrows indicate the estimated point of equivalence between the competitor and experimental samples.

The Journal of Immunology

by guest on September 15, 2017 http://www.jimmunol.org/ Downloaded from

5829
some of which, express MHC class II (25). SE-elicited secretion of IFN-γ and TNF from lymphocytes has been shown to lead to epithelial transport and barrier abnormalities (10). Our results would then suggest that the rapid expansion of mucosal T cells, and concomitant cytokine release, induced by the presence of SEs in the intestinal lumen could contribute directly to the acute intestinal symptoms at the site of SEB uptake, i.e., the diarrhea that is the hallmark of food poisoning. The 50-μg dose used in this study has enabled us to detect mucosal T cell responses in the absence of substantial peripheral T cell activation. At higher doses, or in d-galactosamine treated mice (22, 23), extraintestinal manifestations of enterotoxic shock that involve the central nervous system may result from the uptake of toxin into the bloodstream. Our data indicate that a self-limited local T cell response may explain some acute forms of enterotoxic poisoning.

The response of Vβ8+ T cells in the PP differed markedly depending on the route of toxin administration. Intraperitoneal administration of SEB led to a marked depletion of CD4+ and CD8+ Vβ8+ cells in the PP 12 h later, whereas these subsets are expanded 12 h after i.g. administration of SEB (Fig. 1). It is not yet clear whether this early expansion reflects recruitment of Vβ8+ T cells from other sites (e.g., MLN) or proliferation within the PP itself. The absence of IL-2R (CD25) up-regulation on Vβ8+ T cells in the PP after i.g. SEB (see Fig. 2) may suggest that at least some of this expansion is due to chemokine-induced migration rather than IL-2-driven proliferation.

There have only been a few other studies that have examined Ag-specific T cell responses without in vitro stimulation. Litton et al. showed that i.p. administration of SEB leads to the induction of TNF and IL-2 synthesis in peripheral lymph nodes within 1 h (26). Semiquantitative RT-PCR analysis of lymph node cells showed that IL-2 mRNA peaked at 4 h, while IFN-γ mRNA (produced primarily by CD8+ T cells) peaked at 12 h after i.p. injection. Cytokine expression preceded clonal expansion and deletion, and the estimated frequency of cytokine producing T cells was small (2–3%) (26). In another study, in situ hybridization showed that i.p. administration of SEB led to up-regulation of mRNA for IL-2 and IFN-γ and was limited to T cell areas in the spleen. mRNA for both cytokines peaked at 3–4 h and was gone by 48 h (27). Similar response patterns were noted when we analyzed the induction of IL-2 and IFN-γ mRNA after i.g. administration of SEB, as shown in Fig. 3. The previous study also reported a biphasic response for the induction of TNF-α mRNA by i.p. SEB. At early time points (before 2 h), TNF-α mRNA was restricted to T cells, while at later time points, it was expressed mainly by macrophages. Work from other laboratories has established that SEB can stimulate TNF-α production by macrophages, presumably via binding to MHC class II (28, 29). In agreement with Bette et al. (27), our data on TNF-α expression after i.g. SEB show that TNF-α mRNA peaks at 1.5–4 h in unfractionated PP and MLN (Fig. 3) but is not detectable in purified Vβ8+ T cells examined 4 h after i.g. SEB (data not shown). At the later (4 h) time point, peak expression of TNF-α mRNA may occur in macrophages, which are removed by this T cell purification protocol.

When examined quantitatively (Figs. 4 and 5), the levels of cytokine mRNA induction we see after i.g. administration of SEB are either greater than or of the same order of magnitude as those that have been recently described in another model system. In that study, transgenic OVA-specific T cells were adoptively transferred into normal mice and visualized by in situ hybridization analysis.
after administration of Ag via various routes (30). Subcutaneous immunization of these mice with OVA led to peak induction of IL-2 mRNA in draining peripheral lymph nodes by 8 h, with a return to baseline levels by 24 h. Although the majority of CD4+ transgene-positive cells were activated, as assessed by up-regulation of CD25 (IL-2Rα-chain) and blast transformation, only 8.5% expressed IL-2 mRNA at the peak of the response, i.e., an ~10-fold increase over background levels. Using this immunizing regimen, IL-2 mRNA and IL-2Rα-chain expression coincided. When Ag was administered by a tolerizing route, however, the peak of IL-2 mRNA expression preceded the peak of IL-2Rα-chain up-regulation, and most of the transgene-positive population failed to undergo blast transformation. This led the authors to suggest that the premature induction of IL-2, before up-regulation of IL-2R is complete, would impair the ability of T cells to utilize and produce IL-2 and result in tolerance and/or cell death (30). Although the methodologies are very different, several parallels can be drawn between this study and our results using the superantigen SEB. The kinetics of IL-2 mRNA induction (in each of the tissues examined herein) is similar to that seen by Rogers et al. (30) with a peak between 4 and 12 h. Interestingly, while i.p. administration of SEB resulted in marked up-regulation of the IL-2Rα-chain, with a peak between 4–12 h that coincided with peak induction of IL-2 mRNA, i.e., administration of SEB led to IL-2 mRNA induction in the absence of IL-2Rα-chain up-regulation (see Fig. 2); PP T cells, however, were clearly activated as evidenced by their up-regulation of CD69 and down-regulation of L-selectin. This may suggest that the early cytokine burst seen in response to i.g. administration of SEB ultimately results in tolerance and is in keeping with the observation that repeated oral administration of SEB can induce tolerance to SEB as well as protection against SEB-induced lethal shock in d-galactosamine-sensitized mice (13, 23). Indeed, in our hands, no IL-2 or IFN-γ mRNA was detectable in the spleen or MLN upon challenge of mice rendered tolerant by repeated oral administration of SEB (data not shown).

Superantigen-driven mucosal T cell stimulation has also been implicated in the development of the inflammatory bowel diseases, ulcerative colitis and Crohn’s disease (31–35). The demonstration that luminal presentation of SEB can activate large numbers of mucosal T cells lends support to this hypothesis. The dysregulation that luminal presentation of SEB can activate large numbers of ulcerative colitis and Crohn’s disease (31–35). The demonstration implicates in the development of the inflammatory bowel diseases, repeated oral administration of SEB (data not shown).

Indeed, in our hands, no IL-2 or IFN-γ mRNA was detectable in the spleen or MLN upon challenge of mice rendered tolerant by repeated oral administration of SEB (data not shown).

### Acknowledgments

We thank Paul Alfaro for technical assistance and Drs. Jeanette Thorbecke, Hai Ning Shi, Abhijit Afsalpurkar, and Bobby Cherayil for critical review of the manuscript. We also gratefully acknowledge the assistance of Lisa Steele and Jasper zu Putlitz in setting up the RT-PCR system.

### References