Spotlighting "Neutrophil Elastase Triggers the Release of Macrophage Extracellular Traps": A New Catch in Cystic Fibrosis?

Before 2004, airway disease in cystic fibrosis (CF) was characterized as early bacterial infection associated with neutrophil-predominant inflammation. Data supporting inflammation both as a feature intrinsic to CF and as a response to infection were cited, with either or both contributing to repetitive cycles of tissue destruction leading to clinical bronchiectasis and early mortality. In the airways, copious accumulation of DNA, unopposed neutrophil elastase (NE) activity, reactive oxygen species, and persistent infection were thought to be consequences of ineffective immune responses and cellular necrosis, leading to mucus obstruction. In retrospect, this general formulation appears overly spare given the discovery that leukocytes release extracellular DNA fibers that can entrap and kill various microbes by a process referred to as ETosis. These neutrophil extracellular traps (NETs) and NETosis were first described in 2004 (1) and spawned a distinct area of research focusing on immune cell responses to bacterial infection applicable to multiple diseases, including CF. The process of NETosis includes a well-characterized series of regulated events during neutrophil cell death that alter the microbicidal milieu by stimulating the release of pathogenic enzymes, proteases, histones, and reactive oxygen species. These represent both beneficial and pathologic responses to infection. Similar extracellular structures were soon found to originate from macrophages (termed macrophage extracellular traps (METs; *see* Reference 2 for a contemporary review), albeit with a less extensive body of literature than for NETs and NETosis.

In this issue of the *Journal*, Kummarapurugu and colleagues (pp. 76–85) report that NE promotes MET release in human blood monocyte–derived macrophages (hBMDM) from subjects with and without CF as well as from alveolar macrophages (AMs) harvested from *Cftr*-null and wild-type mouse littermates (3). The study expands the range of diseases affected by METs to include CF. In this study, fluorescently conjugated NE was taken up by both types of macrophages, localized to nuclear domains, and maintained in a proteolytically active form. Because DNA is a major structural component of extracellular traps, NE induction of MET formation was clearly observed in DAPI and antihistone antibody-labeled cells using standard microscopy. An overview of key results confirming defining steps in METosis reported by Kummarapurugu and colleagues are illustrated in Figure 1A.

This research follows, many years later, an investigation of NE and its uptake by macrophages (4). Intervening studies to understand that result led to an enhanced understanding of several macrophage-mediated activities that repurpose NE. Specifically, it was found that internalizing NE might be a clearance mechanism important for resolution of inflammation (4), a step toward an NE-triggered macrophage-mediated amplification of inflammatory signals (5), and a macrophage-mediated method to prolong and direct NE-mediated proteolysis (6, 7). There are other potential pleiotropic activities of NE following degranulation into the airway (and other tissues and spaces) and uptake by macrophages, but the current work initiates a novel branch of investigation into the macrophage-related fate of NE in the airway, specifically the involvement of METs in CF.

The role of METs in the pathogenesis of CF, as implied by the authors, requires additional study. Their initial approach involved classic reductionism, involving the study of hBMDM *in vitro*, which eliminates the possibility of mistaking previously confirmed NETosis *in vivo* for METosis. The authors proceeded to demonstrate that non-CF BMDMs release significantly higher proportions of METs compared with CF BMDMs (as reported in figure 2 of Reference 3).

By including both CF-specific and contrasting non-CF cells *in vitro*, the potential role of abnormal METosis in CF was confirmed and highlighted with certainty. Because the human CF lung phenotype is notorious for hyperinflammation and incomplete clearance of infection, the lack of MET release in response to NE in cultured CF BMDM cells could implicate important antimicrobial roles for METs in lung disease. Deficient METs in CF may simply fail to augment other bacterial clearance mechanisms yet may be sufficient to promote tissue damage. Intriguingly, because the time course by which CF cells undergo METosis *in vivo* remains unknown, we cannot discount the possibility that MET release detrimentally impacts CF lungs by facilitating bacterial growth and aggregation on the long protrusions of scaffolding DNA (8).

The *in vitro* work is convincing, however, a demonstration that CF-specific pathogenicity involving METosis occurs *in vivo*, and is not an artifact of hBMDM culture, is still required. This report takes the first steps to address this critically important issue by treating *Cftr*-null mice with NE; however, this particular animal model may have limited utility in demonstrating relevance to CF because gut-corrected *Cftr*-null mice lack a severe CF lung phenotype (9).

Model studies within a CF-like lung phenotype often use epithelial sodium channel–overexpressing mice. These mice lack a CF genotype but do exhibit an epithelial sodium channel overexpression (*Scnn1b*) genotype, with a resulting phenotype that mimics the human pathophysiology of disrupted CFTR chloride channels in CF and produces inflammatory lung disease (10). Using such mice in the absence of infection, Tucker and colleagues demonstrated key features expected of NETosis earlier this year (11). That study suggests that NE is available in a mouse with a CF-like lung phenotype that could initiate METosis. The work by Kummarapurugu and colleagues leads to the prediction that METosis should occur under these conditions. With similar mice immediately in hand for CF-related investigations and highly intrigued by the

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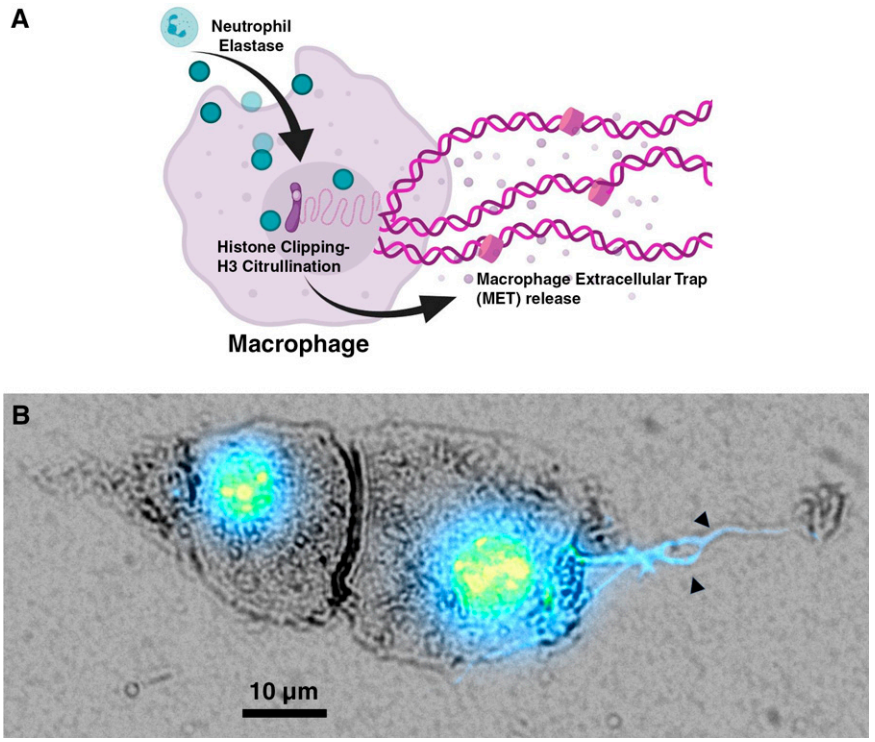

Figure 1. (*A*) A model of neutrophil elastase–activated macrophage extracellular trap (MET) release, as exemplified by recent findings by Kummarapurugu and colleagues. Neutrophil elastase (NE) stimulation induced MET release in *in vitro* studies of human blood monocyte–derived macrophages cells (derived from subjects with and without cystic fibrosis) and *in vivo* after intratracheal administration of NE (into *Cftr*-null and wild-type littermates). NE retained protease activity and associated with partial cleavage of Histone 3. MET-releasing cells maintained viability. (*B*) METosis observed in alveolar macrophages obtained from 6-week-old female Scnn1b-Tr mice with severe cystic fibrosis phenotype (9). An epifluorescent image of DAPI-stained DNA overlayed on a phase contrast image of two AMs is shown. Arrowheads indicate extracellular DNA filaments in a MET. Scale bar, 10 μm.

current study, we could not resist a peek. Recovery and microscopic examination of AMs from 6-week-old female Scnn1b-Tr mice without the addition of NE or other processing revealed AMs that demonstrate key features of METosis (Figure 1B). A single picture is only a beginning, but it shows that the work by Kummarapurugu and colleagues is not a short-lived research story because it implicitly allows for the prediction of further experimental outcomes.

In summary, the findings in Kummarapurugu and colleagues are important and expand our scientific understanding of the complex role that macrophages play in host immune responses. As is often the case with novel work, many new questions now arise, and a feature of their work is an implicit ability to predict experimental outcomes. Such predictions invite investigations in multiple directions using both basic and clinical methods. ■

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