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Different distribution patterns of lymphocytes and microglia in the hippocampus of patients with residual versus paranoid schizophrenia: Further evidence for disease course-related immune alterations?

Stefan Busse [a,1], Mandy Busse [b,1], Kolja Schiltz [a,c], Hendrik Bielau [a], Tomasz Gos [a,d], Ralf Brisch [a,d], Christian Mawrin [c,e], Andrea Schmitt [f], Wolfgang Jordan [a,g], Ulf J. Müller [a], Hans-Gert Bernstein [a], Bernhard Bogerts [a,c], Johann Steiner [a,c,h,*]

[a] Department of Psychiatry, University of Magdeburg, Magdeburg, Germany
[b] Department of Pediatric Pulmonology & Allergology, Medical University of Hannover (MHH), Hannover, Germany
[c] Center for Behavioral Brain Sciences, Magdeburg, Germany
[d] Institute of Forensic Medicine, Medical University of Gdańsk, Gdańsk, Poland
[e] Institute of Neuropathology, University of Magdeburg, Magdeburg, Germany
[f] Department of Psychiatry, Ludwig Maximilians University, Munich, Germany
[g] Department of Psychiatry, Magdeburg Hospital GmbH, Magdeburg, Germany
[h] Pembroke College, University of Cambridge, Cambridge, UK

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ABSTRACT

Certain cytokines have been identified in the peripheral blood as trait markers of schizophrenia, while others are considered relapse-related state markers. Furthermore, data from peripheral blood, cerebrospinal fluid (CSF) and nuclear imaging studies suggest that (1) blood–brain barrier (BBB) dysfunction (e.g., immigration of lymphocytes into brain tissue and intrathecal antibody production) correlates with the development of negative symptoms, while (2) the brain's mononuclear phagocyte system (microglial cells) is activated during acute psychosis.

Based on these neuroinflammatory hypotheses, we have quantified the numerical density of immunostained CD3+ T-lymphocytes, CD20+ B-lymphocytes, and HLA-DR+ microglial cells in the posterior hippocampus of 17 schizophrenia patients and 11 matched controls. Disease course-related immune alterations were considered by a separate analysis of residual (prevailing negative symptoms, $n = 7$) and paranoid (prominent positive symptoms, $n = 10$) schizophrenia cases.

Higher densities of CD3+ and CD20+ lymphocytes were observed in residual versus paranoid schizophrenia (CD 3: left: $P = 0.047$, right: $P = 0.038$; CD20: left: $P = 0.020$, right: $P = 0.010$) and controls (CD3: left: $P = 0.057$, right: $P = 0.069$; CD20: left: $P = 0.008$, right: $P = 0.006$). In contrast, HLA-DR+ microglia were increased in paranoid schizophrenia versus residual schizophrenia (left: $P = 0.030$, right: $P = 0.012$). A similar trend emerged when this group was compared to controls (left: $P = 0.090$, right: $P = 0.090$).

BBB impairment and infiltration of T cells and B cells may contribute to the pathophysiology of residual schizophrenia, while microglial activation seems to play a role in paranoid schizophrenia. The identification of diverse immune endophenotypes may facilitate the development of distinct anti-inflammatory schizophrenia therapies to normalize BBB function, (auto)antibody production or microglial activity.

1. Introduction

Inflammatory processes have been suggested to be prominently involved in the pathogenesis of schizophrenia. A recent meta-analysis of cytokine changes in the peripheral blood has identified interleukin (IL)-12, interferon-gamma (IFN-gamma), tumor necrosis factor-alpha (TNF-alpha), and soluble IL-2 receptor (sIL-2R) as trait markers of schizophrenia because their levels were elevated during acute exacerbations and remission (Miller et al., 2011). In contrast, IL-1beta, IL-6, and transforming growth factor-beta (TGF-beta) appeared to be state markers, as they were increased during acute psychotic episodes and were normalized during antipsychotic treatment (Miller et al., 2011).

* Corresponding author. Address: Department of Psychiatry, University of Magdeburg, Leipziger Str. 44, D-39120 Magdeburg, Germany. Tel.: +49 391 67 15019; fax: +49 391 67 15223.
E-mail address: johann.steiner@med.ovgu.de (J. Steiner).
[1] These authors contributed equally to this work.

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It is not clear yet if corresponding neuroinflammatory responses may bridge the gap between current immune and neurotransmitter hypotheses of schizophrenia (Steiner et al., 2011a). The idea of a low-grade neuroinflammation is supported by studies of cerebrospinal fluid (CSF), which identified slightly increased CSF cell counts and moderate blood-CSF barrier dysfunction in patient subgroups (Bechter et al., 2010; Maxeiner et al., 2009). An infiltration of inflammatory cells and extravasation of inflammatory mediators from vessels into brain tissue due to alterations in the blood–brain barrier (BBB) has been postulated (Axelsson et al., 1982; Torrey et al., 1985). However, a closer reading shows that the term "BBB" was used imprecisely in these publications as they only observed an increased ratio between the amount of albumin in the CSF relative to that in the serum, which reflects the blood-CSF barrier but does not prove a dysfunctional BBB (Reiber, 2003). Anatomically, the BBB is comprised of the vessel wall, the perivascular Virchow-Robin space, and the glia limitans (Bechmann et al., 2007). Conventional gadolinium-enhanced gradient echo magnetic resonance studies have failed to detect an impairment of the BBB in schizophrenia, but current imaging protocols may still be too insensitive for the detection of subtle changes (Shalev et al., 2009; Szymanski et al., 1991). Notably, the BBB should not be misinterpreted as an absolute barrier for leukocyte migration into the brain parenchyma (Galea et al., 2007). A recent electron microscopy study by Uranova et al. (2010) revealed ultrastructural abnormalities in the capillaries, including a vacuolation of endothelial cells and astrocytic endfeet in the prefrontal cortex tissue of patients with schizophrenia. However, to our knowledge, no quantitative analysis on the distribution of lymphocytes in brain tissue has been published in the field of schizophrenia research so far.

Peripheral immune processes may be mirrored in the brain by microglial cells. These antigen-presenting cells (APCs) represent the central nervous system's resident mononuclear phagocyte system (Drexhage et al., 2010; Frank et al., 2007; Steiner et al., 2011a). The "microglia hypothesis of schizophrenia" suggests that the release of proinflammatory cytokines, kynurenines, nitric oxide (NO) and reactive oxygen species (ROS) by activated microglia might cause neuronal degeneration, white matter abnormalities and decreased neurogenesis (Bernstein et al., 2009; Monji et al., 2009; Myint, 2012). In line with this idea, Nikkilä et al. observed a significantly increased proportion of activated CSF macrophages in patients with acute psychosis compared to controls (Nikkilä et al., 1999, 2001). Recent postmortem and positron emission tomography (PET) studies support this finding and point to an activation of microglia during acute psychotic episodes early in the disease course (Doorduin et al., 2009; Steiner et al., 2008, 2006; van Berckel et al., 2008). In contrast, a PET study in chronically ill schizophrenia patients (disease duration of 18.8 ± 12.2 y) did not find microglial alterations (Takano et al., 2010). Therefore, similar to the above mentioned findings in the peripheral blood, these studies suggest that specific immune alterations occur as a function of different stages of the disease course.

Based on these neuroinflammatory hypotheses, we have quantified the numerical density of immunostained CD3+ T-lymphocytes, CD20+ B-lymphocytes, and HLA-DR+ microglial cells in the hippocampus of schizophrenic patients and matched controls. The hippocampus is a particular region of interest because of its involvement in memory and regulation of affect, both of which are compromised in schizophrenia (Bogerts, 1997; Schmitt et al., 2009). We focused on the posterior hippocampus in our study because a large number of magnetic resonance imaging (MRI) studies has shown structural abnormalities in this part of the hippocampus in patients with schizophrenia (Becker et al., 1996; Bogerts et al., 1993; Hirayasu et al., 1998; Narr et al., 2001; Rametti et al., 2007; Velakoulis et al., 2001; Yamasue et al., 2004), while only some others have found pronounced pathological changes in the anterior part (Pegues et al., 2003; Szeszko et al., 2003). Our analysis of lymphocytes in the brain tissue aimed to provide indirect information on the extent to which leukocytes cross the BBB, while the determination of microglial cells should assess the central nervous system's resident mononuclear phagocyte system. Inspired by the above mentioned descriptions of immune alterations specific to the disease stage of schizophrenia, we wanted to explore potential further evidence for disease course-related patterns of immune alteration. Thus, we compared patients with paranoid schizophrenia (with psychotic positive symptoms) to patients with residual schizophrenia (with more prominent negative symptoms) in our study.

2. Materials and methods

2.1. Human brain tissue

Postmortem brains were obtained from the Magdeburg brain bank in accordance with the Declaration of Helsinki and the local institutional review board. Written consent was obtained from the next of kin. Brain tissue of 17 schizophrenia patients and 11 healthy control subjects was used for the present study. The diagnostic main groups were matched with regard to age, gender, and autolysis time, though the diagnostic subgroup of patients with residual schizophrenia had a longer disease duration (25 ± 10 versus 14 ± 9 y; $P = 0.028$) than the paranoid schizophrenia cohort (Table 1).

Information for clinical diagnoses was obtained by the careful study of clinical records and by structured interviews of physicians involved in treatment and persons who either lived with or had frequent contact with the subjects before death. Applying the DSM-IV criteria, final Axis I diagnosis was assigned in year 2004 via consensus meetings of two psychiatrists (HB, JS) using all available information from interviews and clinical records (APA, 2000). The diagnostic subtypes of schizophrenia were defined by the predominant symptomatology according to DSM-IV (Kirkpatrick et al., 2001).

Brains of subjects with lifetime reports of substance abuse, dementia, neurological illness, severe trauma, or chronic terminal diseases known to affect the brain were excluded. Changes due to neurodegenerative disorders, tumors, inflammatory, vascular, or traumatic processes were excluded by an experienced neuropathologist (CM). All patients who received antipsychotic medication during the last 90 days prior to death were treated with typical antipsychotic drugs. The chlorpromazine (CPZ) equivalents of the mean daily dose are shown in Table 1.

The tissue preparation was performed as previously described (Steiner et al., 2008, 2006). Briefly, brains were fixed in 8% phosphate-buffered formaldehyde (pH 7.0) for 3 months. After embedding the brains in paraplast, serial coronal whole brain sections were cut at 20 μm and mounted.

2.2. Immunohistochemistry

Formalin-fixed tissue sections were deparaffinized and antigen demasking was performed by boiling the sections for 4 min in 10 mM citrate buffer (pH 6.0). Preincubation with 1.5% H_2O_2 for 10 min to block endogenous peroxidase activity was followed by blocking nonspecific binding sites with 10% normal goat serum for 60 min and repeated washings with PBS. The primary antibody was diluted in PBS and applied for 48 h at 4 °C (all antibodies from DAKO, Glostrup, Denmark: polyclonal rabbit anti-human CD3, dilution 1:150/monoclonal mouse anti-human CD20, clone L26, dilution 1:50/monoclonal mouse anti-human HLA-DR, clone