ANIMAL MODELS OF HUMAN LUNG DISEASE

Animal models of human pneumonia

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Pneumonia is a medical and public health priority, and advances against this disease will require improved knowledge of biological mechanisms. Human pneumonia is modeled with experimental infections of animals, most frequently mice. Mouse models are leading to important discoveries relevant to pneumonia, but their limitations must be carefully considered. Several approaches to establishing pneumonia in mice have been developed, and each has specific strengths and weaknesses. Similarly, procedures for characterizing microbial and host responses to infection have unique advantages and disadvantages. Mice are not small humans, and the applicability of results from murine models to human disease depends on understanding the similarities and differences between species. Additional considerations such as mouse strain, microbe strain, and prior mouse-microbe interactions also influence the design and interpretation of experiments. Results from studies of pneumonia in animals, combined with complementary basic and translational studies, are elucidating mechanisms responsible for susceptibility to and pathophysiology of lung infection.

species have provided important information about innate immune responses to some of the microbes that cause pneumonia. Nonmammalian species such as birds are studied due to veterinary or zoonotic interest but relatively infrequently. The vast majority of pneumonia studies with animals are performed in mammals. Larger mammalian species, such as rabbits, dogs, and monkeys, are studied to facilitate some types of investigation (e.g., involving extensive physiological monitoring and supportive therapies). Another reason investigators rely on these larger mammalian species is their close phylogenetic relation to the human species. However, rodents and particularly mice are used in pneumonia studies far more frequently than any other animals. This review will focus on using mice to study pneumonia, although many points apply more broadly.

Mice are an important research tool for multiple reasons. Their small size and rapid reproductive rate are conducive to many of the practicalities of laboratory research. Inbred strains allow the study of genetically identical cohorts and facilitate genetic approaches to understanding molecular mechanisms of disease. Genetic engineering of mouse embryonic stem cells is now available to most investigators, and a wide variety of mice have already been generated with loss-of-function, gain-of-function, or reporter genes in their genome, many in cell-specific or condition-specific fashions. Because mice have become such a mainstay of biomedical research, new mouse studies benefit from the vast amount of existing literature regarding murine host defenses and immunology for comparison and from having many relevant resources such as recombinant mouse proteins or antibodies against mouse proteins readily available. With mice so widely appreciated by the biomedical community, we will not emphasize here the many

WORLDWIDE, ACUTE LOWER RESPIRATORY tract infections are the leading cause of premature death and are responsible for a greater burden of disease than cancer, heart attacks, or HIV/AIDS (57, 70). Infectious diseases are more problematic in poorer communities, but lower respiratory infections cause greater morbidity and mortality than any other infection across the socio-economic spectrum (57, 70). In the United States, there has been little or no improvement in rates of death due to pneumonia and influenza for the last 5 decades (4). Further medical and public health advances in this area will require improved knowledge of the underlying biology that results in these infections being so common and so severe.

The biology of lung infections is being elucidated in experiments using diverse approaches (91). Combinations of human population studies, experimental infections of animals, and in vitro analyses are essential to progress on this front. In this review, we discuss the use of experimental infections of animals to elucidate the biology of pneumonia in humans. For the purposes of this review, pneumonia will be defined as acute inflammation in the lung parenchyma induced by microbial infection of the air spaces (Fig. 1).

Diverse animal species provide useful information in pneumonia experiments. Animals without lungs, such as Caenorhabditis elegans, Drosophila melanogaster, or zebrafish, are often used in studies of host-pathogen interactions. While modeling pneumonia only indirectly at best, studies with such
design include the inability to control inoculum, time of infection, or the number of infected animals.

Exposure to aerosolized microorganisms. Many lower respiratory tract infections are acquired by the inhalation of infectious aerosols, including tuberculosis, legionellosis, Mycoplasma and Chlamydia infections, fungal pneumonias, and respiratory viral infections. Aerosol dissemination also is considered the most likely method of delivering a biological weapon, such as anthrax, smallpox, tularemia, or plague (8). The airborne mode of transmission can be mimicked by exposing mice to aerosolized microorganisms in whole-body or nose-only chambers (6, 11, 19, 46, 48, 55, 99, 115, 116, 122, 123). In whole-body exposure systems, animals are placed in fenestrated or mesh compartments within an enclosed chamber. A respirable aerosol, generated by one or more nebulizers designed to produce particles predominantly 1–4 μm in size, is drawn through the chamber by negative pressure. Total airflow is regulated to meet the ventilatory needs of the animals, and humidity can be controlled to optimize microbial viability. The duration of exposure is typically 30–60 min. The targeted inoculum is achieved by adjusting the concentration of microorganisms in the nebulizer. With nose-only systems, mice are positioned in restraining tubes such that only their snouts are exposed to the aerosol chamber. Exposure times are often shorter (5–15 min) with nose-only systems to minimize the restraint time. Whole-body exposure chambers are simpler to operate and less costly than nose-only systems and do not require restraint of the animals. On the other hand, nose-only systems do not coat the entire animal with the infectious agent and thus facilitate biocontainment and reduce the chance of secondary inoculation of experimental animals. Nose-only systems also feature smaller exposure chambers than whole-body systems and thus require smaller volumes of nebulized material to achieve targeted inocula than whole-body systems.

Aerosol exposure systems permit the simultaneous infection of large numbers of unanesthetized animals. The actual inoculum of organisms deposited in the lungs after aerosol exposure can be predicted from the concentration of microorganisms in the nebulizer, estimated by sampling the aerosol, or confirmed by quantitative culture of lung tissue from sentinel animals immediately after exposure (11, 55, 99). Microbial deposition in the lungs of simultaneously exposed animals can vary from as little as 10% to severalfold depending on the infectious agent and the aerosol system. The inhalation of aerosolized microorganisms results in symmetrical deposition throughout both lungs (10, 122). This permits efficient use of tissues from infected animals: as both lungs represent the same process, one lung can be used for one set of measurements and the other lung can be used for distinct purposes. Disadvantages of aerosol models of infection include the costly equipment that must be regularly maintained and tested and the potential exposure of laboratory personnel to airborne biohazards. Deposition of aerosolized organisms in the upper respiratory tract, on the eyes and pelt, and clearance to the alimentary tract may be a confounding factor in some infections (10, 11). Furthermore, some respiratory pathogens (such as Streptococcus pneumoniae and some respiratory viruses) are particularly sensitive to desiccation and/or oxygen toxicity and survive poorly when aerosolized. Finally, there is an upper limit of the inoculum that can be achieved by inhalation; higher dose infections can be achieved by bolus methods.

**Fig. 1.** Pneumonia in the human lung showing acute inflammation in the distal air spaces. Images were collected from autopsy slides of a 50-yr-old man with terminal bacterial pneumonia and widely disseminated adenocarcinoma. A is from an area of early pneumonia showing fluid, fibrin, and neutrophils in the air space and congestion of interstitial vessels. B is from an area of more well-established pneumonia showing dense neutrophil infiltration of the air space and vascular congestion. Images and pathological interpretations were courtesy of Dr. John J. Godleski (Brigham and Women’s Hospital, Boston, MA). Original magnification was ×200.
Intranasal inoculation is a popular method for introducing pathogens into the lungs. This technique allows for the precise dosing of the inoculum to the lower respiratory tract, which is beneficial for studies requiring uniform exposure. However, intranasal inoculation has several limitations, including the requirement for general anesthesia, which can be associated with some contamination of the lower respiratory tract (22, 34, 53). Additionally, nasal instillation is the most variable method of microbial deposition, often exceeding 10-fold (19, 22, 53). Sources of variability include expulsion of inoculation volume from the nose, diversion of the inoculum into the alimentary tract or paranasal sinuses, and variable depth and frequency of respirations during the required anesthesia. Aspirated material after intranasal inoculation is deposited in boluses of variable size, in contrast with the more uniform deposition achieved with aerosol inhalation. Intranasal instillation also may result in coaspiration of upper respiratory flora and microbial products that may elicit a confounding host response. Nasal instillation also may be a more potent stimulus to the mucosal immune system than aerosol exposure (48, 94).

**Direct endotracheal or endobronchial instillation.** Direct inoculation of the lower respiratory tract can be achieved via transtracheal injection or peroral intubation (6, 10, 12, 22, 34, 53, 56, 78, 83). Both methods require general anesthesia. For transtracheal injection, the trachea is surgically exposed and then penetrated caudally with a 25- to 30-gauge needle. The microbial suspension is injected in a volume of 5–50 μl followed by air, after which the incision is sutured or stapled closed. For the peroral route, mice are suspended by their incisors in a semivertical position, and the trachea is accessed via the mouth using a blunt-tipped needle. Intubation can be guided by palpation or transillumination of the trachea (10, 83). With either the transtracheal or peroral approach, it is possible to advance a catheter into the left mainstem bronchus to cause unilateral infection. The peroral technique avoids the surgical wound required for transtracheal injection, which may elicit an acute phase response and cause animal discomfort but is more difficult to perform. Both methods have the advantage of permitting more precise dosing to the lungs of individual animals than other techniques. These methods model oropharyngeal aspiration and deliver a bolus of organisms to a localized area of lung parenchyma, resulting in a higher ratio of infecting organisms to phagocytes and other local defenses at the site of infection than is achieved with aerosol exposure (11, 83). Biocontainment is simpler and the necessary equipment less costly than with aerosol exposures. A caveat with endotracheal injection methods is that the inoculum of microorganisms is deposited predominantly in the lower lung zones in a nonuniform manner (10); this can lead to sampling error if some but not all of the infected lung tissue is processed for a given measurement. Drawbacks of direct instillation techniques include the requirement for general anesthesia, with attendant effects on host defenses and risk of death, and the staggered start time of the experiment, because each animal is infected individually. In addition, peroral intubation is associated with some contamination of the lower respiratory tract with oropharyngeal flora (53).

**Intranasal instillation.** Intranasal inoculation is a popular means of infecting mice with respiratory pathogens (32, 50, 54, 130, 138). For this technique, anesthetized mice are held in the vertical position, and the microbial suspension is deposited in the nares, after which a portion of the inoculum is aspirated into the lungs. Inhalation anesthetics such as isoflurane work best for intranasal inoculation, as the procedure is brief and a stable plane of anesthesia is desirable to minimize variations in the rate and depth of respirations. Inoculation volumes vary from 5–50 μl, but smaller volumes tend to remain localized in the upper respiratory tract. The major attraction of this method is its simplicity. This technique also may have particular relevance for modeling respiratory infections that involve both the upper and lower respiratory tract, such as viral, Mycoplasma, and Chlamydia infections. The major limitation of intranasal instillation is the highly variable deposition of microorganisms in the lungs, with inter-animal differences in microbial deposition often exceeding 10-fold (19, 22, 53). Sources of variability include expulsion of inoculation volume from the nose, diversion of the inoculum into the alimentary tract or paranasal sinuses, and variable depth and frequency of respirations during the required anesthesia. Aspirated material after intranasal inoculation is deposited in boluses of variable size, in contrast with the more uniform deposition achieved with aerosol inhalation. Intranasal instillation also may result in coaspiration of upper respiratory flora and microbial products that may elicit a confounding host response. Nasal instillation also may be a more potent stimulus to the mucosal immune system than aerosol exposure (48, 94).

**The next practical considerations: how to get information out.**

Measuring the microbial burden in mouse lungs. The standard approach to measuring the microbial burden in the lungs and other tissues is quantitative culture. Organs are harvested from euthanized animals, homogenized, serially diluted, and dispersed on suitable media for incubation and subsequent growth of colonies.
enumeration of colony-forming units. In the case of viral infection, tissue homogenates are cultured on appropriate target cells for enumeration of plaque-forming units. In preparing specimens for quantitative culture, care must be taken to use diluents that preserve the viability of fastidious organisms. Pathogens with a tendency to clump, such as staphylococci and mycobacteria, may need to be dispersed sonically or with detergents to ensure that colony-forming units represent single organisms. Especially for intracellular infections, the tissue preparation should be certain to result in lysis of host target cells.

Detection of nucleic acids by real-time PCR has emerged as an alternative approach to measurement of microbial burden in host tissues. This method is particularly valuable with microorganisms that cannot be propagated in artificial media or in tissue culture, such as \textit{Pneumocystis}, for which PCR has replaced quantitative microscopy for enumeration of cysts (2, 131). Nucleic acid amplification techniques also can be less cumbersome than tissue culture methods for enumerating viruses and chlamydiae (80, 132) and for quantitation of organisms that grow very slowly on artificial media, such as \textit{Mycoplasma} and \textit{Mycobacteria}. For pathogens that grow in mycelia, real-time PCR is a more sensitive indicator of microbial mass than quantitative culture (9). Nucleic acid amplification is very sensitive but may not differentiate viable from nonviable organisms.

Bioluminescence is another promising modality for measuring microbial burden (45, 100). This method is based on the detection of light produced by microorganisms that have been engineered to express a luciferase gene and its specific molecular substrate. Useful luciferases for this purpose have been cloned from fireflies, jellyfish, sea pansies, corals, beetles, and bacteria. Bioluminescence can be detected through several centimeters of tissue, and thus it is possible to detect light emitted from the lungs and other organs of mice by viable organisms expressing a luciferase reporter gene. This method has been used to detect bacterial, fungal, and viral infections in mice (Refs. 30, 45, 101; Fig. 2). The great advantage of this approach is the opportunity to quantify and localize microbial burden in living animals that can be imaged repeatedly over time, under brief anesthesia, to follow the course of infection or the impact of therapeutic interventions. The limitations of bioluminescence are the need to use a luciferase-expressing pathogen, the costly imaging system required, and the limited sensitivity and resolution of the current technology. It is expected that advances in this field will rapidly expand the potential

![Bioluminescence imaging of microbial burden in mouse lungs. Luciferase-expressing \textit{Streptococcus pneumoniae} were delivered to the lungs of mice, and light emission was captured using a bioluminescent imaging system. Some mice also received antibiotics, as indicated. Colors indicate a heat map of infection, with red representing greatest microbial numbers. Reprinted from Francis et al. (30) and used with permission.](http://ajplung.physiology.org/)

Fig. 2
applications of bioluminescence to the study of pulmonary infections.

Measuring the inflammatory response to microbes in the lungs. The acute inflammatory response includes neutrophil recruitment and pulmonary edema in humans (Fig. 1) as well as mice (Fig. 3). Both of these processes are essential determinants of outcome during pneumonia (71). Both can be measured in mice to elucidate mechanisms contributing to host defense and pathophysiology.

Measuring neutrophils in infected lungs. In an uninfected lung, neutrophils are in the blood (with increased numbers margined within the pulmonary capillaries compared with other blood vessels) but not the air spaces (24). However, within hours of infection, neutrophils begin to appear in the interstitial compartments and air spaces of the lungs (14, 72).

Perhaps the most widely used method for measuring neutrophil recruitment in the lungs is the enumeration of neutrophils recovered by bronchoalveolar lavage (BAL). This is usually accomplished by cannulating the trachea and injecting and withdrawing a buffered salt solution using a syringe. Some of the cellular contents of the air spaces are recovered by such lavage; the amount and type of materials recovered depend on the numbers of washes, volumes per wash, and characteristics of the solution used for washing (e.g., whether or not divalent cations are present or chelated). The numbers of cells and the percentage that are neutrophils can be determined. All neutrophils so recovered are presumed to have been in the air spaces and to be a reflection of the total quantity of emigrated neutrophils in the lung.

There are distinct strengths to BAL analyses of neutrophils. BAL is a very sensitive and easily quantifiable measure of airway inflammation. Cells including neutrophils recovered by BAL can be subsequently subjected to additional assays or analyses (e.g., flow cytometry). The BAL fluid recovered along with the cells contains other materials such as cytokines or microbes that can be measured to provide additional information about the state of the lungs being studied (albeit in a highly diluted state). However, limitations to BAL analyses should also be considered. The degree of consolidation in pneumonic lungs may influence lavage, with more consolidated regions being less efficiently sampled than healthier regions; if consolidation differs systematically among experimental groups, then sampling efficiency may also differ systematically. Differences in the “lavageability” of cells, due, for example, to cell-cell adhesion, can influence which cells are recovered by lavage; if factors that influence lavageability differ across experimental groups, then sampling efficiency may again differ systematically. By design, only air space neutrophils are sampled, so emigrated but subepithelial neutrophils will not be detected. Finally, no information about where in the tracheobronchial tree or alveoli the neutrophils were before lavage is available. However, due to the strengths detailed above, BAL is often a useful means for quantifying emigrated neutrophils in mouse lungs.

Perhaps the definitive method for quantifying neutrophils in the lungs is morphometric analysis. Neutrophils are morphologically distinct even in standard hematoxylin and eosin-stained sections from paraffin-embedded tissues, so quantifying neutrophils in histological sections is straightforward using stereological techniques. We usually rely on a microscope with a reflecting arm, but other specialized optical attachments or digital imaging with integrated software can be applied. Neutrophils are recognized as emigrated when observed in the air spaces or in the perivascular and peribronchial interstitium and lymphatics. Proper tissue preparation is critical (133); lungs fixed at a consistent transpulmonary pressure with buffered glutaraldehyde are comparably inflated across animals and stable for further processing. If potentially heterogeneous deposition of microbes is of concern, then an inert tracer such as colloidal carbon can be mixed with the suspension of microbes to identify regions of the lung that received the dose. As with all morphometric analyses, an appropriate sampling strategy is critical to collecting interpretable results (133), and the investigator quantifying slides must be blinded to the identities of the mice.

There are advantages to using a morphometric approach to quantify emigrated neutrophils in pneumonic mouse lungs. Neutrophils in all localizations of the lung can be discriminated, rather than only air space neutrophils, and neutrophils will be reliably registered regardless of lung consolidation, cell-cell adhesion, or other tissue and cell properties. Neutro-
phil emigration can be localized within the anatomical context, so variability across different lung lobes, distinct parts of the tracheobronchial tree, and other anatomic compartments can be discriminated. The sections prepared for morphometry are amenable to additional histological and morphometric analyses revealing other aspects of pulmonary pathology. There are also disadvantages. The morphometric approach is slow compared with other techniques for quantifying neutrophils, with results coming days to weeks instead of minutes to hours after the completion of the experiment. It is also labor intensive, with results requiring hours to days of focused attention rather than minutes to hours. However, if neutrophil recruitment is an outcome of particular interest, then morphometry provides insights not attainable with any other approach.

Other methods are also sometimes used to quantify neutrophils in the lungs. Neutrophils can be quantified in single cell suspensions collected after enzymatic digestion of the lungs. This does not measure emigrated neutrophils but rather all neutrophils in the lung sample. Lungs can be perfused before these analyses, but it is difficult to remove all intravascular neutrophils, especially during inflammation when many are firmly adherent to endothelial cells. Markers for neutrophil presence, such as myeloperoxidase activity, can be measured in lung homogenates. As above, this does not measure emigrated neutrophils but rather all neutrophils in the lung sample. Furthermore, the degree to which myeloperoxidase per neutrophil varies during granulopoiesis and inflammation can complicate interpretation.

Measuring pulmonary edema. There are many methods to measure pulmonary edema (86), but, in short, none are fully satisfactory. A poorly met need for investigators studying pneumonia in mice is a reliable, accurate, and precise measurement of edema in the lungs.

The liquid content of the lung can be measured, most often expressed as the ratio of the lung wet weight to dry weight (measured before and after desiccation). This technique reflects total lung liquid accumulation, but it is rather insensitive and has disappointing signal-to-noise ratios. Large differences between groups of mice with severe edema are discernible, but smaller differences, moderate edema, and regional heterogeneity in edema usually are not. Edema accumulation in the alveolar air spaces can be appreciated in lung sections using light microscopy, which can be quantified by morphometry. An advantage of this is that the sites of edema accumulation can potentially be discriminated, but the focal heterogeneity of edema accumulation can present a sampling challenge. Air space filling with protein-rich plasma exudate can also be measured by quantifying concentrations of total protein or plasma proteins such as albumin or IgM in BAL fluids. These methods are, again, rather insensitive since much pulmonary edema can accumulate without pronounced air space flooding.

More sensitive approaches involve tracers injected intravenously and then measured in the lungs. Different types of tracers can be measured using diverse techniques (e.g., radioactivity for radiolabeled albumin, ELISA for human albumin, or spectrophotometric absorbance for Evans blue dye). For all such tracer studies, intravascular tracer content must be differentiated from extravascular tracer content. One method is to measure tracer appearance in the BAL fluid (64). Another method is to perfuse the lungs with tracer-free solutions and measure what remains (139). Yet another method is to quantify the blood volume in the lung and subtract the intravascular tracer content from the total tracer content in the lungs (75, 76). None of these methods are direct measures of edema content but rather measures of the net accumulation of the tracer over a given time period. The timing of tracer injection relative to the time of infection and to the time of tissue collection is a critical determinant of the results; different timings will be necessary to elucidate different types of changes in biology contributing to net edema accumulation. The properties of the tracer are another critical determinant; tracers may interact with tissue proteins, transport proteins, or other factors in the blood or lungs that may cause them to accumulate or disappear from the lungs differently from the liquid surrounding them.

Pulmonary edema accumulation results from changes in many biological variables that exhibit distinct but interacting dynamics. Diverse techniques are available to measure determinants contributing to edema rather than edema accumulation per se. For example, microbes in the air spaces of mouse lungs alter both microvascular permeability and alveolar fluid clearance, each of which can be measured (18, 118, 136).

Measuring sequelae of inflammation. Physiological sequelae of pneumonia can be measured in mice. Spontaneous ventilation, measured in mice using plethysmography (e.g., using the Buxco system), appears to be disparately affected by lung infection in mice and in humans. Whereas human patients with pneumonia typically exhibit rapid shallow breathing, mice instead develop a breathing pattern with a decreased respiratory rate and little or no change in tidal volume, resulting in decreased minute ventilation (20, 75). Changes in lung mechanics in pneumonia mice can be measured using a ventilator (e.g., with the flexiVent system) and are generally consistent with those observed in human patients. For example, inflammatory injury during pneumonia decreases lung compliance in mice (75) as in humans. Arterial blood gases can be measured in mice similar to as in humans, with similar findings; severe pneumonia results in arterial hypoxemia and increased alveolar-arterial oxygen gradients (76, 118).

Noninvasive approaches. Noninvasive measurements are useful for characterizing the dynamic processes of infection and inflammation. Microbial burdens can be measured noninvasively using bioluminescence, as described above (Fig. 2). Bioluminescence can also be used as a noninvasive measurement of gene induction during pneumonia, using mice with luciferase-based promoter-reporter transgenes (101). Limitations described above for bioluminescent detection of microbes apply as well as the degree to which artificial promoters inserted into the chromatin in a nontargeted fashion accurately and specifically report transcription factor activity or endogenous gene expression. PET can be used to noninvasively image relative levels of glucose metabolism in tissue, which correlates with neutrophil recruitment during pneumonia (106). Limitations to PET imaging include the requirements for radioactive probes, the degree to which these probes are specific to biological processes of interest (e.g., glucose metabolism as an indicator of neutrophil recruitment), and millimeter-scale resolution. Pulmonary edema may be measured noninvasively, as with X-ray imaging of the human chest (on which the diagnosis of pneumonia is based). The small size of mice limits the utility of traditional X-ray analyses, but alternative imaging methods are being developed and refined (16, 25, 43, 95). MRI has been used to noninvasively characterize fluid accumulation.
in pneumonic mouse lungs (124), which offers micrometer-scale resolution but relatively low sensitivity. Contrast agents may improve MRI sensitivity (28) in future studies of infected lungs. Ventilation can be measured noninvasively, as described above. Perhaps the most common noninvasive measure used in such studies is body weight, which decreases during pneumonia compared with control mice (39, 119), likely due to changes in behavior and metabolism. Although ventilation and body weight are easily measured in mice, they are very indirect indicators of infection, inflammation, and their physiological sequelae.

THE BIG CONCERN: A MOUSE IS NOT A HUMAN

Mice and humans differ in many important ways relevant to lung infection (Table 2). Differences in the structural anatomy of the murine respiratory tract compared with humans may influence the deposition and clearance of inhaled or aspirated microorganisms. The surface area of the nasal passages is proportionately much larger in mice than in humans and functionally more devoted to olfaction (94). The close apposition of the epiglottis to the soft palate prevents oral inhalation and renders mice obligate nose breathers (94). The tracheobronchial tree has fewer and less symmetrical branches in mice than in humans, and mouse airways terminate abruptly into alveolar ducts without intervening respiratory bronchioles (7, 128). The cellular composition of the murine tracheobronchial epithelium also differs from that of humans. The mouse airway has few mucous and serous cells, and the submucosal glands, which are common in human bronchi (81), are absent below the rostral trachea in mice (47, 85, 87). The absence of nerve endings in murine respiratory epithelium may account for the inability of mice to cough in response to mechanical stimulation of the airway mucosa (85).

Species differences in the structure and function of airway epithelium are reflected in the secretory defenses of the lower respiratory tract. For example, the spatial distribution of lysozyme production, which helps maintain the sterility of the lower respiratory tract, differs in humans and in mice (114). In humans, lysozyme is secreted mainly by the serous cells of the bronchial submucosal glands. In mice, submucosal glands are absent from the conducting airways, and lysozyme is secreted predominantly by type II pneumocytes (114). The production of hypoiodotyrate, another airway epithelial microbicide, appears to be lacking in mice. Unlike human respiratory epithelium, mouse tracheal epithelial cells do not express dual oxidases that generate hydrogen peroxide, a substrate for lactoperoxidase-catalyzed production of hypoiodotyrate (79).

In contrast, both human and murine airway epithelial cells express constitutive and inducible β-defensins with broad antimicrobial activity (107).

Local phagocytic defenses in the mouse lung also differ from those of humans in potentially important ways. Alveolar macrophages exhibit species differences in their capacity to ingest and kill bacteria and fungi and in their susceptibility to intracellular parasitism (36, 109). The roles of inducible nitric oxide synthase (iNOS) and the production of reactive nitrogen intermediates in the antimicrobial defenses of alveolar macrophages also appear to differ between mice and men. Whereas nitric oxide contributes prominently to the antibacterial functions of activated murine alveolar macrophages (126), iNOS is regulated differently in human cells, and its antimicrobial role in human alveolar macrophages remains an area of controversy (29, 42, 104).

Species differences also exist in the pattern recognition receptors that serve as the early warning system for activating pulmonary host defenses against infection. Toll-like receptors (TLRs), which recognize diverse pathogen-associated molecular patterns and are essential for triggering innate and adaptive immune responses, are highly conserved among vertebrate species, but the tissue expression and transcriptional regulation of TLRs differ somewhat in mice and humans (93). For example, TLR3 expression by human leukocytes is restricted to myeloid dendritic cells, but, in mice, TLR3 is strongly expressed by macrophages as well (93). Similarly, TLR9 is primarily expressed by plasmacytoid dendritic cells and B cells in human blood but is more broadly expressed by murine myeloid cells (93). TLR5 also may be differentially expressed by human and murine macrophages. Whereas human monocyte-derived macrophages and dendritic cells express TLR5 and respond to flagellin, murine bone marrow-derived macrophages and dendritic cells do not (67, 129). On the other hand, mouse alveolar macrophages express TLR5 and respond to flagellin (59, 84), but human alveolar macrophages have been reported to lack TLR5 (62). In addition to differences in tissue expression, the ligand specificities and affinities of TLRs and other pattern recognition receptors also differ in humans and mice. Human TLR2, but not mouse TLR2, distinguishes between tripalmitoylated and hexacetylated lipid A structures from *Pseudomonas aeruginosa*, whereas murine TLR4 does not (35). As a result, human cells respond poorly to the hexacetylated lipopolysaccharide found in *P. aeruginosa* isolated from patients with cystic fibrosis, but these structures are strongly recognized by mouse cells (35). Murine TLR5 is more sensitive to the presence of flagellin than human TLR5 (3). Mouse and human TLR9 also recognize distinct CpG DNA motifs (38). There also are differences between humans and mice in the functions of non-TLR pattern recognition receptors. For example, human nucleotide binding and oligomerization domain protein 1 (Nod1), an intracellular receptor for bacterial peptidoglycan, recognizes a muramyl tripeptide, whereas the murine NOD1 is triggered by a muramyl tetrapeptide (21, 60). The development of humanized mice that express human receptors in place of the

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TLRs, Toll-like receptors; Nod1, human nucleotide binding and oligomerization domain protein 1.
murine homologs is an exciting advance in the use of murine models for understanding the roles of these signaling pathways in human disease (112).

There are other differences in the inflammatory and immune responses in mice and humans that may affect pulmonary host defenses. Circulating neutrophil counts are lower in mice than in humans (36), and mouse neutrophils lack α-defensins (27). There is no murine homolog of IL-8, a CXC chemokine with a major role in neutrophil recruitment to sites of infection, although mice express homologs of other CXC chemokines, and CC chemokines in mice are similar to their human counterparts (117). The expression of matrix proteins that regulate the inflammatory response also differ in humans and mice (110). Natural killer cell activity is generally higher in murine than in human lungs, but there is substantial variability among strains of mice in this regard (36).

OTHER WAYS IN WHICH EXPERIMENTAL DESIGN IMPACTS RESULTS AND INTERPRETATION

Although it may be obvious that a mouse is not a human, it is also evident that a mouse cannot be considered truly representative of mice. Different mouse strains demonstrate remarkably different responses to microbes in their lungs. For example, after identical infections of the respiratory tract with pneumococcus, some strains of mice (e.g., BALB/c) demonstrate no bacteremia and virtually no lethality, whereas other strains (e.g., C57BL/6 and DBA/2) demonstrate a modest bacteremia (hundreds of bacteria per milliliter of blood) with ∼50% lethality, and still other strains (e.g., CBA/Ca, C3H/He, and SJL) develop severe bacteremia (millions of bacteria per milliliter of blood) with 100% lethality (31). Of a similar nature, C57BL/6 mice are more susceptible to Klebsiella infection of the lung than are 129/Sv mice, with identical initial infections resulting in log scale differences in bacteria/lung and all of the latter but virtually none of the former strain of mice developing bacteremia and dying (105). Whereas most mouse strains cannot be productively infected with Legionella pneumophila, AJ mice are particularly susceptible and permit replicative infection (12). Thus results of studies in a given mouse strain may or may not be generalizable beyond that strain, and roles of specific molecular pathways in mediating responses in pneumonic mouse lungs may be discernible in some mouse genetic backgrounds but not others. Of course, this “limitation” is also a great strength in the hands of geneticists. For example, distinct responses of inbred mouse strains led to the discovery that mammalian TLR4 triggered innate immune responses to bacterial lipopolysaccharide (89).

Prior interactions of mice with microbes may influence their immune responses to experimental infections. The pathogen-free barrier facilities in which most mice are raised for experiments limits variability in this regard and ensures that investigators can control whether or not mice are naïve to the pathogen of interest during experimental pneumonia studies. However, this environment is quite different from that experienced by other mice or humans, and it raises the risk of systematically influencing results. For example, to the degree that the “hygiene hypothesis” of immunodevelopment applies (103), the diminished pathogen exposures in barrier facilities may alter immune responses of research mice. Furthermore, the commensal microbes colonizing mucosal surfaces influence mammalian physiology and immunology (44), and commensal flora may differ among mice in experimental studies. For example, littermates of different genotypes may host different commensal populations if genotype influences colonization; mice within one inbred colony may have different commensals from other inbred colonies even if genotypes are identical or irrelevant to colonization; and mice in barrier facilities almost certainly have different commensals than do mice or humans that do not live within barrier facilities. The impact of such prior interactions with microbes on pneumonia studies in mice is poorly understood, but matching the prior interactions of control and experimental groups with microbes as best as possible seems advisable.

A main reason that mice have become such a common animal in experimental studies is that they are so readily accessible with specifically targeted genetic mutations. As with any studies using such mice, it must be remembered that the alteration of a single gene product can have complicated results. The prolonged deficiency of a gene product can change the utilization of alternative pathways, making roles for that molecule difficult to discern with such mice. For example, whereas acute inhibition of ICAM-1 by blocking antibodies or antisense oligonucleotides decreases neutrophil recruitment elicited by Gram-negative bacterial stimuli in mouse lungs, the genetic deficiency of ICAM-1 does not (51, 92). In addition, the prolonged deficiency of a gene product can lead to physiological changes that complicate experimentation. For example, CD18 deficiency in mice leads to a pronounced peripheral blood neutrophilia that hinders studies of neutrophil emigration except for when it is completely dependent on this molecule (74); discriminating different roles for CD18 in mediating neutrophil recruitment during different types of pneumonia cannot be accomplished simply by infecting CD18-deficient mice but requires a more complex experimental design (73). Finally, the transgenic systems used to modify gene expression in mice, such as transactivators or recombinases, can themselves have unexpected deleterious effects, independent of the genes they are designed to influence (113, 134).

Many of the same concerns described above as relevant to the mouse are also relevant to the microbe, resulting in different microbes within a single genus and species exhibiting differences in virulence during pneumonias in mice. Just as mouse strain is critical, so also is microbe strain. For example, several thousand S. pneumoniae of serotype 2 or 3 in the lungs result in bacteremia and death of mice, whereas S. pneumoniae of serotype 19 is unable to induce bacteremia after lung infection and results in a nonprogressive course unless tens of millions of bacteria are inoculated (13, 49). Just as prior experiences of the mice are a critical variable, so also is prior experience of the microbes. Culturing pathogens in a particular environment is sometimes essential to virulence, and prior animal passage often increases microbial pathogenicity during lung infection (17, 63, 96, 97, 102, 137). Thus the characteristics of that particular microbe are as important to consider as the characteristics of that particular mouse for experimental studies of pneumonia.

Finally, some factors predisposing to pneumonia in human populations are difficult to study in mice due to limitations inherent to this system of experimentation. Patients in intensive care units (ICUs) are particularly susceptible to nosocomial pneumonias, but the ICU environment has not been fully
replicated for mice. The small size of mice coupled with the high cost of intensive care likely prohibit such a development, so only select influences of the ICU on microbial exposures and host responses of ICU patients (e.g., short-term mechanical ventilation; Ref. 23) can be elucidated using experimental studies of mice. Epidemiology studies demonstrate that elevated air pollution increases pneumonia hospitalizations and pneumonia deaths (68, 98, 108, 140) due to small increases in risk spread across large populations (i.e., everyone breathing). Delivering appropriate air pollution challenges to extremely large numbers of mice may be essential for reliably replicating this finding in the laboratory, which may prove unfeasible. Unfortunately, experimental infections of mice may be ineffective for addressing some of the important questions related to the biology of pneumonia.

**Comparisons of pneumonias in humans and pneumonias in mice.** Overall, there are far more similarities than differences when comparing pneumonias in humans and pneumonias in mice. Both fundamentally involve the same process, the accumulation of pus, a protein-rich exudate containing neutrophils, in the lungs (Figs. 1 and 3). Some similarities will be highlighted briefly here as examples of how studies of experimental pneumonia in mice are relevant to pneumonia in human patients.

Many pneumonias are opportunistic infections, and patients with deficiencies in immune functions are predisposed to infection. Mice with comparable immunodeficiencies are also predisposed to lung infection. For example, human patients with neutropenia of diverse etiologies are susceptible to lung infections, and experimentally induced neutropenia in mice predisposes them to lung infection (69, 121, 125, 127). Similarly, CD4+ T cell depletion in mice renders them susceptible to the same types of lung infections as those that develop in human patients with decreased CD4+ T cells due to HIV/AIDS (65, 111). In addition to direct immunodeficiencies, more complex states of indirect immunocompromise show consistent effects on lung susceptibility to infections in humans and in mice as illustrated with the following examples: respiratory viral infections associate with secondary bacterial pneumonias in humans, and mice with experimental respiratory virus infections have increased susceptibility to bacterial pneumonia (15, 66); alcohol abuse predisposes human patients to bacterial pneumonia, and both acute alcohol intoxication and chronic alcohol exposure are sufficient to compromise pulmonary host defenses in mice (37, 82); obesity is a risk factor for community-acquired pneumonia among humans, and mice rendered obese by leptin targeting have defective innate immune responses to microbes in their lungs (5, 61). These are just a few of the many examples indicating that experimental lung infections in mice model pneumonia in human patients.

Although some differences between species were highlighted above, molecular mechanisms mediating responses during lung infection in mice and humans appear shared more often than disparate. A few examples are listed here. Patients with chronic granulomatous disease due to mutations in the genes for NADPH oxidase components are predisposed to lung infections, and mice in which NADPH oxidase is targeted by genetic engineering show similar susceptibilities (88). Histopathology from humans with leukocyte adhesion deficiency type I resulting from a mutation in the gene for CD18 suggests that neutrophils may use CD18-independent pathways for emigration in the infected lung more than in the skin or soft tissues (40), and mice with CD18 targeted by genetic engineering or blocked by neutralizing antibodies demonstrate that microbial stimuli can induce CD18-independent neutrophil recruitment in the lungs whereas emigration in the skin shows stronger requirements for this adhesion molecule (74, 92). Studies with mice delineated the immune responses triggered by TLR4 stimulation with diverse microbes (52, 89), and polymorphisms in the gene for TLR4 associate with lung infections in human patients (41, 120). The NF-κB protein p50 is essential to preventing lung injury during experimental pneumonia in mice (76), and a recent study suggests that polymorphisms in the human gene for p50 predispose patients to lung injury (1). These examples illustrate only a few of many factors that are essential to the outcome of pneumonia and consistently apply to both humans and mice.

**Conclusion.** Pneumonia is an important public health problem, and the biology of pneumonia must be better understood to diminish the burden of this disease. Mouse models are a very useful tool, but (as with any tool) the limitations must be carefully considered in designing and interpreting experiments. Discoveries from mouse models are already providing valuable insights into the biology of pneumonia and have the potential to further direct future medical approaches to patients with pneumonia.

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**REFERENCES**

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