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Cytokine Secretion Induced by Superantigens in Peripheral Blood Mononuclear Cells, Lamina Propria Lymphocytes, and Intraepithelial Lymphocytes

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Superantigens are potent inducers of T-cell proliferation and induce a broad range of cytokines, including tumor necrosis factor (TNF), gamma interferon, and interleukin 2 (IL-2). In the present study, we compared the abilities of different staphylococcal superantigens (staphylococcal enterotoxin B [SEB], staphylococcal enterotoxin E [SEE], and toxic shock syndrome toxin 1 [TSST-1]) to stimulate distinct cytokine profiles in peripheral blood mononuclear cells (PBMC), lamina propria lymphocytes (LPL), and intraepithelial lymphocytes (IEL). One million PBMC, LPL, and IEL were stimulated with various concentrations of superantigen (10 to 0.001 ng/ml) for 24, 48, and 72 h. Maximum cytokine production by PBMC, LPL, and IEL was observed for all three superantigens at 48 h at a concentration of 1 ng/ml. In PBMC, SEE and TSST-1 stimulated more IL-2 and gamma interferon than SEB. SEE and TSST-1 also stimulated more TNF and IL-4 production than SEB. In contrast, SEB stimulated more IL-6 than either SEE or TSST-1. In LPL, there was no SEE-induced IL-2 or IL-4 production, but IL-6, TNF, and gamma interferon were induced. SEB similarly induced no IL-2 or gamma interferon from the LPL, but IL-4, IL-6, and TNF were detected. TSST-1 stimulation of LPL resulted in IL-2 and TNF production but no IL-4, IL-6, or gamma interferon. In IEL, SEE induced no IL-2, IL-4, or gamma interferon but produced IL-6 and TNF, while SEB stimulation resulted in no IL-2 or gamma interferon but did result in detectable IL-4, IL-6, and TNF. TSST-1 stimulation of IEL produced no IL-2, gamma interferon, or IL-6 but did induce IL-4 and TNF. Taken together, these data indicate that there are significant differences in the cytokine profiles induced by superantigens in LPL and IEL compared with those in PBMC, and these differences may relate to differences in activation requirements.

Staphylococcal enterotoxins are a family of structurally related proteins produced by *Staphylococcus aureus* (19). These compounds, with molecular masses of between 24 and 30 kDa, are classified into six groups (A to E) and toxic shock syndrome toxin 1 (TSST-1) (F) and stimulate subsets of T cells and monocytes on the basis of specific Vβ receptor expression and major histocompatibility complex class II binding (5). These molecules are highly hydrophilic and exhibit low alpha helix and high β pleated-sheet contents, suggesting a flexible, accessible structure (15). Superantigens bind directly to class II molecules on antigen-presenting cells and do not require processing or proteolysis to peptides (11). The alpha-helical regions of the class II molecule are essential for the binding of the superantigens and appear to interact directly with the NH_2-terminal regions of the superantigens (31). Recent studies have shown that a complex of superantigen and major histocompatibility complex class II molecules is required for binding to the Vβ region of the T-cell antigen receptor for T-cell activation (17). Superantigens can also stimulate monocytes by binding class II molecules on the monocyte cell surfaces (37). After binding to T cells, superantigens induce the production of a broad range of cytokines, including tumor necrosis factor (TNF), interleukin 2 (IL-2), IL-4, IL-6, and gamma interferon, in no apparent pattern (24).

Superantigens are among the most common causes of food poisoning and produce a variety of clinical symptoms, including malaise, nausea, vomiting, and diarrhea (6). The illness occurs after the accidental ingestion of contaminated food, is frequently self-limited, and in most cases does not require medical attention. It has been suggested that the effects of staphylococcal enterotoxins—nausea, vomiting, and diarrhea—are secondary to their superantigen activities (19). There is other evidence, however, that the superantigen activities of the enterotoxins may be distinct from their toxic effects on the gastrointestinal tract. Superantigens can stimulate T cells and monocytes in nonprimate animals and yet do not have any enterotoxic effects (17). The enterotoxic effects of the superantigens may be mediated by neural cells, which stimulate gastrointestinal mast cells to release histamine (27). Furthermore, the enterotoxic effects of the superantigens can be separated from the T-cell- and monocyte-stimulating properties by using modified molecules that do not cause gastrointestinal symptoms but that do stimulate T cells (4, 26). Gastrointestinal symptoms also occur in toxic shock syndrome, which is associated with a superantigen (TSST-1) for which the toxin may not directly interact with the gastrointestinal cells (35).

Our laboratory has previously demonstrated that superantigens are potent mitogens for lamina propria lymphocytes (LPL) (2). Since T cells and monocyte-derived cytokines may play a role in the gastrointestinal disease associated with staphylococcal superantigens, we determined if different superantigens stimulate the production of distinct panels of either T-cell-derived or monocyte-derived cytokines from peripheral

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blood mononuclear cells (PBMC), LPL, and intraepithelial lymphocytes (IEL).

MATERIALS AND METHODS

PBMC, LPL, and IEL isolation and stimulation with superantigens. Human mononuclear cells were separated from buffy coats obtained from healthy blood donors at the Mount Sinai Blood Bank by Ficoll-Hypaque (Pharmacia, Piscataway, N.J.) density gradient centrifugation by using previously described methods (32). The cells were washed three times with sterile phosphate-buffered saline (PBS) and resuspended in RPMI 1640 (GIBCO, Grand Island, N.Y.) supplemented with 10% fetal calf serum (GIBCO), 2 mM L-glutamine, and 1% penicillin-streptomycin (GIBCO), henceforth defined as complete medium (CM). IEL and LPL were obtained by a modification (2, 23) of the procedure of Bull and Bookman (8). Briefly, surgical specimens were obtained and washed extensively with Hanks' balanced salt solution (GIBCO) containing 1% penicillin-streptomycin (GIBCO), 50 μg of gentamicin (GIBCO) per ml, and 1% fungizone (GIBCO). The mucosa was dissected free from the submucosa, minced into 2-mm^2 pieces, and treated sequentially for 15 min with dithiothreitol (1 mmol/liter; Sigma, St. Louis, Mo.), and this was followed by three 45-min treatments with EDTA (Sigma) in calcium- and magnesium-free Hanks' balanced salt solution (GIBCO) at 37°C on an orbital shaker. The liberated cells were separated by Percoll (Pharmacia) gradient centrifugation to yield purified IEL (2). To isolate LPL, the tissue fragments received three more treatments with EDTA, and the freed cells were discarded. The remaining tissue was digested for 3 h at 37°C in a 5% CO_2 incubator on an orbital shaker with 20 U of collagenase (Boehringer-Mannheim) per ml in RPMI 1640 (GIBCO) supplemented with 10% fetal calf serum. Liberated cells were obtained by collecting the supernatants and by gently rubbing the tissue fragments against a wire sieve with a sterile rubber policeman. Lymphocytes were then purified by Percoll density gradient centrifugation as described above. Direct surface immunofluorescence and then flow cytometric analysis were performed on PBMC, LPL, and IEL with the commercially available monoclonal antibodies leu 4 (anti-CD3), leu 3 (anti-CD4), leu 2 (anti-CD8), and leu M-3 (anti-CD14) (Becton-Dickinson, Rutherford, N.J.). Indirect immunofluorescence was also performed with anti-α/β and -γ/δ monoclonal antibodies (kindly provided by D. Posnett). The concentrations of the PBMC, LPL, and IEL were adjusted to 10^6 cells per ml, and the cells were cultured in 1-ml cultures in 24-well plates (Falcon, Lincoln Park, N.J.) in the presence or absence of various concentrations (10 to 0.001 ng/ml) of superantigen (staphylococcal enterotoxin E [SEE], staphylococcal enterotoxin B [SEB], or TSST-1) (Toxins Technologies, Phoenix, Ariz.) or phytohemagglutinin (PHA) (1 μg/ml) (GIBCO) for 24, 48, and 72 h. Cell-free supernatants were harvested, aliquoted, and frozen until use. All of the cytokine determinations were performed on the same supernatants. In parallel experiments, 10^5 PBMC in triplicate microwell cultures were stimulated for 24, 48, and 72 h with various concentrations of SEE, SEB, and TSST-1 (10 to 0.001 ng/ml) labeled with [^{3}H]thymidine at 1 μCi per well (ICN, Irvine, Calif.) for 18 h prior to harvest and were counted in a scintillation counter (32).

IL-2 bioassay. The IL-2-dependent murine T-cell line CTLL was used to measure IL-2 (12, 33). CTLL cells were maintained in CM supplemented with 10% concanavalin A-stimulated rat spleen cell supernatant, 2-mercaptoethanol (2×10^{-5} M) (Sigma), and nonessential amino acids (1%) (GIBCO). Prior to the assay, the cells were cultured in IL-2-free medium for 4 h at 37°C. Test T-cell supernatants were added in triplicate microwells for 24 h, 1 μCi of [^{3}H]thymidine was added for the last 4 h of culture, and then the mixture was processed for scintillation counting. Standard curves of IL-2 were generated in each assay by using known concentrations of recombinant cytokine (Boehringer-Mannheim).

IL-4 bioassay. IL-4 induces the expression of CD23 in the B-cell line Ramos (30). Supernatants were incubated with 10^5 Ramos cells for 48 h, then were stained with the anti-CD23 monoclonal antibody BLAST-2 (a generous gift of M. Crow, Hospital for Special Surgery), and were analyzed with a flow cytometer (EPICS C; Coulter, Hialeah, Fla.). Recombinant IL-4 (R and D, Minneapolis, Minn.) was used to generate a standard curve.

IL-6 bioassay. The IL-6-dependent murine B-9 plasmacytoma cell line was utilized to measure IL-6 (1). In these studies, test supernatants were added to triplicate cultures of 5×10^3 B-9 cells in CM for 3 days. [^{3}H]thymidine (1 μCi) was added during the last 16 h of culturing, and the cells were harvested and processed for scintillation counting. Standard IL-6 curves were obtained for each assay by using known concentrations of recombinant IL-6 (kindly provided by E. Siden, Mount Sinai Medical Center). The specificity of the bioassay was determined with a polyclonal anti-IL-6 antibody (Amgen, Thousands Oaks, Calif.) which specifically inhibits IL-6-induced proliferation of the B-9 cell line (38).

TNF bioassay. The L929 bioassay was utilized to measure TNF levels (7). The L929 fibroblast line is a TNF-sensitive line that lyses in the presence of TNF and does not distinguish between TNF-α and TNF-β. In brief, the L929 cells were plated into 96-well, flat-bottomed microtiter plates (Becton-Dickinson, Lincoln Park, N.J.) until the cells were confluent. Test samples were added in triplicate along with 4 μg of actinomycin D (Sigma) per ml, and the plates were incubated for 18 h at 37°C. The wells were washed once with PBS, and 0.05% crystal violet in 20% ethanol was added to each well for 10 min. The crystal violet was removed with tap water. Methanol (100%) was added to each well to elute the stain from the viable cells. The plates were allowed to dry and were read with a microtiter plate reader at A_{570} and A_{630}. Known concentrations of recombinant TNF-α (Cetus, Los Angeles, Calif.) were added to generate a standard curve in each assay.

Gamma interferon. Gamma interferon levels were measured by a direct-binding enzyme-linked immunosorbent assay (ELISA) (Genzyme, Boston, Mass.).

Statistical analysis. Statistically significant differences in cytokine levels induced by the superantigens were determined by the nonparametric Mann-Whitney test as previously described (39).

RESULTS

PBMC, LPL, and IEL. PBMC consisted of 85% ± 12.3% CD3-positive cells, 42% ± 7.8% CD4-positive cells, 28% ± 3.4% CD8-positive cells, and 11% ± 2.5% CD14-positive cells. There were 93% ± 11.2% α/β and 3% ± 2.1% γ/δ cells. The LPL consisted of 83% ± 13.4% CD3-positive cells, 40% ± 7.5% CD4-positive cells, 25% ± 3.0% CD8-positive cells, and 7% ± 2.3% CD14-positive cells. There were 90% ± 12% α/β cells and 7% ± 2.3% γ/δ cells. The IEL consisted of 95% ± 8.9% CD3-positive cells, 2% ± 0.9% CD4-positive cells, and 95% ± 9.0% CD8-positive cells. There were 85% ± 9.2% α/β and 10% ± 2.3% γ/δ cells. There was less than 5% epithelial contamination.

Cytokine assays. We utilized bioassays to measure the cytokine levels of the culture supernatants of the superantigen-stimulated cells since these assays are highly reproducible (33) and measure bioactive cytokines. Triplicate replicates were used in all of the bioassays, and there was a less than 15% experimental error within an individual experiment. Recombinant cytokines were used as standards in all of the bioassays. An ELISA was used to measure gamma interferon because of the highly variable nature of the bioassay. The small numbers of LPL and IEL that were obtained from the surgical specimens did not permit assessment of cytokine mRNA levels.

IL-2 production. To determine if the different superantigens induce distinct cytokine profiles and to compare cytokine production in different lymphoid tissue compartments, we simultaneously measured the levels of IL-2, gamma interferon, TNF, IL-4, and IL-6 in the culture supernatants of PBMC, LPL, and IEL stimulated with SEE, SEB, and TSST-1. Maximal cytokine production for IL-2 occurred 48 h after the stimulation. In PBMC, SEE and TSST-1 stimulated more IL-2 production (51.1 ± 5.4 and 57 ± 5.6 U/ml, respectively) (Table 1) than SEB production (11.5 ± 1.80 U/ml, $P < 0.05$) at 48 h. These differences in IL-2 production were also evident at 24 and 72 h (data not shown), although IL-2 levels at 24 for the SEB- and PHA-stimulated PBMC were comparable. Furthermore, the differences in IL-2 production could not be accounted for by the concentration of superantigen used. The maximal stimulation of cytokine production was seen with each superantigen at a concentration of 1 ng/ml (dose used in Table 1), which is a concentration similar to those found in our previous studies, but differences in IL-2 production were evident at every concentration of SEB (10 to 0.001 ng/ml) (data not shown). There was no IL-2 production induced in IEL by any of the superantigens, and only TSST-1-induced IL-2 production (10.0 ± 2.8 U/ml) was found in LPL. PHA-induced IL-2 production was observed with PBMC (30.1 ± 3.5 U/ml) and LPL (15.1 ± 1.2 U/ml) but not with IEL.

SEE, SEB, and TSST-1 all stimulate equivalent PBMC proliferation. One possible explanation for the findings discussed above may be that there are differences in the abilities of the individual superantigens to stimulate PBMC, LPL, and IEL proliferation. We have previously demonstrated (2) that SEE, SEB, and TSST-1 are mitogenic for LPL. In a result similar to that of a previous study (2), all three superantigens (SEE, SEB,

TABLE 1. Amounts of cytokines induced by different superantigens[a]

Cell type and superantigen	Amt of cytokine (mean ± SD)				
	IL-2 (U/ml)	Gamma interferon (ng/ml)	IL-4 (U/ml)	IL-6 (U/ml)	TNF (U/ml)
PBMC					
SEE	51.1 ± 5.4	4.1 ± 1.1	5.0 ± 1.5	12.1 ± 1.1	58.1 ± 23.0
SEB	11.5 ± 1.8	1.2 ± 0.4	1.1 ± 0.09	25.0 ± 2.3	5.2 ± 0.9
TSST-1	57 ± 5.6	24.1 ± 0.5	7.1 ± 2.3	10.1 ± 0.8	57.2 ± 21.8
PHA	30.1 ± 3.5	1.5 ± 0.03	3.5 ± 1.7	15.6 ± 1.7	51.5 ± 5.7
LPL					
SEE	0	0.1 ± 0.025	0	1.1 ± 0.1	20.0 ± 3.7
SEB	0	0	2.0 ± 0.01	3.5 ± 0.7	2.0 ± 0.05
TSST-1	10.0 ± 2.8	0	0	0	50.0 ± 10.1
PHA	15.1 ± 1.2	0	1.5 ± 0.05	3.0 ± 0.5	20.1 ± 2.1
IEL					
SEE	0	0	9.0 ± 1.1	2.1 ± 0.03	35.0 ± 5.3
SEB	0	0	0	3.5 ± 0.7	9.7 ± 1.0
TSST-1	0	0	2.4 ± 0.4	0	10.1 ± 1.0
PHA	0	0	1.5 ± 0.05	3.1 ± 0.5	31.3 ± 3.1

[a] These data represent pooled data from seven separate experiments.

and TSST-1) were capable of stimulating comparable levels of PBMC and LPL proliferation (Fig. 1). The degrees of stimulation varied from donor to donor but were comparable to the level of response to PHA for all experiments. The maximal proliferation induced by the superantigens at concentrations of 1 ng/ml occurred 48 h after stimulation, while PHA stimulation typically peaked at 72 h. The capacities of all of the superantigens to induce comparable proliferations of PBMC, LPL, and IEL ensured that differences in cytokine production did not relate to differences in mitogenic ability. The optimal dose of superantigen for induction of proliferation corresponded to the optimal dose for cytokine production (1 ng/ml).

IL-4 and gamma interferon production. Since SEE and TSST-1 appeared to induce more IL-2 than SEB, we next assessed the production of gamma interferon and IL-4 by PBMC, LPL, and IEL. In PBMC, SEE (5.0 ± 1.5 U/ml) and TSST-1 (7.1 ± 2.3 U/ml) induced five- to sevenfold more IL-4 than did SEB (1.1 ± 0.09 U/ml, $P < 0.001$) (Table 1) by 48 h after the stimulation. In contrast to the case with IL-2, differences in the kinetics of induction were seen among the various superantigens. SEE-stimulated IL-4 production peaked at 24 h and TSST-1-induced IL-4 production was maximal at 48 h, while PHA and SEB stimulated little or no IL-4 production. In LPL, SEB was the only superantigen that induced any IL-4 production (2.0 ± 0.01 U/ml), while both TSST-1 and SEE induced IL-4 production from IEL (9.0 ± 1.1 and 2.4 ± 0.4 U/ml, respectively) (Table 1). PHA induced IL-4 production from PBMC (3.5 ± 1.7 U/ml), LPL (1.5 ± 0.05 U/ml), and IEL (1.5 ± 0.05 U/ml). In PBMC, SEE and TSST-1 also induced more gamma interferon than did SEB (Table 1), with maximum secretion at 48 h after the stimulation. The values for SEE (4.1 ± 1.1 ng/ml) and TSST-1 (24.1 ± 0.5 ng/ml) were greater than those for SEB (1.2 ± 0.4 ng/ml, $P < 0.04$) and PHA (1.5 ± 0.3 ng/ml). Gamma interferon production was much greater in TSST-1-stimulated cultures. No gamma interferon production was observed in the IEL at any time point while SEE induced gamma interferon at 100 ± 25 pg/ml in the LPL at 48 h (Table 1). PHA induced no gamma interferon from either LPL or IEL at any time point. Thus, in PBMC, the cytokine profile induced by SEE and TSST-1 included IL-2, gamma interferon, and IL-4, while SEB produced lesser amounts of all three cytokines in PBMC (Table 1). In LPL, there was some gamma interferon production induced by SEE at 48 h, IL-4 was induced by SEE, SEB, and TSST-1 at 48 h, while IL-2 was only induced by TSST-1 at 48 h (Table 1). In IEL, no IL-2 or gamma interferon was induced by any of the superantigens while there was IL-4 production induced by both TSST-1 and SEB at 48 h (Table 1). T-cell-derived cytokine production, with the exception of that of IL-4, was reduced in LPL and IEL by comparison with that in PBMC. It appears that there was no clear pattern for the induction of T-cell-derived cytokines by specific superantigens in PBMC, LPL, and IEL.

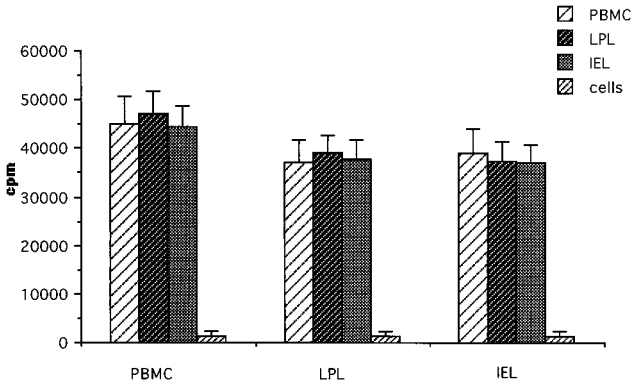

FIG. 1. Proliferation of PBMC, LPL, and IEL in response to superantigen stimulation. PBMC, LPL, and IEL were cultured with medium alone, superantigen (1 ng/ml), or PHA (data not shown) for 48 h. All three superantigens were equally capable of stimulating proliferation of PBMC, LPL, and IEL (pooled data from five separate experiments). "Cells" represents the unstimulated background counts. The error bars represent the standard errors of the means of the five experiments.

IL-6 and TNF production. Since SEB stimulates little in the way of T-cell-derived cytokines in PBMC, LPL, and IEL, we questioned whether the staphylococcal superantigens may preferentially activate monocytes. We therefore measured the production of the predominantly macrophage-derived cytokines, IL-6 and TNF, from the same supernatants analyzed above. In PBMC, SEB stimulated twofold more IL-6 (25 ± 2.3 U/ml) than did SEE (12.1 ± 1.1 U/ml, $P < 0.01$) or TSST-1 (10.1 ± 0.8 U/ml, $P < 0.01$) (Table 1). However, in contrast to the situation with the other cytokines measured, this difference was seen only at 48 h. IL-6 was induced at 24 and 48 h, but the

differences were seen only at 48 h. Since the kinetics of IL-6 production are different for T cells and monocytes (48 and 4 h, respectively) (3), these data might suggest either that the IL-6 is derived from T cells or that induction of IL-6 secretion by monocytes occurs by means of an unmeasured T-cell factor. A monocyte-specific pattern did not hold true when TNF production was assessed, although this result could also be due to T cells, since the L929 bioassay does not distinguish between TNF-α and TNF-β which is produced by T cells. SEE and TSST-1 stimulated more TNF (58.1 ± 23.0 and 57.2 ± 21.8 U/ml, respectively) than did SEB (5.2 ± 0.9 U/ml, $P < 0.001$) (Table 1) at any concentration and at any time point. In findings similar to those with PBMC, SEB induced more IL-6 (3.5 ± 0.7 U/ml) in LPL than did either TSST-1 (0 U/ml) or SEE (1.1 ± 0.1 U/ml) at 48 h. Similar findings with IEL were noted for SEB, which induced more IL-6 (3.5 ± 0.7 U/ml) than either SEE or TSST-1 (2.1 ± 0.03 and 0 U/ml, respectively). With regard to TNF induction in both LPL (SEE, 22.0 ± 3.7 U/ml; SEB, 2.0 ± 0.5 U/ml; and TSST-1, 50.0 ± 10.1 U/ml) and IEL (SEE, 35.0 ± 5.3 U/ml; SEB, 9.7 ± 1.0 U/ml; and TSST-1, 10.1 ± 1.0 U/ml), all the superantigens induced amounts of the cytokine that were nearly comparable to those observed in the PBMC. PHA stimulation of PBMC produced 15.6 ± 1.7 U of IL-6 per ml and 51.5 ± 5.7 U of TNF per ml, while PHA stimulation of LPL resulted in 3.0 ± 0.5 U of IL-6 per ml and 20.1 ± 2.1 U of TNF per ml. PHA stimulation of IEL induced 3.1 ± 0.5 U of IL-6 per ml and 31.3 ± 3.1 U of TNF per ml. In a result similar to the results obtained with the T-cell-derived cytokines, no clear pattern of macrophage-derived cytokine production was observed after SEB stimulation of PBMC, LPL, and IEL (Table 1).

DISCUSSION

The excessive production of cytokines is believed to contribute to the clinical presentation of the diseases associated with the superantigens (36). In this study, PBMC, LPL, and IEL were stimulated with three superantigens, SEE, SEB, and TSST-1, and the cytokine secretion profiles were determined to see if different superantigens stimulate distinct cytokine profiles. Staphylococcal superantigens are known to cause food poisoning, although the pathophysiologic mechanism is unclear. There is some evidence that the emetogenic and mitogenic action of the superantigens may exist on different ends of the protein (4). Acetylation of the superantigen at the C terminus abrogates the emetogenic capacity, but the T-cell stimulatory capacity remains intact (4). Stimulation of nerve fibrils may mediate the nausea and vomiting after staphylococcal enterotoxin ingestion in a process that occurs rapidly.

Preliminary studies performed in our laboratory and the current data demonstrate that the staphylococcal superantigens are mitogenic for LPL and IEL (2). Cytokine production in the LPL and IEL, however, was reduced compared with that in the PBMC (Table 1). IL-4 was produced in the LPL and IEL after they were stimulated with all three superantigens. This is important, since only TSST-1 induced IL-2 production in PBMC but PHA stimulated IL-2 production in LPL but not IEL. Some groups have suggested that LPL are TH-2-like in their cytokine profiles and have demonstrated that IL-2 and IL-4 production is reduced in inflammatory bowel disease LPL after they have been stimulated with mitogens, PHA, and iontomycin (10, 14, 20). LPL have been reported to be unresponsive to antigen stimulation but capable of proliferating to mitogens and anti-CD3 monoclonal antibodies (14). The data demonstrate that IL-2 is produced in LPL after PHA stimulation. Only TNF production was comparable for LPL and PBMC. The reason for the lack of induction of cytokine production in the LPL and IEL is uncertain but may relate to differences in activation requirements. Our data would suggest either that TNF may be a mediator causing the intestinal inflammation associated with food poisoning or that cytokines induced by the superantigens may not play a central role in producing the disease. We speculate that since nausea, vomiting, and diarrhea occur rapidly after ingestion of contaminated food while the induction of TNF occurs maximally 48 h after stimulation, it is unlikely that TNF would be produced early enough in quantities sufficient to cause symptoms.

Differences in cytokine production induced by superantigens have been reported by other groups (18, 25). The mechanism by which superantigens induce cytokine production remains unclear. Since there appear to be differences in the cytokine secretion profiles induced by SEE, SEB, and TSST-1, T-cell and/or monocyte signaling by the three superantigens may be distinct. Less likely is the possibility that cytokine secretions by distinct Vβ subfamilies expressing T cells may be different. Structural differences among superantigens may account for differences in their abilities to stimulate T cells and monocytes. SEE and SEB both contain a disulfide loop which is thought to be critical for mitogenic ability. However, TSST-1 does not contain this loop and yet still can stimulate proliferation of and cytokine secretion in PBMC (19). The interaction between major histocompatibility complex class II molecules and the superantigens appears to involve more than one site (16, 34). SEB and TSST-1 do not compete with each other for binding and instead bind to distinct sites on the HLA-DR molecule (9, 28). However, SEE does compete with both SEB and TSST-1 for class II binding, indicating that there are some common binding sites (29).

From the T-cell side, each of the described superantigens activates distinct TcR Vβ subfamilies. However, there are no data to support the concept that differences in Vβ selection dictate differences in cytokines. Calcium flux and phosphoinositide metabolism have been reported to be absent in T cells stimulated by a superantigen (13, 21, 22). We report similar findings, in that TSST-1, SEE, and SEB were incapable of inducing a calcium flux in T cells (data not shown). Different signal transduction pathways may dictate distinct cytokine secretion profiles, and this may be a reflection of differences in the engagement of the TcR. In line with this possibility, our laboratory has previously demonstrated that stimulating T cells with anti-CD3 monoclonal antibodies directed against distinct chains of the CD3 complex can elicit different cytokine profiles (4).

In conclusion, we report that cytokine production by the LPL and IEL was markedly reduced compared with that by the PBMC, which may reflect significant differences in activation requirements. Our data further suggest that local cytokine production induced by staphylococcal superantigens in the LPL and IEL may not be responsible for the intestinal inflammation seen in staphylococcal food poisoning. Finally, our data support the concept that the cytokine profiles induced by the interaction of T cells and monocytes may depend on the T cell's mode of activation.

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