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Modeling Human Respiratory Viral Infections in the Cotton Rat (*Sigmodon hispidus*)

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Abstract

For over three decades, cotton rats have been a preferred model for human Respiratory Syncytial Virus (RSV) infection and pathogenesis, and a reliable model for an impressive list of human respiratory pathogens including adenoviruses, para influenza virus, measles, and human metapneumo virus. The most significant contribution of the cotton rat to biomedical research has been the development of anti-RSV antibodies for prophylactic use in high-risk infants.

More recently, however, the cotton rat model has been further explored as a model for infection with other respiratory viral pathogens including influenza and rhinovirus.Together with RSV, these viruses inflict the greatest impact on the human respiratory health.This review will focus on the characteristics of these new models and their potential contribution to the development of new therapies.

Keywords: Viral infections; Influenza; Rhinovirus; Cotton rat

Introduction

Cotton rats (*Sigmodon sp*) are "New World" rodents that are abundant from the southern part of the United States to Northern South America. Although many species of cotton rats have been classified in the wild, only two have been inbred and are currently used for biomedical research, *S. hispidus* and *S. fulviventer*. Over the years, *S. hispidus* has been shown to support replication of a broad spectrum of human respiratory viruses including respiratory syncytial virus (RSV) [1], measles virus [2,3], several adenovirus serotypes [4,5], para-influenza virus type 3 [6], and human metapneumo virus [7-9]. Fewer studies have been performed using *S. fulviventer*, although there is some suggestion that they are differences in permissiveness and pathogenesis to certain pathogens when compared to *S. hispidus* [6,10]. The purpose of this review is to update the reader on the benefits of the cotton rat as a preclinical model based on recent infection studies with influenza and rhinovirus.

Influenza Receptors in Cotton Rats

Receptor specificity and the pattern of expression and distribution of influenza sialic acid (SA) receptors dictate, in part, the fate of influenza virus infection. For example, the airway epithelium of mice pre-dominantly expresses receptors with SA linked to cell-surface glycoproteins or glycolipids in a α2-3-linkage (α 2-3-linked SA receptors) [11]. Most human influenza viruses preferentially bind α 2-6-linked SA receptors. Lectin-based staining of the trachea and minor airways of cotton rats shows strong reactivity of α 2-3-linked SA and α 2-6 linked SA. It was established that, in the trachea of cotton rats, α 2-6-linked SA receptors co-localize with ciliated cells, whereas α 2-3-linked SA receptors are more associated with mucin-producing cells [12]. In addition, the lung parenchyma of cotton rats shows a consistent staining of type I and type II pneumocytes with α 2-6-linked SA receptors, whereas α 2-3-linked SA receptors are rarely expressed [12]. Thus, the presence of both α 2-3-linked and α 2-6-linked SA receptors in the airways of cotton rats most likely explains the susceptibility of these animals to infection by a wide variety of human and avian strains of influenza viruses as described below.

Non-Adapted Influenza Strains in Cotton Rats

Cotton rats are susceptible to several low passaged isolates of human influenza *with no previous adaptation*, replicating virus in the upper and lower respiratory tract and producing significant pathology (Table 1) [9,13]. A detailed study of cytokine and interferon (IFN) response to influenza [14] was followed by the characterization of the Type I IFN-activated gene, Mx [15], in the cotton rat that, in contrast to mice,

Type (Subtype)	Strain
B	B/HK/73 B/Sichuan/379/99 B/HK/330/01
A (H1N1)	A/New Caledonia/20/99 A/California/04/09 A/Netherlands/09 A/Argentina/09 A/Brisbane A/Bayern/95 A/Malaya/302/54 A/PR/8/34 (not mouse adapted)
A (H3N2)	X-31 A/Wuhan/359/95 A/Duck/HK/375
A (H5N1)	A/Vietnam/1203/04
A (H9N2)	A/Guinea Fowl/HK/WF10/99

Table 1: Influenza strains tested in cotton rats (*Sigmodon hispidus*).

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is fully functional and most likely helps to circumscribe replication of influenza in the lungs of humans and cotton rats early after infection.

Recently, additional influenza strains of human and avian origin were tested [12]. Cotton rats were susceptible to infection with two different low pathogenicity strains, A/duck/Hong Kong/375/1975 (H3N2) and A/guinea fowl/Hong Kong/WF10/1999 virus (H9N2). Both viruses replicated in the nose and the lung of infected animals. Animals showed clinical signs of disease (*i.e.,* changes in body weight and temperature), local and systemic IFN production, followed by strong lung pathology but no mortality.

Mortality was a unique characteristic only evidenced during infection of cotton rats with the highly pathogenic virus isolate from a lethal human case A/Vietnam/1203/2004 (H5N1)[12]. In fact, mortality in H5N1-infected animals was seen only at high viral inoculum and correlated with the lethal outcome of this virus in humans that has been proposed to be the result of infection with a high viral dose [16].

The first pandemic influenza virus of this century was of swine origin, initially isolated in 2009 [17], and remains today the most common influenza strain circulating among the human population [18]. The prototype isolate A/California/07/09 was tested in the cotton rats and showed replication in the lung and nose on 1 and 2 days post infection (d.p.i), and a peak lung histopathology on day 2 [12]. However, the most characteristic feature of all pandemic H1N1 strains tested was a more robust expression of Mx genes in blood when compared with other seasonal human isolates [12]. A recent study has shown that the RNA transcriptional profile in blood can be used to distinguish influenza, RSV, and rhinovirus infection in children with lower respiratory tract infection [19]. Studies to validate these results in the cotton rat model are currently underway.

Efficacy of a TLR4 Antagonist, Eritoran, against Influenza-Induced Disease

There is a pressing need to develop alternatives to annual influenza vaccines and antiviral agents licensed for mitigating influenza infection. A versatile rodent model susceptible to non-adapted strains of human influenza could greatly accelerate preclinical steps toward developing these drugs for use in the clinic. Eritoran (E5564, Figure 1), a potent, well-tolerated, synthetic TLR4 antagonist [20-22], blocks influenza-induced lethality in mice induced by infection with mouse-adapted influenza A/PR/8/34(PR8) [23]. Eritoran treatment of influenza-induced disease was tested in cotton rats. Animals treated with Eritoran after human H3N2 challenge showed significant reduction in lung pathology on day 4 compared to animals treated with vehicle, that correlated with the decreased lung expression of IL-6 and IL-10 [23]. Future studies will be extended to pandemic viruses in the presence of current antiviral therapies.

Human Rhinovirus Infection in Cotton Rats

Human rhinoviruses (HRV) represent the single most important etiological agent of the common cold and are the most frequent cause of acute respiratory infections in humans. As of today, no animal model is available for protection studies using HRV challenge.

HRV16 infection, replication, and pathogenesis were recently examined in the cotton rat model [24]. Infectious HRV16 was recovered from the nose and trachea of cotton rats infected intra-nasally until 1 d.p.i, and from the lung until 2 d.p.i. No virus was isolated from any of the tissues analyzed 4 d.p.i. Epithelial degeneration was observed in tracheal epithelia of HRV16-infected rats, and extended to some of

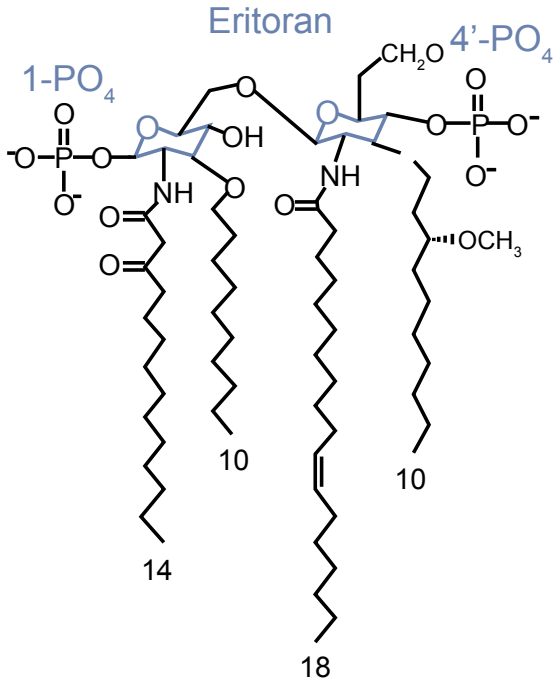

Figure 1: Eritoran (E5564).

the large pulmonary airways. Infection was associated with direct and progressive damage of the ciliated columnar epithelium of the trachea peaking 4 d.p.i., and in many cases, exposing the basal membrane. Analysis of lung pathology revealed mild alveolitis (neutrophilic and histiocytic), and mild peri-bronchiolar infiltrates of neutrophils, macrophages, and lymphocytes. Mucous cell hypertrophy/hyperplasia was another characteristic feature of HRV16 infection in this model.

Vaccine-Induced Protection against HRV16 Challenge

Intramuscular (i.m) vaccination and boosting with live HRV16 induced development of high levels of serum neutralizing antibody in cotton rats [24]. All animals vaccinated intramuscularly showed reciprocal of neutralizing antibody titers >1,280 in the 60% plaque reduction assay and a strong protection from viral replication as evidenced by a marked reduction in pulmonary viral load. In addition, when animals immunized with single i.m. inoculation of HRV16 were challenged 21 days later, virus was not detected in the nasal turbinates or in the trachea, and a > 3 $\log_{10}$ reduction of infectious virus titers was detected in the lung.

Protection against HRV16 Challenge was Conferred by Passive Transfer of Anti-HRV16 Immune Serum

The efficacy of the prophylactic administration of immune (neutralizing antibody titer of 1,280 against HRV16) or normal cotton rat serum to protect against HRV16 challenge was tested [24]. Animals that received 0.5 ml of undiluted sera intraperitoneally showed a reduction in lung viral titers of 2 Log_{10}, whereas those that received normal cotton rat serum, or PBS, remained unprotected. These data indicate that passive transfer of antibodies can be an effective prophylactic therapy against HRV infection and that the cotton rat model could be use to further refine and optimize such a therapy approach.

Conclusion

- The cotton rat model is susceptible to infection with unadapted human and avian influenza strains.
- Therapeutic treatment of influenza disease with Eritoran demonstrated that the drug is safe and efficacious in the cotton rat model.
- Human rhinovirus 16 replicates in the upper and lower respiratory tract of cotton rats.
- The cotton rat is a new animal model option for testing vaccine and antiviral therapies against human rhinovirus infection

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