The American Journal of Pathology, Vol. 182, No. 2, February 2013

ELSEVIER

The American Journal of PATHOLOGY

ajp.amjpathol.org

Imaging Theme Issue

REVIEW

Imaging of Small-Animal Models of Infectious Diseases

Linda A. Jelicks,* Michael P. Lisanti,† Fabiana S. Machado,‡ Louis M. Weiss,§ Herbert B. Tanowitz,§ and Mahalia S. Desruisseaux§

From the Department of Physiology and Biophysics and the Gruss Magnetic Resonance Research Center, and the Departments of Pathology and Medicine,§ Albert Einstein College of Medicine, Bronx, New York; the Departments of Stem Cell Biology and Regenerative Medicine,† Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, Pennsylvania; and the Department of Biochemistry and Immunology,‡ Faculty of Medicine, Institute for Biological Sciences, Federal University of Minas Gerais, Belo Horizonte, Brazil*

Accepted for publication September 13, 2012.

Address correspondence to Linda A. Jelicks, Ph.D., Albert Einstein College of Medicine, 1300 Morris Park Ave, Magnetic Resonance Research Center Bldg, Room 301, Bronx, NY 10461. E-mail: linda.jelicks@einstein.yu.edu.

Infectious diseases are the second leading cause of death worldwide. Noninvasive small-animal imaging has become an important research tool for preclinical studies of infectious diseases. Imaging studies permit enhanced information through longitudinal studies of the same animal during the infection. Herein, we briefly review recent studies of animal models of infectious disease that have used imaging modalities. *(Am J Pathol 2013, 182: 296–304; http://dx.doi.org/10.1016/j.ajpath.2012.09.026)*

Small-animal imaging has become an important research tool in studies of infectious diseases and has significantly contributed to both our understanding of pathogenesis and preclinical investigations on drug development. Noninvasive imaging research permits enhanced information through longitudinal studies of animal models of human diseases. Infectious diseases are important causes of morbidity and mortality in humans worldwide. During the past decade, several different small-animal imaging modalities have been applied to studies of infectious disease, including magnetic resonance imaging (MRI), computed tomography (CT), positron emission tomography (PET), bioluminescence imaging (BLI), and intravital imaging. Multiple-modality imaging has become even more attractive because it permits evaluation of the same animals by different imaging technologies, thus reporting on alterations in anatomical characteristics, metabolism, function, and the location of infectious agents. The development of imaging applications in animal models of infectious diseases using these modalities can quickly move from basic research to the clinic. Herein, we review some recent applications of small-animal imaging technologies to the study of infectious diseases.

http://dx.doi.org/10.1016/j.ajpath.2012.09.026

Overview of Imaging Technologies

MRI is a noninvasive imaging modality with high resolution (approximately 50 to 100 μm for small-animal studies) and excellent intrinsic soft tissue contrast. MRI can be used to image anatomical structures, blood flow, and diffusion in the clinic and in experimental animals. Contrast agents (gadolinium or iron-based agents) can be used to specifically label cells or tissues for diagnostic applications. Although micro-CT is the gold standard for imaging bone in mice, contrast agents are required to enhance soft tissues. CT permits longitudinal studies of anatomical characteristics like MRI. It is a high-resolution (>50 μmol/L), fast (minutes) X-ray–based technique. A

Supported by the National Council for Scientific and Technological Development and Fundação de Amparo à Pesquisa do estado de Minas Gerais (F.S.M.); NIH grants AI93220 and AI31788 (L.M.W.), AI076248 (H.B.T.), and NS069577 (M.S.D.); and a Burroughs Wellcome Fund Career Award for Medical Scientists (M.S.D.).

This article is part of a review series on imaging in small-animal models.

A guest editor acted as editor in chief for this manuscript. No person at Thomas Jefferson University or Albert Einstein College of Medicine was involved in the peer review process or final disposition of this article.

Current address of M.P.L., Breakthrough Breast Cancer Research Unit, The University of Manchester, Manchester, UK.

concern with CT, particularly with longitudinal studies, is the radiation dose, which may be high enough to induce changes in the biological pathways being studied, and the need for contrast agents for soft tissue imaging.

PET is a highly sensitive (pmol/L) molecular imaging technique that can be used to visualize a variety of *in vivo* biological processes. Although the resolution of micro-PET (1 to 2 mm) is not as high as that of CT or MRI, it is adequate for small-animal imaging. The molecule 2-deoxy-2-[^{18}F] fluoro-D-glucose (FDG) is routinely used in the clinical setting for the detection of cancer, based on increased uptake of glucose by malignant cells. In addition to ^{18}F-FDG, many other radiotracers can be used in micro-PET studies.

Single-photon emission CT (SPECT) uses radiotracers that permit visualization of specific physiological information, such as blood flow and perfusion, or to measure biodistribution of a radiolabeled molecule or cell. The radioisotopes used are typically longer lived than those used for PET [eg, technetium-99m (half-life of 6 hours) or indium-111 (half-life of 67 hours)]. SPECT is performed in combination with CT to provide anatomical detail; however, the additional exposure to radiation has to be considered in planning longitudinal studies.

Ultrasonographic (US) imaging has high spatial resolution (approximately 50 μm) and contrast in soft tissue. In addition to being portable, US is a fast and economical technique. US has been extensively used for echocardiographic studies of small animals. Recent advances in technology and in contrast agent development have improved resolution such that US studies of many organ systems of small animals are possible.

In vivo BLI has been applied in many studies of small animals and cells. It can be used to monitor gene expression and to track cells. Bioluminescent and fluorescent probes have been engineered to monitor enzymes and the activity of other biologically important molecules. These probes can be used to follow disease progression or response to treatment. Tumor cell lines and microbial pathogens that express luciferase or fluorescent proteins are commonly used for preclinical studies, as are transgenic animals that stably express bioluminescent or fluorescent proteins.

Noninvasive imaging of animals using these technologies reduces animal numbers by permitting the use of animals as their own controls. In addition, therapeutic agents can be developed and tested using imaging technologies that are directly translatable to the clinic. Longitudinal imaging of chronic diseases permits continuous monitoring of disease progression and response to treatment. Table 1 summarizes the advantages and limitations of imaging technologies commonly used in studies of small-animal models of infectious diseases.

Parasitic Infections

Chagas Disease

Trypanosoma cruzi is the causative agent of Chagas disease, a neglected tropical disease, endemic to Latin America, and is being diagnosed in nonendemic areas as a result of immigration.[1] Cardiac manifestations of Chagas disease include acute myocarditis and chronic dilated cardiomyopathy, accompanied by congestive heart failure, arrhythmias, cardioembolism, and stroke.[2] Approximately 30% of infected individuals develop chronic manifestations, including cardiomyopathy, megasyndromes of the gastrointestinal tract, or both. MRI and echocardiography have been useful in the diagnosis of patients infected with *T. cruzi*.[3–5] An extensive review of advances in imaging animals infected with *T. cruzi* has recently been published.[6] Herein, we will focus on the mouse model that has been extensively studied using a variety of imaging modalities, including MRI, echocardiography, and PET imaging using [18F]-FDG.[7–14]

MRI has been most useful for evaluating the right ventricle of mice (Figure 1), which is difficult to visualize with standard echocardiography,[8,12,14] whereas echocardiography has been effective for evaluating left ventricular function.[7,13,15] SPECT imaging has been applied in human studies[16]; however, it has not been reported in animal studies. On the other hand, PET studies have only been reported in the animal model of *T. cruzi* infection (Figure 1). [^{18}F]-FDG-PET has detected changes in glucose metabolism, presumably due to inflammation, early during the course of *T. cruzi* infection and before significant changes in heart structure or function are detected.[13] Infection can also result in loss of smooth muscle tone and destruction of ganglia throughout the bowel and bladder, resulting in megasyndromes of the esophagus, colon, intestines, and bladder, accompanied by severe constipation, difficulty swallowing, and malnutrition (Figure 1).[17] MRI[18–20] and X-ray methods[21,22] have been useful for studying these organs in mice and for evaluating therapeutic strategies. For example, by using MRI, we demonstrated that the administration of the calcium channel blocker, verapamil, early (but not late) in the murine infection reduces the infection-associated increase in right ventricular internal diameter.[15,23] Treatment of infected mice with an endothelin-converting enzyme inhibitor also reduced right ventricular internal diameter, thus illustrating the role of endothelin in the pathogenesis of *T. cruzi*–induced cardiomyopathy.[24] MRI of the mouse model has also demonstrated a loss of adipose tissue, which is consistent with increased expression of enzymes associated with lipolysis.[25]

African Trypanosomiasis

Another neglected tropical disease (World Health Organization classification) is human African trypanosomiasis (HAT) or sleeping sickness. HAT is a parasitic disease transmitted by the tsetse fly that continues to be an important cause of human morbidity and mortality in sub-Saharan Africa due, in part, to armed conflicts resulting in population shifts. HAT is caused by infection with *Trypanosoma brucei rhodesiense* (East Africa) or *T. brucei gambiense* (West Africa). East African disease is actually a zoonosis among game animals, and humans become the accidental host. Disease progression is rapid, with early invasion of the central nervous system (CNS), and, if untreated,

Table 1 Small-Animal Imaging Modalities

Modality	Optimal use	Advantages: serial studies (all)	Limitations
CT	Anatomical body imaging of bone, lung, and heart	High resolution of anatomical structures <50 μm	Not quantitative Ionizing radiation dose Cost Contrast agents required for soft tissue Contrast, tumors, and angiography
PET	Metabolism, perfusion, cell proliferation, apoptosis, hypoxia, and functional imaging	Quantitative function Picomolar sensitivity Molecular targeting Dynamic imaging	Ionizing radiation (requires injection of a radiotracer) Low resolution Improved anatomical resolution requires hybrid devices (PET/CT or PET/MRI) Cost
SPECT	Functional imaging: metabolism, perfusion, hypoxia, and apoptosis	Qualitative function Picomolar sensitivity Molecular targeting	Ionizing radiation (requires injection of a radiotracer) Not quantitative Low resolution (improved with hybrid CT) Cost
MRI	Soft tissues, angiography, perfusion, functional imaging, and tissue oxygenation	Qualitative function Quantitative function High spatial and temporal resolution with intrinsic soft tissue contrast Nonionizing radiation	Medical contraindications Sensitivity limited for some functional measures Cost Some applications require contrast agents (gadolinium- and iron-based agents are common)
US	Whole body imaging and echocardiography	Nonionizing radiation Anatomical features Molecular targeting	Air and bone produce artifacts Not quantitative
Optical imaging (bioluminescence and fluorescence)	Single-cell and single-gene imaging	Nonionizing Inexpensive Molecular targeting	Not for humans

This table lists the modality, optimal use, advantages, and limitations of commonly used small-animal imaging modalities (CT, PET, SPECT, MRI, US, and optical imaging).

death occurs within 9 months. The Gambian type is a chronic disease, with invasion of the CNS occurring late, with a variety of neuropsychiatric disorders. Rodent and primate models mimic the CNS effects of the human disease. MRI has become an important tool for diagnosis of parasitic diseases of the CNS.[26] MRI studies of human patients with African trypanosomiasis report gadolinium enhancement, suggesting that the blood-brain barrier (BBB) is compromised by infection.[27–30] Recently, Rodgers et al[31] used contrast agent–enhanced MRI to evaluate changes in the BBB integrity associated with the early CNS stage of the disease using a well-established murine model of HAT (Figure 2). They found that T_1- and T_2-weighted MRI with the administration of a gadolinium-based contrast agent (Magnevist; Bayer HealthCare, Uxbridge, Middlesex, UK) could detect significant dysfunction in the BBB of infected mice early in the CNS stage of the disease, when only mild to moderate histopathological changes are apparent. Additional MRI studies designed to evaluate BBB integrity throughout the course of trypanosome infection from the early acute stage, when no histopathological changes are detected, to post-treatment reactive encephalopathy, when the animals exhibit severe meningoencephalitis, are needed to establish the value of MRI for diagnosis and evaluation of therapeutic interventions in humans.

Malaria

Infection with *Plasmodium falciparum*, a causative agent of malaria in humans, accounts for almost one million deaths per year, and cerebral malaria is one of the most severe complications of this infection.[33] The pathogenesis of cerebral malaria is likely multifactorial and includes a reduction in cerebral blood flow associated with vasospasm, adherence of infected red blood cells to the endothelium, up-regulation of inflammatory mediators to the CNS and to the cerebral microvasculature,[32,34–37] and both systemic and cerebral metabolic disturbances.[38,39] Neuroimaging, particularly T_1- and T_2-weighted MRI and MR angiography, has become an important tool in elucidating the underlying mechanisms of cerebral malaria through longitudinal studies in animal models and humans that do not rely on autopsy material.[36,40–43]

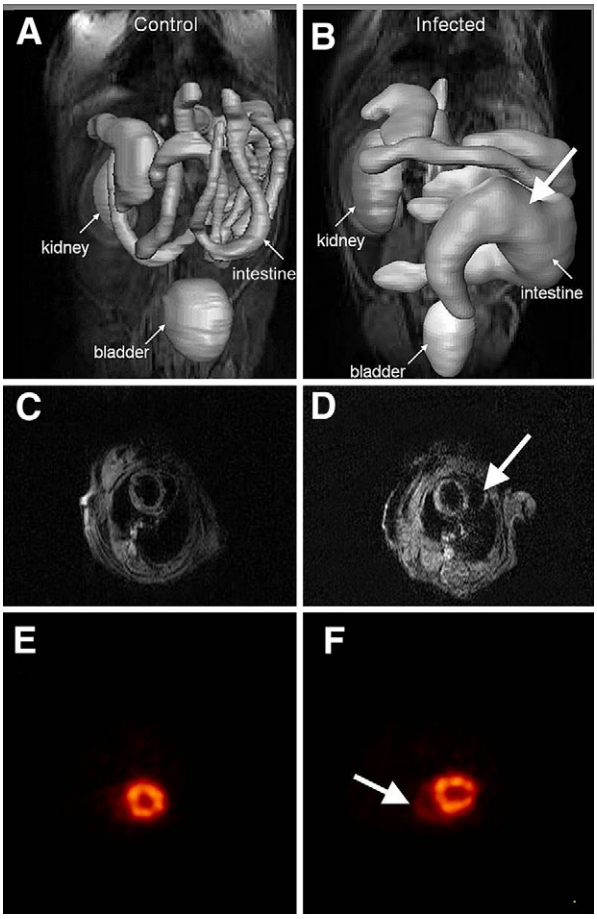

Figure 1 MRI and [^{18}F]-FDG-PET images of control uninfected (**A**, **C**, and **E**) and infected (**B**, **D**, and **F**) mice. MRI of the gastrointestinal (GI) tract (**A** and **B**) and heart (**C** and **D**) and PET of the heart (**E** and **F**). **Large white arrow** indicates enlarged GI tract and right ventricle of the infected mice in **B**, **D**, and **F**. These figures are reproduced from Jelicks et al[12] (**C** and **D**), Prado et al[13] (**E** and **F**), and Ny et al[18] (**A** and **B**), with permission of *The American Journal of Tropical Medicine and Hygiene*.

Although mouse models do not perfectly recapitulate human cerebral malaria, mice infected with *P. berghei*-ANKA (Antwerpen-Kasapa) are an excellent model for imaging. ANKA is a strain of *P. berghei* which confers experimental cerebral malaria in certain rodent strains. These rodents have been invaluable in helping to uncover early markers of the disease that are apparent even before any evidence of swelling in the brain, using T_1- and T_2-weighted MRI and MR angiography.[41,43] Flow-alternating arterial inversion spin-labeling MRI studies and single-voxel proton spectroscopy demonstrated decreased cerebral blood flow, as well as neuronal and axonal injury in mice infected with *P. berghei*-ANKA.[36,43] Saggu et al[41] recently reported the first detection of damage to the optic and trigeminal nerves using T_2-weighted MRI. They previously characterized the disease in mice and observed several characteristic features, including BBB breakdown, hemorrhage, reduced brain perfusion, ischemia, hemodynamic dysfunction, and brain edema.[42,43] In the more recent report, using high-field (11.75-T) MRI, they identified damage to the cranial nerves as the earliest hallmark of the disease before any detectable brain swelling.[41] They observed a hypointense signal in the trigeminal nerves, with significantly reduced dimensions. In addition, they demonstrated that the optic nerves were either hypointense or not visible in images of infected mice.[41] These markers may be clinically relevant and could assist in the early detection of cerebral malaria.

By using MRI and MR angiography to study infected mice deficient in IL-12 receptor β2, Fauconnier et al[44] demonstrated that this molecule is essential for the development of cerebral malaria. Although wild-type mice developed the microvascular pathological characteristics associated with cerebral malaria, the mice deficient in IL-12 receptor β2 developed no neurological signs of the disease.[44]

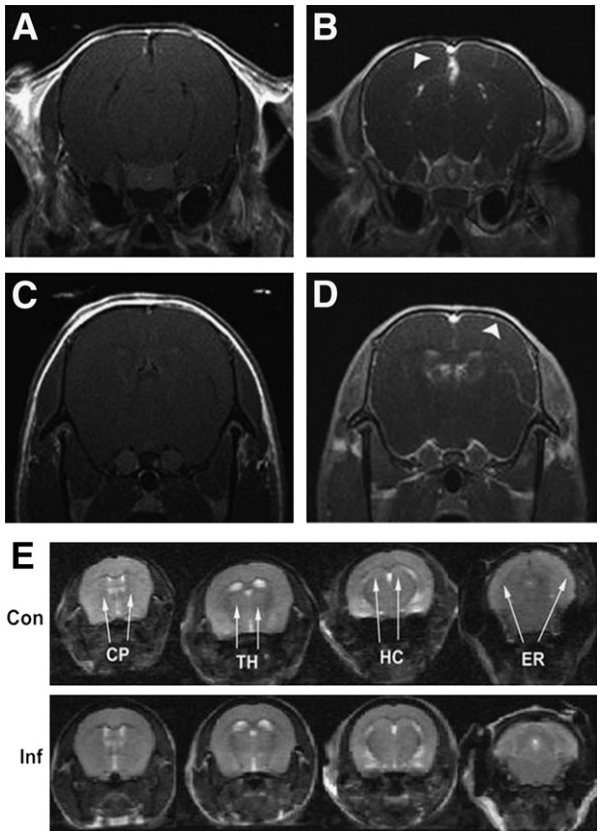

Figure 2 **A**–**D**: MRI scans generated after the administration of contrast agent in an uninfected mouse (**A** and **C**) and an animal scanned 28 days after infection with *T. brucei* (**B** and **D**). **Arrowheads** indicate the presence of clear meningeal enhancement in the infected mouse compared with the uninfected animal imaging to assess BBB damage in murine trypanosomiasis (reproduced from Rodgers et al[31] with permission of the authors and *The American Journal of Tropical Medicine and Hygiene*). **E**: Anatomical images from selected slices in control and infected mice. Slices shown are at the level of the caudate/putamen (CP), thalamus (TH), hippocampus (HC), and entorhinal cortex (ER) (**arrows**). The corresponding anatomical regions are indicated on each slice for the control animals. Con, control; Inf, infected. The images in panel **E** were reproduced from Kennan et al,[32] with permission of Springer Publishing.

Findings of the mouse studies demonstrating the diagnostic value of MRI and the potential for therapeutic applications have been an important basis for the increased use of MRI in patients with cerebral malaria in endemic areas.[40]

[^{18}F]-FDG-PET imaging has been applied in studies of cerebral malaria in a nonhuman primate model and demonstrated decreased cerebral metabolic activity.[38] A diffuse and heterogeneous reduction of metabolic activity in the frontal and temporal lobes before any evidence of neuropathological findings was observed, suggesting that cerebral metabolic changes occur before parenchymal damage in primate cerebral malaria models.[38] A diffuse reduction in activity was postulated to result from decreased blood flow due to sequestration of parasitized red blood cells to the cerebral microcirculation.[38]

Intravital microscopy can examine the living brain through a cranial window. This technique allows for long-term imaging of a single area in the brain for comparison of histopathological alterations and behavioral performances with microvascular changes.[45] Intravital microscopy has emerged as an important tool in determining the underlying pathological features contributing to cerebral malaria. Cabrales and coworkers[37,45,46] have taken advantage of this useful tool to explore the cerebral microvasculature during disease progression. Intravital microcopy was used to visualize the microvasculature after administration of a calcium channel blocker, nimodipine, to mice infected with *P. berghei*-ANKA and demonstrated reversal of the cerebral vascular disturbances during infection associated with improved survival and motor coordination.[37] In other studies, the benefits of treating cerebral malaria in a mouse model with exogenous nitric oxide were demonstrated.[46,47] Intravital imaging will contribute to a greater understanding of microcirculatory hemodynamics and vascular pathological characteristics during the pathogenesis of cerebral malaria in the mouse and will allow researchers to visually assess the function of specific vascular genes by infecting mice deficient in various genes to determine the efficacy of various therapeutic treatments.

Schistosomiasis

Schistosomiasis is a disease caused by several species of the trematode, *Schistosoma*, most notably *S. mansoni*, *S. japonicum*, and *S. hematobium*. The adults are intravascular. The first two species predominantly cause diseases of the liver and mesentery, whereas the latter one causes diseases of the urogenital tract. All three species have invaded the CNS as well. The major lesion is the formation of a granuloma surrounding the egg. This disease is diagnosed by the detection of ova in the feces and urine of infected individuals and by biopsy material. Salem et al[48] used fluorescence molecular tomography, MRI, and [^{18}F]-FDG-PET imaging to evaluate the worm burden in mice infected with *S. mansoni*. [^{18}F]-FDG uptake was correlated with worm burden and was useful for monitoring response to praziquantel treatment. Their results demonstrate the potential for using PET imaging in evaluation of therapeutics for this infection.

Bacterial Infections

Mycobacteria

Tuberculosis is a major public health problem worldwide. The mouse model of *Mycobacterium tuberculosis* has been extensively investigated. Infected mice have been used in a study of superparamagnetic iron oxide nanoparticles conjugated with a surface antibody, developed to improve diagnosis of extrapulmonary *M. tuberculosis*.[49] These *M. tuberculosis* nanoparticles resulted in a 14-fold increase in signal intensity of granulomas on T_2-weighted MRI images and provided a novel noninvasive method for diagnosing extrapulmonary *M. tuberculosis* infections. Although promising, more basic research must be performed to evaluate biodistribution and binding/endocytosis of the particles. In a PET study, Harper et al[50] used copper-64(II)-diacetyl-bis(N4-methyl-thiosemicarbazone), a tracer used to detect hypoxia, to evaluate hypoxia in tuberculosis lesions in mice. During acute infection or in control mice, there was no accumulation of copper-64(II)-diacetyl-bis(N4-methyl-thiosemicarbazone), whereas in chronically infected mice, the tracer accumulated in the lesions in a progressive, time-dependent manner. The accumulated copper-64(II)-diacetyl-bis(N4-methyl-thiosemicarbazone) colocalized with the lesion by CT imaging. [^{18}F]-FDG-PET has been used to evaluate bactericidal activity of drug therapy in mice that were aerosol infected with *M. tuberculosis*.[51] Lesion-specific [^{18}F]-FDG-PET activity correlated with treatment in mice that develop caseating lesions. In another study, Davis et al[52] used SPECT imaging of exogenously labeled *M. tuberculosis*. Mice were infected with wild-type *M. tuberculosis* or *M. tuberculosis* P_{hsp60} thymidine kinase (TK) strains. The *M. tuberculosis* P_{hsp60} TK strain was engineered to express TK using Phsp60, a highly active constitutive mycobacterial promoter. At specific time points, the mice were injected with 1-(2′deoxy-2′-fluoro-β-D-arabinofuranosyl)-5-[^{125}I]-iodouracil ([^{125}I]-FIAU), a nucleoside analog substrate for bacterial TK, and SPECT and CT imaging studies were performed at 3 and 24 hours after injection with the mice in a biocontainment device. SPECT imaging detected and localized the *M. tuberculosis* P_{hsp60} TK strain, but not the wild-type *M. tuberculosis*. Their data suggest that as few as 5 to 10 million *M. tuberculosis* P_{hsp60} TKs inside a granuloma could be detected using SPECT. These studies demonstrate the application of noninvasive imaging to monitor treatment response in small-animal models. The methods can be used to test therapeutics and develop regimens that can be extended to humans, who can then be monitored using the same type of imaging procedures.

Other Bacteria

Listeria monocytogenes is a Gram-positive bacillus that causes a variety of human and animal infections, including gastroenteritis, sepsis, miscarriage, stillbirth, and neonatal meningitis. Hardy et al[53] used US (for viability), MRI (for morphological characteristics), and BLI (for tracking *L. monocytogenes*) to study *Listeria*-induced miscarriage, using a pregnant mouse model. Ultrasonography revealed sustained bradycardia in the infected fetuses, although MRI detected no malformations of the fetuses, even when BLI indicated a high degree of infection. The study demonstrates the potential of using the multimodality approach to study the causes of miscarriage.

Bettegowda et al[54] used SPECT and CT imaging to localize bacterial infections with [^{125}I]-FIAU in mice. *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pneumonia*, *Enterococcus faecalis*, and *Staphylococcus epidermidis* were used to generate localized experimental infections in the thigh of mice; these mice were subsequently injected via the tail vein with [^{125}I]-FIAU imaged by SPECT at specific time points. All five strains of bacteria could be imaged with robust uptake at 4 hours after injection, and a high signal/noise ratio was observed 48 hours after injection. The authors speculate that [^{124}I]-FIAU, a positron emitter, or other positron-emitting nucleosides would be even more sensitive and that novel diagnostics and therapeutics could be developed based on the widespread presence and substrate specificities of bacterial TKs.

In another study of *S. aureus*, an engineered analog of prothrombin was used to study the bacteria in endocarditic vegetations with noninvasive fluorescence or PET imaging.[55] Fluorescent-labeled prothrombin (AF680-ProT) was injected into a mouse model of endocarditis, and imaging was performed using fluorescence molecular tomography fused to X-ray CT. High local concentrations of AF680-ProT were observed in *S. aureus*—induced vegetations 24 hours after injection of the probe. For the PET-CT studies, ^{64}Cu-diethylenetriaminepentaacetic acid-ProT was used, along with a genetically engineered *S. aureus* strain that expressed luciferase at sites of infection. The multimodality approach permitted confirmation of the presence of the bacteria using BLI, which was correlated with the PET imaging of ^{64}Cu-diethylenetriaminepentaacetic acid-ProT and demonstrated that PET imaging could be used to evaluate bacterial load. ^{64}Cu-PET has also been used to study the dissemination of *Francisella tularensis*, the cause of tularemia, when administered intranasally, intratracheally, intragastrically, intradermally, i.p., or i.v. in mice.[56] The results demonstrated that *Francisella* rapidly disseminates within hours to multiple tissues via most routes of administration, although different trafficking patterns were observed. Infection via the pulmonary routes resulted in rapid spread to the lung and gastrointestinal tract.

^{67}Gallium-citrate scintigraphy has been used in routine diagnostics of infections in the clinical setting; however, it has a long (3-day) half-life and is expensive, in addition to having safety concerns. ^{68}Ga, a PET tracer, has a short (68-minute) half-life, a lower cost, and fewer safety concerns. Nanni et al[57] tested ^{68}Ga-chloride as a PET tracer in mice infected with *Chlamydia muridarum.* Although the tracer demonstrated some promise for assessing genital infection, ^{68}Ga uptake was high in control mice with aseptic inflammation caused by the sham procedure and in the infected mice. In another study, *Streptococcus pyrogenes* infection and lipopolysaccharide inflammation in mice were investigated using fluorescent and SPECT/CT imaging with the tracer, ^{111}In-labeled tetraazacyclododecanete tetraacetic acid-biotin, linked to zinc-dipicolylamine-biotin with streptavidin.[58] There was significantly higher accumulation of this tracer in the live bacterial infection in one thigh compared with the sterile inflammation in the other thigh, suggesting that zinc-dipicolylamine may be useful for distinguishing between infection and inflammation. MRI has also been used in longitudinal studies of meningitis in mice.

Viral and Fungal Infections

BLI has been a powerful technique for tracking bacteria, fungi, parasites, and viruses that cause infectious diseases.[59,60] Kang et al[61] have used BLI to monitor virus progression after CNS infection of mice with murine γ-herpesvirus. Murine γ-herpesvirus is similar to human γ-herpesvirus, Epstein-Barr virus, and Kaposi's sarcoma—associated herpesvirus and provides a mouse model for studying the involvement in neurological diseases. After CNS infection, the virus spreads to the spleen, and latent virus could be activated from both the brain and the spleen. Their results suggest a role for the brain as a site for viral persistency after CNS infection. Rift Valley fever is another viral infection that has been studied by BLI in mice.[62] The infection is typically asymptomatic or mild, although a few patients exhibit complications and death is associated with high viral load in blood. Real-time dissemination of the virus in immune-deficient mice was tracked and demonstrated that the thymus, spleen, and liver were infected first, with the liver being the main location for viral replication. BLI can also be used to track fungal infections. The fungus *Aspergillus terreus* is a life-threatening complication in immune-compromised patients. Slesiona et al[63] have used bioluminescent *A. terreus* in mice and demonstrated long-term persistence of the *A. terreus* conidia using BLI. Studies, using the luminescent bacteria strain, *S. aureus* Xen 29, have demonstrated the use of BLI to evaluate therapeutics in mice.[64,65] These studies represent a few recent examples of the potential value of BLI for tracking infection.

MRI has also been valuable for studying viral infections. Diffusion-tensor MRI has been applied to study neuronal loss during HIV-1 infection in a humanized mouse model.[66] Structural changes in gray matter were revealed by imaging and confirmed by immunohistochemistry. To

our knowledge, this study was the first to demonstrate such associations and underscored the potential of humanized mouse models for research in infectious diseases.

Concluding Remarks

Much progress has been made in tracking parasites, bacteria, fungi, and viruses using multimodality small-animal imaging approaches.[59,60] The future development of multimodality molecular imaging studies for investigating the pathogenesis of infection and for evaluating the therapeutics and translation to the clinical setting for diagnostic and theranostic applications will provide researchers and clinicians the tools to have a positive impact on patient care.

References

1. Tanowitz HB, Weiss LM, Montgomery SP: Chagas disease has now gone global. PLoS Negl Trop Dis 2011, 5:e1136
2. Machado FS, Jelicks LA, Kirchhoff LV, Shirani J, Nagajyothi F, Mukherjee S, Nelson R, Coyle CM, Spray DC, de Carvalho AC, Guan F, Prado CM, Lisanti MP, Weiss LM, Montgomery SP, Tanowitz HB: Chagas heart disease: report on recent developments. Cardiol Rev 2012, 20:53–65
3. Viotti RJ, Vigliano C, Laucella S, Lococo B, Petti M, Bertocchi G, Ruiz Vera B, Armenti H: Value of echocardiography for diagnosis and prognosis of chronic Chagas disease cardiomyopathy without heart failure. Heart 2004, 90:655–660
4. Garcia-Alvarez A, Sitges M, Regueiro A, Poyatos S, Jesus Pinazo M, Posada E, Bijnens B, Heras M, Gascon J, Sanz G: Myocardial deformation analysis in Chagas heart disease with the use of speckle tracking echocardiography. J Cardiac Fail 2011, 17: 1028–1034
5. Rochitte CE, Nacif MS, de Oliveira Junior AC, Siqueira-Batista R, Marchiori E, Uellendahl M, de Lourdes Higuchi M: Cardiac magnetic resonance in Chagas' disease. Artif Organs 2007, 31:259–267
6. Jelicks LA, Tanowitz HB: Advances in imaging of animal models of Chagas disease. Adv Parasitol 2011, 75:193–208
7. Chandra M, Tanowitz HB, Petkova SB, Huang H, Weiss LM, Wittner M, Factor SM, Shtutin V, Jelicks LA, Chan J, Shirani J: Significance of inducible nitric oxide synthase in acute myocarditis caused by Trypanosoma cruzi (Tulahuen strain). Int J Parasitol 2002, 32:897–905
8. de Souza AP, Tang B, Tanowitz HB, Araujo-Jorge TC, Jelicks EL: Magnetic resonance imaging in experimental Chagas disease: a brief review of the utility of the method for monitoring right ventricular chamber dilatation. Parasitol Res 2005, 97:87–90
9. Durand JL, Mukherjee S, Commodari F, De Souza AP, Zhao D, Machado FS, Tanowitz HB, Jelicks LA: Role of NO synthase in the development of Trypanosoma cruzi-induced cardiomyopathy in mice. Am J Trop Med Hyg 2009, 80:782–787
10. Durand JL, Tang B, Gutstein DE, Petkova S, Teixeira MM, Tanowitz HB, Jelicks LA: Dyskinesis in Chagasic myocardium: centerline analysis of wall motion using cardiac-gated magnetic resonance images of mice. Mag Reson Imag 2006, 24:1051–1057
11. Goldenberg RC, Jelicks LA, Fortes FS, Weiss LM, Rocha LL, Zhao D, Carvalho AC, Spray DC, Tanowitz HB: Bone marrow cell therapy ameliorates and reverses chagasic cardiomyopathy in a mouse model. J Infect Dis 2008, 197:544–547
12. Jelicks LA, Shirani J, Wittner M, Chandra M, Weiss LM, Factor SM, Bekirov I, Braunstein VL, Chan J, Huang H, Tanowitz HB: Application of cardiac gated magnetic resonance imaging in murine Chagas' disease. Am J Trop Med Hyg 1999, 61:207–214
13. Prado CM, Fine EJ, Koba W, Zhao D, Rossi MA, Tanowitz HB, Jelicks LA: Micro-positron emission tomography in the evaluation of Trypanosoma cruzi-induced heart disease: comparison with other modalities. Am J Trop Med Hyg 2009, 81:900–905
14. Souza AP, Jelicks LA, Tanowitz HB, Olivieri BP, Medeiros MM, Oliveira GM, Pires AR, Santos AM, Araujo-Jorge TC: The benefits of using selenium in the treatment of Chagas disease: prevention of right ventricle chamber dilatation and reversion of Trypanosoma cruzi-induced acute and chronic cardiomyopathy in mice. Mem Instit Oswaldo Cruz 2010, 105:746–751
15. Chandra M, Shirani J, Shtutin V, Weiss LM, Factor SM, Petkova SB, Rojkind M, Dominguez-Rosales JA, Jelicks LA, Morris SA, Wittner M, Tanowitz HB: Cardioprotective effects of verapamil on myocardial structure and function in a murine model of chronic Trypanosoma cruzi infection (Brazil strain): an echocardiographic study. Int J Parasitol 2002, 32:207–215
16. Mahía P, Pineda V, Cuéllar H: Imaging techniques in Chagas' disease, [in English, Spanish]. Rev Esp Cardiol 2009, 62:689
17. Jelicks LA: Imaging the gastrointestinal tract of small animals. J Neuroparasitol 2010, 1:N100504
18. Ny L, Li H, Mukherjee S, Persson K, Holmqvist B, Zhao D, Shtutin V, Huang H, Weiss LM, Machado FS, Factor SM, Chan J, Tanowitz HB, Jelicks LA: A magnetic resonance imaging study of intestinal dilation in Trypanosoma cruzi-infected mice deficient in nitric oxide synthase. Am J Trop Med Hyg 2008, 79: 760–767
19. Boczko J, Tar M, Melman A, Jelicks LA, Wittner M, Factor SM, Zhao D, Hafron J, Weiss LM, Tanowitz HB, Christ GJ: Trypanosoma cruzi infection induced changes in the innervation, structure and function of the murine bladder. J Urol 2005, 173: 1784–1788
20. de Souza AP, Sieberg R, Li H, Cahill HR, Zhao D, Araujo-Jorge TC, Tanowitz HB, Jelicks LA: The role of selenium in intestinal motility and morphology in a murine model of Typanosoma cruzi infection. Parasitol Res 2010, 106:1293–1298
21. Mori T, Yoon HS, Iizuka FH, Myung JM, Sato HR, Silva MF, Okumura M: Intestinal transit and opaque enema study in chagasic mice. Rev Hosp Clin Fac Med Sao Paulo 1995, 50:63–66
22. Guillén-Pernía B, Lugo-Yarbuh A, Moreno E: Digestive tract dilation in mice infected with Trypanosoma cruzi, [in Spanish]. Invest Clin 2001, 42:195–209
23. De Souza AP, Tanowitz HB, Chandra M, Shtutin V, Weiss LM, Morris SA, Factor SM, Huang H, Wittner M, Shirani J, Jelicks LA: Effects of early and late verapamil administration on the development of cardiomyopathy in experimental chronic Trypanosoma cruzi (Brazil strain) infection. Parasitol Res 2004, 92:496–501
24. Jelicks LA, Chandra M, Shirani J, Shtutin V, Tang B, Christ GJ, Factor SM, Wittner M, Huang H, Weiss LM, Mukherjee S, Bouzahzah B, Petkova SB, Teixeira MM, Douglas SA, Loredo ML, D'Orleans-Juste P, Tanowitz HB: Cardioprotective effects of phosphoramidon on myocardial structure and function in murine Chagas' disease. Int J Parasitol 2002, 32:1497–1506
25. Nagajyothi F, Desruisseaux MS, Machado FS, Upadhya R, Zhao D, Schwartz GJ, Teixeira MM, Albanese C, Lisanti MP, Chua SC Jr, Weiss LM, Scherer PE, Tanowitz HB: Response of adipose tissue to early infection with Trypanosoma cruzi (Brazil strain). J Infect Dis 2012, 205:830–840
26. Abdel Razek AA, Watcharakorn A, Castillo M: Parasitic diseases of the central nervous system. Neuroimaging Clin N Am 2011, 21: 815–841, viii
27. Kager PA, Schipper HG, Stam J, Majoie CB: Magnetic resonance imaging findings in human African trypanosomiasis: a four-year follow-up study in a patient and review of the literature. Am J Trop Med Hyg 2009, 80:947–952
28. Braakman HM, van de Molengraft FJ, Hubert WW, Boerman DH: Lethal African trypanosomiasis in a traveler: MRI and neuropathology. Neurology 2006, 66:1094–1096

29. Gill DS, Chatham DS, del Carpio-O'Donovan R: MR imaging findings in African trypansomiasis. AJNR Am J Neuroradiol 2003, 24: 1383−1385
30. Sabbah P, Brosset C, Imbert P, Bonardel G, Jeandel P, Briant JF: Human African trypanosomiasis: MRI. Neuroradiology 1997, 39: 708−710
31. Rodgers J, McCabe C, Gettinby G, Bradley B, Condon B, Kennedy PG: Magnetic resonance imaging to assess blood-brain barrier damage in murine trypanosomiasis. Am J Trop Med Hyg 2011, 84:344−350
32. Kennan RP, Machado FS, Lee SC, Desruisseaux MS, Wittner M, Tsuji M, Tanowitz HB: Reduced cerebral blood flow and N-acetyl aspartate in a murine model of cerebral malaria. Parasitol Res 2005, 96: 302−307
33. World Health Organization, Communicable Diseases Cluster: Severe falciparum malaria. Trans R Soc Trop Med Hyg 2000, 94(Suppl 1): S1−S90
34. Beare NA, Harding SP, Taylor TE, Lewallen S, Molyneux ME: Perfusion abnormalities in children with cerebral malaria and malarial retinopathy. J Infect Dis 2009, 199:263−271
35. Dorovini-Zis K, Schmidt K, Huynh H, Fu W, Whitten RO, Milner D, Kamiza S, Molyneux M, Taylor TE: The neuropathology of fatal cerebral malaria in Malawian children. Am J Pathol 2011, 178: 2146−2158
36. John CC, Panoskaltsis-Mortari A, Opoka RO, Park GS, Orchard PJ, Jurek AM, Idro R, Byarugaba J, Boivin MJ: Cerebrospinal fluid cytokine levels and cognitive impairment in cerebral malaria. Am J Trop Med Hyg 2008, 78:198−205
37. Cabrales P, Zanini GM, Meays D, Frangos JA, Carvalho LJ: Murine cerebral malaria is associated with a vasospasm-like microcirculatory dysfunction, and survival upon rescue treatment is markedly increased by nimodipine. Am J Pathol 2010, 176:1306−1315
38. Sugiyama M, Ikeda E, Kawai S, Higuchi T, Zhang H, Khan N, Tomiyoshi K, Inoue T, Yamaguchi H, Katakura K, Endo K, Suzuki M: Cerebral metabolic reduction in severe malaria: fluorodeoxyglucose-positron emission tomography imaging in a primate model of severe human malaria with cerebral involvement. Am J Trop Med Hyg 2004, 71:542−545
39. Idro R, Ndiritu M, Ogutu B, Mithwani S, Maitland K, Berkley J, Crawley J, Fegan G, Bauni E, Peshu N, Marsh K, Neville B, Newton C: Burden, features, and outcome of neurological involvement in acute falciparum malaria in Kenyan children. JAMA 2007, 297: 2232−2240
40. Potchen MJ, Kampondeni SD, Seydel KB, Birbeck GL, Hammond CA, Bradley WG, Demarco JK, Glover SJ, Ugorji JO, Latourette MT, Siebert JE, Molyneux ME, Taylor TE: Acute brain MRI findings in 120 Malawian children with cerebral malaria: new insights into an ancient disease. AJNR Am J Neuroradiol 2012, 33:1740−1746
41. Saggu R, Faille D, Grau GE, Cozzone PJ, Viola A: In the eye of experimental cerebral malaria. Am J Pathol 2011, 179:1104−1109
42. Penet MF, Kober F, Confort-Gouny S, Le Fur Y, Dalmasso C, Coltel N, Liprandi A, Gulian JM, Grau GE, Cozzone PJ, Viola A: Magnetic resonance spectroscopy reveals an impaired brain metabolic profile in mice resistant to cerebral malaria infected with Plasmodium berghei ANKA. J Biol Chem 2007, 282:14505−14514
43. Penet MF, Viola A, Confort-Gouny S, Le Fur Y, Duhamel G, Kober F, Ibarrola D, Izquierdo M, Coltel N, Gharib B, Grau GE, Cozzone PJ: Imaging experimental cerebral malaria in vivo: significant role of ischemic brain edema. J Neurosci 2005, 25:7352−7358
44. Fauconnier M, Palomo J, Bourigault ML, Meme S, Szeremeta F, Beloeil JC, Danneels A, Charron S, Rihet P, Ryffel B, Quesniaux VF: IL-12Rbeta2 is essential for the development of experimental cerebral malaria. J Immunol 2012, 188:1905−1914
45. Cabrales P, Carvalho LJ: Intravital microscopy of the mouse brain microcirculation using a closed cranial window. J Vis Exp 2010, (45): 2184
46. Cabrales P, Zanini GM, Meays D, Frangos JA, Carvalho LJ: Nitric oxide protection against murine cerebral malaria is associated with improved cerebral microcirculatory physiology. J Infect Dis 2011, 203: 1454−1463
47. Zanini GM, Cabrales P, Barkho W, Frangos JA, Carvalho LJ: Exogenous nitric oxide decreases brain vascular inflammation, leakage and venular resistance during Plasmodium berghei ANKA infection in mice. J Neuroinflammation 2011, 8:66
48. Salem N, Balkman JD, Wang J, Wilson DL, Lee Z, King CL, Basilion JP: In vivo imaging of schistosomes to assess disease burden using positron emission tomography (PET). PLoS Negl Trop Dis 2010, 4: pii: e827
49. Lee CN, Wang YM, Lai WF, Chen TJ, Yu MC, Fang CL, Yu FL, Tsai YH, Chang WH, Zuo CS, Renshaw PF: Super-paramagnetic iron oxide nanoparticles for use in extrapulmonary tuberculosis diagnosis. Clin Microbiol Infect 2012, 18:E149−E157
50. Harper J, Skerry C, Davis SL, Tasneen R, Weir M, Kramnik I, Bishai WR, Pomper MG, Nuermberger EL, Jain SK: Mouse model of necrotic tuberculosis granulomas develops hypoxic lesions. J Infect Dis 2012, 205:595−602
51. Davis SL, Nuermberger EL, Um PK, Vidal C, Jedynak B, Pomper MG, Bishai WR, Jain SK: Noninvasive pulmonary [18F]-2-fluoro-deoxy-D-glucose positron emission tomography correlates with bactericidal activity of tuberculosis drug treatment. Antimicrob Agent Chemother 2009, 53:4879−4884
52. Davis SL, Be NA, Lamichhane G, Nimmagadda S, Pomper MG, Bishai WR, Jain SK: Bacterial thymidine kinase as a non-invasive imaging reporter for Mycobacterium tuberculosis in live animals. PLoS One 2009, 4:e6297
53. Hardy J, Kirkendoll B, Zhao H, Pisani L, Luong R, Switzer A, McConnell MV, Contag CH: Infection of pregnant mice with Listeria monocytogenes induces fetal bradycardia. Pediatr Res 2012, 71: 539−545
54. Bettegowda C, Foss CA, Cheong I, Wang Y, Diaz L, Agrawal N, Fox J, Dick J, Dang LH, Zhou S, Kinzler KW, Vogelstein B, Pomper MG: Imaging bacterial infections with radiolabeled 1-(2′-deoxy-2′-fluoro-beta-D-arabinofuranosyl)-5-iodouracil. Proc Natl Acad Sci U S A 2005, 102:1145−1150
55. Panizzi P, Nahrendorf M, Figueiredo JL, Panizzi J, Marinelli B, Iwamoto Y, Keliher E, Maddur AA, Waterman P, Kroh HK, Leuschner F, Aikawa E, Swirski FK, Pittet MJ, Hackeng TM, Fuentes-Prior P, Schneewind O, Bock PE, Weissleder R: In vivo detection of Staphylococcus aureus endocarditis by targeting pathogen-specific prothrombin activation. Nat Med 2011, 17:1142−1146
56. Ojeda SS, Wang ZJ, Mares CA, Chang TA, Li Q, Morris EG, Jerabek PA, Teale JM: Rapid dissemination of Francisella tularensis and the effect of route of infection. BMC Microbiol 2008, 8: 215
57. Nanni C, Marangoni A, Quarta C, Di Pierro D, Rizzello A, Trespidi S, D'Ambrosio D, Ambrosini V, Donati M, Aldini R, Zanotti-Fregonara P, Grassetto G, Rubello D, Fanti S, Cevenini R: Small animal PET for the evaluation of an animal model of genital infection. Clin Physiol Funct Imag 2009, 29:187−192
58. Liu X, Cheng D, Gray BD, Wang Y, Akalin A, Rusckowski M, Pak KY, Hnatowich DJ: Radiolabeled Zn-DPA as a potential infection imaging agent. Nucl Med Biol 2012, 39:709−714
59. Luker KE, Luker GD: Applications of bioluminescence imaging to antiviral research and therapy: multiple luciferase enzymes and quantitation. Antiviral Res 2008, 78:179−187
60. Hutchens M, Luker GD: Applications of bioluminescence imaging to the study of infectious diseases. Cell Microbiol 2007, 9:2315−2322
61. Kang HR, Cho HJ, Kim S, Song IH, Lee TS, Hwang S, Sun R, Song MJ: Persistent infection of a gammaherpesvirus in the central nervous system. Virology 2012, 423:23−29
62. Gommet C, Billecocq A, Jouvion G, Hasan M, Zaverucha do Valle T, Guillemot L, Blanchet C, van Rooijen N, Montagutelli X, Bouloy M, Panthier JJ: Tissue tropism and target cells of NSs-deleted rift valley fever virus in live immunodeficient mice. PLoS Negl Trop Dis 2011, 5:e1421

63. Slesiona S, Ibrahim-Granet O, Olias P, Brock M, Jacobsen ID: Murine infection models for Aspergillus terreus pulmonary aspergillosis reveal long-term persistence of conidia and liver degeneration. J Infect Dis 2012, 205:1268−1277
64. van Staden AD, Brand AM, Dicks LM: Nisin F-loaded brushite bone cement prevented the growth of Staphylococcus aureus in vivo. J Appl Microbiol 2012, 112:831−840
65. Piper C, Casey PG, Hill C, Cotter PD, Ross RP: The lantibiotic lacticin 3147 prevents systemic spread of Staphylococcus aureus in a murine infection model. Int J Microbiol 2012, 2012:806230
66. Dash PK, Gorantla S, Gendelman HE, Knibbe J, Casale GP, Makarov E, Epstein AA, Gelbard HA, Boska MD, Poluektova LY: Loss of neuronal integrity during progressive HIV-1 infection of humanized mice. J Neurosci 2011, 31:3148−3157