TBK1's Role in Bacterial Pneumonia: Perhaps More than Macrophages and IFNs

Pneumococcal pneumonia continues to be a life-threatening illness. Although an early antibacterial immune response is critical for controlling infection and preserving organ function, excessive and timely unrestricted inflammation can lead to severe lung injury (1). A better understanding of the signaling pathways regulating inflammatory responses is therefore desirable, as it might help us develop novel therapeutic strategies to fine-tune antibacterial immunity during pneumonia.

TBK1 (TANK-binding kinase 1) is a multifunctional kinase that is ubiquitously expressed in both hematopoietic and nonhematopoietic cells. It performs multiple functions related to innate immunity, autophagy, and energy homeostasis (2). In antimicrobial innate defense, TBK1 is best known for its role in inducing type I IFN production through phosphorylation of IRF3 (IFN-regulatory factor 3) (3). This signaling cascade is initiated after sensing of, for example, viral RNA by RIG-I (retinoic acid–inducible gene I)–like receptors or TLR3 (Toll-like receptor 3), microbial DNA by the cGAS (cyclic guanosine monophosphate–AMP synthase)–STING (stimulator of interferon genes) pathway, or LPS by TLR4. cGAS and STING are involved in innate recognition of *Streptococcus pneumoniae* infection (4, 5), and type I IFNs have been shown to influence antipneumococcal defense in mice (6, 7). Moreover, TBK1 was previously shown to directly phosphorylate the transcription factor STAT6 (signal transducer and activator of transcription 6), thereby regulating expression of several chemokines during viral infection (8). In addition to promoting gene expression, TBK1 can also contribute to antimicrobial defense by regulating autophagic degradation of intracellular pathogens (9). TBK1's role in promoting autophagosome formation is dependent on its capacity to phosphorylate several molecules involved in autophagosome formation or regulation (2). Although *S. pneumoniae* has been shown to trigger canonical autophagy in epithelial cells (10), the role of autophagy in immune defense against pneumococci in the lung is not well understood.

In this issue of the *Journal*, Hagan and colleagues (pp. 671–681) report on the role of TBK1 in pneumococcal pneumonia (11). This work extends previous studies in which the investigators described key factors involved in IFN-γ production by neutrophils (12) and upregulation of TBK1 in neutrophils from *S. pneumoniae*–infected mouse lungs (13). In this new publication, the authors demonstrate reduced survival of TBK1-deficient mice upon *S. pneumoniae* infection compared with control animals. Moreover, systemic TBK1 knockouts, but not mice lacking TBK1 in macrophages, showed reduced proinflammatory cytokine production, a trend toward reduced type I IFN induction, and a slight defect in controlling bacterial replication (Figure 1). These data indicate that TBK1 in

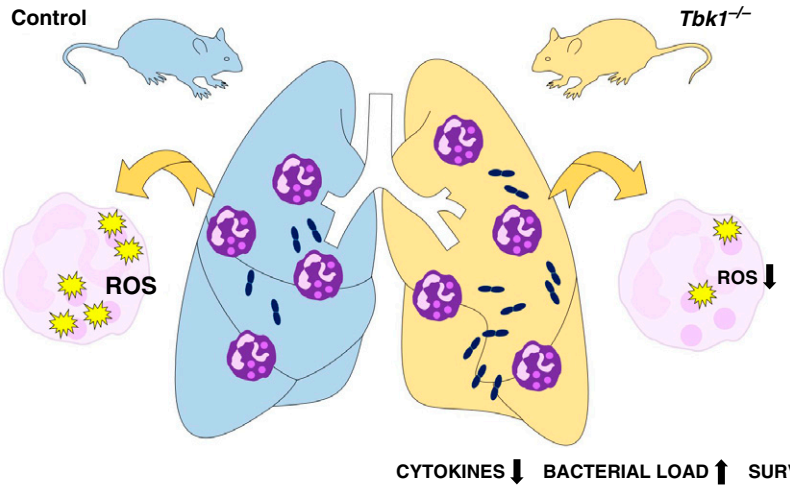

Figure 1. Schematic model representing the role of TBK1 in pneumococcal pneumonia in mice. After intranasal *S. pneumoniae* infection, TBK1-deficient mice show unaltered neutrophil recruitment into lungs but reduced neutrophilic ROS production, decreased pulmonary production of proinflammatory mediators, slightly higher bacterial loads in the lung, and increased mortality compared with control animals. Illustrations were partially created using templates from www.motifolio.com. ROS = reactive oxygen species; TBK1 = TANK-binding kinase 1.

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nonmacrophage cells contributes to antibacterial defense, and the authors focused on neutrophils as a candidate cell type.

They provide some data suggesting that TBK1 in neutrophils is activated upon pneumococcal infection *in vivo* and by TLR, but not STING, agonists *in vitro*. Moreover, neutrophils from *in vivo*–infected TBK1-deficient mice expressed reduced amounts of IFN-γ, IL-12, and reactive oxygen species (ROS) compared with cells from wild-type mice. Similar defects, however, were not observed upon stimulation of TBK1-deficient neutrophils *in vitro*. The authors therefore conclude that the TBK1-mediated cytokine and ROS production by neutrophils *in vivo* is indirectly induced by tissue-derived signals rather than directly stimulated through neutrophil-intrinsic pattern recognition receptors. Despite reduced ROS production by neutrophils from TBK1-deficient animals, the study does not provide direct evidence that this mechanism is relevant for the control of *S. pneumoniae in vivo*, and a previous study indicated that control of pneumococcal infection is not dependent on ROS (14).

These findings related to TBK1's role in antibacterial, perhaps neutrophil-mediated, defense in the lung are interesting. Nonetheless, several conclusions in the paper require further confirmation, particularly regarding the involvement (or lack thereof) of type I IFNs and the contribution of neutrophil-intrinsic TBK1. For example, type I IFN expression appears to be at least partially reduced in mice lacking TBK1 24 hours after infection, and a role for type I IFNs in TBK1-mediated control of pneumococcal infection therefore cannot be completely excluded. Moreover, further studies need to carefully dissect the relative contributions of various cell types (including neutrophils) to TBK1-mediated immune defense against bacterial infection of the lung. This could be achieved by using neutrophil-specific and perhaps other cell type–specific TBK1 knockouts and by further characterizing neutrophils from mice lacking TBK1 (e.g., regarding phagocytosis, bacterial killing, production of neutrophil extracellular traps). Finally, it would be interesting to investigate the role of TBK1 in autophagy and energy homeostasis during *S. pneumoniae* infection, which could play a role in antibacterial resistance and/or tolerance of the host.

The study by Hagan and colleagues proposes a possibly IFN-independent function of TBK1 in regulating the neutrophil's antimicrobial function during bacterial pneumonia. Although many questions about the exact mechanism remain open, this work lays the foundation for further research into the role of TBK1 in neutrophils and other cell types during lung infection. ■

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Facundo Fiocca Vernengo, Ph.D.
Department of Infectious Diseases and Respiratory Medicine
Charité–Universitätsmedizin Berlin
Berlin, Germany

Bastian Opitz, M.D.
Department of Infectious Diseases and Respiratory Medicine
Charité–Universitätsmedizin Berlin
Berlin, Germany
and
German Center for Lung Research
Berlin, Germany

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