ABSTRACT

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Title:
Cathelicidin Anti-Microbial Peptide (CAMP) is toxic during neonatal influenza virus infection

Background:
Respiratory viral infections are a major public health concern for premature infants, who are at increased risk of significant morbidity and mortality. To determine mechanisms of infant infection susceptibility, an age-appropriate pre-clinical model of neonatal influenza virus infection in 3-day old neonatal mice has been established. Previous work demonstrated that influenza-infected neonatal mice treated with *Lactobacillus rhamnosus* GG (LGG) prior to infection had improved survival. Several key anti-viral cytokines were upregulated, compared to sham-treated counterparts. Surprisingly, CAMP, an important innate immune component with a broad spectrum of antimicrobial activity, was down-regulated.

Objective:
To determine the role of CAMP in the innate immune response and subsequent pathogenesis during neonatal influenza virus infection.

Design/Methods:
Wild-type (C57BL/6) and CAMP deficient ($CAMP^{-/-}$) 3-day old neonatal and 8-week old adult mice were infected intranasally with influenza A virus/Puerto Rico/8 (PR8) and tracked for survival. In separate experiments, murine neonatal lungs were harvested at 1-, 3- and 6-days post-infection (DPI). Bronchoalveolar lavage fluid (BALF) and whole lungs were analyzed by flow cytometry for cellular infiltration. Animal work was conducted according to approved IACUC protocols.

Results:
Three-day old $CAMP^{-/-}$ murine neonates had improved survival compared to wild-type (75% versus 14%, $p<0.05$). This is in direct opposition to adult mice, where $CAMP^{-/-}$ and wild-type mice had similar weight loss, an important indicator of adult influenza-related morbidity. At 1- and 6-DPI, neonatal $CAMP^{-/-}$ mice had increased absolute numbers and frequency of alveolar macrophages (AM) compared to wild-type mice ($p<0.05$). However, in the lung interstitium, AMs were increased in wild-type mice compared to $CAMP^{-/-}$ mice at 1 DPI ($p<0.05$). There is a delayed production of pro-inflammatory cytokines IL-6 and IFN-gamma by the wild type mice with a significant difference seen at 6DPI compared to 3DPI ($p<0.05$).
At 1DPI the $CAMP^{-/-}$ mice have increased viral loads compared to the wild type mice ($p<0.05$) but as the disease progresses, the viral loads in the wild type mice become significantly increased by 6 DPI compared to early on in the infectious process ($p<0.05$).

Conclusion:

Neonatal CAMP$^{-/-}$, but not adult, mice are protected during influenza virus infection. CAMP$^{-/-}$ neonates preferentially recruit AMs to the site of infection, compared to their wild-type counterparts with increased AMs in the interstitium. The CAMP$^{-/-}$ mice also seem to have a controlled response to the influenza virus by early production of pro-inflammatory cytokines and chemokines compared to their wild type counterparts who have a persistent pro-inflammatory state most likely driving the increased mortality seen in this subset. This targeted response potentially plays a role in the improved CAMP$^{-/-}$ survival, as AMs are critical to the control of inhaled pathogens. Further studies using our murine model will further investigate the role of AMs as the first-line of defense during neonatal respiratory viral infection.