The Selective α7 Agonist GTS-21 Attenuates Cytokine Production in Human Whole Blood and Human Monocytes Activated by Ligands for TLR2, TLR3, TLR4, TLR9, and RAGE

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The cholinergic antiinflammatory pathway modulates inflammatory cytokine production through a mechanism dependent on the vagus nerve and the α7 subunit of the nicotinic acetylcholine receptor. GTS-21 (3-(2,4-dimethoxybenzylidene) anabaseine), a selective α7 agonist, inhibits inflammatory cytokine production in murine and human macrophages and in several models of inflammatory disease in vivo, but to date its antiinflammatory efficacy in human monocytes has not been characterized. We report here our findings that GTS-21 attenuates tumor necrosis factor (TNF) and interleukin 1β levels in human whole blood activated by exposure to endotoxin. GTS-21 inhibited TNF production in endotoxin-stimulated primary human monocytes in vitro at the transcriptional level. The suppressive effect of GTS-21 was more potent than nicotine in whole blood and monocytes. Furthermore, GTS-21 attenuated TNF production in monocytes stimulated with peptidoglycan, polyinosinic-polyribidylic acid, CpG, HMGB1 (high-mobility group box 1 protein), and advanced glycation end product–modified albumin. GTS-21 decreased TNF levels in endotoxin-stimulated whole blood obtained from patients with severe sepsis. These findings establish the immunoregulatory effect of GTS-21 on human monocytes, and indicate the potential benefits of further exploration of GTS-21’s therapeutic uses in human inflammatory disease.

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study was to characterize the effect of GTS-21 on cytokine production in human whole blood activated with endotoxin ex vivo. Here, we observed that GTS-21 dose-dependently attenuated TNF levels in human whole blood by inhibiting monocyte responses to endotoxin. GTS-21 inhibited TNF transcription in endotoxin-stimulated monocytes and effectively reduced TNF production in monocytes activated by exposure to Toll-like receptor (TLR)2, TLR3, TLR9, and receptor for advanced glycation end products (RAGE) agonists.

### MATERIALS AND METHODS

#### Reagents

Endotoxin from *Escherichia coli* 0111:B4, (−)-nicotine, and peptidoglycan from *Bacillus subtilis* were obtained from Sigma-Aldrich (St. Louis, MO, USA). Polynosinic-polycytidylic acid (poly(I:C)) and type B CpG oligonucleotide were obtained from Invivogen (San Diego, CA, USA). Recombinant HMGB1 and AGE-modified human serum albumin were prepared as previously described (16,17). GTS-21 was provided by Y Al-Abed.

#### Patient Population

A total of 26 subjects (11 patients with severe sepsis and 14 healthy volunteers) were included in the study. Subjects with severe sepsis, diagnosed according to the criteria of the American College of Chest Physicians/Society of Critical Care Medicine (18), were recruited from the Emergency Department at the North Shore Hospital in Manhasset, NY, USA. The study protocol was approved by North Shore-LIJ Institutional Review Board. Informed consent was obtained from all patients. In all subjects, peripheral venous blood was drawn into heparinized tubes. Blood from severe sepsis patients was collected within 24 h of hospital admission.

#### Whole Blood Assay

Phosphate-buffered saline (PBS) (Gibco, Carlsbad, CA, USA), GTS-21 (1, 10, 50, or 100 μmol/L final concentration), or nicotine (100 μmol/L final concentration) was added to heparinized whole blood (500 μL) 15 min prior to addition of endotoxin (1, 10, or 100 ng/mL final concentration). After a 4-h incubation period at 37°C and 5% CO₂, samples were spun down and plasma was collected. Plasma was kept frozen at −20°C until further analysis. Samples were run in duplicates.

#### Cytokine Measurement in Plasma

Concentration of TNF, interleukin (IL)-1β, IL-8, IL-6, IL-10, and IL-12p70 in plasma was determined by cytometric bead array immunoassay (Human Inflammation Kit [BD Biosciences, Franklin Lakes, NJ, USA]) according to the manufacturer’s protocol. Briefly, 50-μL aliquots of diluted plasma samples and cytokine standards were incubated in 12 × 75-mm polystyrene tubes with cytokine capture beads for 1.5 h at room temperature while protected from light. Samples were then washed once and incubated with phycoerythrin detection reagent for 1.5 h at room temperature in the dark. After one washing, samples were resuspended in 150 μL washing solution (PBS, 2% heat-inactivated fetal bovine serum, and 0.1% sodium azide) and transferred to a 96-well plate. Data were acquired with a FACScalibur (BD Biosciences) and cytokine concentrations were calculated using the BD CBA Software (BD Biosciences). Cytokine detection limits were 3.6, 7.2, 2.5, 3.3, 3.7, and 1.9 pg/mL for IL-8, IL-1β, IL-6, IL-10, TNF, and IL-12p70, respectively.

#### Intracellular TNF Staining

Heparinized whole blood (500 μL) was preincubated with PBS, GTS-21 (1, 10, 50, or 100 μmol/L final concentration), or nicotine (100 μmol/L final concentration) plus brefeldin A (final concentration 1 μg/mL) (GolgiPlug, BD Biosciences) prior to addition of endotoxin (final concentration 10 ng/mL) and incubated for 4 h at 37°C and 5% CO₂ in duplicates. Next, red blood cells were lysed (PharMLyse, BD Biosciences) and remaining leukocytes were washed once with staining buffer (PBS, 2% heat inactivated fetal bovine serum, 0.09% sodium azide). Leukocytes were then resuspended in permeabilizing solution (500 μL) (Cytofix/Cytoperm, BD Biosciences) and incubated for 10 min at room temperature protected from light. Cells were washed once in washing buffer (2 mL) (Perm/Wash, BD Biosciences) and resuspended in 40 μL of washing buffer containing phycoerythrin-labeled anti-human TNF antibody (2.5 μL) (BD Biosciences), and incubated for 30 min at room temperature in the dark. Finally, cells were washed once, re-suspended in staining buffer, and acquired with a FACScalibur (BD Biosciences). Unstained samples stimulated with endotoxin or stained nonstimulated samples were used to determine the staining threshold for TNF. Monocytes were defined as CD14-positive cells. Percentage of TNF-positive monocytes was determined.

#### Peripheral Blood Mononuclear Cell and Monocyte Isolation and Culture

Peripheral blood mononuclear cells (PBMCs) were obtained by density gradient fractionation ofuffy coats obtained from anonymous donors through Long Island Blood Services. Whole blood was diluted 1:6 in PBS, and 30 mL of diluted blood was layered over 20 mL of Ficoll-Hypaque (Beckman Coulter) in 50–mL conical tubes and spun down (30 min at 2000 rpm, room temperature). The PBMC-containing interphase was collected by aspiration. PBMCs were washed two times in PBS and resuspended in RPMI (Gibco) supplemented with 10% heat-inactivated human serum (BioWhittaker), 100 U/mL penicillin, and 100 μg/mL streptomycin (Gibco). PBMCs were plated in 24-well plates (Primaria, BD...
Bioscience) at a density of $5 \times 10^6$ cells per well or in 6-well plates at $15 \times 10^6$ cells per well (for TNF mRNA determination) and incubated overnight at 37°C and 5% CO$_2$. Nonadherent cells were washed away with PBS. Serum free OptiMEM (Gibco) was added to remaining adherent cells, which were 80-85% monocytes as determined by flow cytometry. GTS-21 (1, 10, 50, or 100 μmol/L final concentration) or nicotine (100 μmol/L final concentration) was added 15 min prior to endotoxin (10 ng/mL final concentration), and cells were incubated for 4 h at 37°C and 5% CO$_2$ in duplicates. In another set of experiments, monocytes were incubated with GTS-21 (100 μmol/L final concentration) or nicotine (10 ng/mL final concentration) prior to the addition of peptidoglycan (10 μg/mL final concentration), poly(I:C) (10 μg/mL), recombiant HMGB1 (10 μg/mL), AGE-modified albumin (5 μg/mL), and 5% CO$_2$ in duplicates. In another set of experiments, monocytes were incubated with GTS-21 (100 μmol/L final concentration) prior to the addition of peptidoglycan (10 μg/mL final concentration), poly(I:C) (10 μg/mL), CpG (10 μg/mL), GTS-21 (1, 10, 50, or 80-85% monocytes as determined by flow cytometry. GTS-21 (1, 10, 50, or 100 μmol/L final concentration) or nicotine (100 μmol/L final concentration) was added 15 min prior to endotoxin (10 ng/mL final concentration), and cells were incubated for 4 h at 37°C and 5% CO$_2$ in duplicates. In another set of experiments, monocytes were incubated with GTS-21 (100 μmol/L final concentration) or nicotine (100 μmol/L final concentration) prior to the addition of peptidoglycan (10 μg/mL final concentration), poly(I:C) (10 μg/mL), CpG (10 μM), recombiant HMGB1 (1.25 μg/mL), or AGE-modified albumin (5 μg/mL). Monocytes were incubated for 4 h at 37°C and 5% CO$_2$ in duplicates, after which supernatants were collected and kept frozen at 20°C. Concentration of TNF in cell culture supernatants was determined by ELISA (R&D Systems).

Cytotoxicity Assay

Cytotoxicity was determined by measuring lactate dehydrogenase (LDH) activity in supernatants with the Cytotox96 cytotoxicity assay kit (Promega, Madison, WI, USA). Results were expressed as percentage LDH activity by using supernatants of monocytes incubated in medium with 1% Triton X-100 (Sigma) as control.

mRNA Quantification

Monocytes were incubated in duplicates with PBS or GTS-21 (100 μmol/L final concentration) 15 min prior to addition of endotoxin (10 ng/mL final concentration). After 2 h, supernatants were collected and cells were washed 2 times with PBS. Total RNA was extracted from cells by use of the RNeasy Mini Kit (Qiagen, Valencia, CA, USA) and subjected to digestion with DNase (Qiagen). Samples were eluted in RNase-free water and kept frozen at -80°C until further analysis. Relative expression of TNF mRNA was determined by quantitative RT-PCR using TaqMan chemistry. Reactions were set in one-step RT-qPCR mastermix (Eurogentec, Seraing, Belgium), 100 ng of sample RNA, and the following primers (Invitrogen) and probe: Forward primer: 5′-CAC CCT CTT CTT CCT GAT; reverse primer: 5′-GCC AGA GGG CTG ATT AGA GA; probe: UPL#29 (Roche Diagnostics, Indianapolis, IN, USA). Samples were run in duplicates using the ABI PRISM 7700 Sequence Detection System. Thermal cycler conditions were 48°C for 30 min, and 95°C for 10 min at 45 cycles, each consisting of 95°C for 15 seconds, and 60°C for 1 min. Data were analyzed using the Sequence Detection System software version 1.9.1. Results were obtained as threshold cycle (Ct) values. β-Actin was used as reference gene. Relative expression of TNF in samples treated with endotoxin plus GTS-21 was calculated in comparison to samples treated with endotoxin plus vehicle using the delta-delta Ct method. Results are expressed as fold change with respect to vehicle control.

Statistical Analysis

Data are expressed as mean ± SEM. Differences between group means were determined by repeated measures ANOVA followed by Bonferroni’s multiple comparisons test, paired Student t test or ratio t test. P values < 0.05 were considered significant. Analyses were performed in Prism (Graphpad) version 3.0.

RESULTS

GTS-21 Attenuates Endotoxin-Induced TNF and IL-1β in Human Whole Blood

To study the effect of GTS-21 on leucocyte cytokine production, varying concentrations of GTS-21 were added to endotoxin-activated human whole blood obtained from healthy subjects. GTS-21 significantly attenuated TNF levels in whole blood compared with vehicle controls (Figure 1). GTS-21 inhibited TNF levels by 46% as compared with vehicle control at the highest concentration tested (100 μmol/L). We next compared the inhibitory effect of GTS-21 to that of nicotine, the canonical, nonselective nicotinic acetylcholine receptor agonist. Suppression of TNF production by GTS-21 was more effective than an equimolar concentration of nicotine, which did not significantly inhibit TNF levels in whole blood at the concentration tested. GTS-21 significantly attenuated IL-1β levels, but did not reduce IL-6, IL-8, or IL-12p70. IL-10 levels were not significantly reduced either, indicating that GTS-21 inhibition of TNF and IL-1β is not dependent on IL-10 (Figure 1).

GTS-21 Targets Monocytes to Attenuate TNF Levels in Endotoxin-Stimulated Whole Blood

Exposure of whole blood to endotoxin elicits monocytes to release TNF (19,20). Accordingly, we hypothesized that GTS-21 attenuates TNF levels in endotoxin-stimulated whole blood by suppressing TNF production by monocytes. We performed intracellular cytokine staining to directly study the effect of GTS-21 on TNF production by whole blood leukocytes. A robust expression of intracellular TNF was found in monocytes, and only a minor fraction of lymphocytes and polymorphonuclear cells were TNF positive (Figure 2A). Moreover, GTS-21 significantly decreased the number of TNF-producing monocytes, giving direct evidence for decreased TNF synthesis by the principle cell source of TNF in whole blood (Figure 2B). Taken together, these results indicate that GTS-21 attenuates TNF levels in endotoxin-stimulated whole blood by inhibiting TNF production by monocytes.

GTS-21 Attenuates TNF Production by Human Monocytes in vitro

The α7 subunit of the acetylcholine receptor is expressed on monocytes and lymphocytes (21,22). Because it was theoretically possible that GTS-21 attenuates TNF in whole blood by acting indirectly on leukocytes other than monocytes, we ascertained the effect of GTS-21 on iso-
GTS-21 Significantly Attenuates TNF Levels in Human Monocytes

GTS-21 significantly attenuates TNF levels in a dose-dependent manner at 10, 50, and 100 μmol/L. The concentration required to attenuate TNF by 50% (EC50) was ~10 μmol/L. At the highest concentration used, GTS-21 suppressed TNF levels by 76%. Nicotine (100 μmol/L) did not significantly attenuate TNF levels (Figure 3A). Further, reduction of TNF levels by GTS-21 was not found to be attributable to decreased monocyte viability, because we did not observe a significant change in LDH activity in cell culture supernatants (Table 1). Together with the results obtained from whole blood (above), this finding indicates that GTS-21 is significantly more potent than nicotine at inhibiting TNF release by monocytes.

GTS-21 Inhibits TNF Production in Monocytes at the Transcriptional Level

Attenuation of TNF production by GTS-21 in RAW cells and human macrophages is accompanied by inhibition of nuclear factor-κB activation (13). To gain further insight into the GTS-21 action mechanism, we next determined the effect of GTS-21 on TNF mRNA expression in human primary monocytes. GTS-21 significantly reduced TNF mRNA levels by 62% (Figure 3B), indicating that GTS-21 directly attenuates endotoxin-induced TNF protein synthesis in human monocytes by inhibiting TNF gene transcription.

GTS-21 Attenuates Production of TNF by Monocytes Stimulated with TLR2, TLR3, TLR9, and RAGE Agonists

Infectious agents initiate inflammatory responses by binding to pattern recognition receptors (PRRs) expressed on cells of the innate immune system. We wished to examine whether the inhibitory effects of GTS-21 were restricted to TLR4, and therefore studied the effect of GTS-21 on human monocytes activated by exposure to TLR2, TLR3, and TLR9 agonists. Peptidoglycan (TLR2 agonist), poly(FC) (TLR3 agonist), and CpG (TLR9 agonist) elicited a TNF response that was significantly attenuated by GTS-21 by 49%, 68%, and 77%, respectively. The nuclear protein high-mobility group box 1 protein (HMGB1), a proinflammatory mediator released by activated macrophages or by damaged cells (23,24), activates proinflammatory cytokine synthesis in human monocytes via TLR4 and the RAGE (25). GTS-21 significantly attenuated TNF production by 62% in monocytes stimulated with HMGB1. Advanced glycation end products (AGEs), proteins modified by glycosylation and oxidation via nonenzymatic processes, occur as a consequence of protracted hyperglycemia, and activate monocytes by binding to RAGE (26,27). GTS-21 significantly reduced TNF levels by 80% in monocytes activated by exposure to AGE-modified albumin (Figure 4). Thus, GTS-21 significantly attenuates monocyte TNF responses initiated by exogenous and endogenous activators of the innate immune system that signal via TLR2, TLR3, TLR4, TLR9, and RAGE.

GTS-21 Attenuates TNF in Endotoxin-Stimulated Whole Blood from Severe Sepsis Patients

Previous work indicates that the cytokine whole blood milieu from sepsis patients renders monocytes refractory to
the cytokine inhibiting activities of glucocorticoids and norepinephrine (28,29). Accordingly, we asked whether GTS-21 would be capable of inhibiting TNF release from monocytes in whole blood obtained from patients with sepsis. We enrolled 11 patients with severe sepsis and 14 sex-matched healthy volunteers (Table 2). In agreement with previous results, total endotoxin-induced TNF levels were significantly lower in whole blood of severe sepsis patients. We observed that GTS-21 (100 μmol/L) significantly attenuated TNF levels in whole blood of severe sepsis patients stimulated with 1, 10, and 100 ng/mL of endotoxin by 49%, 53%, and 62%, respectively, and in healthy volunteers by 54%, 51%, and 47% (Table 3).

**DISCUSSION**

Circulating leukocytes are an important source of proinflammatory cyto-
In the whole blood model, which resembles closely the physiological milieu in which responses to endotoxin occur, GTS-21 attenuated TNF and IL-1β, but not IL-6, IL-12, or IL-18 levels. IL-6 and IL-8 peak at 6–12 hours, and their production can be induced by TNF (30–32). It is conceivable that the specificity of GTS-21 is related to the time point studied (4 hours), and that GTS-21 indirectly inhibits other cytokines at longer incubation time points by attenuating TNF production. Importantly, GTS-21 did not significantly alter IL-10 levels, indicating that GTS-21 does not inhibit TNF by inducing endogenous IL-10 production.

Our results agree with others that endotoxin-induced TNF in whole blood is primarily produced by monocytes, with negligible contribution from other leukocyte subpopulations (19,20). GTS-21 significantly decreased the percentage of TNF-producing monocytes in whole blood, suggesting that GTS-21 acts directly on monocytes to attenuate TNF levels. It is possible, however, that decreased TNF levels in whole blood are also accounted for by GTS-21 acting on other leukocytes. For example, administration of nicotine is accompanied by an increase in acetylcholine serum concentration, apparently by inducing the release of acetylcholine from blood leukocytes (33). In a similar fashion, GTS-21 might induce acetylcholine release from lymphocytes, which express α7 and synthesize acetylcholine (22,34). Acetylcholine in turn attenuates cytokine production directly or through its metabolite choline, previously shown to attenuate TNF production by macrophages through a mechanism dependent on α7 (35).

Nonselective nicotinic agonists, including acetylcholine and nicotine, attenuate proinflammatory cytokine production by human monocytes and macrophages through and α7-dependent mechanism (3,36). GTS-21 dose dependently decreased TNF levels in isolated human monocytes stimulated with endotoxin in vitro, confirming that monocytes are indeed a GTS-21 target. Notably, GTS-21 was more potent in isolated monocytes (80% inhibition) than in whole blood (46% inhibition). This difference could be explained in part by increased sensitivity of monocytes to cholinergic signaling in vitro. For instance, murine lymphocytes and macrophages upregulate α7 expression when in culture (37). In addition, up to 80% of GTS-21 in blood is bound to serum proteins (38), so it is likely that the free concentration of GTS-21 in whole blood was lower than in culture medium, reducing the amount of GTS-21 able to act upon blood monocytes. Contrary to what has been previously reported (21,36), we observed that nicotine in the concentration used did not significantly reduce TNF levels in monocytes in vitro. This difference could be explained by varied culture conditions including duration of nicotine exposure and endotoxin concentration. It is clear from our study, however, that the suppressive effect of GTS-21 is greater than that of an equimolar concentration of nicotine in

<table>
<thead>
<tr>
<th>LPS, ng/mL</th>
<th>GTS, μmol/L</th>
<th>NIC, μmol/L</th>
<th>% Cytotoxicity, mean ± SEM</th>
<th>P value</th>
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<tr>
<td>10</td>
<td>0</td>
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<td>11.8 ± 2.07</td>
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<tr>
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<td>1</td>
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<td>13.7 ± 1.3</td>
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<tr>
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<td>—</td>
<td>11.9 ± 2.5</td>
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<tr>
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<td>50</td>
<td>—</td>
<td>7.9 ± 1.8</td>
<td>&gt;0.05</td>
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<td>100</td>
<td>—</td>
<td>11.6 ± 1.3</td>
<td>&gt;0.05</td>
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<td>10</td>
<td>—</td>
<td>100</td>
<td>13.9 ± 1.1</td>
<td>&gt;0.05</td>
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*LDH content was measured in cell supernatants and normalized to permeabilized monocytes, which served as 100% cytotoxicity control. GTS-21 and nicotine-treated monocytes were compared with vehicle-treated monocytes.

Figure 4. GTS-21 attenuates TNF production by human monocytes stimulated with TLR2, TLR3, TLR9, and RAGE agonists. Monocytes were incubated with vehicle or GTS-21 (100 μmol/L) 15 min prior to stimulation with peptidoglycan (PGN) 10 μg/mL, poly(I:C) 10 μg/mL, CpG 10 μmol/L, HMGB1 1.25 μg/mL, or AGE-modified albumin 5 μg/mL. TNF levels were determined at 4 h. GTS-21 significantly attenuated TNF production induced by all ligands used. Each drug was tested in duplicate in monocytes obtained from at least five independent donors. Data shown as mean ± SEM. * P < 0.05 versus control; ** P < 0.01 versus control.
whole blood and monocytes in vitro under comparable experimental conditions. Our results add to previous studies reporting attenuation of TNF production by GTS-21 in human placenta cells and macrophages, thus confirming that GTS-21 is an effective modulator of cytokine production in human cells (13,15).

Inflammatory responses by the innate immune system are triggered by pathogen-derived molecules acting on Toll-like receptors. Prior studies addressing the effect of cholinergic agonists had focused almost exclusively on cell activation through TLR4. Our results now indicate that GTS-21 effectively attenuates TNF production elicited in monocytes by activation of TLR2, TLR3, and TLR9. GTS-21 also effectively attenuated production of TNF in response to HMGB1 and AGE-modified albumin. The nuclear protein HMGB1 is passively released from necrotic cells, and induces proinflammatory cytokine production by target cells through activation of TLR2, TLR4, and RAGE (39,40). HMGB1 also plays an important role in various noninfectious inflammatory conditions including trauma (41), ischemia-reperfusion injury (42,43), and rheumatoid arthritis (44). AGEs are molecules modified by glycosylation and oxidation via nonenzymatic processes occurring in environments of oxidative stress and hyperglycemia. AGEs signal through RAGE receptors and induce proinflammatory cytokine production by macrophages and monocytes (26,27). AGEs have been implicated in the pathogenesis of vascular complication of diabetes (45) and Alzheimer's disease (46).

Reduced production of proinflammatory cytokines is a characteristic feature of sepsis. The immune status of monocytes is altered such that whole blood synthesis of TNF, IL-1β, IL-6, and IL-12 is blunted (19,47) and HLA-DR surface expression is reduced (48). We found here that GTS-21 further attenuated TNF levels in whole blood of septic patients, suggesting that α7 signaling in monocytes remains intact in severe sepsis. In summary, we have established that GTS-21 attenuates TNF production in endotoxin-stimulated human monocytes by inhibiting TNF gene transcription. The efficacy of GTS-21 in suppressing TNF production induced by numerous endogenous and exogenous activators of the innate immune system indicates that α7 regulates the critical steps underlying monocyte activation to a wide range of innate immune stimulatory molecules.

### DISCLOSURE

KJ Tracey is inventor on patents related to targeting inflammation using cholinergic agonists.

### REFERENCES


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**Table 2.** Patient demographics.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Severe sepsis (n = 11)</th>
<th>Healthy (n = 14)</th>
<th>P value</th>
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<td>Sex, n</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Males</td>
<td>4</td>
<td>5</td>
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<tr>
<td>Females</td>
<td>7</td>
<td>9</td>
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<td>Age, y</td>
<td>68.7 ± 13.9</td>
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<td>28-Day mortality, n (%)</td>
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<td>Overall mortality</td>
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<td>Male mortality</td>
<td>2 (18)</td>
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<tr>
<td>Female mortality</td>
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<td>Severity of disease</td>
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<tr>
<td>APACHE</td>
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<td>APS</td>
<td>39 ± 12.4</td>
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<td>Clinical manifestation, n (%)</td>
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<td>6 (54.5)</td>
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<td>Urosepsis</td>
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<td>Congestive heart failure</td>
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<tr>
<td>Acquisition of infection</td>
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**Table 3.** Effect of GTS-21 on TNF levels in endotoxin-stimulated whole blood from healthy volunteers and severe sepsis patients.

<table>
<thead>
<tr>
<th>LPS, ng/mL</th>
<th>Healthy</th>
<th>Severe sepsis</th>
<th>% Inhibition</th>
<th>PBS GTS-21</th>
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<td>3680 ± 548b</td>
<td>54</td>
<td>4096 ± 911c</td>
<td>2049 ± 526d</td>
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<td>5268 ± 1026c</td>
<td>2466 ± 633d</td>
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<tr>
<td>100</td>
<td>16760 ± 1789</td>
<td>8788 ± 1271b</td>
<td>47</td>
<td>6006 ± 1431c</td>
<td>2244 ± 433d</td>
<td>62</td>
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</table>

*aWhole blood obtained from healthy donors (n = 14) and from severe sepsis patients (n = 11) on d 1 of hospital admission was stimulated with different endotoxin concentrations plus GTS-21 (100 μmol/L) or PBS. Results expressed as mean ± SEM.

bP < .005 compared with PBS control.

cP < 0.01 compared with healthy individuals.

dP < 0.01 compared with PBS control.
GTS-21 ATTENUATES CYTOKINE PRODUCTION IN HUMAN WHOLE BLOOD AND HUMAN MONOCYTES


