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PERSPECTIVE

The NLRP3 Inflammasome activation in human or mouse cells, sensitivity causes puzzle

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SIGNAL 1 ALONE IS ENOUGH TO ACTIVATE THE NLRP3 INFLAMMAOSME IN HUMAN CELLS

According to our current understanding, the NLR family, pyrin domain containing 3 (NLRP3) inflammasome activation is generally a two-step process. The first step is priming, in which pathogen associated molecular patterns (PAMPs) such as LPS or pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α) induced NF-κB activation provides synthesis of pro-IL-1β and NLRP3 proteins. This priming step is considered as signal 1, which makes the cell ready for a second strike to assemble the inflammasome. Then danger signals such as ATP and MSU provide the signal 2 that promotes formation of the NLRP3 inflammasome and activates caspase-1. Both of these 2 steps are required in mouse macrophages for the NLRP3 inflammasome activation (Dinarello, 2007).

However, in human monocytes and macrophages, PAMPs such as LPS alone can lead to secretion of mature IL-1β without a second signal (Netea et al., 2009; Shenoy et al., 2012). It seems that in human cells signal 1 alone is enough to activate the NLRP3 inflammasome. The reason for this difference may attribute to constitutive caspase-1 activation in primary monocytes in some cases (Netea et al., 2009). Presumably the constitutive caspase-1 activity in primary monocytes isolated from PBMC (peripheral blood mononuclear cell) is resulted from physical or chemical stress during the purification or separation process, because resting monocytic cells such as THP-1 or U937 cells do not carry activated caspase-1 (Zhao et al., 2011). In addition, an early study also nicely showed that freshly isolated human monocytes could respond to LPS stimulation and release IL-1β, but when the monocytes were aged overnight and thus "rested down", low dose LPS (10 ng/mL) were not able to elicit IL-1β secretion anymore (Perregaux et al., 1996). Therefore, the constitutive caspase-1 activation theory may explain a part but not all reasons behind the NLRP3 inflammasome activation with a single PAMP challenge.

HUMAN CELLS ARE MORE SENSITIVE FOR NLRP3 INFLAMMASOME ACTIVATION

We think that different sensitivities between mouse macrophages and human monocytes/macrophages underlie the different requirements for NLRP3 inflammasome activation in human or mouse cells. As evidenced from an early study, low dose LPS treatment of human monocytes led to intracellular synthesis of pro-IL-1β, which was the priming step. Instead of ATP, a higher dose of LPS alone (>100 ng/mL) could serve as signal 2 to induce mature IL-1β release (Chin and Kostura, 1993). Even in mouse BMDMs (bone marrow derived macrophages) that typically need both LPS priming and ATP pulse for NLRP3 inflammasome activation, a very high dose of LPS can also trigger IL-1β release without addition of ATP ((Meng et al., 2009) and Fig. 1). In addition, dynasore, a dynamin inhibitor, activates the NLRP3 inflammasome at the concentration of 5 mmol/L in human monocytic THP-1 cells but not in mouse BMDMs; and only concentrations higher than 80 mmol/L can activate the NLRP3 inflammasome in BMDMs (Fig. 2). Therefore, the human myeloid cells are overall much more sensitive for inflammasome activation. A regular dose of LPS for priming in BMDMs (such as 100 ng/mL) is sufficient to induce both pro-IL-1β synthesis and caspase-1 activation in human cells such as THP-1 cells.

The reason why inflammasome activation is more sensitive in human cells than mouse cells can be multiple. For instance, human monocytes release high amount of ATP upon LPS treatment (Netea et al., 2009), and the ATP-P2X7R signaling pathway is important for NLRP3 inflammasome activation. In addition, the expression level of P2X7R is strongly up-regulated by LPS treatment in human myeloid cells including THP-1, but this specific ATP receptor is not modulated by LPS in mouse mac-

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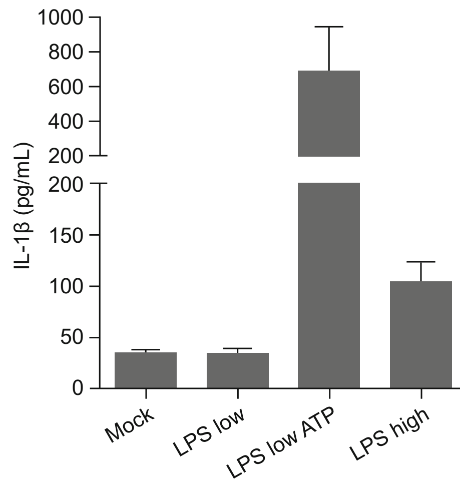

Figure 1. High dose LPS stimulates IL-1β secretion from mouse BMDMs. BMDMs from C57BL/6 mice were treated with low dose (500 ng/mL) or high dose (100 μg/mL) LPS for 10 h, ATP was added for another 30 min as indicated. The supernatants were tested for IL-1β by ELISA.

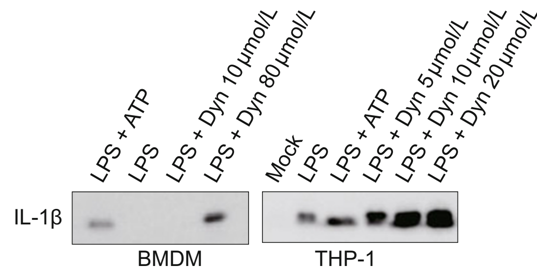

Figure 2. THP-1 is more sensitive than BMDMs upon Dynasore (Dyn) induced inflammasome activation. (Left Panel) BMDMs were primed with LPS (500 ng/mL) for 3 h, ATP (5 mmol/L) or Dynasore (Dyn) (10 mmol/L or 80 mmol/L) were then added as indicated; (Right Panel) PMA differentiated THP-1 cells were treated with LPS (200 ng/mL) plus ATP (5 mmol/L) or increased concentrations of Dynasore (5–20 μmol/L) for 1 h. IL-1β in the supernatants of these BMDMs or THP-1 cells were then detected via immunoblotting.

rophages (Schroder et al., 2012a). This might be the reason why high amount of exogenous ATP has to be added to activate inflammasome in mouse cells unless NLRP3 is mutated (Meng and Strober, 2010). As a matter of fact, there is even considerable difference between different types of mouse cells. Recently Gabriel Nuñez's group found that BMDCs (bone marrow derived dendritic cells) secreted substantial amounts of IL-1β upon stimulation with TLR ligands in the absence of ATP pulse, speaking for the concept that NLRP3 inflammasome can be activated in sensitive cells such as BMDCs with one single LPS challenge (He et al., 2013). Moreover, there are more lines of evidence showing the difference between human and mouse genes related to inflammasome activation. For instance, CARD8 (cardinal) that is present in human genome and suggested to be part of the NLRP3 inflammasome is not found in mouse (Petrilli et al., 2005). Several other proteins that have been proved to be regulators of ASC or caspase-1 are also only present in human genome. For example, POP1 (PYDC1) is identified in human genome , which also have orthologs in lizard (*Anolis carolinensis*) and zebrafish (*Danio rerio*), but not in mouse. Similarly, POP2 (PYDC2) is present in human genome but not in mouse (Schroder and Tschopp, 2010). Besides these Pyrin Only Proteins, some Card Only Proteins such as COP (pseudo-ICE), ICEBERG, and INCA that negatively regulate IL-1β production are also specific for human genome, not present in mouse (Druilhe et al., 2001; Lee et al., 2001; Lamkanfi et al., 2004). Another example along this line is that the human pyrin protein, whose B30.2 domain is mutated in Familial Mediterranean Fever (FMF) patients, but the mouse pyrin doesn't carry this B30.2 domain at all (Chae et al., 2011). The necessity of many negative regulators in humans indicate that the signaling in human cells is more sensitive, so it needs to be controled tightly. Recently, Shao group and Vance group found that NAIP5 and NAIP2 proteins in mouse genome are direct receptors for bacteria flagellin or TTSS proteins, respectively (Kofoed and Vance, 2011; Zhao et al., 2011; Gong and Shao, 2012). However, in humans there is only one Naip protein, which recognizes bacterial needle protein Cprl (Zhao et al., 2011). Although the human receptor for flagellin has not been identified yet, it is not Naip at least, which tells again considerable difference between human and mouse cells for inflammasome composition, activation as well as regulation.

PRIMING IS NOT MERELY A SIGNAL 1 IN NLRP3 INFLAMMASOME ACTIVATION

Another point that should be noted is that in either mouse cells or human cells, the second signal alone (ATP or MSU) cannot activate caspase-1. But short LPS priming plus ATP was sufficient to activate caspase-1 in BMDMs (Fig. 3, upper panel, lane 9 and (Schroder et al., 2012b)), even though IL-1β was not synthesized and secreted in this case (Fig. 3, lower panel, lane 3 and lane 9). This tells that even in mouse cells where inflammasome activation is much less sensitive than in human cells, the LPS stimulation is not only priming cells to synthesize pro-IL-1β and NLRP3 proteins, but also render non-transcriptional changes in cells to respond to ATP stimulation. Recently two reports found that non-transcriptional priming and deubiquitination regulate NLRP3 inflammasome activa-

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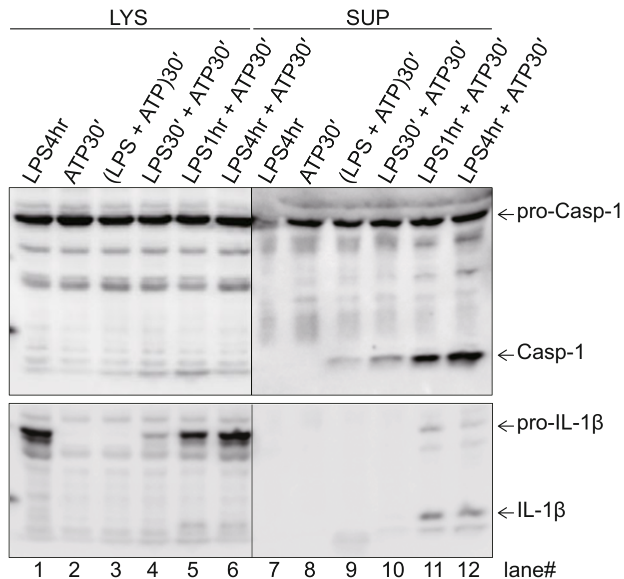

Figure 3. Non-transcriptional priming for NLRP3 inflammasome activation. BMDMs from C57BL/6 mice were treated with various combinations of LPS and ATP as indicated. Cell lysates and culture supernatants were tested for caspase-1 and IL-1β by immunoblotting.

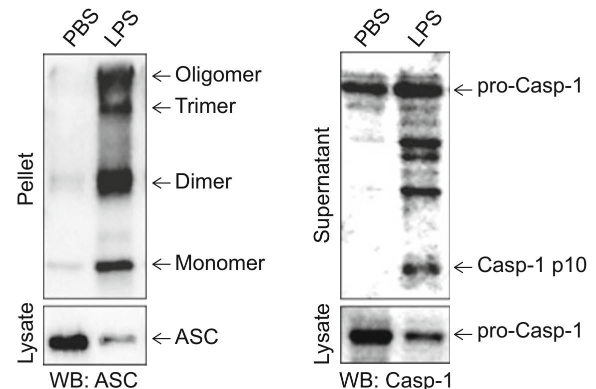

Figure 4. LPS induces the formation of ASC pyroptosome and caspase-1 activation in THP-1 cells. THP-1 cells were treated with PBS or LPS in a concentration of 100 ng/mL for 6 h. Cell lysates and culture supernatants were harvested for Western blot of ASC pyroptosome and caspase-1, respectively.

tion, which awaits further validation in different experimental systems (Juliana et al., 2012; Py et al., 2013). If proved, this unexpected mechanism will be a breakthrough in understanding the way inflammasome get activated under various conditions.

TANGLED PRIMING AND CASPASE-1 ACTIVATION IN THE PROCESS OF NLRP3 INFLAMMASOME ACTIVATION

As described above, the priming step and the caspase-1 activation step are tightly connected. Detailed mechanisms still need to be further characterized. Moreover, in some cases, pathogen infections or danger molecules stimulation alone can activate both signal 1 and signal 2, leading to the secretion of mature IL-1β (Said-Sadier et al., 2010; Niemi et al., 2011). Thus our current two-step model of inflammasome activation should not be misunderstood. Mostly, it is a clear-cut two-step process in mouse cells, whereas in human cells the process is more tangled. Although common dose LPS (such as 100 ng/mL) treatment alone in mouse cells dose not activate the NLRP3 inflammasome, in human cells the same "common" dose of LPS indeed elicits the secretion of IL-1β in NLRP3 dependent manner, as well as activates caspase-1 and renders ASC pyroptosome formation (Fig. 4). Thus, in this scenario, we should not simply say PAMPs like LPS is not an inflammasome activator. Although we still don't know the detailed mechanism to date, the NLRP3 inflammasome is indeed activated. This is evidenced by ASC pyroptosome formation and caspase-1 activation (Fig. 4), which should be the definite readout for inflammasome activation, no matter how the cells are stimulated and what kind of cells they are. Either human cells or mouse cells, either directly activated by PAMPs or indirectly activated through priming-boost steps, the actual downstream signal causing NLRP3 inflammasome assembly and activation is likely a "stress". When this "stress" is strong enough, NLRP3 inflammasome will be activated. To identify and characterize this "stress" signal in detail is a big challenge in this field currently, while with input from a lot of active scientists worldwide, our understanding of inflammasome will be further advanced in the near future.

FOOTNOTES

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Hongbin Wang, Liming Mao and Guangxun Meng declare that they have no conflict of interest.

All institutional and national guidelines for the care and use of laboratory animals were followed.

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