The rat takes the cheese: a novel model of CFTR-dependent chronic bacterial airway infection

Alessandra Livraghi-Butrico [1,3], Taylor B. Franklin [1] and Matthew C. Wolfgang [1,2,3]

[1]Marsico Lung Institute/Cystic Fibrosis Center, University of North Carolina School of Medicine, Chapel Hill, NC, USA. [2]Department of Microbiology and Immunology, University of North Carolina School of Medicine, Chapel Hill, NC, USA. [3]A. Livraghi-Butrico and M.C. Wolfgang contributed equally to this article as lead authors.

Corresponding author: Alessandra Livraghi-Butrico (alessandra_livraghi@med.unc.edu)

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In cystic fibrosis (CF), the absence of cystic fibrosis transmembrane conductance regulator (CFTR)-mediated Cl^- secretion, which normally drives airway surface hydration, results in hyperconcentrated and stagnant mucus, which becomes a niche for bacterial infection. Desirable features of a CF model of infection would be a host that exhibits the main pathophysiological feature of human CF lung disease, *i.e.* airway mucus hyperconcentration and consequent reduced mucus clearance. While currently available large CF animal models appear to recapitulate these aspects of human disease, they also present significant animal husbandry challenges, have limited availability and are cost prohibitive for routine experimentation [1]. Rodent models are usually the preferred preclinical testing platform to understand disease pathology and therapeutic efficacy. So far, modelling of CF-like chronic bacterial infection has been attempted in mice using bacteria embedded in agar beads [2–5], and although this model has proven useful to study mechanism of host and bacterial adaptation, it has not shown robust differences in the establishment of chronic infection between wildtype (WT) and CF mice [6, 7], suggesting that the CF murine models tested failed to fully recapitulate the human phenotype. CF rats, on the other hand, appear to have more relevant features to become the next best candidate to establish a model of CF-like chronic infection, including robust CFTR expression, and possibly reliance on control airway surface hydration, in large and small airways [8], coupled to age-dependent abnormalities in airway mucus secretion and clearance [9, 10].

The study reported by Henderson *et al*. [11] provides the first phenotypic description of CF rats infected with *Pseudomonas aeruginosa* embedded in agar beads. Infections were carried out in young (2 months old) *versus* old (6 months old) rats, two timepoints where the mucus abnormalities are either absent or present, respectively. WT and CF rats [9] were phenotyped longitudinally up to 28 days post-infection (DPI) using a comprehensive array of assays, including bacterial burden, bronchoalveolar lavage differential cell counts, inflammatory mediators, histology, mucin quantification, and micro-optical coherence tomography-based determination of critical mucociliary clearance parameters. The emerging picture is truly encouraging. Infection at the two different timepoints defined by absence and presence of mucus defects resulted in two different outcomes. At a young age, CF rats were indistinguishable from their WT littermates: bacteria were cleared over time and the inflammatory response followed the expected trend of acute elevation and subsequent waning. In contrast, infection of older CF rats yielded an acute drop in bacterial burden followed by a steady increase in colonisation, inflammation and mucus production, all in the face of steadily declining mucociliary clearance, which was otherwise in hyperdrive in WT rats. Henderson *et al*. [11] conclude that "mucus transportability is the overriding factor permitting infection persistence" on the premise that this is the primary difference between 2- and 6-month-old knockout rats. In an interesting final twist, Henderson *et al*. [11] re-infected the rats to recapitulate

recurrent infections in the human population and concluded that the mucosal alterations induced by the previous infection resulted in further reduced bacterial clearance and heightened inflammation in CF compared to WT rats.

This is a very timely and welcome addition to the CF animal models armamentarium, as it provides a solid base from which to tackle unresolved questions in CF pathophysiology and explore novel therapeutic avenues. Some key questions emerge from the study itself. The primary goal of this study was to generate a chronic infection model with CF-like mucus obstruction. While this goal was achieved, a more thorough understanding of the infection microenvironment is still needed. The vehicle for infection in this and related models is *P. aeruginosa* embedded agar beads. This approach is necessary to ensure airway deposition; however, the impact of agar itself was not considered. Key questions remain as to the location of the bacteria, *e.g.* do they remain within the beads or transition to a true mucus infection? The early drop in bacterial burden at DPI 3–7 paralleled by a spike in neutrophil infiltration, followed by a steady increase in bacterial burden along with elevation in MUC5AC, decreased neutrophilia, and mucus obstruction, is intriguing. While speculative, this change in infection dynamics may represent transition of the infection from bead to mucus. Closer histological examination of the infected tissue, however, was not provided and would be a meaningful future study. Similarly, as more specific reagents become available, it would be important to assess the relative role of MUC5AC *versus* MUC5B in disease progression. Moreover, further measures of mucociliary clearance with particular focus on the intrapulmonary compartment, which so far is still outside the reach of micro-optical coherence tomography, would provide additional mechanistic details.

In the era of highly effective modulator therapies, a growing area of interest is the interplay between infection/inflammation and CFTR potentiator/corrector therapies. Potentially conflicting *in vitro* studies report that *P. aeruginosa* infection can reduce potentiator/corrector-stimulated F508del-CFTR Cl^- secretion [12], while a recent study demonstrated that complex inflammatory stimuli collected from infected CF lungs can enhance F508del rescue by CFTR modulators [13]. While these results were derived from different systems and are not directly comparable, it underscores the need for an *in vivo* model that recapitulates the complex CF lung environment during infection. An important future direction of this study will be to use biologically relevant CFTR mutations (*e.g.* F508del), or mutations sensitive to pharmacological (G551D [14, 15],) or genetic [16] correction, as the genetic background.

In summary, Henderson *et al*. [11] introduce a novel rodent model of chronic infection capable of recapitulating hallmark aspects of CF lung disease, *i.e.* mucus hypersecretion and reduced mucociliary clearance, with benefits of scale. This work represents significant progress in the development of *in vivo* models suitable to elucidate the complex relationship between bacterial persistence and eradication strategies in the unique CF lung setting.

Conflict of interest: The authors have no competing interests.

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