The Immune Response of the Human Brain to Abdominal Surgery

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Objective: Surgery launches a systemic inflammatory reaction that reaches the brain and associates with immune activation and cognitive decline. Although preclinical studies have in part described this systemic-to-brain signaling pathway, we lack information on how these changes appear in humans. This study examines the short- and long-term impact of abdominal surgery on the human brain immune system by positron emission tomography (PET) in relation to blood immune reactivity, plasma inflammatory biomarkers, and cognitive function.
Methods: Eight males undergoing prostatectomy under general anesthesia were included. Prior to surgery (baseline), at postoperative days 3 to 4, and after 3 months, patients were examined using [^{11}C]PBR28 brain PET imaging to assess brain immune cell activation. Concurrently, systemic inflammatory biomarkers, ex vivo blood tests on immunoreactivity to lipopolysaccharide (LPS) stimulation, and cognitive function were assessed.
Results: Patients showed a global downregulation of gray matter [^{11}C]PBR28 binding of $26 \pm 26\%$ (mean $\pm$ standard deviation) at 3 to 4 days postoperatively compared to baseline ($p = 0.023$), recovering or even increasing after 3 months. LPS-induced release of the proinflammatory marker tumor necrosis factor-α in blood displayed a reduction ($41 \pm 39\%$) on the 3rd to 4th postoperative day, corresponding to changes in [^{11}C]PBR28 distribution volume. Change in Stroop Color-Word Test performance between postoperative days 3 to 4 and 3 months correlated to change in [^{11}C]PBR28 binding ($p = 0.027$).
Interpretation: This study translates preclinical data on changes in the brain immune system after surgery to humans, and suggests an interplay between the human brain and the inflammatory response of the peripheral innate immune system. These findings may be related to postsurgical impairments of cognitive function.

ANN NEUROL 2017;81:572–582

View this article online at wileyonlinelibrary.com. DOI: 10.1002/ana.24909

Received Nov 10, 2016, and in revised form Feb 15, 2017. Accepted for publication Feb 26, 2017.

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A growing body of evidence suggests that surgical trauma launches a systemic inflammatory response that ultimately reaches and activates the intrinsic immune system of the brain.[1–4] Triggered by surgery-induced damage-associated molecular patterns (DAMPs), an array of proinflammatory mediators and activated blood-borne immune cells orchestrate a rapid spread of this systemic response to the central nervous system (CNS), with inflammatory markers detectable in human cerebrospinal fluid (CSF) within 12 hours.[4–7] In surgical rodent models, this periphery-to-brain pathway seems critically dependent on NF-κB and proinflammatory cytokine signaling (eg, tumor necrosis factor-α [TNF-α]) associated with a short-lasting disruption of blood–brain barrier integrity,[2,3,8] migration of peripheral macrophages into the CNS, and subsequent hippocampal neuronal dysfunction and cognitive impairment.[8] In addition to an acute and transient response, often referred to as a syndrome of sickness behavior including fatigue, anorexia, and fever, surgery-induced immune activation may be associated with prolonged impairments in learning, memory, and concentration termed postoperative cognitive dysfunction.[9–12]

In patients, inflammatory molecules such as TNF-α and interleukins appear in CSF within 12 hours after major surgery.[4,13–15] Although such clinical observations are in line with a series of experimental studies,[2,3,8] the time course pattern beyond the immediate postsurgery phase of immune activation within the human CNS is unknown, and how the systemic pro- and anti-inflammatory response[16–18] is associated with cognitive performance is largely unexplored.

The use of positron emission tomography (PET) and radioligands selective for the translocator protein (TSPO) provide an opportunity for translational studies exploring brain immune activity after surgery. In brain parenchyma, TSPO is primarily expressed in microglia and to a lesser extent in astrocytes. This protein can be viewed as a marker for CNS immune activation, because changes in TSPO levels have been shown to reflect changes in glial cell activity.[19,20] TSPO expression is typically elevated in several acute and chronic CNS disorders involving the immune system[21–25] as well as in animal models of acute inflammation[26] or stroke.[19] With regard to periphery-to-brain interactions, lipopolysaccharide (LPS)-induced acute systemic inflammation is followed by a rapid and transient activation of the brain immune system, as demonstrated using the TSPO radioligand [^{11}C]PBR28 in nonhuman primates[27] and humans.[28]

Here, we examined the impact of major surgery on the human brain immune system by a longitudinal series of PET examinations of TSPO binding in otherwise healthy patients undergoing abdominal surgery and how changes in glial cell activation relate to systemic inflammatory response and cognitive performance.

Patients and Methods

Patients

The study was approved by the Regional Ethics Committee on Human Research at Karolinska Institutet and the local Radiation Safety Committee, Karolinska University Hospital, Stockholm, Sweden. The protocol conformed to the standard of the Declaration of Helsinki, Finland and has been registered at the U.S. National Institutes of Health (NCT01881646; ClinicalTrials.gov).

Eight male patients with American Society of Anesthesiologists physical status 1–2 and scheduled for a robot-assisted radical prostatectomy entered the study after oral and written informed consent. Exclusion criteria included any neurological, metabolic, or cardiovascular disorder, smoking or the use of snuff, or drugs known to interfere with either the immune system or with inflammation. Patients with preoperative cognitive impairment corresponding to a Mini-Mental State Examination score < 25 were excluded. Demographic data are presented in Table 1.

Each subject was examined on 3 separate occasions, that is, 1 to 3 days prior to surgery, on postoperative days 3 to 4, and at 3 months postoperatively. At each occasion, we obtained PET measurements on TSPO binding in brain, systemic biomarkers of inflammation and neuronal injury, ex vivo blood tests on immunoreactivity to LPS stimulation, and assessment of cognitive function.

On the day of surgery, anesthesia was induced between 8:00 and 11:00 AM with thiopental 4 to 7mg/kg intravenously, and after endotracheal intubation using rocuronium 0.6mg/kg, anesthesia was maintained with remifentanil at 0.1 to 0.7 μg/kg/min in combination with desflurane 3.0 to 5.7% in 30 to 50% oxygen in air during mechanical ventilation. Propofol was omitted, as this drug may affect [^{11}C]PBR28 binding.[29]

TABLE 1. Demographic Data for 8 Male Patients Undergoing Robot-Assisted Prostatectomy under General Anesthesia

Characteristic	Value
Age, yr	61 ± 7
Height, cm	176 ± 5
Weight, kg	82 ± 5
Body mass index, kg/m^2	26 ± 2
Duration of surgery, h	3.1 ± 0.8
Blood loss, ml	105 ± 77
Length of stay, days	2.4 ± 1.0

Duration of surgery ranged from 1.5 to 4.5 hours, and the stay in the postanesthetic care unit lasted 4 to 8 hours before transfer to a surgical ward for mobilization. Hospital stay was 2 to 3 days, during which analgesic regimen included oral paracetamol 2 to 4g/day combined with oxycodone 5 to 10mg/day as requested by the patient until discharge; no patient experienced a visual analog scale pain score >4 in the immediate postoperative period, the cutoff level for significant surgical pain. Although most patients had returned to a score of 0 at 3 months, Patient 7 had neck and back pain with a score >4. None of the patients had any postoperative adverse events related to surgery, anesthesia, or infection for the entire 3-month follow-up period.

PET Imaging

For each subject, the 3 PET examinations were conducted either in the morning/before lunch or in the afternoon/after lunch to avoid a possible diurnal influence, with 1 exception due to scheduling conflicts. PET measurements were performed using the High Resolution Research Tomograph (Siemens Molecular Imaging, Knoxville, TN) at the PET center at the Karolinska Institutet, Stockholm, Sweden. Prior to the first PET scan, a preoperative magnetic resonance imaging scan using a 3T Discovery MR750 system (General Electric, Milwaukee, WI) was performed for coregistration with PET and definition of anatomical brain regions. At each study occasion, patients received a radial artery catheter to allow automated arterial blood sampling and a cubital vein catheter in the contralateral arm for intravenous radio ligand administration. Patients were positioned in the PET system using an individually designed helmet placed in a frame holder to minimize head movement during the PET data acquisition. The same helmet and position were used for all 3 study occasions.

[^{11}C]PBR28 was prepared and injected as described previously.[30–32] The average radioactivity administered was 435 ± 50MBq (mean $\pm$ standard deviation [SD]), with a specific radioactivity of 229 ± 82GBq/μmol and an injected mass of 0.77 ± 0.38 μg. PET data were acquired for 63 minutes. Arterial blood was sampled using an automated system for the first 5 minutes. Manual samples were drawn at 2, 4, 6, 8, 10, 15, 20, 25, 30, 45, and 60 minutes. All patients were genotyped for the genetic polymorphism of rs6971, which affects binding of TSPO radioligands, including [^{11}C]PBR28, both in vitro and in vivo.[30,33,34] Six of the subjects were high-affinity binders, and there was 1 mixed-affinity binder, whereas for 1 subject the genotype could not be determined.

PET Image Analysis

Processing of the arterial input function, image processing, and the definition of regions of interest (ROIs) were performed as described previously.[30,31] The primary ROI was brain gray matter (GM). In addition, regional binding in brain areas of relevance for cognitive function was assessed, that is, hippocampus, lateral frontal cortex, lateral parietal cortex, and putamen. A composite volume was defined also for white matter. Distribution volume (V_T) values were calculated using the stationary wavelet-aided parametric imaging (WAPI) approach with optimized parameters of filter kernel and depth of decomposition of 16 and 3, respectively.[35,36] WAPI utilizes Logan graphical analysis with a metabolite corrected plasma input function to fit the regional time–activity data and estimate V_T in each voxel. The estimation of V_T was based on the 6 frames from 27 to 63 minutes.

Importantly, performing full quantification with an arterial input function means that any peripheral changes in [^{11}C]PBR28 plasma concentrations are accounted for. WAPI analysis of TSPO binding has previously been shown to be sensitive to within-subject changes in V_T,[37] and data based on 63-minute acquisition have shown similar reliability compared to longer timeframes.[30] To assess individual rate constants of K_1, k_2, k_3, and k_4, an additional analysis was performed using the 2-tissue compartment model (2TCM).

Ex Vivo LPS Challenge and Systemic Inflammatory Molecules

Immediately prior to each PET examination, 5ml of arterial blood was drawn and instantly used for ex vivo LPS challenge. Another 10ml of arterial blood was sampled and directly centrifuged, and plasma was frozen for later analysis; the inflammatory molecules analyzed included interleukin-1β (IL-1β), IL-6, IL-8, IL-10, TNF-α, TNF-receptor 1 (TNF-R1), C-reactive protein (CRP), serum amyloid A (SAA), and brain injury markers neurofilament light chain (NFL) and tau.[38–40]

For the ex vivo LPS challenge, triplicate blood samples were stimulated with LPS (*Escherichia coli* 0111:B4; Sigma, St Louis, MO; L2630) at a final concentration of 10ng/ml; as a control, phosphate-buffered saline was added to triplicate samples. Blood cultures were incubated on a rocking board at 37°C, 5% CO_2 for 4 hours with 3mM adenosine triphosphate (Sigma, A2383) added for the last hour. Incubation plates were centrifuged, supernatants were collected, and TNF-α and IL-1β content were determined by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (R&D Systems, Minneapolis, MN).

Plasma levels of IL-6, IL-8, IL-10, and TNF-α were analyzed using the MSD V-PLEX Plus Human Biomarker 40-Plex Kit on a MESO QuickPlex SQ 120 instrument according to instructions from the manufacturer (Meso Scale Diagnostics, Rockville, MD). Plasma levels of high-mobility group box 1 protein (HMGB-1) were analyzed using an ELISA assay according to the manufacturer's instructions (Shino Test Corporation, Tokyo, Japan). Plasma NFL protein levels were determined by a novel single molecule array (Simoa) method (Quanterix Corporation, Lexington, MA) based on the same monoclonal antibodies and calibrator as in the CSF NFL assay (UmanDiagnostics, Umeå, Sweden),[41] transferred onto the Simoa platform using a homebrew kit (Quanterix Corporation). All analyses were performed by board-certified laboratory technicians (Department of Radiochemistry, Gothenburg University, Mölndal, Sweden) using 1 batch of reagents, with intra-assay coefficients of variation $<10\%$.

For ex vivo LPS stimulation, the protein levels in unstimulated samples were low at all time points, with no significant differences in TNF-α or IL-1β levels between time points (TNF-α: group average value [$\bar{x}$] $\pm$ SD = 260 $\pm$ 234, 181 $\pm$ 224, and 235 $\pm$ 227, respectively; IL-1β: $\bar{x} \pm$ SD = 432 $\pm$ 419, 319 $\pm$ 341, and 714 $\pm$ 479, respectively). Several samples were below the detection limits. These samples were assigned $\bar{x} + 2$ SD, to preserve the power for detection of differences between time points; this is a conservative estimate of the protein level, as the actual quantity is lower than the entered quantity, and will therefore not inflate differences between time points. To account for differences in leukocyte count in each cell culture, protein levels were normalized to leukocyte count (LPK) for each individual, using the formula: $\text{cytokine}_i/\text{LPK}_i$, where i = individual.

Brain injury markers NFL and tau were measured in plasma samples using ultrasensitive single molecule array.[38–40]

Cognitive Testing

Cognitive function was assessed prior to each of the 3 PET examinations using the International Study Group of Postoperative Cognitive Dysfunction battery as previously described.[11] In brief, the test battery assesses cognitive performance using 4 different tests, providing 7 variables for analysis, that is, the cumulative number of words recalled in 3 trials and the number of words at delayed recall from the Visual Verbal Learning Test, the time (in seconds) and number of errors in part C of the Concept Shifting Test, the time (in seconds) and number of errors from the third part of the Stroop Color-Word Test, and the number of correct answers from the Letter-Digit Coding Test.

Changes in cognitive performance were calculated for each of 7 test variables and corrected for practice effects and variability using data from an age-matched control group that underwent testing using the same battery and with the same intervals.[11] To quantify the change from preoperative test to the postoperative tests and between the 2 postoperative test occasions, z scores were calculated for each variable.[11]

Statistics

All statistical analyses relating to PET data below were performed using SPSS statistics version 22 (IBM, Armonk, NY). Changes of the V_T for [^{11}C]PBR28 binding in GM, hippocampus, lateral frontal cortex, lateral parietal cortex, and putamen were analyzed with repeated measures analysis of variance. Post hoc analyses for individual regions of interest were performed using paired t tests (preoperative, postoperative days 3–4, and 3 months, respectively). Due to the exploratory nature of the regional analysis and the main focus on global changes, no correction for multiple comparisons was performed. Percentage change of V_T between the 3 time points was related to corresponding changes in cognitive test variables and blood biomarkers of inflammation using Pearson correlation analysis. Because the purpose of the analysis was to investigate within-subject changes, and as TSPO binding class has been demonstrated to not influence test–retest reproducibility,[30] the binding class was not included in the analysis.

Statistical analyses of cytokine changes were performed using R version 2.9.2. The preoperative levels of leucocyte count–normalized cytokines obtained after LPS challenge (TNF-α and IL-1β), and the systemic inflammatory markers (IL-6, IL-8, IL-10, TNF-α, and HMGB-1), as well as NFL and tau, were compared to levels at either 3 to 4 days or 3 months after surgery using paired t tests. Due to the exploratory nature of the analysis, no correction for multiple comparisons was performed.

The relative percentage changes in plasma TNF-α, IL-6, and IL-10 were related to corresponding relative percentage change in [^{11}C]PBR28 binding in GM. Absolute and relative differences in LPS-induced TNF-α release and the corresponding change in [^{11}C]PBR28 binding in GM were analyzed using Spearman rank tests. Relative changes in [^{11}C]PBR28 binding in the hippocampus were analyzed in relation to memory function on the Visual Verbal Learning Test (cumulative and delayed recall) and [^{11}C]PBR28 binding in the lateral frontal cortex in relation to tests of executive function (Letter-Digit Coding Test and Stroop Color-Word Test).

Furthermore, because IL-6 has previously been shown to be correlated with postoperative cognitive impairment,[42] we analyzed the association between change in IL-6 and z scores of cognitive change, as well as change in [^{11}C]PBR28; these analyses included acute changes in IL-6 (ie, between baseline and postoperative days 3–4) versus both short- and long-term changes in cognition (ie, between baseline, and both postoperative days 3–4 and 3 months).

Results

PET Imaging

All patients (n = 8) participated in the study according to the protocol. Quantitative PET data for 1 subject was not available at baseline due to technical error during blood sampling. Parametric images for the series of 3 PET examinations in 2 subjects are shown in Figure 1.

Patients showed a global downregulation of brain TSPO binding on the 3rd to 4th postoperative day after abdominal surgery, as demonstrated by a decrease in [^{11}C]PBR28 binding (V_T) to TSPO in GM by 26 $\pm$ 26% compared to baseline ($F = 5.465$; $p = 0.023$). Comparing changes in GM regions, there was a uniform decrease in V_T in all 4 brain regions (Fig 2A, paired t tests).

On the third test occasion 3 months after surgery, 4 of the 7 individuals had numerically higher [^{11}C]PBR28 binding as compared to baseline values, although the group difference was not statistically significant ($p > 0.05$; see Fig 2).

There were no statistically significant differences in the free fraction of [^{11}C]PBR28 in plasma between the 3 time points (preoperative: 6.15 $\pm$ 1.08; postoperative days 3–4: 6.19 $\pm$ 2.29; and 3 months postoperatively:

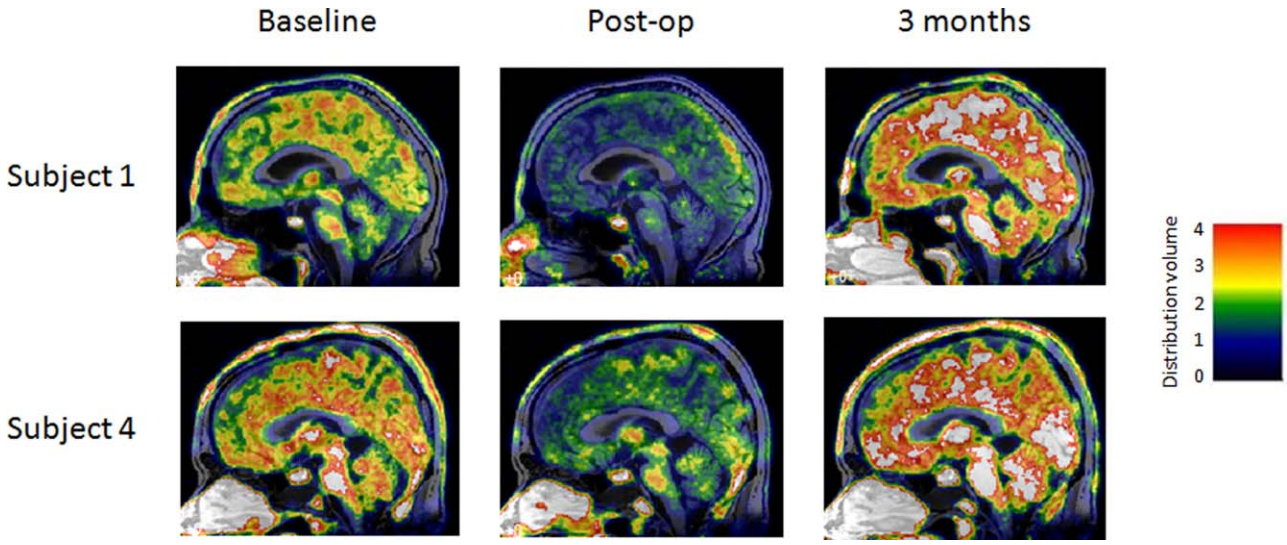

FIGURE 1: Parametric images of [^{11}C]PBR28 binding at 3 occasions preoperatively, days 3 to 4 postoperatively (Post-op), and after 3 months in 2 patients (Subjects 1 and 4) undergoing major abdominal surgery.

5.06 ± 1.73; not significant), nor any difference in the individual rate constants as derived using the 2TCM (Supplementary Table 1; $p > 0.05$).

Ex Vivo Whole Blood LPS Challenge and Systemic Plasma Biomarkers

There was a marked reduction (41 ± 39%) in whole blood LPS-induced release of TNF-α on the 3rd to 4th postoperative day as compared to preoperative level, returning to preoperative level at 3 months after surgery (Fig 3A). Changes in IL-1β release were not significant but directionally similar to those seen with TNF-α.

The time course of changes in [^{11}C]PBR28 binding to TSPO was aligned to the time course of peripheral blood immunoactivity, as assessed by LPS-induced release of TNF-α and IL-1β (see Fig 3A; Supplementary Table 2). At 3 months after surgery, the percentage change in LPS-induced TNF-α production compared to baseline showed a trend toward a positive correlation to change in the V_T for [^{11}C]PBR28 binding ($p = 0.1$).

Systemic plasma levels of TNF-α, IL-6, IL-10, TNF-R1, CRP, SAA, and NFL were significantly increased at postoperative days 3 to 4 compared to preoperatively, whereas plasma IL-1ra was reduced. There was no significant change in plasma HMGB-1 levels at the 2 postoperative time points (see Fig 3B), and plasma levels of tau remained unchanged in all patients at the 2 postoperative time points. All levels of systemic inflammatory mediators and neuronal injury biomarkers had returned to baseline at 3 months (see Fig 3B, C).

There were no associations between changes in [^{11}C]PBR28 binding in brain and systemic levels of either plasma IL-10 or TNF-α ($p > 0.05$), whereas trend level significance was reached for IL-6 ($p = 0.1$).

PET Imaging, Cognition, and Plasma Biomarkers

Only minor changes in the combined z scores for cognitive test results between the 3 test occasions were seen (Supplementary Table 3). However, changes in performance of the Stroop Color-Word Test from postoperative days 3 to 4 to 3 months correlated with changes in GM [^{11}C]PBR28 binding ($p = 0.027$; Table 2). Whereas none of the plasma biomarkers IL-10, IL-6, and TNF-α showed a significant relationship to changes in cognitive performance ($p > 0.05$) during parallel time periods, there were significant correlations between acute changes in IL-6 and long-term changes in 2 cognitive tests (Visual Verbal Learning Test, cumulated, $p = 0.041$ as well as Letter-Digit Coding Test, $p = 0.015$).

Discussion

This exploratory study uncovers a transient yet profound downregulation of the human brain immune system, measured as a decrease in glial activity in the early postoperative period after major peripheral surgical trauma. The reduction in brain TSPO binding coincided with a distinct and transient reduction of immunoreactivity in peripheral blood cells. This early postoperative downregulation was followed by recovery at 3 months after surgery, and in 4 of 7 patients, signs of upregulation of the brain immune system with increased TSPO binding were evident at this time point. Additionally, we found a change in aspects of cognitive function that corresponded to this late change in brain glial cell function. The study is the first to translate results from surgical animal models to humans and suggests an interplay between the brain and the systemic peripheral inflammatory response

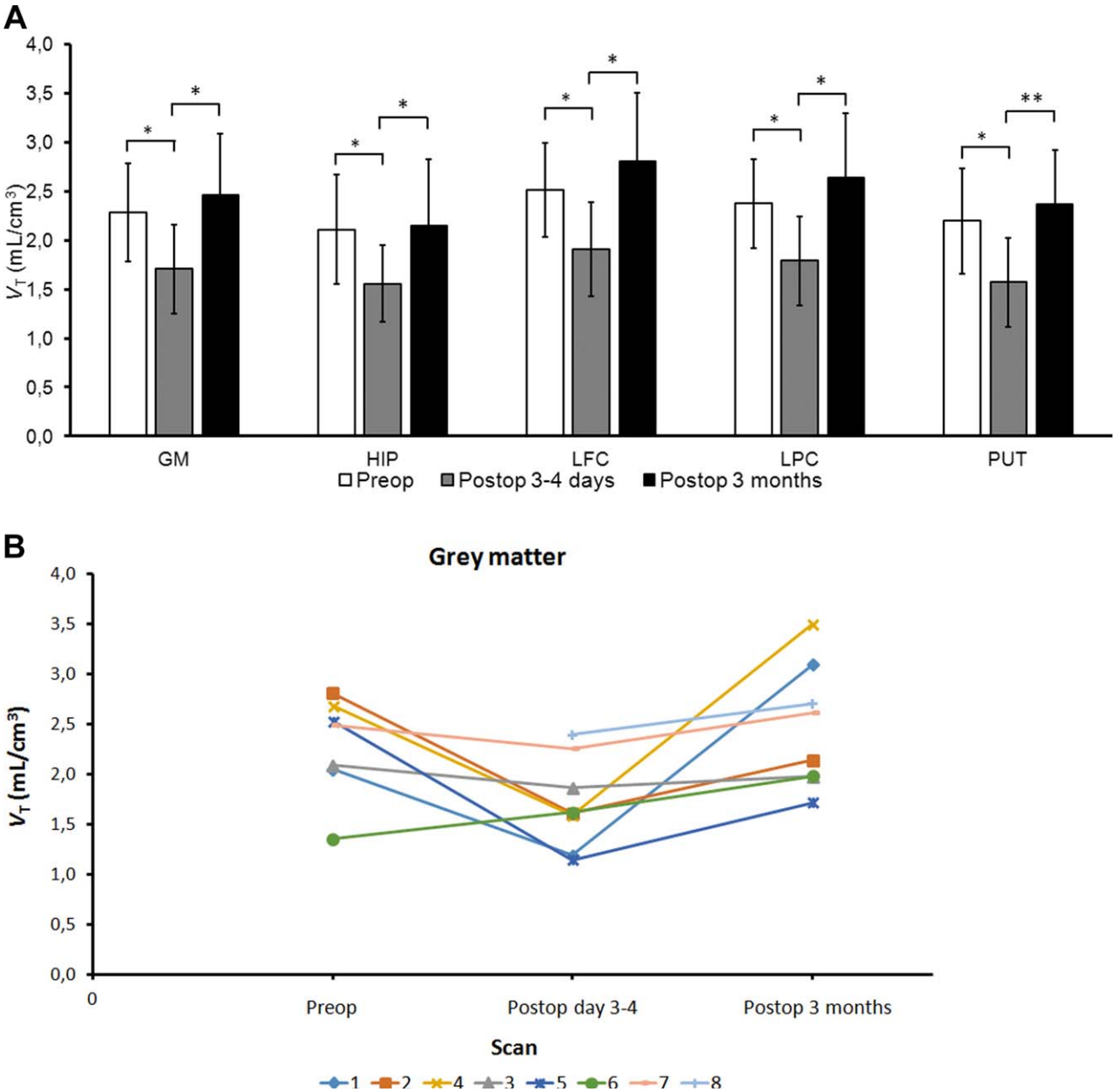

FIGURE 2: Changes in [^{11}C]PBR28 binding. (A) Changes in distribution volume (V_T) across brain regions preoperatively, that is, before abdominal surgery (white), days 3 to 4 postoperatively (gray), and after 3 months (black) by positron emission tomography (PET). Paired *t* test, *$p<0.05$, **$p<0.01$. GM = gray matter; HIP = hippocampus; LFC = lateral frontal cortex; LPC = lateral parietal cortex; PUT = putamen. (B) Individual changes of V_T across brain regions by PET in gray matter at 3 time points: before abdominal surgery (Preop), 3 to 4 days postoperatively (Postop), and after 3 months.

of the innate immune system to peripheral surgical trauma, with possible effects on cognitive function.

Although series of experimental studies in surgical animal models have outlined the periphery-to-brain signaling pathway of the inflammatory cascade,[1–3,9] the impact of acute systemic inflammation due to surgical trauma on the human brain immune system is poorly understood. The natural time course of an acute inflammatory event (eg, in response to infection or trauma) consists of a rapid initial systemic proinflammatory phase triggered by local release of DAMPs or pathogen-associated molecular patterns. Soon thereafter, an anti-inflammatory response opposes the initial proinflammatory response and the aggregate between these opposing mechanisms determines the immune-related outcome for the patient; our findings of increased plasma levels of both TNF-α (proinflammatory) and IL-10 (anti-inflammatory) on postoperative days 3 to 4 reflect these responses. The anti-inflammatory response is generally more protracted than the proinflammatory response, leading to a state of immune suppression

in patients surviving the initial "cytokine storm."[16,43] Upon reaching the brain, the proinflammatory signals interact with the resident brain immune system (eg, microglia and astrocyte populations),[3,9,27] causing a neuroinflammatory reaction and consequent neuronal dysfunction; this was shown in preclinical surgical models to affect CNS plasticity in brain regions relevant for higher cognitive functions.[1–4]

We used plasma tau and NFL as markers of acute neuronal injury[39,40] on postoperative days 3 to 4 and at 3 months. The increase in plasma NFL concentrations with stable plasma tau concentrations over time suggests that no, or very limited, CNS neuronal injury occurred. Therefore, the inflammatory response and changes in brain immune activity we found in this study may have functional rather than structural consequences.

TSPO is a mitochondrial protein expressed in immune cells in both brain and blood.[44,45] Animal studies have shown that the TSPO signal in brain is mainly derived from microglia,[20,46] with a significant contribution from astrocytes during certain conditions.[19,47] Apart from these resident immune cell populations, peripherally derived myeloid cells in the form of infiltrating or perivascular macrophages may also contribute to the signal.[45,48] In primates, systemic LPS exposure has been shown to cause a significant increase in TSPO binding within 1 to 4 hours, and postmortem immunohistochemistry confirmed a correspondence to microglia/macrophage cells, whereas colocalization of TSPO and astrocyte markers was low.[27] Notably, the initial (<4 hours) increase in global [^{11}C]PBR28 binding was followed by a profound decrease in [^{11}C]PBR28 binding at 22 hours postinjection as measured in a subset of

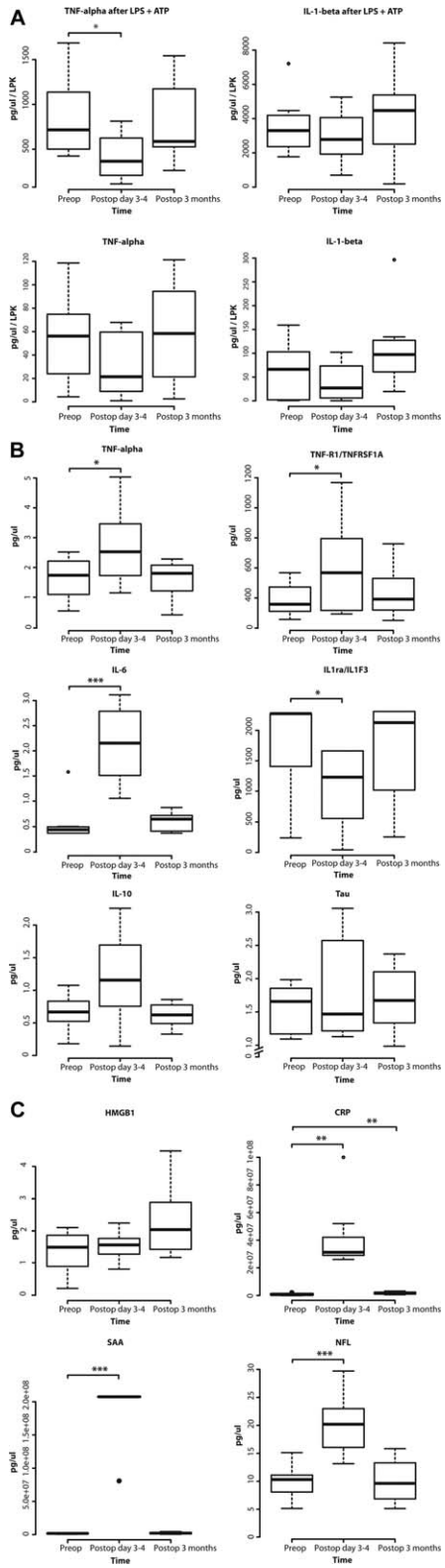

FIGURE 3:

FIGURE 3: Ex vivo and plasma cytokines. (A) Ex vivo cytokine production in abdominal surgery patients. The cytokine responses were measured by tumor necrosis factor (TNF)-α and interleukin (IL)-1β protein levels after lipopolysaccharide (LPS) + adenosine triphosphate (ATP) stimulation of whole blood preoperatively (Preop), postoperatively (Postop) at days 3 to 4, and after 3 months. Protein levels were normalized to number of leukocytes (LPK; TNF-α or IL-1β/leucocyte particle count; top panels). The TNF-α response is dampened 4 days postsurgery despite an increase in leukocytes, but has returned to normal 3 months after surgery. Although similar trends were present for TNF-α and IL-1β in unstimulated blood samples (bottom panels), the differences did not reach statistical significance. Protein levels measured Preop were compared to levels at Postop days 3 to 4 and 3 months Postop using paired *t* test; significant differences are indicated by asterisks. Bars indicate median value, and boxes indicate second and third quartiles. (B, C) Plasma cytokine, high-mobility group box 1 protein (HMGB1), C-reactive protein (CRP), serum amyloid A (SAA), neurofilament light chain (NFL), and tau concentrations following major abdominal surgery in 8 male surgical patients. Data are presented as Preop, Postop days 3 to 4, and after 3 months. Statistical significance is indicated by asterisks (paired *t* test). Bars indicate median value. and boxes indicate second and third quartiles. *$p<0.05$, **$p<0.01$, ***$p<0.001$.

TABLE 2. Correlations of Percentage Change [^{11}C]PBR28 V_T versus Cognitive *z* Scores in Abdominal Surgery Patients

		Region[a]		
Comparison	**Cognitive Test**	**GM**	**HIP**	**LFC**
Baseline vs Postop days 3–4, n = 7	Visual Verbal Learning Test, cumulated	−0.534	−0.54	—
	Visual Verbal Learning Test, delayed recall	0.150	0.211	—
	Letter-Digit Coding Test	−0.147	—	−0.283
	Stroop Color-Word Test, part 3, time	−0.216	—	−0.342
Baseline vs 3 months, n = 7	Visual Verbal Learning Test, cumulated	0.154	−0.012	—
	Visual Verbal Learning Test, delayed recall	−0.018	0.226	—
	Letter-Digit Coding Test	0.385	—	0.334
	Stroop Color-Word Test, part 3, time	0.582	—	0.531
Postop vs 3 months, n = 8	Visual Verbal Learning Test, cumulated	−0.051	−0.114	—
	Visual Verbal Learning Test, delayed recall	−0.221	−0.017	—
	Letter-Digit Coding Test	0.208	—	0.186
	Stroop Color-Word Test, part 3, time	0.650	—	0.736[b]

[a]Pearson correlation.
[b]$p < 0.05$ (2-tailed).
GM = gray matter; HIP = hippocampus; LFC = lateral frontal cortex; V_T = distribution volume.

animals. In the present study, we observed a uniform and marked decrease in [^{11}C]PBR28 binding 3 to 4 days after surgery, which arguably corresponds to this later time point. Guided by available preclinical information, our results may indicate lower numbers or activity of microglia and/or other myeloid cells in brain in the early postoperative period.

[^{11}C]PBR28 has been shown to have a higher uptake and signal-to-noise ratio than the earlier reference ligand [^{11}C]PK11195,[49] and a factor limiting the interpretation of TSPO PET data is a high degree of variability observed also in healthy control subjects. In recent test–retest studies, reproducibility of [^{11}C]PBR28 binding has been shown to be 7 to 18%.[30,50] Potential methodological sources of variance include the use of a metabolite-corrected plasma input that may introduce measurement errors, for instance due to the high rate of radioligand metabolism. To reduce the impact of this source of variability, simplified methods of quantification have been proposed, such as calculating standardized uptake values or V_T, which are then normalized to whole brain,[51,52] or pseudoreference regions such as the cerebellum.[53] However, these approaches will render only relative rather than absolute differences or changes and thus require a hypothesis that only specific regions of the brain are affected, which may not be the case even in disorders with a presumed circumscribed pathology.[54] In the present study, we hypothesized that both global and potentially local changes would be present in brain after surgery and we therefore performed full kinetic modeling, to obtain V_T values.

It may be argued that residual effects of anesthetic agents can contribute to the reduction in glial activity on the 3rd to 4th postoperative day. Although clinical studies on this topic are scarce, an acute reduction of [^{11}C]PBR28 binding has been reported in humans after propofol administration.[29] Consequently, this agent was not used in the present study. Whereas in vitro studies have shown acute effects on microglia cytokine expression by isoflurane, no such effects were reported when examining rodent astrocyte or microglial activity at 1, 3, or 7 days after anesthesia without surgery.[1,2,55,56] To our knowledge, there is currently no experimental evidence for a persistent reduction in microglia activity after 3 to 4 days due to administration of anesthetic agents; however, a contribution of anesthesia to the reductions in TSPO observed in the present study cannot be fully excluded. Another caveat in interpreting our results is the possibility that postoperative pain may have an effect on TSPO binding. As far as we know, only 1 published study has investigated this issue, showing a relative increase in [^{11}C]PBR28 binding in thalamus of patients with chronic pain[51]; it should be noted that this study reported relative changes in regional radioligand uptake and failed to describe any global difference between patients. In the present study, patients did not report

significant pain postsurgery except 1 individual having temporary neck and back pain after 3 months, making an effect of pain on TSPO binding unlikely.

Contemporaneous with [^{11}C]PBR28 PET examinations, serial ex vivo LPS stimulations were performed to assess temporal changes in immune reactivity of blood-borne immune cells after surgery-induced triggering of the innate immune system. Our finding that the release of TNF-α in LPS-stimulated blood cultures was markedly reduced at days 3 to 4 postsurgery and recovered at 3 months after surgery corresponds to the PET data and suggests a suppressed inflammatory phenotype at this time in the postoperative period. The depressed ex vivo response to LPS in blood from surgical patients is in line with previously described peripheral immune cell tolerance, typically triggered by anti-inflammatory mediators such as IL-10 and PGE_2 causing dampening of peripheral immune cell reactivity within a duration of up to 5 days after the proinflammatory triggering event.[17,57–60] In addition to this autocrine peripheral regulation, there might be an additive neuroimmunological link between the CNS and the peripheral immune system as represented by the cholinergic anti-inflammatory reflex pathway, previously described by Tracey.[61]

The analysis of [^{11}C]PBR28 binding and cognitive test data revealed an association between the increase in brain immune activity and an impairment in performance of the highly sensitive Stroop Color-Word Test. This observation is in line with results from earlier animal models[2–4] and supports the hypothesis that the postoperative cognitive dysfunction syndrome is related to surgery-induced activation of the brain immune system.[2–4] This was further supported by an association between acute changes in systemic IL-6 and long-term cognitive performance at follow-up.

The lack of relationship between simultaneous changes in systemic cytokines and brain [^{11}C]PBR28 binding are in agreement with a recent human study showing no correlation between changes in TSPO and systemic cytokine levels after LPS infusion.[28] It may be argued that measured plasma levels of inflammatory mediators reflect the net balance of production and degradation during a prolonged timespan, which is the combined production from multiple cell types, including stromal cells, for example, endothelial cells and hepatocytes, as well as blood-borne immune cells.

Conclusions

This is the first PET study of an immune marker in the human brain after peripheral surgery, revealing a profound downregulation of the brain glial activity, in the early postoperative period that is associated with a marked dampening of the immunoreactivity of peripheral blood. This downregulation of the brain and systemic immunoreactivity is followed by a normalization or upregulation of both brain and peripheral immune systems at 3 months after surgery. These processes may be related to postsurgical impairments of cognitive function.

Acknowledgment

This work was funded by the Vetenskapsrådet (Swedish Research Council Dnr 521-2011-152, Dnr 2015-02776); Torsten Söderberg Foundation, Stockholm, Sweden; Stockholm County Council (ALF grant Dnr 20140188), Stockholm, Sweden; Brain Foundation, Stockholm, Sweden; European Society for Anesthesiology, Brussels, Belgium; Tryg Foundation, Virum, Denmark; and European Union's Seventh Framework Program (FP7/2007-2013; under grant agreement HEALTH-F2-2011-278850; INMIND). S.C.'s contribution was supported by the Vetenskapsrådet (Dnr 523-2014-3467). M.M.'s contribution was supported by the NIH National Institute of General Medical Sciences (GM 104194).

We thank the staff of the Karolinska Institutet PET Science Center for excellent technical assistance.

Author Contributions

A.F., L.I.E., L.F., C.H., S.C., M.J.F., L.S.R., H.Z., K.B., H.E.H., N.T., M.M., J.B., and A.V. contributed to the study concept and design. A.F., L.I.E., L.F., C.H., S.C., M.J.F., L.S.R., H.Z., K.B., H.E.H., P.S., E.C., A.G., A.S., K.D., N.K., N.T., M.M., J.B., and A.V. were involved in data acquisition and analysis. A.F., L.I.E., L.F., C.H., S.C., M.J.F., L.S.R., H.Z., K.B., H.E.H., P.S., E.C., A.G., A.S., K.D., N.K., N.T., M.M., and A.V. took part in drafting the manuscript and figures.

Potential Conflicts of Interest

H.Z. and K.B. are cofounders of Brain Biomarker Solutions in Gothenburg, a GU Ventures–based platform company at the University of Gothenburg. L.F. is an employee of AstraZeneca.

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