Brief report

Bacterial lipopolysaccharide fever is initiated via Toll-like receptor 4 on hematopoietic cells

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Lipopolysaccharide (LPS), a well-known bacterial pyrogen, is recognized by several receptors, including the Toll-like receptor 4 (TLR4), on various cells. Which of these receptors and cells are linked to fever production is unknown. By constructing 4 mouse chimeras and studying their thermoregulatory responses, we found that all 3 phases of the typical LPS fever depend on TLR4 signaling. The first phase is triggered via the TLR4 on hematopoietic cells. The second and third phases involve TLR4 signaling in both hematopoietic and nonhematopoietic cells. (Blood. 2006;107:4000-4002)

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Introduction

Fever is a hallmark of infection and an important host defense response. It is triggered by microbial products, among which the lipopolysaccharide (LPS) of the outer envelope of Gram-negative bacteria is the most studied. In experimental animals, LPS causes a typical polyphasic fever, at least 3 phases of which have been characterized in rats[1] and mice.[2,3] LPS has been shown, mostly in vitro, to signal via several receptors, including Toll-like receptor 4 (TLR4), CD11/CD18 β_2 integrin, and scavenger receptors,[4-7] but which of these receptors is linked to any of the febrile phases is unknown. The present study addressed 2 questions: is TLR4 signaling essential for any of the febrile phases, and if it is, does the signaling originate in hematopoietic or nonhematopoietic cells?

Study design

The experimental protocols were approved by the Saint Joseph's Hospital and National Institutes of Health Animal Care and Use Committees. Chimeric mice were generated from 2 strains: C3H/HeOuJ (expresses a fully functional TLR4) and C3H/HeJ (expresses a nonfunctional TLR4 due to a homozygous missense mutation in the *Tlr4* gene[8]). The C3H/HeOuJ and C3H/HeJ mice (both from Jackson Laboratories, Bar Harbor, ME) had their bone marrow destroyed by ^{137}Cs γ-irradiation (9 Gy). They then received an intravenous transplant of 1 to 4 million bone marrow cells obtained from the femur and tibia of nonirradiated C3H/HeOuJ or C3H/HeJ donor mice (Figure 1). This procedure[9] resulted in 4 chimeric constructs: HeOuJ mice with HeOuJ bone marrow (functional TLR4 in both nonhematopoietic and hematopoietic cells), HeOuJ mice with HeJ bone marrow (functional TLR4 in radioresistant nonhematopoietic cells but nonfunctional TLR4 in transplanted hematopoietic cells), HeJ mice with HeOuJ bone marrow (nonfunctional TLR4 in nonhematopoietic cells but functional TLR4 in hematopoietic cells), and HeJ mice with HeJ bone marrow (nonfunctional TLR4 in both nonhematopoietic and hematopoietic cells). Transplanted hematopoietic progenitors typically do not transdifferentiate into nonhematopoietic cells.[10] Twelve weeks after bone marrow transfusion, that is, when more than 95% of the hematopoietic cells in the recipients are donor-derived,[9] the febrile response of the chimeras to LPS (50 μg/kg, intravenously) derived from a "smooth" *Escherichia coli* colony (055:B5; Sigma-Aldrich, St Louis, MO) was tested according to Rudaya et al.[3] The test involved nonstressful administration of LPS via a preimplanted jugular catheter to freely moving mice kept inside an environmental chamber at a tightly controlled, neutral ambient temperature (31°C). Deep body temperature of the mice was monitored by telemetry (series 4000 E-Mitter; Mini Mitter, Bend, OR). The deep body temperature responses were compared across phenotypes and time points by a 2-way ANOVA for repeated measures followed by the Tukey test (Statistica AX'99; StatSoft, Tulsa, OK).

Results and discussion

As expected,[3] the "controls" (HeOuJ mice with HeOuJ bone marrow) with a fully functional TLR4 responded to the dose of LPS used with a triphasic fever: deep body temperature peaked at about 40, 150, and 300 minutes after injection (Figure 2). In the mice bearing a nonfunctional TLR4 throughout the body (HeJ mice with HeJ bone marrow), all 3 phases of LPS fever were absent (Figure 2; $P < .001$ compared with the controls). This finding indicates that the TLR4 plays an indispensable role in triggering LPS fever. Importantly, the TLR4-deficient mice failed to respond to LPS with fever even though C3H/HeJ mice are capable of rapidly increasing their deep body temperature in response to other stimuli, such as psychologic stressors.[11]

In the past, numerous studies were aimed at identifying the cellular basis of immune-to-brain febrigenic signaling, either by eliminating a selected cellular population and studying fever under

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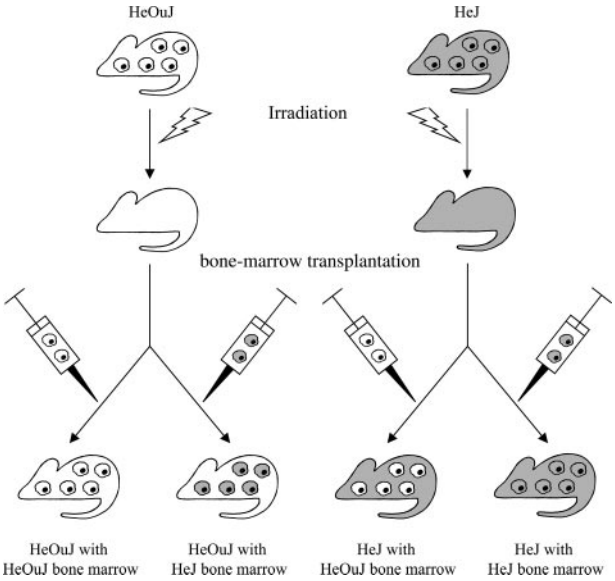

Figure 1. Generation of 4 types of chimeric mice. The images of individual cells refer to hematopoietic (bone marrow–derived) cells; the rest of the mouse body represents nonhematopoietic tissues. The presence of a nonfunctional TLR4 is coded by gray; the presence of a fully functional TLR4 is coded by white.

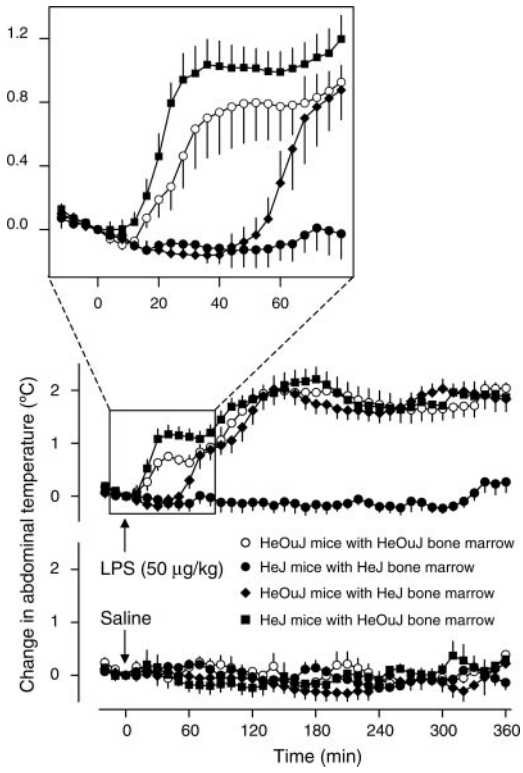

Figure 2. Fever responses of the chimeric mice. Abdominal temperature (mean ± SE) responses of the 4 indicated types of chimeric mice to LPS (50 μg/kg, intravenously) or saline (1 mL/kg, intravenously) are shown. At the time of the injection, the values of abdominal temperature of LPS- and saline-treated mice were, respectively, 35.7 ± 0.2°C (n = 7) and 35.9 ± 0.2°C (n = 6) for the HeOuJ mice with HeOuJ bone marrow, 36.0 ± 0.2°C (n = 7) and 36.2 ± 0.4°C (n = 7) for the HeJ mice with HeJ bone marrow, 35.9 ± 0.2°C (n = 9) and 35.9 ± 0.2°C (n = 7) for the HeOuJ mice with HeJ bone marrow, and 36.2 ± 0.3°C (n = 7) and 35.9 ± 0.2°C (n = 5) for the HeJ mice with HeOuJ bone marrow. These values did not differ statistically from each other.

such conditions,[12,13] or by identifying cells that produce and release febrigenic mediators, such as prostaglandin E_2 (PGE_2), in response to LPS.[14-18] However, no study was able to determine whether the role of certain cells in fever is related to LPS recognition by these cells or to their activation by endogenous mediators induced by LPS. We found (Figure 2) that the first phase of LPS fever was absent ($P < .001$) in the mice bearing a nonfunctional TLR4 selectively in hematopoietic cells (HeOuJ mice with HeJ bone marrow), but was present, and even enhanced ($P = .043$), in the mice bearing a nonfunctional TLR4 in nonhematopoietic cells (HeJ mice with HeOuJ bone marrow). This finding shows that LPS recognition by the TLR4 in hematopoietic cells is pivotal for the development of the first febrile phase. Although future studies are necessary to determine the subpopulation of hematopoietic cells involved, it is tempting to speculate that it consists of macrophages of the LPS-processing organs (such as the liver and lung). Two experimental observations are in line with this scenario. First, the onset of the first phase of LPS fever in rats is associated with up-regulation of PGE_2-synthesizing enzymes in the liver and lung but not in the brain.[19] Second, pretreatment with drugs causing, among other effects, depletion of macrophages in peripheral tissues results in an attenuation of the first phase of LPS fever in rats[12] and guinea pigs.[13]

Surprisingly, the late (second and third) phases of LPS fever were affected neither in the chimeras bearing a nonfunctional TLR4 selectively in hematopoietic cells nor in the chimeras bearing a nonfunctional TLR4 in nonhematopoietic cells (Figure 2). Only when both hematopoietic and nonhematopoietic cells carried a nonfunctional TLR4 (HeJ mice with HeJ bone marrow) were the second and third febrile phases suppressed. Because the absence of the first phase in the HeOuJ mice with HeJ bone marrow did not result in any attenuation of the second or third phase, the triggering mechanisms of the late febrile phases appear to be independent of those of the first phase. Our results also indicate that hematopoietic and nonhematopoietic cells in the chimeras can compensate for each other's inability to sense LPS when it comes to bringing about the late febrile phases. The relative contribution of each cell population remains unknown. In our previous study,[9] the peripheral production of pyrogenic cytokines at the time corresponding to the second and third febrile phases (2-6 hours after LPS) was attributed mostly to hematopoietic cells (eg, macrophages), whereas both nonhematopoietic cells (eg, endotheliocytes, microglia, and astroglia) and hematopoietic cells (eg, perivascular and meningeal macrophages) were found to be responsible for cytokine production within the brain. In the same time window, PGE_2 synthesis is activated both in the periphery and in the brain.[19] Perhaps the late (second and third) phases of fever depend on redundant mechanisms that involve TLR4-dependent activation of hematopoietic cells (in the periphery and brain) and nonhematopoietic cells (mainly in the brain).

We conclude that all 3 phases of the typical polyphasic febrile response to LPS depend entirely on the TLR4. The first phase of LPS fever is triggered by TLR4 signaling in hematopoietic cells. The mechanisms of the second and third phases involve both hematopoietic and nonhematopoietic cells.

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