Disruption of the Transient Receptor Potential Vanilloid 1 Can Affect Survival, Bacterial Clearance, and Cytokine Gene Expression during Murine Sepsis

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ABSTRACT

Background: Previous studies suggest that the transient receptor potential vanilloid 1 (TRPV1) channel has a role in sepsis, but it is unclear whether its effect on survival and immune response is beneficial or harmful.

Methods: We studied the effects of genetic (Trpv1-knockout vs. wild-type [WT] mice) and pharmacologic disruption of TRPV1 with resiniferatoxin (an agonist) or capsazepine (an antagonist) on mortality, bacterial clearance, and cytokine expression during lipopolysaccharide or cecal ligation and puncture–induced sepsis.

Results: After cecal ligation and puncture, genetic disruption of TRPV1 in Trpv1-knockout versus WT mice was associated with increased mortality risk (hazard ratio, 2.17; 95% CI, 1.23–3.81; \( P = 0.01 \)). Furthermore, pharmacologic disruption of TRPV1 with intrathecal resiniferatoxin, compared with vehicle, increased mortality risk (hazard ratio, 1.80; 95% CI, 1.05–3.2; \( P = 0.03 \)) in WT, but not in Trpv1-knockout, mice. After lipopolysaccharide, neither genetic (Trpv1 knockout) nor pharmacologic disruption of TRPV1 with resiniferatoxin had significant effect on survival compared with respective controls. In contrast, after lipopolysaccharide, pharmacologic disruption of TRPV1 with capsazepine, compared with vehicle, increased mortality risk (hazard ratio, 1.92; 95% CI, 1.02–3.61; \( P = 0.04 \)) in WT animals. Furthermore, after cecal ligation and puncture, increased mortality in resiniferatoxin-treated WT animals was associated with higher blood bacterial count (\( P = 0.0004 \)) and higher nitrate/nitrite concentrations and down-regulation of tumor necrosis factor \( \alpha \) expression (\( P = 0.0004 \)) compared with controls.

Conclusions: Genetic or pharmacologic disruption of TRPV1 can affect mortality, blood bacteria clearance, and cytokine response in sepsis in patterns that may vary according to the sepsis-inducing event and the method of TRPV1 disruption.

What We Already Know about This Topic

- Controversy exists regarding the role of transient receptor potential vanilloid 1 (Trpv1) on organ failure and survival during sepsis.

What This Article Tells Us That Is New

- Depending on the sepsis-induced circumstances, genetic (Trpv1-knockout mice) or pharmacologic (intrathecal resiniferatoxin or capsazepine) disruption of TRPV1 affects mortality, blood bacteria clearance, and cytokine response to a varying degree.

Growing body of literature\(^1,^2\) supports the notion that sensory neurons have a role in modulating inflammatory response. In fact, because of the initial description of neurogenic inflammation (i.e., vasodilation, plasma extravasation, and pain) that results from stimulation of sensory neurons, several studies\(^3\) have shed light on the mechanisms underlying the phenomenon. Researchers\(^4,^5\) have shown that...
when sensory neurons expressing the transient receptor potential vanilloid 1 (TRPV1) channels are activated by protons, lipoxigenase products, noxious heat, nitric oxide, or vanilloid compounds, neuropeptides known to generate neurogenic inflammation (i.e., substance P and calcitonin gene-related peptide) are released. The findings that animals genetically lacking the TRPV1 receptor or undergoing ablation of TRPV1 sensory neurons are unable to mount neurogenic proinflammatory responses to noxious stimuli or vanilloid agonists further support the notion that TRPV1 plays a role in inflammation.1–4

With regards to sepsis, some studies implicate TRPV1, calcitonin gene-related peptide, and substance P in its pathophysiology in humans and animals. For example, in septic patients, higher concentrations of calcitonin gene-related peptide and substance P correlate with concentrations of nitrates/nitrites, hypotension, and lethality.7–9 In rats, lipopolysaccharide-induced toxicity is associated with augmented expression of TRPV1 receptors and increased density of calcitonin gene-related peptide-positive mesenteric neurons.10 In mice, genetic disruption of TRPV1 in Trpv1 knockout (KO) mice is associated with worse hypotension, increased cytokine concentrations in the peritoneum, and worse liver injury during lipopolysaccharide-induced toxicity.11 In rats, the TRPV1–competitive antagonist, capsazepine, decreased survival and worsened hypotension in lipopolysaccharide-induced toxicity.12 Thus, taken together, these studies suggest that TRPV1 has a protective role during sepsis.

However, contrary to reports suggesting a protective role of TRPV1, a recent study13 in mice showed that pharmacologic disruption of TRPV1 with capsazepine has beneficial effects during peritonitis and sepsis. This study showed that, in a model of polymicrobial sepsis, one dose of capsazepine, administered 30 min before cecal ligation and puncture (CLP), increased survival and decreased liver and lung damage.13 Although capsazepine can affect receptors other than TRPV1, including nicotinic acetylcholine receptors,14 and other voltage-activated calcium channels,15 the study in capsazepine-treated mice suggests that TRPV1 has a potentially harmful effect during polymicrobial sepsis and that its pharmacologic disruption with capsazepine is protective. Therefore, although animal studies have yielded conflicting results about the role of TRPV1 in survival and multiple organ damage during sepsis, there is ample evidence that TRPV1 plays an incompletely understood role in the pathophysiologic features of sepsis.

The current study investigated the role of TRPV1 in mortality, bacterial clearance, and cytokine expression in murine sepsis induced by bacteria during CLP or lipopolysaccharide challenge. These investigations16,17 are relevant because agents, such as resiniferatoxin, a potent capsaiacin analog and a TRPV1 agonist known to ablate TRPV1-expressing neurons by excessive calcium influx, are being investigated as potential therapies for various pain syndromes and inflammatory states in animals and humans.4,18–20

Materials and Methods

Animals

After approval from the National Institutes of Health Clinical Center Animal Care and Use Committee (Bethesda, MD), we studied female C57BL/6 (wild-type [WT]) and B6.129X1-Trpv1tm1Jul/J (Trpv1-KO) mice (aged 6–8 weeks; weight, 15–20 g) Jackson Laboratories, Bar Harbor, ME). Animals were acclimated to their cages and housed in a temperature-controlled facility with free access to food and water.

Experimental Protocol

Survival. To investigate the role of TRPV1 in sepsis and lipopolysaccharide-induced toxicity, we examined the role of pharmacologic disruption of the receptor with resiniferatoxin (a TRPV1 agonist) and capsazepine (a TRPV1 antagonist) and genetic disruption in Trpv1-KO in two murine models: CLP-induced polymicrobial sepsis and lipopolysaccharide-induced toxicity. Table 1 lists the number of WT and Trpv1-KO animals enrolled in each of the 12 study groups that included combinations of surgery (CLP), lipopolysaccharide administration, genotype (WT or Trpv1-KO), and pharmacological treatment (resiniferatoxin, a potent TRPV1 agonist; and capsazepine, a competitive TRPV1 antagonist, or respective vehicle control). Surgery (CLP) or lipopolysaccharide-challenge experiments were conducted weekly; during each week, animals from the treatment and respective control groups (vehicle or no treatment) were studied simultaneously. In animals undergoing CLP, after isoflurane anesthesia was induced, a small mid-line abdominal incision was made; the cecum was located and exteriorized; and then after a 4–0 silk ligature was placed at 1 cm from its tip, the cecum was perforated once with a 16-gauge needle at the antimesenteric surface. A small amount of fecal material was then expressed through the puncture wound, the cecum was placed back into the abdomen, and the incision was closed with metal clips.21 One investigator (Z.M.N.Q.), unaware of genotype or treatment group, performed all CLP procedures. Saline (50 ml/kg) was administered at the completion of surgery and with each antibiotic dose (imipenem–cilastatin sodium [Primaxin], with 25 mg/kg imipenem; Merck, Whitehouse Station, NJ) starting 2 h after surgery and every 12 h for 72 h (50 ml/kg saline subcutaneously, amounting to 150 ml/kg in the first 24 h and 100 ml/kg per 24 h for 2 more days). After surgery, animals were observed every 2 h for the first 36 h, every 4 h from 36 to 48 h, and once daily from 48 to 168 h.

After CLP (at 6 and 24 h) and lipopolysaccharide challenge (at 3, 6, and 24 h), randomly selected animals were anesthetized (isoflurane) for terminal blood draws for blood bacterial culture, serum cytokine and nitrate/nitrite measurements, or spleen procurement for RNA isolation or peritoneal lavage for flow cytometry (lipopolysaccharide-challenged animals only).

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Pharmacologic Disruption of TRPV1 with Resiniferatoxin or Capsazepine. Resiniferatoxin was administered intrathecally (L5–L6 intervertebral space) 72 h before CLP or lipopolysaccharide challenge to animals assigned to resiniferatoxin groups under light isoflurane anesthesia, as previously described.22 The resiniferatoxin dose (25 ng suspended in Tween 80, 0.25%, and preservative-free saline in a volume of 10 ul) was chosen after pilot studies determined that such dose elicited no morbidity and increased nociceptive behavior latency time on the hot plate (data not shown), indicating successful intrathecal resiniferatoxin injection. Controls received an intrathecal injection of the resiniferatoxin vehicle.

Capsazepine,15 a TRPV1 antagonist, was suspended in dimethyl sulfoxide, 10%, and physiologic saline; 50 µg in 0.1 ml was injected subcutaneously. Animals assigned to the capsazepine experiments received capsazepine or the same volume of vehicle subcutaneously (controls) twice daily for 72 h before and 72 h after lipopolysaccharide challenge.

Lipopolysaccharide (Escherichia coli 0111:B4; Sigma-Aldrich, St Louis, MO) was suspended in sterile lipopolysaccharide-free saline (Hospira Inc., Lake Forest, IL), and mice were injected with 25 mg/kg intraperitoneally.

### Quantitative Real-time Reverse Transcription–polymerase Chain Reaction

RNA was isolated using a kit (RNeasy Mini kit; Qiagen, Valencia, CA). RNA quality was evaluated with an analyzer (Agilent 2100 Bioanalyzer) using kits (RNA 6000 Nano; Agilent Technologies, Santa Clara, CA), and quantity was measured by absorption at 260 nm using NanoDrop (NanoDrop Technologies, Wilmington, DE). Tumor necrosis factor (TNF-α), chemokine (c-c motif) ligand 3, and interleukin (IL) 10 gene expression levels were quantified by real-time reverse transcription–polymerase chain reaction using a kit (One-Step qRT-PCR Kit in the Abi Prism 7500; Applied Biosystems, Foster City, CA). The fold changes in gene expression were calculated after normalization to endogenous glyceraldehyde-3-phosphate dehydrogenase and were expressed relative to that detected in the spleens of WT or vehicle control-injected animals (normalized to 1) using the comparative cycle threshold method.23

### Serum Cytokine and Chemokine Concentrations

Serum TNF-α, CCL3, and IL-10 concentrations were measured using a multiplex sandwich enzyme-linked immunosorbent assay (SearchLight Mouse Cytokine array; ThermoFisher Scientific, Woburn, MA).

### Flow Cytometry Analysis of Peritoneal Cells

In lipopolysaccharide-challenge experiments, peritoneal cells were obtained from peritoneal lavage (5 ml x 1 phosphate-buffered saline; Invitrogen, Carlsbad, CA). Lavage fluid was centrifuged, supernatant was removed, and cell pellet was resuspended in Dulbecco phosphate-buffered saline (Invitrogen), supplemented with BSA, 0.5%. One million cells were stained with CD19 (B-cell marker), F4/80 (macrophage marker), Ly6g (neutrophil marker), or their respective isotype controls (eBioscience, San Diego, CA) for 30 min on ice. After staining, cells were washed and resuspended in 4% w/v paraformaldehyde Cytofix (BD Biosciences, San Jose, CA) and analyzed on flow cytometer FACSCalibur (BD Biosciences) within 48 h.

### Nitrate/Nitrite Measurements

Plasma was deproteinized using 10 kilodalton centrifugal filters (Millipore, Billerica, MA), and the flow through was then mixed with vanadium chloride, 0.4% (5 ml in 1 N HCl), in a purge vessel heated to 90°C, through which helium was continuously bubbled. The resulting nitric oxide from nitrate and nitrite in the samples was detected with an nitric oxide analyzer (Sievers 280; GE Analytical, Boulder, CO). Measurements were based on comparisons to control samples with known concentrations of nitrate.

### Temperature Measurements

To measure body temperature, an electronic thermosensor weighing 120 mg (Bio Medic Data Systems, Seaford, DE) was implanted subcutaneously between the mouse shoulder blades, with general anesthesia, using a sterile trocar (12-gauge needle). Body temperatures were monitored using an electronic thermosensor weighing 120 mg (Bio Medic Data Systems, Seaford, DE) and recorded every 15 min using a temperature recorder (CCL; Denver Instrument, Denver, CO). The maximum body temperature was recorded for each mouse. The survival time was defined as the time between the day of lipopolysaccharide challenge and death of the mouse. Mortality was defined as the inability to maintain body temperature >35°C.
temperature was measured serially before and after lipopolysaccharide challenge.

**In Vitro Effects of Capsazepine in Splenocytes Treated with Lipopolysaccharide**

The WT mice were euthanized under isoflurane anesthesia. Spleens were removed and mechanically dissociated, and erythrocytes were lysed using erythrocyte lysing buffer (ACK, Lysing Buffer). Three experiments were conducted, and each experiment was performed with cells pooled from spleens from two mice. Cells were resuspended in Dulbecco-modified essential medium; supplemented with fetal calf serum, penicillin/streptomycin (10,000 U/ml penicillin and 10 mg/ml streptomycin), and L-alanyl-L-glutamine (GlutaMax) (all solutions from Invitrogen, Grand Island, NY); and then plated at 10 × 10^6 cells/ml in six-well plates. Capsazepine (10 μM) and lipopolysaccharide (10 ng/ml) were added, and cells were incubated for 16 h at 37°C. Cell viability was determined before RNA isolation by trypan blue dye exclusion.

**Statistical Analysis**

The data were analyzed using computer software (SAS, version 9.13; SAS, Cary, NC). Survival analyses were conducted using the Cox proportional hazard model to analyze the effects of Trpv1-KO or resiniferatoxin treatment versus WT control in either a CLP or a lipopolysaccharide-challenge model, the effect of Trpv1-KO animals treated with resiniferatoxin versus Trpv1-KO control in a CLP or lipopolysaccharide model, and the effect of capsazepine versus its vehicle in WT animals. Per the prospective design, WT or Trpv1-KO groups that received no treatment or vehicle in cohort experiments and did not differ in survival were combined as WT or Trpv1-KO controls, respectively, for survival analysis. The hazard ratio (95% CI) of death was used to express the effect of each treatment on mortality compared with its own control. We also performed post hoc analyses to compare the effects of resiniferatoxin on survival between the two sepsis models, CLP versus lipopolysaccharide, and to compare the effects of pharmacologic disruption of TRPV1 in WT control. In contrast to findings observed in WT animals, in Trpv1-KO mice, intrathecal resiniferatoxin injection, compared with vehicle, had no significant effect on mortality (P = 0.47) after CLP (fig. 1B and fig. 2). Therefore, this finding suggests that the harmful effect of resiniferatoxin on mortality after CLP requires integrity of the Trpv1 gene.

**Effect of Resiniferatoxin on Survival after Lipopolysaccharide-Induced Toxicity.** During lipopolysaccharide-induced toxicity, pharmacologic disruption of TRPV1 in resiniferatoxin-treated WT animals was associated with no significant effect on mortality compared with vehicle-treated WT animals (table 1; fig. 1C; P = 0.22).

**Effect of Resiniferatoxin on Bacterial Clearance during Bacterial Infection.** Figure 3 shows blood bacterial counts after CLP. After CLP, resiniferatoxin-treated WT mice had a significantly different pattern of blood bacterial counts at 6 and 24 h (fig. 3A, P = 0.0005) compared with vehicle-treated WT animals. Specifically, by 24 h, there were significantly greater increases (P = 0.0004) in bacteria colony-forming units in the blood in resiniferatoxin- compared with vehicle-treated WT animals; at 6 h, there were no statistically significant differences (fig. 3A).

**Effect of Resiniferatoxin on Serum Nitrate/Nitrite Concentrations and Cytokine Gene Expression Profile after CLP.**

Table 2, table 3, and table 4 show the effects of pharmacologic and genetic disruptions of TRPV1 on cytokine gene expression, survival, blood culture, and nitrate/nitrite concentrations after CLP and lipopolysaccharide. After CLP, resiniferatoxin-treated WT mice had significantly higher serum nitrate/nitrite concentrations (fig. 3C, P = 0.037) at 6, but not at 24 h, compared with WT animals. Regarding gene expression of proinflammatory and antiinflammatory cyto-

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**Effect of Pharmacologic Disruption of TRPV1 with Resiniferatoxin, a TRPV1 Agonist**

**Effect of Resiniferatoxin on Survival after Bacterial Peritonitis and Sepsis.** Table 1 lists experimental groups and respective mortality rates, and figure 1 and figure 2 describe the effect of genetic and pharmacologic disruption of TRPV1 on survival. After CLP-induced polymicrobial sepsis, disruption of TRPV1-sensory neurons with intrathecal resiniferatoxin-treated WT animals was associated with increased risk of mortality (hazard ratio, 1.80; 95% CI, 1.05–3.2; P = 0.03; figs. 1A and 2) compared with WT control. In contrast to findings observed in WT animals, in Trpv1-KO mice, intrathecal resiniferatoxin injection, compared with vehicle, had no significant effect on mortality (P = 0.47) after CLP (fig. 1B and fig. 2). Therefore, this finding suggests that the harmful effect of resiniferatoxin on mortality after CLP requires integrity of the Trpv1 gene.

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kines and chemokines in splenocytes, at 24 h after CLP (table 2), resiniferatoxin-treated WT mice had significantly decreased TNF-α mRNA concentrations compared with vehicle-treated animals (P = 0.004, table 2); at 6 and 24 h, similar CCL3 and IL-10 gene expression levels were found compared with vehicle-treated animals (P = 0.06, table 2).

Effect of Resiniferatoxin on Serum Nitrate/Nitrite Concentrations and Cytokine Gene Expression Profile after Lipopolysaccharide. There was significant up-regulation of cytokine gene expression in all lipopolysaccharide-treated animals compared with phosphate-buffered saline (PBS)—injected controls (data not shown). After lipopolysaccharide, there were no significant differences in nitrate/nitrite concentrations, gene expression or serum concentrations of cytokines, or percentage of B cells, macrophages, and neutrophils in peritoneal lavage comparing resiniferatoxin-treated WT with vehicle-treated animals (data not shown).

Effect of Pharmacologic Disruption of TRPV1 with Capsazepine, a TRPV1 Antagonist

Effect of Capsazepine on Survival after Lipopolysaccharide-induced Toxicity. After lipopolysaccharide-induced toxicity, pharmacologic disruption of TRPV1 in capsazepine-treated WT animals significantly increased mortality compared with vehicle-treated WT animals (hazard ratio of death, 1.92; 95% CI, 1.02–3.61; P = 0.04; figs. 1D and 2). Furthermore, as suggested on post hoc analysis, the pattern of harmful effect of pharmacologic disruption of TRPV1 with capsazepine was significantly different from that of genetic deficiency and pharmacologic disruption with resiniferatoxin (fig. 2, P = 0.03).

Effect of Capsazepine on Serum Nitrate/Nitrite Concentrations, Cytokine Gene Expression Profile, and Peritoneal Cell Trafficking after Lipopolysaccharide. In capsazepine-treated WT mice, the increase in mortality after lipopolysaccharide-induced toxicity, pharmacologic disruption of TRPV1 with capsazepine significantly increased mortality compared with vehicle-treated WT animals (hazard ratio of death, 1.92; 95% CI, 1.02–3.61; P = 0.04; figs. 1D and 2). Furthermore, as suggested on post hoc analysis, the pattern of harmful effect of pharmacologic disruption of TRPV1 with capsazepine was significantly different from that of genetic deficiency and pharmacologic disruption with resiniferatoxin (fig. 2, P = 0.03).
After lipopolysaccharide challenge, capsazepine-treated WT animals had significantly decreased mRNA concentrations of TNF-α at 6 and 24 h and CCL3 at 6 h (tables 2 and 3) and similar serum nitrate/nitrite concentrations at 6 and 24 h (fig. 3D). However, Trpv1-KO mice had significantly higher mRNA concentrations of CCL3 ($P = 0.038$) and IL-10 ($P = 0.026$) at 24 h after CLP compared with WT animals.

**Effect of Genetic Disruption of the Trpv1 Gene on Gene Expression Profile, Serum Nitrate/Nitrite Concentrations, and Peritoneal Cell Trafficking after Lipopolysaccharide.**

At 24 h after lipopolysaccharide, Trpv1-KO animals had significantly higher serum concentrations of IL-10 compared with WT animals (mean ± SEM, 625 ± 485 vs. 22 ± 41 pg/ml for Trpv1-KO vs. WT; $P = 0.02$). After lipopolysaccharide, there were no significant differences in gene expression or serum concentrations of other cytokines, serum nitrate/nitrite concentrations (table 4), and percentage of B cells, macrophages, and neutrophils in peritoneal lavage comparing Trpv1-KO with WT animals (data not shown).

Although no baseline concentrations were obtained, after intraperitoneal injection of phosphate-buffered saline, there were no significant differences in splenocyte TNF-α, CCL3,
and IL-10 mRNA concentrations comparing WT (n = 6) with Trpv1-KO (n = 6) animals (data not shown).

Effect of Genetic Disruption of the Trpv1 Gene on Temperature Changes after Lipopolysaccharide. In a cohort of mice not enrolled in the survival study, we measured body temperature before and after lipopolysaccharide challenge. At baseline, Trpv1-KO mice had significantly higher temperatures compared with WT animals. After lipopolysaccharide, both Trpv1-KO and WT animals had significant decreases up to 15 h and then increases in body temperature (P < 0.0001, fig. 4). However, after lipopolysaccharide, Trpv1-KO animals had less of a decrease in body temperature compared with WT animals (P < 0.0005, fig. 4).

In Vitro Effects of Pharmacologic Inhibition of TRPV1 with Capsazepine

To examine the effects of TRPV1 in lipopolysaccharide challenge in vitro, we measured inflammatory and antiinflammatory cytokine mRNA concentrations using real-time quantitative reverse transcription–polymerase chain reaction in capsazepine- and vehicle-treated WT splenocytes challenged with 1 µg/ml lipopolysaccharide for 16 h (fig. 5). Cell viability was similar among all groups before RNA isolation, as determined by trypan blue dye exclusion (data not show). In basal conditions (without lipopolysaccharide), capsazepine treatment of WT splenocytes significantly decreased mRNA concentrations of TNF-α compared with vehicle treatment (P < 0.025). After lipopolysaccharide, capsazepine-treated WT splenocytes had significantly lower increases in mRNA concentrations of TNF-α (P = 0.015), CCL3 (P = 0.002), and IL-10 (P = 0.003) compared with vehicle-treated WT splenocytes (fig. 5).

Discussion

We found that TRPV1 has an important role in survival, bacterial clearance from the blood, and cytokine expression profile during sepsis. During polymicrobial infection, such as in CLP, the TRPV1 receptor appears to be protective because its disruption (either genetically or pharmacologically with intrathecal resiniferatoxin, resulting in ablation of TRPV1-expressing neurons17,24) increased mortality. More importantly, the detrimental effect of resiniferatoxin on mortality appears to be related to the presence of the Trpv1 gene

Table 3. Overall Effect of Pharmacologic or Genetic Disruptions of TRPV1 on Mortality, Blood Bacterial Culture, Cytokine Gene Expression, and Serum Nitrate/Nitrite Levels after Cecal Ligation and Puncture

<table>
<thead>
<tr>
<th>Perturbation</th>
<th>Mortality at 168 h</th>
<th>Blood Culture, Colon-forming Units/ml, h</th>
<th>Cytokine Gene Expression in Splenocytes, h</th>
<th>Serum Levels of Nitrate/Nitrite, h</th>
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Arrows pointing up or down indicate statistically significant effects (in the direction of the arrow) of given disruptions compared with respective controls, and arrows pointing to the right indicate no significant effect. Cells were left empty when measurements were not obtained.

Table 4. Overall Effect of Pharmacologic or Genetic Disruptions of TRPV1 on Mortality, Cytokine Gene Expression, and Serum Nitrate/Nitrite Levels after Lipopolysaccharide Challenge

<table>
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<th>Perturbation</th>
<th>Mortality at 168 h</th>
<th>Cytokine Gene Expression in Splenocytes, h</th>
<th>Serum Levels of Nitrate/Nitrite, h</th>
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Arrows pointing up or down indicate statistically significant effects (in the direction of the arrow) of given disruptions compared with respective controls, and arrows pointing to the right indicate no significant effect. Cells were left empty when measurements were not obtained.

CCL = chemokine (C–C motif) ligand; IL = interleukin; KO = knockout; TNF = tumor necrosis factor; Trpv = transient receptor potential vanilloid.
because resiniferatoxin increased CLP mortality in WT but not in Trpv1-KO mice. In addition, these increases in mortality with intrathecal resiniferatoxin were associated with decreased bacterial clearance from the blood, down-regulation of TNF gene expression, and increased serum concentrations of nitrate/nitrite early during the infection in WT animals. Therefore, our findings add to the existing literature indicating that the TRPV1 receptor and TRPV1-expressing sensory neurons have an important role in sepsis and inflammatory response.

The findings that genetic disruption of TRPV1 and intrathecal administration of resiniferatoxin increases mortality and decreases bacterial clearance from the blood in a sepsis model are relevant because resiniferatoxin is being pursued as a potential therapy for various pain syndromes and inflammatory states in humans and animals. How might disruption of TRPV1-expressing sensory neurons possibly affect bacterial clearance from the blood in resiniferatoxin-treated animals? One possibility is that intrathecal resiniferatoxin, which ablates 70% of TRPV1-expressing sensory neurons in mice, could ablate a peripheral neural network that is relevant for the inflammatory response. In support of this hypothesis are studies showing that, in addition to TRPV1, sensory neurons coexpress toll-like receptor 4 and CD14, the receptors that recognize bacterial wall products, such as lipopolysaccharide, and trigger the innate immune response to bacterial infections. Other researchers have also shown that the dorsal root ganglia sensory neurons of rats coexpress calcitonin gene-related peptide, TRPV1, and TNF receptors. Therefore, ablation of TRPV1-expressing sensory neurons by intrathecal administration of resiniferatoxin, possibly by altering expression of CD14, TNF receptors, and toll-like receptor 4 (co-expressed in those sensory neurons), could impair the host’s ability to recognize bacterial wall products and mount the immune response to clear bacterial infection.

Our finding that pharmacologic disruption of TRPV1 with resiniferatoxin and genetic disruption of the receptor in Trpv1-KO mice might have different effects on mortality, depending on whether inflammation is caused by polymicrobial sepsis or lipopolysaccharide-induced toxicity, has been previously reported in other mouse models. For example, the C3H/HeJ mice that have a toll-like receptor 4 mutation are resistant to lipopolysaccharide-induced shock and death and highly susceptible to Gram-negative bacterial infection.
Researchers have shown that this susceptibility to Gram-negative infection could be reversed by pretreatment of the C3H/HeJ mice with recombinant TNF and IL-1α, thus suggesting that cytokine generation by the host has an important role in the response to bacterial infection. In the current investigation, we found that after CLP, resiniferatoxin induced decreases in bacterial clearance from the blood that were coupled with down-regulation of TNF expression and increases in nitrate/nitrite concentrations. These findings suggest that the resiniferatoxin ablation of the TRPV1 sensory neurons was detrimental to survival, possibly because of alterations on host response to bacterial infection.

Our findings that capsazepine administered prophylactically (72 h before) and continued for 72 h after lipopolysaccharide challenge significantly increases mortality are in concert with results reported by others. Rats treated with capsazepine before intravenous administration of lipopolysaccharide had significantly decreased survival and worsened lipopolysaccharide-induced hypotension. In that rat study of lipopolysaccharide-induced toxicity, capsazepine’s deleterious effects on survival were associated with attenuation of sepsis-induced sympathetic response, likely explaining worsening lipopolysaccharide-induced hypotension. Conversely, in a study of CLP-induced polymicrobial sepsis in mice, one dose of capsazepine administered 30 min before the onset of sepsis significantly increased survival and decreased sepsis-induced pulmonary and hepatic neutrophil infiltration. Although the discrepancy of these results could possibly be related to differences in species studied, dose, and timing of administration of capsazepine and various TRPV1 agonists and antagonists, these conflicting results further suggest that the overall effect of TRPV1 disruptions during sepsis depends on the sepsis-inducing event and the method of disruption of the receptor and the TRPV1-expressing neurons.

During lipopolysaccharide-induced toxicity, TRPV1 antagonism with capsazepine decreased survival in a pattern that appears to be different from that observed with genetic or pharmacologic disruption (with resiniferatoxin) of the receptor. These discrepant results raise the possibility that the detrimental effects of capsazepine on lipopolysaccharide-induced mortality could result from TRPV1-unrelated effects. In fact, some previous reports showing that capsazepine can affect channels other than TRPV1 support this possibility. For example, capsazepine has reversibly inhibited the effects of nicotine in sensory neurons and blocked other voltage-activated calcium channels in the dorsal root ganglia. Capsazepine’s inhibitory effects on acetylcholine receptors might, in part, have contributed to the increased mortality in lipopolysaccharide-induced toxicity. Recent reports showing that activation of nicotinic acetylcholine receptors (by vagal stimulation) attenuate the inflammatory response to lipopolysaccharide in vitro and in vivo and increase survival in lipopolysaccharide-induced toxicity support that possibility. Further supporting the hypothesis that capsazepine worsens survival in sepsis independently of TRPV1 are findings that capsazepine inhibits the sympathetic response to sepsis and thereby worsens lipopolysaccharide-induced hypotension in septic rats. Therefore, the findings that capsazepine, but not resiniferatoxin, treatment or genetic deficiency of TRPV1 worsened survival after lipopolysaccharide suggest that the detrimental effects of capsazepine during lipopolysaccharide-induced toxicity are possibly TRPV1 unrelated.

Our findings that TRPV1 disruption affects cytokine and chemokine expression during sepsis are of interest and previously undescribed. In vitro, after CLP, resiniferatoxin treatment significantly down-regulated TNF gene expression; in vivo, capsazepine down-regulated TNF, CCL3, and IL-10 in lipopolysaccharide-treated splenocytes. These findings add to the previously reported inhibitory effects of capsazepine and resiniferatoxin on activation of inducible NO synthase and activation of key mediators of the host’s immune response (i.e., the transcription factors nuclear factor κB and activator protein 1). Therefore, taken together, our findings and those of others suggest that TRPV1 has an important role in the host immune response to bacterial infection and lipopolysaccharide-induced toxicity that can affect the expression of proinflammatory and antiinflammatory cytokines.

In summary, disruption of TRPV1, genetically, in Trpv1-KO mice, or pharmacologically (with intrathecal resi

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