Accumulation of Macrophages in the CSF of Schizophrenic Patients During Acute Psychotic Episodes

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Objective: There have been numerous reports of organic or structural abnormalities in the central nervous system (CNS) of patients with schizophrenia. Given that pathological conditions in the CNS are frequently reflected in the cell profiles of CSF, the authors compared the cytology of CSF from schizophrenic patients with that from a reference population in order to find out trails of elementary pathogenetic events in this serious psychiatric disease. **Method:** CSF samples from 35 patients with acute schizophrenia and 46 comparison subjects were prepared by Millipore filtration. The total and differential counts of CSF mononuclear cells were performed by light microscopy. **Results:** At the beginning of treatment, the proportion of mononuclear phagocytes/macrophages in the patients' CSF was significantly higher than that in the comparison subjects. During treatment with conventional neuroleptic medication, the cytology returned to normal in several patients. **Conclusions:** The high proportion of macrophages in schizophrenia without a significantly higher total cell count may reflect neurodevelopmental disorder, a neurodegenerative process, or subtle CNS immunoactivation with mobilization of microglia.

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The etiopathogenesis of schizophrenia has remained elusive. Several findings have, however, suggested that organic factors contribute to the pathogenesis of the disease. Among these, hereditary factors (1), neurodevelopmental disturbances (2, 3), and immunological aberrations (4–7) have been proposed to be of significance. The attractiveness of the dopamine theory has been refreshed by reports on abnormal expression of the D_4 and D_5 receptors (8, 9). Abnormalities in the sites of serotonin (5-HT) uptake (10) and 5-HT receptors (11) have also been implicated. The action of atypical antipsychotic drugs such as clozapine and risperidone have been supposed to be related to their 5-HT_2 antagonism (12). Modern neuroimaging techniques have disclosed low volume (13–15) and gray matter deficits (16, 17), especially concerning limbic structures in the medial temporal lobe surrounding the temporal horn (18, 19), in the brains of schizophrenic patients. Skewings of circulating and CSF cytokine levels and production have been detected; the affected cytokines include interleukin 2 (IL-2) (20, 21), IL-6 (22), and tumor necrosis factor alpha (23).

These findings have raised the question of whether the abnormalities originate from developmental, degenerative, inflammatory, or immunoactive processes in the central nervous system (CNS).

Experimental models of nerve injury have revealed the critical role of macrophages in both degenerative and regenerative actions in the nervous system (24, 25). In certain neurological diseases, where patients suffer from neuronophagia, the activity of resident macrophages (from microglial or perivascular origin) seems to contribute to the disappearance of neurons (26).

In this study we investigated whether reflections of cytological abnormalities in the CNS are detectable in the CSF of patients with acute attacks of schizophrenia, knowing that the CSF cell distribution of normal adults is one-third mononuclear phagocytes/macrophages and two-thirds lymphocytes and that devia-

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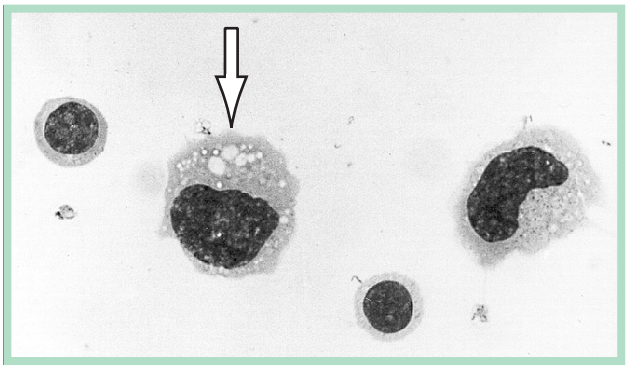
FIGURE 1. Morphological Features of Mononuclear Cells in the CSF of Schizophrenic Patients[a]



[a] Two lymphocytes and two macrophages. The arrow indicates a macrophage containing typical cytoplasmic vacuoles.

tions from normal cell counts or relative cell compositions frequently occur in CNS diseases (27–29).

METHOD

Subjects

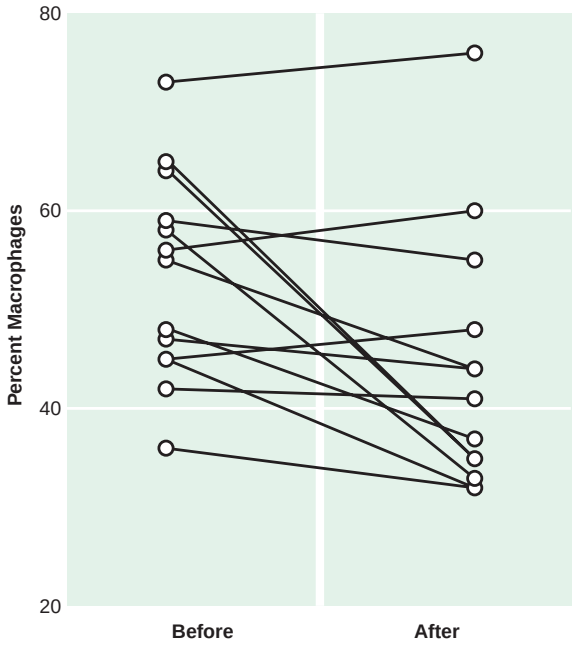
Thirty-five patients, 19 male and 16 female, admitted to Hesperia Hospital (Helsinki City Hospital) in the acute phase of schizophrenia took part in the study. After complete description of the study to the subjects, written informed consent was obtained. The mean age of the male patients was 34 years (SD=9) and that of the female patients was 32 years (SD=11). For 20 patients it was the first admission, and they had not taken neuroleptic medication previously. The remaining 15 patients had been treated previously for at least one psychotic episode but had been drug free for more than 4 months before the current admission. The DSM-III-R diagnostic criteria were applied to confirm that the psychosis was of the schizophrenic type (schizophrenia or schizophreniform psychosis). Acute infections were excluded clinically and by routine laboratory infection markers in peripheral blood and CSF. The CSF-serum ratio for albumin was within reference limits in all subjects, thus excluding significant damage to the blood-brain barrier.

The reference population consisted of subjects previously examined for nonspecific neurological symptoms, such as headache and vertigo, at the Helsinki University Hospital, Department of Neurology. A total of 46 individuals, 21 male (mean age=33 years, SD=8) and 25 female (mean age=32 years, SD=9), were accepted as comparison subjects after a 2-year follow-up period with no evidence of inflammatory or CNS disease (30).

Cytological Techniques

CSF samples were collected within a few days (mean=4, range=0–7) of hospital admission. Thirteen patients each gave a second sample after a few weeks of neuroleptic treatment. One milliliter of CSF was fixed in 1 ml of 96% ethanol, and the specimens were prepared by Millipore filtration (according to 1976 catalogue and purchasing guide from Millipore, Bedford, Mass.). In addition to the Millipore filtration method, cytocentrifuged (800 rpm for 8–10 minutes) CSF samples from patients were stained by the May-Grünwald-Giemsa method and analyzed by light microscopy in order to observe detailed morphological features of the cells and to exclude samples with red blood cell (RBC) contamination; samples with more than 20 RBCs per high power (40×) field were rejected. The methodological details have been previously described (29, 31, 32).

FIGURE 2. Proportion of Macrophages in the CSF of 13 Schizophrenic Patients Before and After Treatment With Typical Neuroleptics



Statistical Methods

The Mann-Whitney U test was used to study differences in the total cell count and lymphocyte/macrophage distributions between the patient and comparison groups. The Wilcoxon matched pairs test was the statistical method applied to compare the patients' CSF cytology before and after treatment. F tests were performed by using rank transforms and analysis of variance (ANOVA).

RESULTS

Cytological examination of the CSF from the 35 psychotic patients revealed that they had a significantly higher proportion of cells morphologically classified as mononuclear phagocytes/macrophages than was present in the CSF of the comparison subjects ($F=47.34$, $df=1, 79$, $p<0.0001$). The total CSF cell count was not, however, significantly higher in the patient group.

The cytological details of the phagocytes frequently included a kidney-shaped or lobulated nucleus and a voluminous cytoplasm with numerous small vacuoles. Overt lipophages with larger cytoplasmic vacuoles, typically seen in the CSF after acute brain injuries, for instance, were rarely seen (figure 1).

A tendency toward normalization of the cytological picture (i.e., decrease in the proportion of macrophages) was observed in the CSF of the 13 psychotic patients who took part in the follow-up section of the study (Wilcoxon matched pairs test, rank transforms, and repeated measures ANOVA: $F=7.83$, $df=1, 12$, $p<0.05$) after a few weeks of treatment with typical neuroleptics (figure 2).

DISCUSSION

There is a growing body of evidence that genetic susceptibility significantly contributes to the etiology of schizophrenia (33). Although the schizophrenia susceptibility genes are still to be identified, several reports have indicated the location of such gene(s) on chromosome 8 (34) or on the short arm of chromosome 6 (35). Incomplete penetrance and environmental forms of phenocopies still leave room also for gene-environment interactions (36, 37), and some associations of human lymphocyte antigens (HLAs) in schizophrenia have been detected (38–40), suggesting that immune mechanisms may contribute to the etiology of the disease. Viewed from the angle of these processes, the accumulation of macrophages in the CSF during acute schizophrenia reported here may reflect a genetically transformed immune response to (or may be caused by) environmental factors.

Abnormalities of the gross anatomy of the brain are frequently found in schizophrenia. Both autopsy studies and modern brain imaging have revealed a loss of brain substance, in particular from the fronto-occipital areas, and enlargement of the CSF space (13–19). These abnormalities may signify dysregulated brain development in schizophrenia. In addition, there are reports of a correlation between the extent of macroscopic brain abnormalities and the duration of the psychotic disease, which implicates a contribution by some chronic degenerative process in the CNS (41). Macrophage dominance is a frequent finding in CNS injuries (42). Consequently, our detection of an excess of vacuolated macrophages in the CSF of patients with schizophrenia may reflect some organic brain destruction of subchronic or chronic type and relate to the neuroradiologically demonstrated low brain volume in schizophrenia.

Most of the macrophages found in the CSF are considered to be of microglial derivation (43, 44). Microglia, which originate from hematopoietic stem cells (45, 46), constitute the main source for the macrophages accumulating in lesions of the CNS. In addition to their phagocytic capacity, microglial cells contribute to the immune network in the CNS by expressing HLA-DR molecules (47) and acting as antigen-presenting cells (48). The absence of neutrophil recruitment and the delay in the increase in macrophage or microglial cells show that the CNS differs from other sites in the body with regard to the kinetics and nature of the myelomonocytic cell responses (49). Coculturing of microglia with T lymphocytes results in clustering of T cells around the microglia and initiation of mixed lymphocyte reaction (50), and activated T lymphocytes induce the cells of the macrophage lineage to produce pro-inflammatory cytokines (51). Moreover, the CNS mononuclear cells express receptors for neurotransmitters and may therefore functionally bridge the CNS and the immune system (52, 53). The macrophage dominance in the CSF of psychotic patients detected in this study may reflect activation or mobilization of the microglial cells, or both, and may also link the previous reports on T lymphocyte deviations and cytokine aberrations in schizophrenia.

Given that 5-HT has been found to act as a modifier of dopamine responses (54) and as an activator of macrophages (55), it is tempting to speculate that abnormal monoamine metabolism in acute schizophrenia may contribute to the mobilization or activation of microglia leading to the relative macrophage dominance in the CSF. Our finding of neuroleptic-induced normalization of the CSF cytology in many patients lends support to this hypothesis.

A macrophage dominance over lymphocytes, as in the CSF of the adult schizophrenic patients in this study, is also typical in the CSF of newborn infants, and normally the cell profile gradually transforms with age into that of normal adults (i.e., lymphocyte dominance of 60%–80%) (27). This phenomenon may have bearing on the neurodevelopmental theories of schizophrenia (2, 3), especially because programmed cell death (apoptosis) and axonal pruning are thought to be essential actions in neurodevelopmental sequences, are substantially affected by macrophages and microglia, and are influenced by both genetic and environmental factors (56).

Macrophages have numerous effects on the CNS mediated by cytokines and neurotoxins (56), and further CSF analysis of, for example, pro- and anti-inflammatory cytokines and excitatory amino acids will be needed to further clarify the more detailed role of macrophages and microglia in the pathogenesis of schizophrenia.

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