Mast cells are necessary for the hypothermic response to LPS-induced sepsis

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Nautiyal KM, McKellar H, Silverman A, Silver R. Mast cells are necessary for the hypothermic response to LPS-induced sepsis. Am J Physiol Regul Integr Comp Physiol 296: R595–R602, 2009. First published December 24, 2008; doi:10.1152/ajpregu.90888.2008.—As central nervous system residents, mast cells contain many cytokines and are localized primarily near large blood vessels in the diencephalon and within the leptomeninges, making them candidates for immune to neural “cross talk.” Using mast cell-deficient KitW-sh/W-sh mice, we assessed the role of these cells in the thermoregulatory component of the immune response to lipopolysaccharide (LPS). KitW-sh/W-sh and wild-type (WT) mice differed in several respects in response to injection of a high dose of LPS (1 mg/kg ip). Core temperature (Tc) of WT mice decreased by ~3°C, whereas KitW-sh/W-sh mice did not become hypothermic but instead exhibited pronounced low-frequency Tc oscillations around their baseline temperature. In addition, KitW-sh/W-sh mice had lower levels of whole brain TNF-α but no differences in IL-1β, IL-6, IFN-γ, or histamine compared with WT mice following injection of the high dose of LPS, consistent with the role of TNF-α in sepsis. KitW-sh/W-sh mice had increased resistance to LPS, and some survived a dose of LPS that was lethal in littermate controls. In contrast, KitW-sh/W-sh and WT mice were similar in other respects, namely, in the hypothermia following injection of TNF-α (1.5 μg icv), reduced nighttime Tc, and locomotor activity (to 1 mg/kg LPS), response to a low dose of LPS (10 μg/kg ip), and response to subcutaneous pertussine injection. These results indicate that mast cells play a role in the regulation of thermoregulatory responses and survival following sepsis induction and suggest a brain site of action.

The hyperthermia induced by low doses of LPS, the mechanisms and physiological role of the hyperthermia resulting from LPS-induced sepsis remain unclear (19). This hypothermic response to LPS, however, is dependent on ambient temperature (3). A dose of LPS that causes hypothermia at room temperature (~21°C) elicits fever when injected in animals housed within their thermoneutral zone (~31°C for C57BL/6 mice) (34). The decline in core body temperature (Tc) that occurs at room temperature, known as anaplepsy, is a regulated response involving the establishment of a new, lower set point in Tc (4) rather than an uncontrolled loss of body heat due to decreased metabolic heat production or hypotension or organ failure. Overproduction of cytokines [such as tumor necrosis factor (TNF)-α, interleukins (IL), and interferons (IFN), among many others], contribute to sepsis-induced lethality (31). In addition, the reduction in Tc further stimulates NF-κB-dependent cytokine production, thereby increasing the severity of sepsis (8).

Mast cells are part of the innate immune system and have long been implicated in allergy and local inflammation (27). It has been shown more recently that mast cells also have a critical and protective role in the initiation of an immune response to pathogens (reviewed in Ref. 21). In response to bacterial infection, mast cells increase the recruitment of neutrophils and aid in bacterial clearance (23). In mounting a host response against LPS, mast cells bind the LPS-LPB complex to their surface TLR4 (39). Upon activation, mast cells synthetize and release mediators including amines, cytokines, and lipid-derived factors, many of which are implicated in the thermoregulatory response to LPS (20, 30, 37).

Mast cells are found at host-environment interfaces and in many vascularized tissues in the body (27). They also are resident in the brain and are localized to the diencephalon, hippocampus, and surrounding leptomeninges (14, 17, 37, 38). Specifically, many mast cells are found on the brain side of blood vessels adjacent to astrocytic and neuronal processes (15). Electrophysiological evidence suggests that mast cell mediators can influence neuron firing (16).

A link has been made between brain mast cells and the thermoregulatory response to LPS. Blocking the degranulation of brain mast cells with intracerebroventricular administration of disodium cromoglycate (cromolyn, a mast cell stabilizer) reduces the hyperthermia to a low dose of LPS in the rat (28). Because cromolyn also affects eosinophils and neutrophils and disrupts the signaling and chemotaxis of other immune cells (29), we sought to clarify the involvement of brain mast cells in the response to LPS-induced sepsis and to further investigate the underlying mechanisms.
In the present study we have used mast cell-deficient Kit<sup>W-sh/W-sh</sup> “sash” mice, which serve as a powerful model for the study of mast cell function in vivo (42). Kit<sup>W-sh/W-sh</sup> mice carry an inversion mutation upstream of the promoter region reducing c-kit receptor function (6, 18). The reduction in c-kit expression on stem cells in the bone marrow inhibits the myeloid progenitor from differentiating into mast cell precursors. This results in a mast cell deficiency but no other known disruptions in the development of hematopoietic stem cell derivatives (12). In addition, c-kit receptor mRNA and protein expression are normal in other tissues, including brain, liver, thymus, and primordial germ cells (6, 12). Thus the Kit<sup>W-sh/W-sh</sup> mouse permits investigation of the role of mast cells in thermoregulatory responses to endotoxemia. We compared Tc, activity, cytokine profiles, and lethality responses of Kit<sup>W-sh/W-sh</sup> and wild-type or littermate control mice to systemic and localized injection of three doses of LPS and turpentine.

**METHODS**

**Animals and Housing**

Homozygous male mast cell-deficient Kit<sup>W-sh/W-sh</sup> mice, C57BL/ Ka-Thy1.1-CD45.1 (Ly5.2) (Jackson Laboratory, Bar Harbor, ME) were bred to establish a colony at the Columbia University Animal Facility. For experiments 1 and 2, wild-type (WT) C57BL/6 mice (Charles River Breeding Facility, Charles River, MA) served as controls. For experiments 3–5, heterozygous and WT littermates of Kit<sup>W-sh/W-sh</sup> mice served as controls.

All mice were housed individually from 10 wk of age in a 12:12-h light-dark cycle. Ambient temperature was 21 ± 0.5°C during the experiment. Purina rodent chow (5001) and water were available ad libitum. All husbandry procedures and experimental methods were reviewed and approved by the Columbia University Institutional Animal Care and Use Committee.

**Transponder Implantation**

Transponders (models ER-4000 or XM-FH; MiniMitter, Bend, OR) were inserted subcutaneously at 2 h after lights on. Activity and T<sub>c</sub> data were collected every 2 min from each mouse. Activity and T<sub>c</sub> data collected over the 3 days before experimental manipulations established baseline responses for within-animal comparisons. All mice were habituated to the handling and injection process with an intraperitoneal injection of pyrogen-free saline (PFS) at 2 h after lights on. Activity was measured as an index of sickness behavior. Furthermore, because activity itself raises body temperature, this measure provided assessment of thermal effects that are not activity dependent. Data were collected continuously starting from the preinjection baseline through 6 days following injection.

**Temperature and Activity Measurements**

Activity and T<sub>c</sub> data were collected every 2 min from each mouse. Activity and T<sub>c</sub> data collected over the 3 days before experimental manipulations established baseline responses for within-animal comparisons. All mice were habituated to the handling and injection process with an intraperitoneal injection of pyrogen-free saline (PFS) at 2 h after lights on. Activity was measured as an index of sickness behavior. Furthermore, because activity itself raises body temperature, this measure provided assessment of thermal effects that are not activity dependent. Data were collected continuously starting from the preinjection baseline through 6 days following injection.

**Intracerebroventricular Cannulations**

For examination of the response to TNF-α, animals were allowed to recover from transponder implantation for 1 wk and then anesthetized and implanted stereotaxically (David Kopf Instruments, Tujunga, CA) with chronic indwelling cannulas (Plastics One, Roanoke, VA). The tip of the cannula was positioned 0.2 mm dorsal to the right lateral ventricle (coordinates: AP, 0.5 mm from bregma; ML, 1.2 mm; DV, 2.5 mm from skull). Screws (McMaster Carr, Princeton, NJ) were anchored to the skull, and dental cement (Jet Brand Acrylics; Lang Dental, Wheeling, IL) was used to secure the guide cannula to the skull. Animals were allowed to recover for 1 wk following cannulation.

Injections of TNF-α (1.5 μg/2 μl; Sigma Aldrich, St. Louis, MO) were made with a 10-μl Hamilton (Reno, NV) syringe attached to polyethylene-50 tubing. The injector cannula was 0.2 mm longer than the guide cannula. Cannula placement was verified following the experiment by injection of india ink.

**Assays**

Trunk blood was collected in K<sub>2</sub>EDTA tubes (BD, Franklin Lakes, NJ). Whole blood was spun at 3,000 g for 15 min, and plasma was collected into centrifuge tubes and stored at −80°C until processed. The brains were rapidly removed from the crania following decapitation and placed on ice for dissection. For assay of TNF-α, the cerebellum and cortex were removed. For all other assays, whole brains were used. The tissue was weighed, put in a solution of phosphate buffer with complete mini protease inhibitor tablets (Roche Diagnostics), homogenized using an electronic pestle, and then centrifuged at 7,500 g for 20 min at 4°C. The supernatant was stored at −80°C until processed.

TNF-α, interleukin (IL)-1β, IL-6, and interferon (IFN)-γ, and histamine levels in plasma and central nervous system homogenates were analyzed using commercially available ELISA kits according to the manufacturer’s directions (Ready-SET-Go ELISA; eBioscience, San Diego, CA for cytokines; Cayman Chemicals, Ann Arbor, MI for histamine). Plates were read on a Dynex spectrophotometer. Dynex Revelation software (v4.06) was used to fit optical densities to the curve generated by the standards provided by the manufacturer. Samples were diluted so that they fell within the linear portion of the standard curve. Brain cytokine and histamine levels are reported per gram of brain weight.

**Experimental Design**

**Experiment 1: Systemic immune response.** To assess systemic responses, LPS was administered intraperitoneally at two doses. WT (n = 7) and Kit<sup>W-sh/W-sh</sup> (n = 9) mice weighing 26.0 ± 0.4 and 26.6 ± 0.6 g, respectively, each received one intraperitoneal injection of PFS and 1 mg/kg LPS from *Escherichia coli* 0111:B4 (lot no. 104K4109; Sigma Aldrich) in a PFS vehicle in counterbalanced fashion. Injections were given 2 h after lights on. Three days intervened between the injections. A second group of WT (n = 9) and Kit<sup>W-sh/W-sh</sup> (n = 10) mice weighing 23.2 ± 0.6 and 20.9 ± 0.3 g, respectively, were similarly injected with PFS and 10 μg/kg LPS.

**Experiment 2: Local immune challenge.** After an interval of 2 wk, animals that had been given a low dose of LPS in experiment 1 were tested to assess their ability to respond systemically to a local immune challenge. Mice of each genotype, WT (n = 6) and Kit<sup>W-sh/W-sh</sup> (n = 6), were given a subcutaneous injection of 0.15 ml of turpentine in the right posterior thigh. WT (n = 3) and Kit<sup>W-sh/W-sh</sup> (n = 4) mice received an injection of the equivalent volume of saline. Activity and T<sub>c</sub> were measured for 6 days following injection.

**Experiment 3: LPS effects on cytokines and histamine.** To evaluate the levels of TNF-α, IL-1β, IL-6, IFN-γ, and histamine, Kit<sup>W-sh/W-sh</sup> mice and littermate controls were injected with LPS (1 mg/kg) or saline 2 h after lights on. Two hours following injection, mice were rapidly decapitated and trunk blood and brains were collected for cytokine and histamine assays. For TNF-α assays, 4 Kit<sup>W-sh/W-sh</sup> mice and 5 littermate controls were used; for IL-1β, IL-6, and IFN-γ assays, 13 Kit<sup>W-sh/W-sh</sup> mice and 13 littermate controls were used; and for histamine assays, 9 Kit<sup>W-sh/W-sh</sup> mice and 8 littermate controls were used.
Experiment 4: Central TNF-α administration. To assess their ability to respond to TNF-α, KitW-sh/W-sh mice (n = 3) and their littermate controls (n = 3) received a 2-μl injection of PFS for habituation to injection. Three days later, each mouse was injected with 2 μl of PFS and TNF-α (1.5 μg/2 μl) in a counterbalanced order with a 3-day intercondition interval. All injections took place 2 h after lights on. Tc and activity were monitored as previously described.

Experiment 5: LPS-induced lethality. For analysis of tolerance to septic lethality, KitW-sh/W-sh mice (n = 16) and littermate controls (n = 14) were injected intraperitoneally with LPS (15 mg/kg) or PFS (n = 6 mice per genotype) at 2 h after lights on. The mice were run in two cohorts, and the data from both runs were combined following statistical verification of no between-cohort differences. The status of the mice was monitored continuously for the first 8 h and thereafter as necessary at a minimum of 8-h intervals by using a clinical checklist that included parameters such as movement and posture as well as symptoms of illness such as mucus discharge or respiratory and gastrointestinal distress.

Data Analysis

Tc over the experimental period was quantified by calculating the area under the curve (AUC) for each animal’s record. Two-way analysis of variance (ANOVA; 2 × 2: treatment × genotype) was performed on activity measures in the form of average counts per 15-min bin (cpb) and on AUC indices of Tc. Post hoc comparisons were done using Tukey’s honestly significant difference, as appropriate. Oscillations in Tc were quantified using the spectrum function in the R statistical language. This calculates the power of the fast-Fourier transform (FFT) based on residuals from a regression line fit to the data. The maximum power and the frequency of that power were noted and compared using a two-way ANOVA (2 × 2: treatment × genotype). Survival curves from the LPS lethality experiment were compared between genotypes with a Mantel-Cox log-rank test. Differences were considered statistically significant when P < 0.05. Data are presented as means ± SE.

RESULTS

Experiment 1: Systemic Immune Response

The response to an injection of LPS at 1 mg/kg ip for the 10 h following injection is shown in Fig. 1. Initially, all mice showed an injection stress-induced hyperthermia. Thereafter, WT mice exhibited a hypothermic response that was significantly different from baseline by −3.5 h after treatment, peaking at 2.3°C below baseline at 4.5 h (Fig. 1A; 34.3 ± 1.2 vs. 36.6 ± 0.3°C; P < 0.01). In contrast, KitW-sh/W-sh mice did not exhibit a hypothermic change in Tc, but rather maintained a mean Tc that was not significantly different from their preinjection baseline (36.1 ± 0.5 vs. 36.6 ± 0.2°C; P > 0.05).

FFT analysis of temperature profiles of individual mice revealed fluctuations in Tc of KitW-sh/W-sh but not in WT mice. As shown in representative records, the WT mouse exhibited a stable Tc profile following injection of saline (Fig. 1Bi) and a hypothermic response following LPS (Fig. 1Bii) as indicated

![Fig. 1](http://ajpregu.physiology.org/)}
above in the group data. The KitW-sh/W-sh mouse also showed a stable Tc profile following injection of saline (Fig. 1Biii). In contrast, following LPS, the KitW-sh/W-sh mouse showed high-amplitude (0.75°C), low-frequency (0.44 cycles/h) fluctuations of Tc around the baseline (Fig. 1Biv). The group means for the power of these low-frequency oscillations reveal that the Tc of KitW-sh/W-sh mice oscillated significantly more at lower frequencies than in WT mice (Fig. 1C).

Tc and activity levels in response to the same dose of LPS above, are shown over a 72-h interval in Fig. 2. Baseline conditions were recorded continuously for 3 consecutive days before injections; there were no significant differences between WT and KitW-sh/W-sh mice in Tc or locomotor activity, and data for the two groups were combined. Both groups showed disrupted long-term regulation of Tc and activity compared with baseline over 3 days following LPS injection. Specifically, WT and KitW-sh/W-sh mice lacked the normal nightly rise in Tc for 2 and 3 nights, respectively, following treatment (P < 0.01; Fig. 2A). Also, activity was reduced for 3 nights after injection in both WT and KitW-sh/W-sh mice (P < 0.01; Fig. 2B).

When injected with a low dose of LPS (10 μg/kg), there were no significant differences in Tc between WT and KitW-sh/W-sh mice (Fig. 3). Both groups showed injection-stress induced short-latency, short-duration hyperthermia and then a return to baseline within 1 h following injection. Subsequently, they exhibited an average increase in Tc of 1.2°C lasting 1–4 h after injection (P > 0.05; Fig. 3).

Experiment 2: Local Immune Challenge

Examination of the systemic response to local immune challenge allowed assessment of other thermoregulatory immune mechanisms. There were no significant differences in Tc or locomotor activity between WT and KitW-sh/W-sh mice following subcutaneous turpentine administration for the 3 days after injection, in either the light or dark phase (Fig. 4A; range over days: P > 0.18 to 0.11). Both genotypes showed normal baseline circadian fluctuation in Tc (low in day and high at night) over the first day following injection. In the next 48-h interval, they showed a low-grade, long-term fever with elevated Tc during the light phase rather than their normal daytime troughs (range over days: P < 0.001 to .04 for WT and P < 0.001 to .002 for KitW-sh/W-sh). Both genotypes recovered to baseline Tc levels by the sixth day following turpentine injection (P > 0.05 for WT and KitW-sh/W-sh). All mice showed reduced nighttime activity, with a 3-day average of 7.9 ± 1.0 and 4.8 ± 0.6 cpb compared with baseline averages of 26.1 ± 0.9 and 21.3 ± 2.3 cpb for WT and KitW-sh/W-sh mice, respectively (Fig. 4B). Nighttime activity levels returned to normal on the sixth dark cycle following injection (P > 0.05 for WT and KitW-sh/W-sh).

Experiment 3: LPS Effects on TNF-α, IL-1β, IL-6, IFN-γ, and Histamine Levels

Because of the observed differences in septic hypothermia, we measured brain and plasma levels of major mast cell derivatives known to mediate hypothermia (19, 20, 40) in littermate controls and KitW-sh/W-sh mice following LPS or saline injection (Fig. 5). For TNF-α, there were significant differences between groups: KitW-sh/W-sh mice had an attenuated rise in brain TNF-α levels 2 h after 1 mg/kg ip LPS injection (Fig. 5A; 43.4 ± 0.2 vs. 26.5 ± 3.2 pg/g of brain tissue).
tissue for littermate controls and Kit<sup>W-sh/W-sh</sup>, respectively; P < 0.01). There were no significant differences between littermate controls and Kit<sup>W-sh/W-sh</sup> mice in brain TNF-α levels following injection of saline (0.3 ± 0.5 and 3.4 ± 4.8 pg/g of tissue, respectively). Examination of levels of plasma TNF-α revealed no significant differences between littermate control and Kit<sup>W-sh/W-sh</sup> mice following saline or LPS injection (Fig. 5B; saline: 3.2 ± 4.5 and 9.5 ± 2.0 pg/ml; LPS: 1,506.5 ± 161.4 and 1,291.8 ± 142.0 pg/ml for littermate controls and Kit<sup>W-sh/W-sh</sup>, respectively).

Of the other cytokines measured, IL-1β, IL-6 and IFN-γ, none were different (P > 0.05) between genotypes in either brain or plasma following saline or LPS treatment (Table 1). For IL-1β and IL-6, there was a main effect of LPS treatment (P < 0.01) for both brain and plasma samples. There were no main effects of LPS treatment on plasma levels of IFN-γ (P > 0.05). However, there was a main effect of LPS treatment (P < 0.05) on brain levels of IFN-γ.

As expected, brain and plasma levels of histamine were higher in control than in sash mice following saline injection (Fig. 5, C and D; P < 0.05 for brain, P < 0.01 for plasma). After LPS treatment, however, there were no differences in brain levels of histamine between genotypes (P > 0.05), although plasma levels were lower in sash mice (P < 0.01).

**Experiment 4: Central TNF-α Administration**

Given the differences in brain TNF-α levels between Kit<sup>W-sh/W-sh</sup> mice and controls following LPS injection, we next examined the response to centrally injected TNF-α. There were no significant differences between littermate controls and Kit<sup>W-sh/W-sh</sup> mice in the T<sub>c</sub> responses to intracerebroventricular injection of LPS (1 mg/kg) on brain and plasma levels of TNF-α (A and B) and histamine (C and D) in littermate control and Kit<sup>W-sh/W-sh</sup> mice. For brain TNF-α (A), 2 h following injection of LPS, control mice showed a larger increase TNF-α compared with Kit<sup>W-sh/W-sh</sup> mice. There was no difference in levels of brain TNF-α following saline injection (P > 0.05). Levels of plasma TNF-α (B) were not different between genotypes following saline or LPS injection. For histamine (C), Kit<sup>W-sh/W-sh</sup> mice had lower brain levels following saline injection compared with littermate controls, as expected. However, following LPS injection, there were no significant differences in brain levels between Kit<sup>W-sh/W-sh</sup> and control mice (P > 0.05). Plasma levels of histamine (D) were lower in Kit<sup>W-sh/W-sh</sup> mice compared with littermate controls following saline and LPS injections. *P < 0.05; **P < 0.01.
TNF-α (Fig. 6). Both genotypes had a short latency rise in Tc, peaking at ~1 h due to injection-induced hyperthermia (Fig. 6); this was also seen in saline-injected animals. Subsequently, both littermate control and KitW-sh/W-sh mice showed a TNF-α-induced rise in Tc, beginning ~3 h following injection, with a return to baseline temperature by 12 h (P < 0.01 and 0.03 for control and KitW-sh/W-sh mice, respectively).

Experiment 5: LPS-Induced Lethality

Given evidence showing that a reduction in core temperature is an indicator of mortality (41), we measured lethality to a 15 mg/kg dose of LPS in littermate control and KitW-sh/W-sh mice. Mast cell-deficient mice have increased resistance to LPS-induced septic lethality compared with their littermate controls. Percent survival is plotted for both groups every 12 h following injection (Fig. 7). Significantly more KitW-sh/W-sh mice survived (30%) induced septic lethality compared with their littermate controls. *P < 0.05, LPS vs. saline within genotype. †P < 0.05, main effect of LPS treatment within the measure.

DISCUSSION

The differences between WT and KitW-sh/W-sh mast cell-deficient mice revealed in the present study implicate mast cells in the thermoregulatory response to LPS-induced sepsis, highlighting a novel element that contributes to the immune response during endotoxic shock. Specifically, we have shown that mast cells mediate the hypothermic response following induction of LPS-induced sepsis. KitW-sh/W-sh mice fail to develop the profound hypothermia seen in WT mice; instead, they show large Tc fluctuations around baseline. These fluctuations of Tc are not present in the control conditions in KitW-sh/W-sh mice. This hysteresis-like variability represents thermoregulatory competence. The results also show that in response to LPS-induced sepsis, KitW-sh/W-sh mice, like their WT counterparts, have reduced nighttime Tc and locomotor activity. In addition, KitW-sh/W-sh mice show an intact systemic immune response following either a low dose of LPS (10 µg/kg) or local turpentine injection. Together, these data demonstrate that KitW-sh/W-sh mice can detect LPS as a pathogen and mount appropriate responses. KitW-sh/W-sh mice have a smaller increase in brain TNF-α compared with littermate controls following high-dose injection of LPS. Nonetheless, their thermal response to central injection of TNF-α is not different from controls, indicating that they have the ability to respond appropriately to TNF-α. Finally, lethality caused by LPS-induced sepsis is attenuated in the absence of mast cells. Therefore, it is possible that both the lack of hypothermia and the smaller elevation in brain TNF-α is beneficial to the survival of KitW-sh/W-sh mice.

Mast cells produce numerous amine-, cytokine-, and lipid-derived mediators implicated in inflammation (25). We assayed TNF-α, IL-1β, IL-6, IFN-γ, and histamine and found differences between KitW-sh/W-sh and littermate controls in response to LPS in TNF-α alone. Not surprisingly, histamine...
levels were lower in Kit<sup>W-sh/W-sh</sup> than in control mice following saline but did not differ between these groups in brain or plasma following LPS. The rise in brain histamine in Kit<sup>W-sh/W-sh</sup> mice following LPS indicates involvement of a non-mast cell-derived source (14). It is unlikely that this rise in histamine mediates the thermoregulatory differences seen between genotypes because central histamine causes hypothermia (11), a response that is absent in the Kit<sup>W-sh/W-sh</sup> mice.

Although it is improbable that TNF-α alone mediates the response observed, previous studies have implicated TNF-α in the cytokine storm that mediates the hypothermic response in sepsis (20). High circulating levels of TNF-α are correlated with reduction in T<sub>c</sub> (20). Treatment with a soluble TNF-α receptor or TNF binding protein (which reduces the availability of circulating TNF-α) increases survival and attenuates the hypothermia following induction of sepsis (31, 40). However, these studies did not differentiate between central and peripheral sources and actions of TNF-α.

The present results point to the possibility of a role for mast cell-derived TNF-α in the brain, rather than in periphery, given that Kit<sup>W-sh/W-sh</sup> mice show an attenuated rise in brain, but not plasma, TNF-α levels (Fig. 5A). This hypothesis is supported by evidence that mast cells in the brain upregulate TNF-α synthesis following immune activation (1) and that TNF-α injection in brain produces changes in T<sub>c</sub> (see Fig. 6; reviewed in Ref. 19). Although the results do not identify the source of TNF-α, mast cell granules contain preformed TNF-α, enabling immediate release (10), and the timing of the response supports the possibility of a mast cell source. Other cells such as microglia or macrophages that produce TNF-α do so upon activation, requiring de novo synthesis and therefore resulting in longer response latency (36). Finally, the lack of differences in the plasma between genotypes does not point to a role of TNF-α in the periphery. In summary, the data support the hypothesis that mast cell-derived mediators are involved in LPS-induced septic hypothermia.

Although the lack of hypothermia in Kit<sup>W-sh/W-sh</sup> mice may, in part, be attributed to reduced brain TNF-α levels, the hysteresis-like regulation of T<sub>c</sub> around baseline likely reflects other thermoregulatory mechanisms. The high-amplitude, low-frequency oscillations in the T<sub>c</sub> of Kit<sup>W-sh/W-sh</sup> mice following LPS compared with saline controls could be mediated by various changes in the thermoregulatory system, both central and peripheral. One possibility is that Kit<sup>W-sh/W-sh</sup> mice fail to monitor T<sub>c</sub> normally, in that the temperature range in which thermal effectors are activated is enlarged (32). Given this scenario, it is plausible that a lack of mast cells is influencing peripheral and/or central thermosensors, thereby disrupting afferent information to central thermoregulatory centers (35). Another possibility is that Kit<sup>W-sh/W-sh</sup> mice have disruptions in autonomic effectors, like blood vessel dilation, brown adipose tissue activation, or skeletal muscle shivering. If this is the case, imprecise effectors would lead to disruption of normal T<sub>c</sub> regulation. Finally, firing of warm- and cold-sensitive neurons in the brain mediating autonomic responses required for precise thermoregulation around a set point could be altered by a lack of mast cell mediators (26). In this case, altered processing in thermoregulatory control centers would compromise efferent signals to thermal effectors of T<sub>c</sub>. We favor this last hypothesis given both our results from brain TNF-α measures and the ability of mast cells to release other mediators implicated in central thermoregulation, such as serotonin and histamine (13, 37). Therefore, the T<sub>c</sub> oscillations in Kit<sup>W-sh/W-sh</sup> mice following LPS may be ascribed to altered efferent commands mediated by the lack of mast cells in the brain.

Kit<sup>W-sh/W-sh</sup> mice have higher survival rates compared with littermate controls, supporting the conclusion that mast cells play a detrimental role during sepsis. However, the role of mast cells in sepsis is controversial (9). Our data are consistent with several converging lines of evidence supporting mast cells’ cytotoxic role in host defense. One study showed that in the absence of a mast-cell-derived cytokine protease (dipeptidyl peptidase I), mice are more likely to survive following onset of sepsis induced by cecal ligation and puncture (24). Other work suggests a detrimental, rather than protective, role of mast cells in sepsis using the W/W<sup>v</sup> mast cell-deficient mouse, which differs from the Kit<sup>W-sh/W-sh</sup> mouse in that it has other immune abnormalities and is anemic and infertile (12). Echtenacher et al. (7) reported that the W/W<sup>v</sup> mouse is more susceptible than WT mice to the lethality caused by sepsis induced by cecal ligation and puncture. Reconstitution of these mice with mast cells from a WT donor provides protection for the host and reduces fatality.

Perspectives and Significance

These data support a broader role of mast cells in physiology beyond immediate hypersensitivity. Mast cells constitute a previously unknown agent in thermoregulatory control following an extreme bacterial infection. In addition, our results showing increased resistance to LPS-induced sepsis in Kit<sup>W-sh/W-sh</sup> mice provide a potential therapeutic target in the clinical treatment of sepsis. Overall, these results contribute to the currently expanding known physiological functions of mast cells.

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MAST CELLS MEDIATE THE HYPOThERMIC RESPONSE TO SEPSIS