Evaluating the NET Influence of Inflammation on Pneumonia Biology

Respiratory infections represent the greatest burden of disease worldwide, with a mortality rate that is virtually unchanged since the dawn of the antibiotic era (1). Although acute pulmonary inflammation is required for defense against invading microbes, it often comes at the expense of disrupted tissue homeostasis. Consequently, pneumonia is the leading cause of acute lung injury (2). At the forefront of this important but dangerous pulmonary immune response are neutrophils, which bear an armament of robust antimicrobial machinery, but can directly exacerbate inflammatory injury (3). In the context of infection, our community has long struggled to understand whether and how this delicate balance can be controlled.

In this issue of the *Journal*, Barletta and colleagues (pp. 1044–1050) aim to determine the influence of adenosine A_{2B} receptor signaling on pneumonia induced by *Klebsiella pneumoniae* (4). Previous reports have almost uniformly supported a tissue-protective role for signaling downstream of the A_{2B} receptor in settings of lung injury, including that elicited by lipopolysaccharide (5, 6), mechanical ventilation (7), bleomycin (8), and hypoxia (9). Moreover, antiinflammatory phenotypes have coincided with evidence that adenosine (via A_{2B} receptor) limits fundamental neutrophil functions such as migration (5, 6), oxidative burst (5), and phagocytosis (10). Based on the reciprocal relationship observed between A_{2B} receptor signaling and neutrophilic inflammation, Barletta and coworkers hypothesized that deletion of this receptor would improve antibacterial host defense during pneumonia, a condition during which the role of A_{2B} receptor signaling has never been explored. After all, it seems only natural that enhanced inflammation would generate a less favorable environment for bacteria.

As anticipated, mice devoid of A_{2B} receptor exhibited significantly less mortality in association with decreased bacterial burdens in the lungs and blood. Perhaps the most compelling finding of this study, however, is not what was different, but rather, what was not. By all accounts (airspace leukocytosis, cytokine expression, plasma extravasation, etc.), typical indices of innate immunity were unaffected by A_{2B} receptor deficiency in pneumonic mice. At first glance, this contradicts the aforementioned reports of exaggerated pulmonary inflammation and worsened outcomes in A_{2B} receptor-deficient mice (5–9). However, the pathology in those studies was solely driven by sterile inflammation, as opposed to here where the pathological response to live infection was subject to the complex dynamics of bacterial viability. In this particular instance wherein bacterial burdens were reduced, one may predict fewer neutrophils as a consequence of fewer inflammatory signals. As this is not the case, the results are in fact consistent with the antiinflammatory roles previously ascribed to this receptor. Interestingly, the authors provide several pieces of evidence that unequivocally implicate neutrophils in A_{2B} receptor-dependent outcomes, despite equivalent neutrophil numbers. First, A_{2B} receptor expression was far greater for lung neutrophils compared with other lung cells and even resting bone marrow neutrophils. Second, improved host defense was reproducible in chimeric mice lacking A_{2B} receptor in hematopoietic cells alone. Last, neutrophil depletion significantly reduced the benefit of A_{2B} receptor deficiency on antimicrobial defense. So although the quantitative aspects of neutrophilic alveolitis were insufficient to explain the protection afforded by A_{2B} receptor deficiency, this cell type was nonetheless at the heart of the phenotype.

To explain the improved bacterial killing due to A_{2B} receptor deficiency, the authors turned their attention toward neutrophil function. *In vitro* phagocytosis, intracellular killing, and oxidative burst were all unchanged in neutrophils exposed to *K. pneumoniae*. Interestingly, A_{2B} receptor deficiency caused a remarkable increase in extracellular bacterial killing by cultured neutrophils, which was mirrored by a marked increase in extracellular DNA content. Therefore, in contrast to more traditional indices of neutrophil function, neutrophil extracellular traps (NETs), as indicated by DNA content, were found to be under exquisite control of the A_{2B} receptor. Increased DNA levels and NET formation in the bronchoalveolar lavage fluid of A_{2B} receptor-null mice also corroborated these findings *in vivo*, raising the interesting question of whether or not NETs were causally linked to improved host defense in *K. pneumoniae*-challenged mice. NETs were fairly recently discovered as an antimicrobial effector strategy for neutrophils (11). These chromatin-based lattices provide an efficient scaffold for antimicrobial proteins (12), but our understanding of their contribution to pulmonary host defense is rudimentary at best. Here, the authors found that *ex vivo* killing by A_{2B} receptor-deficient neutrophils was completely abrogated by DNase. Given the other findings of this study, this result strongly suggests that NET formation is mechanistically linked to improved bacterial killing in A_{2B} receptor-deficient mice.

These findings coincide with another recent study in the *Journal* linking NETosis to pulmonary host defense (13). Yamada and colleagues found that mice lacking a functional gene for IFN γ had impaired clearance of *Streptococcus pneumoniae* in association with decreased NET formation (13). Although different in several important ways, this study had an interesting resemblance to the current work by Barletta and coworkers. Neither airspace neutrophil content nor acute lung injury was associated with bacterial killing in either study. In fact, IFN γ -deficient mice actually had increased numbers of airspace neutrophils, despite impaired host defense (13). These findings urge caution for the interpretation of neutrophil recruitment data, as it is easy to dismiss important roles for this cell type when their numbers do not align with biological outcomes.

Although the present study was explicitly designed to interrogate the influence of adenosine signaling through the A_{2B} receptor during pneumonia, the data implicate NETosis as an important aspect of innate immunity, regardless of its relationship with adenosine. But many questions remain unanswered, not the least of which relate to generalizability. The degree to which A_{2B} receptor and/or NETs influence other types of lung infection is unknown. It is also far from certain that NETs are universally beneficial during pneumonia. For instance, a recent study by Caudrillier and colleagues convincingly demonstrates a contribution of NETs to transfusion-related lung injury in mice and humans (14). With regard to adenosine signaling, it is also possible that infections other than *K. pneumoniae* elicit an entirely different landscape for signaling, with varying receptor expression and/or different degrees of adenosine release. This is particularly important considering the functional diversity of the four distinct adenosine receptors, all of which are expressed on neutrophils (15). Therefore, although it is attractive to consider the therapeutic potential of A_{2B} receptor antagonism in patients with lower respiratory infection, only time will tell whether these experimental findings translate to clinical utility. Regardless, this report provides important insights regarding the innate immune response

to gram-negative pneumonia, and emphasizes the need to look beyond neutrophil number as an assessment of function. Most compellingly, this study substantively improves our understanding of the signaling pathways involved in lung NETosis, including the fact that these pathways are here demonstrated to be critical to the outcome of pneumonia.

Author disclosures are available with the text of this article at www.atsjournals.org.

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