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Full-length Article

# *In vivo* evidence of a functional association between immune cells in blood and brain in healthy human subjects

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ABSTRACT

Microglia, the resident macrophages in the central nervous system, are thought to be maintained by a local self-renewal mechanism. Although preclinical and *in vitro* studies have suggested that the brain may contain immune cells also from peripheral origin, the functional association between immune cells in the periphery and brain at physiological conditions is poorly understood.

We examined 32 healthy individuals using positron emission tomography (PET) and [$^{11}$C]PBR28, a radioligand for the 18-kDa translocator protein (TSPO) which is expressed both in brain microglia and blood immune cells. In 26 individuals, two measurements were performed with varying time intervals. In a subgroup of 19 individuals, of which 12 had repeat examinations, leukocyte numbers in blood was measured on each day of PET measurements. All individuals were genotyped for TSPO polymorphism and categorized as high, mixed, and low affinity binders. We assessed TSPO binding expressed as total distribution volume of [$^{11}$C]PBR28 in brain and in blood cells.

TSPO binding in brain was strongly and positively correlated to binding in blood cells both at baseline and when analyzing change between two PET examinations. Furthermore, there was a significant correlation between change of leukocyte numbers and change in TSPO binding in brain, and a trend-level correlation to change in TSPO binding in blood cells. These *in vivo* findings indicate an association between immunological cells in blood and brain *via* intact BBB, suggesting a functional interaction between these two compartments, such as interchange of peripherally derived cells or a common regulatory mechanism. Measurement of radioligand binding in blood cells may be a way to control for peripheral immune function in PET studies using TSPO as a marker of brain immune activation.



## 1. Introduction

The brain was long considered to be an immunologically privileged organ due to the blood brain barrier (BBB) preventing entry of immune cells from the peripheral circulation. However, during the last decades it has become clear that circulating blood monocytes, progenitors of macrophages, can move into the brain through the intact BBB (Lawson et al., 1992; Persidsky et al., 1997; Fiala et al., 1997). Furthermore, in addition to parenchymal microglia the brain hosts also other myeloid populations such as macrophages in the choroid plexus, perivascular space and meninges (Prinz and Priller, 2014). Very recently, a lymphatic system has been identified in the dural sinuses, further suggesting transfer of immune cells between the periphery and the central nervous system (Louveau et al., 2015). Although brain microglia and peripheral macrophages have similar morphology and

*Abbreviations:* AUC, area under the curve; BBB, blood brain barrier; HAB, high affinity binder; HCT, hematocrit value; LAB, low affinity binder; MAB, mixed affinity binder; PET, positron emission tomography; ROI, region of interest; TAC, time activity curve; TSPO, 18-kDa translocator protein; $V_T$, distribution volume.

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phagocytic functions, microglia are thought to have distinct ontogenesis and kinetic features. In contrast to peripherally derived cells, microglia are long-lived and are considered to be maintained primarily by local self-renewal mechanisms (Ginhoux et al., 2010; Prinz and Priller, 2014). Importantly, most reports of interactions between central and peripheral cell populations are based on preclinical or *in vitro* studies. Whether blood monocytes can reach the human brain through intact BBB at physiological conditions, or if there is an indirect link between immune cells in brain and blood in humans is still largely unknown.

The translocator protein (TSPO), formally named the peripheral benzodiazepine receptor, is a mitochondrial protein highly expressed in brain microglia and macrophages, and to some extent astrocytes (Papadopoulos et al., 2006; Zavala et al., 1984; Venneti et al., 2013). In blood, the protein is also expressed in monocytes and polymorphonuclear neutrophils, as well as to a lesser extent in other cell types (Canat et al., 1993). Quantitative imaging of TSPO with positron emission tomography (PET) may provide a biomarker for the activity of brain immune cells, and several TSPO radioligands have accordingly been developed during recent years. Among those, the second generation TSPO radioligand [$^{11}$C]PBR28 shows an increased signal-to-noise ratio compared to the first-generation TSPO radioligand [$^{11}$C]PK11195 (Brown et al., 2007; Fujita et al., 2008; Imaizumi et al., 2008; Kreisl et al., 2010; Jučaite et al., 2012) and has been used to demonstrate changes in the brain of patients with inflammation-related diseases (Oh et al., 2011; Kreisl et al., 2013a).

There are three different TSPO binding phenotypes in human subjects, i.e. high, mixed and low-affinity binders (HAB, MAB and LAB, respectively), for which the difference in binding affinity is caused by a polymorphism of the *Tspo* gene (rs6971) leading to an amino-acid substitution (Ala147Thr) (Owen et al., 2011, 2012). Since TSPO is expressed also in peripheral immune cells, binding to this protein also outside of the brain might be affected by the *Tspo* polymorphism. However, thus far there are no published quantitative studies on TSPO binding in peripheral blood cells *in vivo*, and its relationship to TSPO binding in brain has not been explored.

As is the case for other TSPO radioligands, there is a high degree of inter-individual variability in [$^{11}$C]PBR28 binding, also after taking TSPO genotype into account (Fujita et al., 2008; Kreisl et al., 2010; Narendran et al., 2014; Collste et al., 2016). Furthermore, we have recently reported a sizeable test–retest variability of 18.2% in [$^{11}$C]PBR28 binding in healthy control subjects (Collste et al., 2016). In that study, a diurnal effect was observed where [$^{11}$C]PBR28 binding measured in the afternoon was higher compared to that in the morning, suggesting that part of the variability may have a biological source. This is in line with experimental evidence showing that the monocyte/microglia system is highly dynamic, as demonstrated for instance in studies showing rapid recovery after experimental microglia depletion (Elmore et al., 2014). If there is a link between the peripheral and central immune cells, such that changes in the peripheral immune system may influence brain TSPO levels, this relationship could contribute to the intra- and inter-individual variability of [$^{11}$C]PBR28 binding in brain. In that case, controlling for change of peripheral TSPO binding in brain PET measurements may allow for a more sensitive detection of brain-specific immune activation both in case-control and longitudinal studies.

The primary aim of this study was to examine the association between TSPO-binding in peripheral blood cells and brain. A secondary aim was to investigate whether accounting for TSPO binding in blood could reduce variability in measurements of TSPO levels in brain. For that purpose, we analyzed [$^{11}$C]PBR28 PET data from 32 healthy individuals, of which 26 underwent repeated measurements. We calculated the distribution volume of [$^{11}$C]PBR28 binding in blood cells ($V_{T\ Blood\ cells}$) from blood and plasma radioactivity and compared that to brain TSPO binding expressed as $V_{T\ Brain}$. Based on preclinical and *in vitro* experiments, we hypothesized that there would be an association between these two parameters. Assuming that TSPO levels and blood immune cell numbers may change also during physiological conditions, we furthermore compared the change of $V_{T\ Brain}$ and $V_{T\ Blood\ cells}$ between two PET measurements, as well as the correlation between [$^{11}$C]PBR28 binding and blood leukocyte numbers.

## 2. Materials and methods

### 2.1. Subjects

This study was approved by the Regional Ethical Review Board in Stockholm, Sweden, and the Radiation Safety Committee at Karolinska University Hospital, Stockholm, and was performed in accordance with the current amendment of the Declaration of Helsinki and International Conference on Harmonization/Good Clinical Practice guidelines. Two cohorts of healthy subjects were included in this study (Table 1). Cohort 1 included 7 males and 6 females aged 24.1 ± 3.0 (mean ± SD), and cohort 2 consisted of 9 males and 10 females aged 42.2 ± 13.4. All subjects were genotyped for the rs6971 polymorphism using a Taqman SNP genotyping assay and classified as HAB, MAB and LAB ($n$ = 18, 13 and 1, respectively).

### 2.2. PET and MR measurements

For 26 subjects (13 from cohort 1 and 2, respectively. See Table 1), two PET measurements were performed either on the same day ($n$ = 6, 3 HABs and 3 MABs, average time interval of 202 ± 62 min between injections), on separate days with short intervals ($n$ = 7, 3 HABs, 3 MABs and 1 LAB, average interval 4.0 ± 2.1 days), or on separate days with long intervals ($n$ = 13, 8 HABs and 5 MABs, average interval 158 ± 73 days). Examinations were performed using the High Resolution Research Tomograph (HRRT, Siemens Molecular Imaging, Knoxville, TN, USA). For each subject, a plaster helmet was made that fixated the subject's head to the PET system in order to prevent head motion during examinations. A transmission scan was performed using a rotating $^{137}$Cs source in order to correct the emission data for signal attenuation.

[$^{11}$C]PBR28 was prepared as described previously (Collste et al., 2016) and was administered intravenously as a bolus injection. Mean injected radioactivity in PET1 for all subjects ($n$ = 32) was 408 ± 52 MBq, for PET2 ($n$ = 26) 404 ± 42 MBq. Mean injected mass in PET1 ($n$ = 32) was 0.52 ± 0.26 μg, in PET2 ($n$ = 26) 0.61 ± 28 μg. The differences in injected radioactivity and specific activity for those subjects that underwent two PET measurements were not statistically significant. PET images were acquired in list mode for 93 min except for two individuals where data acquisition was limited to 60 min for PET2 in cohort 1 (HAB, different day with short intervals), and 50 min for PET1 in cohort 2 (HAB, different day with long intervals). For all subjects, the radioactivity level in arterial blood ($C_{Blood}$) was measured *via* an automatic blood sampling system (ABSS; Allogg Technology, Mariefred, Sweden) for the first 5 min, and by manual sampling. Manual sampling time was at 1, 3, 5, 7, 9, 10.5, 20, 30, 40, 50, 60, 70, 80, and 90 min in cohort 1, and at 2, 4, 6, 8, 10, 15, 20, 25, 30, 40, 50, 70, and 90 min in cohort 2. Radioactivity level in arterial plasma ($C_{Plasma}$) was measured after centrifugation of the blood sample. To calculate plasma radioactivity for the ABSS phase (0–5 minutes), the plasma-to-ratio for two first manual samples were linearly interpolated to create a continuous time-curve, which was then