

Bruton tyrosine kinase inhibition: Clinical relevance beyond B cells



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Key words: Nucleotide-binding leucine-rich repeat receptor family pyrin domain containing protein 3, Bruton tyrosine kinase, ibrutinib

Ask a clinical immunologist for their first thought about Bruton tyrosine kinase (BTK) and the answer most likely will be related to B cells and humoral immunodeficiency. Ask the same question about the pattern recognition receptor nucleotide-binding leucine-rich repeat receptor family pyrin domain containing protein 3 (NLRP3) and the probable response will reveal a wholly separate compartmentalization linked to periodic fever syndromes and autoinflammatory diseases. Although an association linking the 2 proteins has been described *in vitro* and in a mouse model,¹ whether this finding translates to a clinically relevant interaction was unknown and had been unexplored.

In this issue of the *Journal*, for the first time, Liu et al² show that loss of or interference with BTK in patients profoundly affects NLRP3 function. This is an important and clinically relevant finding, revealing an unexpected effect of a classically adaptive immune modulator on an ancient innate immune pathway.

The nucleotide-binding domain and leucine-rich repeat containing receptors are a family of cytosolic receptors that stimulate innate immune responses. NLRP3 is the most studied member and is activated in the context of a multiprotein complex termed an inflammasome.³ The NLRP3 inflammasome consists of the receptor NLRP3, the adapter molecule apoptosis-associated speck-like protein containing a CARD (ASC), and the proenzyme procaspase-1. Numerous diverse activators trigger NLRP3 inflammasome activation through an incompletely defined signaling pathway. Although there is disagreement as to the precise pathway by which the NLRP3 inflammasome is activated, it has been reported to require an influx of calcium, efflux of potassium, and mitochondrial dysfunction generating mitochondrial reactive oxygen species. Downstream of this signaling cascade, the NLRP3 inflammasome assembles at mitochondria, followed by autocatalysis of caspase-1, which then cleaves pro-IL-1 β and pro-IL-18 into mature secreted IL-1 β and IL-18. IL-1 β and IL-18 are strongly proinflammatory cytokines with profound systemic effects that include triggering

fever, production of acute-phase reactants, upregulation of chemokines and adhesion molecules by endothelial cells, and recruitment and activation of innate and adaptive immune cells. Activation of the NLRP3 inflammasome has been linked to many inflammatory conditions, including the response to microbial infections, but also sterile inflammatory states, including gout, atherosclerosis, metabolic syndromes, diabetes, obesity, and silicosis. Spontaneous and inappropriate activation of NLRP3 results in the periodic fever syndrome known as cryopyrin-associated periodic syndromes (CAPS), which are associated with genetic mutations in *NLRP3*.⁴ Given the association of NLRP3 activation as a key driver of pathologic inflammatory states, its blockade is attractive therapeutically. However, despite years of research, a specific target for NLRP3 inflammasome regulation has not been defined, and its clinical blockade remains through prevention of signaling through the IL-1 receptor. Because both IL-1 α and IL-1 β signal through this receptor and are also produced by NLRP3-independent processes, this nonspecific approach is likely to block both beneficial and pathologic IL-1 signaling. It is clear that a better understanding of the mechanism through which NLRP3 is activated is needed to develop new molecular regulators that specifically target NLRP3 without impairing all IL-1-mediated responses.

BTK is best known as a critical regulator of B-cell development in patients with X-linked agammaglobulinemia (XLA), which is caused by mutations in *BTK*. It has been shown that BTK is required for propagation of the B-cell receptor signals that are necessary for B-cell development.⁵ Absent or dysfunctional BTK results in blockade of B-cell development and a lack of mature B cells with an associated failure of antibody responses. BTK is a cytosolic protein kinase of the Tec family of nonreceptor tyrosine kinases and consists of a pleckstrin homology domain and a catalytically active kinase domain flanking a central Tec homology domain involved in protein-protein interactions. Lyn or Syk phosphorylates BTK at a critical tyrosine (Y551), which activates the catalytic domain and results in BTK autophosphorylation at a second tyrosine (Y223). Resultant functional BTK then activates phospholipase C- γ , generating Ca²⁺ influx.^{6,7} More recently, constitutively active BTK has been described in certain B-cell malignancies, and its blockade with the US Food and Drug Administration–approved inhibitor ibrutinib in patients has delivered promising results.⁸ Although BTK function is linked clinically to adaptive immune responses, it is also expressed by innate immune cells, including macrophages, neutrophils, and dendritic cells, and has been shown to have a prominent role in Toll-like receptor signaling.⁹

The relevance of BTK to NLRP3 was first suggested in a study by Ito et al¹ in which they screened a panel of pharmacologic kinase inhibitors and found BTK inhibition specifically prevented NLRP3 inflammasome activation *in vitro*. They also found through coimmunoprecipitation that BTK physically associated first with the adaptor ASC and subsequently with NLRP3. Models of cerebral ischemic injury in mice with BTK inhibitors or mice

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Supported by an American Lung Association/American Academy of Allergy, Asthma & Immunology foundation grant and National Institutes of Health grant AI104706 (to S.L.C.).

Disclosure of potential conflict of interest: The authors declare that they have no relevant conflicts of interest.

Received for publication February 21, 2017; revised March 9, 2017; accepted for publication March 23, 2017.

Available online April 26, 2017.

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J Allergy Clin Immunol 2017;140:985-7.

0091-6749/\$36.00

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<http://dx.doi.org/10.1016/j.jaci.2017.03.041>

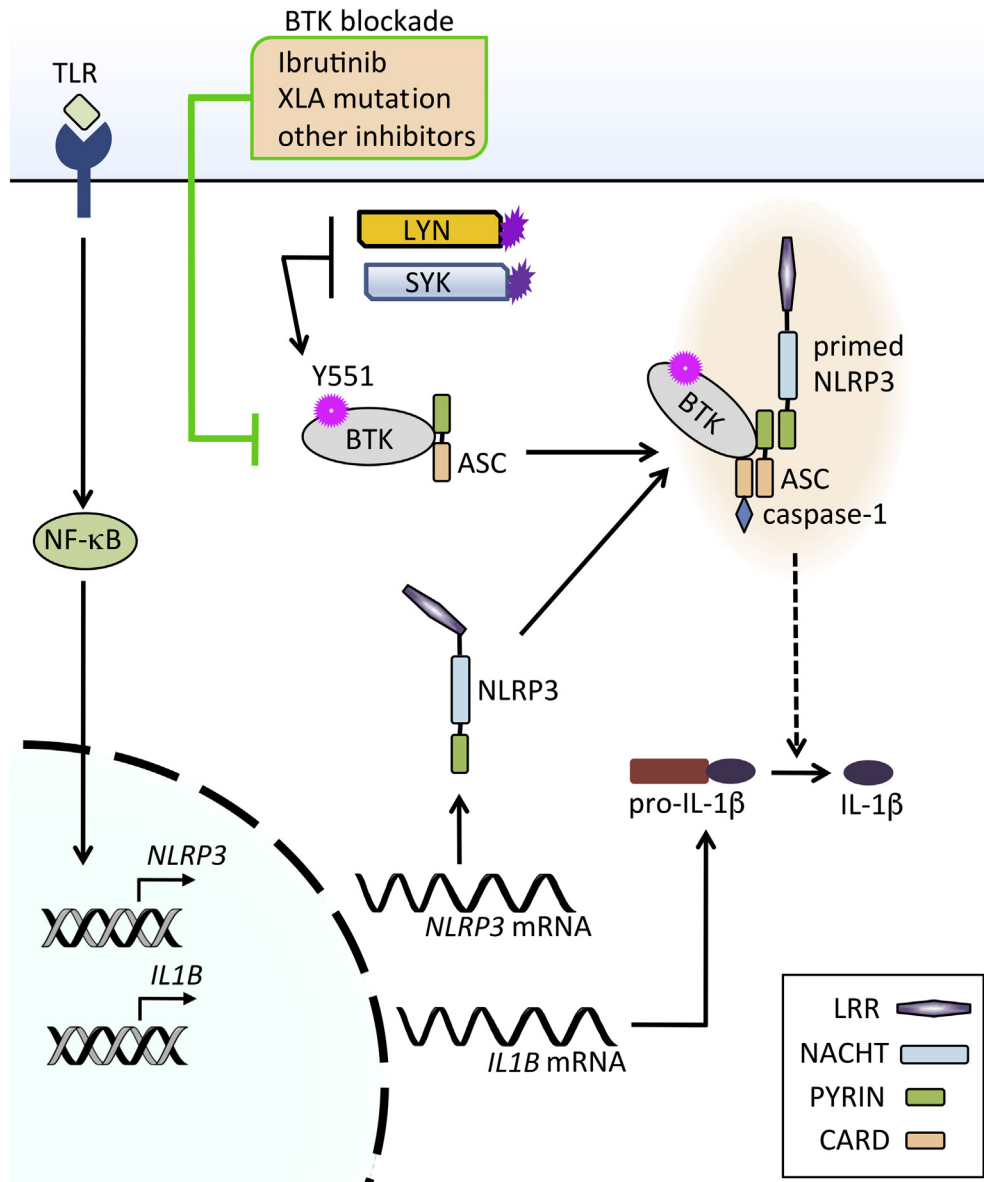


FIG 1. BTK-mediated regulation of NLRP3 inflammasome complex activation. Toll-like receptor (TLR) signaling primes the NLRP3 inflammasome for activation and upregulates *NLRP3* and *IL1B* mRNA. During NLRP3 inflammasome activation, BTK is activated through phosphorylation at tyrosine 551 and physically interacts with the NLRP3 inflammasome components NLRP3 and ASC. Interference with BTK, whether through mutation or chemical inhibition, results in failure of NLRP3 inflammasome activation with loss of IL-1β secretion and caspase-1 activation, showing BTK is necessary for NLRP3 inflammasome activation in patients. CARD, Caspase activation and recruitment domain; LRR, leucine rich repeat; NACHT, nucleotide binding and oligomerization domain; NF-κB, nuclear factor κB.

with genetic defects in *BTK* resulted in neuroprotection and concomitant downregulation of inflammation.

Although the findings of Ito et al¹ are an important start, it is unfortunately common that seemingly important pathways defined through *in vitro* studies and mouse models do not translate to relevance in human systems. In this issue Liu et al² build on these original findings and define the human and clinical relevance of the interaction of BTK and NLRP3. They first used an unbiased proteomics approach to show BTK is rapidly phosphorylated at the activating tyrosine Y551 residue during NLRP3 activation. They then confirm earlier *in vitro* studies,

this time in primary human mononuclear cells, by inhibiting BTK and showing abrogation of NLRP3 inflammasome activation.

The authors then resourcefully took advantage of the clinical use of the BTK inhibitor ibrutinib for malignancy to ask whether BTK inhibition is relevant to NLRP3 activation in a patient population. This is a particularly important finding because it circumvents the standard shortcomings related to attempts to extrapolate results from *in vitro* or mouse studies to human disease. They took blood from the patients undergoing ibrutinib therapy, stimulated NLRP3 in the cells, and assessed the competency

of activation of the NLRP3 inflammasome. They found that ibrutinib-treated mononuclear cells from patients had appreciably impaired NLRP3 activation, as shown by inhibition of IL-1 β release and failure to cleave caspase-1 to its active form. The authors also stimulated cells from patients with XLA and again found NLRP3 inflammasome activation was impaired, nicely confirming the specificity of ibrutinib inhibition to BTK function (Fig 1).

In another clever approach, the authors investigated the relevance of BTK inhibition to spontaneous activation of the NLRP3 inflammasome in patients with CAPS. Taking PBMCs from patients with CAPS, they added BTK inhibitors and assessed NLRP3 inflammasome activation. Here they again found that BTK inhibition markedly downregulated NLRP3 inflammasome activation, suggesting the BTK-NLRP3 interaction might be a valid therapeutic target in this population.

As with many studies, the relevance of these findings is found less within the actual data but more in the implications of those data. Here there are 2 primary findings that relate directly to patient care. First is the important insight that BTK function is required for NLRP3 inflammasome activation in the patient population. Although global inhibition of BTK is unlikely to translate into a useful treatment for inappropriate NLRP3-mediated inflammation, this understanding opens a new door for investigation. Perhaps a novel inhibitor could be generated that specifically blocks the BTK binding site on NLRP3, preventing the activation of NLRP3 but leaving other BTK-dependent functions intact.

The second important finding is that it is clear that patients with impaired BTK function, whether caused by a genetic mutation or pharmacologic inhibition, have a previously unappreciated defect in their innate immune function that is clinically relevant. That patients with XLA have been shown to have autoimmune disease despite their markedly impaired B-cell levels and function

suggests there is some degree of redundancy that preserves these pathways.¹⁰ Similarly, that fever and local inflammation are primary side effects associated with the use of ibrutinib indicates that not all innate immune pathways are compromised despite interference with NLRP3 activation. However, it will be important for these patients and their care teams to recognize this extended immunodeficient state and consider whether increased surveillance for infection or modification to current therapeutic approaches is indicated.

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