Interleukin 4 and Interleukin 10 Levels Are Elevated in the Cerebrospinal Fluid of Patients With Creutzfeldt-Jakob Disease

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Background: In neurodegenerative diseases, increasing attention has been focused on inflammatory mediators such as pro-inflammatory and anti-inflammatory cytokines and their potential influence in the process of neurodegeneration. In prion diseases, much data has been gained on the cell culture and animal disease models level, but only limited information is available on humans affected by Creutzfeldt-Jakob disease (CJD).

Objective: To obtain data on anti-inflammatory cytokines interleukin 4 and interleukin 10 in the cerebrospinal fluid of patients with CJD, patients with other dementia, and nondemented neurological patients and controls.

Design: Cerebrospinal fluid samples were collected from CJD patients and control subjects, and concentrations of the anti-inflammatory cytokines interleukin 4 and interleukin 10 were determined using an enzyme-linked immunosorbent assay.

Patients: Cerebrospinal fluid samples from 61 patients were analyzed. The group was composed of patients with CJD (n=20), patients with other dementia (n=10), patients with motoneuron disease (n=6), patients with normal pressure hydrocephalus (n=5), and control subjects (n=20).

Results: Interleukin 10 levels were significantly elevated in the cerebrospinal fluid of CJD patients (median, 9.8 pg/mL). The elevation was significant to other dementia (median, 7.9 pg/mL, P<.05), motoneuron disease (median, 7.9 pg/mL, P<.05), normal pressure hydrocephalus (median, 7.0 pg/mL, P<.05), and controls (median, 1.3 pg/mL, P<.001). Levels of interleukin 4 were significantly elevated in cerebrospinal fluid of patients with CJD (median, 26.4 pg/mL) compared with control subjects (median, 6.2 pg/mL, P<.001) and patients with a motoneuron disease (median, 10.5 pg/mL, P<.001).

Conclusions: Elevated levels of the anti-inflammatory cytokines interleukin 4 and interleukin 10 in cerebrospinal fluid of patients with CJD are new findings. The data of the present study provide a clue toward the possible role of cytokines as immunological modifiers in the neurodegenerative process of CJD.

Arch Neurol. 2005;62:1591-1594

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CREUTZFELDT-JAKOB DISEASE (CJD) is a rare and fatal neurodegenerative disease that occurs worldwide. Creutzfeldt-Jakob disease belongs to the human prion diseases which are characterized by the accumulation of an infectious protein, termed prion (PrP\textsuperscript{Sc}). This represents the pathological isoform of the protein PrP\textsuperscript{c}, expressed physiologically and predominantly in the central nervous system (CNS).\textsuperscript{1}

Recently, detailed analysis of the function of the immune system in the peripheral prion invasion has been performed. It revealed that major components of the immune system, such as secondary lymphoid organs and B-cells as well as follicular dendritic cells are necessary for prion replication and propagation.\textsuperscript{2-4}

Research concerning the involvement of the immune system in the CNS in prion diseases has focused mainly on infectivity of microglia and the release of pro-inflammatory cytokines.\textsuperscript{5-7} Expression of pro-inflammatory cytokines was described earlier in scrapie-infected mice.\textsuperscript{8} Also, elevated levels of the pro-inflammatory cytokines interleukin (IL) 1\textbeta and tumor necrosis factor \alpha have been found in the CSF of patients with CJD.\textsuperscript{9}

The question of anti-inflammatory cytokines has not been addressed so far. We used the CSF as a testing platform to determine levels of anti-inflammatory cyto-
kines in CSF in patients with CJD, since in many neurological diseases, changes in the CSF protein pattern are closely linked to pathological processes of the brain. The detection of elevated levels of 14-3-3 and other neuronal proteins in the CSF in patients with CJD exemplifies how the rapid brain damage and release of neuronal proteins into the CSF reflect the changes of affected brain tissue.\(^{10}\) We intended to first obtain information on CSF levels of IL-4 and IL-10 in patients with CJD, since there are no data available on this subject in the literature.

### CLINICAL CHARACTERISTICS OF CJD PATIENTS

This group consisted of 20 patients who were reported as suspected CJD cases to the German CJD surveillance unit. These patients, 11 men and 9 women, were classified as sporadic CJD cases based on established clinical criteria (probable sporadic CJD, n=15) and post-mortem examination (definite sporadic CJD, n=5). The average age was 64 years (age range, 54-79 years).

### PATIENTS WITH OTHER FORMS OF DEMENTIA

This group consisted of 10 patients, 8 men and 2 women with the following diagnoses: Alzheimer disease, vascular dementia, and dementia of unknown etiology. The average age was 65 years (age range, 35-79 years).

### PATIENTS WITH NORMAL PRESSURE HYDROCEPHALUS

Here we included a group of patients with normal pressure hydrocephalus (n=5, median age, 70 years, age range, 63-78 years).

### PATIENTS WITH MOTONEURON DISEASE

This group included 6 patients (median age, 52 years, age range, 35-80 years).

### CONTROL PATIENTS

This group included 20 patients, 15 women and 5 men. The average age was 39 years and the age range was 16 to 78 years. These patients received inpatient treatment at the Neurological Clinic of the University Hospital Göttingen, Göttingen, Germany, had a lumbar puncture and normal CSF findings, and normal results from a neurological examination, neuropsychological tests, and brain magnetic resonance imaging. The diagnoses in this group of patients were determined on the basis of medical records. The following diagnoses were made: psychiatric diseases (eg, depression), pain syndromes (eg, headache), and vertigo (eg, benign paroxysmal positional vertigo). In all cases, an inflammatory CNS disease was excluded.

### CSF SAMPLES

Cerebrospinal fluid samples from patients with CJD were collected at the respective external hospitals. These samples were sent to the laboratory of the CJD surveillance unit at the Department of Neurology, University Hospital Göttingen, for determination of protein 14-3-3. Only CSF samples that had been sent on dry ice and that had been immediately stored at −80°C were used for this study.

Cerebrospinal fluid samples from patients of the other test groups were obtained from the Neurochemical Laboratory of the University Göttingen and immediately frozen at −80°C. These patients had either been admitted to the Neurological Clinic of the University Hospital Göttingen for inpatient treatment or had received treatment at the outpatient clinic for clarification of their diagnoses by lumbar puncture.

### ENZYME-LINKED IMMUNOSORBENT ASSAY

Commercially available enzyme-linked immunosorbent assay test kits (Bender MedSystems; MedSystems Diagnostics GmbH, Vienna, Austria) were used for quantitative analysis of the anti-inflammatory cytokines IL-4 and IL-10. Tests were performed in accordance with the manufacturer’s instructions. The concentration of the first standard was 7.8 pg/mL for IL-4 and 3.2 pg/mL for IL-10.

### STATISTICS

Statistical evaluation of the data was performed using Statistica version 6.0 (Statsoft Inc, Tulsa, Okla). The Mann-Whitney U test was used to determine the statistical significance of the differences between the 2 independent data sets. Differences were considered significant at an error probability of P<.05.

### RESULTS

#### INTERLEUKIN 4 IN CSF

Table summarizes the levels of IL-4 in CSF. The level of IL-4 was significantly increased in the CSF of patients with CJD vs normal controls (P<.001), patients with normal pressure hydrocephalus (P<.02), and patients with motoneuron disease (P<.001) (Figure 1). There was no statistical difference between patients with CJD and patients with other forms of dementia (P=.4).

#### INTERLEUKIN 10 IN CSF

Levels of IL-10 are summarized in Table. The level of IL-10 was significantly elevated in the CSF of patients with CJD compared with controls (P<.001), patients with normal-pressure hydrocephalus (P<.05), patients with motoneuron disease (P<.05), and—most interestingly—compared with patients with other forms of dementia (P<.05) (Figure 2).
The exact mechanisms leading to the neurodegeneration in prion diseases are not known in detail and the involvement of microglia is subject for discussion. Various studies have recently been performed to identify immunological mechanisms. These have contributed mainly to an understanding of the involvement of the immune system during the peripheral prion invasion in experimental models, in which the lymphoreticular organs are necessary for accumulating and replicating prions. However, it still remains unclear to what extent the CNS is immunologically involved in CJD.

Cerebrospinal fluid changes as seen in other infectious and inflammatory diseases are absent in CJD. In histopathological examination, inflammatory infiltrates are absent. One important histological finding relates to activated microglia, which represent resident CNS macrophages that can react as immunologically potent cells in case of stress to the CNS. In their activated state, they are supposed to play a predominant role in the so-called atypical inflammatory process in prion diseases with expression of major histocompatibility complex class II molecules and release of inflammatory and tissue-toxic mediators such as pro-inflammatory and anti-inflammatory cytokines. However, it remains unclear whether the neurodegenerative process in CJD is primarily caused by prion accumulation alone or whether tissue-toxic mediators released by activated microglia may contribute to the neurotoxicity—and subsequently to neurodegeneration. Another hypothesis posits that even neurons may release cytokines, especially anti-inflammatory cytokines after damage. In CJD and also in Alzheimer disease, the anti-inflammatory cytokine transforming growth factor β2 could be localized intraneuronally in degenerative neurons.

The potential role of anti-inflammatory cytokines (IL-4, IL-10, and IL-13) in prion disease has recently been investigated in IL-10−/−mice. These animals developed disease earlier in comparison to wild type mice, suggesting an important modifying function for anti-inflammatory cytokines in the development of prion disease.

The main purpose of this study has been to investigate the anti-inflammatory cytokines IL-4 and IL-10 in CSF of patients with CJD. Interleukin 4 and IL-10 are potent anti-inflammatory cytokines which are produced by a variety of cells, specifically T02 cells, and by macrophages in the periphery. In the CNS, they are produced by microglial and astroglial cells under various conditions, eg, multiple sclerosis, ischemia, and also in neurodegenerative diseases such as Alzheimer disease. We show in our study that IL-4 and IL-10 are significantly elevated in the CSF of patients with CJD compared with controls and patients with other neurodegenerative CNS diseases. To date there are no studies in the literature describing levels of IL-4 or IL-10 in the CSF or brain tissue of patients with CJD.

In the literature, a similar investigation has been carried out on the ventricular CSF of patients with juvenile parkinsonism and Parkinson disease. The authors have detected elevated levels of IL-4 (among other interleukins) in patients, but not in controls, and speculate that alterations of cytokines in the CSF may reflect changes of cytokines in the brain during the process of neurodegeneration. They also speculated that the increase in cytokines might not only be due to a compensatory response, but might itself trigger neurodegeneration. Another study has shown that IL-4 and IL-10 differentially regulate microglial responses to Aβ and may play a role in the observed pathology surrounding senile plaques in Alzheimer disease. Unfortunately, no data on the levels of these cytokines in CSF in Alzheimer disease were given.

Our own data are in line with these observations, since we could also detect elevated levels of IL-4 and...
IL-10 in the CSF of patients with dementia other than CJD. In our article we focused on CJD, but our group of patients with dementia also included patients with Alzheimer disease and elevated levels were observed in this subgroup as well.

Cerebrospinal fluid changes may reflect changes of the CNS to a major degree. Cerebrospinal fluid analysis is performed routinely for the purpose of diagnosis in a majority of CNS diseases, especially in those of an inflammatory nature, and may therefore represent a valuable platform for investigating pathological CNS processes. Because elevated levels of IL-4 and IL-10 were also found in other neurodegenerative diseases, one can assume that the levels of those cytokines might be related to the severity or acuity of the neurodegenerative process. Subsequent studies on brain material or animal disease models are needed to clarify the potential involvement of cytokines in the neurodegenerative process in general and their possible involvement in a defined pathway in prion diseases.

Accepted for Publication: May 6, 2005.

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Funding/Support: This study was supported by grants from the German Bundesministerium für Gesundheit (GZ: 325-4471-02/15), the European Commission (QLG3-CT-2002-81600), and the German Bundesministerium für Bildung und Forschung (KZ: 0312720).

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