Microglial Activation and Neuroinflammation

17

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Introduction

Microglia are immunocompetent cells in the central nervous system with various physiological functions, such as synaptic pruning during neural development, providing trophic support, and clearing neuronal debris. In response to cellular injury, microglia undergo morphological changes, with reduction in the size and number of their processes, and accompanying shift toward pro-inflammatory activities [1]. These include release of cytokines, activation of complement, and generation of reactive oxygen species. Chronic, dysfunctional inflammatory microglial activity, possibly in response to aggregation of misfolded proteins, has been considered a pathogenic contributor to neurodegenerative disease.

PET imaging of microglial activation, targeting proteins expressed by microglia, allows in vivo quantification of neuroinflammation. Unlike measurement of soluble inflammatory biomarkers found in cerebrospinal fluid, such as sTREM2 or YKL-40, PET imaging allows spatial information regarding neuroinflammatory changes in the brain.

The most commonly employed microglial target for PET imaging of neuroinflammation is the 18 kDa translocator protein (TSPO). TSPO is a mitochondrial protein (coded by nuclear DNA, not mitochondrial DNA) with proposed roles in cholesterol transport, steroid synthesis, and heme metabolism. Alternative pathways may compensate for loss of TSPO function, as null knockout mice have been shown to be viable and fertile [2]. TSPO is expressed in several tissues, including peripheral monocytes, heart, lung, kidneys, adrenals, gonads, and vascular epithelium. In the brain parenchyma, TSPO is mainly expressed by microglia but also by astrocytes [3]. Under physiological conditions, TSPO expression is low in the brain. However, in response to cellular injury, the density of TSPO increases. Whether this increase in TSPO density is due to increased TSPO expression per microglia, or increased number of microglia by migration to the site of injury, may depend on the species and mechanism of injury involved [4]. Recruitment of peripheral myeloid cells into the brain may also contribute to TSPO density [5]. The exact role of TSPO in neuroinflammation is not well established, but evidence suggests this protein may influence mitochondrial reactivity, release of reactive oxygen species, and activation of the inflammasome [6].

PET Radiotracers

The first PET radioligand developed for TSPO was ^{11}C-*(R)*-PK 11195, an isoquinoline carboxamide. Studies have demonstrated increased ^{11}C-*(R)*-PK 11195 signal in neurodegenerative disorders such as Alzheimer's disease, frontotemporal dementia, Huntington's disease, and parkinsonian disorders (see [7] for review). Increased ^{11}C-*(R)*-PK 11195 binding has also been shown in patients with multiple sclerosis, HIV infection, stroke, and epilepsy. However, ^{11}C-*(R)*-PK 11195 has the disadvantage of low ratio of specific-to-nonspecific binding, resulting in low signal-to-noise on imaging [8].

Second-generation TSPO radioligands with improved ratio of specific-to-nonspecific binding include ^{11}C-PBR28, ^{11}C-DPA-713, ^{18}F-DPA-714, ^{11}C-DAA1106, and ^{18}F-FEPPA. A shared limitation of second generation radioligands is their sensitivity to a common polymorphism on the *TSPO* gene that variably affects the binding affinity [9]. Therefore, individuals who have two gene copies of the rare allele (low-affinity binders) have lower binding than those who only express the common allele (high-affinity binders), while those heterozygous have intermediate binding since they express TSPO with both high- and low-affinity binding sites (mixed affinity binders). In Caucasians and African-Americans, the allelic frequency of the polymorphism is about 30%, resulting in approximately 9% of the population

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being low-affinity binders. Exclusion of low-affinity binders (identified by genetic analysis or binding assays performed on peripheral blood cells), and statistical correction for variance in binding caused by presence or absence of one copy of the rare allele, has allowed use of second-generation TSPO radioligands in clinical PET studies [10].

A recently developed "third-generation" TSPO radioligand, ^{11}C-ER176, has reduced sensitivity to the *TSPO* polymorphism, allowing inclusion of low-affinity binders but still requiring statistical correction of variance created by the presence of the polymorphism [11]. ^{11}C-ER176 also has higher signal-to-noise than some second-generation radioligands and does not generate brain-penetrant radiometabolites [12]. ^{18}F-GE-180 has demonstrated high signal-to-noise in rodent studies [13]. However, its poor brain uptake in clinical studies has raised concerns about the ability of ^{18}F-GE-180 to effectively measure TSPO density in humans [14, 15].

While TSPO is the most studied target for inflammatory PET radioligands, other proteins have been proposed. Monoamine oxidase B (MAO-B) is an enzyme expressed by astrocytes and serotonin-releasing neurons. MAO-B not only is involved in monoamine metabolism but also has pro-oxidative functions by generating hydrogen peroxide. Increased MAO-B expression has been demonstrated in reactive astrocytosis, similar to the elevation seen in glial fibrillary acidic protein [16]. Radioligands used to target MAO-B include ^{11}C-deuterium-L-deprenyl, ^{11}C-SL25.1188 [17], and ^{18}F-SMBT-1 [18].

Radioligands for several other inflammatory targets are currently under development (Table 17.1). These targets include cyclooxygenase 1 (COX-1), colony stimulating factor 1 receptor (CSF1R), and the purinergic P2X7 receptor. Each is predominantly expressed by microglia in the brain. However, to date, radioligands for these novel targets are in early developmental stages and have not yet been well validated in human disease models.

Image Interpretation

TSPO is diffusely expressed throughout the brain, and therefore, no true reference region exists. Accordingly, accurate quantification of TSPO density in the brain requires kinetic modeling using the metabolite-corrected arterial input function. While this method allows absolute measurement of TSPO, the need for arterial sampling and plasma analysis is not practical for clinical use. Alternative methods that obviate the need for arterial catheterization have therefore been developed; however, these come with trade-offs such as underestimation bias. The use of a "pseudo-reference" region, one where pathological expression of TSPO is expected to be low or absent (e.g., the cerebellum in patients with Alzheimer's disease), has been used to measure relative binding, similar to the standard uptake value ratio approach commonly applied to amyloid and tau PET imaging [19]. Similar strategies have been used for kinetic modeling of PET data, which provides more robust quantification, either using an a priori pseudo-reference region or a supervised cluster analysis, in which voxels that behave as if they are devoid of specific binding are identified and grouped to create a data-driven reference region [20]. Currently, no consen-

Table 17.1 Neuroinflammatory proteins with currently available PET radioligands

Target protein	Cell expression in the brain	Role in inflammation	Current radioligands
18 kDa translocator protein (TSPO)	Microglia Astrocytes Also expressed by peripheral monocytes	Possible role in mitochondrial reactivity and activation of the inflammasome	^{11}C-*(R)*-PK 11195 ^{11}C-PBR28 ^{11}C-DPA-713 ^{11}C-DAA1106 ^{11}C-ER176 ^{18}F-FEPPA ^{18}F-DPA-714 ^{18}F-GE180
Monoamine oxidase B (MAO-B)	Astrocytes Serotonin-releasing neurons	Generation of hydrogen peroxide	^{11}C-deuterium-L-deprenyl ^{11}C-SL25.1188 ^{18}F-SMBT-1
Cyclooxygenase 1	Microglia	Facilitates pro-inflammatory prostaglandin upregulation	^{11}C-PS13
Colony stimulating factor 1 receptor (CSF1-R)	Microglia Also expressed by peripheral macrophages	Controls the activation and survival of macrophages and microglia	^{11}C-CPPC
Purinergic P2X7 receptor (P2X7R)	Microglia Oligodendrocytes Astrocytes Also expressed by peripheral macrophages	Inflammatory cytokine release	^{11}C-JNJ-54173717 ^{18}F-JNJ-64413739 ^{11}C-SMW139

sus exists on which is the preferred method of quantification, as this may vary depending on which radioligand is used and for what clinical application.

Clinical Application

TSPO PET studies have been performed in several neurological and psychiatric diseases, often with disparate results, the latter likely resulting from differences in methodologies used across laboratories. The most consistent positive results have been demonstrated in patients with Alzheimer's disease, major depressive disorder, and traumatic brain injury.

Alzheimer's Disease

Several studies using the prototypical TSPO radioligand ^{11}C-*(R)*-PK 11195 have shown increased binding in patients with Alzheimer's disease than in age-matched control participants [21–23]. Most studies using second-generation radioligands, when correcting for the *TSPO* polymorphism, have shown similar results [24, 25]. Studies have additionally shown that increased TSPO binding is associated with worse cognitive performance, cortical volume loss, and the extent of tau pathology (Fig. 17.1) [26, 27]. While multiple longitudinal studies have shown overall increase in TSPO binding in patients with Alzheimer's disease over time [28, 29], results from one study demonstrated an interval decrease in TSPO signal in patients with mild cognitive impairment [30]. A meta-analysis of 28 TSPO PET studies showed increased binding in mild cognitive impairment and Alzheimer's disease patients, particularly in neocortical regions, with inverse association between TSPO and Mini-Mental Status Exam score in the parietal lobe [31].

A small number of PET studies have used non-TSPO radioligands to detect inflammation in patients with Alzheimer's disease. One study showed increased MAO-B binding in patients with mild cognitive impairment, while decreased in patients with Alzheimer's disease, suggesting that increased astrocytosis might be limited to early clinical stages of the disease [32]. A study using arachidonic acid PET imaging showed increased radioligand turnover in Alzheimer's disease patients [33].

Major Depressive Disorder

Several studies have shown increased TSPO binding in patients with major depressive disorder, particularly in untreated patients during major depressive episodes (Fig. 17.2) [34–39]. These include studies using ^{11}C-*(R)*-PK 11195 and second-generation radioligand studies using either ^{11}C-PBR28 or ^{18}F-FEPPA. One study using ^{11}C-PBR28 in nine patients with depression (eight untreated, one treated) and ten healthy control participants reported no difference in TSPO binding between groups [40]. However, this negative study included

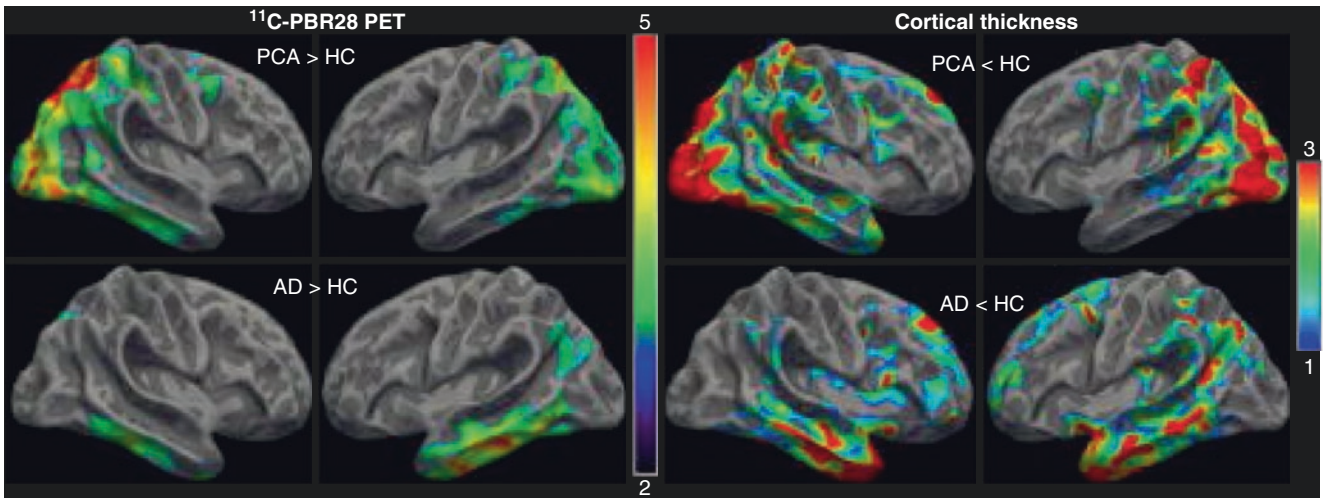

Fig. 17.1 Patterns of increased TSPO binding mirror those of neurodegeneration in patients with different clinical variants of Alzheimer's disease. Surface-based projection maps showing differences in ^{11}C-PBR28 binding (left) and cortical thickness (right) among patients with posterior cortical atrophy (PCA) and amnestic Alzheimer's disease (AD) and in healthy controls (HC). Voxels with threshold $P < 0.05$ correcting for family-wise error are displayed and color bars denote *T*-values. (Adapted from Kreisl et al., Distinct patterns of increased translocator protein in posterior cortical atrophy and amnestic Alzheimer's disease, Neurobiology of Aging, 2017)

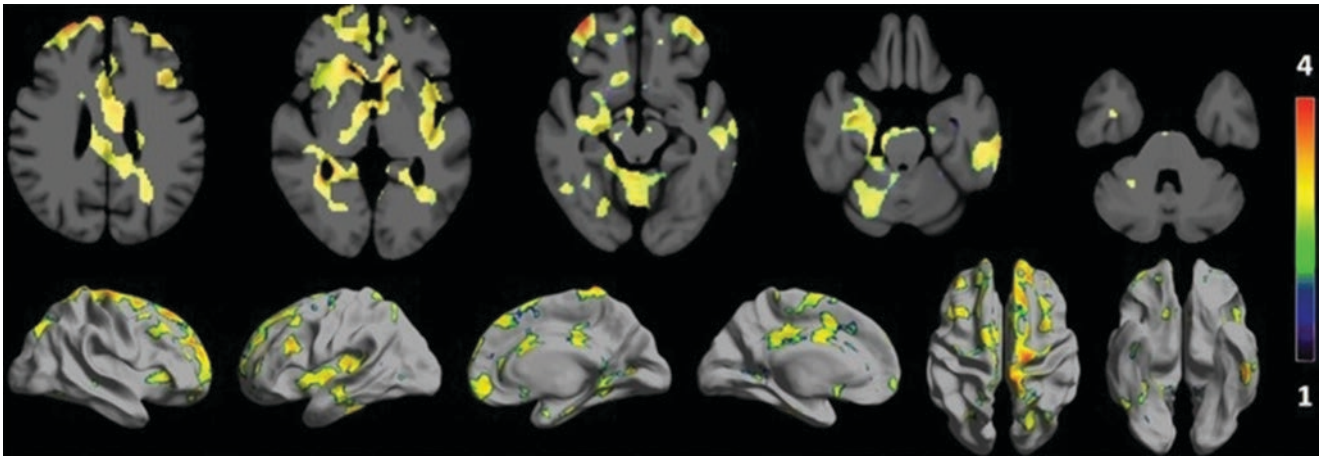

Fig. 17.2 Statistical significance maps rendered on axial slices and semi-inflated surface showing increase in 11C-PBR28 binding in unmedicated depressed patients compared to healthy controls. Only clusters with threshold $p<0.05$ for cluster-wise multiple comparisons are displayed. The color bar represents T values. (Adapted from Richards et al. PET radioligand binding to translocator protein (TSPO) is increased in unmedicated depressed subjects. EJNMMI Res. 2018; 8: 57)

fewer participants than the mentioned positive TSPO studies. Moreover, patients experiencing a major depressive episode who also had elevated ^{18}F-FEPPA binding were more likely to experience reduction in mood symptoms after treatment with the anti-inflammatory drug celecoxib [41].

Traumatic Brain Injury

Increased TSPO binding has been detected with both ^{11}C-*(R)*-PK 11195 and ^{11}C-PBR28 in participants with prior history of traumatic brain injury [42, 43]. Two studies using ^{11}C-DPA-713 were performed in players from the National Football League, one that included active and recently retired football players and one that included former players 57–74 years of age. Both studies showed widespread increase in TSPO binding, with particularly high signal in the supramarginal gyrus [44, 45]. However, elevated TSPO signal was not associated with differences in cognitive performance between NFL players and controls. Therefore, the clinical significance of these neuroinflammatory changes detected in active and retired professional football players remains to be seen.

PET/MRI

Clinical TSPO studies using simultaneous PET/MRI modalities are limited. However, those studies using TSPO PET/MRI demonstrate both the feasibility of this technique in human research and potential advantages. For example, one study performed dual PET/MRI using ^{18}F-DPA-714 in nine patients with suspected primary angiitis of the central nervous system, a rare inflammatory disorder that affects the cerebrovasculature and is associated with changes on MRI, such as multifocal ischemic and hemorrhagic lesions, vessel wall and leptomeningeal enhancement, and stenosis of the blood vessels [46]. Two patients in this study showed focal increase in TSPO binding that extended beyond the areas of contrast enhancement or restricted diffusion on MRI and were confirmed to have primary angiitis of the central nervous system on biopsy. Three patients with primary angiitis of the central nervous system did not have increased ^{18}F-DPA-714 binding despite MRI changes. However, these three were on immunosuppressive therapy for a longer period of time than the TSPO-positive patients, which may have affected the PET results. Four patients were determined to have strokes not associated with vasculitis, and those cases showed variable amounts of ^{18}F-DPA-714 uptake, not extending beyond the area of infarction seen on MRI.

In another TSPO PET/MRI study, ^{11}C-PBR28 detected increased TSPO binding in veterans with Gulf War illness in several cortical regions, including precuneus, prefrontal, primary motor, and somatosensory cortices [47]. TSPO binding was not related to clinical symptoms or concentrations of peripheral inflammatory markers.

In a PET/MRI study of patients with Huntington's disease, ^{11}C-PBR28 binding was greater in patients than controls in the putamen and pallidum [48]. Increased TSPO signal was also detectable at the single subject level, with some participants demonstrating binding in the thalamic and brain stem nuclei thought to be related to visual and motor function.

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