

Extra View

Cells That Trigger Fever

Andrej A. Romanovsky^{1,*}

Alexandre A. Steiner¹

Kiyoshi Matsumura²

¹Systemic Inflammation Laboratory; Trauma Research; St. Joseph's Hospital and Medical Center; Phoenix, Arizona USA

²Osaka Institute of Technology; Hirakata-City, Osaka Japan

*Correspondence to: A.A. Romanovsky; Trauma Research; St. Joseph's Hospital; 350 W. Thomas Rd.; Phoenix, Arizona 85013 USA; Tel.: 602.406.5059; Fax: 602.406.4113; Email: aromano@chw.edu

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ABBREVIATIONS

COX	cyclooxygenase
LPS	lipopolysaccharide
PAMP	pathogen-associated molecular pattern
PG	prostaglandin
TLR	toll-like receptor

ABSTRACT

Bacterial lipopolysaccharide (LPS) is recognized by several receptors, including the toll-like receptor (TLR) 4, on various cells. Among many biological responses to LPS is fever, an often polyphasic rise in body temperature that is thought to be mediated by prostaglandin (PG) E₂. Which receptors on which cells are linked to fever production is unknown. It is also unknown which cells produce PGE₂ that triggers the earliest (first) phase of fever. Two recent studies from our group answer these questions. In the first one, we studied LPS-induced fever in mouse chimeras selectively lacking the TLR4 in hematopoietic or nonhematopoietic cells. We found that the first phase of fever is triggered via the TLR4 on hematopoietic cells. In the second study, we investigated LPS fever in rats. We found that the number of cells expressing cyclooxygenase (COX)-2, a PGE₂-synthesizing enzyme, surged at the onset of fever in the lung and liver (but not in the brain), and that most of these cells were macrophages. Because LPS-induced PGE₂ production in macrophages is TLR4-dependent, it is tempting to speculate that the TLR4-bearing, bone marrow-derived cells implicated in fever pathogenesis by the first study are the same as the COX-2-positive macrophages identified in the second study. Hence, pulmonary and hepatic macrophages that recognize LPS via the TLR4 and rapidly produce PGE₂ are likely triggers of the fever response.

Phagocytic cells were noted by microscopists in the 19th century, but, according to Rogoff and Lipsky,¹ the early microscopists thought that these cells promoted spreading of microorganisms and aided infection. Élie Metchnikoff² (a more common transliteration from Russian is Ilya Mechnikov) first recognized that these amoeba-like cells attracted to the site of injury to engulf foreign bodies play a crucial role in host defense responses, a discovery for which he was awarded the 1908 Nobel Prize in Physiology or Medicine. After phagocytic cells were found in the sinusoids of the liver and in the reticulum of other tissues, Karl Ludwig Aschoff³ coined the term reticulo-endothelial system. Later, morphological characteristics of these phagocytes and their lineage from a bone marrow precursor cell were established, and the cells were termed collectively the mononuclear phagocyte system.⁴ The present Extra-view article is an auto-commentary on two recent studies^{5,6} showing that, in addition to their many other functions, mononuclear phagocytes are responsible for the initiation of fever.

Fever is a hallmark of infection and an important host defense response. It is triggered by a variety of endogenous and exogenous agents, including the so-called pathogen-associated molecular patterns (PAMPs), which are specific, structurally conserved components of certain broad groups of microorganisms. A typical PAMP is lipopolysaccharide (LPS) of Gram-negative bacteria. For LPS signaling, the most studied, and in many cases the most important, PAMP receptor is the toll-like receptor (TLR) 4, but other receptors also exist.^{7,8} In mammals, a typical thermoregulatory response to LPS is polyphasic fever; at least three febrile phases have been characterized in rats and mice.⁹ The first phase occurs within 30 min of LPS administration; it is one of the earliest manifestations of systemic inflammation. Because this phase is sensitive to ambient temperature and other methodological factors,^{10,11} it is often overlooked and remains poorly characterized.

The two studies discussed in the present Extra-view paper were aimed at identifying the cellular basis of the first phase of LPS fever. The first study⁵ was conducted in mouse chimeras that possess the TLR4 in bone marrow-derived ("hematopoietic") cells only, non-hematopoietic cells only, both cell types, or no cell type.¹² By using the recently developed methodology of studying LPS fever in mice,¹¹ we questioned whether TLR4 signaling is essential for the first febrile phase, and if so, what are the cell types involved. We found that the first phase of *Escherichia coli* LPS-induced fever is triggered exclusively by

TLR4-bearing cells of the hematopoietic lineage. The two largest cell populations of the hematopoietic lineage that play pivotal roles in innate immunity are monocytes/macrophages and polymorphonuclear leukocytes (mostly neutrophils). Monocytes express high levels of the TLR4 on their surface and readily respond to LPS; neutrophils express low levels of the TLR4, and their responses to LPS are now thought to be secondary (i.e., orchestrated by monocytes).¹³ Furthermore, hepatic macrophages (Kupffer cells) and alveolar macrophages effectively detoxify LPS, whereas polymorphonuclear leukocytes do not.¹⁴ It is, therefore, tempting to speculate that the TLR4-bearing cells that trigger fever are macrophages of the major LPS-processing organs, the liver and lung.^{15,16} Indeed, pretreatment with cytotoxic drugs that, among other effects, deplete macrophages in peripheral tissues results in an attenuation of the first phase of LPS fever in rats¹⁷ and guinea pigs.¹⁸

Our second study in focus⁶ determined which prostaglandin (PG)-synthesizing cells trigger fever. It is known that all phases of fever are PGE₂-dependent,¹⁹ and that the late (second and third) phases are mediated by PGE₂ originated in endotheliocytes and perivascular cells of the brain.^{20,21} However, the phenotypes and tissue location of the cells that produce PGE₂ triggering the first febrile phase are unknown. It is also unknown whether the PGE₂ that triggers the first phase originates within the brain, as thought by many, or in the periphery, as proposed by Anthony Milton and others in the 1970–80s.^{22–25} More recently, this question resurfaced at the 1999 invitational symposium on Antipyretic Pharmacotherapy (Parsons Island, MA, USA). There, the predominant hypothesis of the brain origin of febrigenic PGE₂ (presented by Blatteis et al.²⁶) was challenged by the peripheral origin hypothesis (presented by Romanovsky et al.²⁷). Since then, our studies have supported the peripheral origin hypothesis. We showed that intravenous PGE₂ causes fever when administered in a monomeric form (e.g., as a complex with albumin).^{6,28} Furthermore, we showed that the onset of the first phase of LPS fever is accompanied by transcriptional upregulation of PGE₂-synthesizing enzymes in the liver and lung, but not in the brain.²⁹ The study in focus⁶ also included an important experiment with peripheral administration of an anti-PGE₂ antibody (first reported at the 2004 Physiology and Pharmacology of Temperature Regulation meeting in Rhodes, Greece³⁰ and recently reproduced by Li et al.³¹). This experiment showed directly that fever is triggered by PGE₂ synthesized in the periphery. The same study in focus⁶ also found that the protein level of the PGE₂-synthesizing enzyme cyclooxygenase (COX)-2 is increased in the periphery (but not in the brain) already at the onset of the first phase, and that these events coincide with a surge in the number of cells displaying COX-2 immunoreactivity in the lung and liver (but not in the brain).

To identify the pulmonary and hepatic producers of PGE₂, we determined how the cells that become COX-2 positive at the onset of LPS fever relate to the histological elements revealed by hematoxylin staining. These data, which were not included in the original paper,⁶ are presented in this paper (Fig. 1). In the lung, COX-2-positive cells were found to cluster around alveoli, often forming what looked like cell chains (Fig. 1A). In the liver, the parenchyma did not stain for COX-2, and most COX-2-positive cells were located in the stroma, often close to sinusoids (Fig. 1B). Some COX-2-positive cells were also found around the central vein (a small vein that gathers blood from sinusoids) and in the visceral peritoneum covering the liver. We then double-stained lung and liver tissue for COX-2 and either the macrophage marker ED2 or the endothelial marker RECA1. Almost 90% of COX-2-positive cells in the lung and more than 80% in the liver were found to be macrophages (ED2-positive).

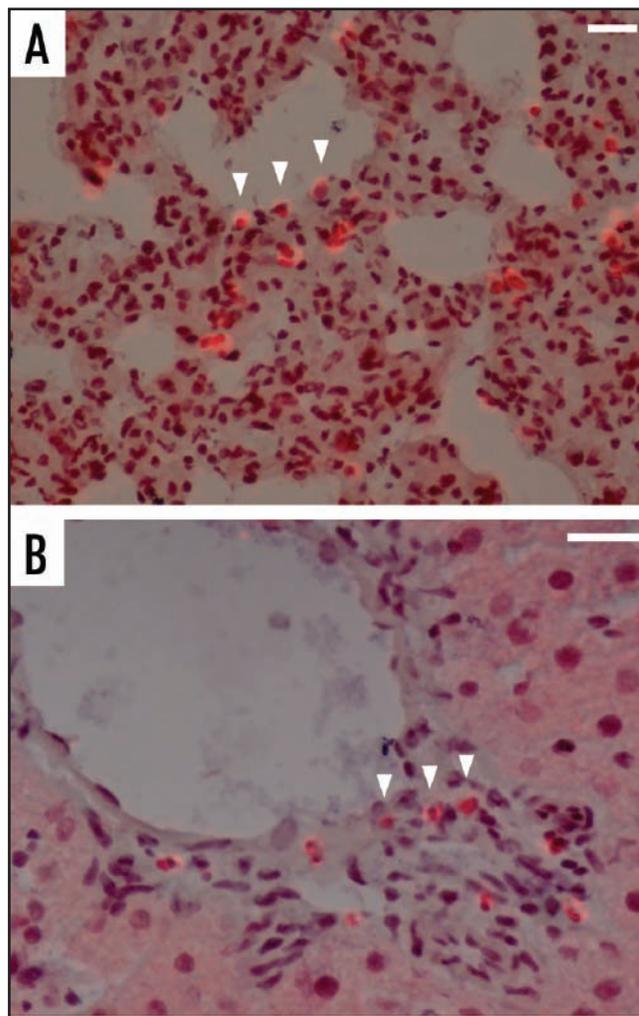


Figure 1. Cells that trigger fever. COX-2-positive cells in the lung (A) and liver (B) of rats at the onset of the first febrile phase, i.e., 40 min after intravenous injection of LPS (10 $\mu\text{g}/\text{kg}$) at thermoneutrality. For each tissue, a freshly frozen section (14 μm) was stained with an anti-COX-2 antibody (red fluorescence), and the image was captured by a digital camera coupled to a microscope. Then, the section was restained with hematoxylin (stains nuclei purple/brown), and a second image was acquired. The two images were superimposed in Adobe Photoshop. Scale bars, 20 μm .

In summary, our first study in focus⁵ shows that the first phase of LPS fever critically depends on TLR4-positive cells of the hematopoietic lineage (presumably hepatic and pulmonary macrophages). Our second study⁶ shows that this phase is mediated by peripherally synthesized PGE₂ and coincides with activation of PGE₂ production in hepatic and pulmonary macrophages. The question then arises: are the TLR4-bearing cells shown to trigger fever in the first study the same as the COX-2-positive macrophages revealed in the second study? Because LPS-induced upregulation of COX-2 in macrophages is TLR4-dependent,³² the answer to this question is expected to be positive. Hence, the likely triggers of the fever response are the hepatic and pulmonary macrophages that recognize LPS via the TLR4 and produce PGE₂ via a mechanism involving transcriptional upregulation of COX-2. Because fever is a disease symptom and a host defense response, both the early microscopists and Mechnikov appear to have been right: by promoting fever, macrophages promote simultaneously a clinical manifestation of the underlying disease and a defense against this disease.

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