Environmental Health Perspectives
Vol. 35, pp. 107-112, 1980

Experimental Models for Study of Common Respiratory Viruses

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Numerous epidemiological studies have shown that there is excess respiratory disease morbidity in areas of high atmospheric pollution, implying an interactive effect on the clinical illness associated with these common infections. The principal etiologic agents of human respiratory infections are respiratory syncytial virus (RSV), influenza viruses (IV), parainfluenza virus types 1 and 3 (P1, P3), adenoviruses (AD), rhinoviruses (RV) and *Mycoplasma pneumoniae* (Mpn). Understanding the pathogenesis of the excess morbidity related to pollutants would facilitate detection of undesirable human health effects and provide a basis for intervention strategies. Through use of experimental model systems the mechanism of toxic effects could be defined (whether microbiological, immunological, pathological or physiological) to provide direction for appropriate studies in the human host. Small animal models of IV and Mpn infections have been available for many years; recently, experimental models of several more common viral diseases have been developed. A parallel to human RSV infections is provided by the ferret: virus replicates in the lungs of infant animals, but only in the noses of adults. The common cotton rat infected with RSV develops small airways lesions which may mimic the pathophysiologic changes of bronchiolitis. Both guinea pigs and Syrian hamsters are susceptible to human P3 virus, developing peribronchiolar and interstitial lesions. Practical small animal models for human AD and RV infections are not available because of the high host-specificity of these agents. Both the RSV and P3 model infections are nonlethal which enables study for long-term sequelae. Recent reports of pulmonary function abnormalities among children suffering bronchiolitis in infancy underscores the importance of defining toxic influences which could play a role by making the initial infections more severe.

Introduction

Many epidemiologic investigations have shown that there is excess mortality (*1*) and respiratory disease morbidity (*2*) in areas of high atmospheric pollution. These adverse health effects can be categorized in part on the basis of the toxic substance(s) involved; however, the mechanisms through which the pollutants exert their effects are poorly understood. While elimination of cause in a cause-and-effect relationship can be curative, this is not always possible or practical. A more complete understanding of the effect limb can suggest other approaches to modulate the end result of the cause-and-effect equation for benefit of the human host.

It is recognized that infections with common viruses are responsible for the vast majority of acute respiratory diseases. The viral agents involved are similar in nature and distribution in areas of both low and high atmospheric pollution. It follows, therefore, that an interactive effect of toxic exposure and infectious agent may be involved in the excess respiratory disease morbidity which has been described in polluted areas. Research on this problem has been limited by the ethical/moral constraints of human experimentation, and further by lack of clear indications for approaches to this issue. Use of experimental models of the common respiratory virus infections could provide the necessary foundation, by suggesting hypotheses to test in humans.

The purpose of this presentation is to review information concerning the development of practical animal models for important human respiratory viral infections. Following a brief discussion of the relative importance of different respiratory viruses, four examples of experimental model systems will be described. In the context of this discussion consideration will be given to study parameters which could be meaningful for examination of the interactive effects of viruses and toxic substances.

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Important Human Respiratory Viruses

While the list of viruses that can infect the human respiratory tract is extensive, a relatively small number constitutes those that are encountered most frequently (Table 1) (*3*). The relative importance of the different viruses varies according to host age and other epidemiologic factors. In infancy, respiratory syncytial virus (RSV) is of paramount importance and is the principal etiologic agent of epidemic bronchiolitis. Natural immunity to RSV appears to be minimal, and annual reinfections are frequent during the first years of life (*4*). Parainfluenza type 3 virus (P3) is another common cause of bronchiolitis; infections with this agent are seen in both epidemic and endemic patterns (*5*). Among preschool children, parainfluenza type 1 (and type 2, to a lesser extent) virus infections are related to the occurrence of epidemic croup. The influenza viruses assume relatively greater importance as causes of respiratory disease morbidity in older children and adults. Experimental models for investigation of influenza virus infections have been available for many years and will not be considered further in this presentation. Although outside the scope of this discussion, it should be noted that *Mycoplasma pneumoniae* infections are a very common cause of tracheobronchitis and pneumonia in older children and young adults. Experimental models of mycoplasma infection are described elsewhere in this symposium (*6, 7*). Adenovirus respiratory diseases occur at all ages, although the types involved differ: in children, types 1, 2, 5, and 6 are encountered most frequently; in adults, types 3, 4, and 7 are seen particularly as causes of acute respiratory disease (ARD) of military recruits. Rhinoviruses assume importance as agents of significant respiratory disease morbidity primarily in adults.

From the foregoing it is apparent that research related to the interactive effects of pollutants and respiratory viruses should be targeted on those agents which have the greatest human health impact. Toward this end, information will be summarized on experimental models of respiratory syncytial virus, parainfluenza type 3 virus, adenovirus and rhinovirus infections.

Models of Respiratory Syncytial Virus Infections

Respiratory syncytial virus is an enveloped RNA-containing virus sharing some biologic properties of the paramyxovirus group. The agent is difficult to handle in the laboratory because of its lability and the limited range of susceptible cell culture types available for isolation and propagation of the virus. The natural host range includes at least the higher primate and bovine species, in addition to humans. Initial work in search of animal models for RSV had only limited success (*8*). It was found that infection could be established in ferrets, although disease was limited to the nasal passages; hamsters and guinea pigs showed little evidence of disease manifestations after infection, although immune responses were measured. More recently, further studies of ferrets of differing ages and of the cotton rat have yielded promising results.

The Ferret-RSV Model

Coates and Chanock first demonstrated susceptibility of the ferret to RSV in a search for animal

Table 1. Nonbacterial agents of importance in human respiratory diseases.

Agent	Host	Disease syndrome
Respiratory syncytial virus	Infants	Bronchiolitis, pneumonia
	Children, adults	Rhinitis
Parainfluenza viruses		
Type 3	Infants	Bronchiolitis, pneumonia
	Children, adults	Rhinitis
Types 1, 2	Infants, children	Croup
	Adults	Rhinitis
Mycoplasma pneumoniae	Older children, adults	Tracheobronchitis, pneumonia
Influenza viruses	Children, adults	Influenza
	Adults	Pneumonia
Adenoviruses		
Types 1, 2, 5, 6	Infants	Rhinitis, bronchiolitis, pneumonia
Types 3, 4, 7, 14, 21	Adults	ARD, pneumonia
Rhinoviruses	Adults	Rhinitis

models involving several other species (*8*). After intranasal inoculation, ferrets yielded virus from their tracheas for 4 days and nasal turbinates for 1 week. Maximum viral replication occurred within 3-4 days, and the animals demonstrated brisk serologic responses in 3-4 weeks by the neutralization and complement fixation techniques. Histopathologic changes were limited to the nasal passages; after 1 week there was destruction of the ciliated epithelium, formation of multinucleated cells and appearance of intracytoplasmic inclusions. Healing took place in 30 days, but submucosal cellular hyperplasia and irregularities in cartilage were seen as residua. Little use was made of this model subseqently due to the lack of significant pulmonary involvement.

In 1976, Prince and Porter (*9*) re-examined the possible use of ferrets for experimental RSV infections. They employed animals of various ages and reproduced the findings described above in adult animals. Moreover, it was found that virus replicated in the lungs of infant ferrets and that this event was age-dependent, disappearing by age 4 weeks. Viral antigen was demonstrated by immunofluoresence in the surface epithelium of the nose, and in alveolar cells within the lungs. Lack of bronchiolar involvement makes the infant ferret model an imperfect human counterpart; however, the age-dependent organ localization mimics the lower respiratory tract involvement with RSV seen in infants, and the upper respiratory tract disease of older children and adults. The model thus has usefulness for long-term studies and for examination of the determinants of virus host-cell tropism.

The Cotton Rat-RSV Model

In 1971, Dreizen and co-workers (*10*) explored use of the common cotton rat (*Sigmodon hispidus*) as an experimental host for RSV infection. In contrast to the ferret, RSV replicated to high titer throughout the respiratory tract of the cotton rat. The time course of viral replication was similar to that seen in the ferret nasal turbinates, with maximal production around the fourth day following inoculation. Pathologic changes of rhinitis, bronchitis and bronchiolitis were present which predominantly involved the surface epithelium.

Recently Prince and co-workers (*11*) confirmed Dreizen's findings and added new information about the cotton rat model. It was found that age of the animal had no influence on susceptibility of the lung to RSV, unlike the ferret. Ciliated epithelial cells showed morphologic evidence of injury, with changes including cytoplasmic ballooning, loss of cilia, nuclear contraction and cellular desquamation. Epithelial injury was most prominent in the nasal turbinates, but was present also in bronchioles. Viral antigen was present throughout the nasal epithelium as shown by immunofluorescence; additionally antigen was identified in bronchial and bronchiolar epithelium, but not in the trachea.

Although information on human lung pathology in RSV disease is not extensive (*12*), the cotton rat observations appear to supply a reasonable counterpart in terms of the areas involved in the respiratory tract. Autopsy material reveals a component of interstitial pneumonia and extensive peribronchiolar round cell infiltration, which has not been characteristic of the cotton rat lesions. However, the microbiologic similarities and host cell specificity provide a basis for many kinds of meaningful experiments; the increased volume of the bronchiolar epithelium may even compromise flow through small airways, making pathophysiological studies possible. Together, the cotton rat and the infant ferret provide the basic tools required to initiate an understanding of the pathogenesis of RSV disease. Unfortunately, cotton rats are not available commercially at the present time.

Other RSV Models

The initial recovery of RSV was from a chimpanzee with rhinitis; for a time the virus was called the chimpanzee coryza agent. Apparently this animal is naturally infected with RSV and thus could serve as an experimental host. However, usefulness of the model is limited by cost and the difficulties attending studies with large primates. Limited experience also suggests that chimpanzees do not have pulmonary disease after inoculation with RSV. Serologic surveys have shown that Cebus monkeys regularly possess RSV antibodies, suggesting another animal which experiences natural disease with the virus (*13*).

Recently, a bovine RSV has been described, and experimental infection of calves is reported (*14*). Inoculated animals develop fever, serous rhinitis, peribronchiolitis, and pneumonia. While the calf could be valuable for certain kinds of studies, there are practical limitations for extensive laboratory investigations with animals of this size. Bovine antiserum against RSV is available commercially (Burroughs Wellcome, Ltd.) and has been used successfully for rapid immunofluorescence diagnosis of human infections; this suggests close similarity or identity of the human and bovine viruses.

Models of Parainfluenza Type 3 Virus Infections

As the name implies, P3 virus resembles influenza virus in some features; notable is possession of a

hemagglutinin and neuraminidase, but these do not show the antigenic diversity of influenza virus. The virus grows readily in a number of cell culture types commonly used in the diagnostic laboratory. It can be recognized by characteristic cytopathic effects or by adsorption of erythrocytes, and can be identified by hemadsorption inhibition with type-specific antiserum. The natural host range includes man, guinea pigs and monkeys; a similar but antigenically distinct virus is the etiologic agent of shipping fever in cattle and is widespread through many cloven-hoofed species. Practical experimental models to be discussed are the Syrian hamster and the guinea pig.

The Hamster P3 Model

Experimental P3 virus infections using hamsters were described in detail by Buthala and Soret (*15*), who examined virologic, serologic, and pathologic parameters. In initiating these studies they found variable susceptibility to infection of animals from different sources. This variability was unrelated to age, sex, or weight of the animals and was thought to be due to partial immunity. Although P3 virus is not known to cause natural disease in the hamster, it is possible that human to hamster transmission occurs, or that they have endemic infection with a related agent such as Sendai virus (murine parainfluenza type 1). In any event, source of supply is a critical matter requiring careful control and monitoring.

Optimal infection of the hamster with P3 was achieved with intranasal inoculation of cell culture-passaged material. Virus replication reached a peak in the lung at 3 days, clearing spontaneously by 1 week. Pathologic changes were maximal at 5-7 days, resolving by 2 weeks. Necrotizing nasal lesions were observed first, followed by extension of changes into the lower respiratory tract. The airway epithelium became hyperplastic with formation of multinucleated cells. Peribronchial and perivascular leukocytic infiltration was pronounced, but alveolar involvement was seen infrequently. Intralumenal exudates of leukocytes and desquamated epithelial cells were noted. Infected animals developed a brisk serologic response detectable with the complement fixation method which rose between the first and second weeks post-inoculation. The pattern of changes which accompanies P3 infection of the hamster is reproducible with regularity in susceptible animals. There are close similarities between the pathologic findings and information concerning P3 bronchiolitis and pneumonia in humans (*12*). Until animal models of RSV infections are developed further, the P3 hamster model can serve as a good system for studies on the pathogenesis of acute viral bronchiolitis.

The Guinea Pig P3 Model

Guinea pigs are known to be susceptible to P3 infection, and have been used extensively as donors of antiserum for studies on interrelationships of the paramyxoviruses. Details of the experimental disease produced in this animal have not been described; however, unpublished observations (*16*) indicate a virologic and pathologic course very similar to that in the hamster. The guinea pig offers several advantages over the hamster for use as an experimental model. Many technical manipulations are done more readily in the guinea pig because of its size. Commercial reagents are available for immunologic studies. Also, the animals are highly sensitive to many pharmacologic agents, which could make them good models for pulmonary physiologic changes in consequence of infection. As with the hamster, guinea pigs are commonly immune to P3, which probably reflects a combination of their sensitivity to infection and the ubiquitous distribution of the virus in nature. It is possible to produce colonies free of P3 experience, and methods for this purpose have been reported (*17*).

Models of Adenovirus and Rhinovirus Infections

Although adenoviruses cause less respiratory disease morbidity than some other agents which have been discussed, they were among the first respiratory viruses discovered and have been studied extensively. These DNA-containing viruses constitute a large family of specific serotypes whose natural hosts include man, monkeys, cattle, rodents, dogs and chickens. Generally there is species specificity between host and given serotypes such that experimental models are not readily available. Considering the types most frequently encountered in humans (see Table 1), hamsters, dogs, and rabbits may be infected but show no overt signs of illness. Latent infections may become established in hamsters; several serotypes are carcinogenic, resulting in sarcomas at the site of inoculation. Pulmonary lesions can be produced in pathogen-free, colostrum-deprived piglets with serotypes 1, 2, 5, and 6 (*18*). A fatal disease can be produced in newborn hamsters with serotype 5 (*19*).

The rhinoviruses are etiologic agents of many common colds, especially among adults, and thus are important for study in the context of this symposium. These RNA-containing viruses demonstrate a multiplicity of serotypes, which complicates their identification and the performance of serologic testing. Special conditions are required for rhinovirus isolation, including selected cell cultures, and low

incubation temperature and medium pH relative to other groups of respiratory viruses. The host range of these viruses appears rather limited (*3*). Cattle experience natural infection with biologically similar agents; chimpanzees and gibbons can be infected with some of the human serotypes. Experimental infection of a variety of common laboratory animals has been unsuccessful.

Progress in the development of techniques to maintain organ or specialized cell cultures (*7*) provides the potential for use of human cells as hosts for the adenoviruses and rhinoviruses of interest. These model systems provide easily controlled circumstances for detailed study of the host-parasite interaction, but obviously lack the ability to respond like the intact host. Nevertheless, simplified models could prove very useful in dissecting the cellular basis of the interactive effects of toxic substances and infectious agents.

Research Strategies Using Experimental Models of Respiratory Virus Infections

The foregoing discussion has emphasized the development of animal models of the more common human respiratory virus infections. Consideration will now be given to meaningful ways in which these model systems might be used to study the interactive effects between the viruses and pollutants of interest. This symposium has addressed a wide variety of host defense mechanisms and pharmacologic reactions, all of which could be evaluated in the experimental infections described.

Information accumulating on the pathogenicity of different infectious agents suggests many mechanisms by which host defenses are bypassed. Depending upon the target cell for a given agent within the respiratory tract, the microbial offense can relate primarily to evasion of mucociliary clearance, avoidance of phagocytosis, exertion of immunosuppressive effects, and the like. It is probable that effects of toxic substances have a similar specificity; accordingly, the most appropriate study parameters must be chosen considering both infectious agent and pollutant. Concerning respiratory viruses, the microbiologic, immunologic, pathologic, and physiologic dimensions of the experimental models could be explored to determine the superimposed effects of toxic exposures.

The virologic aspects of the experimental models have been defined in terms of the time course of infection, amount of viral replication, and principal host cell types involved. Adverse effects of toxins might be reflected in longer viral persistance, higher titers and more extensive involvement of host cells. The role of toxins in these effects could include alterations in host cell receptor sites for the viruses, and in the immune and phagocytic mechanisms required for control and elimination of infection.

Studies on the immunologic aspects of the experimental infections are facilitated by the information developed in recent years concerning the respiratory tract. The presence of virus-specific secretory IgA in the upper respiratory tract correlates well with the presence of protective immunity. Antibodies of other classes can be produced locally by the bronchus-associated lymphoid tissue, and probably function in neutralization of free virus, prevention of host cell attachment or opsonization for phagocytosis of infected cell debris. Since intracellular virus is protected from the effects of antibody, another important mechanism concerns cellular immunity provided by thymus-derived lymphocytes. Immune cells which are specific for both virus and host cell type are capable of lysing virus-infected cells, thereby contributing to control of the infection. The immune mechanisms described are operative in or on the respiratory mucosal surface, and thus may be particularly vulnerable to the effects of atmospheric pollutants.

The pathology of experimental infections could be altered in a variety of ways by the super-imposed effects of toxic exposure. Attention could be focused on the degree, extent, and persistence of histopathologic changes in the lung. The models described in this presentation are self-limited diseases, so that the occurrence of mortality would provide meaningful information. Sublethal effects in regard to the healing and repair processes also should receive attention, since the interaction of infection and toxin might produce long-term sequelae.

Lastly, physiologic studies are important to accomplish in the experimental model systems. Correlates are needed for the pathophysiologic changes in humans which have been an important indicator of the health effects of pollutants. Methods are available to perform respiratory physiologic studies in small laboratory animals, but there has been limited application of these procedures to models of infectious diseases thus far. Some of the anatomic considerations that have been described suggest that pathophysiologic changes could be a very sensitive measure of the modulation of disease by toxic exposure.

In summary, several practical laboratory animal models for common human respiratory virus infections have been described. These models lend themselves to studies on the interactive effects of pollution and infection, thus addressing an important human health problem. Although findings from such studies cannot be extrapolated directly to the human

situation, they can serve as indicators of health effects which should be sought and of appropriate methods of study that could be employed.

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