Interaction of staphylococcal toxic shock syndrome toxin-1 and enterotoxin A on T cell proliferation and TNF secretion in human blood mononuclear cells

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BACKGROUND: The majority of menstrual toxic shock syndrome (MTSS) cases are caused by a single clone of Staphylococcus aureus that produces both toxic shock syndrome toxin-1 (TSST-1) and staphylococcal enterotoxin A (SEA).

OBJECTIVE: To determine whether the two superantigens interact to cause an enhancement of biological activity in human peripheral blood mononuclear cells (PBMCs).

DESIGN: PBMCs from nine healthy donors were stimulated with TSST-1 or SEA, either alone or in combination at their minimum effective concentrations.

SETTING: In vitro study.

INTERVENTIONS: Human PBMCs were stimulated in vitro with TSST-1 (1 pg/mL), SEA (0.1 pg/mL) or combination for 20 to 72 h. Mitogenic response was determined by [3H]-thymidine incorporation. PBMC culture supernatants were assayed for the presence of tumour necrosis factor-alpha (TNFα), interleukin (IL)-1 and IL-6 by ELISA.

MAIN RESULTS: The combination of TSST-1 and SEA induced significantly greater mitogenesis in human PBMCs compared with either toxin alone (P<0.05, paired Student’s t test, two-tailed). Similarly, the production of TNFα in culture supernatants was significantly greater in the combination of TSST-1 and SEA compared with either TSST-1 or SEA alone (P<0.05). In contrast, no enhancement in the levels IL-1 or IL-6 was observed.

CONCLUSIONS: These data suggest that the co-production of TSST-1 and SEA by S aureus may provide some biological advantage to the organism through an enhanced effect of these superantigens on T cell activation and TNF secretion.

Key Words: Cytokines; Mitogenic response; Staphylococcal enterotoxins; Toxic shock syndrome toxin-1

Pour le résumé, voir page suivante
Interaction de la toxine-1 et de l’entérotoxine A staphylocoques du syndrome de choc toxique sur la prolifération des cellules T et sur la sécrétion du TNFα dans les cellules mononucléaires du sang humain

CONCLUSIONS : Ces données permettent de croire que la coproduction de la TSST-1 et de la SEA par les CMSP humains augmente les niveaux d’IL 1 ou d’IL 6. Par contre, on n’a pas observé une augmentation des niveaux d’IL 1 ou d’IL 6.

MATERIALS AND METHODS

Purification of TSST-1 and SEA: TSST-1 was purified from culture supernatants of S. aureus MN8 by preparative isoelectric focusing and chromatofocusing as previously described (17). SEA was purchased from Toxin Technology (Sarasota, Florida) and further purified by chromatofocusing using a pH 6 to 8 gradient polybuffer exchanger (PBE 94; Pharmacia Fine Chemicals, Uppsala, Sweden). The purity of TSST-1 and SEA preparations was verified by the presence of a single band of approximately 22 kD and approximately 28 kD, respectively, following SDS-PAGE or immunoblotting with polyclonal antisera (17). SEA was purchased from Toxin Technology (Sarasota, Florida) and further purified by chromatofocusing using a pH 6 to 8 gradient polybuffer exchanger (PBE 94; Pharmacia Fine Chemicals, Uppsala, Sweden). The purity of TSST-1 and SEA preparations was verified by the presence of a single band of approximately 22 kD and approximately 28 kD, respectively, following SDS-PAGE or immunoblotting with polyclonal antisera (17).
Mitogenicity assay: Mitogenesis of human PBMCs stimulated by TSST-1 or SEA was assessed by [3H]-thymidine incorporation as previously described (17). A 1:1 ratio of T cells to monocytes (3×10⁵ cells/well) in supplemented RPMI 1640 were cultured in 0.2 mL volumes with various concentrations of TSST-1, SEA, or combination for three days at 37°C, 5% carbon dioxide in 96-well round-bottom plates (Falcon Labware, Becton-Dickinson Canada Inc, Mississauga, Ontario). Cells were pulsed with 1 Ci of [3H]-thymidine (6.7 Ci/mmol; ICN Flow Laboratories, Irvine, California) 18 h before completion of incubation, and harvested onto glass-fibre filter paper with an automatic harvester (Skatron, Sterling, Virginia). Samples were counted in a liquid scintillation counter (LS1800, Beckman, Mississauga, Ontario).

Cytokine assays for tumour necrosis factor-alpha, interleukin-1β and interleukin-6: A 1:1 ratio of T cells to monocytes (2×10⁶ cells/well) in supplemented RPMI 1640 were cultured in 1.0 mL volumes in conjunction with various concentrations of TSST-1, SEA, or combination at 37°C and 5% carbon dioxide in 24-well culture plates (Becton-Dickinson). After approximately 20 h, supernatants were harvested by centrifugation at 800 g for 5 mins, and stored at −70°C until analysis. Tumour necrosis factor-alpha (TNF α), interleukin (IL)-1β, and IL-6 present in the culture supernatants were assayed by ELISA previously developed in the authors’ laboratory (17). The sensitivity limits for detection were 250 pg/mL for TNF α, 500 pg/mL for IL-1β, and 625 pg/mL for IL-6.

Statistical analyses: All quantitative data were expressed as means ± standard error of the mean (SEM). Differences in mitogenic responses and cytokine secretion between different groups were compared using the Student’s t test for paired samples (two-tailed). P<0.05 was considered significant.

RESULTS

Minimum effective dose of TSST-1 and SEA for inducing mitogenesis in human PBMCs: PBMCs (1:1 ratio of T cells to monocytes) from one healthy donor were stimulated with various concentrations of TSST-1 and SEA (0.001 pg/mL to 100 μg/mL) to generate dose-response curves for mitogenesis (Figure 1). This was performed to select a minimum effective concentration of TSST-1 and SEA for the interaction studies. The concentration range of the dose response to TSST-1 and SEA observed in this donor was similar to that observed by other investigators (17-20), although the amplitude of the proliferative responses varied from one subject to another (18). Based on these dose-response curves, 1 pg/mL of TSST-1 and 0.1 pg/mL of SEA, respectively, were chosen for further study of the mitogenic responses in human PBMC. Two higher doses of TSST-1 (10 pg/mL and 100 pg/mL) were also tested in some cases.
Interaction of TSST-1 and SEA on mitogenesis of human PBMC: The mitogenic response of human PBMCs to the combination of TSST-1 at 1.0 pg/mL and SEA at 0.1 pg/mL from nine healthy donors was significantly greater than either toxin alone (P<0.05, paired Student’s t test, two-tailed) (Figure 2). In addition, PBMCs from three donors simulated with TSST-1 at 10 pg/mL and at 100 pg/mL, each in combination with 0.1 pg/mL SEA, also exhibited significantly greater T cell mitogenesis than either toxin alone (data not shown). The increased mitogenic effect appeared to be additive rather than synergistic, because the mean proliferative response observed upon co-incubation with TSST-1 and SEA did not significantly exceed the sum of the proliferative response with either toxin alone.

Minimum effective dose of TSST-1 and SEA on cytokine secretion from human PBMCs: The dose responses of human PBMCs to various concentrations of TSST-1 and SEA (ranging from 0.001 to 10 pg/mL) in the induction of TNF-α, IL-1β, and IL-6 were studied in one healthy donor. Although dose-dependent effects of TSST-1- and SEA-induced TNF-α and IL-6 production were observed with this donor (data not shown), IL-1β was detected only at the highest concentration of TSST-1 and SEA tested (10 pg/mL). In light of this, and the inherent variability in cytokine induction from different donors (21,22), the minimum effective dose of TSST-1 and SEA, chosen arbitrarily for further study of cytokine secretion in human PBMCs, were 10 pg/mL and 1.0 or 10 pg/mL, respectively.

Interaction of TSST-1 and SEA on TNF-α, IL-1β and IL-6 secretion in human PBMCs: PBMCs (1:1 ratio of T cells to monocytes) of eight healthy donors were stimulated with TSST-1 (10 pg/mL) and SEA (1 pg/mL), either alone or in combination. Considerable intersubject variability in cytokine responses was observed (Table 1). A significant increase in TNF-α was observed in the culture supernatants harvested from five donors stimulated with the combination of TSST-1 and SEA (1.65 ± 0.27 ng/mL) compared with either TSST-1 alone (0.85 ± 0.22 ng/mL, P<0.05, paired t test, two-tailed), or SEA alone (0.56 ± 0.35 ng/mL, P<0.05) (Figure 3A). In contrast with TNF-α, the induction of IL-1β from eight donors stimulated with the combination (5.34 ± 2.4 ng/mL) was not significantly greater than either toxin alone (P<0.05, paired Student’s t test, two-tailed) (Figure 3B). The induction of IL-6 was significantly greater in seven of eight donors stimulated with the combination (23.71 ± 11.50 pg/mL) compared with either toxin alone (P<0.05, paired Student’s t test, two-tailed) (Figure 3C).

<table>
<thead>
<tr>
<th>Donor</th>
<th>TSST-1 (10 pg/mL)</th>
<th>SEA (1 pg/mL)</th>
<th>TNF-α (pg/mL)</th>
<th>IL-1 (pg/mL)</th>
<th>IL-6 (pg/mL)</th>
</tr>
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<tr>
<td>1</td>
<td>1149 ± 45</td>
<td>1539 ± 63</td>
<td>11,550 ± 750</td>
<td>14,250 ± 210</td>
<td>1085 ± 5</td>
</tr>
<tr>
<td>2</td>
<td>914 ± 5</td>
<td>UD</td>
<td>18,000 ± 1380</td>
<td>UD</td>
<td>666 ± 81</td>
</tr>
<tr>
<td>3</td>
<td>1010 ± 32</td>
<td>UD</td>
<td>UD</td>
<td>UD</td>
<td>2601 ± 201</td>
</tr>
<tr>
<td>4</td>
<td>UD</td>
<td>UD</td>
<td>UD</td>
<td>UD</td>
<td>UD</td>
</tr>
<tr>
<td>5</td>
<td>1178 ± 32</td>
<td>1266 ± 72</td>
<td>UD</td>
<td>UD</td>
<td>UD</td>
</tr>
<tr>
<td>6</td>
<td>NT</td>
<td>NT</td>
<td>936 ± 64</td>
<td>603 ± 2</td>
<td>673 ± 7</td>
</tr>
<tr>
<td>7</td>
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<td>NT</td>
<td>14,130 ± 450</td>
<td>3725 ± 5</td>
<td>2993 ± 5</td>
</tr>
<tr>
<td>8</td>
<td>NT</td>
<td>NT</td>
<td>1210 ± 175</td>
<td>487 ± 2</td>
<td>1270 ± 20</td>
</tr>
</tbody>
</table>

Human PBMCs from eight donors were stimulated with TSST-1 (10 pg/mL) and SEA (1 pg/mL) for approximately 20 h. TNF-α, IL-1β and IL-6 in the culture supernatants were measured by ELISA. Sensitivity limits of detection were 250, 500, and 625 pg/mL, respectively. Results for each donor are expressed as the mean ± SEM cytokine concentration (pg/mL) in duplicate determinations. NT Not tested; UD Undetectable.

**Figure 3** Induction of tumour necrosis factor-alpha (TNF-α), interleukin (IL)-1 and IL-6 in culture supernatants of human peripheral blood mononuclear cells (PBMCs) from healthy donors following stimulation with TSST-1 and SEA.
greater than with TSST-1 alone (5.77 2.8 ng/mL) or SEA alone
(3.65 2.0 ng/mL) (Figure 3B). Increasing the concentration of
SEA to 10 pg/mL did not alter the results (data not shown).
Similarly, the induction of IL-6 from eight donors stimulated
with the combination (1.19 0.43 ng/mL) was not significantly
greater than with TSST-1 alone (0.99 0.41 ng/mL), although a
significant increase was observed in comparison with SEA alone
(0.53 0.28 ng/mL; P<0.05) (Figure 3C). Again, increasing the
concentration of SEA to 10 pg/mL did not change the re-
sults (data not shown).

DISCUSSION
The objective of our study was to determine if the combina-
tion of minimum effective concentrations of TSST-1 and SEA
would induce greater T cell proliferation and cytokine secre-
tion from human PBMCs with compared either toxin alone.
This might be suspected because TSST-1 and SEA have similar
biological activities, and both bind to MHC class II molecules
on human PBMCs (23,24), an event critical for both T cell mi-
togenesis and cytokine secretion (11,25-27). Furthermore, be-
cause TSST-1 and SEA share a common MHC class II receptor
in human PBMCs (28-30), a greater response in mitogenesis or
cytokine secretion, at minimum effective concentrations,
could be expected from the combination of these two toxins
rather than with either toxin alone. Our finding that the co-
incubation of SEA with TSST-1 induced significantly greater
T cell proliferation and TNF secretion supports this notion.
The enhanced effects of these toxins in combination are rele-
vant because TSS-associated S aureus frequently co-produce
both TSST-1 and SEA (7,31,32) and because these two bio-
logical activities are believed to be pivotal to the pathogenesis of
TSS (15,16). However, one must be cautious in extrapolating
these in vitro results to the clinical setting because the precise
amount of TSST-1 and SEA produced in vivo by S aureus dur-
ing TSS is not known.

In contrast with TNF induction, we were unable to dem-
onstrate an enhancement in either IL-1ß or IL-6 production by
the toxin combination compared with both TSST-1 and SEA
alone. There are a number of possible explanations for this ap-
narent disparity. First, it is likely that suboptimal concen-
trations of TSST-1 and SEA were used for some of the donors
studied. Ideally, a dose-response curve for IL-1ß and IL-6 in-
duction should be determined for each PBMC donor, so that
the minimum effective dose of TSST-1 and SEA could be more
precisely defined for each individual donor. Secondly, the se-
cretion of IL-1ß and IL-6 in PBMCs is known to appear later
than TNF after superantigen stimulation (16). Because cyto-
kine secretion in our study was determined at approximately
20 h after stimulation, there may not have been sufficient time
for IL-1ß and IL-6 synthesis and secretion to occur in the
PBMCs of some donors in contrast with TNF. In support of this,
IL-1ß was below the limits of detection in four of eight cul-
ture supernatants, while IL-6 was undetectable in three of
eight donors (Table 1). However, because these cytokines ap-
pear to be differentially regulated via distinct signal transduc-
tion pathways (33-35), it would not be totally surprising if
this apparent disparity in cytokine secretion between TNF
and the other cytokines was still observed when tested under
more optimal conditions. Whether the different cytokine acti-
vation pathways induced by these toxins are related to the
unique MHC Class II-binding sites of these toxins (36-38) is
not known.

Considerable intersubject variability in cytokine secretion
patterns was observed in our healthy donors following su-
perantigen stimulation, as has been documented by others
(21,22,39). For example, among the eight donors examined
for cytokine secretion in the current study, the amount of
IL-1ß induced with 10 pg/mL of TSST-1 ranged from less than
500 to 18,000 pg/mL, whereas IL-6 levels ranged from less
than 625 to 2955 pg/mL (Table 1). A similar finding was ob-
served following stimulation with 1 pg/mL of SEA. Donor 1
produced high amounts of all three cytokines in response to
both TSST-1 and SEA, whereas donor 4 did not produce any
detectable cytokine in response to either TSST-1 or SEA. In
general, donor PBMCs that did not respond to TSST-1 (eg,
donors 4, 5 and 6) also did not respond to SEA in the secretion
of identical cytokines. Several factors, including isotypic and
allic polymorphism of MHC Class II in different subjects, could
account for the variations observed in the secretion of dif-
ferent cytokines by the same donor or the same cytokine by
different donors. First, different superantigens preferentially
bind to distinct MHC class II isotypes (40,41) and alleles
(36,41), thereby possibly leading to variability in the induc-

tion of different cytokines in the same individual. In this re-
gard, the activation of certain T cell subsets by SEA was
found to have an influence on the type of cytokine released
from human monocytes (42,43). The finding that human
TNF genes are linked to the MHC gene cluster on chromo-
some 6 (44) also supports the concept that genetic factors
may regulate the cytokine response, at least for TNF. Ge-
etic factors may also provide an explanation for the obser-
vation that some individuals appear to be consistently high
or low cytokine producers (21). Variation in the Vß determi-
nant for the TCR repertoire among different individuals may
also account for some intersubject variability in cytokine re-
sponses to different superantigens (10).

CONCLUSIONS
The combination of TSST-1 and SEA induces a significantly
greater mitogenic response and TNF secretion in human
PBMCs compared with stimulation with either toxin alone.
Whether these in vitro observations also occur in vivo and
whether the coproduction of TSST-1 and SEA in TSS-
associated S aureus renders this strain more virulent than an-
other that produces only TSST-1 or SEA clearly warrant fur-
ther investigation.

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**CLINICAL VIGNETTE**

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**DIAGNOSIS**

Sinus X-rays were performed (single view in Figure 2), which showed complete opacification of both maxillary sinuses, air fluid levels in both frontal sinuses, opacification of the ethmoid sinuses and a questionable air fluid level in the sphenoid sinus. A computed tomography scan of the sinuses and orbits with infusion showed the soft tissue swelling of the face without extension into the orbits. The nasal cavity was completely occluded, with uptake of radiocontrast into the nasal, ethmoid and maxillary mucosa. The computed tomographic scan also showed complete occlusion of the maxillary sinuses by hypodense material, and partial occlusion of the sphenoid and frontal sinuses as well. Air fluid levels were again seen in the frontal sinuses. A diagnosis of facial erysipelas secondary to pansinusitis was made.

The patient was started on intravenous meropenem (Merrem, AstraZeneca, Mississauga, Ontario) with gradual improvement in the fever and facial swelling over the next four days. No sinus lavage was felt to be indicated, due to the adequate response to antibiotics alone. Blood cultures were negative, and he was changed to oral amoxicillin/clavulanic acid (Clavulin, SmithKline Beecham, Oakville, Ontario) and discharged home on day 5. A follow-up visit with the otolaryngologists was arranged.

**DISCUSSION**

Facial erysipelas used to be the most common form of erysipelas, now much more common in the lower extremity. It is almost always caused by *Streptococcus pyogenes*, but may be produced by beta-hemolytic streptococci of other types (ie, groups C or G), as well as other bacteria in more rare circumstances. Preceding skin trauma or portals of entry are common, but facial erysipelas may also be caused by extension of orofacial foci such as odontogenic infections. Sinus infections as a source of facial erysipelas, without orbital involvement, are quite rare. The exact microbial etiology of facial erysipelas when caused by an underlying sinusitis is unknown, but may be polymicrobial in nature. Cultures of erysipelas are infrequently positive, even if performed by ‘leading edge aspiration’ of the rash. Bacteremia occurs less than 5% of the time. Therapy usually consists of antistreptococcal antibiotics (unless the condition is suspected to be caused by a polymicrobial infection) and local wound care. Blistering and desquamation are common during the healing process.

The two unusual aspects of this patient’s presentation are the sinus infection as the likely source of the erysipelas, and the lack of facial pain or pressure in the presence of such an extensive pansinusitis. When encountering a facial erysipelas without evidence of a portal of entry, a search for an underlying infectious source is mandatory. An examination of the teeth and sinuses are necessary. If no source is still evident, sinus x-rays should be completed in all such patients, regardless of symptoms.

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