

## LETTER TO THE EDITOR

# TLR5: beyond the recognition of flagellin

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*Cellular and Molecular Immunology* (2017) 14, 1017–1019; doi:10.1038/cmi.2017.122; published online 20 November 2017

The innate immune system plays an essential role in the host defense against infections by initially sensing and recognizing diverse microbial pathogens and directing adaptive immune responses to their infections. How does the innate immune system sense and recognize pathogens? The identification of pattern recognition receptors (PRRs) during the past decades has brought us into a new era to explore this fundamental question. PRRs are an array of germline-encoded multifunctional proteins that do not recognize specific pathogens but rather recognize conserved molecular patterns associated with various classes of pathogens, leading to pathogen-associated molecular patterns (PAMPs).<sup>1</sup> Among the PRRs, there is a family of Toll-like receptors (TLRs), including TLR5, that recognize a wide variety of PAMPs and elicit innate immune responses. TLR5 is known to specifically sense and recognize flagellin, the major structural protein of bacterial flagella.<sup>2</sup> It is conceivable that the immune system is equipped with TLR5 for the detection of flagellin in different cells, tissues and organs. However, the

fundamental functions of TLR5 beyond the recognition of flagellin are only beginning to be appreciated. Studies on TLR5 in recent years have revealed different scenarios after the recognition of flagellin by TLR5 in the respiratory tract, gastrointestinal tract, liver or even in the dorsal root ganglion neurons.

TLR5 is expressed constitutively in epithelial cells and immune cells, such as monocytes and immature DCs. TLR5 is preferentially expressed on the apical side of respiratory epithelia in both mice and humans.<sup>3,4</sup> Thus, TLR5 can induce early signaling dedicated to protective innate immune responses against respiratory infection. For example, airway epithelium TLR5 can sense *Pseudomonas aeruginosa* and initiate an early host inflammatory reaction to clear the invading pathogen.<sup>5</sup> Moreover, the early clearance of *P. aeruginosa* from the airways depends on flagellin-TLR5-MyD88-dependent signaling in respiratory epithelial cells.<sup>6</sup>

In contrast with the respiratory tract, TLR5 is expressed mostly on the basolateral side of intestinal epithelial cells and recognizes the bacterial locomotion component flagellin for detecting whether bacteria have crossed the gut epithelia<sup>1</sup> (Figure 1a). The expression profile and subcellular distribution features of TLR5 in the gastrointestinal tract are in line with the prominent location of bacteria in the gastrointestinal tract and the requirements of the gut immune system to address gastrointestinal microbiota, in which commensal

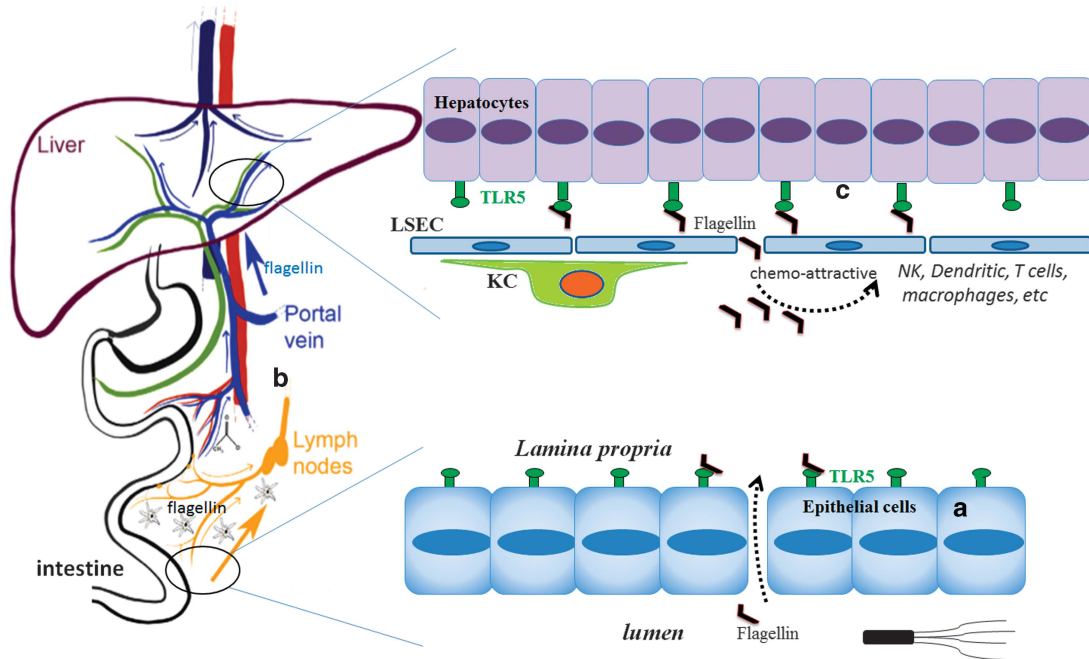
dissemination to extraintestinal organs, the overgrowth of toxigenic members or opportunistic pathogenic bacterial invasion and infection need to be constantly monitored and prevented. Accordingly, TLR5 is used by the mucosal immune system of the gut to detect flagellin for the surveillance of gastrointestinal microbiota. Thus, intestinal epithelial cell TLR5 senses the composition and localization of the intestinal microbiota to prevent diseases associated with intestinal inflammation.<sup>7</sup> Aberrantly elevated TLR5 activation in response to bacterial flagellin might result in an impairment of the epithelial barrier integrity or the exacerbation and perpetuation of chronic gut inflammation.<sup>8</sup> Improper bacterial flagellin recognition by TLR5 is also linked to changes in the gut microbiota composition, distorted adipose tissue metabolism and inflammation.<sup>9</sup> As an ancient innate immune receptor, TLR5 is crucial for both immune homeostasis and protection against bacterial infection in mammals, birds, amphibians, fish and reptiles.<sup>10</sup> Further investigation of the most primitive function of TLR5 will shed new light on the role of TLR5 in both sustaining gut immune homeostasis and the clearance of intestinal pathogenic bacteria.

The liver is a unique anatomical and immunological site because of its physiological roles in the metabolism of nutrients and the confluence of hepatic arterial and venous blood (Figure 1b). The liver is continually exposed to various pathogens with both systemic and

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Received: 6 August 2017; Accepted: 8 October 2017



**Figure 1** TLR5 is in the intestine and liver for the recognition of flagellin and beyond. (a) TLR5 is expressed on the basolateral side of intestinal epithelial cells and functions in the normal surveillance of flagellated bacteria. (b) The liver receives the intestinal venous blood circulation and arterial supply and acts as a functional vascular firewall that clears commensals that have penetrated either the intestinal or systemic vascular circuits. (c) TLR5 is expressed at a low level by hepatocytes, which can be activated by flagellin specifically in the liver. TLR5 signaling in the liver modulates local immunity by secreting cytokines and chemo-attracting immunocytes, such as neutrophils, macrophages, NK cells, dendritic cells and T cells. These cells may help to eliminate bacteria directly and regulate specific immune responses.

gut origins. In light of this situation, the liver plays a role in immune surveillance for pathogen detection and host defense.<sup>11</sup> The ability of liver immune sentinels to detect pathogens is also dependent on the array of PRRs expressed by liver sinusoidal endothelial cells (LSECs), hepatocytes, Kupffer cells (KCs), DCs and liver-resident lymphocytes. The liver expresses a wide array of PRRs, including TLRs. Given the anatomical location and functional features of the liver, the response to a number of TLR ligands in the liver often differs from the response observed in other tissues. This is represented mostly as the immunosuppressive response, which is speculated to be a mechanism used to avoid a constant state of liver inflammation induced by continuous exposure to bacteria-derived molecules, such as LPS, flagellin and so on. Although TLRs are expressed by most cells in the liver, including hepatocytes, LSECs, HSCs, KCs and lymphocytes, TLR5 is only expressed at low levels in hepatocytes, KCs and hepatic DCs; however, it is not

expressed on LSECs.<sup>12</sup> The role of TLR5 in the liver has been largely neglected because of its low expression in this organ, but in recent years, increasingly important activities of TLR5 in the liver have been observed (Figure 1c). For example, hepatocyte TLR5 was found to detect flagellin in the liver, promote bacterial clearance from the liver and protect the liver against chronic inflammatory diseases, although its precise mechanism has not yet been defined. In this detection and clearance process, the liver may act as a functional vascular firewall that clears commensals that have penetrated either the intestinal or systemic vascular circuits.<sup>13</sup> Another study showed that the liver is the major organ that responds to the TLR5 agonist.<sup>14</sup> Moreover, hepatocytes were found to be the key liver cell type that responds rapidly and directly to a flagellin analog but not to LPS, and the activation of liver TLR5 has been shown to play a central role in flagellin's radioprotective and anticancer properties.<sup>14</sup> The following study further demonstrated that the

systemically administered flagellin analog generates protective antitumor T-cell immunity in the liver through coordination with CXCR3-expressing NK cells.<sup>15</sup> These studies reveal that the effects of flagellin-TLR5 activation in the liver go far beyond the recognition of flagellin. In light of this, the protective effects and possible side effects of the activation of the TLR5-signaling pathway in the liver by flagellin should be evaluated more carefully, especially in regard to its proposed systemic use for tumor patients. At the least, the over-activation of TLR5 signaling in the liver should be avoided because liver injury induced by high doses of flagellin was observed in mice.<sup>16</sup>

In addition to the roles played by TLR5 in the respiratory tract, gastrointestinal tract and liver, a recent study on mechanical allodynia in neuropathic pain showed that the effect of TLR5 activation in neurons even works beyond its recognition of flagellin.<sup>17</sup> This study found that TLR5 is expressed by peripheral sensory neurons, in which TLR5

mediates touch sensation and permits the rapid transport of certain small molecules across the neuronal membrane through an unknown mechanism.<sup>17</sup> This introduces a promising new therapeutic strategy to treat persistent pain by targeting TLR5. On the other hand, it also suggests a more intriguing role for TLR5 as a gate-keeper for cellular ion channels or as a regulator of infection-caused tissue injury and pain.

It seems that more functional roles of TLR5 are waiting to be revealed. Many open questions regarding TLR5 beyond its recognition of flagellin remain to be answered. First, how does TLR5 act in the bidirectional communication between the intestine and liver under physiological conditions and in different diseases? Second, is there any immune communication between the lung and liver? If yes, does TLR5 play a role in the lung–liver axis? Third, what is the physiological role of TLR5 expressed in peripheral sensory neurons? Does TLR5 play roles in detecting tissue injury and related pain? These answers will open new doors for our further understanding of TLR5 and other TLRs.

#### CONFLICT OF INTEREST

The authors declare no conflict of interest.

#### ACKNOWLEDGEMENTS

This work was supported by grants from the National Natural Science Foundation of China (Nos. 31300717 and 81461130019)

and the Grants of Deutsche Forschungsgemeinschaft (TRR60 and GK1949).

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