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Commentary

Humoral and cellular immune responses to AAV delivery in the airway

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Mutations in the gene encoding the cystic fibrosis (CF) transmembrane conductance regulator (CFTR) lead to CF, an autosomal recessive disorder characterized by progressive respiratory dysfunction.[1] CFTR is an ion channel that transports chloride and bicarbonate across the epithelial cell membrane. CFTR deficiency leads to increased mucus viscosity and bacterial colonization in the airway. As a result, CF patients experience chronic airway obstruction and infection. Adeno-associated virus (AAV)-mediated delivery of a functional CFTR gene to the airway holds promise in treating CF lung disease irrespective of underlying gene mutations.[2,3]

Due to the turnover of the airway epithelial cells,[4] a sustained CF therapy would require periodic re-administration. However, most studies have failed to achieve successful redosing.[3] On a few occasions where re-delivery worked, the initial dose of AAV was either administrated under transient immune suppression or delivered to neonates. These findings suggest that the humoral response from initial exposure to the AAV vector is a major hurdle to achieving durable AAV CF gene therapy.

In a study published in this issue of *Molecular Therapy Methods & Clinical Development*, Tang et al. reported a rather unexpected but clinically highly important finding.[5] They demonstrated persistent transgene expression for up to 5 months in adult ferret lungs and successful redosing without immune modulation.

Historically, investigators have examined airway AAV redosing 1 month after the initial administration.[2,3] However, whether this is an appropriate time window has never been clear. Theoretically, redosing should be applied when expression from the initial AAV delivery falls below the therapeutic threshold. To determine the durability of a single administration in a clinically relevant setting, Tang et al. performed their study in adult ferrets using AAV2.5T, an evolved AAV variant with superior apical tropism in both ferret and human airway epithelial cells.

Ferrets have several advantages over other animal models. They are one of the closest species to humans in regard to the airway cytoarchitecture and composition of the chloride channel. Ferret CF models have been developed, and importantly, they faithfully reproduce the pulmonary phenotype of human patients.[6] Ferrets have been widely used to study human respiratory virus infection and in vaccine development. Their ability to mount a robust immune response enables a more accurate evaluation of the efficacy and safety of a gene transfer vector.

The authors employed the AAV2.5T-fCFTRΔR for the initial dosing. In this vector, a ferret CFTR minigene was expressed from a robust synthetic promoter. Because this vector expressed an endogenous ferret protein, the elicited host immune responses would theoretically be against the AAV capsid proteins and genomes, not the transgene product. It should be mentioned that a human version of this vector is at the advanced stage of preclinical development. It effectively corrected chloride transport defects in polarized human CF airway epithelium *in vitro*.[7]

To understand the kinetic profiles of pulmonary AAV delivery, the authors harvested lung, plasma, bronchoalveolar lavage fluid (BALF), and splenocytes at 10 and 21 days, and at 1, 3, 4, and 5 months after intratracheal administration. They evaluated AAV transduction, transgene expression, and the immune response. High levels of the AAV vector genome (>60 copies per cell) were detected at the 10-day time point but dropped to ∼1–2 copies per cell in subsequent time points. Supraphysiological CFTR minigene expression was achieved in the first 3 weeks. It reached a level ∼2- to 3-fold higher than the endogenous CFTR mRNA. While AAV-derived expression declined afterward, it stabilized at ∼20% of the endogenous CFTR level. This is a significant finding because this expression level is considered therapeutically relevant.[5,6] On immunological assays, neutralizing antibodies in the plasma and BALF peaked at 21 days. Evaluation of the AAV capsid-specific T cell response by the interferon-γ ELISpot assay revealed the highest response in the same time frame. The persistent AAV expression is intriguing because aerosolized AAV particles can be cleared by macrophages and dendritic cells in the lung. Furthermore, the cellular response to the AAV capsid should eliminate AAV-transduced cells. Clearly, a subset of AAV-transduced cells survived these clearance mechanisms.

At the 5-month time point, the BALF neutralizing antibody level dropped to one-third of the peak level. The authors previously showed that the peak-level neutralizing antibody blocked redosing at a 1-month interval in juvenile ferrets.[7,8] Hence, they next tested whether the reduced neutralizing antibody level was sufficient to prevent

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re-administration. To examine the capsid-specific immune response, they used an AAV2.5T vector that expressed secreted Gaussia luciferase (AAV2.5T-gLuc) at redosing. Surprisingly, redosing at a 5-month interval resulted in an expression similar to that of single-dosed animals. However, redosing induced a more robust immune response to the AAV capsids. Notably, AAV2.5T-gLuc administration resulted in a >2-fold loss of the vector genome and CFTR minigene expression of the first dosed AAV2.5T-CFTRΔR vector.

The findings from Tang et al. provide a new path for testing AAV gene replacement therapy for CF. They highlight the complexity of the immune response to AAV delivery in the airway. While the findings from Tang et al. are certainly encouraging, several important questions remain to be addressed. First, CFTR expression is highly heterogeneous and regulated in airway epithelial cells and submucosal glands.[6] This study quantified the transgene expression by the mRNA transcript (for CFTR minigene) or enzymatic assay (for gLuc). Future studies using intracellular reporters or *in situ* hybridization are needed to determine whether treatment-relevant cells (e.g., pulmonary ionocytes) are effectively targeted and retained at the late time point. Second, the redosing was performed with an AAV vector that expressed a different transgene. While such a design is appropriate for studying capsid-specific responses, the findings are less informative in guiding clinical trials that use the same vector for redosing. The use of the barcoded vector may help address this issue. Third, expression from the second administration was examined 14 days after redosing. It is unclear how long the expression will last. The answer to this question is critical given the robust immune response induced by redosing. Fourth, it is intriguing that redosing with the AAV2.5T-gLuc induced a partial clearance of the first dosed AAV2.5T-fCFTRΔR vector. Further investigation is warranted to elucidate how the boosted immunity from redosing the same AAV capsid vector led to the destruction of the previously transduced cells. Lastly, the present study was performed in normal ferrets. The inflammatory microenvironment in diseased subjects is expected to aggravate the immune response to the AAV vector. Future studies are needed to determine the durability and repeated administration of AAV2.5T-CFTRΔR in a ferret CF model.

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DECLARATION OF INTERESTS

D.D. is a member of the scientific advisory board and an equity holder in Solid Biosciences and is a member of the scientific advisory board for Sardocor Corporation.

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