Published in final edited form as:

Immunol Allergy Clin North Am. 2010 November ; 30(4): 497–511. doi:10.1016/j.iac.2010.08.005.

Animal models of virus-induced chronic airway disease

Louis A. Rosenthal, Ph.D.^{a,b}

^a Division of Allergy, Pulmonary and Critical Care Medicine, Department of Medicine, University of Wisconsin School of Medicine and Public Health, Madison, Wisconsin

Keywords

viral respiratory tract infections; asthma; animal models; respiratory tract viruses; airway dysfunction; asthma onset

Introduction

Physicians recognized a link between the common cold and asthma long before the discovery of the viral causation of colds. The great physician-philosopher Moses Maimonides wrote in the 12th century that with regard to asthma, “I conclude that this disorder starts with a common cold, especially in the rainy season, and the patient is forced to gasp for breath day and night, depending on the duration of the onset, until the phlegm is expelled, the flow completed and the lung well cleared” [1]. In 1698, the pioneering English physician and clinical investigator Sir John Floyer, who himself had asthma, stated in his classic work, *A Treatise of the Asthma*, “I cannot remember the first occasion of my asthma; but have been told that it was a cold when I first went to school” [2]. Although physicians observed associations between colds and asthma, it is only relatively recently that improved methods of respiratory tract virus detection have permitted intensive investigation of these links and the potential role of early life viral respiratory tract infections in the inception of asthma.

Despite the growing evidence that experiencing viral wheezing illnesses early in life is an important risk factor for the development of asthma [3–6], a detailed understanding of the role of these infections in the onset of asthma remains to be elucidated [7]. It appears likely that a complex interplay of viral, host, environmental, and developmental factors influences the processes by which viral respiratory tract infections contribute to asthma inception. A central issue in asthma research is the role that acute insults to the airways, such as viral infections, play in the eventual development of chronic airway abnormalities. Animal models of virus-induced chronic airway dysfunction can serve as important investigative tools to address this issue because they permit control and manipulation of host genetic factors through the use of defined inbred, transgenic, and knockout animal strains and developmental factors through the capacity to perform viral inoculations and evaluate the outcomes of viral respiratory tract infections at specific stages of development. Furthermore, the nature of the airway insult, i.e., an infection with a respiratory tract virus, is known, and the animals can be inoculated with respiratory tract viruses in a specific and controlled manner. In this chapter, the potential

^bCorresponding author for proofs and reprints: Louis A. Rosenthal, Ph.D., Department of Medicine, University of Wisconsin School of Medicine and Public Health, 600 Highland Avenue, K4-948 CSC-9988, Madison, WI 53792, (608) 262-4470, (608) 263-3104 (fax), lar@medicine.wisc.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

advantages and disadvantages of using animal models for investigating the mechanisms by which early life viral respiratory tract infections could initiate a process leading to chronic airway dysfunction and the asthmatic phenotype will be discussed. A variety of useful animal models have been developed to study various aspects of viral respiratory tract infections of relevance to asthma. These models have utilized a variety of animal species, including mice [8–15], rats [16–24], guinea pigs [25], cotton rats [26], sheep [7], cattle [28], and nonhuman primates [29]. This review will discuss the potential advantages and disadvantages of models in mice and rats because most work has been carried out in these species.

There is increasing evidence that viral wheezing illnesses in early life, especially those caused by infections with human rhinovirus (HRV), are associated with an increased risk of developing asthma in childhood [3–6]. Viral respiratory tract infections also are the main cause of asthma exacerbations in children and adults [30]. In addition, repeated viral respiratory tract infections during childhood might be a factor in the generation of the persistent airway changes associated with asthma [31]. There is also evidence of interaction between early life viral respiratory tract infections, atopy (the genetic predisposition to produce high levels of IgE in response to allergen) and allergic sensitization (a manifestation of atopy) in relation to the risk for developing asthma. Children who experience viral respiratory tract infections with wheezing in early life and develop allergic sensitization are at substantially greater risk for developing persistent wheezing and childhood asthma than are children who experience one or the other or neither [3,5]. Therefore, there is a strong need for the development of animal models that can mimic these early life viral respiratory tract infections; in particular, those early life viral infections that appear to have long-lasting consequences for respiratory health. Consequently, it is important to develop rodent models of asthma that include this feature, i.e., an early life respiratory infection that results in persistent airway sequelae. It would be especially useful if these models also had the capacity to incorporate components of allergic sensitization and allergic airway inflammation, which appear to be important factors in the development of childhood asthma. Because the inception of childhood asthma might depend on interactions between the immune and pulmonary systems in early life, it would be important for the models to have the capacity to examine both the immunologic and physiologic consequences of relevant viral respiratory tract infections. To date, many of the viral respiratory tract infection models in rodents, while highly useful, have focused on the acute effects of the viral infection and the acute interactions with allergic sensitization and acute allergic airway inflammation. A more limited number of models have also addressed chronic, long-term effects of viral respiratory tract infections in the context of attempting to better understand the origins of asthma [8–10,16–23].

What Might an “Ideal” Rodent Model Look Like?

In order to consider the potential advantages and disadvantages of using rodent models to study virus-induced chronic airway dysfunction, it would be useful to consider what features might be incorporated in an “ideal” model. Recent evidence indicates that viral wheezing illnesses and atopy, as demonstrated by allergic sensitization, may have additive or perhaps even synergistic effects with regard to the associated risk for the development of persistent wheezing and asthma in childhood [3–6]. Therefore, an “ideal” rodent model would allow investigators to examine important aspects of these interactions from multiple perspectives. The ability to study relevant host genetic factors would be important. For example, it would be of great value to be able to study genetic aspects of atopy and susceptibility to allergic sensitization, as well as genetic factors regulating the susceptibility to viral respiratory tract infections and postviral airway sequelae. The capacity to manipulate environmental factors, such as exposures to respiratory tract viruses and allergens, would be necessary. The ability to investigate developmental factors, such as the effects of the maturational states of the immune and pulmonary systems on the responses to respiratory tract virus and/or allergen exposure and the

subsequent development of chronic airway sequelae, would be of great importance as well. Overall, an “ideal” model of asthma inception would allow for the investigation of mechanisms that could potentially tie together the host immunologic responses to environmental insults to the airways (in this case respiratory tract virus infections), atopy and allergic sensitization, and the subsequent development of persistent structural and functional changes to the airways that might lead to development of the asthmatic phenotype. To further this discussion of the potential advantages and disadvantages of rodent models of virus-induced chronic airway dysfunction, the capacity of these models to investigate relevant viral, host, environmental, and developmental factors will be considered.

Viral factors

Despite the increasing evidence that early life viral wheezing illnesses are strongly associated with increased risk for the development of persistent wheezing and asthma in childhood, the actual mechanisms by which viral wheezing illnesses could mediate these affects are poorly understood. In addition, a causal role for these viral wheezing illnesses in the onset of asthma has yet to be established. Viral wheezing illnesses might trigger long-term airway sequelae in susceptible infants and children leading to the eventual development of asthma. However, it is also possible that infants and children who will subsequently go on to develop asthma because of abnormalities in their lungs or immune systems are particularly susceptible to wheezing brought on by viral respiratory tract infections. That is, are viral wheezing illnesses a contributing factor to the development of asthma or are they a marker for individuals who will develop asthma for other reasons? Or can both of these scenarios occur in different individuals? Rodent models of virus-induced chronic airway dysfunction could be very useful in addressing the relative importance of viral factors as mechanisms by which viral respiratory tract infections could cause chronic structure-function changes in the airways that could lead to long-lasting physiologic abnormalities. These mechanisms could then be tested under the more limited conditions of what can be studied directly in children. That is, rodent studies can yield testable hypotheses that could be investigated in new prospective asthma cohort studies or through the use of banked clinical specimens from previous and ongoing asthma cohort studies. Box 1 summarizes potential advantages and disadvantages of using rodent models to investigate mechanisms of virus-induced chronic airway dysfunction

Important unresolved questions include whether specific respiratory tract viruses might be “asthmagenic” and if so, whether different respiratory tract viruses vary in their “asthmagenic” potential. That is, are certain viruses more or less likely to cause respiratory tract infections that can trigger the chronic alterations in airway function observed in asthma. Rodent models can provide a useful way to investigate mechanisms by which respiratory tract viruses could vary in their “asthmagenic” potential. For example, investigators have demonstrated that different strains of respiratory syncytial virus (RSV) can differ in their pathogenicity and ability to induce mucus production in a well-defined mouse model [32]. They have been able to define genetic differences among these RSV strains that account for this diversity in mucogenicity and pathogenicity.

An important issue with regard to rodent models of viral respiratory tract infections is the permissiveness of the host for the virus of interest. Some of the advantages and disadvantages of these rodent models can be a consequence of which respiratory tract virus is employed in the model. For example, an advantage of the recent establishment of mouse models of HRV infection is that these models use one of the viruses that cause HRV infections and illnesses in humans [33,34]. The disadvantage is that HRV does not replicate nearly as efficiently in mice as it does in the human host. Consequently, relatively high doses of HRV must be used to establish transient infections in the mice [33,34]. Even though the replication of HRV in mice is limited, these models will continue to be useful for studying the mechanisms of HRV-induced

inflammation [33,34]. A mouse-adapted strain of HRV has been generated in vitro [35], and it would be interesting to see if this virus replicates more efficiently in mice than nonadapted HRV strains. Because there are no known rodent rhinoviruses, we have used a related rodent picornavirus (HRV is a member of the picornavirus family), mengovirus, to establish respiratory infection models in rats [36] and mice (L.A. Rosenthal, unpublished data), which has the advantage of using a natural rodent virus in a natural host. Therefore, the required viral dose is smaller and viral shedding occurs for a longer time than in the HRV infection model in mice [36]. Rodent models using rodent parainfluenza 1 (Sendai) virus, which like RSV is also a paramyxovirus, have been particularly useful because this virus replicates well in the rodent host and more closely resemble a natural virus exposure [8–10,16–23]. A potential disadvantage to consider when using rodent respiratory tract viruses is that they might exhibit differences with regard to viral virulence mechanisms compared to the corresponding human respiratory tract viruses.

Another important consideration with regard to viral factors is the so-called “hit-and-run” hypothesis [37]. That is, it is hypothesized that a viral respiratory tract infection occurs early in life and even after the virus has been cleared by the host, an array of processes have been set in motion that will lead to long-term airway sequelae that are dependent on the initiating viral infection. It is likely that the viral respiratory tract infection must be moderate-to-severe and involve the lower airways for it to have significant enough effects to set such a process in motion, i.e., the virus would have induced a spectrum of disorders ranging from wheezing illnesses to bronchiolitis. How a single respiratory tract virus infection could have such long-lasting consequences remains to be elucidated, but rodent models of virus-induced chronic airway dysfunction are providing an important path for investigating this possibility and the mechanisms that could contribute to this process [8–10,16–23]. The results from these rodent models can inform the design of studies in humans, allowing the hypotheses generated from the animal model data to be tested in human clinical studies. The “hit-and-run” hypothesis remains a viable one, and an ideal rodent model of virus-induced chronic airway dysfunction would almost certainly have characteristics that would enable the investigation of this hypothesis. Two rodent models studying the postviral airway sequelae following Sendai virus infection have been very useful for looking at these questions [8–10,16–23] and have directly led to new hypotheses and to studies in humans to test them [10,38].

The binding specificity of human respiratory tract viruses for their cellular receptors is also an important consideration and a potential disadvantage when designing rodent models of viral respiratory tract infection. Differences in receptor-binding capacity to rodent cells can limit the ability of human respiratory tract viruses to be studied in rodent models, i.e., the human virus is unable to bind to and enter rodent host cells. A notable example is the inability of major group HRV to infect rodent cells because of the requirement for binding to ICAM-1 [35]. HRV cannot use murine ICAM-1 for entry into cells. This difficulty has been overcome in one model by using transgenic mice that constitutively express human ICAM-1 as the host for inoculation with a major group HRV [33]. Thus, one advantage of using rodent respiratory tract viruses in the design of rodent models of virus-induced chronic airway dysfunction is that the virus and its receptor are compatible.

Another distinct advantage of rodent models is that the entire progression of the viral infection can be observed because the initiation of the viral infection is controlled by the investigator. Thus, in rodent models all stages of the viral respiratory tract infection as well as any sequelae identified during the chronic phase after the clearance of the virus can be studied. In addition, cells, tissues, and organs from the immune and pulmonary systems of rodents are readily available for analysis of viral titers, viral gene and protein expression, and the expression of viral virulence factors at any time after inoculation with the respiratory tract virus.

In studies involving human subjects, the only instance when this is true is in experimental inoculation studies, which are of great value but are technically challenging, and relatively few research centers have the capacity to perform them. Otherwise, most human studies involve subjects who present with viral respiratory tract infections. Even in large cohort studies where subjects are monitored prospectively, infections only become apparent when presented to the investigators by the subjects or their parents/caregivers in the case of infants and children. A potential disadvantage in rodent models is that the course of infection in rodents is unlikely to be identical to that in humans, e.g., the kinetics are likely to be somewhat different. Also, most inoculations of rodents deposit the virus both in the nasopharynx and the lungs, whereas the initiation of viral respiratory tract infections with typical cold viruses in humans is more likely to occur with deposition of virus in the nose and/or eyes which then infects the lower airways.

In rodent models, it is possible to administer respiratory tract viruses that have been genetically modified to investigate viral virulence factors. In experimental inoculations of humans this usually would not be possible, with the exception of inoculation with experimental or approved vaccine strains of reduced virulence. Inoculation of human subjects with respiratory tract viruses that have been genetically modified to increase virulence would not be ethically acceptable. However, this would be possible to do in rodent models as long as appropriate precautions were taken and appropriate containment procedures were employed.

When new respiratory tract viruses of potential relevance to asthma are discovered, it is possible to attempt experimental inoculations of rodents right away if stocks can be prepared; a notable example is the relatively rapid development of mouse models of metapneumovirus-induced respiratory infection [39,40]. In contrast, experimental inoculations of humans would involve a long safety testing and regulatory process, including an investigational new drug application to the U.S. Food and Drug Administration, and may not be ethically possible at all depending on the virulence of the virus in humans. In addition, such clinical respiratory tract virus challenges in human subjects require a relatively large infrastructure of investigators, research coordinators, and laboratory personnel and relatively few research centers have the capacity to do this.

An instructive example of the ability of rodent models to be useful for the investigation of viral factors involves the recent advance in understanding the important association between HRV-induced wheezing illnesses and the development of childhood asthma. HRV had been implicated as the most common viral infection associated with asthma exacerbations in children and adults [30]. However, recent birth cohort studies in the U.S. and Australia have also shown that HRV infections are a significant cause of wheezing illnesses in infants and children and that these HRV wheezing illnesses are associated with an increased risk for the development of asthma in childhood, especially if the children are sensitized to allergen [3–6]. Because viral challenge studies, even with safety-tested strains of HRV, are not feasible in children, rodent models provide an avenue for investigating the effects of HRV in younger animals in order to model what may be going on in infants and children.

The available animal models include those using minor group HRV which binds to the low density lipoprotein receptor on both human and rodent cells [33,34], those using major group HRV which binds to ICAM-1 [33], requiring the use of mice transgenic for human ICAM-1 because HRV does not bind to mouse ICAM-1, and our use of a related murine picornavirus, mengovirus, to model HRV infections [36]. To date, all of the published studies have used young adult rodents for the development of these models. Inoculation experiments on young adult animals provide data that are relevant to colds and cold virus-induced asthma exacerbations. However, it is likely that investigators will take advantage of these novel models to examine effects on younger rodents.

Rodent models also can be advantageous for preclinical testing of novel antiviral therapeutic strategies and for investigating the mechanisms of action of these therapies [41]. However, a caveat is that the viral virulence factors targeted by these therapeutics might differ in rodents compared with those in humans.

Host factors

Host factors are a crucial consideration in understanding the potential basis for the development of chronic airway dysfunction as a consequence of early life viral respiratory tract infections. The use of rodent models provides a variety of potential advantages and disadvantages in this regard (Box 2). One distinct advantage is that the genetics of the rodent models can be controlled and manipulated to facilitate mechanistic studies. The use of inbred rodent strains dramatically reduces the genetic variability of the experimental subjects. Sophisticated breeding strategies are available that could be used for mapping and characterizing genetic loci associated with host responses to viral respiratory infections [42]. The availability of knockout mouse strains, and the recent but more limited availability of knockout rat strains, enables studies of subjects with specific genetic deficiencies, permitting examination of the effects of specific genes on these processes. In addition, the use of transgenic tools has permitted the development of rodents constitutively expressing or overexpressing genes of potential interest. Of even greater potential value is the development of mouse models in which genes of interest are either conditionally knocked out or expressed, which markedly increases the ability of researchers to control the experimental conditions [43]. A further refinement of these methods has permitted investigators to conditionally knock out or express genes in a cell or tissue specific manner through use of cell and tissue specific promoters in the constructs that are introduced into the mouse lines. Thus, investigators are able to derive mice that at a time of their choosing can be triggered to knock out, downregulate, or turn on the expression of specific genes of interest, while controlling the cell specificity, tissue specificity, and the temporal specificity of this modulation of gene expression. That is, investigators are increasingly capable of controlling the levels, location, and timing of gene expression in these models. Transgenic technology also allows investigators to label and track specific cell types and cell subtypes in vivo by expressing fluorescent marker proteins via appropriate promoters with appropriate cell- or tissue-specificities [44], which will enable cell tracking experiments that attempt to define the mechanisms regulating the host responses to viral respiratory tract infections.

Another important aspect of host factors that can be studied advantageously in rodent models is the role of gender. Studies can be readily performed in males and females and the results compared and contrasted. This capacity is of importance because of the evidence of gender-related differences in wheezing and asthma in childhood [45]. Rodent models can investigate these issues in a mechanistic way and generate hypotheses to be tested in human studies. In addition, rodent studies can be designed to investigate the mechanisms of gender-related differences observed in human clinical studies.

Another important area of investigation that is becoming amenable to study in rodent models are epigenetic effects, which include heritable changes in gene expression without changes to the underlying genomic sequence [46,47]. The use of rodent models to study the role of epigenetic effects in regulating gene expression during host responses to viral respiratory tract infections would be advantageous because it is possible to modulate epigenetic-relevant DNA modifications through pharmacologic methods. It also would be of interest to investigate whether long-term changes in airways following early life viral respiratory tract infections could be due in part to epigenetic modifications induced during the host response to the viral infection. The well-established rat and mouse models of Sendai virus-induced chronic airway dysfunction would be attractive candidates for this type of investigation because airway dysfunction in these models continues to persist for the entire period of observation, up to one

year after viral clearance [8–10,16–23]. Therefore, it is likely that the persistent airway inflammation and dysfunction is due to host factors that were initiated by the viral infection and then persisted even after viral clearance, i.e., the “hit-and-run” hypothesis. If epigenetic effects could be defined in these rodent models, focused hypotheses could be generated for testing in human studies using the much more limited array of biological samples that can be obtained from infants and children enrolled in prospective cohort studies.

Rodent models also can play an important role in addressing the mechanistic role of atopy, the genetic predisposition to produce high levels of IgE in response to allergen exposure, in the development of virus-induced chronic airway dysfunction. Rodent strains differ with regard to their capacity to generate IgE and Th2-type immune responses, which is useful for investigating the interaction of viral respiratory tract infections and subsequent antiviral host responses with the atopic status of the host. This has been an important aspect of the brown Norway rat (BN) model of virus-induced chronic airway dysfunction in which only rats of the atopic BN strain are susceptible to the development of a postbronchiolitis, asthma-like phenotype after experiencing parainfluenza-1 (Sendai) virus-induced bronchiolitis as weanlings. F344 rats, after undergoing the same viral inoculation under the same conditions experience a resolution of the viral bronchiolitis and have normal airways after several weeks [16–23,48]. One significant difference between these strains is their atopic status. The BN rat has Th2-biased immune responses, whereas the F344 rat mounts Th1-biased immune responses. BN rats can make substantial amounts of IgE after allergen sensitization, whereas it is difficult to generate IgE responses in F344 rats. While these global differences in the ability to mount the Th2-type responses that promote allergic inflammation are highly useful, transgenic and knockout rodents permit even finer distinctions with regard to atopy to be investigated: the effects of individual genes can be investigated with regard to their effects on susceptibility to allergic sensitization and the subsequent development of allergic inflammation. Information derived from these studies can then inform studies in humans where genes identified in rodent models can be investigated, e.g., by examining associations between genetic polymorphisms in these genes of interest and the development of allergic sensitization and asthma, as well as how they influence susceptibility to the early life viral respiratory tract infections that might contribute to the development of chronic airway dysfunction.

Another issue to consider is that there are notable differences in pulmonary physiology between rodents and humans [49]. For example, the airway caliber in rodents, especially in mice, is larger relative to their body size compared to humans. This is one reason why it can be difficult to detect airway obstruction in mice with viral respiratory tract infections in the absence of a challenge with a cholinergic agent, such as methacholine. These rodent models are still highly useful, but it is important to keep these differences in mind when interpreting physiology data from rodent models of human airway disease.

Environmental factors

Rodent models present both potential advantages and disadvantages with regard to the investigation of the influences of environmental factors on host responses to viral respiratory tract infections and the development of chronic airway dysfunction (Box 3). One advantage is that it is possible to design controlled exposures to defined environmental factors, such as microorganisms, microbial products (endotoxin, microbial DNA, glucans, etc.), dusts, allergens, diesel exhaust, ozone, and tobacco smoke and then assess how these exposures affect the responses of the rodents to a challenge with a respiratory tract virus. That is, do these environmental exposures act as co-morbid factors to increase the likelihood of developing or the severity of virus-induced chronic airway dysfunction? Alternatively, do these environmental exposures boost the capacity of the host to resolve the viral challenge in a way that prevents the development of chronic airway dysfunction? It is also advantageous that

exposures can be made during specific developmental windows encompassing different maturational stages of the immune system and lungs. Defined prenatal and/or postnatal exposures can be performed as well to study in utero effects. Foster nursing approaches can be used to investigate effects of breast milk consumption.

The dosage, frequency, and duration of the exposures can be controlled and quantified. This has been shown to be important, for example, with regard to the effects of environmental exposures to microbial products, such as endotoxin. Studies in rodent models and epidemiological studies in humans have shown substantial dosage effects in which lower levels of exposure may increase the risk of allergen sensitization, whereas higher levels of exposure may have protective effects in this regard [50,51]. This is further complicated by interactions with host factors, especially the genetics of the host, whereby gene-by-environment interactions can modulate the effect of exposures to microbial products, e.g., endotoxin [51]. The use of rodent models can permit detailed mechanistic studies of these types of gene-by-environment interactions through the use of knockout mice or transgenic mice overexpressing the genes of interest and by the ability to do prospective dose response experiments. A disadvantage of rodent models is that the environmental exposures will not be as complex as those in the human environment; therefore, important interactive effects among different types of environmental exposures might not be appreciated. To address, this issue there has been interest among some investigators to take a somewhat less reductionist approach and expose the rodent to more complex mixtures, such as house dust extracts [52]. It seems likely that both approaches, exposure to one or two specific environmental agents vs. exposure to more complex mixtures of environmental agents will yield complementary findings that will inform each other.

Another issue to consider is the differences in dosages and timing of environmental exposures between rodent models and the experiences of humans. One of the major values of using rodents as research models is that their relatively short lifespan permits contraction of the time that would be required to do similar studies in human subjects. However, this often results in the need for a more compressed schedule of exposures with larger amounts of material to achieve effects which can be studied in these shorter time frames. Thus, it is important for the research community as a whole to keep going back and forth between rodent data and human data to periodically assess the relevance of the rodent models and to use data from clinical research to design novel mechanistic studies in rodent models and vice versa to use mechanistic studies in rodents to suggest hypotheses to be tested in clinically-oriented studies.

The difficulty of exposing rodents to the complexity of environmental factors encountered by humans is being alleviated by the development of new technologies that permit the characterization of this complexity. This allows investigators to replicate aspects of these complex environmental exposures to study potential interactive effects within their rodent models of virus-induced airway dysfunction. For example, multiplex technologies have been developed that can assay for multiple allergens within environmental samples [53]. The development of microarray technologies to detect practically all known bacterial species will allow the careful delineation of bacterial species within different environments [54], such as farming environments, that could have an impact on the outcome of early life viral respiratory tract infections. This will allow investigators to better define how these complex mixtures can affect the development of host antiviral responses, the characteristics of which may have profound effects on the likelihood that chronic airway sequelae will develop after a viral respiratory tract infection in early life.

Developmental factors

Rodent models of virus-induced airway dysfunction also exhibit advantages and disadvantages with regard to studying developmental factors (Box 4). A major advantage is the shorter lifespan of the rodent, which enables longitudinal studies encompassing all ages to be done within a relatively convenient time period. These types of longitudinal studies are also done in human subjects but require relatively large teams of investigators working many years. While these pediatric cohort studies are invaluable, the rodent models permit similar questions to be addressed in a mechanistic manner in a condensed time frame. The data derived from these rodent studies can then be used to generate focused hypotheses to be addressed in clinical studies. A disadvantage of rodent models is that harmonizing human and rodent maturational stages with regard to the immune system and pulmonary system can present some challenges. For example, in rodents alveolarization occurs postnatally; rodent pups are born with saccules. Therefore, respiratory viral challenges must take this into account. For example, Sendai virus infection of neonatal rats yielded an interesting model [55], but it was unsatisfactory for the kinds of changes associated with the development of asthma because it induced alveolar dysplasia, i.e., the viral infection interfered with the normal course of postnatal alveolar development, which in humans would have occurred prenatally. Thus, the BN rat model of the post-bronchiolitis, asthma-like phenotype was developed by infecting weanling (3–4 week old) BN rats with Sendai virus, which induced bronchiolitis without alveolar dysplasia because alveolarization had already occurred in these weanling animals [16–23].

An important aspect of rodent models is the ability to perform manipulations prenatally as well as postnatally under defined conditions. For example, this can help to distinguish *in utero* influences from other effects. With the growing interest in regulatory mechanisms such as epigenetics, investigation of *in utero* effects is likely to become an important area of research.

The availability of knockout and transgenic rodent strains allows investigators to look at the effects of specific genes during development on the response to viral respiratory tract infections. While these studies do not replace pediatric clinical studies, they permit a mechanistic focus and are complementary to studies involving human subjects.

Another advantage of developmental studies in rodents is the capability to sample cells and tissues. Many cell and tissue samples are difficult to obtain from children because of ethical, safety, and logistical reasons. For example, the ability to obtain approvals for experimental bronchoscopy of children is markedly different in the different regions of the world and even at different institutions within the same country. The ability to do these types of procedures ranges from almost never to sometimes with sufficient justification. The tissue availability from the rodent models does provide the capacity to do certain type of mechanistic studies that cannot be done in children. These mechanistic studies can then serve as a basis for designing focused studies in pediatric subjects where access to biological samples can be limited and the amount of material in the samples can also be limiting.

Synopsis

There is now increasing evidence that experiencing viral wheezing illnesses early in life, especially in conjunction with allergic sensitization, is an important risk factor for the onset of asthma. In this review, the potential advantages and disadvantages of using rodent models of virus-induced chronic airway dysfunction to investigate the mechanisms by which early life viral respiratory tract infections could initiate a process leading to chronic airway dysfunction and the asthmatic phenotype are discussed. The potential usefulness of rodent models for elucidating the viral, host, environmental, and developmental factors that might influence these processes are emphasized. Overall, there is a need for the continued development of rodent

models of early life viral respiratory tract infections that include the development of chronic airway dysfunction, the capacity to add components of allergic sensitization and allergic airway inflammation, and the ability to address both immunologic and physiologic consequences. Investigation of these rodent models should complement the research from pediatric cohort studies and begin to bring us closer to understanding the role of viral respiratory tract infections in the inception of childhood asthma.

Acknowledgments

This work was supported by grant nos. AI070503 and HL097134 from the National Institutes of Health.

References

1. Maimonides, M. Treatise on asthma. Munter, S., editor. Philadelphia: Lippincott; 1963.
2. Floyer, J. A treatise of the asthma. London: Printed for Richard Wilkin; 1698.
3. Kusel MM, de Klerk NH, Keadze T, et al. Early-life respiratory viral infections, atopic sensitization, and risk of subsequent development of persistent asthma. *J Allergy Clin Immunol* 2007;119(5):1105–1110. [PubMed: 17353039]
4. Lemanske RF Jr, Jackson DJ, Gangnon RE, et al. Rhinovirus illnesses during infancy predict subsequent childhood wheezing. *J Allergy Clin Immunol* 2005;116(3):571–577. [PubMed: 16159626]
5. Jackson DJ, Gangnon RE, Evans MD, et al. Wheezing rhinovirus illnesses in early life predict asthma development in high-risk children. *Am J Respir Crit Care Med* 2008;178(7):667–672. [PubMed: 18565953]
6. Sly PD, Kusel M, Holt PG. Do early-life viral infections cause asthma? *J Allergy Clin Immunol* 2010;125(6):1202–1205. [PubMed: 20304476]
7. Rosenthal LA, Avila PC, Heymann PW, et al. Viral respiratory tract infections and asthma: the course ahead. *J Allergy Clin Immunol* 2010;125(6):1212–1217. [PubMed: 20513518]
8. Walter MJ, Morton JD, Kajiwar N, et al. Viral induction of a chronic asthma phenotype and genetic segregation from the acute response. *J Clin Invest* 2002;110(2):165–175. [PubMed: 12122108]
9. Grayson MH, Cheung D, Rohlfing MM, et al. Induction of high-affinity IgE receptor on lung dendritic cells during viral infection leads to mucous cell metaplasia. *J Exp Med* 2007;204(11):2759–2769. [PubMed: 17954569]
10. Kim EY, Battaile JT, Patel AC, et al. Persistent activation of an innate immune response translates respiratory viral infection into chronic lung disease. *Nat Med* 2008;14(6):633–640. [PubMed: 18488036]
11. Matsuse H, Behera AK, Kumar M, et al. Recurrent respiratory syncytial virus infections in allergen-sensitized mice lead to persistent airway inflammation and hyperresponsiveness. *J Immunol* 2000;164(12):6583–6592. [PubMed: 10843718]
12. Hashimoto K, Graham BS, Ho SB, et al. Respiratory syncytial virus in allergic lung inflammation increases muc5ac and gob-5. *Am J Respir Crit Care Med* 2004;170(3):306–312. [PubMed: 15130904]
13. Hashimoto K, Durbin JE, Zhou W, et al. Respiratory syncytial virus infection in the absence of STAT1 results in airway dysfunction, airway mucus, and augmented IL-17 levels. *J Allergy Clin Immunol* 2005;116(3):550–557. [PubMed: 16159623]
14. Estripeaut D, Torres JP, Somers CS, et al. Respiratory syncytial virus persistence in the lungs correlates with airway hyperreactivity in the mouse model. *J Infect Dis* 2008;198(10):1435–1443. [PubMed: 18828742]
15. Mejias A, Chavez-Bueno S, Gomez AM, et al. Respiratory syncytial virus persistence: evidence in the mouse model. *Pediatr Infect Dis J* 2008;27(10 Suppl):S60–S62. [PubMed: 18820580]
16. Rosenthal, LA.; Sorkness, RL.; Lemanske, RF, Jr. Origin of respiratory virus-induced chronic airway dysfunction: exploring genetic, developmental, and environmental factors in a rat model of the asthmatic phenotype. In: Johnston, SL.; Papadopoulos, NG., editors. *Respiratory Infections in Allergy and Asthma. Lung Biology in Health and Disease*. Vol. 178. New York: Marcel Dekker; 2003. p. 365–88.

17. Uhl EW, Castleman WL, Sorkness RL, et al. Parainfluenza virus-induced persistence of airway inflammation, fibrosis, and dysfunction associated with TGF- β 1 expression in Brown Norway rats. *Am J Respir Crit Care Med* 1996;154:1834–1842. [PubMed: 8970378]
18. Kumar A, Sorkness R, Kaplan MR, et al. Chronic, episodic, reversible airway obstruction after viral bronchiolitis in rats. *Am J Respir Crit Care Med* 1997;155:130–134. [PubMed: 9001301]
19. Sorkness RL, Castleman WL, Kumar A, et al. Prevention of chronic post-bronchiolitis airway sequelae with interferon- γ treatment in rats. *Am J Respir Crit Care Med* 1999;160:705–710. [PubMed: 10430749]
20. Sorkness RL, Gern JE, Grindle KA, et al. Persistence of viral RNA in 2 rat strains differing in susceptibility to postbronchiolitis airway dysfunction. *J Allergy Clin Immunol* 2002;110(4):607–609. [PubMed: 12373269]
21. Sorkness RL, Tuffaha A. Contribution of airway closure to chronic postbronchiolitis airway dysfunction in rats. *J Appl Physiol* 2004;96(3):904–910. [PubMed: 14594863]
22. Rosenthal LA, Mikus LD, Tuffaha A, et al. Attenuated innate mechanisms of interferon- γ production in rats susceptible to postviral airway dysfunction. *Am J Respir Cell Mol Biol* 2004;30(5):702–709. [PubMed: 14592927]
23. Sorkness RL, Herricks KM, Szakaly RJ, et al. Altered allergen-induced eosinophil trafficking and physiological dysfunction in airways with preexisting virus-induced injury. *Am J Physiol Lung Cell Mol Physiol* 2007;292(1):L85–L91. [PubMed: 16905639]
24. Piedimonte G, Hegele RG, Auais A. Persistent airway inflammation after resolution of respiratory syncytial virus infection in rats. *Pediatr Res* 2004;55(4):657–665. [PubMed: 14711892]
25. Adamko DJ, Yost BL, Gleich GJ, et al. Ovalbumin sensitization changes the inflammatory response to subsequent parainfluenza infection. Eosinophils mediate airway hyperresponsiveness, m(2) muscarinic receptor dysfunction, and antiviral effects. *J Exp Med* 1999;190(10):1465–1478. [PubMed: 10562321]
26. Hassantoufighi A, Oglesbee M, Richter BW, et al. Respiratory syncytial virus replication is prolonged by a concomitant allergic response. *Clin Exp Immunol* 2007;148(2):218–229. [PubMed: 17335559]
27. Olivier A, Gallup J, de Macedo MM, et al. Human respiratory syncytial virus A2 strain replicates and induces innate immune responses by respiratory epithelia of neonatal lambs. *Int J Exp Pathol* 2009;90(4):431–438. [PubMed: 19659901]
28. Gershwin LJ, Gunther RA, Hornof WJ, et al. Effect of infection with bovine respiratory syncytial virus on pulmonary clearance of an inhaled antigen in calves. *Am J Vet Res* 2008;69(3):416–422. [PubMed: 18312142]
29. Mohapatra SS, Boyapalle S. Epidemiologic, experimental, and clinical links between respiratory syncytial virus infection and asthma. *Clin Microbiol Rev* 2008;21(3):495–504. [PubMed: 18625684]
30. Friedlander SL, Busse WW. The role of rhinovirus in asthma exacerbations. *J Allergy Clin Immunol* 2005;116(2):267–273. [PubMed: 16083778]
31. Jartti T, Lee WM, Pappas T, et al. Serial viral infections in infants with recurrent respiratory illnesses. *Eur Respir J* 2008;32(2):314–320. [PubMed: 18448489]
32. Moore ML, Chi MH, Luongo C, et al. A chimeric A2 strain of respiratory syncytial virus (RSV) with the fusion protein of RSV strain line 19 exhibits enhanced viral load, mucus, and airway dysfunction. *J Virol* 2009;83(9):4185–4194. [PubMed: 19211758]
33. Bartlett NW, Walton RP, Edwards MR, et al. Mouse models of rhinovirus-induced disease and exacerbation of allergic airway inflammation. *Nat Med* 2008;14(2):199–204. [PubMed: 18246079]
34. Newcomb DC, Sajjan US, Nagarkar DR, et al. Human rhinovirus 1B exposure induces phosphatidylinositol 3-kinase-dependent airway inflammation in mice. *Am J Respir Crit Care Med* 2008;177(10):1111–1121. [PubMed: 18276942]
35. Harris JR, Racaniello VR. Amino acid changes in proteins 2B and 3A mediate rhinovirus type 39 growth in mouse cells. *J Virol* 2005;79(9):5363–5373. [PubMed: 15827151]
36. Rosenthal LA, Amineva SP, Szakaly RJ, et al. A rat model of picornavirus-induced airway infection and inflammation. *Virol J* 2009;6(1):122. [PubMed: 19671179]
37. Holtzman MJ, Shornick LP, Grayson MH, et al. “Hit-and-run” effects of paramyxoviruses as a basis for chronic respiratory disease. *Pediatr Infect Dis J* 2004;23(11 Suppl):S235–S245. [PubMed: 15577579]

38. Lemanske RF Jr. The childhood origins of asthma (COAST) study. *Pediatr Allergy Immunol* 2002;13 (Suppl):1538–43.
39. Williams JV, Crowe JE Jr, Enriquez R, et al. Human metapneumovirus infection plays an etiologic role in acute asthma exacerbations requiring hospitalization in adults. *J Infect Dis* 2005;192(7):1149–1153. [PubMed: 16136455]
40. Guerrero-Plata A, Baron S, Poast JS, et al. Activity and regulation of alpha interferon in respiratory syncytial virus and human metapneumovirus experimental infections. *J Virol* 2005;79(16):10190–10199. [PubMed: 16051812]
41. Kong X, Zhang W, Lockey RF, et al. Respiratory syncytial virus infection in Fischer 344 rats is attenuated by short interfering RNA against the RSV-NS1 gene. *Genet Vaccines Ther* 2007;54.
42. Flint J, Valdar W, Shifman S, et al. Strategies for mapping and cloning quantitative trait genes in rodents. *Nat Rev Genet* 2005;6(4):271–286. [PubMed: 15803197]
43. Hausding M, Sauer K, Maxeiner JH, et al. Transgenic models in allergic responses. *Curr Drug Targets* 2008;9(6):503–510. [PubMed: 18537589]
44. Mohrs K, Wakil AE, Killeen N, et al. A two-step process for cytokine production revealed by IL-4 dual-reporter mice. *Immunity* 2005;23(4):419–429. [PubMed: 16226507]
45. Lemanske RF Jr, Busse WW. Asthma: clinical expression and molecular mechanisms. *J Allergy Clin Immunol* 2010;125(2 Suppl 2):S95–102. [PubMed: 20176271]
46. Holgate ST, Davies DE, Powell RM, et al. Local genetic and environmental factors in asthma disease pathogenesis: chronicity and persistence mechanisms. *Eur Respir J* 2007;29(4):793–803. [PubMed: 17400878]
47. Barnes PJ. Targeting the epigenome in the treatment of asthma and chronic obstructive pulmonary disease. *Proc Am Thorac Soc* 2009;6(8):693–696. [PubMed: 20008877]
48. Sorkness RL, Castleman WL, Mikus LD, et al. Chronic postbronchiolitis airway instability induced with anti-IFN- gamma antibody in F344 rats. *Pediatr Res* 2002;52(3):382–386. [PubMed: 12193672]
49. Bates JH, Rincon M, Irvin CG. Animal models of asthma. *Am J Physiol Lung Cell Mol Physiol* 2009;297(3):L401–L410. [PubMed: 19561139]
50. Eisenbarth SC, Piggott DA, Huleatt JW, et al. Lipopolysaccharide-enhanced, toll-like receptor 4-dependent T helper cell type 2 responses to inhaled antigen. *J Exp Med* 2002;196(12):1645–1651. [PubMed: 12486107]
51. Martinez FD. CD14, endotoxin, and asthma risk: actions and interactions. *Proc Am Thorac Soc* 2007;4 (3):221–225. [PubMed: 17607003]
52. Lam D, Ng N, Lee S, et al. Airway house dust extract exposures modify allergen-induced airway hypersensitivity responses by TLR4-dependent and independent pathways. *J Immunol* 2008;181(4):2925–2932. [PubMed: 18684984]
53. Earle CD, King EM, Tsay A, et al. High-throughput fluorescent multiplex array for indoor allergen exposure assessment. *J Allergy Clin Immunol* 2007;119(2):428–433. [PubMed: 17196246]
54. DeSantis TZ, Dubosarskiy I, Murray SR, et al. Comprehensive aligned sequence construction for automated design of effective probes (CASCADE-P) using 16S rDNA. *Bioinformatics* 2003;19(12):1461–1468. [PubMed: 12912825]
55. Castleman WL, Sorkness RL, Lemanske RF Jr, et al. Neonatal viral bronchiolitis and pneumonia induce bronchiolar hypoplasia and alveolar dysplasia in rats. *Lab Invest* 1988;59:387–396. [PubMed: 3411939]

Box 1

Use of rodent models for investigating the role of viral factors in virus-induced chronic airway dysfunction

Potential Advantages	Potential Disadvantages
1. The timing of viral inoculations is at the investigator's convenience, permitting reproducible observation of the entire natural history of the respiratory tract virus infection.	1. Human respiratory tract viruses, when inoculated into rodents, often replicate inefficiently in rodent airway cells and require relatively high viral doses to achieve airway infection and inflammation.
2. Respiratory tract viruses can be genetically modified before inoculation to identify and characterize viral virulence factors.	2. Rodents might not express a receptor for the human respiratory tract virus.
3. Mechanisms by which viral virulence factors subvert host defenses can be investigated by inoculating rodents with genetic deficiencies in antiviral response pathways.	3. Viral virulence factors might operate differently in rodents than in humans due to host-related differences in antiviral responses.
4. A variety of viruses can be directly compared in experimental inoculation studies.	
5. Useful models can be established by infecting rodents with rodent respiratory tract viruses, which replicate efficiently in rodent airway cells and require lower viral doses to achieve airway infection and inflammation in rodents than do human respiratory tract viruses.	
6. Models can be useful for preclinical studies of therapeutic interventions and antiviral medications.	
7. Rodents can be inoculated with emerging respiratory tract viruses, which would be unsafe to do in human subjects.	
8. Cells, tissues, and organs are available for analysis of viral titers, viral gene and protein expression, and expression of viral virulence factors at any time after viral inoculation.	

Box 2

Use of rodent models for investigating the role of host factors in virus-induced chronic airway dysfunction

Potential Advantages	Potential Disadvantages
1. Use of inbred rodents for viral inoculation studies reduces host genetic variability.	1. There are differences in airway structure/function and physiology between rodents and humans.
2. Use of knockout and transgenic rodent strains for viral inoculation studies facilitates the investigation of the roles of specific host genes in responses to viral respiratory infections.	2. The antiviral responses of rodents and humans are similar but not identical.
3. Specialized breeding strategies can be used to map and characterize genetic loci associated with host responses to viral respiratory infections.	3. The immune systems of rodents and humans have significant differences.
4. The role of atopy in host responses to viral respiratory infections can be investigated by performing viral inoculations in inbred rodent strains that differ in this regard or in knockout or transgenic strains with genetic modifications that influence atopy.	
5. Cells, tissues, and organs are available for analysis at any time after viral inoculation.	

Box 3

Use of rodent models for investigating the role of environmental factors in virus-induced chronic airway dysfunction

Potential Advantages	Potential Disadvantages
1. Timing, dose, and frequency of environmental exposures that are being tested for their ability to modulate host responses to viral respiratory tract infections can be controlled.	1. In rodent models, it can be difficult to replicate the complexity of the environmental factors encountered by humans.
2. Gene-by-environment interactions that might influence host responses to viral respiratory tract infections can be studied under conditions in which both the environmental and host genetic factors are controlled.	2. The time frame of rodent studies often dictates that the timing of environmental exposures is accelerated and that higher doses of environmental agents are required to generate biological effects.
3. Prenatal and postnatal environmental exposures can be performed in a controlled manner.	3. Rodents and humans might respond differently to environmental exposures.
4. Cells, tissues, and organs are available for analysis at any time during and after environmental exposures and viral inoculations.	

Box 4

Use of rodent models for investigating the role of developmental factors in virus-induced chronic airway dysfunction

Potential Advantages	Potential Disadvantages
1. Shorter lifespan of rodents allows study of chronic postviral airway sequelae within a convenient period of time.	1. Prenatal and postnatal developmental progress over time is scaled differently in rodents compared to humans.
2. The developmental window in which viral inoculations are performed can be chosen.	2. There are differences in prenatal and postnatal lung maturation between rodents and humans.
4. Knockout and transgenic rodent strains can receive viral inoculations at any age to define the effect of specific genes on developmental factors regulating host responses to viral respiratory tract infections.	3. There are differences in the prenatal and postnatal maturation of the immune system between rodents and humans.
3. Cells, tissues, and organs are available for analysis at any age.	