Neutrophil extracellular trap release driven by bacterial motility: Relevance to cystic fibrosis lung disease

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ABSTRACT
Neutrophil extracellular trap (NET) formation represents a unique effector function of neutrophils (PMN). The mechanism of NET release in response to bacteria is largely unknown. We studied the process by which Pseudomonas aeruginosa, an opportunistic pathogen, interacts with primary PMNs, and found that flagellar swimming motility of the bacterium is essential for inducing NET extrusion. Cystic fibrosis (CF) lung disease is associated with P. aeruginosa infection and PMN-dominated inflammation. Although NETs are abundant in CF airways, the main factors triggering NET release in CF remain unclear. Our study implicates that motile P. aeruginosa is a strong NET-inducer in CF. In early stages of CF lung disease flagellated, motile isolates of P. aeruginosa are characteristic and their interactions with PMNs could lead to NET formation. In chronic CF, P. aeruginosa down-regulates its flagellum expression to avoid recognition by the immune system and forms biofilms. Flagellated bacteria, however, are released from biofilms and could interact with PMNs to form NETs. Although flagellated forms likely represent only a small fraction of the total P. aeruginosa load in chronic CF, NET release induced by them could have a significant impact on inflammation and lung function since flagellated forms trigger the most robust response of the immune system including PMNs.

Overall, we speculate that NET formation driven by motile P. aeruginosa could be a novel, significant contributor to pathogenesis at both, early and late stages of CF lung disease.

CF airways contain large numbers of PMNs. CF sputum PMN counts, levels of extracellular DNA, myeloperoxidase and human neutrophil elastase all correlate with CF lung disease severity.1-6 PMNs are the clinically most important leukocyte in chronic CF airways and PMN-mediated inflammation contributes to lung disease. Extracellular DNA is derived from the host,7 mainly PMNs.5,7,8 Although PMNs were thought to die by necrosis in CF airways, recently other mechanisms have been proposed. NET formation provides an attractive alternative explanation since PMNs simultaneously release lung-damaging extracellular DNA and primary granule components by forming NETs, and NETs are abundant in the airways of adult CF patients. We and others reported robust NET release from human PMNs induced by laboratory strains and CF clinical isolates of P. aeruginosa.9,14 Since NETs could mediate the release of lung-damaging PMN cargo but could also be important in fighting pathogens, their exact, potentially complex, role in CF airway disease remains to be elucidated (Fig. 1).

Flagellar motility drives P. aeruginosa-induced NET release
In our current study, we found that early exponential growth phase cultures of P. aeruginosa elicited the most robust NET release and presence of a functional flagellum was essential for this process.14 Immotile bacterial mutants without flagellum or with nonfunctional flagellum are weak NET-inducers.14 Forced contact of immotile P. aeruginosa with PMNs restored their ability to trigger maximal NET extrusion.14 P. aeruginosa flagellin alone was unable to induce NET release.14 In a genetic complementation study we found that both, motAB and motCD loci of P. aeruginosa flagellar motor genes are needed for maximal NET induction in human PMNs.14 Thus, we identified flagellar swimming motility as a novel microbial factor crucial to PMN activation and NET formation.

Flagellated P. aeruginosa in CF
Although it is undocumented whether NETs are present at early stages of CF lung disease, bacterial motility-
fueled NET formation likely occurs at this initial phase because early CF clinical isolates of *P. aeruginosa* typically express flagellum\(^{15,16}\) and PMNs are also present\(^{17}\) (Fig. 1). The interaction of PMNs with early forms of *P. aeruginosa* must be critical to determine later progression of CF lung disease. Important questions to be answered are why NETs released at this early stage would be incapable of clearing *P. aeruginosa* infection and instead drive bacterial adaption toward an aflagellated, biofilm-forming phenotype.

Over the course of CF lung disease, *P. aeruginosa* down-regulates its flagellum expression\(^{15,16,18-20}\). In chronic CF airways, *P. aeruginosa* mainly exist in 3-dimensional, “suspension biofilms” also called non-attached aggregates (Fig. 1).\(^\text{21-23}\) These suspension biofilms surrounded by PMNs represent the characteristic clinical picture in chronic CF airways.\(^\text{21-24}\) Biofilms are dynamic structures, and motile, flagellated bacteria likely break free from biofilms in chronic CF, possibly interacting with PMNs (Fig. 1). This is supported by recent data showing that *P. aeruginosa* flagelin is detected in sputa of chronic CF patients.\(^\text{25}\) PMNs phagocytosing planktonic *P. aeruginosa* have also been observed in chronic CF.\(^\text{26,27}\) Nonmucoid revertant cells of *P. aeruginosa* have also been documented in chronic CF airways.\(^\text{15,18}\)

A minor population of flagellated *P. aeruginosa* in chronic CF airways could also have been marginalized so far because this topic has not been intensely investigated yet, and this population is hard to study and could have been overlooked in the presence of much more abundant biofilm-bound bacteria accumulating well-characterized mutations (mucA, lasR).\(^\text{28,29}\) Conclusions with respect to the population structure of *P. aeruginosa* in CF have largely been generalized based on results obtained on single bacterial isolates, although high levels of phenotypic diversity among *P. aeruginosa* isolates within individual CF patients have already been noted.\(^\text{30}\) A small population of flagellated *P. aeruginosa* could be found in CF airways while most *P. aeruginosa* are present in form of alginate-producing, elastase-negative bacteria. *MucA* mutations drive the mucoid, biofilm-forming phenotype, *lasR* mutations contribute to PMN recruitment\(^\text{28}\) while outbreaks of flagellated bacteria from biofilms could be mainly responsible for PMN activation and NET release.\(^\text{14}\) PMNs quickly and easily recognize motile, flagellated forms of *P. aeruginosa* and launch their robust effector mechanisms including NET release in response to them.\(^\text{14}\) On the other hand, bacterial biofilms likely provide a much weaker stimulus for PMN activation. Therefore, the way motile *P. aeruginosa* interacts with PMNs, the cell type representing most of cells found in chronic CF airways, is likely an important factor in influencing the progression of CF lung disease despite the fact that planktonic forms of *P. aeruginosa* are outnumbered by those found in biofilms (Fig. 1). Overall, we speculate that *P. aeruginosa* motility-driven PMN activation has clinical relevance not only at initial but also later stages of CF airway disease.\(^\text{14}\)

**Disclosure of potential conflicts of interest**

No potential conflicts of interest were disclosed.

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