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Magnetic resonance imaging characterization of microbial infections☆

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ABSTRACT

The investigation of microbial infections relies to a large part on animal models of infection, if host pathogen interactions or the host response are considered. Especially for the assessment of novel therapeutic agents, animal models are required. Non-invasive imaging methods to study such models have gained increasing importance over the recent years. In particular, magnetic resonance imaging (MRI) affords a variety of diagnostic options, and can be used for longitudinal studies. In this review, we introduce the most important MRI modalities that show how MRI has been used for the investigation of animal models of infection previously and how it may be applied in the future.

1. Introduction

Magnetic resonance imaging (MRI) has developed into the most versatile and widely used clinical diagnostic tool over the last three decades. MRI is an essential part of the clinical diagnostic routine in acute injury, musculoskeletal disease, pathologies of the brain such as stroke or neurodegenerative disease, detection and staging of cancer, and in cardiac imaging. However, for the diagnosis of infectious disease, both bacterial and viral, its application is limited and it is primarily used to detect local inflammation, edema formation and other manifestations of the immune response, but not to detect the infectious agent directly or follow the spread of metastatic infections [1–3]. This lack of application is astonishing, since infectious disease is still one of the major unsolved problems in health care worldwide.

Research in medical microbiology consists of two main fields of inquiry. The first investigates the pathology and host interactions important to understand the mechanisms of the disease and develop novel therapeutic strategies. Second resistance mechanisms have to be explored and novel agents tested. In addition to basic scientific investigation research is also prompted by the urgent clinical need for more specific and efficient diagnostic tools for infectious diseases. Many infections can lead to severe diseases such as endocarditis, osteomyelitis, or encephalopathies, which often result in devastating conditions. In particular, early or chronic infections are difficult to detect and, for example in osteomyelitis, it is often not possible to distinguish non-infected from infected tissue intra-operatively. A diagnostic imaging tool that non-invasively detects pathogens and discriminates between bacterial infections and other sources of inflammation would greatly enhance diagnostic and therapeutic options and thus aid disease management. To this end different imaging techniques such as bioluminescence (BLI) and fluorescence imaging, positron emission tomography (PET), single photon emission computed tomography (SPECT) and MRI have been developed and applied to both patients and animal models of infections [1,3–5].

PET methods usually detect the dissemination of pathogens indirectly via changes in cellular processes and metabolic turn over. With SPECT a number of promising specific radiotracer molecules based on antibiotics, antimicrobial peptides, cytokines, or monoclonal antibodies have been suggested to specifically label bacterial infections. However, due to the low resolution of this technique further and improved imaging modalities are required [6]. For preclinical or basic microbiological research purposes BLI, fluorescence imaging (fluorescence reflectance imaging (FRI)) and fluorescence mediated tomography (FMT) represent promising alternative technologies [4,7]. BLI studies of viral or bacterial infections are typically performed using recombinant pathogens, genetically engineered to express a luciferase enzyme. Low sensitivity of a single bacterium, however, limits the application of BLI. By contrast, fluorescence imaging using green or red fluorescent proteins allows in vivo detection of single cells when invasive methods are applied. When used for non-invasive imaging, however, autofluorescence and scattering become significantly limiting issues. To this end, near-infrared fluorescent probes have been developed, which possess

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great potential for this purpose, since red fluorescent dyes increase penetration depth and reduce problems caused by green autofluorescence of tissue [8]. Recently, the feasibility of detecting inflammation in the murine lung by FMT has been shown [7]. However, due to the poor spatial resolution and the lack of intrinsic contrast FMT requires co-registration with other modalities, such as MRI.

While BLI, FMT, and also FRI are valuable tools for research in animal models and may contribute to our understanding of pathogenicity mechanisms of microorganisms, their application in patients is most unlikely. Finally, pathogens have been indirectly detected by MR diagnostic imaging through edema formation and changes in local tissue parameters such as relaxation time, water content, or diffusivity. Both for the development of novel imaging technologies and for the understanding of disease mechanisms and immune response the use of small animal models of infections is important to simulate the complex interactions in the living organism. A general review of imaging techniques with respect to their application in small animal models of infection has recently been published [3]. This review will focus on current MRI techniques and recent MRI developments, which are applicable to microbiology research using animal models of infection.

2. Endogenous MRI contrast

The strength of MRI lies in its excellent intrinsic soft tissue contrast, unlimited penetration depth, and high anatomical resolution. Small local differences in tissue relaxation time, water content, or diffusivity can be detected readily by T_1, T_2, proton density, or diffusion weighted spin echo or gradient echo MR sequences, and can subsequently be related to defined anatomical structures with high accuracy. Usually both spin echo and gradient echo sequences can be used to create the desired contrast, however, gradient echoes are preferred when susceptibility differences are expected as major source of contrast. These effects are most pronounced in gradient echo images, since the detected echo is created by matching bipolar gradients, which do not refocus magnetic field inhomogeneities [9]. To some extent all of these parameters affect the contrast in the acquired MR image. However, by choice of the appropriate echo times (TE) and repetition times (TR), a particular type of contrast can be emphasized. Table 1 lists the typical TE and TR values used for spin echo and gradient echo sequences to achieve a specifically weighted image [10]. Corresponding spin echo images of the mouse brain are shown in Fig. 1A.

T_1 weighted images are less sensitive to subtle differences in soft tissue composition and may be suitable to depict anatomical structures with high resolution. T_2 weighted images are usually more sensitive to water content and molecular composition of soft tissue, and therefore T_2 weighting is the most common technique to describe and recognize pathologically altered tissue. Proton density-weighted images are mostly used to resolve the gross anatomical structure. In infections the pathogenicity of bacteria, viruses, fungi, and parasites usually originates from a single class or a small number of molecules such as toxins, exoenzymes, adhesins, or immune-modulating proteins, which are released from the pathogen. Many of these PAMPs (pathogen associated molecular pattern) are recognized by members of the Toll-like receptor (TLR) trans-membrane protein family. These proteins play a key role in mediating the systemic responses to invading pathogens, and trigger automatic inflammation processes including dilated blood vessels and increased blood flow [11] as one of the first responses to an infection [12]. The increased blood flow helps defensive immune cells such as leucocytes (macrophages, neutrophils, dendritic cells, mast cells, eosinophils, basophils, and natural killer cells) and lymphocytes (B and T cells) reach the place of infection. Additionally, complement activation is often involved in inflammation and leads to a high concentration of a variety of different proteins and antibodies in the region of infection [13]. The combination of all immunological reactions leads to a high proton density and prolonged tissue relaxation times at the site of infection. These can be detected easily as hyperintensities in T_2 weighted images [14,15]. Thus, lesions in the brain with bright MRI signals have been detected and characterized successfully with respect to Shiga toxin-producing *Escherichia coli* infections [16], *Herpes simplex* encephalitis (Fig. 2) [17–19], Cryptococcal meningoencephalitis [20], *Citrobacter koseri* brain abscesses [21], sepsis-associated encephalopathy [22], Lymphocytic Choriomeningitis Virus infections [23], and *Toxoplasma gondii* brain infections [24]. Recently, endogenous T_2 contrast was also defined as early marker of cerebral malaria, one of the most severe complications caused by *Plasmodium falciparum*. This disease results in damaged optical and trigeminal nerves, depicted as hypointensities in T_2 weighted imaging [25–27]. In addition to animal models of cerebral infections lesions of inflammation were successfully investigated by endogenous T_1 and T_2 contrast in *Staphylococcus aureus* induced osteomyelitis [28], *Helicobacter bilis*-induced colon cancer [29], LPS-induced sepsis [30], *Aspergillus fumigates*, *Candida albicans* and *S. aureus*-induced muscle infections [31–33] as well as in *Streptococcus* or *Klebsiella*-induced pneumonia [34,35], and pulmonary alveolar Echinococcosis [36].

Proton MRI of the lung is known to be especially challenging since air-tissue interfaces produce strong susceptibility artifacts leading to very fast T_2^* relaxation. In the work of Marzola et al. it was shown that edema formation in lung infections resulted in significantly modified lung tissue, leading to a significant increase in T_2^* which allowed for the detection of hyperintense lesions in both T_2^* and T_1 weighted gradient echo images [35]. The applicability of this technique to *Pseudomonas aeruginosa*-induced cystic lung fibrosis [37] and poststroke pneumonia [38] were also studied. Recent developments are based on the application of hyperpolarized ^{3}He and ^{129}Xe gases to assess the lung function during an infection [39].

The excellent soft tissue contrast of MRI can also detect abnormalities of the gastrointestinal tract [40,41]. Recent studies focused on the spleen, which is the most important organ in filtering infectious microorganisms. Structural and functional changes of the spleen in a Plasmodium infection were analyzed using T_2 maps and T_1 weighted steady-state imaging [42].

Besides the strong tissue contrast induced by differences in the relaxation times, changes in the self-diffusion of water molecules can be used as a parameter to detect and characterize zones of inflammation. Molecular diffusion is usually a three-dimensional motion and free water diffuses randomly in all spatial directions. In tissue, however, random Brownian motion of water molecules is restricted by surrounding tissue, leading to preferred water diffusion along the orientation of neuronal or muscular fibers. The diffusion anisotropy represents a reasonable measure of the anisotropy of tissue structure. The diffusivity of water molecules is usually measured as the apparent diffusion coefficient (ADC) and the preferred diffusion directions are characterized by the fractional anisotropy (FA). Both parameters are usually measured three-dimensionally using DTI with diffusion encoding along 30 or more directions (Fig. 1B) [43,44]. Thus, the local diffusion anisotropy and diffusion coefficient can be calculated and modeled by a tensor, providing data of diffusion directions in the tissue of interest. When tissue is inflamed or destroyed by injuries, the cell density is diminished due to large amounts of infiltrating water or cell swelling. The result is dramatic changes in water diffusivity and the resulting tissue ADC and FA values [45,46]. This approach has been applied to the study of the development and progression of pneumococcal meningitis [47,48], sepsis-associated