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Can GPR43 sensing of short chain fatty acids unchain inflammasome-driven arthritis?

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Colonization of the intestine by trillions of bacteria plays important roles in gut cell physiology and in metabolism, and increasingly recognized roles in immune system homeostasis, as well as multiple major organ and rheumatologic diseases driven by innate and adaptive immune responses (1). The human intestinal microbiome vastly differs from individual to individual in both health and disease, including intestinal microbiome imbalance (“dysbiosis”) recently identified in ankylosing spondylitis (2). Moreover, differences between individuals, via factors including diet, lifestyle, use of antibiotics, prebiotics, or probiotics, and through gut microbiota-generated metabolites, further influence intestinal homeostasis, metabolic, nervous system and immune and inflammatory response pathways (3).

Many of the signals generated by diet and intestinal microbiota that modulate immune and inflammatory responses system are sensed by cells in and beyond the gut, via pattern recognition receptors. These include the Toll-like receptors (TLRs) TLR2 and TLR4, which respond to stimuli including not only microbial components but also certain free fatty acids (4). Several G protein-coupled receptors (GPRs) also recognize short, intermediate, and long chain fatty acids, including omega-3 fatty acids, in a complex array of functionally important interactions (5–7).

GPR120 and GPR40, initially characterized by the terms free fatty acid receptor 4 (FFAR4), and FFAR1, respectively, modulate inflammatory responses by sensing medium and/or long chain fatty acids, and omega-3 fatty acids (6). Notably, GPR120 and GPR40 act additively to transduce the capacity of omega-3 fatty acids to suppress NLRP3 inflammasome activation by urate crystals and other stimuli, in a process involving internalization of the omega-3 fatty acid complex with GPR120 and/or GPR40 (6).

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GPR41 and GPR43 (FFAR3 and FFAR2, respectively), and the niacin and butyrate receptor GPR109A sense short chain fatty acids (SCFAs) generated in large part by colonic microbiota as fermentation metabolites of cellulose from non-digestible dietary fiber (5). Levels of the SCFAs acetate, butyrate, and propionate generated by gut commensal microbiota affect gut epithelial cell bio-energetics, proliferation, and barrier and inflammatory functions. SCFA levels vary not only due to microbiome composition but also dietary and lifestyle factors. Significantly, butyrate and propionate enter the systemic circulation, and acetate can reach particularly high levels in the systemic circulation. Moreover, blood acetate levels markedly rise in response to alcohol ingestion. Contrariwise, all SCFAs are markedly depleted in the gut and blood of germ free mice.

Leukocyte SCFA sensing has inhibited inflammatory responses, including adhesion molecule and inflammatory mediator expression, and leukocyte chemotaxis in multiple experimental systems (5,7) (Figure 1A). SCFAs can inhibit inflammatory transcriptional NF- κ B signaling, and butyrate, at relatively high concentrations suppresses class I histone deacetylases, possibly via changes in CpG methylation, and effects on histone methylation and acetylation by multiple mechanisms (7,8). SCFAs also have the capacity to stimulate apoptosis in neutrophils, macrophages, and lymphocytes. Signaling through GPR43 (Figure 1A), which may be the preferential receptor for acetate, and which interacts and is expressed with multiple other molecules mediating innate immunity, appears to be a critical transducer of such damping effects of SCFAs on inflammation (5).

The capacity of GPR43 to modulate inflammation is rooted in part by expression not only by colonic epithelial cells, but also by leukocytes, including lymphocytes, monocytes, macrophages, and, at particularly robust levels, in neutrophils (5,7). *GPR43*^{-/-} mice manifest increased leukocyte-driven severity or impaired resolution of multiple disease models, including K/BxN serum transfer inflammatory arthritis, ovalbumin-induced allergic airway inflammation, and dextran sulfate sodium-induced colitis (5). Conversely, gut microbiota as a whole, and SCFAs through GPR43, also exert stimulatory effects on host defense and inflammation. Gut microbiota promote phagocytic capacity, microbial killing activity, NADPH oxidase activity in phagocytes, validated *in vivo* by decreases in these functions in phagocytes from germ-free compared to conventional mice. Furthermore, gut commensal microbiota appear to support full expression of NLRP3 inflammasome-induced inflammatory changes in the lung, and IL-1 β production and skin disease in neonatal mice with a cryopyrin associated periodic syndromes (CAPS)-associated NLRP3 mutation (9).

In this issue of the journal, work by Vieira et al, extending to a mouse model of gouty arthritis (10), breaks new ground by highlighting how GPR43 sensing of acetate has the potential to prime and amplify acute inflammatory arthritis driven by the NLRP3 inflammasome (Figure 1B). IL-1 β release, via NLRP3 inflammasome assembly and activation particularly in macrophage lineage cells, plays a major role in not only CAPS, but also in particulate-induced inflammatory disorders induced by urate, CPPD, hydroxyapatite, and silica crystals and asbestos, as well as periprosthetic osteolysis at sites of orthopedic joint implants. In the paradigm of urate crystal-induced inflammation, Vieira et al further characterize the heterogeneity of priming signals for the NLRP3 inflammasome complex to fully execute proteolytic activation of caspase-1 and consequent maturation of pro-IL-1 β and

secretion of mature IL-1 β (10) by macrophages (Figure 1B). In essence, for urate crystals to induce robust mature IL-1 β release in mononuclear phagocytes, NLRP3 inflammasome priming signals are provided in monocytes by C5a (generated by C5 endoproteolysis on the crystal surface)(11), and in macrophages by GM-CSF (12) and/or by TLR4 or TLR2 ligands (eg, provided experimentally by lipopolysaccharide (LPS), and potentially in nature by dietary long chain fatty acids such as palmitate or stearate)(4). These priming signals collectively affect differentiation, inducing responses that include pro-IL-1 β , pro-caspase-1, and NLRP3 expression, and the capacity for NADPH oxidase expression. Vieira et al demonstrate that GPR43 sensing of acetate provides a complementary set of priming signals *in vitro* to increase the capacity of macrophages to ingest particulates, activate the NADPH oxidase, and cleave pro-caspase-1 (Figure 1B) (10). The consequences of the priming signals are marked gain in the extent of activation of the NLRP3 inflammasome complex in cultured mononuclear phagocytes in response to the second signal, crystalline monosodium urate (4,10,11,12), which, via ingestion, induces phagolysosome perturbation, osmotic effects, and oxidative stress (Figure 1B)(13). There is partial selectivity of the effects of GPR43 on SCFA sensing responses that modulate tissue inflammation and host defense, since macrophage inflammatory priming responses to ATP but not LPS also are impaired by GPR43 deficiency (10). Whether GPR43-mediated macrophage priming signals are potentially limited by constitutive and therapeutic anti-priming mechanisms is an open question, and also related in part to the gut, nutrition, and lifestyle. For example, AMP activated protein kinase (AMPK), a nutritional biosensor and master energy regulator, naturally suppresses macrophage priming and NLRP3 inflammasome-mediated inflammatory responses to urate crystals (14). AMPK is not only upregulated by physical fitness and restraints in caloric and alcohol intake, but also transduces the capacity of low concentrations of colchicine to inhibit macrophage pro-inflammatory priming and effector responses (14).

Vieira et al observed that in germ free mice with decreased SCFAs, and/or in *GPR43*^{-/-} mice, NLRP3 inflammasome activation and pain and inflammatory responses, including neutrophil influx, IL-1 β and CXCL1 chemokine release, are decreased in knees joints injected with urate crystals (10). For germ free mice *in vivo*, the urate crystal-induced inflammatory responses were restored by acetate administration in drinking water (10). SCFAs can promote neutrophil migration *in vitro*. However, injection into mouse knee joints of IL-1 β together with urate crystals restored the neutrophil recruitment response to baseline in *GPR43*^{-/-} mice (10). This suggests that acetate and GPR43 can contribute to gout-like acute inflammation much more by promoting macrophage NLRP3 inflammasome activation than by affecting the primary functions of neutrophils.

The provocative implication of both GPR43 and acetate in model acute gouty inflammation (10) adds to experimental evidence that gut microbiota can significantly support NLRP3 inflammasome-driven inflammatory responses outside of the gut (9). The results of Vieira et al are derived from a broad round of experiments with germ free mice (with and without acetate feeding), comparisons to conventionally housed mice (with and without antibiotic treatment), and studies using *GPR43*^{-/-} and wild type control mice (10). Translationally, at a minimum, the work adds further support to the recommendations for avoidance of excess

alcohol intake in triggering acute gout flares, here pointing out potential salutary effects via prevention of increased acetate generation from alcohol. The results suggest the potential value of further investigation into the effects on gout and other NLRP3 inflammasome-mediated disorders of recognized gut microbiome dysbioses in obesity, diabetes, chronic kidney disease, chronic alcohol abuse, and antibiotic use, as well as distinctions in the microbiome related to diet and other environmental exposures.

GRP43 represents an interesting potential target for investigation of novel strategies to limit inflammatory macrophage priming and thereby to help suppress gouty inflammation, let alone limit flares of CAPS and suppress other NLRP3 inflammasome-driven disorders. However, the promise of the findings of Viera et al, using *GPR43*^{-/-} mice and acetate feeding (10), needs to be tempered by caution in both interpreting the work, and weighing potential translational significance in humans. In particular, proving causality in studies of complex ecosystems such as gut microbiota is fundamentally challenging, due in part to differences in microbiomes between not only mouse strains but also experimental laboratory environments. Moreover, one wonders, in the case of butyrate, whether differences in butyrate uptake and β -oxidation by inflammatory cells may explain differing experimental results for inflammation regulation by gut microbiota fermentation-derived SCFAs. In this light, butyrate inhibited the capacity of a combination of urate crystals and the long chain fatty acid palmitate (C16:0) to induce pro-inflammatory responses in unfractionated human peripheral blood mononuclear cells (7). However, in a different study, butyrate failed to significantly inhibit urate crystal-induced caspase-1 activation in LPS-primed mouse bone marrow derived macrophages, but under the same conditions, β -hydroxybutyrate (BHB), at millimolar concentrations, was a broad inhibitor of macrophage NLRP3 inflammasome activation *in vitro* (15). BHB, a ketone, body, is robustly produced as a transportable energy source by hepatic mitochondria in response to caloric restriction, strenuous exercise, and glucose deprivation; though subject to rapid clearance from blood, BHB reaches millimolar concentrations range when hepatic fatty acid oxidation is ramped up under such conditions. BHB broadly suppresses NLRP3 inflammasome activation *in vitro*, and administration of BHB *in vivo*, via relatively stable lipid nanolipogels, suppressed inflammation both in response to urate crystals and in a murine CAPS model (15). BHB limits NLRP3 inflammasome activation via inhibition of cytosolic K⁺ efflux and oligomerization of the NLRP3 inflammasome adaptor protein Apoptosis-Associated Speck-Like Protein Containing CARD (ASC) in macrophages (15). The capacity of BHB to suppress class I histone deacetylases and limit oxidative stress likely adds to BHB anti-inflammatory activities. Though GPR109A is not necessary, we need to understand if GPR43 could mediate anti-inflammatory activities of BHB (15).

Additional considerations in weighing impact of the findings of Vieira et al (10) first include the possibility of subtle changes in intestinal GPR43 expression that could alter gut microbiota by feedback loops between the two. Second, many mouse inflammatory model systems fail to provide reproducible constructs in human inflammatory diseases. Third, the time point of inflammation in the study urate crystal-induced inflammation of Viera et al was very short (10). It is now recognized that neutrophils, dependent on NADPH oxidase activation, are required for development and maximal early intensity of urate crystal-

induced inflammation, but also critically mediate spontaneous resolution of this inflammatory response by neutrophil extracellular trap formation (NETosis)(16). Fourth, it remains to be determined if potential long-term risks of loss of GPR43 homeostatic gut epithelial barrier function, and constitutive anti-inflammatory and host defense functions in the gut and beyond, outweigh potential short-term benefits of suppression of NLRP3 inflammasome-driven inflammatory processes. In this context, manipulating the intestinal microbiome can result in substantial deleterious systemic effects on leucocytes, including induction of a state of IL-10-mediated hypo-responsiveness, involving neutrophils and macrophages, that increases susceptibility to infections and certain immune disorders due to altered and weakened inflammatory responses (3).

Essentially, intestinal epithelial cells and gut and peripheral leucocytes need to encounter “healthy microbiota” and their metabolites, as articulated in the “hygiene hypothesis” underlying increases in certain allergic, inflammatory, and autoimmune diseases in Western societies, whereas failure to do so can be hazardous to development and long term health of the human gut and immune system, as well as host defense (3). As such, more work is required to define and understand the potential translational significance and risk:benefit ratio of the paradoxical ability of GPR43 sensing of short chain fatty acids to help unleash NLRP3 inflammasome-driven inflammatory arthritis.

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Figure 1. Leukocyte sensing of gut microbiota fermentation-derived SCFAs has paradoxical effects on inflammatory responses in mononuclear phagocytes

Panel A schematically illustrates that sensing of the gut microbiota fermentation-generated SCFAs acetate, butyrate, and propionate, via G protein receptor coupled GPR43, signaling limits multiple inflammatory responses by suppressing adhesion molecule and inflammatory mediator expression, and leukocyte survival and chemotaxis. These gut microbiota fermentation-derived SCFAs act in part by inhibiting transcriptional NF-κB signaling, and at relatively high concentrations, butyrate suppresses class I histone deacetylases. An open question is how gut microbiota-generated butyrate NLRP3 anti-inflammatory effects potentially relate to those of the transportable ketone body β-hydroxybutyrate (BHB), generated primarily by hepatic mitochondrial fatty acid oxidation, and a broad suppressor of NLRP3 inflammasome-driven disorders, as discussed in the text but not depicted here.

Panel B highlights paradoxical findings, in which GPR43 sensing of gut microbiota fermentation-derived SCFAs has the potential to unleash acute inflammatory arthritis driven by NLRP3 inflammasome-induced IL-1β maturation and release in macrophage lineage, exemplified by the urate crystal-induced model acute gouty arthritis in new work of Vieira et al published in this issue of the journal (10). Here, GPR43 sensing of acetate provides a complementary set of priming signals to C5a (generated by C5 endoproteolysis on the urate crystal surface) in monocytes, and GM-CSF and TLR2 and TLR4 ligands in macrophages *in vitro*, to increase the capacity to phagocytose particulates, activate the NADPH oxidase, and cleave pro-caspase-1. Together, these heterogeneous priming signals transduce robust activation of the NLRP3 inflammasome complex in response to the illustrated second signals provided via urate crystal ingestion in mononuclear phagocytes.