Human NACHT, LRR, and PYD domain–containing protein 3 (NLRP3) inflammasome activity is regulated by and potentially targetable through Bruton tyrosine kinase

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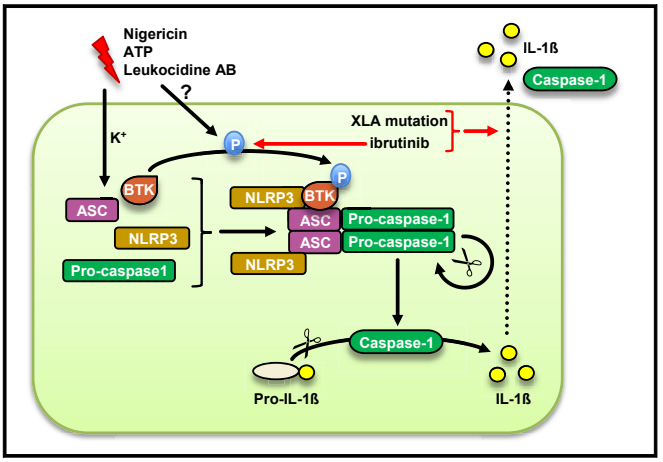
GRAPHICAL ABSTRACT



Background: The Nod-like receptor NACHT, LRR, and PYD domain–containing protein 3 (NLRP3) and Bruton tyrosine kinase (BTK) are protagonists in innate and adaptive immunity, respectively. NLRP3 senses exogenous and endogenous insults, leading to inflammasome activation, which occurs spontaneously in patients with Muckle-Wells syndrome; *BTK* mutations cause the genetic immunodeficiency X-linked agammaglobulinemia (XLA). However, to date, few proteins that regulate NLRP3 inflammasome activity in human primary immune cells have been identified, and clinically promising pharmacologic targeting strategies remain elusive.

Objective: We sought to identify novel regulators of the NLRP3 inflammasome in human cells with a view to exploring interference with inflammasome activity at the level of such regulators.

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Supported by the Else-Kröner-Fresenius Stiftung (to A.N.R.W.), the University of Tübingen, and the University Hospital Tübingen (Fortüne Grant 2310-0-0 to X.L. and A.N.R.W.).

Disclosure of potential conflict of interest: X. Liu has received a PhD stipend from the China Scholarship Council. T. Pichulik is employed by Merck Serono GmbH. A. Stutz has received grants from the European Research Council and the German Research Council, is employed by IFM Therapeutics GmbH, and has received stock/stock options from IFM Therapeutics GmbH. E. Daiber, L. Munzenmayer, B. Schulte, and C. Wolz have received grants from Deutsche Forschungsgemeinschaft. J. Kümmerle-Deschner is a board member for Novartis, has consultant arrangements with and has received travel support from Novartis and Sobi, has received a grant from Novartis, and has received payment for lectures and development of educational presentations from Novartis. M. Radsak has received a grant from Deutsche Forschungsgemeinschaft (CRC156/1 Project A05). E. Latz has consultant arrangements with IFM Therapeutics and Pfizer and has stock/stock options with IFM Therapeutics. S. Stilgenbauer has consultant arrangements with and has received payment for lectures and development of educational presentations from Janssen and Pharmacyclics. B. Grimbacher has received grants from BMBF, the European Union, Helmholtz, DFG, DLR, and DZIF; is employed by University College London and University Medical Center Freiburg; and has received payment for lectures from CSL Behring, Baxalta, and Biotest. L. Miller has consultant arrangements with Nantworks; has received grants from MedImmune, Regeneron Pharmaceuticals, Pfizer, and Nantworks; and has received stock/stock options from Noveome Biotherapeutics. A. N. R. Weber has received grants from Else-Kröner-Fresenius-Stiftung and Tübingen University Fortüne Program and is employed by the University of Tübingen. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication April 8, 2016; revised December 23, 2016; accepted for publication January 11, 2017.

Available online February 16, 2017.

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0091-6749/$36.00

http://dx.doi.org/10.1016/j.jaci.2017.01.017

Methods: After proteome-wide phosphoproteomics, the identified novel regulator BTK was studied in human and murine cells by using pharmacologic and genetic BTK ablation. Results: Here we show that BTK is a critical regulator of NLRP3 inflammasome activation: pharmacologic (using the US Food and Drug Administration–approved inhibitor ibrutinib) and genetic (in patients with XLA and *Btk* knockout mice) BTK ablation in primary immune cells led to reduced IL-1β processing and secretion in response to nigericin and the *Staphylococcus aureus* toxin leukocidin AB (LukAB). BTK affected apoptosis-associated speck-like protein containing a CARD (ASC) speck formation and caspase-1 cleavage and interacted with NLRP3 and ASC. *S aureus* infection control *in vivo* and IL-1β release from cells of patients with Muckle-Wells syndrome were impaired by ibrutinib. Notably, IL-1β processing and release from immune cells isolated from patients with cancer receiving ibrutinib therapy were reduced. Conclusion: Our data suggest that XLA might result in part from genetic inflammasome deficiency and that NLRP3 inflammasome–linked inflammation could potentially be targeted pharmacologically through BTK. (J Allergy Clin Immunol 2017;140:1054-67.)

Key words: *IL-1, ibrutinib, Bruton tyrosine kinase, NLRP3, inflammasome, macrophage, X-linked agammaglobulinemia,* Staphylococcus aureus*, Muckle-Wells syndrome, inflammation*

Abbreviations used

APC: Allophycocyanin
ASC: Apoptosis-associated speck-like protein containing a CARD
BMDM: Bone marrow–derived macrophage
BTK: Bruton tyrosine kinase
CFU: Colony-forming unit
DMSO: Dimethyl sulfoxide
DSS: Disuccinimydyl suberate
FDA: US Food and Drug Administration
FITC: Fluorescein isothiocyanate
GFP: Green fluorescent protein
HEK: Human embryonic kidney
IVIG: Intravenous immunoglobulin
KO: Knockout
LukAB: Leukocidin AB
M-CSF: Macrophage colony-stimulating factor
MoMac: Monocyte-derived macrophage
MWS: Muckle-Wells syndrome
Ni-NTA: Nitrilotriacetic acid
NLR: Nod-like receptor
NLRP3: NACHT, LRR, and PYD domain–containing protein 3
PE: Phycoerythrin
PIP_3: Phosphatidylinositol (3,4,5)-trisphosphate
PMA: Phorbol 12-myristate 13-acetate
PVL: Panton Valentine Leukocidin
SCX: Strong cation exchange
SH: Src homology
shRNA: Short hairpin RNA
TBP: TATAA-box binding protein
TH: Tec homology
TLR: Toll-like receptor
Xid: X-linked immunodeficiency
XLA: X-linked agammaglobulinemia

The human immune system relies on both innate and adaptive mechanisms to defend the host against infections (eg, from pathogenic bacteria, such as *Staphylococcus aureus*). Initial pathogen sensing by the innate immune system uses so-called pattern recognition receptors, such as Toll-like receptors (TLR) and Nod-like receptors (NLRs). Their activation by microbe-associated molecular patterns or invader-induced cellular insults leads to production of proinflammatory cytokines, which establish an inflammatory state and are critical in activating adaptive immunity.[1] Conversely to other cytokines, the important proinflammatory cytokine IL-1β is induced ("primed") at the mRNA level but requires processing and secretion initiated by NLR activation.[2] NACHT, LRR, and PYD domain–containing protein 3 (NLRP3), the most prominent NLR member, is activated by various pathogenic, environmental, and endogenous stress–related insults and thus plays a role in both microbe-elicited and sterile inflammation.[3,4] On activation, NLRP3 assembles a so-called inflammasome complex, with the adaptor apoptosis-associated speck-like protein containing a CARD (ASC) and caspase-1 leading to caspase autoactivation and consequent proteolytic cleavage of pro–IL-1β into bioactive IL-1β for subsequent secretion.[2] The latter is a vital step in host defense against infectious agents, such as *S aureus*.[5] Interestingly, the IL-1 axis also has pathophysiologic significance, as exemplified by autoinflammatory periodic fever syndromes, such as Muckle-Wells syndrome (MWS), in which rare gain-of-function polymorphisms in NLRP3 lead to spontaneous inflammasome activation.[4,6] Furthermore, NLRP3 inflammasome activity has been implicated in a diverse range of complex human diseases, including gout, rheumatoid arthritis, type 2 diabetes, atherosclerosis, and neurodegeneration.[4] Thus the NLRP3 inflammasome can be considered an attractive therapeutic target, but efforts to develop inflammasome inhibitors have been hampered by incomplete knowledge regarding the identity and role of the molecular steps involved in activating and regulating the inflammasome. Additionally, how the thus far proposed experimental inhibitors work is unclear. Thus identification of well-defined and therapeutically tractable regulatory proteins would be highly desirable.

Bruton tyrosine kinase (BTK) has long been regarded as a protagonist in adaptive antimicrobial defense because in the 1990s *BTK* mutations were discovered to be the cause for X-linked agammaglobulinemia (XLA),[7] the first described primary immunodeficiency.[8] XLA, also termed Bruton disease, is characterized by the almost complete absence of B cells and, consequently, antibodies, leading to severe immunodeficiency. More recently, BTK has also become appreciated as an important therapeutic target, such as in patients with B-cell malignancies, which are characterized by continuous B-cell receptor signaling through BTK.[9] Promising results have been obtained by using US Food and Drug Administration (FDA)–approved ibrutinib (PCI-32765), an orally administered, selective, and covalent BTK inhibitor, in patients with mantle cell lymphoma and chronic lymphocytic leukemia trials. Ibrutinib was both efficacious and well tolerated.[10,11]

BTK encodes a cytoplasmic protein tyrosine kinase expressed at high levels in B cells, controlling development, survival, differentiation, and activity from very early stages of development.[12,13] Additionally, *BTK* is also expressed in cells of the myeloid lineage, including macrophages, neutrophils, mast cells, and dendritic cells,[1,2] so that a contribution of the myeloid compartment to the overall phenotype of XLA cannot