Cystic fibrosis, gene therapy, and lung inflammation: for better or worse?

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THE STUDY by van Heeckeren and colleagues, one of the current articles in focus (Ref. 20, see p. L717 in this issue), addresses the question of whether adenoviral delivery of cystic fibrosis transmembrane conductance regulator (CFTR) to the cystic fibrosis airways attenuates inflammation and mortality associated with a chronic bacterial infection. This is an intriguing question because adenoviral vectors themselves can invoke a substantial immune response that prohibits their repeated administration to the lung (2). In their article, van Heeckeren and coworkers report that, within the infected cystic fibrosis lung, CFTR expression and/or adenoviral vector-induced inflammation yields a significant survival advantage. This promising observation may have profound implications for the utility of adenoviral vectors in the gene therapy treatment of cystic fibrosis.

**CFTR mutations and cystic fibrosis lung pathophysiology.** Cystic fibrosis is the most common autosomal recessive disorder in the Caucasian population, with a frequency of ~1 in 2,500 live births. It is caused by defective function of the cftr gene product (14), which encodes a 170- to 180-kDa protein that functions as a cAMP-regulated chloride channel. The CFTR protein is expressed normally in those tissues affected in cystic fibrosis, including airway submucosal glands and airway epithelial cells. The cystic fibrosis respiratory disease phenotype includes thick mucus secretion and bacterial colonization of the lung with a select few bacterial pathogens, including *Pseudomonas aeruginosa* (13). Repeated *P. aeruginosa* infections compounded by an inability to clear this organism result in a profound neutrophil migration into the airways and secretion of a variety of inflammatory mediators, including neutrophil elastase, reactive oxygen species, and inflammatory cytokines, which cause irreversible damage to the airways and gas-exchange components.

Cystic fibrosis and adenoviral vector-mediated gene therapy. Correction of the defective cftr gene in vivo has long been the holy grail of the cystic fibrosis research community. The use of adenoviral vectors in this pursuit has been problematic (reviewed in Ref. 2). In early clinical trials, the administration of adenoviral vectors encoding CFTR demonstrated great promise, with transient correction of chloride transport defects. Subsequent studies, however, failed to repeat this initial finding and, moreover, revealed that adenoviral vector-mediated delivery of CFTR to the cystic fibrosis airways was limited by the local immune response associated with multiple vector administrations. Similarly, van Heeckeren and colleagues (19) reported previously that *P. aeruginosa*-induced bronchopulmonary inflammation also limited the efficacy of adenoviral vector-mediated gene transfer. Despite this limitation, several studies suggest that the extent of CFTR correction required for a normal lung phenotype may be quite low (2), thereby valuing the use of adenoviral vectors in cystic fibrosis gene therapy.

Until the current study by van Heeckeren et al. (20), the impact of adenoviral vector-mediated gene therapy had not been examined in the context of a cystic fibrosis lung chronically infected with bacteria (19). In this ambitious study by van Heeckeren and coworkers (20), the authors determined whether CFTR delivery improved the outcome of a cystic fibrosis murine model infected with *P. aeruginosa*. Specifically, the authors reported that gut-correction cystic fibrosis mice treated with an adenoviral vector encoding CFTR and inoculated with *P. aeruginosa*-laden agarose beads exhibited a significantly greater cumulative 10-day survival compared with sucrose controls; survival of empty vector controls, however, was not significantly different. Importantly, these authors observed that there were no differences between mice receiving the CFTR vector or controls with regard to the extent of lung inflammation. From these data, the authors concluded that the observed survival advantage in mice receiving adenoviral delivery of CFTR to the cystic fibrosis lung may be due to CFTR expression and/or proinflammatory effects of the adenoviral vector.

**CFTR expression vs. adenoviral vector-induced inflammation.** Because cumulative 10-day survival was not significantly different between mice receiving CFTR-encoding vectors vs. empty vector controls, it is likely that CFTR expression plays a small role in this response. As the authors note (20), it is possible that the administration of an adenoviral vector may have induced a protective immune response that improved cumulative survival. In support of this possibility, the authors observed a persistent lung lymphocytosis in response to adenoviral vector administration; this response was further enhanced upon challenge with *P. aeruginosa*-laden agarose beads. Interestingly, the authors did not observe increased bacterial killing in mice treated with either the CFTR-encoding adenoviral vector or the empty vector control.

Early studies by Markham and coworkers (8, 12) suggest that T cell-mediated immune responses are protective against *P. aeruginosa* infections. For example, these authors have shown that adoptive transfer of T cells from mice immunized with *P. aeruginosa* to granulocytopenic mice resulted in nearly 100% survival upon challenge with *P. aeruginosa* (12). Similar findings have been reported by others (3). Despite these reports, the exact nature of T cell-mediated immunity in *P. aeruginosa* infections remains unclear. Hoiby and colleagues have reported that, in a rat model of *P. aeruginosa* pneumonia (4) and in cystic fibrosis patients (11), chronic *P. aeruginosa* infection induces a T helper type 2 (Th2)-like response. Th2 responses are characterized by the production of IL-4 and IL-5 and are important in the generation of humoral immunity. These results are further supported by their observation that chronic *P. aeruginosa* lung infection is more severe in a Th2 responding mouse strain (BALB/c) (10). With regard to protective T cell immunity, Hoiby and coworkers (4, 5, 9) have demonstrated that induction of a Th1-dominated cytokine re-

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sponse improves the outcome of chronic *P. aeruginosa* lung infection. Th1 responses are characterized by the production of IFN-γ and are instrumental in the generation of cell-mediated immunity. Interestingly, previous studies have shown that adenoviral vector administration can trigger a Th1 response in the lungs of mice (18, 21) and humans (1). In light of these findings, it is tempting to conclude that the improved cumulative survival observed in the van Heeckeren study (20) is a function of an adenovirus-induced Th1 response; however, this conclusion may be too simplistic.

Several recent studies call into question the beneficial nature of a Th1 response in *P. aeruginosa* infections. Reports examining the role of the virulence factor alginate (17) and the quorum-sensing molecule N-(3-oxododecanoyl)homoserine lactone (16) in *P. aeruginosa* pathogenicity indicate that each of these factors induces a Th1-predominated immune response that may contribute to *P. aeruginosa* pathogenesis. Moreover, Schultz and colleagues (15) have reported recently that, in a rat model of *P. aeruginosa* pneumonia, endogenous IFN-γ impairs bacterial clearance and host defense. Clearly, differences in the strain of *P. aeruginosa* (nonmucoid vs. mucoid), type of infection (acute vs. chronic), route of administration, and model system of analysis must be taken into account when considering the role of Th1/Th2 responses in *P. aeruginosa* infections.

Despite the ongoing debate of T cell responses in *P. aeruginosa* infections, it should be noted that these studies were performed in non-cystic fibrosis model systems. Because recent evidence indicates that T cell responses may be dysregulated in cystic fibrosis (6, 7), such studies need to be performed in the context of the cystic fibrosis lung. This is underscored by the van Heeckeren study (20), which suggests that adenoviral vector-induced inflammation may promote survival of cystic fibrosis mice during a chronic *P. aeruginosa* infection. As with all well-conceived studies, this study precipitates a number of unanswered questions. For example, in the absence of *P. aeruginosa* infection, does the nature of the inflammatory response differ between mice receiving an adenoviral vector encoding CFTR vs. empty vector controls? Does CFTR play a role in the T cell immune responses triggered by *P. aeruginosa* infection? How does the hyperimmune response of the cystic fibrosis lung contribute to adenoviral vector- or *P. aeruginosa*-mediated inflammation? Future studies examining immune responses triggered upon adenoviral vector administration of the *P. aeruginosa*-infected cystic fibrosis airways should provide new and important information regarding the utility of adenoviral vectors in cystic fibrosis-related gene therapy.

**REFERENCES**


