Toxic Shock Syndrome Toxin–1 Challenges the Neuroprotective Functions of the Choroidal Epithelium and Induces Neurotoxicity

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To probe encephalopathy pathogenesis during toxic shock syndrome (TSS), we investigated the fate of blood-borne TSS toxin–1 (TSST-1) as it moves through the choroid plexus epithelium that forms the main blood–cerebrospinal fluid (CSF) barrier and the effect that TSST-1 has on choroidal barrier properties and on cultured neuronal cell viability. TSST-1 showed a slow, diffusional movement across a cellular model of the blood-CSF barrier but did not compromise the integrity of the barrier. Relevant to the acute symptoms of TSS, a combination of human leukocytes and the toxin induced a decrease in CSF clearance of the pyrogenic prostaglandin E2 (PGE2). The direct effects that TSST-1 had on primary cortical neuron cultures and a neuronal cell line involved elevated caspase 3/7 levels, which correlated with an increase in neuronal cell death. The results of the present study suggest that TSST-1 can affect the brain, by inducing both an intracerebral increase in PGE2 concentration and caspase-dependent neuronal death, which are possibly relevant to long-term intoxication.

Toxic shock syndrome (TSS) is a well-described illness induced by Staphylococcus aureus, characterized by fever, rash followed by desquamation, hypotension, and multiple organ failure [1]. It involves TSS toxin–1 (TSST-1), as well as, in some cases, staphylococcal enterotoxins [2–5]. All these toxins exhibit superantigen activity, which induces the production of antigen-presenting and T cell–derived cytokines, such as tumor necrosis factor (TNF)–α, which are believed to cause the manifestations of TSS [4]. Experiments have shown that superantigens act synergistically with lipopolysaccharides (LPSs) of gram-negative bacteria [6, 7]. LPS has been detected in patients with TSS, and it has thus been suggested that it participates in the pathogenesis of TSS [6], although this remains controversial.

In clinical settings, central nervous system (CNS) effects of TSS are prominent. Tofte and Williams found that, in the acute phase, toxic encephalopathy is characterized by confusion, disorientation, agitation, or somnolence without focal neurological signs and that dizziness and headache are among the primary symptoms [8]. These symptoms are usually reversible, but persistent neuropsychological impairments have been observed in several patients with a history of TSS [9]. The pathogenesis of TSS-related neurological symptoms and their aftermath are still unknown. The symptoms may result from a direct effect that staphylococcal toxin has on the CNS, subsequent to its putative cerebral penetration.

Exchanges between the brain and the external environment are strictly regulated by the blood-brain barriers, which comprise the choroid plexuses (CPs), the cerebral microvessels, and the arachnoid membrane [10]. CPs form the main barrier between the blood and the cerebrospinal fluid (CSF). They are composed of a...
simple, tight epithelium, bearing the effective barrier function, and protrude in the ventricles of the brain, while delimiting a conjunctive stroma that contains large, fenestrated blood vessels [11]. CPs are potential gates of entry into the CNS for bloodborne bacteria and toxins [12, 13]. Furthermore, the notion that they play a role in the control of neuroinflammatory events has been suggested by the rapid and strong induction of proinflammatory mediators, such as cytokines, occurring in the context of neuroinfection [11, 14]. The notion that CPs play a role is also supported by the involvement of the choroidal epithelium in the active cerebral elimination of inflammatory modulators such as eicosanoids, the pyrogenic prostaglandin E 

In the present study, we used a polarized in vitro blood-CSF barrier model that reproduces the specific properties of the barrier in vivo [15, 17, 18], as well as primary cortical neuron cultures and a neuronal cell line, to investigate the cerebral penetration of TSST-1, the effects that it has on the blood-CSF barrier properties, and its neurotoxicity.

MATERIALS AND METHODS

Toxins and chemicals. TSST-1 was obtained from Toxin Technology. Salmonella typhimurium LPSs and Phaseolus vulgaris agglutinin were obtained from Sigma. The biological activity of purified TSST-1 was checked by measuring its ability to induce dose-dependent (from 5 ng/mL to 10 μg/mL) CD69 expression on human leukocytes from different donors [19]. [14C]-sucrose (350 mCi/mmol) and [14C]-polyethylene glycol (PEG) 4000 (0.5 mCi/g) were obtained from Amersham Biosciences, and [3H]-PGE₂ (200 Ci/mmol) was obtained from PerkinElmer Life Sciences.

Cell isolation and cell culture. Animal care and procedures were conducted in accordance with the guidelines approved by the French Ethical Committee and by the European Community. OFA rats were obtained from Harlan. Choroidal epithelial cells from lateral ventricle CPs were prepared and cultured on permeable Transwell polycarbonate inserts (6.5-mm diameter, 0.4-μm pore size) (Costar-Plastics), coated with laminin as described in detail elsewhere [17]. Laminin-coated filters without cells were kept in the same conditions and used in transfer experiments to estimate flux across the filter alone. Experiments were performed 7 days after plating.

Peripheral blood was collected from adult volunteers by venipuncture, using lithium heparinate (Becton Dickinson) as anticoagulant. Erythrocytes were removed by hypotonic lysis with 0.9% NH₄Cl for 15 min. The cells were washed 3 times and resuspended in Dulbecco’s modified Eagle medium/Ham’s Nutrient mixture F12 (1:1) (Invitrogen).

Fresh medium was added to epithelial cells the day before starting the experiment (figure 1A). In a first set of experiments, epithelial cells were exposed to TSST-1 to investigate the movement of this toxin across the cell monolayer. TSST-1 was added to the basolateral or apical compartment at various concentrations for 24 or 48 h. At the end of the incubation period, medium was recovered from both chambers and kept frozen until being processed for TSST-1 quantification. Cell-covered filters were recovered to assess the paracellular permeability of the choroidal monolayers.

In a second set of experiments, epithelial cells were co-cultured with freshly isolated human leukocytes. These cells were added to the basolateral compartment to a final concentration of 2 × 10⁶ cells/mL. This conformation impeded direct contact between both types of cells and enabled only soluble factors to diffuse through the microporous filters. TSST-1 and, in some experiments, LPSs were added to the basolateral com-

Figure 1. Flux of toxic shock syndrome toxin–1 (TSST-1) across an in vitro model of the blood-cerebrospinal fluid barrier. A, Schematic representation of the blood-cerebrospinal fluid barrier model. At confluence, the epithelial choroidal cells cultured on a porous filter form a barrier delimiting an apical compartment (Ap), representing the cerebrospinal fluid, and a basolateral compartment (Bl), corresponding to the stroma/blood space. B, Bidirectional transfer of TSST-1 across the choroidal epithelium. Choroidal epithelial monolayers were exposed to TSST-1 (275 ng/mL) for 24 h and added to either the apical or the basolateral chamber. Aliquots of the acceptor (opposite) chamber were sampled at 10 and 24 h and analyzed for TSST-1 content. Values represent mean ± SD (n = 3). At either time of analysis, the amounts of TSST-1 that crossed the choroidal epithelium in each direction were not statistically different. The paracellular permeability to sucrose was determined at the end of the incubation period and was similar in both conditions of TSST-1 exposure (data not shown).
partment. Filters with unchallenged leukocytes were used as controls, with the same blood cell suspension. After 24 or 48 h of coincubation, medium was sampled from both compartments for TSST-1 analysis, and cell-covered filters were recovered for permeability studies. When present, human leukocytes were removed by centrifugation before sample collection.

Rat primary cortical cultures were prepared as described elsewhere [20]. Cultures were maintained for 8 days before experimentation and were exposed to various concentrations of TSST-1 for either 1 or 6 days. Human neuroblastoma SH-SY5Y cells (European Collection of Cell Cultures) (passages 14–20) were trypsinized and washed, and 5 × 10⁴ cells/well were plated into 96-well plates (Falcon) and maintained for 24 h before experimentation. Cultures were exposed to various concentrations of TSST-1 for 24 h. Assays monitoring neurotoxicity (MTT assay) or apoptosis (caspase 3/7 assays) were performed immediately after TSST-1 exposure.

**Analysis of TSST-1 flux and modelization of expected CSF concentrations.** TSST-1 concentration was determined in samples by use of TSST-1–specific ELISAs (Toxin Technology), with minor modifications [21] as recommended by the manufacturer. The movement of TSST-1 across the choroidal monolayer was expressed either as the amount of molecule recovered from the acceptor chamber (in nanograms per unit of surface area of cell monolayer) or as clearance volume (in microliters per unit of surface area of cell monolayer). The latter was calculated by dividing the amount of TSST-1 found in the acceptor chamber at the end of the incubation by the toxin concentration present in the donor chamber.

Considering a rate of CSF production (equal to the rate of CSF clearance, QCSF) of 120 µL/h in the rat [22] and assuming a constant concentration of TSST-1 in the blood GCblood—which is a reasonable assumption, given the time frame needed for TSST-1 concentration to build up in the CSF, as calculated below (equation [4])—the maximal concentration CCSF of TSST-1 that will be reached in the CSF is

\[
C_{\text{CSF}} = \frac{I}{Q_{\text{CSF}}}. \tag{1}
\]

The variable I represents the rate of influx of TSST-1 and can be defined as

\[
I = C_{\text{blood}} \times CR_{\text{TSST-1}} \times S. \tag{2}
\]

\(CR_{\text{TSST-1}}\) is the clearance rate measured across the choroidal epithelium for TSST-1 (µL/h · cm²), and \(S\) is the surface area developed by the choroidal cell monolayer. In the rat, we assigned this surface an area of 2.8 cm² (not taking into account the development of the apical and basolateral membranes; N. Strazielle and J. F. Ghersi-Egea, unpublished data); therefore, from equations (1) and (2),

\[
C_{\text{CSF}} = 23 \times 10^{-5} C_{\text{blood}} \times CR_{\text{TSST-1}}. \tag{3}
\]

The time \((t_{1/2})\) by which half of the maximal concentration is reached in the CSF (volume \([V]\) of 200 µL in rat) is equal to the time required to renew half of the CSF. This time, calculated as

\[
0.5 = e^{-Q_{\text{CSF}}x_{\text{CSF}}/V}, \tag{4}
\]

is 70 min.

**Permeability studies for sucrose, PEG-4000, and PGE, and calculation of flux.** Apical-to-basolateral (CSF-to-blood) transfer measurements were performed in Ringer-Hepes over a 1-h period as described elsewhere in detail [18]. Sets of at least 3 filters were studied for each experimental condition. The paracellular diffusion pathway was assessed on each individual filter by measuring the flux of \([1^4C]\)-sucrose or \([1^4C]\)-PEG-4000 (tracer concentration, 0.2 nCi/ml). Laminin-coated filters without cells were run in parallel, in similar conditions. In some experiments, the apical-to-basolateral flux of \([3^H]\)-PGE \(_2\) (radiolabeled tracer supplemented with unlabeled PGE \(_2\) (Sigma) to a final concentration of 100 nmol/L in the donor compartment) was assayed simultaneously to that of sucrose. Aliquots from the donor and acceptor media were counted by liquid scintillation, by use of Ultima Gold scintillation liquid (Packard) in a beta counter (Packard Tri-Carb TR1600-LS analyzer).

The flux of radiolabeled compounds across the monolayer was estimated as the amount cleared from the donor compartment. The rate of active clearance of PGE \(_2\) across the cell-filter system \((P_e)\), and the passive permeability coefficient of the cell monolayer alone \((P_i)\) for sucrose and PEG-4000, both in cm/min, were calculated as described in detail elsewhere [15, 23].

**Neurotoxicity assays.** Cell death was assessed using the MTT cytoxicity assay, as described elsewhere [20]. Absorbance at 570 nm was recorded using an Anths 2020 plate reader. Positive controls were cultures treated with 0.3% hydrogen peroxide for 30 min. Cell viability data are expressed as the percentage of the mean value obtained for untreated cells.

**Caspase 3/7 assays.** Apoptotic induction was assessed by measuring the activity of caspase 3/7, by use of the Apo-ONE Homogeneous Caspase-3/7 Assay (Promega), in accordance with the manufacturer’s instructions. Fluorescence was recorded using a Fluoroskan Ascent fluorescence plate reader (Labsystems). In some experiments, a 10 µmol/L concentration of the pan caspase inhibitor z-VAD-fmk (Bachem) was coincubated with TSST-1. Blanks consisted of medium with Apo-ONE Caspase reagent alone (without cells); caspase 3/7 activity was determined as the net fluorescence (test value minus blank value). Data are ex-
pressed as a percentage of the mean value obtained for untreated controls.

RESULTS

Passage of TSST-1 and sucrose across choroidal epithelial cell monolayers. In the first set of experiments, the transcellular flux of TSST-1 per se was measured. For 3 increasing doses of TSST-1 (36 ng/mL to 5.7 μg/mL), the basolateral-to-apical clearance measured during a 24-h period did not vary significantly (table 1), indicating that TSST-1 transfer is not saturable in this range of concentration. Similarly, the permeability of cells to [14C]-sucrose measured at the end of the TSST-1-exposure period was not statistically different from that of control cells (without TSST-1), demonstrating the maintenance of the barrier integrity. Analyzing the rate of TSST-1 transfer in both apical-to-basolateral and basolateral-to-apical directions indicated that the movement of the toxin across the choroidal epithelium monolayer was not polarized (figure 1B).

Because it has been proposed that TSST-1–exposed leukocytes, as well as bacterial LPS, may exacerbate the effects of TSST-1, we then tested whether such an association could increase the flux of the toxin across the choroidal epithelium. Neither 24 h nor 48 h of incubation with leukocytes and/or LPS modified the basolateral-to-apical clearance rate of TSST-1 across the choroidal epithelial cells (figure 2A). With regard to the barrier integrity, only the coinoculation of all 3 factors—TSST-1, LPS, and leukocytes—induced a statistically significant increase in sucrose P_e, which was, however, very modest (less than 2-fold), by comparison to the 30-fold increase observed on dissociation of tight-junction proteins (via Ca^{2+} deprivation) (figure 2B). Furthermore, this alteration in the paracellular pathway was not detected when using PEG-4000, another paracellular marker of higher molecular weight. P_e values of 2.9 × 10^{-3} ± 1.0 × 10^{-7} cm/min and 3.6 × 10^{-3} ± 0.1 × 10^{-3} cm/

![Figure 2](image)

**Figure 2.** Basolateral-to-apical clearance of toxic shock syndrome toxin–1 (TSST-1) across the choroidal epithelium exposed to leukocytes and lipopolysaccharides (LPS). The basolateral side of choroidal epithelial monolayers was exposed to TSST-1 (mean concentration, 385 ng/mL), alone or in the presence of 2 × 10^{8} leukocytes/mL with or without 1 μg/mL LPS, for either 24 or 48 h. A, Clearance values, determined for TSST-1 over the total period and expressed as percentage values of the clearance measured on control filters (i.e., in the absence of leukocytes and LPS) from the same cell preparation. B, Paracellular permeability to sucrose, determined at the end of the coinoculation period, and permeability coefficients (P_e) expressed as percentage values of control cells. For comparative purposes, the effect that a complete tight-junction dissociation after calcium removal has on paracellular permeability is illustrated by the hatched bar. Values are mean ± SD (n = 3–10 different filters from 1 or 2 cell preparations). *P < .01, vs. control (1-way analysis of variance, followed by “a posteriori” Dunnett’s test).

### Table 1. Basolateral-to-apical flux of toxic shock syndrome toxin–1 (TSST-1) across the choroidal epithelium.

<table>
<thead>
<tr>
<th>TSST-1 concentration (ng/mL)</th>
<th>Basolateral-to-apical clearance, μL/cm² × 10^{-3} cm/min</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td>5.6 ± 0.2</td>
</tr>
<tr>
<td>36</td>
<td>3.4 ± 0.2</td>
</tr>
<tr>
<td>520</td>
<td>3.3 ± 1.5</td>
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<tr>
<td>5.7</td>
<td>3.4 ± 0.4</td>
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**NOTE.** Basolateral-to-apical flux of TSST-1 across the choroidal epithelium is dose independent and does not affect the paracellular permeability. Choroidal epithelial cells were exposed to various concentrations of TSST-1 in the basolateral compartment for 24 h. The flux of TSST-1 was measured at the end of this period, and the paracellular pathway was then evaluated by measuring the clearance of sucrose over a 1-h transfer experiment. Values are mean ± SD (n = 3). P_e, passive permeability coefficient of the cell monolayer alone.
Figure 3. Inhibition of apical-to-basolateral transport of pyrogenic prostaglandin E₂ (PGE₂) across the choroidal epithelium by toxic shock syndrome toxin–1 (TSST-1)–activated leukocytes. The basolateral side of choroidal epithelial monolayers was exposed to TSST-1 (500 ng/mL), 2 × 10⁴ leukocytes/mL, or both for 48 h. A, Apical-to-basolateral transport of PGE₂ (100 nmol/L), determined and expressed as percentage values of the permeability measured on control cell–covered filters (Pₜ) in the absence of leukocytes and TSST, at the end of the incubation period. PGE₂ transport by the choroidal epithelium is a saturable process for which the nonsaturable component (represented by the solid area of bars) was determined on similar control filters using the saturating 250 μmol/L concentration of PGE₂ [15]. B, Paracellular permeability to sucrose, determined simultaneously in the apical-to-basolateral direction, and permeability coefficients (Pₑ), expressed as percentage values of control cells without leukocytes and TSST-1. Values are mean ± SD (n = 4–12 different filters from 1–3 cell preparations). *P < .01, vs. control (1-way analysis of variance, followed by “a posteriori” Dunnett’s test).

Decreasing the concentration of TSST-1 to which leukocytes were exposed in the basolateral compartment still resulted in an inhibition of the transporter-mediated flux of PGE₂ (figure 4). A significant decrease of 30% in the saturable transport component was achieved in the presence of 5 ng/mL TSST-1, indicating that low concentrations of toxin in blood can affect the clearance of PGE₂ from CSF and brain. Leukocytes exposed to a combination of both TSST-1 and LPS (100 pg/mL) produced an inhibition in PGE₂ choroidal active efflux that was higher than the leukocyte-mediated effect induced in the presence of LPS and TSST-1 alone (data not shown).

Effect of TSST-1 on caspase 3/7–dependent neurotoxicity. To investigate whether the neuropathological aspects of TSS involve a direct effect that TSST-1 has on neuronal cells, we studied the effect that this toxin has on cell death in rat primary cortical neuron cultures and on the SHSY5Y cell line, using assays monitoring both neuronal viability and induction of apoptosis via the activation of effector caspase 3/7. Long-term (6 days) exposure to TSST-1 caused a reduction in primary cell viability that was detectable at a toxin concentration of 450 ng/mL (cell viability, 76.5% ± 5.1%) and that became highly significant at 1 μg/mL of toxin (cell viability, 51.5% ± 3.4% of the control value) (figure 5A). Concurrent with this toxic effect, the level of effector caspase 3/7 showed an increase at TSST-1 concentrations of ≥45 ng/mL (figure 5B). No significant change in control cells to 0.88 × 10⁻³ ± 0.11 × 10⁻³ cm/min in the presence of toxin and leukocytes can be estimated to represent a 55% ± 7% decrease in the saturable—that is, transporter-mediated—component of PGE₂ flux. This decrease occurred despite the slight, though statistically significant, increase in the paracellular permeability, which was observed after the 48-h incubation period, as illustrated by the 1.3-fold change in the sucrose Pₑ value (figure 3B).

Figure 4. Toxic shock syndrome toxin–1 (TSST-1) dose dependency of leukocyte-induced inhibition of pyrogenic prostaglandin E₂ (PGE₂) transport across the choroidal epithelium. The basolateral side of choroidal epithelial monolayers was exposed to 2 × 10⁴ leukocytes/mL in the presence of various concentrations of TSST-1 (5, 50, or 500 ng/mL) for 48 h. At the end of this incubation period, the apical-to-basolateral transport of PGE₂ was determined. The results are expressed as percentages of inhibition of the saturable fraction of the transport measured on control cell–covered filters (incubated in the absence of leukocytes and TSST-1). Values are mean ± SD (n = 4–16 different filters from 1–4 cell preparations). *P < .01, vs. control (1-way analysis of variance, followed by “a posteriori” Dunnett’s test).
either in neuronal cell viability or in caspase level was observed after a shorter (24 h) exposure of the cortical neurons to TSST-1 (figure 5A and 5B). In the more sensitive SHSY5Y cell line, the toxic effect of TSST-1 was detected 24 h after initiating the treatment (figure 5C). Cell survival was reduced significantly at TSST-1 concentrations \( \geq 450 \) ng/mL, resulting in close to 30% cell death. These results were correlated with an increase in caspase 3/7 levels, which was initiated at a toxin concentration of \( 0.45 \) ng/mL and reached an almost 1.7-fold change at \( 1 \) \( \mu \)g/mL. Longer exposure of SHSY5Y cells to TSST-1 was not tested, because of the rapid growth rate of this cell line. In both cultures, the addition of the pan-caspase inhibitor z-VAD-fmk at \( 10 \) \( \mu \)mol/L inhibited this increase in caspase 3/7 levels, which was correlated with increased viability (table 2).

**DISCUSSION**

In contrast to long-term neuropsychological impairments from TSS, those from bacterial meningitis are well documented [24, 25] and include the loss of higher integrative and cognitive functions [9]. One of the factors implicated in these impairments is direct neuronal injury of cortical and subcortical regions (including the dentate gyrus of the hippocampus), which results from caspase 3–dependent apoptosis in these neurons [25–27]. We investigated whether a similar pathway may account for neuropathological symptoms associated with TSS. Data from this study support the hypothesis that TSST-1 can directly affect neurons through caspase 3/7–dependent cell apoptosis, because both primary cortical cultures and the SHSY-5Y neuronal cell line were sensitive to the toxin.

For TSS to exert such deleterious effects, blood TSST-1 would have to enter the brain either through the capillary endothelial cells forming the blood-brain barrier or through the blood-CSF barrier. We measured the transfer rate of TSST-1 across the latter, which is considered to be relatively more permissive to polar compounds and macromolecules [28], using a cellular model that reproduces the transport and polarity properties of the choroidal epithelium [17, 23]. A low basolateral-to-apical (i.e., blood-to-CSF) transfer rate of TSST-1 across the choroidal epithelial barrier was detected, and TSST-1–activated leukocytes with or without LPS did not significantly modify TSST-1 flux across the choroidal epithelium. TSST-1 transfer was observed in both directions, at similar magnitudes, and choroidal epithelium permeability was constant at all TSST-1 concentrations tested (\( \leq 500 \) ng/mL), which indicates that the toxin flux does not occur via unidirectional receptor-mediated transcytosis. The comparison of the \( P_r \) value for PEG-4000 with the clearance rate of TSST-1 calculated from table 1 shows that toxin transfer is 10 times lower than PEG-4000 transfer. Given the respective molecular weights of PEG-4000 and TSST-1 (4000 and 22,000 kDa, respectively), this finding suggests that TSST-1 crosses the choroidal epithelium only by restricted paracellular diffusion.

Previous studies have suggested that purified TSST-1 is able to cross monolayers of human crypt-like colonic epithelial cell line T-84 and human adenocarcinoma cell line Caco-2, by receptor-mediated transcytosis [29, 30]. However, the saturation concentration was \( >50 \) \( \mu \)g/mL, and the permeability coefficients that could be deduced from the data were of the same order of magnitude as those we report in the present study.

From the average clearance rate measured across the rat choroidal epithelium for TSST-1 (0.15 \( \mu \)L/h \( \cdot \) cm\(^2\)), equation (3) (see Materials and Methods) can be used to approximate the toxin concentration achieved in the CSF during toxemia as \( 3.5 \times 10^{-3} \) times the blood concentration. Although this value...
is based on a simplified CSF compartment model, it indicates that the concentration reached by TSST-1 in the CSF after its entry through an intact blood-CSF barrier remains lower than the blood concentration by 2 orders of magnitude. The concentration in the CSF estimated using human brain fluid system parameters was also a hundred times lower than the concentration in blood. TSST-1 has been quantified in human serum samples from patients with TSS or TSS-like symptoms, but not in CSF. Toxin concentrations up to 5000 pg/mL (mean, 440 pg/mL) were measured in serum samples [31], potentially leading to CSF concentrations of approximately several tenths of a picogram per milliliter, calculated on the basis of the above entry through an intact blood-CSF barrier remains lower than the concentration reached by TSST-1 in the CSF after its accumulation of TSST-1 within the CNS.

Leukocytes activated by either TSST-1 or LPS did not significantly modify the flux of paracellular markers across the choroidal epithelium, which corroborates previous evidence that the paracellular permeability of this barrier is not altered under conditions of cytokinetic challenge [33]. By contrast, TSST-1–activated immune cells induced a strong decrease in the apical-to-basolateral transfer of PGE2, at TSS-relevant concentrations in blood. PGE2 is known as a key factor in the development of the clinical symptoms associated with TSST-1–activated immune cells induced a strong decrease in the apical-to-basolateral transfer of PGE2, at TSS-relevant concentrations in blood. It is thus very likely that the influx of PGE2 is a critical factor in TSS neurotoxic effects, which is in line with the rarity of persistent neurological impairment occurring in patients after TSS. For those individuals who experience persistent impairment after TSS, TSST-1 neurotoxic effects could be relevant to these persistent symptoms only if higher serum concentrations have been attained or if the integrity of the other interface—that is, the blood-brain barrier—has been challenged in the course of the shock. Such a difference in reactivity between blood-brain barriers has already been reported, on challenge by another bacterial toxin, the pertussis toxin, which altered only the blood-brain barrier paracellular permeability [32]. Finally, neural cell binding cannot be ruled out as a concentrative mechanism leading to the accumulation of TSST-1 within the CNS.

Thus, the choroidal epithelium appears to efficiently limit the intracerebral concentration of TSST-1 to levels that preclude direct neurotoxic effects, which is in line with the rarity of persistent neurological impairment occurring in patients after TSS. For those individuals who experience persistent impairment after TSS, TSST-1 neurotoxic effects could be relevant to these persistent symptoms only if higher serum concentrations have been attained or if the integrity of the other interface—that is, the blood-brain barrier—has been challenged in the course of the shock. Such a difference in reactivity between blood-brain barriers has already been reported, on challenge by another bacterial toxin, the pertussis toxin, which altered only the blood-brain barrier paracellular permeability [32]. Finally, neural cell binding cannot be ruled out as a concentrative mechanism leading to the accumulation of TSST-1 within the CNS.

Leukocytes activated by either TSST-1 or LPS did not significantly modify the flux of paracellular markers across the choroidal epithelium, which corroborates previous evidence that the paracellular permeability of this barrier is not altered under conditions of cytokinetic challenge [33]. By contrast, TSST-1–activated immune cells induced a strong decrease in the apical-to-basolateral transfer of PGE2, at TSS-relevant TSST-1 concentrations in blood. PGE2 is known as a key factor in the development of the clinical symptoms associated with inflammation and infection that are collectively referred to as “sickness behavior syndrome” [34]. The concentration of PGE2 in CSF is increased in patients with bacterial meningitis [35] as well as in experimental models of bacterial meningitis [36]. When injected intracerebrally, this prostanooid induces the febrile response [37, 38] and, possibly, other neurological nonspecific manifestations of infection, such as agitation [39]. Interestingly, some reversible adverse effects of administering misoprostol, an oral prostaglandin analog, include agitation, headache, and confusion [40–42]. Thus, the intracerebral increase in prostaglandin concentration, sustained by a deficient Leukocytes activated by either TSST-1 or LPS did not significantly modify the flux of paracellular markers across the choroidal epithelium, which corroborates previous evidence that the paracellular permeability of this barrier is not altered under conditions of cytokinetic challenge [33]. By contrast, TSST-1–activated immune cells induced a strong decrease in the apical-to-basolateral transfer of PGE2, at TSS-relevant TSST-1 concentrations in blood. PGE2 is known as a key factor in the development of the clinical symptoms associated with inflammation and infection that are collectively referred to as “sickness behavior syndrome” [34]. The concentration of PGE2 in CSF is increased in patients with bacterial meningitis [35] as well as in experimental models of bacterial meningitis [36]. When injected intracerebrally, this prostanooid induces the febrile response [37, 38] and, possibly, other neurological nonspecific manifestations of infection, such as agitation [39]. Interestingly, some reversible adverse effects of administering misoprostol, an oral prostaglandin analog, include agitation, headache, and confusion [40–42]. Thus, the intracerebral increase in prostaglandin concentration, sustained by a deficient
choroidal clearance, is expected to contribute to the neurological symptoms observed during the acute phase of TSS. Moreover, a dysregulation in central PGE\(_2\) concentration may also be relevant to the long-term impairments associated with TSS, including learning disability. Although growing evidence demonstrates that PGE\(_2\) could play a role in normal hippocampal neuronal functions, memory acquisition, or retention [43], additional studies demonstrate PGE\(_2\)-induced neurotoxicity, via either a direct apoptotic effect or an indirect glutamate-mediated toxicity [44, 45]. The mechanism leading to the decrease in PGE\(_2\) clearance is likely to be mediated by proinflammatory cytokines known to be released from leukocytes on TSST-1 stimulation [46]. Indeed, TNF-\(\alpha\) and interleukin-1 significantly decreased organic anion transport at the CPs [33] and are involved in the reduced PGE\(_2\), choroidal efflux induced by virally activated lymphocytes [15].

In conclusion, the results of the present study suggest that TSST-1 affects the brain in different ways, starting with an alteration of PGE\(_2\) clearance from the brain, with PGE\(_2\) being a likely mediator of the reversible neurological symptoms of the acute phase of TSS. During strong or extended intoxication, TSST-1–induced caspase-dependent neuronal death may be responsible for irreversible brain injury, provided that the brain permeation of TSST-1 increases in the course of the pathophysiological process.

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