Molecular Imaging of Inflammation: Current Status

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Running Title: molecular imaging of inflammation

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ABSTRACT

The ability to image inflammation in vivo can improve our understanding of the pathophysiology underlying various disease etiologies including cancer, atherosclerosis and neurodegeneration. A great wealth of preclinical and translational research has been and is currently being developed to help decipher the involvement of the immune system in disease pathophysiology, quantify disease course as well as visualize the potential detrimental effects of excessive inflammation. Down the road, the ultimate goal is to have clinical non-invasive in vivo imaging biomarkers of inflammation that will help diagnose disease, establish prognosis and gauge response to preventative and therapeutic strategies.

KEY WORDS

Inflammation, molecular imaging, PET, MRI, iron oxide nanoparticles
INTRODUCTION

Imaging tissue inflammation using conventional imaging methods such as computed tomography (CT) and magnetic resonance imaging (MRI) mainly provides information about structural changes in the involved tissues. Those changes can include edema (accumulation of fluid in the extracellular space), contrast enhancement (endothelial disruption) and organ damage. Nuclear medicine techniques on the other hand, such as radiolabeled white blood cells and $^{18}$F-Fluorodeoxyglucose (FDG), provide functional information about the inflammatory reaction, based on chemotaxis and glucose metabolism respectively. More recently, more specific imaging of the inflammatory reaction has been sought, using various molecular imaging techniques, mainly positron emission tomography and single-photon emission computed tomography (PET/SPECT) and MRI. Those attempts are generally based on targeting specific elements of the immune system (e.g. macrophages, lymphocytes), but are often tailored differently for various organs, based on the organs’ individual properties.

TARGETING THE IMMUNE SYSTEM

One way of imaging inflammation is by targeting the upregulation and trafficking of immune system cells as they interact with noxious stimuli, whether infectious, autoimmune or degenerative. For many years, targeting immune cells was mostly isotope-based, with SPECT and PET applications. More recently, MRI based approaches gained momentum, especially with the advent of different types of magnetic nanoparticles that can be functionalized for specific targeting of surface markers and other cell constituents. The
accumulation of magnetic nanoparticles results in shortening of the T2 and T2* relaxation times of surrounding tissues which causes signal reduction (negative contrast) on MR images. For more information about the physics of detecting paramagnetic nanoparticles in vivo, please refer to (1).

**Imaging Macrophages/Monocytes**

Macrophages/monocytes have a very important and diverse role in the innate immune system response to injury/pathogens, which includes antigen presentation (to lymphocytes), cytokine/chemokine secretion (pro and anti-inflammatory) and most prominently, phagocytosis (2).

Targeting monocytes/macrophages in the setting of inflammation has been achieved using a variety of nanomaterials including magnetic nanoparticles such as the small and ultrasmall paramagnetic iron oxide nanoparticles (SPIO, USPIO) (3). The phagocytic abilities of the monocyte/macrophage system are generally assumed to account for the process of nanoparticle uptake (4). This uptake process depends on many factors, most importantly the size and shape of the nanoparticle as well as its surface coating characteristics and opsonization. By modifying the size of the nanoparticles (< or >100 nm), their charge (positive versus negative), surface coating characteristics (glycine increases uptake by activated macrophages) and the addition of specific targeting ligands (e.g. specific macrophage receptors such as CCR2 and CX3CR1), the degree of uptake as well as potentially the specific population uptake, can be regulated (4).
Imaging macrophages can also be done using PET/SPECT ligands (e.g. modified dextran nanoparticles labeled with $^{89}$Zr), optical imaging (quantum dots and fluorochrome labeled nanoparticles), and computed tomography. For more information, the reader is referred to an excellent review on this topic (4). Examples of targeting macrophages/monocytes in various organ systems will be described later in this paper.

**Imaging Lymphocytes**

B lymphocytes play a major role in the humoral immunity component of the adaptive immune system by producing antibodies in response to various antigens. Multiple subtypes of B cells are generally identified by flow cytometry through specific cellular markers. B cell dysfunction/dysregulation, on the other hand, can lead to various autoimmune diseases making those pathogenic B cells the main targets for corresponding therapeutic approaches. Imaging biomarkers can be developed based on those therapies. Perhaps the best example is the use of radiolabeled rituximab, an anti-CD20 antibody. $^{99m}$Tc and $^{124}$I Radiolabeled rituximab have been successfully used in patients with various diseases such as rheumatoid arthritis, psoriatic arthritis and sarcoidosis (5). Limitations of this approach include potential for immunogenicity and sequestration within the spleen which might necessitate pre administration of unlabeled rituximab (6). Discussion of radioimmunotherapies using other types of humanized B cell antibodies such as Yttrium labeled ibritumomab tiuxetan and epratuzumab tetraxetan is beyond the scope of this article.

T lymphocytes play an important role in cell-mediated immune response. Various cellular markers are responsible for activating the T cell population in response to antigen
presentation. Imaging T lymphocytes thus becomes important both for delineation of inflammatory abnormalities as well as monitoring autoimmune disorders associated with their dysregulation. Longstanding attempts at labeling T cells with isotopes such as $^{111}$In, $^{51}$Cr and $^{99m}$Tc were mostly evaluated in preclinical models of disease and faced various levels of success with some reported problems such as the negative effect of certain labels like $^{99m}$Tc on cell trafficking and migration (7). More recently, labeling with PET isotopes such as $^{64}$Cu and $^{18}$F were attempted, including labeling with $^{18}$F-FDG, $^{18}$F-FBEM and $^{64}$Cu-PTSM (7). Successful reporter gene labeling has been described recently as well and a comparison between different reporter gene systems showed that the combination of human norepinephrine transporter and $^{18}$F-fluorobenzylguanidine (MFBG) reporter system is the most sensitive for detection of lymphocytes in a mouse model (8). In contrast to the in vitro approach to labeling T-lymphocytes, an alternative approach is to target those cells in vivo with labeled cytokines (e.g IL-2, IL-12, IL-1, IL-1ra) and/or chemokines (e.g. IL-8). The majority of those experiments were evaluated in pre-clinical models of cancer and infection/inflammation with few human applications, namely using radiolabeled IL-8 and IL-2 (7). Finally, extensive probing of radiolabeled antibodies to surface markers of T lymphocytes such as CD25, CD3, CD4, CD45, CD5 and CD2 has been done since the 1980s, generally in animal models and patients with hematopoietic malignancies and lung cancer with only a few applications targeting inflammatory entities such as rheumatoid arthritis (9). Limitations to this approach are not specific to this type of antibodies but rather common to most antibody-based approaches and include delayed imaging requirements due to long plasma half-lives and the potential development of human antimurine antibodies (10). A recent paper described the novel use of cys-
diabodies (bivalent antibody fragments with shorter serum half-life) against CD4 and CD8 for the visualization of lymph nodes and spleen (11) in mice (Figure. 1). For more information about imaging T lymphocytes, the reader is directed to an excellent review paper (7).

**IMAGING INFLAMMATION AT THE ORGAN LEVEL:**

**Central Nervous System Inflammation**

Neuroinflammation is a suspected culprit in a large number of diseases including chronic infections (e.g. human immunodeficiency virus), neurodegenerative diseases (e.g. Parkinson and Alzheimer's dementia), brain tumors (including primary and metastatic disease) as well as psychiatric diseases (e.g. depression, anxiety and schizophrenia. Currently, the most common approach to imaging neuroinflammation is through targeting the translocator protein (TSPO), an outer mitochondrial membrane receptor that gets upregulated in activated microglia, astrocytes and blood derived macrophages in response to central nervous system injury (12). The classical ligand is 11C-PK11195, however a multitude of newer ligands designed to overcome the limitations of PK11195 (high lipophilicity resulting in decreased bioavailability, high non-specific binding and short half-life of 11C) have been recently developed (12). Along with the improved binding characteristics of the second generation ligands, however, new problems emerged. The most important problem is a polymorphism (rs6971) located in exon 4 of the TSPO gene that is believed to result in nonconservative amino-acid substitution at position 147 from alanine to threonine (Ala147Thr) in the fifth transmembrane domain of the TSPO protein
This polymorphism results in three levels of binding necessitating, in most cases, the genotyping of subjects prior to quantitative assessments of TSPO density using PET. Another problem in non-focal brain diseases is the lack of a reference region due to diffuse involvement of the brain. Recent description of TSPO ligands that are not sensitive to polymorphism are encouraging (14). Review articles about TSPO imaging in neuroinflammation exist (12).

Other potential targets for neuroinflammation imaging are currently evaluated namely the cannabinoid receptors CB1 and CB2 which together constitute the endocannabinoid system (15). Multiple ligands targeting CB2 are currently being developed (16). Animal applications in neuroinflamatory disease models are encouraging (16). Alternative imaging approaches to neuroinflammation include targeting the arachidonic acid cascade (17) and cyclooxygenase -2 expression (18).

Targeting neuroinflammatory changes with Iron oxide magnetic nanoparticles has been done in animal studies of ischemia however no correlation was found between iron oxide based enhancement and infarct size in human studies (19). Imaging multiple sclerosis with magnetic nanoparticles, on the other hand, identified more active lesions than gadolinium enhancement alone. Interestingly, the vast majority of USPIO positive lesions show no concomitant Gd-DTPA enhancement, indicating more severe disease hiding behind intact or repaired blood brain barrier (20) (Figure. 2). Enhancement with both types of contrast (paramagnetic nanoparticles and Gd-DTPA) reflected more aggressive nature of lesions compared to those enhancing with one or the other (20).

Vascular Diseases/Atherosclerosis
Imaging the inflammatory component of atherosclerosis has become a major target in the last couple decades due to the discovered relationship between inflammation (macrophage activity) and the risk of plaque development and rupture (vulnerable plaque) (21). An extensive body of literature is available using FDG PET imaging as a marker of macrophage infiltration in the identification of inflammatory vascular plaques with excellent review articles (22). In one study, FDG uptake was found to positively correlate with macrophage density (23). TSPO ligands such as $^{125}$I-DPA-713 (24) and $^{11}$C-PK11195 (25), as well as labeled DOTATATE ($^{68}$Ga and $^{64}$Cu) (26) (Figure 3) have also been used in the evaluation of plaques. While the former ligands target TSPO expression in the infiltrative macrophages present in vulnerable plaques, the latter (labeled DOTATATE) seems to target the same cell population (macrophages) but through a different cellular component, the somatostatin receptor subtype 2 (26). Still at the preclinical levels, $^{18}$F-lableing of anti vcam1 nanobodies seemed to show promise in apoE-/- mice (27).

The use of SPIO nanoparticles both in animal models of atherosclerosis and in patients has been performed. The magnitude of T2 signal intensity reduction on high-resolution MRI after administration of superparamagnetic nanoparticles can theoretically quantify the macrophage burden in plaques, allowing for better characterization of the plaque and monitoring for therapy-mediated changes (28).

Used together in a rabbit model of atherosclerosis imaged using a combined MRI/PET scanner, FDG PET was found to be more sensitive than USPIO for the detection of early changes in plaque inflammation although correlations between mean standardized uptake value or Change in R2* value and macrophages density (RAM-11 staining) were
good (29). For a detailed in-depth description of nanoparticle usage in imaging cardiovascular disease, the reader is referred to an excellent review (30).

**Extracranial Organs: Chest, Abdominal organs, Joints**

Imaging inflammation of the lungs and various abdominal organs has also been done. As with most other targets, FDG-PET was found to be useful for imaging of post radiation pneumonitis (31), pulmonary complications of cystic fibrosis (32) and allergen induced asthma (33). More specific than FDG, a novel tracer (18F-NOS) that binds to Inducible nitric oxide synthase was recently found to accumulate in subjects with endotoxin induced acute lung inflammation (34). Also of interest is recent description of cysteine cathepsin-targeted imaging probes that can be used to monitor the contribution of macrophages to fibrotic disease progression in a murine model of pulmonary fibrosis and in patients with idiopathic pulmonary fibrosis (35) (Figure.4). Cathepsins are known to be highly expressed in activated macrophages (35).

Accumulation of USPIOs in the infarcted myocardium was visualized, increasing significantly over time, suggesting recruitment of inflammatory cells over time. The exact clinical significance of this finding is still unclear (36). Similarly, accumulation of USPIO in experimental septic arthritis provided means for identifying early synovitis and monitoring of therapy (37). The role of FDG in the evaluation of peripheral inflammatory arthritis was reviewed by Brujinien (38) who concluded that although promising, more imaging in larger cohorts is still necessary before clinical application. On the other hand, targeting macrophages using 11C-PK11195 to identify subclinical joint inflammation prior to clinical symptomology in rheumatoid arthritis patients was found to be more predictive of the
development of clinical flare than MRI (39). Finally imaging renal inflammation in animals and in patients using FDG PET and USPIO generally was able to identify regions/extent of macrophage infiltration, especially in the setting of autoimmune diseases such as SLE and Sjogren’s (40).

**CONCLUSION**

Molecular imaging of inflammation is a rapidly growing field with multiple applications in a vast array of disciplines. Using MR based as well as nuclear medicine techniques will certainly add to our understanding of the role of inflammation in cancer, neurodegeneration, infection and psychiatric diseases. More targets, and corresponding ligands, within the immune system and various organs need to be established for the field to reach its potential.
REFERENCES


Figure 1: Imaging T-lymphocytes. Anti-CD4 small-animal PET (using $^{89}$Zr-malDFO-GK1.5 cysDiabodies) in wild-type (first row), CD4-blocked (second row), and CD4-depleted (third row) mice, imaged at 4, 8, and 22 h after injection. Images are represented as 25-mm maximum-intensity projections. Note the lack of axillary lymph nodes (ALN), cervical lymph nodes (CLN), inguinal lymph nodes (ILN), popliteal lymph nodes (PLN) and spleen (SP) uptake in both the CD4 blocked and CD4 depleted animals compared to the wild-type (B=bone; K=kidney; Li=liver) (Modified and reproduced with permission from (11)).
Figure 2: Imaging inflammation in the brain using MRI. Axial unenhanced T2-weighted MR image in a young woman with multiple sclerosis showing multiple hyperintense lesions (A). While axial gadolinium-enhanced T1-weighted image (B) shows three enhanced lesions (arrows), axial T1-weighted image obtained 24–48 hours after injection (C) of Ultrasmall superparamagnetic iron oxide (USPIO) nanoparticles shows the same three lesions along with three additional active lesions that enhanced only with USPIO (arrows) (Modified and reproduced with permission from [20]).
Figure 3: $^{64}$Cu-DOTATATE PET of two patients with Framingham risk score of 30 (A) and Framingham risk score of 2 (B). Images show high focal uptake in thoracic aorta in the first patient (A) compared to lower and more diffuse uptake in the second patient (Modified and reproduced with permission from (26)).
Figure 4: Imaging lung inflammation. $^{68}$Ga-BMV101 PET-CT probe (targeting cysteine cathepsins, which are highly expressed in activated macrophages) was injected intravenously and images were collected at 2.5 hours post probe administration. There is increased accumulation in regions of lung fibrosis in the patient with idiopathic pulmonary fibrosis (third row), with no significant increase in probe accumulation in the unclassified fibrosis patient (second row) when compared to the normal lung controls (first row) (Modified and reproduced with permission from (35)).
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