Let’s get this pyrin started!

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Inflammasomes enable cells to respond to pathogens or biological damage, but the specific signals being used to convey these messages are not always clear. A new paper identifies two potential microbiota-derived metabolites, the bile acid analogues BAA485 and BAA473, as the first small molecules to activate the pyrin inflammasome. These results suggest that microbiota may be able to modulate this inflammatory process which, in turn, may contribute to the maintenance of intestinal homeostasis.

Inflammasomes are intracellular protein complexes that form in response to pathogen- or damage-associated molecular patterns, thereby informing host cells that something bad is going on. Inflammasome formation leads to caspase-1 activation; caspase-1 cleaves the proinflammatory cytokines interleukin (IL)\(^3\)-1β and IL-18 as well as the pore-forming protein gasdermin D, facilitating cytokine secretion and an inflammatory response more broadly, and ultimately results in a form of cell death known as pyroptosis. There are several putative pattern recognition receptors (PRRs) capable of forming inflammasomes, including AIM2, NAIP-NLRC4, NLRP1, NLRP3, NLRP6, and pyrin (1). However, specific ligands are known for only some of these PRRs. Given that inflammasome components are found in the gut, the microbiome, with all its accoutrements, presents a whole world of potential ligands. There are bacteria, their cell walls, their DNA, their metabolites, gut metabolites that bacteria transform, and the list goes on. A new study by Alimov et al. (2) explores this connection, finding two potential gut microbial compounds that initiate inflammasome signaling in a pyrin-dependent manner. These provocative results identify the first small-molecule activators of the pyrin inflammasome, and by extension underscore a potentially important role for pyrin in the modulation of intestinal homeostasis and autoinflammation.

The inflammatory response must be carefully balanced throughout the body, but especially so in the gut, where some baseline inflammasome activation is actually host-protective (3, 4), but disruptive bacteria are ready to take advantage of insufficient monitoring, and overactive inflammatory responses can lead to diseases such as inflammatory bowel disease. It is likely that the overall effect of specific activator–inflammasome partnerships may vary depending on the activating ligand, as well as the host cell type (5). Some exciting examples of these activator–inflammasome partnerships have already come from the study of the microbiome and their metabolites in the context of NLRP3 and NLRP6. Colonic microbes provide signals that drive both NLRP3- and NLRP6-mediated secretion of IL-18, which in turn not only induces expression of antimicrobial peptides that modulate the colonic microbiota, but also indirectly increases IL-22, which supports wound healing. Several microbe-derived metabolites (e.g., taurophil, pinotol, sebacate, undecanediol) have been identified as NLRP6 activators. In contrast, metabolites derived from a dysbiotic microbiota (e.g., histamine, spermine) can suppress NLRP6 activation, decreasing the production of microbiota-modifying anti-microbial peptides (3). Similarly, short-chain fatty acids can activate the NLRP3 inflammasome (4), while lactate negatively regulates NLRP3-mediated inflammation (food for thought when you are considering whether to buy a lactobacillus-laden or, in other words, lactate-producing, probiotic supplement) (6).

Alimov et al. (2) hypothesized that additional secondary metabolites from the microbiome could be playing a role in inflammasome biology. To test this idea, the authors used a targeted screen of predicted microbiota-derived metabolites to identify two bile acid analogues (BAA485 and BAA473) as inflammasome activators. They first demonstrated that BAA485 could weakly induce IL-18 production in primed peripheral blood mononuclear cells (PBMCs). Structure–activity relationship studies led them to identify BAA473 as a more potent compound that induces selective secretion of IL-1β and IL-18 (but not IL-6 or IL-8, which are induced by other pathways) in primed PBMCs, a human macrophage cell line, as well as a human-derived gut monolayer culture.

In order to gain a better understanding of how BAA473 activates the inflammasome, the authors tested inhibitors of proteins involved in IL-1β and IL-18 secretion. They observed that the effects of BAA473 were blocked by a caspase-1 inhibitor but not by an NLRP3 inhibitor, suggesting that BAA473-induced inflammasome activation is independent of NLRP3. To define the inflammasome pathway activated by BAA473, the authors performed a whole-genome pooled CRISPR screen.

The authors declare that they have no conflicts of interest with the contents of this article.

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3 The abbreviations used are: IL, interleukin; PBMC, peripheral blood mononuclear cell; PRR, pattern recognition receptor.
using BAA473-mediated pyroptosis as a readout. The gRNAs enriched in nonpyroptotic cells were analyzed by next generation sequencing, which showed that the strongest hits in the screen were ASC (a common adaptor for several inflammasome pathways) and pyrin. To validate this finding, the authors knocked out ASC and pyrin in THP-1–Cas9 cells using separate gRNAs, confirming that these two components are essential for BAA473-induced secretion of IL-1β and IL-18 and cell death. The authors further extend their findings by demonstrating that treatment with colchicine (a microtubule network disruptor and by extension an inhibitor of pyrin activation) blocked inflammasome activation by BAA473 (Fig. 1). Conversely, the use of a cell line stably expressing pyrin led to enhanced secretion of IL-18 and more cell death upon BAA473 treatment compared with cells expressing empty vector.

In contrast to NLRP3 and NLRP6, relatively little is known about pyrin inflammasome activators in the intestine, especially from a metabolic perspective. Yet, pyrin is expressed in intestinal epithelial and immune cells, and pyrin-deficient mice have intestinal dysbiosis and increased susceptibility to chemical colitis (7). It has been suggested that pyrin does not recognize danger molecules per se, but rather disturbances in cytoplasmic homeostasis. For example, GTPase-modifying bacterial toxins, such as Clostridium difficile enterotoxin A (TcdA), can drive pyrin inflammasome activation, partly due to their effect on GTPase-driven modification of the cytoskeleton (8). The study by Alimov et al. (2) therefore provides important new information by identifying specific pyrin inflammasome–activating ligands. It is not clear whether BAA485 or BAA473 is actually present in the human intestine, as existing studies of bile acid conversion have not identified BAA485 or BAA473. The intestinal microbiota, however, is likely to metabolize bile acids into similar compounds, and the authors’ establishment of a stable pyrin-expressing cell line represents a useful in vitro model for the evaluation of additional candidate metabolites.

Further studies are now needed to determine whether the bile acid metabolites directly bind to pyrin, and, if so, characterize their molecular interactions. Additionally, it will be important to search for bile acid metabolites produced in situ that activate pyrin, and to characterize the impact of metabolic acid-driven pyrin inflammasome activation in vivo. It might also be useful to evaluate the impact of bile acid sequestrants such as cholestyramine, in humans and mouse models, on the composition of the microbiota, host inflammation, and, of course, pyrin inflammasome activation. While inflammasomes may be known as reporters of something bad, these new results definitely point to a lot of good and exciting new questions at the interface of immunology and the microbiome.

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