Degeneration of microglial cells in frontal and temporal lobes of chronic schizophrenics

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Schizophrenia is a social disease that occurs in 0.5–1% of the population. It shows a high variability in both clinical picture and theory of its pathogenesis. Its clinical manifestations are accompanied by biochemical, immunological and structural changes.

A pivotal role in the development of psychotic disorders is attributed to the impaired limbic system. The aim of this study was to find out whether, and if so, to what extent immunocompetent cells of the central nervous system (microglia) are involved in the process of degeneration occurring in these structures.

The study was carried out on 12 brains of female chronic schizophrenics. Sections of frontal and temporal cortex were subjected to ultrastructural as well as histochemical and immunohistochemical examinations by light microscopy.

In the structures under study, a large number of ramified microglial cells showing on their surface the expression of the major histocompatibility complex class II (MHC II) was observed. Most cells showed degenerative traits (cytoplasm shrinkage, thinning, shortening and fragmentation of their processes) up to apoptotic changes. Perivascular microglia displayed the lowest intensity of degenerative changes. Ultrastructurally, some damaged microglial cells contained phagosomes and/or degenerated mitochondria. Most abnormal microglia showed morphological signs of the former normal function of immunocompetent and phagocytosing cells.

Degeneration of microglial cells, resulting most likely from the primary impairment of the neuron-glia communication that damages their immunocompetent function, may lead to the exacerbation of structural damage and psychotic symptoms. Treatment of chronic schizophrenics should involve the supply of agents to prevent degeneration of microglia and/or long-term immunotherapy.

**key words:** microglia, schizophrenia, frontal, temporal lobes, ultrastructure

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**INTRODUCTION**

Schizophrenia is a social disease that occurs in 0.5–1% of the population mostly among young people, aged 15–30 years, leading very often to their long-term disability [35, 42]. It is characterised by extensive clinical heterogeneity and variability [6, 69]. Its clinical manifestations are accompanied by more or less documented aberrations in biochemical (the amount of neurotransmitters: dopamine, glutamate, gamma-aminobutyric acid (GABA)) and immunological (HLA antigen as well as IgG, IgA and IgM antibodies) examinations [20, 41, 43, 48, 54]. In addition, the map of damaged genes is expanding, which has been reported in both family and sporadic cases of...
These abnormalities were also observed in neuroimaging examinations (6–60% of cases). They mostly involved cerebral ventricular enlargement and reduced volume of specified structures: in particular, temporal and frontal lobes, thalamus and cerebral cortex. Similar observations were made in position emission tomography (PET) and functional magnetizing resonance imaging (fMRI) applied to investigate blood flow and metabolic activity in these regions of the brain [19, 34, 35, 38, 60].

Histochemical, immunohistochemical, ultrastructural and morphometric methods were used for thorough observation of structural changes at the microscopic level [1, 14, 15, 32, 49, 53]. Analysis of neuropathological observations of the brains of schizophrenic patients showed that the following changes described in the neocortex seemed to be most specific: the differences in the density and shape of neurones, the number of synapses, changes in the number and course of neuronal processes in the superior temporal gyrus (Brodmann’s areas 22, 41, 42), prefrontal cortex (Brodmann’s areas 8–10, 41–47), and anterior cingulate cortex (Brodmann’s area 24). Studies have also shown changes in archicortex that forms the pes hippocampi and cingulate gyrus. Some abnormalities have also been reported in paleocortex, namely in the entorhinal cortex and septum pellucidum. Researchers have often observed the damage to the whole limbic system or its individual segments (hippocampus gyrus, cingulate gyrus) [4, 7, 16, 22, 23, 29, 37, 59, 61–63, 73, 74]. Sometimes differences in the volume of investigated structures between male and female schizophrenics have been observed [3, 27].

Abnormalities in the pool of glia cells have been reported rather rarely. Astroglisis was sporadic contrary to microgliosis observed in many forms of schizophrenia [5, 9, 10, 21, 24, 30, 50–52]. A significant increase in the number of macrophages in cerebrospinal fluid of patients with acute schizophrenia, as well as activated microglia cells in some brain structures in chronic schizophrenics have also been reported [46, 47, 50, 51].

A homogenous etiology of schizophrenia does not exist as neurodevelopmental morphological (neurone migration disorders, formation of synapses, neuronal links, myelination, glial development) and/or functional (production of hormones, neurotransmitters, immunological hallmarks) disorders may be only a sequence of genetic aberrations or effects of pathological factors in the pre- and perinatal period (e.g., virus infections, toxins, traumas, ischaemia/hypoxia). The effect of pathological factors in early youth, leading to neurodegeneration and/or psychosocial pathologies as a possible mechanism responsible for triggering the process of the disease development, is also possible [2, 28, 45, 64, 65, 67, 70, 72].

It has also been reported that immunological mechanisms play a prominent role in the generation of psychotic disorders in schizophrenia [9, 43, 51]. Cytokines secreted by activated microglia are also essential for the regulation of the dopaminergic, noradrenergic and serotonergic systems, and also exert their effect on neurotransmission [10, 12, 44]. The study of morphological structure of microglia and the assessment of their activity may considerably contribute to better understanding of schizophrenia etiopathogenesis.

**MATERIAL AND METHODS**

The light microscope morphological examination of microglia was performed on sections of temporal lobes, encompassing the hippocampal cortex and dental gyrus and frontal lobes with the cingulate gyrus (Brodmann’s area 24) of both right and left sides. The study material was obtained from 12 brains of female schizophrenics. In six cases, schizophrenia was diagnosed as paranoid schizophrenia according to the criteria adopted in the Diagnostic Statistical Manual of Mental Disorders (DSM-IV) [6] (Table 1). The control group comprised brains of 7 patients (of the same age group) who died from cerebral diseases (Table 2) with the exception of neurological disorders.

The brains were fixed in 4% paraformaldehyde in 0.1 M phosphorane-buffer saline, pH 7.4. Frontal and temporal lobe slices were cut serially at 8 μm. Brain tissue slices were routinely stained with hematoxylin-eosin, and lectin RCA-1. To visualise immunohistochemical reactions, the following antibodies were used: anti-human macrophage CD 68 (DAKO 1:40), CD 45 (LCA, Immunotech 1:50), myeloid/histocyte antigen Mac 387 (DAKO 1:100), anti-human necrosis factor α, TNFα (Boeringer 5 μg/ml), anti-human HLA-DR, DQ, DR (DAKO 1:50), GFAP (DAKO 1:2000). All antibodies were tested for optimal dilution and ways of epitop disclosure (trypsin, microwave oven, incubation in 0.1 M citrate buffer).

Ultrastructural analysis was carried out on the fragments of cortex and white matter of the frontal (cingulate gyrus) and temporal lobes, obtained from five patients covered with immunohistochemical/immunohistochemical survey, embedded in paraffin blocks (Table 1, numbers with asterisk). Fragments of cerebral lobes, selected after an analysis of histopreparations, were removed from paraffin blocks and fixed in 2.5% glutaraldehyde with post-fixation in osmium tetroxide. After
dehydration, they were immersed in Spur. Microglial cells were identified on semi-thin preparations stained with toluidine blue, and ultra-thin preparations were cut from selected blocks. Following the contrasting with uranyl acetate and lead citrate, the slices were examined by Turbo DPS 109 transmission electron microscopy.

**RESULTS**

Morphological examinations of microglia were performed on the frontal and temporal lobes of brains obtained from female schizophrenics suffering from the disease for 14–40 years (no data in one case). The presence of activated ramified microglia (RM) was observed in all sections obtained from the patients’ brains, regardless of the age and illness duration. Compared to activated RM observed in control brain sections, microglia were less ramified in both frontal and temporal lobes (independent on the side of the brain); their processes were thinner, and cytoplasm was not so abundant (Fig. 1A, B).

It was quite often observed that distributions of processes were not uniform, or they were short and fragmented (Fig. 2A, B and 3A, B). Sometimes, microglial cells almost completely devoid of processes and cytoplasm were visible; their nuclei were small and hyperchromatic with irregular shapes (Fig. 4A, B). When compared to the control brain slices, a stronger expression of the major histocompatibility complex class II (MHC II) was noted around vessels and on the pericyte surface (Fig. 5A, B, 6A, B). RM cells seemed to undergo a degenerative process. The most intensified changes were

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age (years)</th>
<th>Duration of disease (years)</th>
<th>Cause of death</th>
<th>Concomitant diseases</th>
</tr>
</thead>
<tbody>
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<td>1*</td>
<td>32</td>
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<td>Pneumonia</td>
<td>No data</td>
</tr>
<tr>
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<td>20–30</td>
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<td>54</td>
<td>35</td>
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<td>54</td>
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<td>Hyperthyroidism</td>
</tr>
<tr>
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<td>32</td>
<td>Sudden circulatory arrest</td>
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<td>8*</td>
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<td>14</td>
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<td>Hepatitis C</td>
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<tr>
<td>11</td>
<td>67</td>
<td>10</td>
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<td>12*</td>
<td>46</td>
<td>21</td>
<td>Sudden circulatory arrest</td>
<td>No data</td>
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</tbody>
</table>

Table 1. Cases of schizophrenia in women (numbers marked with asterisk indicate cases studied also under electron microscopy)

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age (years)</th>
<th>Duration of disease (years)</th>
<th>Cause of death</th>
<th>Concomitant diseases</th>
</tr>
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<tbody>
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<td>Pulmonary embolism</td>
<td>Miocardiopathy</td>
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<td>51</td>
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<tr>
<td>3</td>
<td>38</td>
<td>Sudden circulatory arrest</td>
<td>Pulmonary embolism</td>
<td>Hyperthyroidism</td>
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<tr>
<td>4</td>
<td>57</td>
<td>Pneumonia</td>
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<tr>
<td>5</td>
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<td>Miocardiopathy</td>
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<tr>
<td>7</td>
<td>44</td>
<td>Circulatory failure</td>
<td>Coronary heart disease</td>
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</table>

Table 2. Female controls
Figure 1. Temporal cortex. **A.** Control (case no. 1). MHC II expression on the surface of activated, ramified microglia (MR). The bar represents 12 μm. **B.** Schizophrenia (case no. 10). MHC II expression on the surface of ramified microglia (MR). The bar represents 10 μm.

Figure 2. Control. **A.** Dentate gyrus (case no. 7). Cells of activated ramified microglia with well-developed ramification. MHC II. The bar represents 12 μm; **B.** Ammon’s horn (case no. 2). MHC II expression on the surface of activated, ramified microglia. The bar represents 12 μm.

Figure 3. Schizophrenia (case no. 7). **A.** Dendate gyrus. Microglia indicating MHC II expression with short fragmented processes. The bar represents 10 μm. **B.** Cingulate gyrus (case no. 10). Microglia with narrow cytoplasm and abnormal processes. MHC II. The bar represents 18 μm.

Figure 4. Schizophrenia (case no. 11). **A.** Hippocampus. Microglial cells almost completely devoid of processes. MHC II. The bar represents 8 μm; **B.** Cingulate gyrus. Microglia devoid of processes with narrow cytoplasm. MHC II, RCA-1. The bar represents 8 μm.

Figure 5A. Control (case no. 4). Frontal lobe. Ramified perivascular microglia. MHC II. The bar represents 10 μm; **B.** Schizophrenia (case no. 1). Temporal lobe. MHC expression on the surface of perivascular microglia. The bar represents 10 μm.

Figure 6. Schizophrenia. **A.** Hippocampus gyrus (case no. 7). MHC II expression on the surface of pericytes. The bar represents 12 μm. **B.** Cingulate gyrus (case no. 2). MHC II expression on the surface of the vascular pericyte. The bar represents 12 μm.

Figure 7. Schizophrenia. **A.** Cingulate gyrus (case no. 6). Ramified microglia with damaged processes. MHC II. The bar represents 12 μm; **B.** Cingulate gyrus (case no. 2). Ramified microglia with atypically distributed processes. The bar represents 10 μm.

Figure 8. Control (case no. 5). MHC II expression on the surface of rod microglia. The bar represents 12 μm.

Figure 9A. Control (case no. 7). Temporal lobe, subependymal microglia. RCA-1. The bar represents 30 μm; **B.** Schizophrenia (case no. 5). Temporal lobe. Subependymal microglia without processes, displaying morphological traits of ameboid microglia. MHC II. The bar represents 30 μm.
observed in the hippocampal gyrus, in both pyramidal and granular layers as well as in the cingulate gyrus (Fig. 7A, B). In the brains of schizophrenics, rarely rod microglial cells or macrophages were observed. They also occurred sporadically in control brains (Fig. 8). Subependymal microglia were also less ramified and their morphological picture bore rather resemblance to ameboid microglia, but most frequently without expanded cytoplasm (Fig. 9A, B). Microglial cells present in the brains of schizophrenics reacted most frequently to lectin RCA-1 and the antibody that visualised MHC II molecules; the reactions with the other antibodies were frequently non-specific and sporadic.

Ultrastructurally, microglial cells of different phenotypes that exhibited traits of apoptotic changes of varied intensity were observed in the grey matter of the frontal and temporal lobes in all studied cases. Some microglial cells, morphologically resembling ameboid microglia, contained in their cytoplasm vacuoles, which were frequently empty or with small amount of osmiophilic material (Fig. 10). Most likely, some of those vacuoles were a reminder of degenerated mitochondria. In addition, the rough endoplasmic reticulum (RER) and clusters of dense material, probably corresponding with phagosomes of different size, were visualised in cytoplasm of those cells (Fig. 11). The nuclei of microglial cells were located centrally or on the cellular pole (Fig. 12). Strongly condensed chromatin was found on the nuclear periphery or within whole cross-section area, forming a compact ring or separate clusters (Fig. 12, 13). In some

Figure 10. Schizophrenia (case no. 2). Ameboid microglia with vacuoles, some of them contain amorphous material. The bar represents 1 μm.

Figure 11. Schizophrenia (case no. 7). Ameboid microglia with electron-dense material accumulated in cytoplasm. The bar represents 1 μm.

Figure 12. Schizophrenia (case no. 8). Microglial cell containing polarly located nucleus with a clumped structure of heterochromatin. The bar represents 1 μm.

Figure 13. Schizophrenia (case no. 8). Microglia showing strongly condensed chromatin along the nuclear membrane and the osmiophilic material in cytoplasm. The bar represents 1 μm.
nuclei, their envelopes were wrinkled, like in an apoptotic process (Fig. 14). There were also microglial cells with small volume of dark cytoplasm, or only with cytoplasm in a form of a dark rim with scarce cytoplasmic structures and strongly condensed nuclear chromatin in oval nuclei, phenotypically corresponding with resting microglia (Fig. 15). Only rare microglial cells with a medium volume of cytoplasm and RM morphology looked normal (Fig. 16).

**DISCUSSION**

Cells of activated ramified microglia were observed in all slices obtained from the cerebral frontal and temporal lobes of female schizophrenics. Limited knowledge of differences in the development, morphoarchitecture, and functioning of the brain in men and women do not allow for an explicit statement whether the patient’s gender may have some impact on his or her morphological picture of impairments generated by schizophrenia [3, 27]. The assessment of the study material obtained only from women or only from men renders it possible to obtain more clear-cut study material, at least with respect to the gender. In the hippocampal gyrus and cingulate gyrus, the largest number of microglial cells with the appearance of degenerative cells or with definite traits of atrophy in the mechanism of apoptosis. Degeneration was manifested by changes in the appearance of cytoplasm cells, shortening and fragmentation of processes and their abnormal distribution. These abnormalities were intensified mostly in the cingulate gyrus, hippocampus, and structures of the limbic system with their decreased volume due to the reduced number of neurones and their dysfunction attributed to psychotic symptoms observed in schizophrenia [1, 14, 23, 28, 29, 39, 41], whereas structural anomalies are noticed even in first episode patients [19, 59]. They may induce or result from (according to the glutamatergic theory of schizophrenia) specific dysfunction of glutamatergic, ionotropic receptors (e.g., N-methyl-D-aspartate (NMDA); L-α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMDA) and kainate receptors), which are found in large quantities in neurones of layers II and III of the cingulate cortex. The disorders of serotonergic and GABAergic transmissions whose receptors for γ-aminobutyric amid are present in the whole entorhinal cortex (Brodmann’s area 28) and play as well an essential role in the development of psychotic disorders are also taken into account. Changes in GABAergic and glutamatergic neurotransmission are responsi-
ble for the decreased activity of GABAergic neurons and reduced function of glutamate receptors, leading finally to dysfunction of synapses in those structures [25, 26, 38, 41, 48, 74]. Such functional disorders together with a decreased number of neurons do not remain neutral in the function/number of receptors (trk, p 75) to neurotrophins (e.g., nerve growth factor (NGF), brain-derived growth factors (BDNF), neurotrophin-3-NT-3), which regulate normal differentiation and functioning of neurones and glial cells [8, 12, 66].

The rapture of the transmission chain for this neurotrophins may lead to death of cells in the mechanism of apoptosis.

Despite greatly advanced degeneration of microglial cells, expression of MHC II was observed on their surface, which may indicate rather secondary than primary damage to these cells. A strong expression of MHC II on the surface of perivascular RM may evidence their ability at least in an early phase to generate complexes of MHC II molecules. Activation and proliferation of glia have traditionally been described in neurodegenerative diseases such as Alzheimer’s disease, Parkinson’s disease or multiple sclerosis. In schizophrenia, regardless of its course, the patient’s age and the applied therapy, gliosis has not been reported; the authors rather observed reduced astroglia or even oligodendroglia, which inclined them to accept rather neurodevelopmental (encephalopathy model) than neurodegenerative model of schizophrenia pathogenesis [13, 24, 30, 45, 60, 67, 70, 71].

Microglia in the pool of immunocompetent cells usually reacted by multiplication and activation, which have been associated with their direct cytotoxic effect on schizophrenia pathogenesis [44, 46, 50, 51]. Our observations revealed a strong microglial expression of MHC II and ultrastructural traces of primary phagocyto-sis, which would suggest that degeneration of microglial cells is rather influenced by factors developed in the course of the disease (pharmacology should also be taken into account), leading among others to mitochondrial dysfunction [11, 34]. Only Munn [44] hypothesised that the destruction of microglia cells may also be regarded as an essential cause of abnormalities observed in schizophrenia. His considerations were based on the studies indicating that microglia: 1) play an important role in the migration of neurones, their growth and differentiation, and also in phagocytosis of additional neuroblasts or abnormally shaped processes; 2) participate in infection control; 3) protect the brain during perinatal ischemia/hypoxia; and 4) produce cytokines essential for normal functioning of cerebral nervous and glial cells. This hypothesis has directly been used by Wank [68], who applied a long-term adoptive immunotherapy in patients suffering from different mental diseases (autism, bipolar disorder, schizophrenia) and observed considerable improvements in the health of his patients. In his opinion, the afore-said therapy renders it possible to recover “a sixth sense”, namely the cerebral immune system.

CONCLUSIONS

Degeneration of microglial cells in the frontal or temporal lobes of the brain in schizophrenics may result from primary disorders in the morphology and function of neurones and neuroglia, or their interrelation. A secondary disorder of microglial function that disturbs and decreases the capacity of the patients’ immune system may exacerbate structural damages and psychotic symptoms. Treatment of chronic schizophrenia should involve the supply of agents that directly prevent degeneration of microglia and/or long-term immunotherapy.

REFERENCES