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 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...
the pathophysiologic mechanisms responsible for such changes. In particular, it is not known whether influenza and other viral infections are initially limited 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 radiotracer 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°C 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...
a requirement for positron decay. Moreover, as a cross-sectional technique, SPECT provides significant advantages over planar nuclear medicine imaging for locating sites of disease. For example, whole antibodies or antibody fragments labeled with 99mTcTechnetium or 111Indium can be imaged with SPECT for a broad range of potential infectious disease applications.

The most widely used PET radiotracer is 18F-fluorodeoxyglucose (FDG). Because deoxyglucose is taken up by cells and phosphorylated but not further metabolized, the radiotracer is selectively trapped in cells with high rates of glycolysis. FDG - PET imaging has a flourishing oncologic literature, based on the detection of active neoplasms with elevated glycolysis by combining PET with CT, which has greater sensitivity and specificity than either method alone. In contrast, relatively few data have yet been published to support a role for FDG - PET and/or CT in infectious diseases imaging. In clinical practice, FDG - PET and/or CT can also visualize inflammatory processes, both infectious
and noninfectious, via nonspecific up-
take 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 re-
sponses to infection [38]. For example,
FDG has been used to visualize the re-
sponse 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 in-
flammatory 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 con-
ditions [47–51].

FDG-PET and/or CT were recently
used to study a patient with severe in-
fluenza, 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 in-
fection, 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 com-
pared with measurements of viral repli-
cation, cellular markers, and gene
expression to identify relationships
among these factors and pulmonary
parenchymal injury.

Because intravenously administered
FDG is taken up and trapped non-
specifically 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 re-
sponses [24, 54]. For example, a 64Cu-
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

<table>
<thead>
<tr>
<th>Target</th>
<th>Potential imaging methods</th>
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<tbody>
<tr>
<td>Binding of virion to cell-surface receptor</td>
<td>PET or SPECT imaging using radiolabeled lectins or viral HA molecules as probes. BLI of infection by a GFP- or luciferase-encoding virus [32].</td>
</tr>
<tr>
<td>Fusion and entry into cell</td>
<td>PET or SPECT imaging using radiolabeled adamantanes, which bind specifically within the M2 ion channel [34].</td>
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<tr>
<td>Transcription and genome replication</td>
<td>PET or SPECT imaging using a recombinant virus encoding the HSV TK, or using a radiolabeled non-nucleoside RNA polymerase inhibitor as tracer.</td>
</tr>
<tr>
<td>Processing of viral proteins and virion assembly</td>
<td>PET or SPECT imaging using a radiolabeled protease inhibitor as a tracer [36].</td>
</tr>
<tr>
<td>Budding and exit of virus particles</td>
<td>PET or SPECT imaging using radiolabeled antibodies that bind to viral antigens on the cell surface, or a radiolabeled neuraminidase inhibitor.</td>
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</tbody>
</table>

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 18FDG-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 radio-labeled 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 radio-labeled tracers to detect sites of replication or recombinant TK expression [56]. The method has been used to image experimental herpesviral infections of

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**Figure 7.** Chest CT (A), 18FDG-PET scan (B), and fused image (C) of a patient with severe swine-origin H1N1 influenza and ARDS. The PET image shows increased glucose metabolism, both in areas that contain infiltrates by CT and in regions of apparently normal aeration. Red indicates the highest and blue the lowest level of radiotracer activity. (Courtesy of Giacomo Bellani.)

**Figure 8.** Identification by MRI of areas of inflammation in the lungs of a mouse 24 and 48 hours after an intratracheal inoculation of lipopolysaccharides, based on the uptake of 19F-labeled emulsified perfluorocarbons, which are phagocytized by monocytes and macrophages. A and B, 1H gradient echo images of the thorax. C, 19F MRI. D, Superimposed images, with sites of perfluorocarbon uptake highlighted in red. (From [67], with permission.)
tumors, but its use for visualizing her-
pesviral encephalitis has been limited by
the requirement for the probe to pene-
trate the blood-brain barrier [57–59].
The published literature has not yet
established the use of radiolabeled anti-
viral 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 experi-
mental drugs that inhibit the SARS co-
roronavirus protease as imaging agents
[34, 36]. PET has been used to image the
distribution of radiolabeled oseltamivir
in uninfected mice and of inhaled za-
amivir 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 ra-
diolabeled ciprofloxacin in experimental
bacterial infections [62].

Magnetic Resonance Imaging
In MRI, exposure of the body to a com-
bination 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 pro-
cesses 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 tis-
sues. The use of MRI for the study of
pulmonary disease has been significantly
more limited than CT because of several
challenges, including cardiac and re-
spiratory motion, the low water content
of pulmonary parenchyma, and the
variable magnetic susceptibility at alve-
olar and bronchial structure interfaces,
which produces short T2 and T2* re-
 laxation times, lowering the signal-to-
to-noise ratio [65]. However, technical in-
novations, 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 in-
fluenza on the central nervous system
and the heart, it has yet to be applied in
published research on pulmonary in-
fluenza or other respiratory viral in-
fec tions. However, advances in
pulmonary MRI and MRI-compatible
cell labeling are providing a unique op-
portunity 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 arti-
facts, are particularly useful for studying
inflammatory processes, because they
are detectible as dark (hypointense) re-
gions much larger than the particles
themselves, rendering them visible even
when only a few cells are labeled (Figure
8B) [73]. Although promising for iden-
tifying 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 re-
semble normal lung.

Bioluminescence Imaging
Because it uses relatively inexpensive
detection equipment and does not in-
volve 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 1 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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**References**


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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.)


