Potential Applications of Conventional and Molecular Imaging to Biodefense Research

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Imaging methods that visualize the structure and function of the living body are widely used in patient care and biomedical research, but their full potential has not yet been applied to the study and treatment of the severe illnesses caused by pathogens of biodefense concern. “Conventional” imaging techniques (e.g., radiography, computed tomography, ultrasound, or magnetic resonance imaging) delineate anatomic changes in tissues, whereas “molecular” methods employ magnetic resonance, positron emission tomography, single-photon emission computed tomography, or optical (fluorescence or bioluminescence) imaging to detect biochemical reactions that accompany pathogen replication or host responses. We review the basic principles of these methods, describe the diseases caused by 6 pathogens classified as category A or B bioterror agents (anthrax, plague, tularemia, filoviral hemorrhagic fever, smallpox, and aerosolized equine encephalitis virus infection), and discuss how imaging could be used to study their pathogenesis in laboratory animals and to diagnose and monitor infection in humans.

The threat of bioterrorism has made clinicians and scientists aware of limitations in our current understanding of the diseases caused by a number of virulent pathogens. For a variety of reasons, most illnesses of biodefense concern are rarely or never seen by physicians: anthrax, plague, and tularemia occur only sporadically and in rural areas; filoviral hemorrhagic fevers are limited to central Africa; smallpox has long been eradicated; and the infections caused by aerosolized encephalitis viruses do not occur under natural conditions [1]. Although a number of laboratory animal models have been developed to study their pathogenesis, these diseases remain poorly characterized.

Modern imaging technologies could help to close this knowledge gap and provide new tools for early diagnosis and patient treatment. Two general approaches are in use. “Conventional” techniques, such as radiography, CT, and MRI, delineate structural changes in tissues associated with a disease process (table 1). These methods have been employed to a limited extent in the diagnosis and management of some illnesses of biodefense concern [2]. “Molecular” imaging methods, by contrast, make use of radiolabelled probes or molecules with specific optical signatures or activation chemistries to detect biochemical markers or enzymatic reactions, visualizing them by MRI, positron emission tomography (PET), single-proton emission CT (SPECT), or optical detectors of fluorescence or bioluminescence (table 2) [3–5]. In contrast to conventional methods, these techniques have rarely been applied to acute infectious processes and have not been used to study biodefense-related diseases.

Here, we review the basic principles of conventional and molecular imaging and suggest how these techniques could be employed to study diseases of biodefense concern. The discussion focuses both on studies in laboratory animals (table 3) and on potential uses of clinical imaging for early diagnosis and recognition of a disease outbreak and the evaluation and monitoring of patients.

OVERVIEW OF IMAGING TECHNIQUES

Images may be obtained by either sending energy through a subject (as in radiography and ultrasound), detecting emissions originating within a subject (as in radionuclide imaging, including PET, and detection of emitted light), or using an external energy source to induce signal production within the subject (as in MRI and fluorescence). Plain-film radiography
Table 1. Components of an infectious process that can potentially be visualized by conventional imaging methods on the basis of anatomic alterations in tissues.

<table>
<thead>
<tr>
<th>Process</th>
<th>Physical change</th>
<th>Imaging modality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue damage</td>
<td>Necrosis, hemorrhage, edema, effusions</td>
<td>Radiography, CT, MRI</td>
</tr>
<tr>
<td>Inflammation</td>
<td>Increased perfusion, elevated temperature, decreased pH level</td>
<td>MRI, magnetic resonance spectroscopy</td>
</tr>
<tr>
<td>Involvement of regional lymph nodes</td>
<td>Cellular influx, necrosis, apoptosis</td>
<td>CT, MRI</td>
</tr>
<tr>
<td>Altered systemic vascular function</td>
<td>Dilatation, increased permeability</td>
<td>Contrast CT or MRI</td>
</tr>
<tr>
<td>Coagulation</td>
<td>Decreased perfusion, increased blood clotting</td>
<td>Contrast CT or MRI</td>
</tr>
</tbody>
</table>

Table 2. Components of an infectious process that could potentially be visualized by molecular imaging methods on the basis of biochemical activity associated with pathogen replication or host responses.

<table>
<thead>
<tr>
<th>Process</th>
<th>Biochemical reaction</th>
<th>Imaging modality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pathogen replication</td>
<td>Antigen expression, pathogen-specific enzymes, receptor binding</td>
<td>MRI, PET, SPECT, bioluminescence or fluorescence</td>
</tr>
<tr>
<td>Toxin action</td>
<td>Interruption of intracellular pathways</td>
<td>MRI, PET, SPECT, bioluminescence or fluorescence</td>
</tr>
<tr>
<td>Response to infection</td>
<td>Apoptosis, cytokine/chemokine expression</td>
<td>MRI, PET, SPECT, bioluminescence or fluorescence</td>
</tr>
<tr>
<td>Cellular immune responses</td>
<td>Trafficking of labelled cells</td>
<td>MRI, PET, SPECT, bioluminescence or fluorescence</td>
</tr>
<tr>
<td>Changes in vascular function</td>
<td>Up-regulation of endothelial cell-surface receptors</td>
<td>MRI, PET, SPECT, bioluminescence or fluorescence</td>
</tr>
</tbody>
</table>

**NOTE.** PET, positron emission tomography; SPECT, single-photon emission CT.
Table 3. Models of bioterror-related diseases in laboratory animals.

<table>
<thead>
<tr>
<th>Human disease (pathogen[s]), animal</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anthrax (Bacillus anthracis)</td>
<td></td>
</tr>
<tr>
<td>Small animals</td>
<td>Aerosolized spores are lethal for mice, guinea pigs, and rabbits; injection of attenuated Sterne strain is lethal for mice.</td>
</tr>
<tr>
<td>NHP</td>
<td></td>
</tr>
<tr>
<td>Plague (Yersinia pestis)</td>
<td></td>
</tr>
<tr>
<td>Small animals</td>
<td>Aerosolized, intranasal, or injected bacilli of various strains are lethal for mice and guinea pigs.</td>
</tr>
<tr>
<td>NHP</td>
<td></td>
</tr>
<tr>
<td>Tularemia (Franciscella tularensis)</td>
<td></td>
</tr>
<tr>
<td>Small animals</td>
<td>Injected or aerosolized LVS causes lethal disease in mice.</td>
</tr>
<tr>
<td>NHP</td>
<td>Aerosolized bacilli of the Schu4 strain cause lethal disease.</td>
</tr>
<tr>
<td>Filoviral hemorrhagic fever (Ebola and Marburg viruses)</td>
<td>Mouse-adapted Ebola and guinea pig–adapted Ebola and Marburg viruses cause rapidly lethal systemic infection.</td>
</tr>
<tr>
<td>Small animals</td>
<td></td>
</tr>
<tr>
<td>NHP</td>
<td>All Ebola and Marburg isolates cause severe or fatal disease in nonhuman primates.</td>
</tr>
<tr>
<td>Smallpox (cowpox, vaccinia, ectromelia, monkeypox, rabbitpox viruses)</td>
<td>Low doses of ectromelia and rabbitpox virus cause lethal systemic infection in mice and rabbits, respectively; larger intranasal doses of cowpox or vaccinia virus cause lethal respiratory tract infection in mice.</td>
</tr>
<tr>
<td>Small animals</td>
<td></td>
</tr>
<tr>
<td>NHP</td>
<td>Intravenous monkeypox or variola virus cause smallpox-like illness; aerosolized virus causes lethal bronchopneumonia.</td>
</tr>
<tr>
<td>Alphavirus encephalitis (Venezuelan, eastern, western encephalitis viruses)</td>
<td>All viruses cause systemic illness with encephalitis in mice and hamsters; Sindbis infection of mice is also used.</td>
</tr>
<tr>
<td>Small animals</td>
<td></td>
</tr>
<tr>
<td>NHP</td>
<td>All viruses cause febrile illness by injection or aerosol, with varying percentage of progression to CNS infection.</td>
</tr>
</tbody>
</table>

NOTE. LVS, live vaccine strain; NHP, nonhuman primate.

copy has been used to detect the presence of specific organic compounds such as lactic acid or ATP, combining anatomic and functional analyses, and a magnetic resonance–based gene reporter system has also been developed [12, 13].

Radionuclide-based techniques incorporate short-lived radioactive atoms into molecular probes, then use them to detect a variety of targets, including cell surface markers and metabolic reactions. Planar γ imaging can provide 2-dimensional localization of a specific process (figure 4A). In SPECT, by contrast, emissions detected by linear collimators revolving through 360° provide depth information that can be integrated into a 3-dimensional image [14]. PET scanning takes advantage of the fact that the decay of a positron produces 2 high-energy photons that travel off in opposite directions; consequently, “coincidence detection” by a ring of sensors allows the source of emission to be identified in 3 dimensions (figure 4B and 4C) [15]. Superimposing a PET image on a simultaneously obtained radiographic CT scan provides data that can be used for attenuation correction and optimal anatomic localization.

Optical signals are composed of photons in the visible or near-infrared spectrum that can be used to obtain information in 2 basic ways. The first employs an external source, in a manner similar to radiography, and generates images based on the scatter or absorption of photons or induced fluorescence. Some images obtained during endoscopic procedures fall within this category. Image quality can be enhanced by introducing contrast agents that alter the absorption, fluorescence, and scattering properties of tissues. The second approach, more commonly used in research than in clinical practice, utilizes a fluorescent or bioluminescent signal originating within the subject. Genes encoding fluorescent proteins, such as green fluorescent protein [16, 17] or bioluminescent proteins (i.e., luciferases), can be used as reporters to reveal sites of gene expression and detect biological processes in laboratory animals (figure 5) [18–20]. The sensitivity of these methods is determined by the optical properties of mammalian tissues, which limit the transmission of visible light to short distances [21]. However, because red and near-infrared light can pass through several cen-
imeeters of tissue, it is now possible to examine almost every organ in small animals (figure 5). Some clinical applications of this approach are under development [22].

IMAGING DISEASES OF BIODEFENSE CONCERN

Conventional techniques and emerging molecular methods can be used to visualize infectious processes in complementary ways (tables 1 and 2). For example, anthrax can be recognized by chest radiography or CT on the basis of necrosis, edema, and hemorrhage centered in mediastinal lymph nodes (figure 1A–1C), but the infection might also be identified before significant tissue damage occurs by using molecular probes to detect bacterial antigens, pathogen-specific enzymatic reactions, patterns of cell trafficking, or cytokine expression. Similarly, altered vascular function can be visualized by tracking the distribution of injected contrast medium by CT or MRI but might also be detected in its early stages by using molecular imaging methods to demonstrate altered expression of endothelial cell-surface adhesion molecules. By incorporating reporter genes into pathogens or laboratory animals, imaging may help to develop new noninvasive methods of measuring pathogen burden and assessing host responses and could help to accelerate and refine studies of disease mechanisms, vaccine efficacy, and intervention strategies [4, 23–27]. Laboratory research may also facilitate the development of new probes and contrast agents for use in clinical settings.

In the following sections, we discuss how imaging methods could be used to answer a variety of questions in biodefense research and to assist in early diagnosis and patient care. Although we focus on infections caused by category A and B bioterror agents [28], the same principles and methods could apply to any infectious process.

Anthrax. Anthrax results from the germination of Bacillus anthracis spores in tissues, replication of the vegetative bacilli, and the effects of plasmid-encoded lethal and edema toxins. In cutaneous anthrax, the introduction of spores through the skin produces a necrotic lesion with surrounding edema at the site of entry [29]. In inhalational disease, by contrast, spores inhaled into the respiratory tract are taken up by pulmonary macrophages and carried to mediastinal lymph nodes, where their replication results in necrosis, hemorrhage and edema, com-
Radiography and CT have been used to visualize structural changes resulting from inhalational anthrax in humans (figure 1A–1C) and could potentially be used in a similar manner to study the disease in animals (table 3) [2, 31, 32]. The classic chest radiograph finding, mediastinal widening (figure 1A), becomes evident only after significant tissue damage has occurred, but the use of CT or MRI would allow earlier recognition of typical features of illness, including alterations in lymph node morphology and focal pulmonary hemorrhage in the relative absence of parenchymal disease (figures 1B and 1C) [33, 34].

The use of new types of contrast media, such as superparamagnetic iron oxide nanoparticles that are taken up by macrophages, might further improve MRI of lymph nodes and other tissues in anthrax and other infections [35]. Conventional imaging methods could also be used to detect structural changes associated with the spread of infection to the CNS. As noted above, molecular imaging approaches might also be used to facilitate the early diagnosis of anthrax and the clinical monitoring of the disease. Infection might be recognized either through the binding of labeled antibodies to bacterial antigens or the detection of chemical reactions specific to spore germination, replication of bacilli, the intracellular action of lethal and edema toxins, responses of infected macrophages, or the recruitment of immune cells to sites of infection.

**Plague.** The most common naturally occurring form of the disease, bubonic plague, results from the bite of a flea carrying *Yersinia pestis* from an infected rodent. Replication of bacilli in regional lymph nodes causes inflammation, necrosis, and painful swelling (a bubo). The further spread of bacilli through the bloodstream (septicemic plague) may initiate pulmonary infection (secondary pneumonic plague) that can be transmitted to close contacts by the airborne route. The lung infection that develops in these secondary cases (primary pneumonic plague) is the uniformly lethal form of illness that is expected to result from a bioterrorist release of aerosolized *Y. pestis* [36]. Bacterial replication in the lung causes local tissue damage and progresses rapidly to septic shock as endotoxin and other bacterial products stimulate macrophages to release proinflammatory cytokines and chemokines and to synthesize tissue factor, causing diffuse vascular dilatation, increased permeability, and disseminated intravascular coagulation. Gangrene resulting from fibrin deposition in peripheral vessels is a prominent feature of plague.

In contrast to anthrax, the initial pathologic processes following inhalation of *Y. pestis* are localized to the lung parenchyma, where necrosis, edema, and inflammatory infiltrates produce foci of consolidation that can be seen on chest radiography (figure 1D) [2]. More precise information on the progression of pulmonary disease, spread of infection to regional lymph nodes, and other aspects of pathogenesis could be obtained using CT or MRI. As in the case of anthrax, labelled ligands, substrates, or antibodies could be used to detect bio-

**Figure 2.** Ultrasound imaging. A, Experimental ultrasound imaging of the liver in a rabbit before (upper) and after (lower) injection of a microbubble contrast agent, showing marked enhancement because of the organ’s high vascularity. (Reprinted with permission from [53].) B, Endoscopic ultrasound image demonstrating an enlarged lymph node (arrow). (Courtesy of Loren Ketai.)
chemical processes specific to *Y. pestis* replication, monitor the course of infection, and assess the effects of therapy in animal models (table 3). Molecular imaging techniques might be especially useful for visualizing alterations in vascular function, such as expression of endothelial adhesion molecules, changes in permeability, extravasation of leukocytes, initiation of coagulation, and remodelling of fibrin deposits.

**Tularemia.** The agent of tularemia, *Francisella tularensis*, produces a wide variety of syndromes in humans, depending on the subspecies of bacterium and the route by which it enters the body. Handling an infected animal may result in initiation of infection in the skin, with local ulceration and regional lymphadenopathy. Ingestion of contaminated food or water can produce a similar process in the oropharynx. Biodefense concerns focus on airborne transmission, because the organism is stable and highly infectious when aerosolized [37]. Evidence from natural outbreaks of tularemia indicates that inhalation often results in both localized infection of the upper respiratory tract and multifocal pneumonitis. In contrast to plague, however, the ensuing disease is not rapidly progressive and is not accompanied by severe coagulopathy or the early onset of hypotension. Instead, the principal manifestations of disease reflect the fact that macrophages can slow the spread of infection by ingesting bacilli but are unable to kill them. The secretion of cytokines and chemokines by persistently infected cells attracts neutrophils and additional macrophages to the site of infection, leading to the release of toxic mediators, local tissue injury, and granuloma formation. Untreated patients may undergo months of debilitating febrile illness until the organism is finally eliminated or die of the cumulative effects of multifocal infection.

As in the case of plague, conventional imaging can be used to visualize morphologic changes in the pulmonary parenchyma, alterations of regional lymph nodes, and other structural changes resulting from *F. tularensis* infection (figure 1E). The use of CT or MRI would provide additional information and might facilitate early diagnosis of persons exposed to the aerosolized pathogen [2]. Molecular imaging techniques, especially the tracking of immune cells, could be used in research studies in animals to define the nature of innate and adaptive responses to *F. tularensis* and to characterize the effort of the immune system to eliminate the organism. Serial monitoring of biochemical activities specific to the pathogen and the host could be particularly important in evaluating responses to therapy, including interventions with immunomodulatory agents.

**Filovirus infection.** In contrast to the bacterial diseases described above, Ebola and Marburg virus infections do not begin with a localized stage of illness. Instead, the filoviruses first infect macrophages and dendritic cells at the site of entry, then spread to similar cells in regional lymph nodes, the spleen, and other tissues throughout the body [38]. Rapid dissemination is apparently aided by suppression of type I IFN responses. Infected cells release proinflammatory cytokines, chemokines, and other mediators and synthesize tissue factor, producing a syndrome of fever, hypotension, and disseminated intravascular
coagulation resembling septic shock seen in septicemic plague and other bacterial infections [39, 40]. The further spread of virus to parenchymal cells in the liver, adrenal glands, and other organs causes additional tissue injury. Massive bystander apoptosis of lymphocytes contributes to immunosuppression [41].

No clinical images have been obtained of patients with Ebola or Marburg virus infections. However, filovirus infections of nonhuman primates and mice have many points in common with the human disease (table 3) and could be used for imaging studies. Virus-induced necrosis of macrophages and dendritic cells in lymphoid tissues results in a distinct pattern of injury that could potentially be recognized by both conventional and molecular imaging methods. Similarly, the numerous morphologic changes and biochemical processes that accompany multifocal hepatic injury, lymphocyte apoptosis, altered vascular

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**Figure 4.** Radionuclide imaging. A, Superimposed radiograph and planar γ imaging scans showing ex vivo 111In-labeled CD8+ T cells in a mouse. (Courtesy of Mark Williams, University of Virginia, Charlottesville, VA). B, positron emission tomography (PET) scan showing 18F-labeled bone-marrow-derived dendritic cells in a mouse. (Reprinted with permission from [55]). C, PET scan of a patient with sarcoidosis injected with 18F-fluorodeoxyglucose, showing markedly increased metabolic activity in lymph nodes and spleen. (Courtesy of Esther Lim, National Institutes of Health Clinical Center, Bethesda, MD).

**Figure 5.** Optical imaging. A, Unilateral (left and center) and bilateral (right) pneumonia in mice inoculated with Pseudomonas aeruginosa labeled through expression of the lux operon from Photorhabdus luminescens. (Reprinted with permission from [4]). B, Dorsal (left) and ventral (right) views of the distribution of T cells labeled ex vivo by retroviral transduction, using a viral vector encoding both luciferase and green fluorescent protein. (Reprinted with permission from [20]). C, MRI (upper) and bioluminescence images (lower) of a glioblastoma in a mouse. (Courtesy of Brian Ross, Center for Molecular Imaging, University of Michigan Medical School, Ann Arbor).
function, and disseminated intravascular coagulation could be detected both by conventional techniques, including MRI and ultrasound, and by molecular imaging methods [42]. A variety of techniques could also be used to track the migration of immune cells to sites of infection. Techniques based on emitted light would be ideal for studying Ebola virus infection in mice, using either a genetically modified virus encoding a bioluminescent marker or transgenic animals in which infection triggers the expression of a reporter molecule.

**Smallpox.** Smallpox is initiated by the inhalation of variola virus into the respiratory tract [43, 44]. No symptomatic lesions develop at the site of entry; instead, studies in animals suggest that local replication is followed by the spread of virus first to macrophages and related cells in local lymph nodes, then to similar cells in the liver, spleen, and other organs, and finally throughout the body in a “secondary viremia” [45]. The 10–14-day incubation period ends when the release of cytokines from infected cells causes fever and malaise. Replication and dissemination are aided by a battery of virus-encoded proteins that block apoptosis and inhibit innate immune responses, whereas the development of lesions in the skin and oropharynx is favored by the release of a virus-encoded epidermal growth factor from infected cells. The ability of host responses to control viral replication during the incubation period appears to be critical in determining the outcome of illness. The majority of patients apparently develop a low secondary viremia, resulting in scattered lesions separated by areas of normal skin (“ordinary smallpox”). In a few cases, however, high levels of circulating virus, presumably accompanied by massive release of cytokines, produce a rapidly lethal hemorrhagic syndrome resembling septic shock [45]. Human monkeypox infection resembles ordinary smallpox but features more-prominent lymphadenopathy (figure 1F) [46].

Smallpox was not associated with any characteristic findings by radiography, the only imaging method available in regions of endemicity before the disease was eradicated in 1977 [2]. Even though smallpox no longer exists, various aspects of its pathogenesis can be examined in laboratory animals. The mouselike and rabbitpox models are most useful for studying viral dissemination during the incubation period, because very small quantities of aerosolized virus cause lethal disseminated infection (table 3) [4, 47]. Recombinant viruses encoding a bioluminescent or fluorescent marker would be especially useful in tracking this process. Nonhuman primates are a less appropriate choice for studying airborne-transmitted disease, because large amounts of aerosolized monkeypox or variola virus are required to induce severe or fatal illness, and the resulting diffuse pneumonitis was not seen in human smallpox [48]. However, the same spectrum of illness seen in humans, ranging from discrete skin lesions through fulminant hemorrhagic disease, can be generated in macaques by producing an artificial secondary viremia through the intravenous injection of a range of doses of those viruses [49]. Molecular imaging approaches might be most appropriate to visualize the pathologic processes underlying these syndromes, especially because the detection of fluorescent or bioluminescent signals could be used to study the initiation and development of skin lesions.

**Equine encephalitis virus infection.** Venezuelan, eastern, and western equine encephalitis viruses pose a threat as bioterror weapons because they are stable and highly infectious when released as aerosols [1]. In contrast to the severe diseases described above, most infections would probably be characterized by an incapacitating but nonfatal flulike illness. Natural transmission of these agents by mosquitoes results in infection of lymphoid cells, release of proinflammatory cytokines, and rapid development of high virus loads; in a small percentage of cases, infection spreads to the CNS [50]. Laboratory accidents during the middle third of the 20th century demonstrated that inhalation of these viruses results in a similar syndrome without causing symptoms localized to the respiratory tract. However, studies in laboratory animals suggest that airborne transmission could favor the development of neurological disease by providing a “short cut” into the brain through infection of the olfactory epithelium and spread along the olfactory nerve [51].

The principal goals of research on aerosolized encephalitis viruses are to mitigate the inflammatory phase of illness and prevent neurologic disease. Conventional imaging methods may not be especially useful for diagnosing or studying the former, because the cytokine responses induced by viral infection are apparently not accompanied by significant tissue injury. However, molecular imaging methods could be employed to characterize innate and adaptive responses to infection in animals and to track the spread of virus, especially because the addition of a gene encoding a luminescent marker would be relatively easy for these agents. Both conventional and molecular imaging techniques could be used to study the mode of viral spread to the brain and to develop methods for the early diagnosis of encephalitis [2].

**CONCLUSIONS**

The urgent need for improved understanding of bioterrorism-related diseases comes at a time when a synthesis of chemistry, biology, and physics is leading to major advances in imaging. This unprecedented convergence of technologies has produced a range of new techniques with tremendous potential for studies in laboratory animals and for detecting, monitoring, and studying infections in humans.

The incorporation of modern imaging methods into biodefense research will require special efforts to adapt equipment and procedures to the unique environment of biocontainment laboratories. On the clinical side, new protocols may be needed
to ensure that imaging can be performed safely and effectively in the event of a bioterror attack, especially in the potentially important role of early diagnosis. Because each imaging method has limitations in sensitivity, resolution, and practical application and because each pathogen induces a characteristic pattern of structural changes and biochemical reactions, the effective application of these powerful techniques to biodefense research will require close communication between the imaging and infectious disease communities.

Acknowledgments

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References