Models of Respiratory Infections: Virus-Induced Asthma Exacerbations and Beyond

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Respiratory infections are one of the main health problems worldwide. They are a challenging field of study due to an intricate relationship between the pathogenicity of microbes and the host’s defenses. To better understand mechanisms of respiratory infections, different models have been developed. A model is the reproduction of a disease in a system that mimics human pathophysiology. For this reason, the best models should closely resemble real-life conditions. Thus, the human model is the best. However, human models of respiratory infections have some disadvantages that limit their role. Therefore, other models, including animal, in vitro, and mathematical ones, have been developed. We will discuss advantages and limitations of available models and focus on models of viral infections as triggers of asthma exacerbations, viral infections being one of the most frequent causes of exacerbating disease. Future studies should focus on the interrelation of various models.

Key Words: Respiratory tract infections; theoretical models; animal models; in vitro diagnostic devices; mathematical computing

INTRODUCTION

Respiratory infections are important clinical conditions because of their diffusion and also because of their potential severe expression and clinical consequences. For these reasons, in the past decade, many studies have deeply investigated the mechanism of respiratory infections to gain information for more effective treatment/prevention of these clinical events. Many different models have been developed. A model is a system that mimics the complexity of relationship between microbes and the host’s defenses. This can be reproduced in vivo in humans and animals, and also in vitro. Moreover, some mathematical models have been created, with the aim of describing, in formulas, the principles of respiratory infections and their diffusion. Each model has advantages and limitations, which are described in this review. Among various models here considered, we will detail the models of viral infections causing acute clinical worsening of obstructive lung diseases, and in particular asthma and obstructive lung diseases, and in particular asthma and chronic obstructive pulmonary disease (COPD) exacerbations.

We will start describing the experimental setting that is considered the optimal modeling condition, i.e. human models, and then consider conditions more imperfect progressively less closely reproducing the complexity of human wild infections. The integration/association of different modeling will be finally considered.

Human models

Since a model endeavored to reproduce human pathophysiology, human models are most accurate. Clearly, the main advantages of human models include the possibility to evaluate the role of genetics, the exact reproduction of anatomical and physiological characteristics of the respiratory tract, and the possibility of direct analysis of inflammatory and immune responses, including pulmonary host defenses. Currently available human models are limited, for obvious safety and ethical reasons, to few viral infections of the respiratory tract.

DeVincenzo et al.1 described a human model of respiratory syncytial virus (RSV) infection. The rationale of their work is based on the results of in vitro and animal models, which portray RSV disease severity as linked more to immunologic responses than to viral load.1 However, in contrast to these premises, they demonstrated that, in humans, RSV infection symp...
toms depend on viral load and are not related to immunologic responses. The study suffers from some limitations. In particular, the model reproduced a mild infection in adults, while usually RSV causes a severe disease in children. These aspects could be considered intrinsic limits of human models and are linked to ethical considerations, which do not allow the reproduction of severe infections (e.g., bacterial infections) or the inclusion of children. For these reasons, in the young population, pneumonia caused by *Mycoplasma pneumoniae* have been retrospectively studied.

**Human models of virus-induced asthma exacerbations**

There is much evidence of the causal role of respiratory viruses in asthma exacerbations. Respiratory viruses have been detected in >80% of asthma exacerbation cases of children and in 44% in adults, and rhinoviruses (RVs) is most common, accounting for 60%-66% of viral cases. A strong epidemiological association of RVs with asthma exacerbations is sided by evidence supporting that RVs spread not only in the upper but also in the lower airways, inducing inflammation. Both infected bronchial and pulmonary epithelial cells produce inflammatory factors that enhance airway inflammation and obstruction. In a human model, RV infection in asthmatics induced the most typical characteristics of exacerbating asthma: lung function impairment, worsening symptoms, increased bronchial reactivity, and eosinophilic airway inflammation. The exacerbation severity was related to immunological changes, in particular to deficient interferon (IFN)-γ and interleukin (IL)-10 and to increased IL-4, IL-5, and IL-13. The viral load related to clinical and functional parameters in asthmatic patients, but not in healthy controls, revealing relationships between viral infections and severity of asthma exacerbations.

Bronchial mucosal biopsies derived from bronchoscopies of experimental RV infections in asthmatics revealed an increase in epithelial eosinophils. Moreover, during the RV colds there are a burden in submucosal lymphocytes and a peripheral blood lymphopenia, both related to the increase in histamine responsiveness. A further model of RV-induced asthma exacerbations demonstrated a more important increase in neutrophils and CD68+ macrophages in bronchial mucosa of asthmatics compared to healthy controls. This inflammation was related to viral load and correlated with a decrease in lung function, underlying a role of RV-induced inflammation in the severity of asthma exacerbations.

**Human models of virus-induced COPD exacerbations**

Similarly, a human model demonstrated a connection between viral infections and COPD exacerbations. Since it is very difficult to examine naturally occurring COPD exacerbations, in order to better understand and study the mechanisms of virus-induced COPD exacerbations, experimental RV infection in COPD patients has been developed. The experimental RV6 infection produced typical COPD exacerbations’ clinical features associated with worse airflow obstruction and respiratory symptoms/inflammation (both local and systemic) in COPD patients compared to controls. In COPD patients, as in asthmatics, exacerbation severity related to immunologic alterations, such as an increase in neutrophils.

Symptoms and inflammatory markers were associated with viral load. Interestingly, viral load was higher, and IFN production was lower in COPD patients compared to healthy controls. Thus, impaired IFN-γ production and neutrophilic inflammation may play an important role in RV-induced COPD exacerbations.

Even if human models are one of the truest representations of infectious respiratory diseases and offer answers to many clinical questions, they have many weak points. In addition to those already described, like ethical limitations, further disadvantages of human models consist of poor adherence to therapy and confounding factors, such as comorbidities, that are common in real life but do not contaminate the “sterile control” conditions of an experimental model. Moreover, human models are often difficult to manage, requiring particular facilities that are available only in a few specialized centers. For these reasons, until now other models have been more extensively used.

**Animal models**

Animal models of respiratory infections have been widely studied. There are many models, and each has some advantages and disadvantages; thus, the choice of model is based on the question that is being examined (Table 1). For example, chimpanzees have anatomic, genetic, and immunological similarities with humans, so their use is essential in the development of vaccines. Unfortunately, chimpanzees are expensive, are difficult to manage, and involve emotional aspects and ethical implications.

Instead, cattle are used for modeling respiratory infections because they are the natural host of *Mycobacterium tuberculosis bovis* and usually develop a disease very similar to the human disease, so they have provided important information for tuberculosis vaccine development. However, their most important disadvantage is also their largest limitation: they are affected by a specific *Mycobacterium (M. bovis)* that is similar to, but not the same as, the main human pathogen (*M. tuberculosis hominis*). Moreover, they require a large amount of space and expensive equipment.

Sheeps have been used to evaluate the role of infections in the development of the respiratory system and in lung function because these aspects are easily measurable in these animals. Unfortunately, limited molecular tools and difficult handling limit studies on such animals.

Rabbits and guinea pigs are easy to handle and particularly useful for studies examining the role of the immune system. Nevertheless, high costs, a lack of suitable reagents, and diffi-
Table 1. Role of animal models*

| Advantages                             | Limits                   | Answer on                  |
|----------------------------------------|--------------------------|----------------------------|
| Immunologic features                   | Expensive                | Anatomic role (chimpanzees) |
| Genetic manipulation                   | Logistically difficult   | Role of immune system      |
|                                        |                          | (chimpanzees, rabbits, guinea pigs) |
| Availability of reagents               | Ethical implications     | Infection effect on lung function (sheeps) |
| Pulmonary-function tests feasibility   | Emotional burden         | Vaccines studies (cattle and M. bovis vaccine) |
| Relevant sample size                   | Immunologic differences  | Genetic role by genetic manipulation (mice) |
| The smallest are easy to handle        | The largest are difficult to handle |
| The largest have anatomic features similar to humans | The smallest have huge difference from humans |

*describes the advantages and disadvantages of animal models, from the most similar to humans, but least used, to the most common, but more dissimilar to human conditions.

Animal models of virus-induced asthma exacerbations

Many small-animal models of RV infection have been attempted and failed, probably because among known RV serotypes, the majority (90%) use human intercellular adhesion molecule-1 (ICAM-1) as their cellular receptor and do not bind mouse ICAM-1. However, a minor RV group (10%) use a different adhesion molecule and can bind the mouse equivalent. Using this minor-group of RV has been possible to create a mouse model of asthma exacerbation caused by RV infection. An important study evaluated 3 mouse models of RV infections in BALB/c mice, in transgenic BALB/c mice with a mouse-human ICAM-1 chimera, and in a mouse model of asthma (BALB/c mice sensitized to ovalbumin [OVA] challenged with OVA or PBS), inducing exacerbation. These animal models induced inflammatory changes, such as increases in mucus and some cytokines (IL-4 and IL-13 in the mouse model of asthma), which reproduce humans’ responses, so they can be potentially useful in the study of both infectious and allergic conditions, as asthma exacerbations triggered by colds. Another animal model, using OVA-sensitized and -challenged mice (which reproduce allergic airway inflammation), showed that RV infection induced type 2 cytokine production from airway macrophages, including eotaxin-1, IL-4, and IL-13. These molecules increased eosinophil inflammation and airway hyperresponsiveness. The IL-4 signaling pathway is fundamental in regulating macrophage activation and the consequent pattern in the mouse model of RV-induced asthma exacerbation. Indeed, without IL-4/IL-13 signaling, RV infection produces type 1 cytokine production, with subsequent increases in neutrophils; instead, in the presence of IL-4/IL-13 signaling, RV infection produces type 2 cytokine production from macrophages, with subsequent eosinophilic inflammation. Interestingly, different viral infections have different consequences in mouse-models of asthma exacerbations. RSV infection in the house dust mite (HDM) mouse model of asthma increases all inflammatory cell types in BAL, but reduces eosinophils, and does not increase cytokines, while influenza infection produces an increase in BAL lymphocytes, neutrophils, IFN-γ, IL-1β, IL-4, IL-5, IL-10, and IL-12. Moreover, infection by RSV reduces steroid sensitivity, while influenza infection is sensitive to this treatment, revealing novel pharmacological challenges for asthma exacerbation therapy. Mouse models have also been able to reproduce corticosteroid-resistant asthma exacerbations, by using the relevant aeroallergen HDM and the viral mimic poly(I:C) (viral mimetic/toll-like receptor 3 [TLR3] agonist). The inflammation enhanced in this model is characterized by neutrophils, without any increase in either Th2 cytokines or eosinophil chemoattractant, and is insensitive to oral prednisone therapy. Thus, this model may provide a useful tool to better understand mechanisms of severe asthma exacerbations and to explore a novel therapeutic target.

Animal models of virus and bacteria-induced COPD exacerbations

Many different approaches have been explored in order to reproduce COPD features in animals: exposure to noxious inhalants (usually cigarette smoke), use of tissue-degrading proteinases (i.e. elastase), and genetic manipulation. The advantage of cigarette smoke-induced models is the use of the most important COPD causative agent. Nevertheless, in mice, even long-lasting exposure to this agent is able to induce only mild respiratory problems, while use of proteinases induces more
severe features reproducing also advanced COPD.20

These animal models are particularly useful to study and describe COPD exacerbations and their mechanisms and causes, including viral and bacterial respiratory infections.21

In a mouse model of COPD, RV infection induced airway inflammation and impaired production of IFNs compared to controls.22 In another cigarette smoke-induced guinea-pig model of COPD, latent adenoviral infection produced an increase in lung volume and a reduction in the surface/volume ratio, typical of emphysema lung destruction.23 In some cigarette smoke-induced mouse models of COPD, mice were also infected with bacteria, such as Haemophilus influenzae or Pseudomonas aeruginosa, with subsequent increased pulmonary inflammation and clinical symptoms.24 Instead, an elastase-treated mouse model of COPD showed a reduction in ICAM-1 in airway epithelium, suggesting that this impairs bacterial clearance.25 Other studies will be necessary to assess similarities and discrepancies among bacterial flora of animal and human airways, which could potentially affect usability of bacteria-induced COPD exacerbation models.26

Animal models of pneumonia

Studies on mouse models are not limited to viral and bacterial common infections, but also to complicated infections like pneumonia. Animals can be manipulated in different ways to investigate various aspects of diseases. For instance, immunological insights into pneumonia have been obtained by photon-irradiation (non-lethal doses) of mice then challenged intracheally with Klebsiella pneumonia.27 These mice developed bronchopneumonia, and the lung inflammatory cellular reaction was inversely related to the irradiation dose, while Gram-negative amount (in colony-forming-unit) and mortality were directly related to the irradiation dose.28 Interestingly, the irradiation mimicked immunodeficiency conditions providing us with an animal model of hospital-acquired pneumonia.29

Marquette et al.27 developed a model of experimental spontaneous pneumonia in mechanically ventilated piglets. Even though these pneumonia infections were due mainly to common airway colonizing microorganisms,27 while the majority of human ventilator-acquired pneumonia (VAP) infections are due to Pseudomonas aeruginosa,28 this model provided vast insights into the pathophysiology of pneumonia. In order to obtain a correct model of VAP, Luna et al.29 intubated piglets, treated them with mechanical ventilation, and induced pneumonia by direct inoculation with Pseudomonas aeruginosa. Even if this model did not reproduce the common pathophysiological sequence of events leading to VAP, this study gave important information about local and systemic responses in VAP, different patterns of mechanical ventilation during lung infections, and responses to antimicrobial therapy. An obvious limitation of this as of any “pure” animal model is that animals do not have comorbidities, which are common in ventilated patients and play a relevant role in the overall clinical condition and evolution/prognosis.

The immune/inflammatory airway responses are very important to the clinical outcome of the infection. Unfortunately, they cannot be exactly reproduced in animal models. Thus, in order to overcome this limitation, humanized models have been developed. Humanized mice are immunodeficient mice engrafted with functional human cells, tissues, and immune systems that are used to study human inflammatory defences.30

Respiratory epithelium has been successfully engrafted and reconstructed in mice.31 Several applications, including the study of host-pathogen interactions, have been described. In particular, a study on humanized mice demonstrated that Staphylococcus aureus adhering to human airway epithelial cells is strongly linked to fibronecrtin.32 Indeed, a FN-binding protein-deficient strain of S. aureus had a 5-fold lower adherence level to airway epithelial cells compared to the parental strain.32 The study also showed that 97% of S. aureus clinical strains, isolated from the airway secretions of Cystic Fibrosis (CF) patients and patients with nosocomial pneumonia, possessed the FnBP.33 The strains from patients affected by nosocomial pneumonia have higher FN-binding capacity than did those from CF patients. These results suggested a major role of FnBP in the development of pneumonia and a role also in colonization of airways by S. aureus in patients affected by CF.32

Even though humanized mice are very interesting and useful animal models that open new research possibilities for the future, they do have disadvantages. Some limitations include a lack of HLA molecules required for appropriate T-cell selection, a lack of appropriate HLA APCs, and species-specificity of growth factors and other molecules,31 low levels of humoral immune responses34 and residual innate immunity of the host.30 Thus, further studies are needed before these models can be extensively used.

In vitro models

In vitro studies are important research tools in the field of respiratory infections. They have many advantages, including being quite inexpensive, easily controlled, well standardized, and not being influenced by comorbidities (Table 2). Moreover, they permit answers to be quickly obtained and reduce the exposure of animals and humans to potentially toxic or ineffective drug therapy.35

In vitro models are the first step to study a respiratory infection mechanism. They consist in a basic environment, with a controlled setting, which simplify the system to be studied. They allow us to evaluate the role of every single element/condition in order to clarify the pathway that leads to respiratory infections. After this first step, the interrelationship between individual elements can be studied in a complex setting as an animal/human model. A more complex setting is represented by the ex-vivo models, which consist of human samples that are
infected in vitro.

In vitro models of RV infection

In vitro studies have been very important in studying which are molecular/inflammatory mechanisms of RV infection on the respiratory tract. They were able to demonstrate that RV increases airway inflammation, inducing the secretion of proinflammatory cytokines like IL-1β and IL-6.\textsuperscript{43,47} Furthermore, models also revealed that, among proinflammatory mediators, RV infections induced TNF-α\textsuperscript{36} which amplifies the viral infection because TNF-α is able to increase vulnerability of bronchial cells to RV.\textsuperscript{3,37} Moreover, in vitro studies also showed that RV increases intracellular oxidant production, enhancing the production of inflammatory interleukins.\textsuperscript{3,38} These virus-induced inflammatory mediators are reduced by β₂-agonists and glucocorticoids, as revealed by another in vitro study which evidenced the synergic effect of the combined therapy.\textsuperscript{3,39} In vitro studies also showed that corticosteroids reduce RV-induced ICAM-1 upregulation in bronchial epithelial cells.\textsuperscript{40}

Together with in vitro studies, ex vivo studies has also been extremely important in revealing immunologic mechanisms involved in respiratory RV infections. A defective innate immune response has been found in asthmatic primary bronchial epithelial cells and alveolar macrophages expressed as deficient interferon induction after an experimental infection. This impairment was related to increased viral replication and more severe clinical expression of the infection (exacerbation) severity in asthmatic subjects.\textsuperscript{41} Similarly, considering acquired immunity, ex vivo data on peripheral blood mononuclear cells (PBMC), exposed to RV, revealed that asthmatics have a defective type 1 and a higher type 2 response to RV infection compared to healthy controls.\textsuperscript{42} Indeed, RV induced the production of inflammatory mediators in both groups of patients, but IFNγ and IL-12 levels were lower, while IL-10 levels were higher in asthmatics than in controls, and IL-4 is induced only in asthmatics.\textsuperscript{42}

Moreover, it has recently been shown that RV infection of bronchial and nasal epithelial cells cocultured with eosinophils results in the impaired innate immune response and the enhanced inflammatory cascade.\textsuperscript{43} These ex vivo data suggest a negative interplay between eosinophilic inflammation (a hallmark of asthmatic airway inflammation) and the outcomes of RV infection.\textsuperscript{43}

An in vitro study on nasal polyp epithelial cells also demonstrated that RV-16 increases mucin production in the upper respiratory tract and that this secretion increases even more if there is a co-stimulation with fungi and eosinophils.\textsuperscript{44} It would be interesting to study if these respiratory infections also have the same effects on the lower respiratory tract, potentially exacerbating the inflammatory process.

Recently, the effect of viral infection on the mechanism of action of glucocorticoids, the mainstream of asthma therapy, has been investigated. Indeed, despite optimal treatment, asthma exacerbations still occur. Cultured primary human bronchial or transformed (A549) respiratory epithelia were infected with RV16 and then exposed to dexamethasone.\textsuperscript{45} This approach revealed that RV infection reduces binding of the glucocorticoid receptor to glucocorticoid response elements in airway epithelial cells, due to impaired GR nuclear translocation, i.e. it impairs the limiting step of the mechanism of action of glucocorticosteroids inducing a sort of steroid resistance.\textsuperscript{45} RV-16 infection alters all outcomes depending from dexamethasone, including inhibition of CXCL8 release induced by IL-1β and expression of genes for mitogen-activated protein kinase phosphatase 1.\textsuperscript{45} RV-16 infection induces nuclear factor κB activation and GRα phosphorylation, so the proinflammatory pathways JNK and NF-κB are both activated by RV and are key elements in RV-induced steroid insensitivity.\textsuperscript{45} However, inhibitors of IKK2 and JNK are able to prevent these consequences restoring dexamethasone effects.\textsuperscript{45}

RV-induced corticosteroid insensitivity may explain why virus-associated asthma exacerbations occur despite optimal anti-inflammatory treatment and require an increase in inhaled or systemic corticosteroid treatment.

New pathways have recently been targeted in vitro, in search of new therapeutic options against respiratory viruses most commonly involved in human respiratory infections, including virus-associated exacerbations of obstructive lung diseases. Kinases are implicated in signal transduction pathways involved in steroid responsiveness. Thus, some of the novel compounds focus on them. These compounds, called narrow spectrum kinase inhibitors, are RV568 and RV1088, and in vitro studies showed that they are able to inhibit both HRV16-induced inflammation and HRV16 replication.\textsuperscript{46,47} Moreover, it is demon-

### Table 2. Role of in vitro models*

| Advantages | Limits | Answers on |
|------------|--------|------------|
| Not influenced by comorbidities | Unable to evaluate host defenses | Gene role |
| Inexpensive | Unable to evaluate inoculum effect | Pathogenetic mechanisms |
| Easily controlled | For pathogens more virulent in vivo | Molecular mechanisms |
| Quick answer | Unable to evaluate drugs distribution | Therapies |
| Well standardized | | |

*lists the roles of in vitro models describing their positive and negative aspects.
strated that RV1088 significantly increases HRV-induced IFN-β and IFN-λ mRNA induction, which are innate immune responses to viral respiratory infection. These data suggest that RV1088 and RV568 could be new therapies for viral exacerbations of COPD.\textsuperscript{46,47}

In vitro models of bacterial infection and interactions with antibiotics

In vitro studies can help clarify relevant clinical questions. For instance, it is known that ventilated patients in the ICU carry a significant risk of developing VAP caused by \textit{Pseudomonas aeruginosa}, but the same \textit{Pseudomonas} rarely causes pneumonia outside the ICU.\textsuperscript{29,46} \textit{In vitro} studies demonstrated that catecholamine/stress hormones can stimulate the growth and infectivity of some bacteria.\textsuperscript{49} Since these drugs are frequently prescribed in the ICU,\textsuperscript{50} these data suggested the hypothesis that inotropes could affect \textit{Pseudomonas} infection.

Catecholamines are potent stimulators of \textit{Pseudomonas} growth (50-fold increases); they increase \textit{Pseudomonas} biofilm formation on endotracheal tubes, enhance \textit{Pseudomonas} toxicity in its interaction with the respiratory epithelium, and facilitate a rapid recovery of \textit{Pseudomonas} from a tobramycin antibiotic challenge.\textsuperscript{51} In contrast, the non-catecholamine inotropes vasopressin and phenylephrine have no stimulatory effect on \textit{Pseudomonas aeruginosa}.\textsuperscript{51} These results suggest inotropes to be a risk factor for ventilator-associated pneumonia induced by \textit{Pseudomonas aeruginosa} in patients at intensive care units, proposing new therapeutic approaches to severe patients.\textsuperscript{51} Thus, even if \textit{in vitro} studies sometimes appear to be distant from clinical needs, they may be able to raise and answer some clinical questions.

Moreover, since animal models closely imitate the characteristics of human respiratory infections but have the important disadvantage of metabolic differences which imply significant discrepancies in pharmacokinetics (PK)\textsuperscript{52,53}. \textit{In vitro} models can help investigate antibiotic activities.\textsuperscript{52,54} Indeed, \textit{in vitro} models are more flexible and adaptable to different conditions from animal models; therefore, they are able to recapitulate \textit{in vivo} drug clearance profiles and the time course of an antimicrobial agent, mimicking human PK.\textsuperscript{35,55}

In addition to the inherent limitation of any \textit{in vitro} condition, i.e. the artificial “unnatural” setting, \textit{in vitro} models have specific shortcomings (Table 2), including the risk of contamination of the culture with external bacteria\textsuperscript{56} and the need for special conditions, such as a temperature-controlled environment.\textsuperscript{55} Indeed, elevated temperature/fever in humans during infection contributes to bactericidal activity, which must be taken into account when designing \textit{in vitro} studies.\textsuperscript{57} Since bacteria grow faster \textit{in vitro} than they do in animal models or in human serum,\textsuperscript{58} and the antimicrobial activity of some drugs is related to the rate of bacterial growth,\textsuperscript{59} growth speed may be a major limitation of \textit{in vitro} models. In addition, \textit{in vitro} models cannot incorporate all variables seen \textit{in vivo},\textsuperscript{60} especially immunological factors, the inoculum effect of respiratory pathogens,\textsuperscript{61} and the virulence and metabolic behavior of a pathogen.\textsuperscript{52} Therefore, derived pharmacodynamic (PD) parameters cannot be directly transferred to the \textit{in vivo} situation. However, although \textit{in vitro} pharmacodynamic models cannot reproduce all \textit{in vivo} conditions, they provide valuable data for the development and assessment of antimicrobial therapies.\textsuperscript{35}

Mathematical models

PK and PD properties of antibiotics have also been analyzed by mathematical models, which define rules that guide drug behavior.\textsuperscript{62} Moreover, in order to gain better insights into the dynamics of viral infections, a mathematical model of the \textit{in vitro} dynamics of viral infection has been developed.\textsuperscript{63} This model describes the disease as battle between the virus and the immune system, a combat between invading viral particles and the ability of the inflammatory system to answer by producing substances conferring resistance to the virus.\textsuperscript{63} Thus, this model includes infection, cell death, interferon production, and the development of resistance.\textsuperscript{63}

Mathematical models are also developing as essential tools to understand the transmission and control of contagions, the progression of epidemics, and the roles of public health interventions (Table 3). The so-called SIR model is one of the simplest epidemiological models that describes the progression of an epidemic. It is based upon calculating the proportion of the population in each of the 3 classes (susceptible, infected, and recovered) and upon determining the rates of transition between them.\textsuperscript{64} Mathematical models are also able to analyze some complex situations; they examine the possibilities of spreading diseases, taking into consideration various determi-

\begin{table}[h]
\centering
\begin{tabular}{|l|l|}
\hline
\textbf{Pharmacokinetic-pharmacodynamic} & PK/PD analyses and PK/PD modeling\textsuperscript{52} \\
\hline
\textbf{Viral infection dynamics} & \begin{tabular}{l}
Infection, cell death, production of interferon and development of resistance\textsuperscript{63}
\end{tabular} \\
\hline
\textbf{Progress of an epidemic} & \begin{tabular}{l}
Calculating proportion and transition between three classes: susceptible, infected, recovered people\textsuperscript{64}
\end{tabular} \\
\hline
\textbf{Guide public health interventions} & \begin{tabular}{l}
Pharmaceutical (drugs, vaccines) and non-pharmaceutical (social distancing)\textsuperscript{66}
\end{tabular} \\
\hline
\end{tabular}
\caption{Role of mathematical models*}
\end{table}

*Illustrates the main applications of mathematical models in the field of respiratory infections starting from PK and PD analyses to guide for public health interventions.

PK, pharmacokinetic; PD, pharmacodynamic.

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Allergy Asthma Immunol Res. 2015 November;7(6):525-533. http://dx.doi.org/10.4168/aair.2015.7.6.525
nents of infectious diseases, such as virulence, spatial distances, and risk factors. Finally, mathematical models could be powerful tools to develop prevention and containment strategies for mitigating the severity of a new influenza pandemic, a top global public health priority. These models also attempt to understand and schematize underlying principles of immunization, in order to develop effective vaccination strategies, addressing major public health interventions. However, estimates of policy effectiveness will change if characteristics of future pandemic strains differ substantially from those seen in past pandemics.

Integration of different models

Finally, every model, even the theoretical mathematical model, has some limitations; thus, the best results are derived from combining all models and integrating their outcomes.

A good example is the integration of studies regarding interferon-inducible transmembrane proteins (IFITM). It has been documented in vivo that IFITM3 restricts the replication of multiple pathogenic viruses, including influenza. Deletion of the IFM locus leads to increased severity of influenza A virus disease, while restoration of this protein decreases viral infectivity. These results were confirmed by animal models. Indeed, mice lacking IFITM3 display fulminant viral pneumonia when challenged with a normally low-pathogenicity influenza virus. The role of IFITM3 in the protection from influenza was also demonstrated in humans. Indeed, severe infections requiring hospitalization occur in patients bearing a IFITM3 allele called "C," which has functional defects and causes reduced influenza restriction. Taken together, these data indicate that the action of a single intrinsic immune effector, IFITM3, profoundly alters the course of influenza virus infection.

Other well-defined examples of different model integration are studies on Th1/Th2 ratio in asthmatics. An important in vitro study, based on primary bronchial epithelial cells and alveolar macrophages, demonstrated impaired interferon production by RV in asthmatics. In the human model of RV infection, this deficit is involved in reduced ability of asthmatics to eliminate viruses, as revealed by relationship between viral load and severity of RV-induced asthma exacerbations. The impaired interferon production characterizes not only asthma, but also other conditions, such as atopy, and it is also present in children as demonstrated ex vivo in bronchial biopsy specimens and epithelial cells obtained from children undergoing bronchoscopy. Similarities in antiviral responses among different conditions characterized by Th2/Th1 imbalance is associated with similar pathologic findings. Indeed, an ex vivo study on bronchial biopsies, obtained from children undergoing bronchoscopy, demonstrated that epithelial damage and basement membrane thickening, which are typical of adult asthma, are also observed in childhood asthma. Moreover, airway eosinophilia and angiogenesis appear even in atopic non-asthmatic children.

The strength of these data are derived from accumulating evidence, in vivo, ex vivo, and in vitro. The integration of information derived from different settings provides additive, complementary, reciprocally potentiating pieces of evidence at different levels of system biology, supporting the robustness of the overall structure of the hypotheses. When in vitro results are confirmed in animal and human models, and vice-versa, evidence supports each other, evaluating different aspects and mechanisms. In vitro studies permit us to evaluate in detail the role of different molecules without confounding factors; animal studies permit us to assess aspects closer to the real condition; human models explore real-life and clinical conditions; mathematical models analyze all information for obtaining general rules to predict the progression of epidemics and to plan public health interventions. Thus, different models are complementary in their attempt to clarify disease pathogenesis, and they could inform new perspectives for intervention (pharmacological and non-pharmacological) strategies.

CONCLUSION

Human disease models are the closest approximations to real-life conditions but difficult to realize; therefore, animal models have been developed. However, animals are similar, but not exact models of humans; therefore, these models may have important differences. Thus, in vitro models have been widely used, and their results have been further elaborated in order to obtain theoretical schemes by mathematical models. Nevertheless, every model, even the abstract mathematical model, has some limitations; thus, the best results can be obtained by examining all models collectively and integrating their outcomes.

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