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Imaging Macrophage-associated Inflammation

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Abstract

Macrophages belong to the mononuclear phagocyte system comprising closely related cells of bone marrow origin. Activated macrophages are critical in several diseases such as tuberculosis, sarcoidosis, Crohn's disease, and atherosclerosis. Noninvasive imaging techniques that can specifically image activated macrophages could therefore help in differentiating various forms of inflammatory diseases and to monitor therapeutic responses.

Introduction

Macrophages mediate an innate immune response of inflammation, being one of the first lines of defense against the invasion of various pathogens, as well as in several chronic inflammatory diseases.[1] Various nuclear imaging techniques are currently in development, and we discuss some of the promising targets and agents in this review.

Imaging Folate Receptor (FR)-β

FRs are cell surface glycoproteins of molecular weights in the range of 38–45 kDa and attach to the plasma membrane by a glycosylphosphatidylinositol anchor.[2] Several isoforms are present in humans and include FR-α, FR-β, and FR-γ. FR-β is a marker protein in normal hematopoiesis in the lineage of myelomonocytic cells,[2] and is also found in pathogenic cells, such as acute and chronic myelogenous leukemia cells.[3] FR-β is overexpressed on activated but not normal macrophages, and its expression on other cells is undetectable,[4] making this receptor a good target for imaging macrophage-mediated inflammatory diseases. The diagnostic and therapeutic implications of this target were demonstrated by Chandrupatla et al, where they highlighted the role of ^{18}F-fluoro-PEG-folate PET as a therapeutic monitoring tool for methotrexate therapy.[5]

Small molecule ligands that specifically target FR-beta have several advantages, including high affinity to its target even after conjugation, easy conjugation with imaging agents, and the low or undetectable expression of the target receptor on normal cells.[4] Folic acid has

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been linked to a variety of dyes or radiopharmaceuticals, such as ^{99m}Tc, gallium-67, and indium-111, and have been used for imaging in the detection of FR-positive activated macrophages and cancer cells.[1,6–8] In addition, in carotid endarterectomy specimens of 20 patients, higher numbers of M2-like macrophages were present in areas of high ^{99m}Tc-folate accumulation than in areas with low accumulation, suggesting that this technique can also detect pathologies caused by M2-like macrophage phenotypes.[9] Imaging studies of macrophage-mediated inflammatory diseases using FR-β have been performed in animals models and in human patients (Table).

Imaging CD206, the Mannose Receptor

The mannose receptor (MRC-1, CD206) is a C-type lectin primarily present on the surface of macrophages and immature dendritic cells. CD206 mediates the endocytosis of glycoproteins by macrophages but also acts as a phagocytic receptor for bacteria, fungi, and other pathogens.[17,18] However, mannose receptor expression is not macrophage restricted; it is also expressed by hepatic and lymphatic endothelia, and dendritic and kidney mesangial cells.[19] MRC-1-targeting agents are mostly designed for imaging tumor-associated macrophages and other macrophage-mediated diseases like rheumatoid arthritis. Nanobodies against the macrophage mannose receptor have been developed and were successfully used to specifically target a subpopulation of tumor-infiltrating macrophages in SPECT-micro-CT imaging studies.[20,21] However, Bala et al found no significant uptake of MRC-specific nanobodies in atherosclerotic lesions in a mouse model.[22]

Imaging the Translocator Protein (TSPO)

Monocytes and their progeny (macrophages, microglia, and dendrocytes) express the TSPO along with many other cell types, notably the heart, lung, kidney, endocrine tissues, and endothelium. TSPO is an 18-kD transmembrane protein arranged as a pentamer of alpha helices, which form a channel for ligand binding. In monocytic lineages, TSPO is upregulated during inflammation and is reduced in the presence of anti-inflammatory pharmacotherapies. TSPO has been used as a biomarker particularly for imaging microglia because background TSPO expression in the rest of the central nervous system (CNS) is low. Nevertheless, others have also targeted peripheral macrophage-expressing TSPO to image diseases outside the CNS. A variety of small-molecule TSPO-targeted ligands for PET and SPECT have been developed, such as the first-generation compounds ^{11}C-PK11195 and ^{11}C-Ro 5-4864, as well as subsequent radioligands, such as ^{11}C-DAA1106,[23] ^{11}C-PBR28,[24] and ^{11}C-DPA-713.[25] *N,N*-Diethyl-2-[2-(4-methoxy-phenyl)-5,7-dimethyl-pyrazolo[1,5-α]pyrimidin-3-yl]-acetamide, DPA-713, is a pyrazolopyrimidine that is twice as potent as the first generation, archetypal ligand, PK11195, and 10 times more hydrophilic,[26] allowing for better clearance from nontarget tissues. A drawback of using several second-generation ligands is that binding affinity to TSPO is determined by the subject's genotype (SNP rs6971). High affinity is observed in individuals with two copies coding for alanine at amino acid position 147. Medium affinity is observed in heterozygous individuals, each expressing TSPO containing alanine or threonine, and low affinity is observed in individuals expressing TSPO coding for threonine at position 147.[27] This drawback certainly limits imaging in T/T expressing individuals and reduces sensitivity in individuals expressing A/T when using these radiotracers. (*R*)-^{11}C-PK11195 exhibits uniform affinity for TSPO

regardless of genotype, but suffers from a characteristic highly lipophilic biodistribution profile in CNS as well as in periphery and leads to high nonspecific background uptake.

Neuroimaging using radiolabeled TSPO ligands is by far the most pursued application of TSPO imaging.[28,29] This technique relies upon the restricted expression of TSPO within the CNS, which is limited to microglia, astrocytes, choroid plexus, and ependymal cells of the ventricles and the olfactory bulb. Disease states involving gliosis, either early-phase microglial activation or chronic astrogliosis, for which TSPO imaging has been employed, include inflammation secondary to contact sport head trauma,[30] inflammation in multiple sclerosis,[31] and inflammation in neuropsychiatric disorders[32] and in a rabbit model of tuberculous meningitis.[33]

Peripheral macrophage imaging via TSPO is possible and has been reported in experimental models of rheumatoid arthritis,[34] atherosclerosis,[35,36] and tumor-associated macrophage imaging.[37] Because of relatively rapid tracer kinetics (0–2 hours post injection), largely because of short radionuclide half-life and high densities of TSPO expression in the lungs, heart, adrenal glands, and kidney, imaging is largely confined to either the extremities or the brain, where background signal is low. One exception to this restriction is the discovery that radioiodinated DPA-713 is almost exclusively retained by macrophages at a longer uptake time (24 hours post injection) while washing out of TSPO-expressing tissues, allowing imaging of macrophages almost anywhere within the CNS or periphery. ^{125}I-Iodo-DPA-713 was found to accumulate specifically in tuberculosis (TB)-associated inflammatory lesions in murine models.[38] Serial ^{125}I-DPA-713 SPECT was also able to correctly identify the bactericidal activities of the two TB treatments as early as 4 weeks after the start of treatment.[39] Furthermore, ^{124}I-DPA-713 PET was able to localize TB lesions with high signal-to-noise ratios (Fig.). However, gastrointestinal clearance of radiotracer makes imaging challenging in this tissue. Lastly, pharmacotherapies applied to the patient may also affect imaging data. Using anti-inflammatory or steroidal medications regardless of source or molecular target may upregulate or downregulate macrophage or microglial inflammation and may result in altered radiotracer uptake.

Oxidized LDL Receptor (LOX-1)

LOX-1 is a 52 kDa type II lectin-like inducible membrane protein that forms a homodimer at the plasma membrane.[40] It is conditionally expressed on endothelial cells, macrophages, neutrophils and smooth muscle cells where the C-terminus binds to oxidized low-density lipoprotein (oxLDL) species and other ligands, such as damaged cells, platelets, and bacteria. Ligand binding subsequently activates several signaling molecules, including nuclear factor-κB, resulting in reactive oxygen species release. In the case of endothelial cells, release of reactive oxygen species causes vessel damage as well as stimulates neovascularization. In the case of macrophages and neutrophils, reactive oxygen species release causes tissue damage and innate immune response against bacteria, fungi, and parasites, and in smooth muscle, tissue damage is the primary result.

LOX-1 has served as a biomarker primarily for initiation and progression of atherosclerosis because of its upregulation in a pro-atherogenic environment, including the presence of hypertension and dyslipidemias.[41] In an oxidizing host environment, low-density lipoprotein

(LDL) transforms into oxLDL within tissues or blood either through acetylation of B100 or direct oxidation of the cholesterol ligand. When oxycholesteryl oxLDL binds to LOX-1 on macrophages, it is internalized via a distinct endosomal pathway from LDL and acetylated oxLDL.[42] Oxycholesteryl oxLDL is metabolized much more slowly than LDL, and as a result, endosomes containing oxLDL fuse with lysosomes that expand to contain substantial stores of oxLDL and LDL, which are visible in foamy macrophages using acid red stain. Increasing oxLDL binding promotes upregulation of LOX-1 expression. Efforts to target and noninvasively visualize densities of LOX-1-expressing foam cells, inflamed endothelium, and inflamed cardiac muscle have exclusively employed LOX-1-targeted antibodies. The first reported study described ^{99m}Tc-labeled anti-LOX-1 IgG in a rabbit model of atherosclerosis.[43] Ex vivo autoradiographic and fluorescent data showed strong tropism for plaque-associated macrophages, and in vivo SPECT-CT data showed focal uptake in descending aorta. Similarly, another group using indium-111-labeled (and also Gd^{3+}) anti-LOX-1 IgG conjugated to liposomes to image plaques in a mouse ApoE−/− model of atherosclerosis reported similar findings.[44] This group's antibody-guided probe was similarly bound to macrophages within plaques and showed focal vascular uptake in vivo, as well as very high liver uptake. Small-molecule ligands to LOX-1 have been reported,[45] although none have been labeled for imaging.

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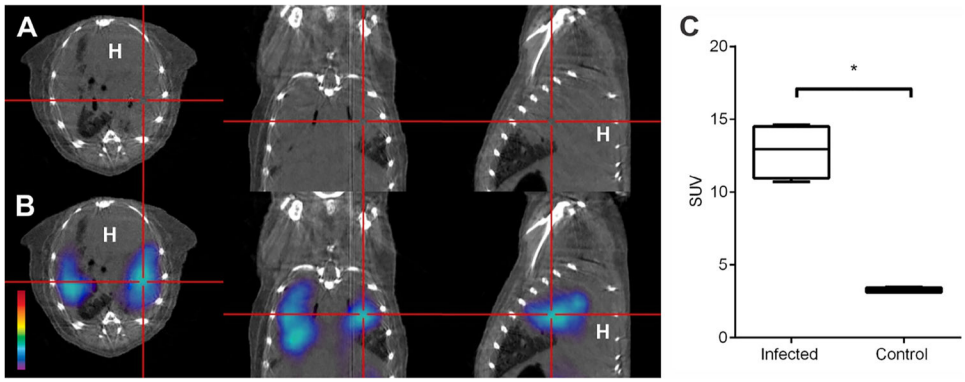

Figure.

SUV, standardized uptake value; H, heart; * = $P < 0.05$. ^{124}I-DPA-713 PET imaging of a *Mycobacterium tuberculosis*-infected mouse 24 hours after injection of the tracer. PET signal colocalizes with the pulmonary lesion visualized by CT (panels A and B). PET signal in the pulmonary lesions of infected mice is significantly higher than that in the lungs of uninfected mice. (Adapted from Ordonez et al.[39])

Table

Folate Receptor-β-targeted In Vivo Imaging

Diseases	Animal Model/Human	Reference
Rheumatoid arthritis	Rat	[10]
	Dog	[11]
	Human	[12]
Osteoarthritis	Human	[4,13]
Atherosclerosis	Mice	[14]
Asthma	Mice	[15]
Ovarian cancer	Human	[16]