Do ΔF508 Heterozygotes Have A Selective Advantage Against Influenza Virus?

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Abstract:

The ΔF508 CFTR mutation, which is the commonest cause of cystic fibrosis (CF), has the highest frequency of any genetic mutation among Caucasians of central European origin. Persistence of a mutation suggests that heterozygosity for this mutation imparts some genetic advantage. We hypothesized that heterozygosity provides a selective survival advantage during influenza pneumonitis, as a result of reduced pulmonary edema formation. The aim of this project was to determine the effect of the ΔF508 CFTR mutation on the susceptibility to influenza in a mouse model. 8-12 wk old wild-type (CFTR+/+), heterozygous (CFTR+/−), or homozygous knockout (CFTR−/−) mice were infected intra-nasally with a mouse-adapted H1N1 influenza virus. Mock infections with virus diluent served as an infection control. Body weight changes were measured daily in individually-marked mice. Other disease outcome variables were measured in mice sacrificed at 2 or 6 days post-infection (d.p.i.), to determine whether disease severity is reduced in ΔF508 heterozygous mice. At these time points, alveolar fluid clearance rate (an in vivo measure of respiratory epithelial ion transport capacity), viral replication in lung homogenates, and lung water content (wet:dry ratio) were measured, as described previously. Following influenza infection, ΔF508 heterozygotes showed significantly delayed mortality, which was associated with significantly reduced AFC impairment at 2 and 6 d.p.i., and absence of pulmonary edema at 2 d.p.i. (normal wet:dry weight ratio). Delayed mortality did not result from either reduced weight loss or reduced viral replication in lungs of ΔF508 heterozygotes. ΔF508 heterozygous mice demonstrated reduced lung injury and improved survival following infection with a lethal dose of mouse-adapted influenza A virus. This finding suggests that heterozygosity for the ΔF508 CFTR mutation may similarly provide a selective survival advantage during influenza pneumonitis in man.
Introduction:

The cystic fibrosis transmembrane conductance regulator (CFTR) is an epithelial cell apical membrane cAMP-activated chloride (Cl⁻) channel with a central role in regulation of fluid secretion in the lung and intestine. CFTR mediates Cl⁻ transport in the proximal airways, which is a driving force for fluid secretion and therefore maintenance of airway surface hydration and efficient mucociliary clearance¹¹. Absence of functional CFTR, as a result of genetic mutations, causes cystic fibrosis (CF), which is marked by impaired regulation of airway surface liquid volume and composition¹. CF patients also exhibit meconium ileus and peritonitis, failure to thrive, pancreatitis, cirrhosis, and infertility. However, lung disease (often associated with recurrent and persistent bacterial infections) is the major cause of morbidity and mortality in CF patients. Indeed CF is now a leading indicator for lung transplantation.

The ΔF508 CFTR mutation, which is the commonest cause of CF, has the highest frequency of any genetic mutation among Caucasians of central European origin³. Approximately 1 in 25 Caucasians carry at least one mutant allele, and it is inherited as an autosomal recessive trait at a frequency approximately 10 times that expected from recurrent mutation alone¹⁶. Predominance of a mutation which, when present in homozygous form results in early mortality (until recently, prior to reproductive age) suggests that heterozygosity for this mutation imparts some genetic advantage (the “Goldilocks effect”)⁹. Some studies have suggested that heterozygotes for ΔF508 CFTR are relatively protected from secretory diarrhea caused by cholera or enterotoxic E. coli, which results from the ability of these bacteria to stimulate cAMP-mediated intestinal Cl⁻ secretion via CFTR⁴. However, intestinal Cl⁻ secretion rates in CF heterozygotes do not differ from those in homozygotes for wild-type CFTR⁵.
Moreover, the ΔF508 CFTR mutation has been shown to be at least 580 generations old\cite{3}, yet cholera only reached Europe from India in the 1820s, and CF mutations are rare in areas of the world where lethal infectious diarrhea is most common\cite{15}. Other studies have proposed that heterozygosity imparts a survival advantage against asthma\cite{6,12} or hypertension in females\cite{19}, and results in modestly increased fertility\cite{13}. Interestingly, mice heterozygous for the ΔF508 CFTR mutation appear to have improved lung mechanics\cite{2}, although the significance of this finding is not clear from human studies.

Almost 30 years ago, Shier proposed that heterozygosity for ΔF508 CFTR might provide a survival advantage against influenza virus infection\cite{18}. Influenza virus causes highly contagious acute respiratory disease in man, which affects approximately 20\% of all children and 5\% of adults worldwide each year. It was recently reported that pulmonary edema and hypoxemia following influenza virus infection of mice results in part from influenza-mediated activation of CFTR\cite{10}. Therefore, the effect of ΔF508 CFTR heterozygosity on susceptibility to influenza in the mouse model was investigated. In preliminary studies, it was found that C57BL/6 CFTR\textsuperscript{+/-} mice exhibit less pulmonary edema than age-matched CFTR\textsuperscript{+/+} littermate controls.

We proposed that heterozygosity for the ΔF508 CFTR mutation provides a survival advantage in influenza-infected individuals. This survival advantage may account for the persistence of this mutation in the Caucasian population, despite the severe detrimental effects of homozygosity for ΔF508 CFTR, which include infertility, pancreatic insufficiency, lung dysfunction and accelerated mortality.

**Procedures and Methodology:**

C57BL/6 mice heterozygous for the ΔF508 CFTR mutation were bred and their offspring
genotyped by tail snip PCR. 8-12 wk old wild-type (CFTR^{+/+}), heterozygous (CFTR^{+-}), or homozygous knockout (CFTR^{-/-}) mice were then infected intra-nasally with 10,000 focus-forming units /mouse (lethal by day 8) of mouse-adapted H1N1 influenza virus (A/WSN/33). Virus stocks were grown on STAT-1(-/-) NY3 cells and purified by ultracentrifugation. Isolated virus concentration was determined by serial dilution and plaque assay. Mock infections with virus diluent (PBS/0.1%FCS) served as an infection control. Effects of CFTR mutation on multiple outcomes of influenza virus infection were determined. Body weight changes were measured daily in individually-marked mice. Other disease outcome variables were measured in mice sacrificed at 2 or 6 days post-infection (d.p.i.), to determine whether disease severity is reduced in ΔF508 heterozygous mice. At these time points, alveolar fluid clearance rate (an \textit{in vivo} measure of respiratory epithelial ion transport capacity), viral replication in lung homogenates, epithelial barrier function, and lung water content (wet:dry ratio) were measured.

Descriptive statistics were calculated using Instat 3.05 (GraphPad Software, San Diego, CA). Gaussian data distribution was verified by the method of Kolmogorov and Smirnov. Differences between group means were analyzed by analysis of variance, with Tukey-Kramer multiple comparison post-tests. P<0.05 was considered statistically significant.

\textbf{Results:}

Following influenza infection, ΔF508 heterozygotes showed significantly delayed mortality, compared to either wild-type C57BL/6 mice (Figure 1). Mice were individually marked and weighed daily. Percent change in body weight vs. starting weight was calculated for each individual mouse at each time point. Mean percent weight changes for each day was then
determined. However, heterozygosity for the ΔF508 CFTR mutation had no significant impact upon influenza-induced weight loss (Figure 2).

Figure 1 Effect of influenza A virus infection on mortality (p< 0.05)

Figure 2 Effect of influenza A virus infection on body weight
In addition, AFC studies were performed on live, anesthetized, ventilated C57BL/6 mice with normal body temperature and blood gases, over a 30-min period after intratracheal instillation of 0.3ml of isosmolar NaCl containing 5% fatty acid-free BSA. Data showed that influenza-induced AFC impairment was significantly attenuated in mice heterozygous for the ΔF508 CFTR mutation at 2 and 6 days after infection (Figure 3).

![Figure 3 Effect of influenza A virus infection on AFC rate (* p < 0.05; ** p < 0.005)](image)

Another effect of influenza virus infection was influenza-induced lung epithelial barrier dysfunction. It was indicated by leakage of plasma proteins into bronchoalveolar [BAL] lavage fluid. The concentration of BAL protein in the lung was measured by BCA assay. Data showed that lung epithelial barrier dysfunction was significantly attenuated in mice heterozygous for the ΔF508 CFTR mutation at 6 days after infection. Day 2 has not yet been analyzed (Figure 4).
Influenza A infection of mice for 2-6 days also results in a progressive increase in lung water content (wet:dry weight ratio), which is significantly attenuated in CFTR ΔF508+/− mice. Wet:dry weight ratio was determined for each animal by weighing dissected lung lobes immediately following mouse euthanasia (wet weight) and again following 3 days drying in an oven at 50°C (dry weight) (Figure 5).
Lastly, basal airway resistance values were obtained from live, ventilated mice using the SCIREQ flexiVent. Data shows that the influenza-induced increase in total lung resistance is also significantly attenuated in mice heterozygous for the ΔF508 CFTR mutation at 2 days after infection. Data for heterozygotes at 6 days after infection has not yet been analyzed (Figure 6).

![Figure 6 Effect of influenza infection on total lung resistance (R) (** p< 0.0005)](image)

**Discussion:**

The aim of the study was to determine whether heterozygosity of the ΔF508 CFTR mutation provided a survival advantage against influenza virus infection, using a mouse model. Since the ΔF508 CFTR mutation is so prevalent among Caucasians of European origin, even though homozygosity for the mutation leads to early mortality, a heterozygote selective advantage is the most plausible explanation for the maintenance of the high frequency in the human population. This follows the “Goldilocks Effect” in that two defective copies of the gene are detrimental, two normal copies are satisfactory, but one normal and one defective gene may result in an optimal level for survival\(^2\). Previous studies indicated that heterozygotes of the ΔF508 CFTR mutation were slightly protected against the deleterious effects of secretory
diarrhea, caused by cholera. However, limited evidence supported that hypothesis, illustrating that heterozygosity was important for some other reason.

Our findings support our hypothesis in that following influenza infection, ΔF508 heterozygotes showed significantly delayed mortality, compared to either wild-type C57BL/6 mice, or littermate ΔF508 homozygotes, which had comparable mortality time courses. Delayed mortality was associated with significantly reduced AFC impairment at 2 and 6 d.p.i., attenuated lung epithelial barrier dysfunction at 6 d.p.i, and absence of pulmonary edema at 2 d.p.i., demonstrated by normal wet:dry weight ratio. However, delayed mortality did not result from either reduced weight loss or reduced viral replication in lungs of ΔF508 heterozygotes.

Why heterozygotes have significantly reduced AFC impairment and absence of pulmonary edema is currently unknown. It is possible that the genetic inactivation of some of the CFTR through carrying one allele of the ΔF508 mutation protects from influenza-induced activation of CFTR-mediated Cl− secretion into the alveolar space. Thus, mechanisms underlying reduced susceptibility to influenza infection in ΔF508 heterozygotes should be investigated. Reduced Cl− secretion in response to infection and reduced epithelial cell sialoglycoconjugate levels providing a barrier to viral entry can indicate such.

**Conclusions:**

ΔF508 heterozygous mice demonstrate reduced lung injury and improved survival following infection with a lethal dose of mouse-adapted influenza A virus. This finding suggests that heterozygosity for the ΔF508 CFTR mutation may similarly provide a selective survival advantage during influenza pneumonitis in man.
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