Current Cardiovascular Imaging Reports (2019) 12:38
https://doi.org/10.1007/s12410-019-9515-3

MOLECULAR IMAGING (J WU AND P NGUYEN, SECTION EDITORS)

Recent Advances in Imaging Inflammation Post-Myocardial Infarction Using Positron Emission Tomography

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Abstract

Purpose of Review Despite advances in medical and surgical therapy, some patients after a myocardial infarction still develop heart failure, a devastating disease that has a high rate of morbidity and mortality. Differences in the immune response to the myocardial damage may affect the healing process. Molecular imaging enables the interrogation of immune cell subsets that can aid or impair left ventricular recovery post-myocardial infarction. Visualization of patient-specific differences in the cellular and molecular process among patients post-myocardial infarction may lead to the development of personalized therapy to prevent remodeling and promote myocardial recovery.

Recent Findings Positron emission tomography (PET) has emerged as a commonly used modality for imaging myocardial inflammation in both pre-clinical and clinical studies. While ^{18}F-FDG continues to be investigated for its ability to track inflammation in small clinical trials, its relative short half-life and nonspecific uptake have prompted investigation into other PET tracers, including agents that image amino acid metabolism, mitochondrial transport proteins, cell surface receptors, and integrins. Two of the most innovative approaches include the use of reporter gene imaging and nanoparticles. Of these, the latter offers the added advantage of delivering a therapeutic payload. Finally, recent advances in PET instrumentation such as total-body imaging can allow the study of multi-organ dysfunction simultaneously (e.g., cardiac and brain inflammation after a heart attack) with high sensitivity. Moreover, with multi-isotope PET scanners, simultaneous observation of multiple positron emitting isotopes has enabled the examination of different biological processes with distinct molecular probes in a single scan setting.

Summary The development of advanced PET tracers for the visualization of the inflammatory cascade post-myocardial infarction may enable the development of immune modulating agents to promote left ventricular recovery.

Keywords Positron emission tomography · Inflammation · Myocardial infarction · Ischemic heart disease

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This article is part of the Topical Collection on *Molecular Imaging*

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Introduction

Between 2013 and 2016, about 6.2 million patients in the U.S. suffered from heart failure, a devastating disease with an estimated 50% mortality risk within 5 years of diagnosis [1, 2]. Despite advances in urgent revascularization and medical therapy to prevent remodeling, myocardial infarction remains a leading cause of heart failure [1, 2]. Interestingly, some patients fare better than others [3•], even after controlling for the location and size of infarction as well as time to revascularization, suggesting that factors that dictate whether the left ventricle recovers or remodels are patient-specific. While previous studies [4–6], mostly in animal models, have shown in detail how immune cells that infiltrate the myocardium post-myocardial infarction actively participate in both repair and injury, it remains unclear how to harness the immune system for therapeutic benefit. The failure of clinical trials in the early

Published online: 04 September 2019

2000s despite promising preclinical data highlights this knowledge gap [7–10]. Molecular imaging enables the noninvasive, in vivo visualization, and serial analysis of how immune cells contribute to remodeling or recovery post-myocardial infarction. Imaging these molecular and cellular events in vivo may provide a better understanding of the complexities of the immune response in individual patients, the identification of patients at greatest risk for adverse remodeling, and a strategic road map for the development of patient-specific immune modulating therapy.

Myocardial Inflammation Post-Myocardial Infarction

A sudden complete or near complete obstruction of a coronary artery results in an acute loss of oxygen and vital nutrients to the myocardium, leading to localized necrosis, apoptosis, and the recruitment of leukocytes (white blood cells), primarily from the bone marrow and spleen, to the injured area [11, 12•]. These events can be characterized by three phases distinguishable by the function of the recruited immune cells: (1) A pro-inflammatory phase that attracts immune cells to the site of injury to isolate the damage and clean up dead tissue, (2) a proliferation phase characterized by inflammation, and (3) a maturation phase that leads to the development of a healing scar.

During the initial pro-inflammatory phase that occurs within minutes of injury [13], neutrophils and other granulocytes infiltrate the damage tissue where they secrete pro-inflammatory cytokines and proteases that further injure the vasculature and myocardium. Monocytes (e.g., $Ly6^{high}$) expressing the C-C chemokine receptor type 2 (CCR2+) are then recruited by the release of a chemokine (e.g., CCL2: C-C motif ligand 2) from damaged or dying myocardial cells [13, 14]. These monocytes differentiate into pro-inflammatory macrophages (e.g., M1-like macrophages) that phagocytose cell debris and limit the extent of apoptosis.

The pro-inflammatory phase continues for 3 to 5 days [15], until the release of another chemokine (e.g., CX3CL1: C-X3-C motif ligand from damaged or dying cells, which attracts a different monocyte subset ($Ly6^{low}$) that infiltrates the injured site and differentiates into macrophages that secrete chemokines supporting tissue repair (e.g., M2-like macrophages). During this proliferation phase, these secreted cytokines and growth factors stimulate angiogenesis, fibroblast reorganization, and extracellular matrix synthesis [16]. Finally, during the maturation process, apoptosis of fibroblasts and vascular cells lead to the development of a collagen scar. In addition to M2-like macrophages, lymphocytes, especially CD4 helper T cells, are thought to play a role in the transition between the inflammation and repair phases [17].

Maintaining the balance between the pro-inflammatory and reparative phase is required to achieve an optimal outcome post-myocardial infarction (Fig. 1) [18]. If the duration of the inflammatory response or the composition of leukocyte subpopulations changes, the rate of ventricular wall rupture, size of the infarct, and extent of late remodeling can be adversely affected, as has been shown previously in mouse models [14]. While the above aforementioned findings have been well validated in murine models, further molecular imaging studies in larger animal models including humans are needed to develop and monitor immune modulating therapies to shift the balance toward recovery rather than adverse remodeling, a maladaptive process that results in ventricular dilatation and reduced contractile performance with a decreased LV ejection fraction.

PET Imaging

Positron emission tomography (PET) can quantitatively measure and monitor the molecular expression of immune cells, making it one of the most promising imaging modalities for interrogating the role of the immune system in myocardial recovery or remodeling post-myocardial infarction. A common strategy for PET is to use a radiotracer, which is a small molecule "tagged" with a positron emitter, that either attaches to, is taken up, or is enzymatically acted upon by molecular targets in the body [19]. Alternatively, biomolecules such as radiolabeled monoclonal antibodies, engineered antibody fragments, or synthetic peptides designed to bind to molecular targets can be used, commonly referred to as immuno-PET imaging [20]. Although immuno-PET imaging has emerged as an important biomarker for the effectiveness of immune-modulating therapy in cancer, its application in cardiovascular disease remains limited. As the field of cardio-immunotherapy grows, development of immuno-PET imaging should follow. The challenge with molecular imaging, however, remains maximizing the uptake of tracers in immune cells while minimizing the uptake by other damaged cells including myocytes, fibroblasts, and endothelial cells that often dampen the specific inflammatory signal. Moreover, it may be difficult to differentiate between immune subsets with unique roles in the inflammatory cascade without the development of highly specific tracers.

Glucose

Many previous studies have used the fact that macrophage subsets are both metabolically active but express different receptors on their surface. Compared to reparative M2 macrophages, M1 macrophages accumulate a higher amount of 2-[^{18}F]Fluoro-2-deoxy-D-glucose (^{18}F-FDG) due to higher expression of glucose transporters [21]. While imaging with

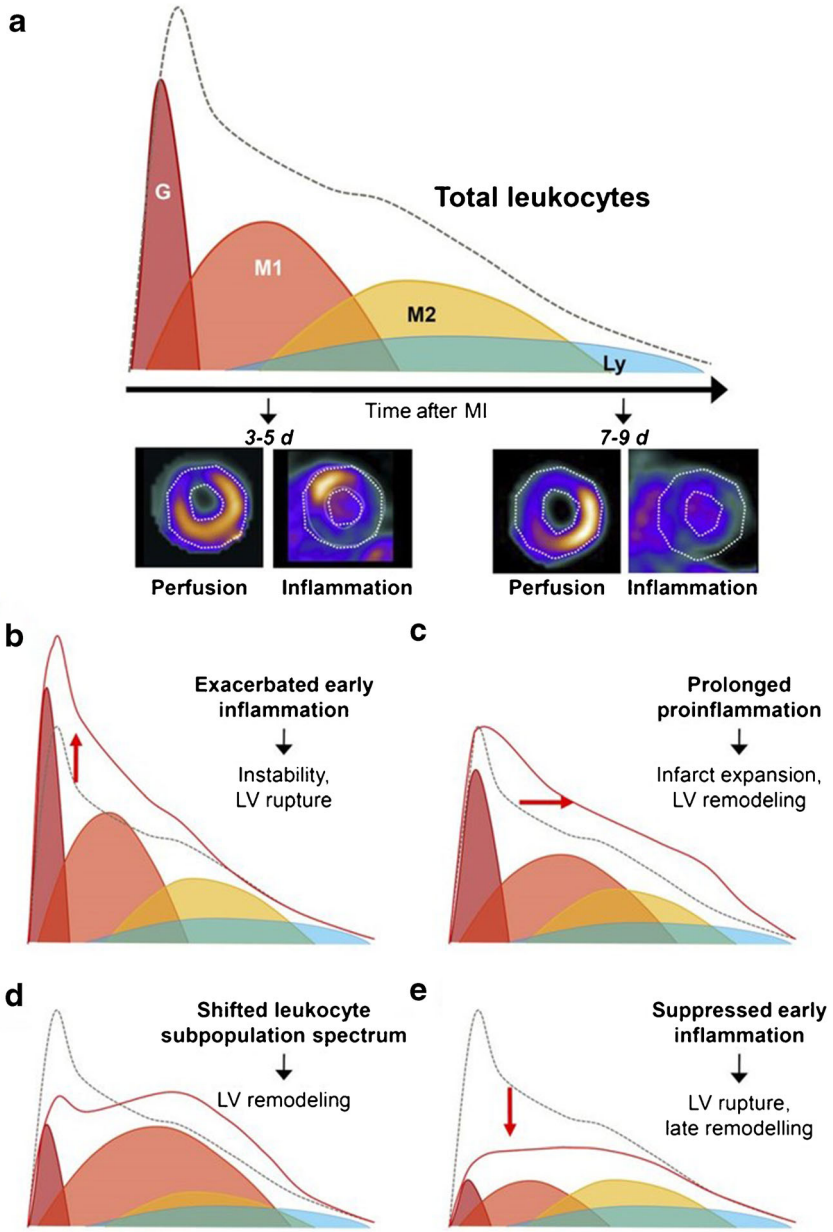

Fig. 1 Schematic of how leukocyte subsets can infiltrate the myocardium after myocardial infarction, favoring rupture, remodeling and recovery **a)** PET perfusion imaging shows decreased myocardial blood flow in the anterior septal territory, leading to infarct and increased inflammation. Granulocytes (G) and pro-inflammatory M1-like macrophages (M1) infiltrate, followed by anti-inflammatory M2-like macrophages (M2) and then lymphocytes (Ly). **b)** If there is an exuberant early, pro-inflammatory response, this leads to instability and potential left ventricular (LV) rupture. **c)** If there is prolonged inflammation, infarct expansion and further LV remodeling can occur. **d)** A shift in leukocyte subsets may also affect late LV remodeling. **e)** If the remodeling is suppressed early, LV rupture and late remodeling may occur. (This figure is originally published in the *Journal of Nuclear Medicine* [18]. Thackeray JT, Bengel FM. Gauging cardiac repair and regeneration with new molecular probes. *Journal of Nuclear Medicine.* 2018;59 [4]:549–550)

^{18}F-FDG has been validated by flow cytometry of monocytes and macrophages in mice, cardiomyocyte glucose suppression is required to reduce background signal. In mice, cardiac suppression can be achieved with prolonged fasting or administration of ketamine/xylazine for anesthesia, the latter of which cannot be translated into clinical application [22].

To address the feasibility of heparin pre-treatment as a method to promote myocardial fatty acid metabolism at the expense of glucose, Wollenweber et al. evaluated the utility of PET/MRI in 15 patients using ^{18}F-FDG with heparin pre-treatment within 7 days of myocardial infarction [23]. Compared to five controls with old infarcts, increased glucose utilization was noted in infarcted regions defined by late gadolinium enhancement compared to edematous and remote areas, suggesting that this strategy can be used to define inflammatory activity post-myocardial infarction. However, there was no correlation with other parameters of myocardial damage including perfusion defect size, left ventricular ejection fraction, and peak level of creatinine kinase or other markers of systemic inflammation including blood leukocyte count or C-reactive protein, which may reflect the limitations of glucose as a nonspecific marker of inflammation or the small sample size of the study.

In a larger cohort of 49 patients who suffered an ST elevation myocardial infarction, Rischpler et al. evaluated the value of ^{18}F-FDG PET/MRI as a marker of left ventricular functional outcome [24]. Simultaneous PET/MR imaging was performed 5 days post-percutaneous coronary intervention,

followed by a cardiac MRI after 6 to 9 months as well as serial evaluation of monocyte subsets in the peripheral blood using flow cytometry. The study showed a significant relationship between the size of the PET signal, a subset of monocytes in the peripheral blood, and infarct size on univariate analysis. A stepwise multivariate regression analysis to evaluate whether these factors were associated with global left ventricular ejection fraction, however, failed to show significance for any of these factors. Taken together, it appears that ^{18}F-FDG is not the ideal agent for correlating inflammation with left ventricular recovery or cardiac remodeling post-myocardial infarction.

While several clinical protocols to suppress cardiomyocyte glucose uptake have been evaluated, which are reviewed elsewhere [22], other investigators have turned to additional targets such as imaging mannose [25], whose receptor is predominantly present on M2 macrophages. A single study demonstrated the feasibility of imaging ^{18}F-mannose in atherosclerotic plaque, but its ability to image M2 subsets and its advantages over ^{18}FDG were unclear [26]. Improved targeting of M2 receptor subtypes may be achieved by using microantibodies; however, this has been restricted to oncological applications. Further study is needed to evaluate the utility of mannose imaging post-myocardial infarction.

Amino Acids

Because amino acids are only a minor metabolite for normal cardiomyocytes, amino acid imaging may serve as an alternative to glucose imaging. One promising PET agent is ^{11}C-methionine, a tracer taken up by M1 macrophages in cell uptake studies in vitro [27]. Using a radiocarbon version of this tracer in a small animal model of myocardial infarction, Taki et al. reported an increase in myocardial uptake of ^{14}C-methionine as early as 1 day after infarction followed by detection at 3 and 7 days post-infarction; histological analysis confirmed that the tracer was a marker of infiltrating macrophages (Fig. 2) [28]. Importantly, the tracer kinetics was similar to a previous study using ^{11}C-methionine to track inflammation post-myocardial infarction in patients [29]. A head-to-head comparison between PET tracer agents that image the glucose vs. amino acid metabolism of immune cells has yet to be performed.

Mitochondrial Translocator Proteins

An alternative strategy to metabolic imaging is to image proteins that may be expressed on immune cells after activation. Mitochondrial translocator proteins (TSPOs), for example, are up-regulated on the mitochondrial cell surface of macrophages during inflammation [30], serving as a potential ligand for PET tracers. In a murine study

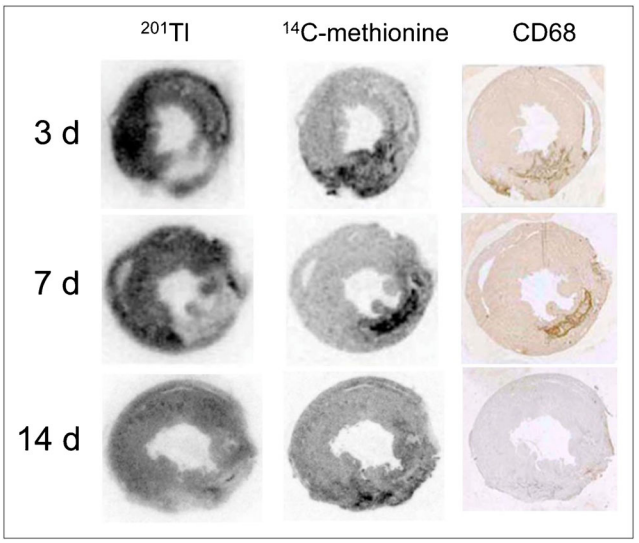

Fig. 2 Autoradiograms of ^{14}C-methionine correlate with ^{201}Tl and anti-CD68 antibody staining (marker of macrophages). At days 3–14 after reperfusion, ^{14}C-methionine uptake was observed predominantly in areas with reduced ^{201}Tl uptake, which denotes the injured area. ^{14}C-methionine uptake, however, only positively correlated with anti-CD68 antibody (a marker of macrophages) at 3 and 7 days. At 14 days, ^{14}C-methionine uptake did not correlate with anti-CD68 antibody but correlated with anti-SMA staining (anti-smooth muscle α), which may be a marker of myofibroblasts and neo-vessel formation late in the healing process (data not shown). These findings help to clarify the ^{11}C-methionine PET uptake in patients with acute myocardial infarction after successful reperfusion. Thus, methionine imaging may be useful for inflammatory imaging early after myocardial infarction. (This figure is originally published in the *Journal of Nuclear Medicine* [28]. Taki J, Wakabayashi H, Inaki A, et al. ^{14}C-Methionine uptake as a potential marker of inflammatory processes after myocardial ischemia and reperfusion. *Journal of Nuclear Medicine.* 2013;54 [3•]:431–436)

demonstrating how the autonomic nervous system can worsen remodeling post-myocardial infarction [31•], Thackeray et al. performed serial whole-body PET imaging of TSPO to image activated macrophages in the heart and microglia in the brain post-coronary artery ligation. Using the third-generation TSPO PET tracer flutriciclamide (^{18}F-GE180) whose safety has been recently evaluated in healthy controls [32], the study found that ^{18}F-GE180 localized to the infarct 1-week post-myocardial infarction and predicted subsequent remodeling at 8 weeks ($r = -0.687$; $p = 0.001$) (Fig. 3). Similarly, ^{18}F-GE180 localized to the microglia in the brain 1-week post-MI. Eight weeks post-MI, TSPO signal increased in the remote infarct area as well as the brain but did not correlate with CD68$^+$ inflammatory cells, which may reflect mitochondrial dysfunction in the myocardium rather than expression on inflammatory cells. Importantly, in a subset of animals, administration of Enalapril, which is known to reduce inflammation, attenuated the TSPO signal in the brain and heart and resulted in less remodeling. Although these findings were validated in three patients who had myocardial

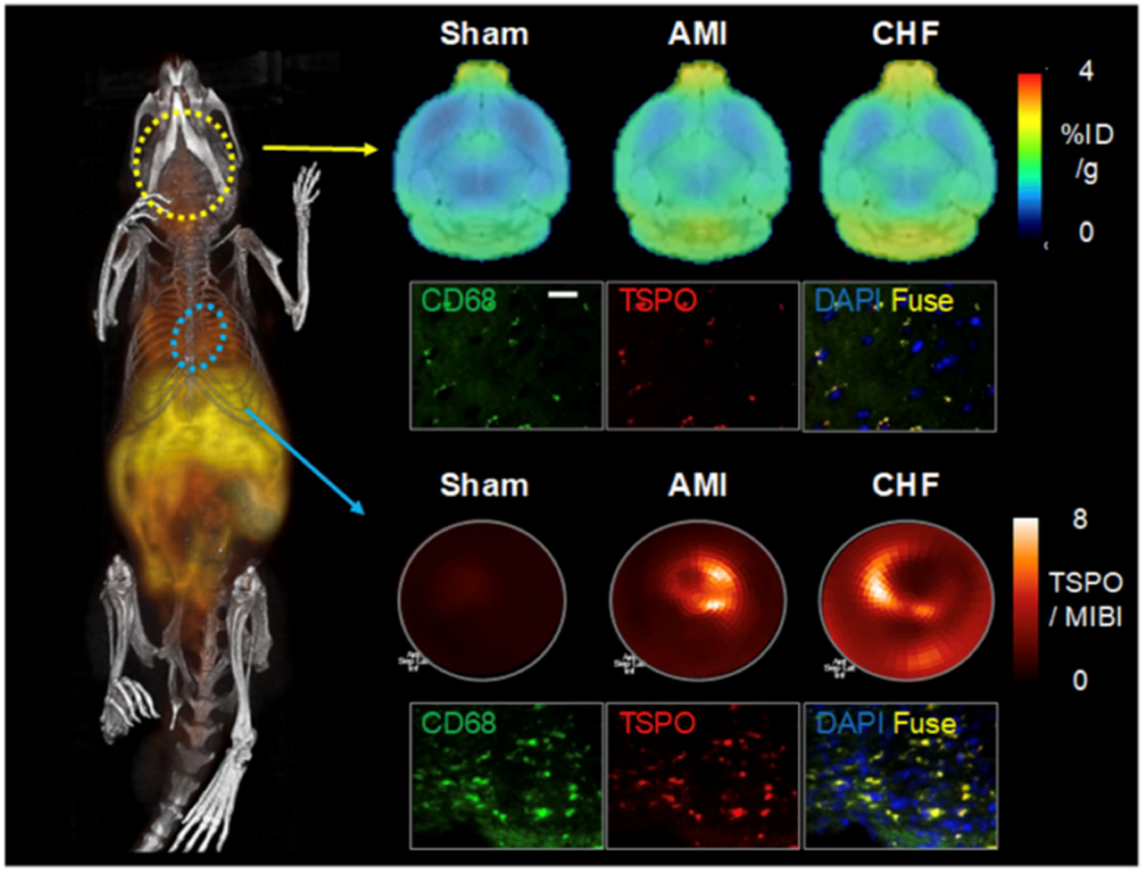

Fig. 3 Simultaneous tracer uptake of mitochondrial translocator protein (TSP) in heart and brain after myocardial infarction. Representative images of uptake of ^{18}F-GE180, which binds TSPO, in heart and brain after myocardial infarction. Uptake of ^{18}F-GE180 in the left ventricle appears in regions with diminished ^{99m}Tc-sestamibi, which defines the area of injury. Interestingly, neuro-inflammation concurrently occurs with heart inflammation post-myocardial infarction. (Reprinted from Thackeray et al. [31•] with permission from Elsevier)

infarction, further studies are needed to evaluate the potential translation of this imaging strategy.

Somatostatin Receptor

Leukocytes also express somatostatin receptor type 2 (SSTR2), which can be targeted by ^{68}Ga-DOTATATE and ^{68}Ga-DOTATOC [33]. Compared to other leukocyte subsets, activated M1-like macrophages have a two to five-fold higher expression [34], based on RNA sequencing data. In a study comparing ^{68}Ga-citrate, ^{68}Ga-DOTATATE, or ^{18}F-FDG with ketamine/xylazine suppression of myocyte glucose uptake in mice post-myocardial infarction [35], Thackeray et al. found that both ^{68}Ga-citrate and ^{68}Ga-DOTATATE did not display any specific myocardial uptake. Interestingly, in a small subset of patients ($n = 6$) who had a myocardial infarction, uptake of ^{68}Ga-DOTATOC correlated with areas of infarct defined by MRI [19]. Reasons behind the discrepancy between animal and human data remain unclear and need further investigation because unlike ^{18}F-FDG, gallium-68–labeled tracers do not require a cyclotron on the premises for their synthesis, which makes it a more viable candidate for clinical translation (especially in developing countries).

Chemokine Receptors

As discussed above, inflammatory cells recruit other immune cells during the pro-inflammatory and proliferation phase by releasing chemokines, making the associated chemokine receptor an attractive target for imaging. The PET imaging agent [^{68}Ga]Pentixafor, for example, targets chemokine receptor type 4 (CXCR4). In a murine mouse model of myocardial infarction [36], Thackeray et al. demonstrated that [^{68}Ga]Pentixafor identified CXCR4 up-regulation, which corresponded to leukocytes, specifically CD68+ macrophages and Ly6G+ granulocytes, in the infarct area. Importantly, blockade of CXCR4 extinguished the signal. Although authors provide preliminary validation of their findings in 12 patients imaged shortly after MI, further studies are needed.

Another subset of macrophages, some of which are tissue resident, express C-C chemokine receptor (CCR2+) and are recruited by damaged cardiomyocytes, as detailed above, and promote further monocyte recruitment, driving inflammation

[37]. Heo et al. recently developed a strategy to image CCR2+ accumulating in the heart tissue with high sensitivity and specificity using the PET radiotracer ^{68}Ga-DOTA [1,4,7,10-tetraaza-cyclododecane-1,4,7,10-tetraacetic acid]-ECL1i [extracellular loop 1 inverso] that binds allosterically to CCR2 [38•]. Unlike control animals and CCR2$^{-/-}$ mice, PET signal localized to sites of tissue injury in mice in vivo and in human heart tissue sections ex vivo, correlating with both histology and autoradiography. While the field of chemokine receptor imaging remains in its infancy, these studies suggest that it can be a promising approach to visualize events that occur early in the inflammatory cascade.

Glucagon-Like Peptide-1 Receptor

Glucagon-like peptide-1 (GLP-1) and its cell surface receptor, the GLP-1 receptor (GLP-1R), have been shown to exhibit cardioprotective effects after myocardial ischemia and reperfusion injury in both animal models and clinical trials [39]. Cardiac imaging of GLP-1R may be an interesting target because its activation has been shown to induce differentiation of human macrophages into an anti-inflammatory or reparative M2 phenotype, increase the amount of M2 macrophages after MI, and attenuate myocardial inflammation and interstitial fibrosis post-MI by modulation of signaling pathways in macrophages [40]. As a result, GLP-1 agonists such as exendin-4 have been radiolabeled with positron emitters for imaging with PET. Interestingly, exendin-4 is a 39-amino acid peptide that was isolated from the venom of the Gila monster lizard *Heloderma suspectum*. Gao et al. at the National Institutes of Health applied the GLP-1R–specific imaging tracer ^{18}F-FBEM-Cys40-exendin-4 to visualize and quantify the temporal changes of GLP1-R expression in a rat myocardial ischemia/reperfusion model [39]. They found that increased focal tracer retention was observed as early as 8 h after the onset of ischemia, and that tracer accumulation decreased along with time in the infarcted and peri-infarct zones.

Moreover, Ståhle and colleagues evaluated whether dynamic PET imaging of a ^{68}Ga-labeled exendin-4 tracer can detect up-regulation of GLP1R expression in the rat heart after MI and if the tracer uptake was associated with histological markers of myocardial repair [40]. They showed that ^{68}Ga-NODAGA-exendin-4 PET/CT imaging detects up-regulated cardiac GLP-1R expression after MI in rats that underwent permanent ligation of the left coronary artery. Additionally, Ståhle et al. reported that the level of ^{68}Ga-NODAGA-exendin-4 uptake in the infarct region positively correlated with the presence of CD68+ macrophages during the healing phase of MI by both PET and autoradiography [40]. Collectively, these studies highlight that fluorine-18 or gallium-68 labeled exendin-4 biomolecules for PET imaging may be a useful translational tool for monitoring the activated repair mechanisms after an ischemic myocardial injury as well as for assessing the response to potential therapies that aim to modulate inflammation (e.g., by enhancing endogenous healing and attenuating adverse remodeling).

Integrins

Cell adhesion molecules, including $\alpha_v\beta_3$ integrins that mediate the rolling and extravasation of leukocytes, have served as another molecular target for PET imaging. Perhaps, the most common target is the arginine-glycine-aspartic acid (RGD) peptide sequence expressed by $\alpha_v\beta_3$ integrins, which has been shown to correlate with macrophage infiltration in animal studies [41]. The major drawback of targeting integrins, however, is that endothelial cells up-regulate the expression of this surface protein in angiogenesis [42].

In a small clinical study, Jenkins et al. [43] evaluated the utility of the novel $\alpha_v\beta_3$-selective radiotracer ^{18}F-Fluciclatide in 21 patients 2 weeks after ST elevation MI. The authors found the PET signal was increased in areas of hypokinesis and subendocardial infarct but did not correlate with infarct size or markers of inflammation (e.g., C-reactive protein). Biopsy specimens revealed that integrin expression was highest in endothelial cells and not macrophages. Consistent with findings in this small clinical study, in a rat PET imaging study of myocardial infarction comparing ^{18}F-galacto-RGD, ^{68}Ga-NODAGA-RGD, or ^{68}Ga-TRAP(RGD)$_3$ [44], Laitinen et al. showed that areas of tracer uptake correlated with areas of angiogenesis after only 1-week post-MI. The advantage of gallium tracers is that a cyclotron is not needed. Because integrin expression is higher after 7 days post-MI in endothelial cells than inflammatory cells, restricting imaging to earliest days after MI to image inflammation may be a potential strategy.

Direct and Reporter Gene Labeling of Immune Cell Subsets Ex Vivo

A commonly used way to image inflammation is to directly label immune cells ex vivo rather than develop tracers to target in vivo processes. In fact, ex vivo white blood cell labeling with ^{18}F-FDG has been used clinically to diagnose infection. Because of the poor labeling efficiency of ^{18}F-FDG and its relative short half-life, research in the utility of longer-lived PET imaging agents like those labeled with ^{89}Zr are being initiated. In both in vitro and in vivo studies [45], Charoenphun et al. demonstrated imaging with ^{89}Zr produces comparable retention and biodistribution to ^{111}In labeling, which is a tracer for single photon emission tomography, suggesting that this agent may be a promising alternative to ^{18}F-FDG for PET imaging. One of the limitations of direct cell labeling, however, is that radiotracers can get diluted and wash out over time, making longitudinal monitoring difficult. This limitation could be addressed by the use of reporter gene imaging.

By incorporating a "label" into the cell genome, reporter gene imaging can permit long-term labeling of cells and their progeny. Using this technology, Thunemann et al. generated PET reporter mice (e.g., R26-mT/sr39tk) that carry a Cre-activatable herpes simplex virus type I thymidine kinase (HSV1-tk) [46•], which phosphorylates ^{18}F- or ^{124}I-labeled nucleoside analogs, under control of a cell type specific promotor (e.g., Pf4 — platelets, CD4—T cells, and Myh6 — cardiomyocytes) after integration into the murine Rosa26 (R26) locus. Using a combination of the ^{18}F-FHBG reporter probe, which images the expression of the HSV1-tk gene, in addition to ^{18}F-FDG, the authors were able to image both myocardial cell viability and inflammation, respectively, in a murine infarct model, suggesting this imaging approach can be used as an alternative to PET/MRI in acute MI, where late gadolinium enhancement may reflect areas of edema rather than actual infarct. Although the authors were able to demonstrate successful T cell tracking in an animal model of T cell–mediated skin allergy, the authors did not evaluate the feasibility of T cell imaging in myocardial infarction. A downside to relying on ex vivo labeling for imaging is the requirement that peripheral blood cells that are administered via injection are recruited quickly to the area of injury, which may be variable and unpredictable.

Nanoparticles

In recent times, radiolabeled nanoparticles have emerged as an effective strategy for molecular imaging due to their unique advantages including amplification of the target signal, a large surface area that facilitates target binding, and the ability to deliver therapeutic agents (e.g., loading carbon nanotubes with angiotensin receptor blocker-neprilysin inhibitor therapy to attenuate myocardial remodeling and improve infarct perfusion or administering pro-efferocytic anti-CD47 therapies to specifically and safely reduce atherosclerotic plaque burden) [47–50], in addition to their diagnostic capabilities. Using a nanoparticle conjugated to folate, whose expression is up-regulated in activated macrophages, Ni et al. [51] demonstrated that macrophages selectively took up the tracer at days 1 through 7. Using histological analysis, the authors confirmed a shift from pro-inflammatory to anti-inflammatory phenotypes on day 1 and day 3, respectively. Macrophages at day 7 did not appear to express any distinguishable markers. In another study, Keliher et al. [52] showed feasibility of PET imaging of ^{18}F-Macroflor, a modified polyglucose nanoparticle with high affinity for macrophages. Unlike other nanoparticles, Macroflor can be excreted renally, which improved target background ratios in the vascular system. PET signals generated from ^{18}F-Macroflor in atherosclerotic plaques of mice and rabbits as well as infarcted myocardium of mice showed robust correlation with macrophages detected on histology.

One of the main advantages of using nanoparticles is their unique ability to additionally deliver a payload unlike the other tracers. Nano-drug delivery systems have been proposed for immune modulation in cancer [53], neurological disorders [54], and rheumatoid arthritis [55]. While such nano-based payloads for cardiovascular disease have not yet been

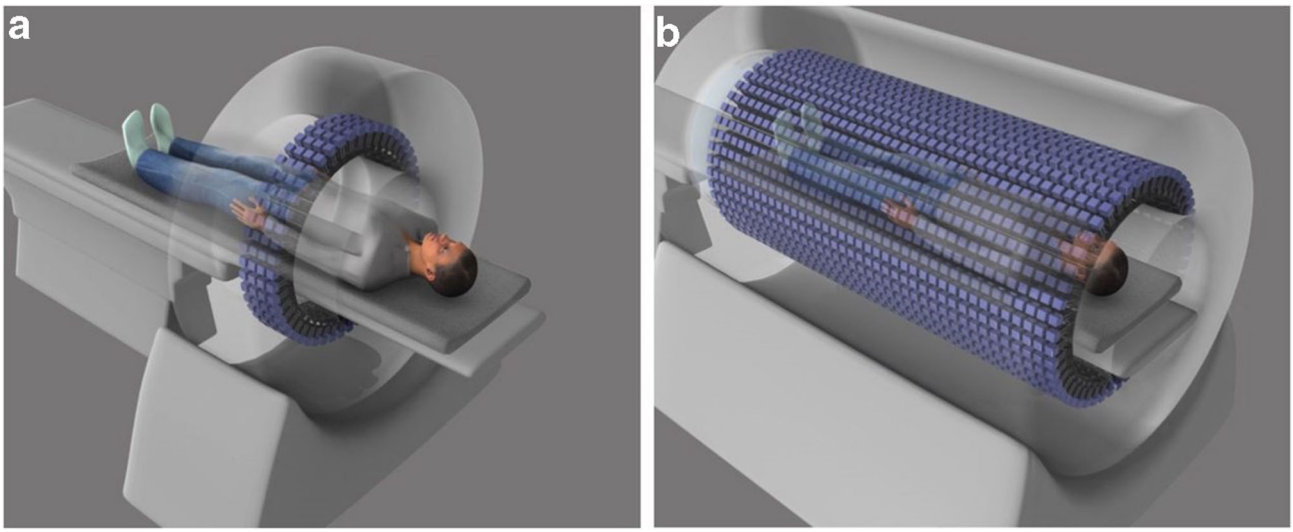

Fig. 4 Illustration of a **a)** conventional PET scanner and **b)** total-body PET scanner. The 194-cm axial field of view of the EXPLORER PET/CT scanner is sufficient to cover the entire human body in a single acquisition .EXPLORER is short for EXtreme Performance LOng REsearch scanneR. An x-ray computed tomography scanner is mounted on the front of the total-body PET gantry to ensure fusion of anatomical data with functional/molecular data. Total-body PET imaging shows potential for better, faster, and lower-dose images. For multi-system diseases and other complex conditions, the simultaneous study of multiple body organs (e.g., heart-brain axis or gut-brain-heart axis) would advance our understanding of pathophysiology and spur translation of basic science research. (This figure is originally published in the *Journal of Nuclear Medicine* [57]. Cherry SR, Jones T, Karp JS, et al. Total-body PET: maximizing sensitivity to create new opportunities for clinical research and patient care. *Journal of Nuclear Medicine* Jan 2018;59 [1]:3–12)

published, a major obstacle for the application of nanoparticles in any disease is the potential toxic side effects in vivo.

Total-Body PET Imaging — A Quantum Leap for Medical Imaging

With the recent construction of the world's first total-body PET/CT scanner, called EXPLORER, we can now encompass the entire human body within the field of view (FOV) of the PET scanner and allow imaging of all tissues and organs of the body simultaneously [56–58] (Fig. 4). This is in contrast to whole-body PET scanners, which cover most or all of the body as a series of image sets acquired at discrete bed positions. Total-body PET imaging would have significant applications for studying systemic disease (e.g., cancer, inflammation, vascular disease, and infectious disease), monitoring cellular and nanoparticle-based therapies, assessing drug pharmacokinetics and toxicology, examining normal tissue physiology and metabolism, and investigating multi-organ diseases or conditions that involve the interplay of one organ with other organs or systems (e.g., the heart-brain axis after myocardial infarction or stroke). Furthermore, the increase in geometric coverage of the total-body PET system produces a sensitivity increase of about 40× for imaging the entire body. Thus, total-body PET images could be obtained with a six-fold better signal-to-noise ratio (SNR), or in 1/40th the scanning time, or with 1/40th the injected activity while maintaining the existing SNR [57].

Multi-tracer PET Imaging — The Next Big Thing?

One of the greatest strengths of PET is its ability to image a wide array of molecular targets for disease evaluation using different radiotracers. However, multi-tracer PET imaging has been hindered because conventional PET imaging systems permit only a single PET tracer to be imaged at a time, as all PET isotopes give rise to indistinguishable 511 keV annihilation photon pairs. As a result, multiple scanning sessions need to be scheduled, often on different days, resulting in high costs and a long and arduous experience for the patient. Several methods have been proposed for examining different biological processes with PET using multiple tracers in a single scanning session [59–65]. These include 1) utilizing the differences in radioactive half-lives of various positron emitters to distinguish between tracers separately tagged with them, 2) staggering the injections of different tracers in time while acquiring in dynamic imaging mode and then implementing signal-recovery techniques to separate the multi-tracer dataset into individual tracer components, or 3) making use of a specialized PET scanner that can simultaneously observe two positron emitting radioisotopes from two distinct molecular probes by placing additional prompt gamma ray detectors around the normal PET detector array.

Concerning the last method, one of the administered tracers needs to be labeled with a radionuclide that emits positrons (e.g., fluorine-18 or carbon-11) to provide double coincidence events for the PET system to detect, while the other tracer is labeled with a radionuclide (e.g., sodium-22, copper-60, or manganese-52) that emits positrons plus an additional prompt gamma ray to provide triple coincidence events for the PET system to detect. For imaging the inflammation cascade post-MI, these multi-tracer techniques could prove useful in capturing the different immune players involved as well as the viability and perfusion of the heart tissue in a single scan setting, offering great potential for image-guided personalized medicine.

Conclusion

Findings from preclinical and clinical cohort studies suggest that the immune system plays a critical role in left ventricular healing after myocardial infarction. Because of limited access to myocardial tissue post-myocardial infarction in patients, molecular imaging is needed for the non-invasive visualization of immune cells recruited to the damaged cardiomyocytes so that we can better understand the unique role of immune subsets in myocardial recovery post-injury and develop agents for immune modulation. While recent advances in PET imaging suggest that we are closer to clinical translation, there is still a long, exciting road ahead.

Compliance with Ethical Standards

Conflict of Interest All authors declare no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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