Thermoregulatory responses to lipopolysaccharide in the mouse: dependence on the dose and ambient temperature

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IN RAT AND RABBIT MODELS OF bacterial LPS-induced systemic inflammation, the dynamics of the associated thermoregulatory responses have been well characterized and are known to depend on several factors, including ambient temperature (Ta) and LPS dose (for a review, see Ref. 24). At a neutral Ta, intravenous LPS typically causes fever; low, just suprathreshold doses, cause a single monophasic rise in deep body temperature (Tb), whereas moderate to high doses trigger several sequential Tb rises, named febrile phases (19, 26, 35). At least three febrile phases have been identified in the rat (29, 32) and at least two in the rabbit (19). At a subneutral Ta (cool environment), intravenous LPS evokes fever at low doses, mild hypothermia followed by fever at intermediary doses, and deep hypothermia at high, shock-inducing doses (27, 28, 30, 32, 33).

The second pitfall is that many experiments in mice were performed at a poorly controlled Ta; it fluctuated within −2°C in a single study and varied by almost 10°C among studies (6, 13, 16, 21). For example, Ta was 22–25°C in the studies by Li et al. (16) and Oka et al. (21), but it was 29–31°C in the studies by Gourine et al. (6) and Kozak and Kozak (13). Whereas the former range is clearly below the thermoneutral zone of the mouse, the latter range is neutral or near-neutral (5). In several species, especially small rodents such as the rat, the thermoregulatory response to LPS strongly depends on Ta, and a difference of a few degrees centigrade is sufficient to change the response drastically, e.g., from hypothermia to fever (27).
Hence, poor control of $T_a$ was likely responsible for the overall inconsistency in the $T_b$ patterns observed in mice.

The third pitfall is that the thermoregulatory responses to LPS strongly depend on its dose. As explained above, both rats (27) and rabbits (19) mount qualitatively different responses to different LPS doses. However, with the exception of a few studies (e.g., Refs. 12 and 21), such dependence was largely ignored in the mouse.

The present study was aimed at identifying and characterizing the murine thermoregulatory responses to LPS under experimental conditions free of the three pitfalls described above. In this study, the procedure of LPS injection was painless and nonstresstful, $T_a$ was tightly controlled, and a wide range of LPS doses was tested.

MATERIALS AND METHODS

Animals. Ninety-five C57BL/6 mice (Charles River Laboratories, Wilmington, MA) weighing 27–30 g at the time of the experiment were used. The mice were initially housed two per cage; after surgery, they were housed individually. Standard mice chow (Purina, Richmond, IA) and tap water were available ad libitum. The room was maintained on a 12:12-h light-dark cycle (lights on at 7:00 AM) and had $T_a$ of 27°C. Each mouse was handled daily for 5 days and then systematically habituated (5 training sessions, 3–4 h each) to staying in either a Plexiglas enclosure (length, 15 cm; width, 15 cm; height, 25 cm) or a wire-mesh conical confiner (diameter at base, 3 cm; base-to-vertex distance, 10 cm). The enclosure did not limit the animal’s movement; it was used for experiments involving telemetric thermometry. The confiner prevented the animal from turning around without limiting its back-and-forth movements; it was used for experiments involving thermocouple thermometry. All experiments were started between 8:00 AM and 9:00 AM. Each mouse was used for experiments involving thermocouple thermometry. The confiner prevented the animal from turning around without limiting its back-and-forth movements; it was used for experiments involving telemetric thermometry. The animal image is a roentgenogram of a mouse chronically implanted with a jugular vein catheter and an intraperitoneal telemetry probe. The mouse was injected with a small amount of a contrasting solution through the catheter.
not only on Ta, but also on other physical factors, including air humidity and velocity, effective radiant field, thermal conductivity of the housing structure, and the way the animal’s body contacts the housing structure. Hence, the thermoneutral zone often varies among experimental setups (for detailed analysis, see Ref. 25). In this setup, thermoneutrality was determined by measuring radiant tail skin temperature (Tsk) by infrared thermography. An infrared camera (ThermoVision A20M; FLIR Systems, N. Billerica, MA) was positioned on top of the enclosure with a mouse freely moving inside the enclosure; the camera was programmed to acquire images every second. When the mice were exposed to a low Ta (e.g., 26°C; Fig. 2A), they presented maximal skin vasoconstriction (Tsk at a near-Ta level), indicating that this Ta was subneutral (for explanations, see Ref. 25). We then gradually increased Ta in a stepwise fashion (step height, 0.5°C; step duration, >2 h) until moderate tail skin vasodilation (an increase in Tsk) was observed, which occurred at 31°C (Fig. 2B). Thus Ta of 31°C was found to be neutral for mice in this setup.

**Thermocouple thermometry.** This experimental setup permitted simultaneous measuring of both colonic temperature (index of Tb) and Tsk (index of tail skin vasomotor tone) in loosely confined mice. Each mouse was placed in a confiner and equipped with two copper-constantan thermocouples. The colonic thermocouple was inserted 2 cm beyond the anal sphincter and fixed to the base of the tail with a loop of adhesive tape. The skin thermocouple was positioned on the lateral surface of the tail (at the boundary of the proximal and middle thirds) and insulated from the environment with tape. Thermocouples were plugged into a data logger (Dianachart, Rockaway, NJ) connected to a personal computer. The mouse in its confiner was transferred to a climatic chamber. The jugular catheter was extended with a length of PE-50 tubing filled with the drug of interest (for details, see LPS preparation and administration below). The extension was passed through a wall port and connected to a syringe filled with saline. This thermocouple setup (Fig. 3) also permitted intravenous administration of the drug from outside the chamber, without disturbing the mouse.

To determine a neutral Ta in this setup, Ta, Tsk, and Tb were monitored with thermocouples while the mice were exposed to different Tas. At Ta of 32°C or lower, Tsk was close to Ta, and exhibited no fluctuations (Fig. 4), thus indicating maximal tail skin vasoconstriction typical for a subthermoneutral environment. At Ta of 34°C or above, Tsk was maintained at a relatively high level without marked fluctuations, which is characteristic of a supraneutral environment. At Ta of 33°C, two signs of thermoneutrality were observed: the average level of Tsk was intermediate, and the dynamics of Tsk was characterized by large fluctuations (for explanations, see Ref. 25). Hence, Ta of 33°C was found to be neutral for mice in this experimental setup.

**LPS preparation and administration.** E. coli 0111:B4 LPS was purchased from Sigma-Aldrich (St. Louis, MO). A stock suspension of LPS (10^4 µg/ml) in saline was stored at −20°C. At the time of the experiment, this stock was used either undiluted or diluted to a final concentration of 10^4, 10^1, 10^1.5, 10^1.75, 10^2, or 10^3 µg/ml. For intravenous injection, the PE-50 extension of the jugular catheter was prefilled with 27–30 µl (1 ml/kg) of a suspension of LPS in such a way that the suspension stayed near the mouse end of the extension; the remainder of the extension was filled with saline. The LPS suspension was separated from saline with a ~0.5 µl air bubble. At the time of injection, LPS (10^0–10^4 µg/kg) was flushed into the mouse’s circulation by replacing the LPS suspension at the mouse end of the PE-50 tubing with a 0.9% saline solution through the catheter.

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**Fig. 2.** Thermoneutrality test for mice in the telemetric thermometry setup. The effect of ambient temperature on tail skin temperature (Tsk) of mice was determined by infrared thermography. Thermograms were taken at an ambient temperature of 26.0°C (A) or 31.0°C (B); see Telemetric thermometry in MATERIALS AND METHODS for explanations.

**Fig. 3.** Schematic of the thermocouple thermometry setup. The animal image is a roentgenogram of a mouse chronically implanted with a jugular catheter, instrumented with colonic and tail skin thermocouples, and injected with a small amount of a contrasting solution through the catheter.
of the extension with 0.3 ml of saline injected from the syringe end. In a control, the extension was prefilled with saline only, and the injection was performed by the same procedure. This procedure was executed without touching the animal or causing pain or stress. In a separate experiment, LPS (10 ml/kg: 10–10^4 μg/kg) or saline was injected intraperitoneally by a common, stressful procedure that involved pricking the abdominal wall with a needle.

Data processing and analysis. The absolute values of Tb and, when available, Tsk were used to evaluate the thermoregulatory responses to LPS. In most cases, the responses were compared across treatments and time points by a two-way ANOVA, and F and P values (for the entire observation period/response duration) were reported. When the effect of treatment was evaluated on two parameters simultaneously (the effect of LPS dose on the latency and amplitude of the first phase of the day at a subneutral or neutral Ta, presumably reflecting the circadian rhythm of Tb (15, 20, 21, 31, 36). Compared responses were considered significantly different at P < 5.0 × 10^-2. Results are reported as means ± SE.

RESULTS

At Tsk of 31°C (which was neutral for mice in the telemetry setup), intravenous saline caused a slight (~0.5°C) decrease in Tsk over the course of the experiment (Fig. 5A). Such a decrease often occurs in untreated or saline-treated mice during the light phase of the day at a subneutral or neutral Tsk, presumably reflecting the circadian rhythm of Tsk (15, 20, 21, 31, 36). Compared with saline, all doses of LPS (10^9–10^4 μg/kg) induced a significant rise in Tsk (10^9 μg/kg: F1,429 = 1.6 × 10^3, P = 9.4 × 10^-5; 10^1 μg/kg: F1,390 = 1.3 × 10^2, P = 1.0 × 10^-7; 10^1.5 μg/kg: F1,429 = 7.2 × 10^1, P = 1.0 × 10^-7; 10^2 μg/kg: F1,507 = 3.6 × 10^2, P = 1.0 × 10^-7; 10^2.5 μg/kg: F1,507 = 1.1 × 10^3, P = 1.0 × 10^-7; 10^3 μg/kg: F1,507 = 7.3 × 10^3, P = 1.0 × 10^-7). The changes of the response were Tsk dependent. LPS induced a monophasic fever at 10^1–10^1 μg/kg (Fig. 5, B and C) and a biphasic fever at 10^1.5 μg/kg (Fig. 5D). Similar to what occurs in rats at a narrow dose range around 10 μg/kg (24), there was a drastic change in the response of the mice when the dose increased from 10^1.5 to 10^1.75–10^2 μg/kg: the biphasic fever was replaced by a qualitatively different polyphasic fever (Fig. 5, E and F). Three distinct febrile phases were observed over the time of the experiment; the third phase was the most pronounced (Fig. 6, A and C). When the data were plotted in a phase plane (rate of Tsk change vs. Tsk) for further explanations, see Ref. 29), these three phases were visualized as three separate loops (Fig. 6, B and D). As the dose of LPS was further increased (10^3–10^5 μg/kg), the second and third phases merged together and became indistinguishable (Fig. 5, G and H). Similar to LPS fever in the rat (26, 27, 30, 35), both the latency and amplitude (R0.38 = 1.3 × 10^1; P = 5.9 × 10^-8) of the first phase of polyphasic fever in the mouse decreased as the LPS dose increased (Fig. 7).

Because a low-amplitude first phase is often overlooked (for review, see Ref. 24), it was important to confirm its existence in the mouse by measuring the underlying effector activity. At neutral and supraneutral Tsk, the first febrile phase in rats (29, 35) and rabbits (19) is brought about, at least partially, by skin vasoconstriction (revealed as a decrease in Tsk). Tsk and Tsk in the same animal were simultaneously monitored by thermocouple thermometry in LPS-treated mice at Tsk of 33°C, which was neutral for mice in the telemetry setup (see MATERIALS AND METHODS). The dose of 10^1.75 μg/kg was selected for this experiment because it caused the most pronounced first phase in the telemetry setup (Fig. 5E). A similarly pronounced first febrile phase was caused by this dose in the thermocouple setup (Fig. 8A). The Tsk rise was associated with a significant decrease in Tsk from −34°C to a near-Tsk value (R16.52 = 3.3 × 10^-5, P = 6.0 × 10^-4; Fig. 8B), thus indicating tail skin vasoconstriction.

The thermoregulatory responses of mice to LPS (10^9, 10^1.75, or 10^3 μg/kg) or saline were also studied at Tsk of 26°C, which was subneutral for this species in the telemetry setup. The mice injected intravenously with saline showed a gradual (−0.5°C) decrease in Tsk over the course of the experiment (Fig. 9A). Compared with saline, every dose of LPS tested induced significant changes in Tsk (10^9 μg/kg: F1,468 = 2.2 × 10^2, P = 1.0 × 10^-7; 10^1.75 μg/kg: F1,507 = 2.1 × 10^2, P = 1.0 × 10^-7; 10^3 μg/kg: F1,290 = 1.2 × 10^2, P = 1.0 × 10^-7; 10^5 μg/kg, hypothermia: F1,221 = 9.2 × 10^2, P = 1.0 × 10^-7). These changes differed from those observed at a neutral Tsk in two ways. First, the animals appeared more sensitive to LPS in a cool environment. Indeed, the first febrile phase caused by low doses (10^9 and 10^1.75 μg/kg) had a substantially shorter (by ~20 min) latency at a subneutral Tsk (Fig. 9, B and C) than at a neutral Tsk (Fig. 5, B and E). Furthermore, the response to 10^5 μg/kg was transformed from a monophasic to biphasic fever (compare Figs. 5B and 9B). Second, the febrile response in a cool environment was followed by a late decrease in Tsk. At higher doses (>10^2 μg/kg), this decrease led to hypothermia, which started at ~180 min postinjection and was pronounced (~3°C at 10^4 μg/kg; Fig. 9D). At lower doses (e.g., 10^1.75 μg/kg), this decrease resulted in the disappearance of the otherwise prominent third febrile phase (compare Figs. 5E and 9C).

Under no circumstances did the mice exposed to a subneutral Tsk respond to a stress-free intravenous injection of LPS with early (latency, ~30 min) hypothermia, although such hypothermia reportedly (4, 7, 21, 23) occurs in the same species in response to a stressful injection of moderate-to-high doses of LPS. The mice in the present study failed to develop early hypothermia even when high doses of LPS (10^3–10^4
Fig. 5. Effects of intravenous LPS (doses indicated) or saline on the T\textsubscript{b} of mice at thermoneutrality. T\textsubscript{b} was monitored by telemetric thermometry at a neutral T\textsubscript{a} of 31°C. The vertical dotted lines show the time of injection. The horizontal dotted lines show the T\textsubscript{b} at the time of injection. Here and in Figs. 8–10, the number of animals (n) is indicated.
μg/kg) were injected at Tₚ as low as 20, 18, or even 16°C (data not shown). It was, therefore, important to test whether a stressful intraperitoneal injection of LPS would cause early hypothermia in the setup used. At a subneutral Tₚ of 26°C (telemetry setup), the mice were injected with intraperitoneal LPS (10³ or 10⁴ μg/kg) or saline via a common procedure involving animal handling and needle pricking. When injected this way, saline caused an immediate small increase in Tₚ.

Fig. 6. The polyphasic febrile responses of individual mice to intravenous LPS (doses indicated) at thermoneutrality. A and C: plots of Tₚ vs. time (time plots). B and D: plots of the rate of change in Tₚ (temperature velocity) vs. Tₚ (phase-plane plots). In the phase-plane plots, the onset of each phase is indicated with a circle symbol, and the end of the recording is shown with a triangle. The time elapsed after the injection (in minutes) is shown next to each symbol. The 3 febrile phases can be identified as 3 increases in the abdominal temperature (A and C) and 3 complete loops (shown in different colors) in the phase plane (B and D). To eliminate a high-frequency noise, the curves were smoothed by the Savitzky-Golay method (Origin 6.0; Microcal Software, Northampton, MA).

Fig. 7. Relationship between the amplitude of the first phase of polyphasic fever and the latency to its onset. A: an individual plot is used to show how the amplitude and latency were determined. B: a plot of the means is used to reveal the effect of LPS dose (shown next to each symbol).

Fig. 8. Ta response to intravenous LPS (dose indicated) during the initiation of fever at thermoneutrality. Tₚ (A) and Tₕ (B) were monitored by thermocouple thermometry at a neutral Tₚ of 33°C. The vertical dotted line shows the time of injection.
(stress hyperthermia) followed by a slow decline in $T_b$. In response to LPS, the mice developed a similar small increase in $T_b$ followed by hypothermia; compared with saline, hypothermia was significant only in response to the highest dose of LPS ($F_{1,96} = 8.7 \times 10^3$, $P = 4.1 \times 10^{-3}$; Fig. 10). In contrast to the late hypothermic response to stress-free intravenous administration of LPS (Fig. 9), the response to stressful intraperitoneal administration was early (latency, ~30 min).

**DISCUSSION**

Murine thermoregulatory responses to LPS at thermoneutrality. Although it has been repeatedly shown that mice can develop fever in response to LPS, the results obtained were often contaminated by stress hyperthermia (due to a painful injection procedure) and by a thermoregulatory response to external cooling (due to a poorly controlled, subneutral $T_a$). As a result, many authors (e.g., Refs. 3, 11, 15, 17, 18, 38) have described a response that consisted of rapid hyperthermia of variable magnitude and duration followed by either fluctuations of $T_b$ without a clear pattern or by a steady decline of $T_b$ to a hypothermic level. Importantly, the responses to the same or a similar dose of LPS differed drastically among studies and sometimes even within a study. The present research identified the dynamics of the thermoregulatory responses of mice to LPS administered in a stress-free fashion via a chronically implanted intravenous catheter. The mice were kept in an environmental chamber with tight control of $T_a$, and the relationship between $T_a$s used and the thermoneutral zone was verified.

We have found that mice exposed to a neutral $T_a$ respond to LPS with fever in a dose-dependent fashion: as the dose is increased from $10^0$ to $10^6 \mu g/kg$, the fever is transformed from monophasic to biphasic to polyphasic. The polyphasic character of the febrile response of mice to LPS has been recognized by Oka et al. (21). By assessing thermoeffector activity (vasomotor tone of the tail skin) and using several modes of data presentation, including the phase-plane plot, the present study has identified and characterized three phases of the polyphasic response. The present study has also established that many
characteristics of LPS fever in mice are similar to those reported for rats. For example, the second and third phases of the febrile response in mice (Fig. 5) and rats (27) become less distinct and fuse when the dose of LPS approaches 10^3–10^4 µg/kg. Another similarity is that both the amplitude and the latency of the first phase of polyphasic fever in mice (Fig. 7) and rats (26, 35) are inversely proportional to the LPS dose. Whereas it is not surprising that the latency of the response decreases as the dose of LPS increases, the fact that the amplitude of the first phase in both species decreases with an increase in the dose is counterintuitive and remains unexplained.

Thermoregulatory responses of mice to LPS in a cool environment. The present study has found that exposure of mice to a cool environment changes the thermoregulatory responses to LPS in two ways. First, it has a sensitizing effect on the febrile response. Indeed, responses to low (10^9 µg/kg) and intermediate (10^{1.75} µg/kg) doses of LPS start earlier in a cold environment. Furthermore, what is a monophasic fever in a neutral environment turns into a biphasic fever in the cold. Such a paradoxical, sensitizing effect has not been described before. Second, the febrile response of mice in a cool environment is followed by a late (starts at ~180 min postinjection) decrease in T_b. At lower doses of LPS (e.g., 10^{1.75} µg/kg), this decrease is seen as the disappearance of the otherwise prominent third febrile phase. At higher doses (>10^4 µg/kg), this decrease results in a pronounced (~3°C) hypothermic response. In other words, T_b-decreasing mechanisms are turned on at later stages of the response to LPS in a cool environment. These mechanisms seem to compete with T_b-increasing mechanisms, and such a competition may result in the disappearance of late febrile phases and/or in the development of hypothermia. A late (latency >120 min) hypothermic response to LPS in mice has been previously reported (1, 12, 21, 22). Such a response may last for up to 48 h and seems to be associated with ongoing inflammation, as evident from the sensitivity of late hypothermia to both dexamethasone and inhibitors of eicosanoid biosynthesis (22).

The most surprising observation of the present study is that mice do not respond to LPS with an early hypothermia, whereas, in rats, the early (10–30 min, latency; ~90 min, nadir) hypothermia is a highly reproducible and pronounced febrile response. Indeed, responses to low (10^9 µg/kg) and intermediate (10^{1.75} µg/kg) doses of LPS start earlier in a cold environment. Furthermore, what is a monophasic fever in a neutral environment turns into a biphasic fever in the cold. Such a paradoxical, sensitizing effect has not been described before. Second, the febrile response of mice in a cool environment is followed by a late (starts at ~180 min postinjection) decrease in T_b. At lower doses of LPS (e.g., 10^{1.75} µg/kg), this decrease is seen as the disappearance of the otherwise prominent third febrile phase. At higher doses (>10^4 µg/kg), this decrease results in a pronounced (~3°C) hypothermic response. In other words, T_b-decreasing mechanisms are turned on at later stages of the response to LPS in a cool environment. These mechanisms seem to compete with T_b-increasing mechanisms, and such a competition may result in the disappearance of late febrile phases and/or in the development of hypothermia. A late (latency >120 min) hypothermic response to LPS in mice has been previously reported (1, 12, 21, 22). Such a response may last for up to 48 h and seems to be associated with ongoing inflammation, as evident from the sensitivity of late hypothermia to both dexamethasone and inhibitors of eicosanoid biosynthesis (22).

The most surprising observation of the present study is that mice do not respond to LPS with an early hypothermia, whereas, in rats, the early (10–30 min, latency; ~90 min, nadir) hypothermia is a highly reproducible and pronounced (up to several degrees centigrade) response to moderate or high doses of intravenous LPS in a subthermoneutral environment (27, 28, 30, 32, 33). The present study shows that C57BL/6 mice do not develop early hypothermia even in response to very high doses of LPS (up to 10^4 µg/kg) at T_a as low as 16°C. Furthermore, another strain of mice (wild-type mice with a mixed B6;129P2 background; Taconic, Germantown, NY) has also been found to be incapable of responding with early hypothermia to LPS (10^3–10^4 µg/kg iv) at a subneutral T_a of 26°C (A. A. Steiner, A. Y. Rudaya, and A. A. Romanovsky, unpublished observations). However, the early hypothermic response did occur in C57BL/6 mice in the present study when LPS was administered by using a common, stressful procedure of intraperitoneal injection. Such a response has also been observed by others in different mouse strains when LPS was administered by a stressful procedure involving either pricking the abdomen (21, 23) or tail (4, 7) with an injection needle for intraperitoneal or intravenous administration, respectively. That early hypothermia occurs in the mouse exclusively under stressful conditions suggests that this is not an intrinsic response to LPS in this species. This finding may be another example of how stress interferes with thermoregulatory responses (10, 27, 34).

In summary, the present investigation identifies experimental conditions to study LPS-induced mono- b-and polyphasic fevers and late hypothermia in mice and provides detailed characteristics of these responses. It also highlights the importance of methodological issues in thermoregulation research. While studying the febrile and hypothermic responses of mice to LPS, caution should be taken to administer LPS in a stress-free fashion, to account for the intricate dependence of the response on LPS dose and to run experiments at a controlled T_a.

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Innovative Methodology


