Cell Cycle 11:2, 343-349; January 15, 2012; © 2012 Landes Bioscience

Aging reverses the role of the transient receptor potential vanilloid-1 channel in systemic inflammation from anti-inflammatory to proinflammatory

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Key words: TRP channels, sepsis, systemic inflammation, endotoxin shock

Abbreviations: CLP, cecal ligation and puncture; LPS, lipopolysaccharide; SIRS, systemic inflammatory response syndrome; T_b, body temperature; TNF, tumor necrosis factor; TRPV1, transient receptor potential vanilloid-1

Studies in young rodents have shown that the transient receptor potential vanilloid-1 (TRPV1) channel plays a suppressive role in the systemic inflammatory response syndrome (SIRS) by inhibiting production of tumor necrosis factor (TNF)α and possibly by other mechanisms. We asked whether the anti-inflammatory role of TRPV1 changes with age. First, we studied the effect of AMG517, a selective and potent TRPV1 antagonist, on aseptic, lipopolysaccharide (LPS)-induced SIRS in young (12 wk) mice. In agreement with previous studies, AMG517 increased LPS-induced mortality in the young. We then studied the effects of TRPV1 antagonism (AMG517 or genetic deletion of TRPV1) on SIRS in middle-aged (43–44 wk) mice. Both types of TRPV1 antagonism delayed and decreased LPS-induced mortality, indicating a reversal of the anti-inflammatory role of TRPV1 with aging. In addition, deletion of TRPV1 decreased the serum TNFα response to LPS, suggesting that the suppressive control of TRPV1 on TNFα production is also reversed with aging. In contrast to aseptic SIRS, polymicrobial sepsis (induced by cecal ligation and puncture) caused accelerated mortality in aged TRPV1-deficient mice as compared with wild-type littermates. The recovery of TRPV1-deficient mice from hypothermia associated with the cecal ligation and puncture procedure was delayed. Hence, the reversal of the anti-inflammatory role of TRPV1 found in the aged and their decreased systemic inflammatory response are coupled with suppressed defense against microbial infection. These results caution that TRPV1 antagonists, widely viewed as new-generation painkillers, may decrease the resistance of older patients to infection and sepsis.

Introduction

Systemic inflammatory response syndrome (SIRS) is the leading cause of death in hospitalized patients.[1,2] SIRS is considered a disease of the aged: its incidence and mortality are substantially higher in the older population.[3] SIRS can be either triggered by non-infectious insults, such as blunt trauma, or associated with an infection (in which case it is called sepsis). In the laboratory, systemic administration of lipopolysaccharide (LPS, a cell-wall constituent of Gram-negative bacteria) in mice and rats is often used to induce SIRS aseptically, whereas polymicrobial sepsis is often studied in rodents subjected to cecal ligation and puncture (CLP). In either model, shock and death can occur, largely as the result of the "cytokine storm," an overt production of proinflammatory cytokines, including TNFα,[4] and other mediators, cumulatively referred to as the "inflammatory soup."[5-7] In both LPS-induced SIRS and CLP-induced sepsis, proinflammatory cytokine production and mortality rate are much lower in young animals.[8-11] Furthermore, sepsis in young animals is much more responsive to treatment.[11]

Recent studies have brought attention to the role that the transient receptor potential vanilloid-1 (TRPV1) channel may play in SIRS. Abundant on small-diameter sensory nerve fibers, TRPV1 is activated by diverse stimuli, including several ingredients of the inflammatory soup.[12,13] Activation of TRPV1 on sensory nerves potently inhibits LPS-induced TNFα production.[14] Studies using either knockout (*Trpv1*$^{-/-}$) mice, a pharmacological blockade with capsazepine (TRPV1 antagonist) or desensitization with resiniferatoxin (TRPV1 agonist) have shown that TRPV1 plays an anti-inflammatory role in LPS-induced SIRS by, among other mechanisms, limiting the production of TNFα, possibly via sensory nerves.[15-17] However, all studies cited above were conducted

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Submitted: 10/20/11; Revised: 11/14/11; Accepted: 11/15/11
http://dx.doi.org/10.4161/cc.11.2.18772

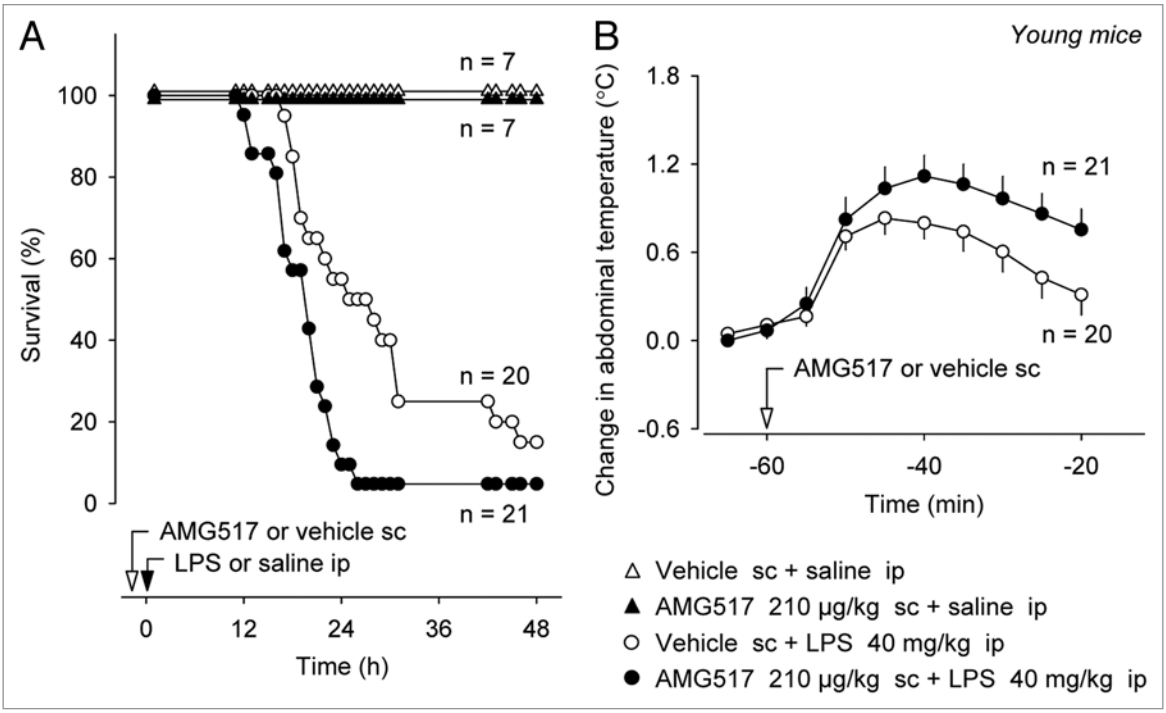

Figure 1. Systemic pretreatment with AMG517 (dose indicated) decreases survival of young mice in LPS-induced SIRS (A). Confirming an effective blockade of TRPV1 channels, the AMG517 pretreatment increases deep T_b in young mice (B).

Table 1. Effects of age and TRPV1 antagonism on mortality in LPS-induced SIRS and CLP-induced sepsis

Model	Variable	Cox survival regression analysis			Logrank test		Time to death	
		Hazard ratio of death	95% confidence interval	p	Score	p	Mean ± SE (h)	p
LPS	Aged vs. young	2.2	1.3 ÷ 3.1	<0.001	20.5	<0.001	16 ± 1 vs. 26 ± 2	<0.001
LPS in young	AMG517 vs. vehicle	0.9	0.2 ÷ 1.6	<0.010	4.1	<0.05	19 ± 1 vs. 26 ± 2	0.003
LPS in aged	AMG517 vs. vehicle	-1.0	-1.8 ÷ -0.2	<0.015	5.4	<0.05	19 ± 1 vs. 16 ± 1	<0.040
LPS in aged	*Trpv1*$^{-/-}$ vs. *Trpv1*$^{+/+}$	-1.3	-2.4 ÷ -0.2	<0.020	5.4	<0.05	24 ± 3 vs. 19 ± 1	<0.100
CLP in aged	*Trpv1*$^{-/-}$ vs. *Trpv1*$^{+/+}$	0.7	-0.5 ÷ 1.9	0.239	1.4	>0.05	20 ± 2 vs. 52 ± 11	<0.007

in young rodents. Whether TRPV1 channels play a similarly prominent anti-inflammatory role in the aged is unknown.

Results and Discussion

Effects of a TRPV1 antagonist on LPS-induced systemic inflammation in young mice. First, we verified whether pretreatment with AMG517, a potent and selective TRPV1 antagonist,[18,19] decreases the mortality of young adult (12 wk) C57BL/6 mice in LPS-induced SIRS. Mice responded to LPS (40 mg/kg, ip) with a marked, rapidly progressing SIRS (**Fig. 1A**). Pretreatment with AMG517 (210 µg/kg, sc) profoundly decreased the survival rate at multiple time points (e.g., from 50% to 5% at 26 h, $p < 0.001$), overall (48 h) survival rate (from 15% to 5%, $p < 0.05$) and increased the risk of mortality (hazard ratio of death of 0.9, $p = 0.01$, **Table 1**). AMG517 pretreatment also shortened the mean time to death from 26 ± 2 to 19 ± 1 h ($p = 0.003$). All LPS-treated mice, both in this experiment and in other experiments within the present study, developed profound hypothermia: deep (abdominal) body temperature (T_b) decreased from ~36°C to ~33°C at 10 h. However, no inter-treatment differences in the hypothermic response occurred during the short time period before mice started dying (data not shown). Similar to our previous studies in references 18 and 19, AMG517 caused a short-lasting increase in T_b compared with the vehicle ($p < 0.01$, **Fig. 1B**), thus confirming an effective systemic blockade of TRPV1 channels. Overall, the results of our experiment show that pharmacological blockade of TRPV1 increases mortality of young mice in LPS-induced SIRS. Similar observations have been made in adolescent (6–8 wk) mice and in rats treated with capsazepine.[16,17] It should be noted, however, that capsazepine is not a highly selective TRPV1 antagonist and has a low potency of blocking the proton mode of TRPV1 activation in the rat and mouse.[20] In fact, a non-TRPV1-mediated effect of capsazepine

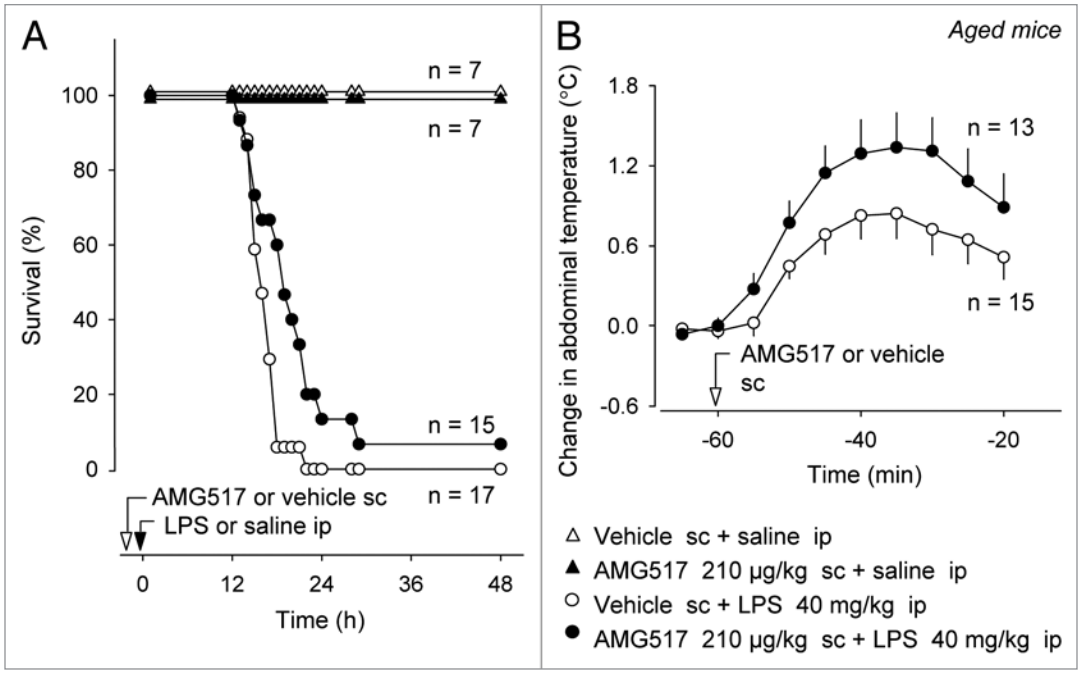

Figure 2. Systemic pretreatment with AMG517 (dose indicated) increases survival of aged mice in LPS-induced SIRS (A). Confirming an effective blockade of TRPV1 channels, the AMG517 pretreatment increases deep T_b in aged mice (B).

on the outcome of systemic inflammation has been proposed recently in reference 17. The present results also agree with the exaggerated symptoms of LPS-induced shock found in young mature (13–20 wk) *Trpv1*$^{-/-}$ mice.[15]

As reviewed by Steiner et al.[21] and Guptill et al.[17] many treatments affect mortality in LPS-induced aseptic inflammation and CLP-induced sepsis in opposite ways, confirming that the systemic inflammatory response per se can be harmful to the host, even though it is crucial for defending the host against infection.[6,7] For example, mice with a dysfunctional toll-like receptor 4 are resistant to LPS but are highly susceptible to Gram-negative bacterial infection.[22-24] This susceptibility to infection can be reserved by pretreating the toll-like receptor 4-deficient mice with TNF and interleukin-1α.[22] Interestingly, antibiotic treatment makes sepsis less different from aseptic SIRS, as it eradicates the infectious agent. For example, acute nicotine administration increases survival of mice in LPS-induced SIRS[21,25-27] and in antibiotic-treated CLP-induced sepsis,[25,26] but it worsens the outcome of untreated CLP-induced sepsis in the same species.[21] From this point of view, two studies of the role of TRPV1 in CLP-induced sepsis in adolescent (5–8 wk) mice[17,28] agree with the effects observed in LPS SIRS. The first study[17] has found that antibiotic-treated CLP-induced sepsis causes a higher mortality when TRPV1 channels are absent (*Trpv1*$^{-/-}$ mice) or desensitized (with intrathecal resiniferatoxin). The second study[28] has found that capsazepine increases survival in untreated CLP-induced sepsis. Overall, prior literature data obtained in young rodents (adolescents to mature adults) and our present experiment with AMG517 in young adult mice show that the effects of TRPV1 blockade on both LPS-induced SIRS and antibiotic-treated sepsis vary from none to strong exaggeration of severity and mortality,[15-17] whereas the effect of TRPV1 blockade on mortality in untreated sepsis is the opposite: attenuation.[28]

Effects of AMG517 on LPS-induced systemic inflammation in aged mice. To study whether the anti-inflammatory role of TRPV1 in SIRS is preserved with aging, we conducted experiments in middle-aged (44 wk) C57BL/6 mice (**Fig. 2A**). The outcome of LPS-induced SIRS in these older mice was more severe than in young mice (hazard ratio of 2.2, $p < 0.001$, **Table 1**). The mean time to death in vehicle-pretreated aged mice was 16 ± 1 h, and none of the vehicle-pretreated aged mouse survived for longer than 24 h. Pretreatment of aged mice with AMG517 (210 µg/kg, sc) increased the survival rate ($p < 0.05$), delayed the mean time to death (19 ± 1 h, $p < 0.05$) and decreased the risk of mortality (hazard ratio of -1.0, $p < 0.05$)—effects directly opposite of those observed in the young. Survival rate of AMG517-pretreated aged mice at 18 h was 10 times higher than that of vehicle-pretreated aged mice (60% vs. 6%, $p < 0.001$). Confirming a systemic blockade of TRPV1 channels in this experiment, AMG517 increased T_b as compared with the vehicle ($p < 0.05$, **Fig. 2B**). Hence, whereas the effect of AMG517 on LPS-induced systemic inflammation in aged mice was the opposite to that found in young mice (**Figs. 1A and 2A**), the effect on T_b was qualitatively the same (**Figs. 1B and 2B**). It is possible that the role of TRPV1 in different functions changes with age in a different way. In the regulation of locomotor activity[29,30] and inflammation (present results), the role of TRPV1 reverses with age. In the modulation of T_b (for mechanisms, review ref. 31), it does not. In the regulation of body mass, TRPV1 channels are either uninvolved[29] or counteract obesity[32] in the young but promote obesity in the aged.[29,30]

Effects of genetic deletion of TRPV1 channels on LPS-induced systemic inflammation in aged mice. We then

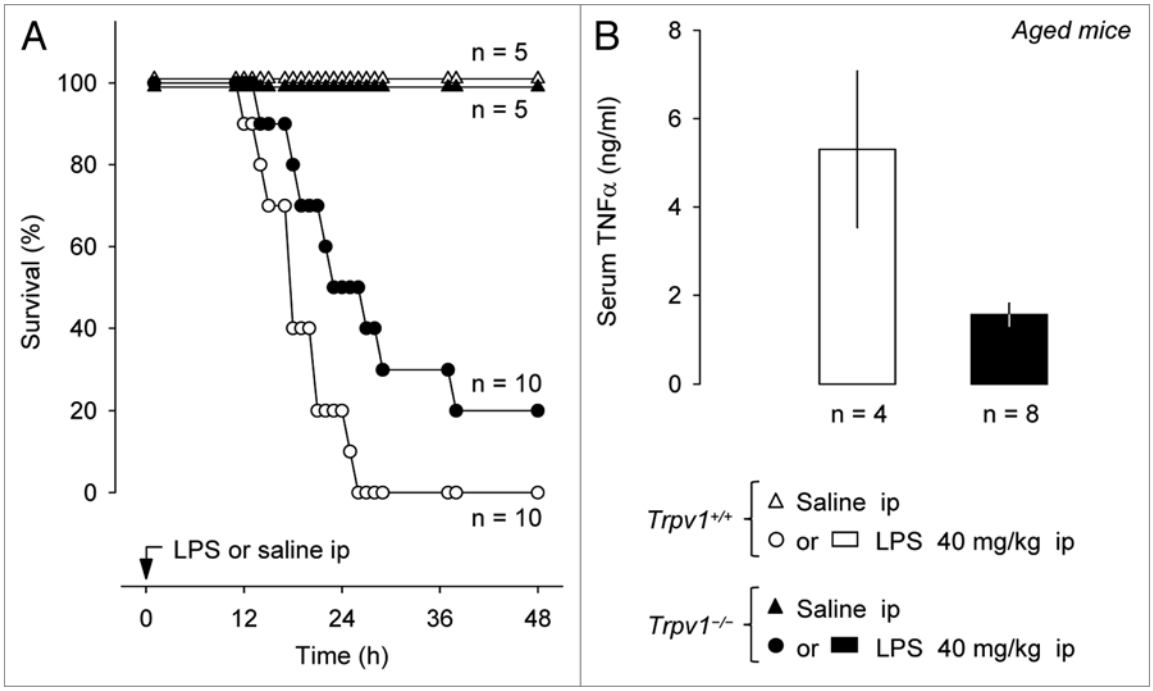

Figure 3. Compared with their age-matched wild-type littermates, middle-aged *Trpv1*$^{-/-}$ mice have a higher survival rate (A) and a lower serum TNFα concentration (B) during LPS-induced SIRS.

tested whether genetic deletion of TRPV1 would have the same effects on SIRS in middle-aged mice as a pharmacological blockade. Experiments were conducted in 43–44 wk-old *Trpv1*$^{-/-}$ C57BL/6 x 129 mice of both sexes and in their age- and sex-matched *Trpv1*$^{+/+}$ littermates. LPS caused death in all *Trpv1*$^{+/+}$ mice but only in 80% of *Trpv1*$^{-/-}$ mice ($p < 0.05$, **Fig. 3A**). Survival rate of *Trpv1*$^{-/-}$ mice at 21 h was 3.5 times higher than of wild-type controls (70% vs. 20%, $p < 0.001$). Genetic deletion of TRPV1 channels decreased the risk of mortality (hazard ratio of -1.3, $p < 0.05$) and tended to delay death ($p < 0.1$, **Table 1**). Importantly, *Trpv1*$^{-/-}$ mice exhibited a 70% suppression of the TNFα response at 12 h post-LPS ($p < 0.05$, **Fig. 3B**). It should be noted that aged *Trpv1*$^{-/-}$ mice in this study (see Materials and Methods) and in our previous studies in references 29 and 30 were overweight compared with their wild-type littermates. Obesity[33-36] and hyperlipidemia[37] associated with various mutations in rats do not seem to affect the febrile response to low, non-shock-inducing doses of LPS, and obesity does not seem to increase the risk of sepsis even though it increases the risk of an infection.[38] Nevertheless, systemic inflammation and obesity are intimately interconnected,[39,40] and we cannot rule out that obesity could have been a contributing factor to at least some of the effects found in aged *Trpv1*$^{-/-}$ mice.

Mechanisms of the age-associated reversal of the anti-inflammatory role of TRPV1 are unknown. Our TNFα data suggest that the reversal occurs at initial stages of the pathogenesis of SIRS—at or upstream of TNFα production. The TNFα response to LPS has been shown to be under suppressive control of TRPV1 channels on sensory nerves.[14] Loss of TRPV1-mediated suppression of TNFα production in aged animals may reflect reduced translation of the TRPV1 protein and its reduced transport to the periphery,[41] possibly due to age-associated decline in neurotrophic support to ganglionic neurons.[42] Changes in TNFα production may be central to aging-related changes in the pathobiology of sepsis: elderly patients respond to infection, including septic shock, with higher TNFα,[43,44] and inflammatory cytokine production in intensive-care-unit patients with sepsis is affected by TNFα-related genetic polymorphisms.[45]

Effects of genetic deletion of TRPV1 channels on CLP-induced sepsis in aged mice. Next, we tested whether the attenuation of aseptic SIRS (of LPS-induced TNFα response and mortality) observed in middle-aged *Trpv1*$^{-/-}$ mice would result in attenuation of the body's defense against CLP-induced polymicrobial infection. CLP sepsis caused substantial mortality in aged mice of both genotypes (**Fig. 4A**). However, *Trpv1*$^{-/-}$ mice died significantly faster than their *Trpv1*$^{+/+}$ littermates (**Table 1**). The mean time to death in *Trpv1*$^{-/-}$ mice was 20 ± 2 h, as compared with 52 ± 11 h in *Trpv1*$^{+/+}$ mice ($p < 0.01$), and the 30 h survival rate in *Trpv1*$^{+/+}$ mice was 3.9 times higher than in *Trpv1*$^{-/-}$ mice (86% vs. 22%, $p < 0.001$). Further confirming the higher severity of sepsis in *Trpv1*$^{-/-}$ mice, recovery of deep T_b after the CLP procedure (and the related anesthesia) was delayed ($p < 0.001$, **Fig. 4B**).

Conclusions. The present study shows that the anti-inflammatory role firmly established for TRPV1 channels in young rodents[15-17] is reversed with aging. Whereas pharmacological or genetic TRPV1 antagonism decreases the survival rate in aseptic SIRS and in antibiotic-treated sepsis in the young, both types of TRPV1 antagonism have the opposite effect on aseptic SIRS in middle-aged mice. The age-dependent reversal of the

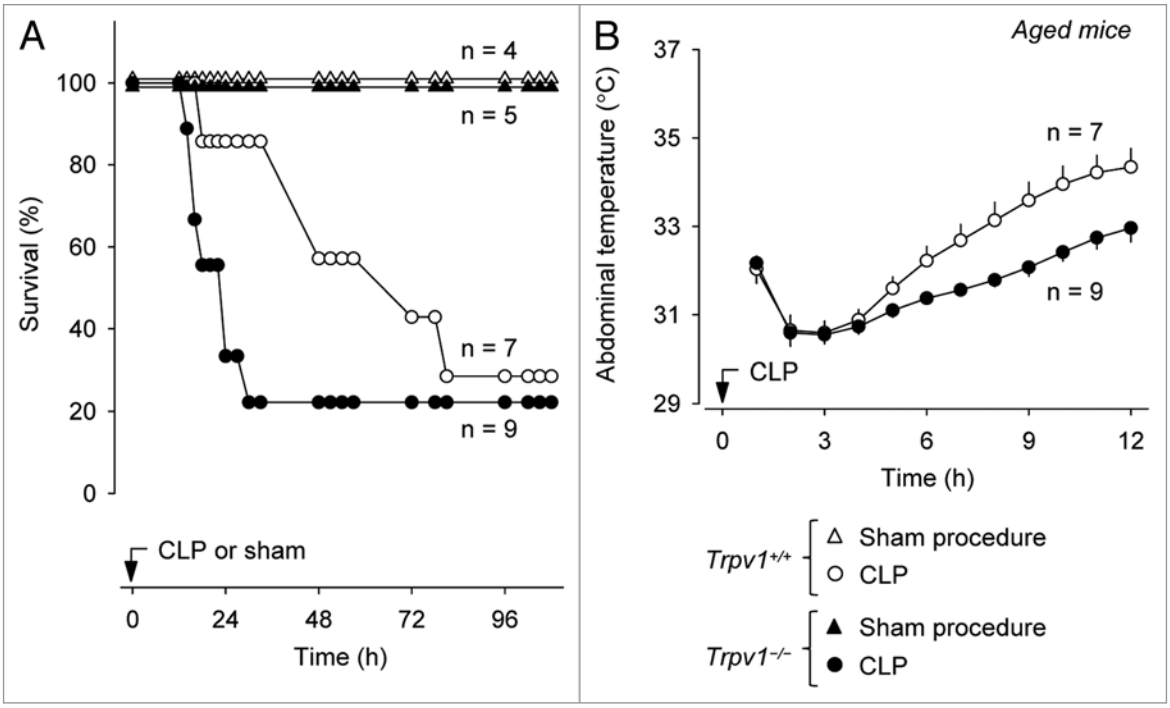

Figure 4. Compared with their age-matched wild-type littermates, middle-aged *Trpv1*$^{-/-}$ mice have a shorter survival time (A) and a slower T_b recovery (B) during CLP-induced sepsis.

anti-inflammatory role of TRPV1 to proinflammatory is likely due, at least in part, to a reversal of the suppressive control of TRPV1 on TNFα production. These pathobiological changes are highly important, as evident from the decreased ability of aged *Trpv1*$^{-/-}$ mice to resist polymicrobial sepsis. The reversal of the anti-inflammatory role of TRPV1 reported in this study is another example of profound effects of aging on the pathobiology of systemic inflammation.[11,46,47]

Significance. Our findings may influence development of TRPV1 antagonists, widely viewed as new-generation painkillers.[18,20,48] If what we found for murine models applies to human sepsis, anti-TRPV1 therapy may suppress the systemic inflammatory response in the previously uninfected (untreated with antibiotics) elderly and, hence, decrease their resistance to bacterial infection and sepsis. This potential side effect is especially serious, because recognition of infection is often complicated in older patients by a variety of factors, including the absence of fever, which often delays treatment.[49,50]

Materials and Methods

The study was conducted in 168 adult mice (either young or middle-aged) of both sexes, including 101 *Trpv1*$^{+/+}$ C57BL/6 mice (Charles River Laboratories) and 67 *Trpv1*$^{-/-}$ or *Trpv1*$^{+/+}$ C57BL/6 x 129 mice (Amgen colony at Charles River Laboratories). Several phenotypic properties of *Trpv1*$^{-/-}$ mice from the Amgen colony have been characterized in our recent studies in references 29 and 30. At the time of experiments, young adult C57BL/6 mice were 12 wk-old, and their body mass was 25 ± 0 g (n = 55); middle-aged C57BL/6 mice were 44 wk-old, and their body mass was 32 ± 1 g (n = 46). The ages of C57BL/6 mice listed are approximate; the vendor filled orders for a specified age, but did not provide the date of birth for each mouse. Middle-aged *Trpv1*$^{+/+}$ C57BL/6 x 129 mice were 43 ± 1 wk-old (n = 30), and their body mass was 40 ± 1 g. Middle-aged *Trpv1*$^{-/-}$ C57BL/6 x 129 mice were 44 ± 1 wk-old (n = 37) and significantly heavier (46 ± 1 g; $p < 0.001$) than their wild-type littermates. The ages of C57BL/6 x 129 mice were calculated based on the dates of birth, which were known for all mice. Mice were maintained, surgically prepared and habituated to experimental setups as described in our earlier studies.[21,29] All surgeries were performed under ketamine-xylazine-acepromazine (81.7, 9.3 and 1.2 mg/kg, ip) anesthesia. Antibiotic protection (enrofloxacin, 1.1 mg/kg, sc) was provided, except for animals subjected to CLP. For deep T_b measurements, all mice were implanted intraperitoneally with telemetry transmitters (G2 E-Mitter series, Mini Mitter). For CLP, under the same anesthesia, the cecum was pulled out of the abdominal cavity, filled with the intestinal content (by gently squeezing the content from the ascending colon) and ligated with 3-0 silk just distal to the ileocecal junction. The cecal wall was punctured through at the antimesenteric side with a 26-gauge needle. All protocols were approved by the St. Joseph's Hospital and Medical Center Animal Care and Use Committee.

During all experiments, mice were housed singly in their home cages placed inside a climatic chamber (model 3940, Forma Scientific) with an ambient temperature maintained at 28.0°C, i.e., within the thermoneutral zone for this experimental setup.[29,51] Cages were kept on top of telemetry receivers (model ER-4000, Mini Mitter). In the experiments with LPS-induced

SIRS, the survival rate and T_b were monitored for 48 h. To this end, mice were periodically examined for the presence of spontaneous movements and cardiac and respiratory activities. Because deep T_b decreases steeply toward the ambient temperature when an animal dies, T_b curves were also examined to identify mortality events. In the experiments with CLP-induced sepsis, the same parameters were monitored for 108 h. At the end of experiments, any survivors were euthanized with sodium pentobarbital (200 mg/kg, ip).

In a separate series of experiments (under the same anesthesia as for surgery), blood (1 ml) was collected by cardiac puncture at 12 h after LPS administration, and mice were euthanized with sodium pentobarbital. The 12 h time point for blood collection was chosen because aged mice in this model start dying shortly after this point (**Fig. 3A**). Serum concentration of TNFα was determined by ELISA according to the manufacturer's instructions (SABiosciences, catalog number MEM-004A).

A suspension of *E. coli* 0111:B4 LPS (Sigma-Aldrich, L2630, 2.5 mg/ml) in saline was prepared in advance and stored at 4°C. To induce SIRS, LPS (40 mg/kg ip) was injected as a bolus; controls received saline.

AMG517 (gift from Amgen) was used to block TRPV1 receptors pharmacologically. This is a highly potent and selective TRPV1 antagonist that had been tested in human patients.[18] Aliquots of an ethanolic solution of AMG517 (3 mg/ml) were stored at -80°C. This stock was diluted with ethanol and saline ex tempore to achieve a 21 μg/ml final concentration of AMG517 in 3.3% ethanol. AMG517 (210 μg/kg sc) or its vehicle was administered as a bolus, 1 h before the administration of LPS (or saline).

Survival data were analyzed by the χ^2 test for individual time points and by the logrank test[52] for the entire observation period. The Cox proportional hazard survival regression model was used to determine hazard ratios of death.[53] Unpaired student's t-test was used to compare times to death and TNFα concentrations. Deep T_b values were compared across treatments and time points by two-way ANOVA; p-values for the entire duration of response are reported. A difference was considered significant at $p < 0.05$. Results are reported in the format mean ± SE.

Disclosure of Potential Conflicts of Interest

N.R.G. is employed by Amgen Inc. A.A.R. has consulted for TRP programs at Amgen, Inc. and several other pharmaceutical companies, and his TRP-related research has been supported by Amgen, Inc. and Abbott Laboratories.

Acknowledgements

We thank Tatiane B. Nucci and Justin Eales for their help with animal work, Drs. Junwei Hao and Fu-Dong Shi for help with the ELISA assay, and Catherine M. Krall and Julie M. Turko for editing the manuscript. A.G.'s and E.P.'s present address is University of Pécs Medical School, Pécs, Hungary. This research has been supported in part by the National Institutes of Health (grant R01NS41233 to A.A.R.) and Amgen, Inc., (study agreements with A.A.R.). S.P.W. was a Fellow of the National Council for Scientific and Technological Development, Brazil. Author contributions: S.P.W., C.C.C. and A.A.R. designed the study. S.P.W., A.G., E.P. and D.L.O. performed experiments. N.R.G. provided reagents and materials. S.P.W. and A.A.R. analyzed data and wrote the manuscript.

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