Alleviation of gram-negative bacterial lung inflammation by targeting HECTD2

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Lung injury remains a significant clinical problem worldwide. The nature and pathogenesis of the injury is highly multifactorial as it can be acute or chronic, triggered by bacteria, viruses, fungi, transfusions, sepsis, multiple fractures, aspiration and several other factors. In the case of invading pathogens and sepsis, the innate immune system may get overwhelmed, resulting in the secretion of large amounts of proinflammatory cytokines which mediate pulmonary edema, shock and potentially, multi-organ failure (1,2).

From this perspective, Coon and colleagues have unraveled a novel pathway in murine models of pneumonia induced by either the gram-negative bacterium *Pseudomonas aeruginosa* (PA) or the gram-negative endotoxin lipopolysaccharide (LPS) (3). This pathway centers around protein inhibitor of activated signal transducers and activators of transcription (STATs) (PIAS). The PIAS protein family members all play important roles in the regulation of various cellular events such as cell survival, cell migration and mediation of several signal transduction pathways (4). The cellular effects of PIAS1 are generally translated to down-regulation of distinct inflammatory pathways (4) and interestingly, PIAS1 knockout mice have been shown to display significantly elevated levels of proinflammatory cytokines (5). In addition to inhibiting STAT, PIAS1 also inhibits nuclear factor κB (NF-κB)-dependent gene activation (5-9). The paper by Coon *et al.* uncovered a previously unappreciated role for HECTD2, a ubiquitin E3 ligase, which is able to ubiquitinate and mediate the degradation of PIAS1 thereby rescuing STAT and NF-κB signaling and enabling pulmonary inflammation in mice. Furthermore, the authors performed a single-nucleotide polymorphism (SNP) database analysis and discovered a naturally occurring nonsynonymous G/C polymorphism (rs7081569) within HECTD2 (A19P) with an allele frequency of 8.5%. This HECTD2^{A19P} was found to predominantly reside in the cytosol and had lost the ability to degrade PIAS1 protein in vitro as nuclear entry of HECTD2 was shown to be required for interaction with PIAS1. To further assess the contribution of HECTD2^{A19P} *in vivo*, mice were infected with a lentivirus encoding HECTD2^{WT} (wildtype) or HECTD2^{A19P} followed by challenging the mice with PA. In contrast to mice infected with HECTD2^{WT}, mice infected with HECT2D2^{A19P} failed to induce PA-mediated lung injury. As PA-induced pneumonia is also implicated in acute respiratory distress syndrome (ARDS) (10), the authors evaluated the HECT2D2^{A19P} polymorphism in a cohort of 63 patients with or at-risk for ARDS. The results indicated that not a single patient carried the HECT2D2^{A19P} polymorphism. They next investigated the inflammatory effects induced by HECTD2 *in vivo* and found that PIAS1 knockdown induced significant lung injury in mice as assessed by bronchial lavage protein concentrations, lavage cell counts, lavage cytokines and cell infiltrates. Subsequently, knockdown of HECTD2 ameliorated the PA-inflicted lung

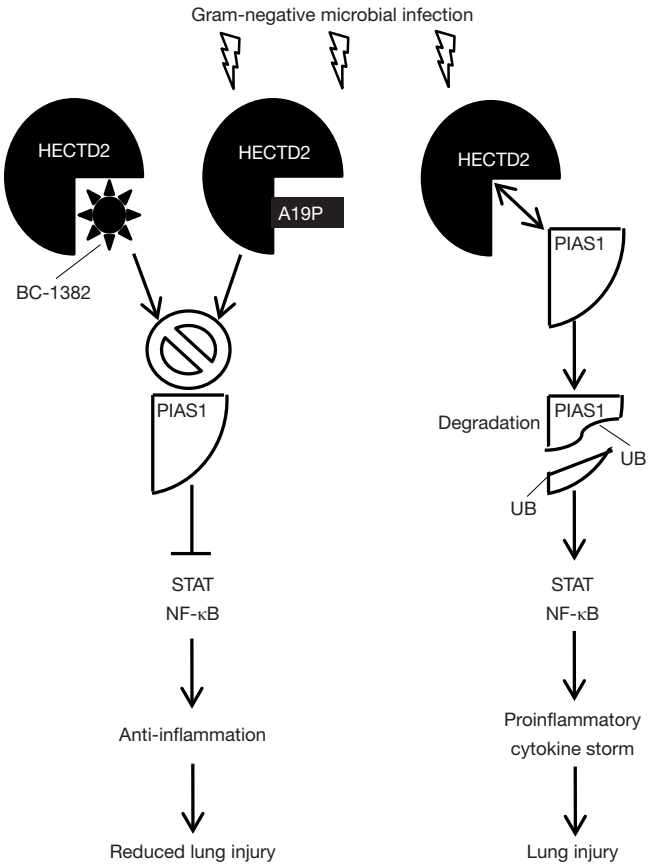

Figure 1 Proposed mechanism by Coon and colleagues of gram-negative microbial infection-induced lung injury based on HECTD2-PIAS1 interaction. During gram-negative bacterial infection, HECTD2 interacts with PIAS1 resulting in PIAS1 UB and subsequent degradation. This leads to increased inflammation via elevation of STAT and NF-κB levels and release of proinflammatory cytokines which enables lung injury. HECTD2^{A19P} mainly resides in the cytosol and is incapable of interacting with PIAS1 in the nucleus. Similarly, the small molecule inhibitor BC-1382 targets HECTD2 also preventing signaling which leads to PIAS1-degradation. Consequently, HECTD2^{A19P} as well as BC-1382 promote anti-inflammatory signaling via inhibition of STAT and NF-κB which alleviates and protects from lung injury. NF-κB, nuclear factor κB; STAT, signal transducers and activators of transcription; UB, ubiquitination; PIAS, protein inhibitor of activated signal transducers and activators of transcription (STATs).

injury *in vivo*. Next, the authors cleverly searched for a small-molecule inhibitor of HECTD2 and as such identified compound BC-1382. They confirmed the binding as well as the inhibitory effect of BC-1382 towards HECTD2 and showed that BC-1382 also improved PIAS1 protein stability by increasing its half-life and by suppressing LPS-induced PIAS1 degradation. Importantly, BC-1382 also decreased the severity of the lung injury and cytokine levels in both murine LPS- and PA-induced pneumonia models as was assessed 18 hours after IP injection of the compound.

The findings by Coon *et al.* are summarized in *Figure 1*, which schematically shows that during microbial infection with gram-negative bacteria, HECTD2 targets PIAS1, resulting in PIAS1-ubiquitination (UB) and degradation. As a result, cytokine-driven inflammation is promoted resulting in lung injury. Compound BC-1382 can bind and inhibit HECTD2 and prevents PIAS1 degradation thereby shifting the balance towards PIAS1-induced anti-inflammatory signals which suppress secretion of proinflammatory cytokines and alleviates the lung injury. The mutated HECTD2^{A19P}, however, mainly resides in the cytosol and is incapable of interacting with PIAS1 in the nucleus thus protecting individuals from lung injury induced by PIAS1-degradation.

The study by Coon *et al.* has greatly advanced the field and should be seen as a stepping stone towards deciphering the contribution of the HECTD2-PIAS1 pathway in other models of experimental lung injury. For instance, would this model also be relevant and therapeutically exploitable in a setting of gram-positive bacterial lung inflammation such as when inflicted by *Streptococcus pneumonia* which is in fact one of the main pathogens responsible for community-acquired pneumonia worldwide (11-14)? Similarly, the setting of viral or fungal-related pneumonia would also be interesting to assess the effects and the relevance of the HECTD2-PIAS interaction. Likewise, the contribution of the acute phase protein C-reactive protein (CRP) in this context would be interesting as it was recently shown that CRP can enhance transfusion-related acute lung injury (TRALI) in mice in part through the enhancement of the pro-inflammatory cytokine macrophage inflammatory protein (MIP)-2 (15), the murine ortholog of interleukin (IL)-8. Although the authors did assess HECTD2 polymorphisms in patients at-risk or suffering from ARDS, it would be insightful to perform these studies in other patient populations as well such as patients with or at-risk for (recurrent) pneumonia. Therapeutic compounds targeting HECTD2 may prove to be a valuable intervention especially in light of the increasing resistance to antibiotic regimens. As the authors noted, however, the safety profile and *in vivo* kinetics of such therapeutic compounds will need to be extensively characterized.

Overall, the paper by Coon *et al.* provides compelling data on the pathogenesis of gram-negative bacterial pneumonia with novel therapeutic insights. This may have the potential to eventually result in an attractive alternative for antibiotic-resistant pneumonia. These findings should stimulate further investigations into various disease models and patient cohorts of different types of lung injury.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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