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Animal models of respiratory syncytial virus infection and disease

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The study of human respiratory syncytial virus pathogenesis and immunity has been hampered by its exquisite host specificity, and the difficulties encountered in adapting this virus to a murine host. The reasons for this obstacle are not well understood, but appear to reflect, at least in part, the inability of the virus to block the interferon response in any but the human host. This review addresses some of the issues encountered in mouse models of respiratory syncytial virus infection, and describes the advantages and disadvantages of alternative model systems.

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Introduction

Respiratory syncytial virus (RSV), a member of the paramyxovirus family, remains a major clinical problem for which there is no effective vaccine or treatment. This is a ubiquitous respiratory pathogen that infects 100% of humans by the age of 2 [1], and is the most common cause of lower respiratory tract infection in infants and young children [2]. The most severe disease afflicts infants experiencing a primary infection. RSV infection in older children and adults generally produces a relatively mild illness limited to the upper respiratory tract, but infection can cause fatal pneumonia in immunocompromised hosts, a cohort that includes young infants with immature immune systems and the fragile elderly. Most interesting is the capacity of this virus for frequent reinfection of the human host [3], a phenomenon that is not well understood. Unlike other acute respiratory virus infections, the ability of RSV to reinfect human patients does not appear to be due to rapid virus evolution, a trait common to many RNA viruses. Although there is published evidence suggesting that circulating viral clades change with respect to predominance in a given population, there is no evidence of progressive viral evolution resulting in emergence of new strains [4]. These observations are all the more interesting given that this virus has no known animal reservoir, and the source of the inevitable yearly epidemics is unclear.

Rodent models of RSV infection

This exquisite specificity of RSV for the human host has made it challenging to develop small animal models of RSV pathogenesis, and therefore difficult to understand the basis of the relatively ineffective human immune response to this infection. This dilemma has been a major hurdle for vaccine development, which has been unsuccessful despite a half century of intensive research. Cormier et al. [5] have estimated that 77% of published RSV studies have been carried out in mice, a species with well-characterized genetics, for which a host of immunological techniques and reagents are available. Many important studies have been carried out in mouse models of RSV infection (recently reviewed by Openshaw [6]), but the limitations of this model leave open to question our ability to translate information gained by these studies into clinical practice.

A major issue in animal model development is the relative resistance of rodent species to human RSV infection. Although the commonly used BALB/c mouse has been shown to be among the most susceptible mouse strains [7], inoculation of these mice with very large doses of virus produces minimal microscopic disease and a total viral yield on the order of 1000-fold below virus input. The high degree to which RSV is adapted to its only natural host (*Homo sapiens*) presents a complicated challenge to the development and interpretation of animal models. Even in the phylogenetically most closely matched hosts — nonhuman primates — RSV replication and pathogenesis poorly reflects human RSV infections [8]. Two approaches toward an improved mouse model have been contemplated: [9] adaptation of hRSV to nonhuman hosts, and [10] use of related cognate virus/host pairs. The first approach is exemplified by the adaptation multiple human pathogens to mice by serial passage, examples being influenza A virus [11], SARS [12], and ebolavirus. Adult mice are resistant to infection

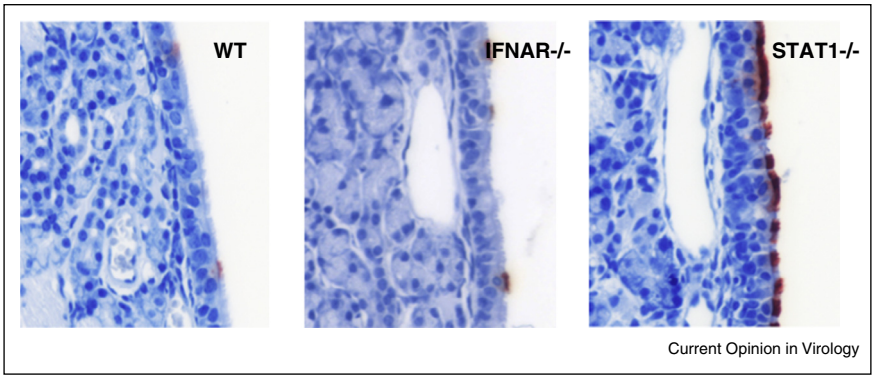
with strains of ebolavirus isolated from humans, though suckling mice are susceptible. Bray et al. [13] passaged virus through successively older mice and recovered, after six such cycles, a 'mouse-adapted' ebolavirus. The key mutations accounting for virulence in mice were determined to be mutations that conferred resistance to the interferon response [14]. Attempts to adapt hRSV to the mouse have not been successful. The very low ratio of progeny to inoculum virus in *in vivo* passage represents an insurmountable hurdle to this approach. We have passaged the virus in cultured mouse cells over hundreds of cycles and, despite the accumulation of genotypic and phenotypic (i.e. plaque morphology) changes, we have seen no apparent shift in the ability of the passaged virus to replicate in the mouse (unpublished data). Mice lacking signal transducer and activator of transcription 1 (STAT1), and therefore interferon responsiveness, are in fact more susceptible to hRSV [15] (see Figure 1), but even in this model the host can be described at best as 'semipermissive' for RSV replication. The ratio of progeny to inoculum is still $>1/100$. Thus there are likely mismatches between hRSV's innate immunity counter-defenses and corresponding molecular targets in mice, independent of STAT1 pathways. The RSV gene products that have been associated with inhibiting the interferon response are NS1 and NS2 [16••,17]. These proteins have been implicated in the accelerated degradation of STAT2 in human cells [18,19] (critical in the response to type I and type III interferons) and in the inactivation of RIG-I, TRAF3 and IRF-3 [10] — all essential to the induction of both type I and type III interferons. The extent to which these viral products are 'tuned' to their natural hosts was best demonstrated by Bossert and Conzelmann [20••], who constructed rhabdoviruses with either human or bovine RSV NS1 or NS2, and found the resistance of the resulting recombinant viruses to type I interferon was host specific, even for these closely related viruses.

A more permissive rodent model of RSV disease, and the species used with increasing frequency for testing RSV vaccines and therapeutics, is the cotton rat (*Sigmodon hispidus*) [21•], recently reviewed in detail by Boukhvalova et al. [22]. In addition to the increased virus replication, respiratory tract infection in the cotton rat more closely resembles that seen in human subjects, with diffuse infection of the nasal mucosa, and replication limited to bronchiolar rather than alveolar mucosa as seen in the mouse lung. See Figure 2. Also similar to human patients, cotton rats are susceptible to reinfection of the upper airway beginning 8 months after primary infection [23]. Unlike other new world rodents, such as guinea pigs, which have also been used for RSV studies, cotton rats are available as an inbred strain, and a growing number of reagents are available for the study of cytokines and chemokines in this species.

Bovine RSV in cattle

The alternative approach to animal model development is to explore the utility of related cognate virus/host pairs. The only such pair involving rodents identified to date is pneumonia virus of mice (PVM). In this system — similar to infection of humans by hRSV — a small inoculum produces orders of magnitude more progeny, and substantial pathogenesis [24]. One severe drawback to this system, however, is the very remote relationship between PVM and hRSV (see Figure 3), rendering any extrapolations between distantly related gene products and their mechanisms tenuous. The pneumovirus most closely related hRSV is bovine RSV (bRSV) which was first

Figure 1



In mice, the uppermost airway is resistant to hRSV infection. Photomicrographs taken here show the nasal septum four days after intranasal delivery of hRSV to wild type (WT) mice, or animals lacking the IFN-α/β receptor chain 1 ($IFNAR^{-/-}$), or the transcription factor signal transducer and activator of transcription 1 ($STAT1^{-/-}$). The presence of viral proteins is demonstrated here by immunohistochemistry with RSV infected cells staining red. In WT or $IFNAR^{-/-}$ mice, only rare cells are infected, and infection appears not to spread to adjacent cells. The nasal respiratory mucosa in the $STAT1^{-/-}$ animals is relatively permissive, suggesting that IFN signaling restricts virus spread in this mouse. While $IFNAR^{-/-}$ mice cannot respond to type I, or $-\alpha/\beta$ IFNs, the recently discovered type III, or $-\lambda$, IFNs also play a role in antiviral protection and are STAT1 dependent.