Molecular Imaging of Influenza and Other Emerging Respiratory Viral Infections

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Research on the pathogenesis and therapy of influenza and other emerging respiratory viral infections would be aided by methods that directly visualize pathophysiologic processes in patients and laboratory animals. At present, imaging of diseases, such as swine-origin H1N1 influenza, is largely restricted to chest radiograph and computed tomography (CT), which can detect pulmonary structural changes in severely ill patients but are more limited in characterizing the early stages of illness, differentiating inflammation from infection or tracking immune responses. In contrast, imaging modalities, such as positron emission tomography, single photon emission CT, magnetic resonance imaging, and bioluminescence imaging, which have become useful tools for investigating the pathogenesis of a range of disease processes, could be used to advance in vivo studies of respiratory viral infections in patients and animals. Molecular techniques might also be used to identify novel biomarkers of disease progression and to evaluate new therapies.

The public health importance of novel respiratory viral infections has been underlined during the past decade by the emergence of H5N1 avian influenza in Southeast Asia, the epidemic of severe acute respiratory syndrome (SARS), and the pandemic of swine-origin H1N1 influenza that began in early 2009. As each new disease has been identified, physicians have made use of chest radiography and computed tomography (CT) to characterize pulmonary morphologic changes in patients at the time of presentation and in cases of severe illness and to identify prognostic markers of disease progression [1–5]. However, although these radiographic methods are useful for clinical management, their ability to elucidate underlying pathologic mechanisms is much more limited. Research would, therefore, benefit from techniques that could image physiologic processes in patients or in laboratory animals over the course of illness. In this regard, the use of molecular imaging methods could markedly enhance the ability to study the pathophysiology of influenza and other emerging respiratory viral infections.

Molecular imaging has been defined by the Society of Nuclear Medicine as “the visualization, characterization, and measurement of biological processes at the molecular and cellular levels in humans and other living systems” [6]. It may use multiple imaging modalities, with either 2-dimensional or 3-dimensional images, and can involve quantification of data over time. The key factor is that data are obtained through the interaction of the imaging mechanism with cellular or subcellular processes, such as enzymatic modification or receptor binding. [7, 8]. Single-photon emission CT (SPECT) and positron emission tomography (PET) can be used for molecular imaging by using radiolabeled probes to detect the presence of specific target molecules or biochemical reactions in sites of interest. In addition, some applications of magnetic resonance imaging (MRI) use differences in susceptibilities to magnetic fields to label cells and differentiate surrounding tissues. Bioluminescence imaging (BLI) can also elucidate pathophysiologic processes in murine models of human illness, using visible light produced by luciferase or other recombinant reporter molecules.

In this article, we describe how molecular imaging methods could be used to study respiratory viral infections in patients and laboratory animals. The first section assesses the capabilities and limitations of current radiographic methods, by reviewing typical chest...
radiograph and CT findings in swine-origin H1N1 influenza, H5N1 avian influenza, and SARS. We then describe how SPECT, PET, MRI, and BLI could be used to enhance our understanding of the underlying mechanisms of these disease processes.

CHEST RADIOGRAPH AND CT IMAGING OF RESPIRATORY VIRAL INFECTIONS

Clinical radiography assesses the physical characteristics of body tissues based on the attenuation of high-energy photons (X-rays) as they pass through regions of differing density. Soon after X-rays were discovered in 1895, the human thorax was found to be an excellent substrate for imaging, because the air-filled lungs provided a low-density background against which areas of consolidation and other structural changes could be visualized. The value of chest radiography for assessing the severity of respiratory tract infections was firmly established during the 1918 influenza pandemic, in which the new radiologic capabilities were widely implemented to study patients and to correlate imaging with pathologic and clinical findings [9]. At the same time, it was recognized that the radiographic changes typical of severe influenza were also observed in other conditions, both infectious and noninfectious. The ability of chest radiographs to detect and discriminate disease processes was also restricted by overlapping projections of soft tissues. Although this problem was partly mitigated by the development of CT, which enabled major advances in image resolution, the sensitivity and specificity of radiographic imaging remain limited by the absence of unique features to distinguish infection from inflammation and by the lack of standardized scoring systems for disease severity [9, 10]. The value of chest radiographs and CT for research is also restricted by the difficulty of obtaining direct radiologic-pathologic correlation, because lung tissue samples from patients with influenza are only examined when a case ends in death, followed by autopsy.

The capabilities and limitations of radiography for the study of respiratory viral infections are illustrated by reports from past disease outbreaks and the swine-origin H1N1 influenza pandemic [10–14]. In cases of mild, self-limited illness, in which lesions apparently remain localized to the upper respiratory tract (Figure 1D), the chest radiograph is either normal or displays only minor changes. The development of more severe disease is typically marked by the appearance of multiple patchy densities in one or both lungs, in a multilobar peripheral distribution (Figure 1A). By CT, these areas are termed ground-glass opacities (GGO) because of the hazy density of the lesions, in which the margins of bronchi and blood vessels are partially visible (Figure 1B). Autopsy findings suggest that GGO correspond to foci of viral pneumonitis and/or diffuse alveolar damage (Figure 1E). However, GGO are also seen in a variety of other pulmonary diseases, both infectious and noninfectious, in which they may reflect partial airspace filling, interstitial cellular infiltration or edema, capillary congestion, or a combination of these factors [10, 12, 14]. CT imaging of some patients with swine-origin H1N1 influenza has also shown consolidative patterns, in which the increased lesion density obscures all

Figure 1. Radiographs and histopathologic examination of lung tissues at autopsy of patients fatally infected with pandemic swine-origin H1N1 influenza. A, Chest radiograph on day 5 of illness, showing multiple, bilateral opacities (arrows). B, CT of the same patient, showing multifocal, patchy ground-glass opacities. C, CT of a different patient, showing areas of denser consolidation consistent with bacterial pneumonia. D, Section of trachea showing inflammation of submucosal glands. E, Section of lung showing diffuse alveolar damage with hyaline membrane formation. F, Section of lung showing a massive infiltrate of neutrophils, consistent with bacterial pneumonia. (From [12] and [14], with permission.)
parenchymal structures except for some traversing open airways (air bronchograms), consistent with either severe viral injury or bacterial pneumonia (Figure 1C, 1E) [12, 15].

In contrast to influenza, in which fatal disease generally occurs only in infants, older persons, and persons with underlying medical conditions, severe cases of SARS frequently occurred in previously healthy individuals. However, the spectrum of radiographic changes seen in the epidemic largely resembled that observed in the swine-origin H1N1 influenza pandemic (Figure 2A, B) [16]. In both influenza and SARS, patients with severe illness show patchy, bilateral infiltrates by chest radiograph and multifocal GGO on CT. Because the radiographic findings are not specific, a diagnosis of SARS could only be made if a patient gave a history of contact with a person with a known case. However, radiography still contributed to medical management by identifying patients in need of intensive supportive care and by assessing their response to therapy [1, 17]. In contrast to severely ill patients with influenza, in many of whom influenza progressed to bacterial pneumonia, fatal cases of SARS were typically characterized by the progressive expansion of areas of GGO to involve both lungs [17]. In the only radiographic study in laboratory animals, pulmonary airspace opacities were observed in cynomolgus macaques infected with the SARS coronavirus by the respiratory route (Figure 3), but a radiologic-pathologic correlation was not obtained [18].

Similar to the situation with SARS and severe swine-origin H1N1 influenza, chest radiographs of patients with H5N1 avian influenza show the early development of multiple, bilateral patchy opacities, which in fatal cases, progress rapidly to acute respiratory distress syndrome (ARDS) (Figure 4) [3]. The rapid development of severe, diffuse pulmonary parenchymal dysfunction is thought to reflect the propensity of the H5N1 virus to infect alveolar lining cells, in contrast to the tropism of seasonal influenza A viruses for the tracheobronchial epithelium, coupled with an intense inflammatory response [19].

The above discussion has shown that, although radiographic methods are capable of providing detailed images of the morphologic alterations that accompany severe respiratory viral infections, they provide little or no information on...
to the upper respiratory tract or whether severe pulmonary involvement is the result of a more diffuse initial disease process. Similarly, the mechanisms responsible for the development of pulmonary infiltrates and GGO have not been identified, and it is not known to what extent respiratory dysfunction is the result of viral infection and injury of the respiratory tract epithelium or is induced by the local or systemic release of inflammatory mediators. The factors that predispose some patients to the development of fatal bacterial superinfection are also poorly understood. Imaging methods that are not based on tissue structural changes, but on the detection of specific biochemical processes, may be able to answer such questions.

**MOLECULAR IMAGING**

Historically, a major goal of imaging has been to facilitate medical treatment decisions by differentiating infectious from inflammatory processes (eg, osteomyelitis from aseptic inflammation) [20]. In recent years, much of this effort has focused on the characterization of host immune responses through molecular applications of SPECT, PET, and MRI; many of these approaches could potentially be used to image respiratory viral infections (Table 1). Ideally, researchers would also use molecular imaging to directly visualize viral infections, in the way that SPECT is now being used to detect experimental mycobacterial infections with use of a bacterial thymidine kinase (TK) enzyme as the reporter molecule (Figure 5) [30].

In the case of viral infections, pathogen-specific imaging would rely either on the construction of recombinant viruses encoding reporter molecules, as has been accomplished for BLI, or on the development of radiolabeled probes that are selectively retained at sites of infection (Table 2). If techniques can be devised to specifically image viral infections in large animals or humans, the eventual goal will be to visualize host responses and viral replication simultaneously, to determine the relationship between the spread of the pathogen and processes, such as the release of proinflammatory mediators, the influx of inflammatory cells, apoptosis of infected or bystander cells, and changes in vascular function. In the following sections, we review the basic principles of SPECT, PET, MRI and BLI and discuss how they could be used to enhance our understanding of influenza and other respiratory viral infections.

**Radionuclide Imaging**

SPECT and PET are based on the selective retention of radiotracers molecules at sites of biological interest through a broad range of mechanisms including either as a result of high-affinity binding to a chosen molecular target or through trapping in cells by mechanism pathways such as modification by a specific enzyme. PET imaging probes emit positrons, which travel a short distance in tissues before undergoing annihilation, producing 2 high-energy photons traveling in 180° opposite directions that strike surrounding detectors simultaneously. By using highly energetic photons of a single frequency, PET reduces soft-tissue attenuation, scatter, and noise. However, a limiting factor in PET resolution is the distance that the positron must travel through tissues before undergoing annihilation. In contrast, the various radiotracers used for SPECT imaging release single photons, with a range of energies depending on the decay characteristics of the radionuclide.

Although SPECT has traditionally been considered to be less quantitative and more subject to soft-tissue attenuation than PET, its use for infectious disease research is being enhanced by improved collimation, the use of CT for attenuation correction, and evolving quantitative techniques. Although not all SPECT radiotracers are used for molecular imaging, SPECT offers a wide variety of probes, because it is not limited by

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**Figure 4.** Sequential radiographs of a 22-year-old man with H5N1 avian influenza. The film on hospital admission (A) shows bilateral perihilar patchy opacities with air bronchograms. Two days later (B), there was bilateral multifocal consolidation. On day 4, the patient showed worsening respiratory failure with features suggestive of ARDS. (From [3], with permission.)
a requirement for positron decay. Moreover, as a cross-sectional tech-
nique, SPECT provides significant ad-
vantages over planar nuclear medicine
imaging for locating sites of disease. For
example, whole antibodies or antibody
fragments labeled with 99mTc or 111In can be imaged with SPECT
for a broad range of potential infectious
disease applications.

The most widely used PET radiotracer
is 18F-fluorodeoxyglucose (FDG). Be-
cause deoxyglucose is taken up by cells
and phosphorylated but not further
metabolized, the radiotracer is selectively
trapped in cells with high rates of gly-
colysis. FDG - PET imaging has a flour-
ishing oncologic literature, based on the
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| Host response | Imaging methods | Current status |
|---------------|-----------------|---------------|
| Apoptosis resulting from infection or inflammation | SPECT imaging with 99mTc radiolabeled annexin-V for pulmonary disease [21] | Laboratory animals |
| Triggering of type I interferon response | BLI imaging of transgenic mice in which luciferase expression is driven by an IFN-beta promoter [22] | Laboratory animals |
| Identification of sites of inflammation | Intravenous infusion of 67Ga-citrate, which binds to transferrin and lactoferrin and localizes to sites of increased vascular flow and permeability [23]. Intravenous infusion of 111In-labeled immunoglobulin, followed by planar gamma camera and SPECT imaging [23]. Intravenous infusion of 18F-FDG, followed by PET imaging to detect foci of increased glycolytic activity [24]. In vitro labeling of leukocytes with 111In oxine or leukocyte labeling with 99mTc-exametazime (also known as 99mTc-HMPAO), for planar gamma camera imaging or SPECT [25]. Identification of activated monocytes/macrophages by infusion of superparamagnetic iron oxide particles, followed by MRI [25]. | Clinical practice, Preclinical research, Laboratory animals |
| Alterations in the size and structure of regional lymph nodes | Characterization of lymph node structure by infusion of superparamagnetic iron oxide particles, followed by MRI [26]. | Laboratory animals |
| Activation of vascular endothelium | Detection of increased cell-surface ICAM-1 or E-selectin expression with radiolabeled antibodies [27] [28]. Detection of increased E-selectin expression by MR, using superparamagnetic iron oxide nanoparticles labeled with specific antibody [29]. | Laboratory animals |

NOTE. Column 3 indicates whether the procedures are in clinical use, are being employed in preclinical studies in humans, or are limited to imaging of laboratory animals.

Figure 5. SPECT/CT images of the thorax of a mouse 6 weeks after low-dose aerosol infection with recombinant M. tuberculosis, in which a bacterial TK gene is under the control of the strong, constitutive P_hsp60 promoter. The probe is 1-(2’deoxy-2’-fluoro-β-D-arabinofuranosyl) - 5[125I]-iodouracil (125I-FIAU), which is preferentially phosphorylated by the bacterial TK over the host cell enzyme to produce SPECT signal localizing to pulmonary granulomas (arrows). (From [30].)
and noninfectious, via nonspecific uptake of the tracer by multiple types of white blood cells, including lymphocytes and neutrophils. Therefore, FDG-PET and/or CT can theoretically be a useful tool for measuring inflammatory responses to infection [38]. For example, FDG has been used to visualize the response to the introduction of endotoxin into the lungs of human volunteers (Figure 6) [39, 40]. This suggests that FDG-PET could be particularly useful for imaging the inflammatory response to severe respiratory viral infections, because studies of human cases and experimental models in mice and ferrets indicate that acute inflammatory responses play a critical role in the severe respiratory dysfunction induced by the 1918 H1N1 and the H5N1 avian influenza viruses and by the SARS coronavirus [19, 41–46]. Radionuclide imaging of experimental influenza or SARS coronavirus infections could help to resolve the question of whether anti-inflammatory therapies are beneficial or harmful for these conditions [47–51].

FDG-PET and/or CT were recently used to study a patient with severe influenza, showing that areas of GGO seen by CT were characterized by increased radiotracer uptake (Figure 7) [52]. The same authors have also found that, in patients with acute lung injury and ARDS from causes other than viral infection, the metabolic rate of pulmonary tissues is markedly increased, even when CT shows them to be normally aerated [53]. This work suggests that FDG-PET quantitation of inflammation in infected animals could be experimentally compared with measurements of viral replication, cellular markers, and gene expression to identify relationships among these factors and pulmonary parenchymal injury.

Because intravenously administered FDG is taken up and trapped nonspecifically by a wide range of white blood cells, efforts are under way to label specific populations of white blood cells in vitro. In the case of acute infection, in vitro labeling of neutrophils could be a useful method of tracking host responses [24, 54]. For example, a $^{64}$Cu-labeled peptide that targets the formyl peptide receptor on neutrophils has been

### Table 2. Hypothetical Targets for Molecular Imaging in the Replication Cycle of Influenza A Viruses and the SARS Coronavirus

| Target                                | Influenza A viruses                                                                 | SARS coronavirus                                                                 |
|---------------------------------------|------------------------------------------------------------------------------------|----------------------------------------------------------------------------------|
| Binding of virion to cell-surface receptor | PET or SPECT imaging using radiolabeled lectins or viral HA molecules as probes. BLI of infection by a GFP- or luciferase-encoding virus [32]. | BLI of infection by a GFP- or luciferase-encoding virus [33].                   |
| Fusion and entry into cell            | PET or SPECT imaging using radiolabeled adamantanes, which bind specifically within the M2 ion channel [34]. | PET or SPECT imaging using radiolabeled peptide inhibitors of viral membrane fusion [36]. |
| Transcription and genome replication  | PET or SPECT imaging using a recombinant virus encoding the HSV TK, or using a radiolabeled non-nucleoside RNA polymerase inhibitor as tracer. | PET or SPECT imaging using a recombinant virus encoding the HSV TK, or using a radiolabeled non-nucleoside RNA polymerase inhibitor as tracer. |
| Processing of viral proteins and virion assembly | PET or SPECT imaging using radiolabeled antibodies that bind to viral antigens on the cell surface, or a radiolabeled neuraminidase inhibitor. | PET or SPECT imaging using a radiolabeled protease inhibitor as a tracer [36]. |
| Budding and exit of virus particles   | PET or SPECT imaging using radiolabeled antibodies that bind to viral antigens on the cell surface, or a radiolabeled neuraminidase inhibitor. | PET or SPECT imaging using radiolabeled antibodies that bind to viral antigens on the cell surface [37]. |

**NOTE.** For BLI, sites of replication can be identified through the expression of a virus-encoded luciferase, GFP or other reporter molecule, as has been shown for an influenza virus encoding a chimeric NS1-GFP protein [31]. Virus-specific imaging with PET or SPECT might make use of a radiolabeled probe that binds with high affinity to a virus-specific molecule, or is selectively modified by a virus-encoded enzyme, causing it to be retained within infected cells.

**Figure 6.** CT (upper row) and $^{18}$FDG-PET images of the lungs of a human volunteer before and 24 hours after an intrabronchial installation of endotoxin. The subtraction image shows an area of tracer retention representing an acute inflammatory response. Red indicates the highest and blue the lowest level of activity. (From [39], with permission.)
used to image bacterial infections in mice [55]. Key issues include demonstrating the stability of the neutrophil-peptide complex, which affects the localization of a molecular process if the radioactive molecule fails to remain attached to the target peptide, and studying the effect on tracer uptake of increased perfusion in areas of infection [55].

Because the techniques just described cannot reliably distinguish between infection and other inflammatory processes, there is a need for methods that could specifically detect viral replication and track the spread of a pathogen in the respiratory tract. One possible approach would be to design radiolabeled probes that bind with high affinity to virus-encoded molecules or that are substrates for a virus-encoded enzyme (Table 2). An example of the latter is the herpesviral TK, which phosphorylates thymidine analogues that are not substrates for the host enzyme. Compounds, such as acyclovir and its analogues, can therefore be used both as antiviral drugs for the treatment of herpesviral infections and as radiolabeled tracers to detect sites of replication or recombinant TK expression [56]. The method has been used to image experimental herpesviral infections of...
tumors, but its use for visualizing herpesviral encephalitis has been limited by the requirement for the probe to penetrate the blood-brain barrier [57–59]. The published literature has not yet established the use of radiolabeled antiviral drugs as probes to image influenza or SARS, but opportunities might exist to use amantadine and rimantadine, which inhibit influenza viral replication by selectively occupying the M2 ion channel; oseltamivir and zanamivir, which bind to the active site of the influenza neuraminidase; and experimental drugs that inhibit the SARS coronavirus protease as imaging agents [34, 36]. PET has been used to image the distribution of radiolabeled oseltamivir in uninfected mice and of inhaled zanamivir in healthy human volunteers [60, 61]. It is interesting to speculate that these radiolabeled drugs could also be used to identify the distribution of influenza virus in the respiratory tract of patients with influenza, unless their distribution would essentially localize nonspecifically to areas of increased perfusion, as has been observed for radiolabeled ciprofloxacin in experimental bacterial infections [62].

Magnetic Resonance Imaging

In MRI, exposure of the body to a combination of a strong magnetic field and pulses of radio-frequency energy induces signals that reflect the local molecular environment. In addition to providing detailed structural images, MRI can obtain information on physiologic processes through the use of contrast agents, such as superparamagnetic iron oxide (SPIO) particles, to label immune cells and the analysis of spectral data to detect and quantify specific substances in tissues. The use of MRI for the study of pulmonary disease has been significantly more limited than CT because of several challenges, including cardiac and respiratory motion, the low water content of pulmonary parenchyma, and the variable magnetic susceptibility at alveolar and bronchial structure interfaces, which produces short T2 and T2* relaxation times, lowering the signal-to-noise ratio [65]. However, technical innovations, such as respiratory gating and adjusted MRI sequences, are addressing these challenges to image pulmonary infections [64–66].

MRI is now being used to track the development of pulmonary lesions and characterize inflammatory responses in murine models of bacterial infection (Figure 8A) [67–70]. Although MRI has been used to study the effects of influenza on the central nervous system and the heart, it has yet to be applied in published research on pulmonary influenza or other respiratory viral infections. However, advances in pulmonary MRI and MRI-compatible cell labeling are providing a unique opportunity for these studies. For example, inflammatory cells loaded ex vivo with antiviral drugs or nanoparticles have been demonstrated to localize to sites of infection [71, 72]. SPIO particles, which cause local magnetic susceptibility artifacts, are particularly useful for studying inflammatory processes, because they are detectible as dark (hypointense) regions much larger than the particles themselves, rendering them visible even when only a few cells are labeled (Figure 8B) [73]. Although promising for identifying areas of inflammation in solid organs, such as the brain, these methods may prove to be less useful for studying respiratory tract infections, because dark areas of diminished signal intensity at sites of SPIO particle uptake may resemble normal lung.

Bioluminescence Imaging

Because it uses relatively inexpensive detection equipment and does not involve radioactivity, BLI is the molecular imaging method most accessible to
bench researchers. The technique is based on the emission of photons of visible or near-infrared light by firefly luciferase when it is exposed to an injected bolus of its natural substrate, luciferin [74]. Because photons in this energy range are quickly absorbed as they pass through tissues, BLI studies can only be performed in small animals (predominantly in mice) with organs of interest within a few millimeters of the skin surface.

Most investigators who have used BLI to visualize viral replication have inserted a luciferase gene into the genome of the agent of interest, a procedure that is quite straightforward for large DNA viruses, such as the poxviruses. In the case of vaccinia virus infections of the respiratory tract, the photon flux from the lungs has been shown to correlate directly with tissue viral titers, making it possible to both visualize and quantitate viral replication in living animals over the entire course of illness (Figure 9) [76, 77]. Thus, BLI has the potential to supplement or even replace some traditional research methods in which data on viral replication could only be obtained by infecting a large number of mice, sacrificing cohorts at various time points, collecting tissue samples, and determining viral titers.

The insertion of a reporter gene is more challenging for a small RNA virus such as influenza. However, a recombinant influenza A virus encoding a chimeric NS1-green fluorescent protein (GFP) molecule has been successfully generated and used to image the distribution of virus in the lungs of infected mice [31]. Because of the short tissue penetration of UV light and the presence of background autofluorescence, imaging could only be performed ex vivo, on excised lungs; however, the findings still provide a clear demonstration of the effect of antiviral therapy (Figure 10). A GFP-encoding SARS coronavirus has also been constructed, but luciferase-encoding coronaviruses have not been described [33, 78]. Of interest, BLI can also be used to study the bacterial pneumonia that often accompanies viral infection of the respiratory tract. By inserting a lux operon into the genome of Streptococcus pneumoniae, researchers were able to image pulmonary bacterial replication and demonstrate that pneumonia was more severe in mice that had undergone preliminary influenza virus infection than in control animals [79].

In addition to using BLI to visualize pathogen replication, it can also be used to study host responses to infection, by creating reporter mice, in which a luciferase gene is inserted into the animal’s genome under the control of a promoter of interest [80]. For example, mice in which luciferase expression is driven by the IFN-β promoter have been used to characterize type I IFN responses to wild-type and recombinant influenza A viruses, (Figure 11) [22, 81]. Thus, BLI makes it possible to perform a variety of studies that could serve as proofs of concept for further investigation in large animals and in humans.

CONCLUSION

Molecular imaging has the potential to complement traditional radiographic methods, by characterizing pathophysiologic processes that underly pulmonary structural changes and impaired respiratory function during the course of illness. SPECT, PET, MRI, and BLI offer molecular imaging techniques that could potentiate the discovery of new biomarkers for predicting disease progression and supporting the development of novel therapies targeting viral replication or damaging host responses. Although new diseases, such as novel H1N1 influenza and SARS, will inevitably continue to emerge, molecular imaging can help to reduce their impact on public health by elucidating their pathogenetic mechanisms.
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Figure 11. Response of “reporter mice” homozygous (left) or heterozygous (right) for firefly luciferase under the control of an IFN-β promoter to infection with an NS1-deficient influenza A virus. Red indicates the highest and blue the lowest level of photon flux. (From [81], with permission.)
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