Animal Models of Respiratory Syncytial Virus Infection

Linda G. Byrd and Gregory A. Prince

Over the past two decades, animal models of respiratory syncytial virus (RSV) infection have been developed using primates, cotton rats, mice, calves, guinea pigs, ferrets, and hamsters. Use of these models has shed light on the mechanisms of vaccine-enhanced disease seen in clinical trials of a formalin-inactivated RSV vaccine and has provided a means for testing efficacy and safety of candidate prophylactic and therapeutic strategies. The development of multiple animal models has coincided with the realization that RSV disease in humans is a multifaceted disease whose clinical manifestations and sequelae depend upon age, genetic makeup, immunologic status, and concurrent disease within subpopulations. There is no single human subpopulation in whom all forms of RSV disease manifest, nor is there a single animal model that duplicates all forms of RSV disease. The choice of an experimental model will be governed by the specific manifestation of disease to be studied.

Respiratory syncytial virus (RSV) has been enigmatic since its discovery >40 years ago. Initially isolated from chimpanzees during an epizootic of upper respiratory tract disease [1], RSV was subsequently found to be the most important cause of infectious pulmonary disease in human infants. More recently, RSV has been shown to be a major pathogen in immunosuppressed and elderly individuals.

RSV is a member of the family Paramyxoviridae, subfamily Pneumovirinae, and has a nonsegmented negative stranded genome of 15,222 nucleotides (strain A2) [2]. The virion consists of a nucleocapsid within a lipid envelope, and the virions are irregular in size and shape. There are two major glycoproteins expressed on the virion surface: the fusion protein and the attachment protein. A small hydrophobic protein is also expressed on the virion surface. Two major antigenic groups (A and B) have been described; these groups are distinguished primarily by dissimilarities in the attachment protein (1%–7% relatedness). Other related viruses include bovine RSV, ovine RSV, caprine RSV, pneumonia virus of mice, and turkey rhinotracheitis virus. RSV is structurally and functionally similar to parainfluenza viruses, although there is little antigenic or sequence relationship. RSV is much more distantly related to the family Orthomyxoviridae (influenza virus), which have a segmented genome.

In one prospective study of children [3], the rate of RSV infection was 68.8 cases per 100 children during the first year of life and 82.6 cases per 100 children during the second year of life. By 24 months of age, all of the children had been infected at least once, and one-half had had two infections. It is estimated that the incubation period for RSV infection is 4 or 5 days, and transmission probably occurs via fomites rather than aerosols. There is no viremia.

Pulmonary involvement occurs in 25%–40% of initial infections but is uncommon in subsequent infections, except those in recent bone marrow transplant recipients or elderly patients. Virus may be shed as long as 20 days. There is no evidence of persistent infection in immunocompetent individuals. Premature infants or those with cyanotic congenital heart disease or bronchopulmonary dysplasia are especially likely to develop severe pulmonary infections with RSV. The immune response of infected individuals does not appear to be protective for longer than a few months; whether the temporary protection is due to antibody or to cellular immunity is unclear.

The development of the first vaccine (employing the same technology—formalin inactivation—as the highly successful Salk vaccine) and subsequent vaccination of human children ended with disastrous results when natural RSV infection developed in those children (up to 80% of children were hospitalized, and two children died) [4]. These results have overshadowed efforts to develop an RSV vaccine for three decades and still stand as a barrier to licensure.

Interpretation of the vaccine trials was severely hampered by the lack of a small animal model in which vaccine-enhanced disease could be reproduced and studied experimentally. In the absence of such a model, the vaccine’s sponsors—observing that the moderate serum antibody responses in the vaccinees provided no apparent protection—concluded that serum antibody to RSV actually enhanced disease rather than protecting against it [5]. This hypothesis gained widespread acceptance for nearly 15 years until the advent of small animal models, whereupon studies in those models (particularly the cotton rat model) disproved the hypothesis and established the foundation for antibody-based immunoprophylaxis.

The development of models of RSV infection and disease, particularly in the cotton rat [6, 7] and the inbred mouse [8], marked turning points in research efforts to prevent RSV disease and to understand the mechanisms of vaccine-enhanced disease. Studies in the cotton rat model showed that serum IgG...
with RSV-neutralizing activity (RSV1g) could prevent pulmonary infection and attenuate nasal infection [9–11], and these studies established the rationale for clinical trials of RSV1g prophylaxis with plasma-derived IgG for infants at high-risk [12, 13]. These trials, in turn, led to the licensure of the first RSV preventive agent, RespiGam (MedImmune, Inc., Gaithersburg, MD), in 1996.

At the same time, results obtained with animal models challenged prevailing wisdom concerning RSV1g; they provided the foundation for dissecting the mechanisms of vaccine-enhanced disease [14]. These mechanisms are complex, involve many arms of the immune response, and are not restricted to formalin-inactivated (FI) RSV formulations [15]. As it becomes apparent that various types of formulations, in addition to FI RSV vaccine, have the potential of stimulating an inappropriate immune response, the issue of vaccine safety becomes increasingly important. Although this issue will not be resolved completely until an RSV vaccine has been tested in seronegative human infants, the decision to proceed to that stage will be based in large measure upon studies in animal models. Several pragmatic issues relating to animal modeling in general are listed in table 1. We now describe the most significant animal models of RSV infection and disease, emphasizing the relative strengths and weaknesses of each model without attempting to summarize all of the experimental data derived from their use.

**Chimpanzee**

Although no other laboratory animal approaches the genetic relatedness of chimpanzees to humans, practical and biological considerations severely limit the utility of chimpanzees in RSV research and perhaps cast doubts upon the relevance of published experimental studies. Chimpanzees are scarce, extremely expensive, and available in the United States only through primate breeding programs. The already small numbers of animals available to investigators will be reduced even further due to a recent reduction in funding for the breeding programs, raising the purchase cost even further. Current costs of leasing and caring for chimpanzees are also extremely high. Maintenance costs are further inflated because terminal experimentation in chimpanzees is generally not permissible, and thus the investigator must either maintain the animals for the duration of their natural lives or trade or sell them to other investigators for other purposes.

The net effect of these expenses has been that only one laboratory (Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health) has published studies of RSV infection in chimpanzees. Primary RSV infection was initially described in only four animals [23], in whom the study of infection was restricted to nasal tissues. Subsequent studies have been limited to four or fewer animals per group, and studies have often used historical rather than concurrent controls [17–21, 24]. The genetic heterogeneity to be expected among outbred animals and the statistical insignificance of data from so limited a number of observations raise concerns about the scientific validity of these experiments. Furthermore, the scarcity of chimpanzees precludes verification of published observations by other laboratories.

Biological considerations also suggest that the utility of the chimpanzee model is limited. Although advocates of the model emphasize the dramatic rhinorrhea accompanying experimental infection, no studies have documented pulmonary disease following primary infection [18]. Furthermore, no evidence exists that FI RSV vaccine can produce enhanced disease in the chimpanzee, thus raising questions about the relevance of the chimpanzee as a model of vaccine safety.

**Other Primates**

Experimental RSV infection has been described in the owl monkey [25, 26], rhesus monkey [23], African green monkey [27], cebus monkey [28], squirrel monkey [23], bonnet monkey [29], and baboon [30]. Purchase and maintenance costs for all of these species (in the United States), although considerably lower than those for the chimpanzee, are still high and tend to result in the use of statistically insignificant numbers of animals. Unlike chimpanzees, all of these species may be used in terminal experimentation, thus allowing more detailed virological and histological studies of pulmonary RSV disease.

Although these species all are closer genetically to humans than are rodents, it has yet to be demonstrated that their use provides data of greater or even equal relevance to the understanding, prevention, and treatment of human disease. Lack of

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**Table 1. Criteria to consider when developing an animal model of respiratory syncytial virus infection.**

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<thead>
<tr>
<th>Genetics</th>
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<td>The wide divergence of patterns of RSV disease among different human subpopulations [16] suggests a strong connection between host genotype and disease phenotype that may influence the choice of animal models.</td>
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<th>Availability</th>
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<td>Some types of studies require specialized host strains (inbred, transgenic, or knockout) and specific reagents for identification and quantitation of immunoglobulins, immune cells, and cytokines.</td>
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<th>Husbandry</th>
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<td>Practical issues include maintenance costs, ease of handling, anesthesia, surgery, dosing, and tissue sampling.</td>
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<td>Statistically significant numbers of animals per study are essential, and the use of one animal [17] or of historical rather than concurrent controls [18–21] may lead to statistically insignificant and potentially misleading data.</td>
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<th>Dosage</th>
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<td>An excessive dose of challenge virus may either obscure an otherwise effective prophylactic or therapeutic approach [22] or produce experimental disease that is not analogous to its human counterpart.</td>
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**NOTE. RSV = respiratory syncytial virus.**
inbreeding in any of these species limits immunologic studies, and relative unrelatedness to humans (compared with the chimpanzee) has meant that few human immunologic reagents cross-react. None of these species has been shown to develop either clinical or radiological signs of pulmonary RSV disease, and pulmonary infection has been documented (though not well-characterized) only in the owl monkey. Owl monkeys and baboons develop mild rhinorrhea, whereas the other species show no signs of nasal disease.

The most useful of these species may be the African green monkey because the enhanced pulmonary disease it develops subsequent to immunization with FI RSV vaccine [27] is histologically similar to that seen in humans who died after vaccination [4]. However, the large number of animals that would be necessary to characterize fully the enhanced disease, the lack of inbreeding, and the scarcity of immunologic reagents suggest that African green monkeys will be, at best, a secondary model of vaccine-enhanced disease and vaccine safety.

**Cotton Rat**

RSV infection in the cotton rat was first demonstrated by Dreizin and co-workers [6], who described the kinetics of viral replication and histological changes in the rats’ lungs. The cotton rat remains uniformly susceptible to pulmonary infection through adulthood, thus establishing it as a useful model for long-term studies [7]. In comparison with the mouse, the cotton rat is 100-fold more permissive (per input dose of virus) and more responsive immunologically, developing titers of serum antibody that are 10-fold or more higher [8, 31]. In addition to its greater permissiveness, the cotton rat develops vaccine-enhanced pulmonary disease that appears to parallel that in humans and other primates [14], whereas the mouse does not [32]. The candidate vaccines of Wyeth-Lederle Vaccines and Pediatrics (Pearl River, NY) [33], Upjohn (Kalamazoo, MI) [34], Pasteur Mérieux Connaught (North York, Ontario, Canada) [35], SmithKline Beecham Biologicals (Rixensart, Belgium) [36], and Institut De Recherche Pierre Fabre (Boulogne, France) [37] have been tested extensively in the cotton rat, and it currently serves as the primary model for the determination of vaccine safety.

Studies in the cotton rat showed that ribavirin (Virazole, ICN Pharmaceuticals, Costa Mesa, CA) effected a modest reduction in pulmonary virus (about 10-fold), but no histological data that might have given clues to the drug’s ability to reverse the disease process were provided [38]. Subsequent studies in our laboratory failed to demonstrate an ameliorating effect on histopathologic changes [39], and recent clinical studies showing marginal or undetectable clinical benefit [40–42] are consistent with the data for the cotton rat. Studies with the cotton rat model also showed that serum neutralizing antibody was highly effective in preventing pulmonary infection [10, 11]. Subsequent clinical trials confirmed this observation [12, 13] and formed the basis of licensure of RSV Ig as a preventive agent in 1996. Therapeutic studies in the cotton rat [9, 43] served as the basis for clinical trials of aerosolized [44] and intravenous [45] IgG treatment of hospitalized infants with RSV disease. Although IgG treatment reduced titers of virus, none of these studies showed a significant effect on clinical outcome. Our more recent work examining a combined antiviral/antiinflammatory approach [46] suggests that modulation of lung inflammation, in addition to clearance of virus, will be required for rapid reversal of clinical disease; clinical trials are currently being planned to test this approach.

Although there are several inherent advantages of the cotton rat model, there have been factors that have limited its use. Despite the fact that inbred cotton rats are now available commercially (Virion Systems, Rockville, MD), one major factor still limiting their use is the lack of reagents for characterization and quantitation of immunoglobulins, complement and other plasma proteins, cell surface antigens, and cytokines. Another disadvantage of the cotton rat is that, unlike the mouse, there are no congenic, transgenic, or knockout strains. The availability of such strains of mice and the unlikelihood of similar strains of cotton rats being developed suggest that RSV studies requiring such resources will continue to be done in the mouse.

**Mouse**

Shortly after RSV was discovered, Coates and Chanock [47] examined several species of laboratory animals to determine if any were permissive for pulmonary viral infection. Among the animals were four strains of inbred mice (DBA/2, BALB/c, AKR, and C3H). None of these mice developed CF antibody, and only one strain (AKR) developed neutralizing antibody; no attempt to recover infectious virus from the lungs was reported. Prince and co-workers [8] found that each of 20 inbred strains, including the four tested in the earlier report, was permissive for RSV infection in the lungs and nose. Levels of viral replication varied by two orders of magnitude from the least permissive (CBA/CaHN) to the most permissive (DBA/2N) strains, yet even the most permissive strain was about 100-fold less sensitive than the cotton rat [7].

The mouse model has several advantages over all other species, including a vast array of inbred, congenic, transgenic, and knockout strains; an unmatched library of specific reagents allowing identification and quantitation of cell types, immunoglobulins, cytokines, and other antigens; and relatively low purchase and maintenance costs. Although no prophylactic or therapeutic formulations have yet been licensed on the basis of studies in mice, many insights into the immunology of RSV disease have emerged that could not yet have been obtained from other models (for reviews of such studies, see [48, 49]). Of particular interest are studies describing different cytokine profiles in animals undergoing various types of immunization, including that with FI RSV vaccine [50]. However, although such profiles would be of great value in defining the parameters of safe vaccines, distinctly different cell types are obtained.
from different inbred strains with use of bronchoalveolar lavage [51], and such differences (combined with the histological dissimilarity of vaccine-enhanced disease in the mouse and human [32]) have the potential of confusing rather than clarifying the pathogenesis of FL RSV vaccine–enhanced disease. Further research on FL RSV immunization of other inbred strains may clarify this issue.

Calf

Thirteen years after the discovery of RSV as a human pathogen, a related virus was recovered from cattle with epidemic respiratory tract disease [52]. Subsequent studies showed that bovine RSV was a ubiquitous pathogen of cattle throughout the world [53]. The diseases caused by RSV and bovine RSV have several common characteristics, suggesting that the calf may be a useful model of human RSV disease. For example, both viruses cause acute disease that is limited to the respiratory tract, induce an incomplete immune response permitting repeated infections throughout life, cause epidemic disease clustered during winter months, are attenuated by high levels of maternally derived antibody, and cause severe pulmonary disease primarily in neonates [53]. The calf model of bovine RSV infection, however, has many of the same practical drawbacks of primate models: the cost of purchasing and maintaining animals, lack of inbred strains, and lack of specific reagents.

Two characteristics of clinical disease caused by bovine RSV in calves raise concerns about the usefulness of the calf model. First, bovine RSV commonly causes fever, while human RSV does not [54]. It is not known whether this circumstance is due to differences in the viruses or in host responses. Second, bacterial (particularly pasteurella and haemophilus [55]) and mycoplasmal [56] coinfections are common complications of bovine RSV infection, whereas such coinfections with human RSV have rarely been identified.

Recent studies have shown that calves immunized with FI bovine RSV vaccine and then challenged intranasally with live homologous virus develop enhanced pulmonary disease similar to that seen in humans, African green monkeys, and cotton rats [57]. Although a model employing a viral pathogen in its natural host might have an advantage over a human pathogen in an unnatural host, there are several other factors to consider that might dampen enthusiasm for a calf model of human RSV infection. The limited homologies of amino acids in several RSV and bovine RSV proteins and the greater difficulty in propagating and titrating bovine RSV in vitro underscore the fact that these viruses, while related, are not identical. It is likely that the most sound approach to animal modeling would include the human virus in the most permissive experimental host plus a nonhuman but related virus in its natural host.

Guinea Pig

Coates and Chanock [47] reported that RSV infection in the guinea pig caused a moderate neutralizing antibody response, but no attempt to quantitate viral replication in the lungs or nose was reported. Indeed, no systematic description of viral replication has yet been published. The only study to quantitate infectious virus in the lungs [58] reported a single time point (6 days after infection) and a very low titer (10^1.2 pfu/g).

The chief advantage of the guinea pig model derives from the extensive knowledge of airway physiology, particularly reactive airway disease, in this species. A causal relationship between RSV infection early in life and subsequent development of asthma has been suggested for many years, but this relationship has not been proven. Heightened responsiveness to acetylcholine was observed 7 days after RSV infection in guinea pigs but not 14 days after infection [59], suggesting either that the guinea pig is not a suitable model or that other noncholinergic effectors of airway hyperreactivity might be modulated by RSV. Recent reports of the persistence of viral genome and protein, but not infectious virus, for up to 60 days provide an intriguing backdrop for further studies [60, 61].

The chief disadvantages of the guinea pig model are its apparent limited permissiveness for RSV infection, the general unavailability of inbred strains, and the scarcity of immunologic reagents.

Ferret

RSV replicates in high titers in the nasal tissues of ferrets of all ages [47], but the virus replicates in the lungs only of infant animals [62]. Although the rapidly decreasing permissiveness of the lung severely limits the utility of ferrets, it provides an intriguing model for the age dependence of severe RSV disease in humans (in whom the severity of primary pulmonary disease is inversely proportional to age); nasal disease in humans does not vary with age [63]. Subsequent studies of RSV infection in ferret lung tissue and monolayer cultures confirmed in vivo findings, thereby suggesting a local, immunologic mechanism [64]. Despite the fact that the ferret model has disadvantages similar to those of the guinea pig and primate models (lack of inbred strains and immunologic reagents), it is the only model in which age-dependent pulmonary infection has been described and thus offers a potential tool for dissecting the mechanisms of age-dependent disease in humans.

Hamster

The only other animal for which quantitative virological studies have been reported is the Syrian hamster [65]. Although RSV replicated in both the lungs and nose, the relative permissiveness of the hamster was equivalent to that of the mouse and was ~100-fold less than that of the cotton rat. No follow-up reports have extended the quantitative studies of Wright et al. [65], and no studies describing RSV infection in hamsters have been published since 1983.
Summary

No single animal model has been shown to duplicate all aspects of primary RSV disease in the human infant at normal risk, much less in the increasing number of subpopulations for whom RSV poses threats ranging from inconvenience to death. Thus, there is no single answer to the question, "What is the best animal model of RSV disease?" Rather, the selection of a model will depend upon the type of RSV disease being studied. Problems of cost, availability, and opposition from animal rights activists will likely continue to restrict the study of RSV in primate models, particularly the chimpanzee. The primary advantage of the models are as follows: the calf, an RSV species in its natural host; the cotton rat, high permissiveness and reliability as a predictor of prophylactic and therapeutic strategies; the mouse, availability of specialized strains and reagents; the guinea pig, parallels in reactive airway disease; and the ferret, age-dependent pulmonary infection. Utilization of the calf, cotton rat, mouse, guinea pig, and ferret models likely will increase as the relative strengths of each model become better defined.

Acknowledgment

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