Interleukin-12 is reduced in cerebrospinal fluid of patients with Alzheimer’s disease and frontotemporal dementia

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Abstract

Interleukin-12 is a heterodimeric cytokine produced by activated blood monocytes, macrophages and glial cells. It enhances differentiation and proliferation of T cells and increases production of proinflammatory cytokines, such as Interferon-γ and Tumor Necrosis Factor-alpha. There is little information about the involvement of IL-12 in the pathophysiology of Alzheimer’s disease (AD) and other tauopathies.

Objectives: The objective of our study was to assess the role of IL-12 as a potential marker of immune reactions in patients with AD and frontotemporal dementia (FTD).

Patients and methods: We measured by immunoassay cerebrospinal fluid (CSF) IL-12 levels in 19 patients with AD and 7 patients with FTD in comparison with CSF IL-12 levels in 30 patients with non-inflammatory neurological diseases served as neurological control patients (NCTRL). IL-12 levels were correlated with age, age of disease onset, disease duration, MMSE score, and rate of dementia progression. Aβ42 and Total tau (τ) levels in CSF were also measured.

Results: Patients with AD had significantly lower CSF IL-12 levels compared with NCTRL patients (p<0.001). Patients with FTD had also lower CSF IL-12 levels compared with NCTRL patients (p<0.05). Age, sex, disease duration and MMSE score did not affect IL-12 levels in any of the groups. In AD a significant positive correlation was noted between IL-12 levels and τ levels (Rs=0.46, p=0.048).

Conclusions: Our findings may suggest a reduced inflammatory reaction during the course of AD and FTD. A neurotrophic role of IL-12 and other proinflammatory cytokines cannot be excluded.

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Keywords: IL-12; CSF; Alzheimer’s disease; Frontotemporal dementia; Inflammatory reactions

1. Introduction

Alzheimer’s disease (AD) is a dementing neurodegenerative disorder characterized by tau protein deposition in the form of neurofibrillary tangles and β-amyloid deposition in the form of senile plaques and cerebrovascular amyloidosis [1,2]. Inflammatory responses have been implicated in the pathogenesis of AD and deposition of β-amyloid. The presence of the acute phase proteins a-1-antichymotrypsin (ACT), a2- macroglobulin (a2-M), C-reactive protein (CRP) and proinflammatory cytokines IL-6 and IL-1 in senile plaques was shown [3–6]. Adhesion molecules are presumably involved in brain tissue lesions of patients with AD [2,7,8]. Cultured neural cells can be induced to express intercellular adhesion molecule-1 (ICAM-1) by tumor necrosis factor (TNF), interleukin-1 (IL-1) and interferon-gamma (INF-γ) [9]. Microglial cells agglomerating in the center of senile plaques are highly activated expressing leukocyte function associated-antigen-1 (LFA-1) which is dramatically upregulated [10]. Its ligand, (ICAM-1) is expressed by reactive astrocytes in human brain and its
expression is dramatically intense in astrocytes marginating senile plaques. This pathway suggests a close association between microglia and astrocytes in AD and probably a neural ICAM-1 production. LFA-1 and ICAM-1 are very important in the initiation of immune responses as can be demonstrated by the ability of antibodies against them to block antigen presentation [11]. There is substantial evidence that low-grade, sustained inflammatory responses are present in the Alzheimer’s disease brain [12–14].

Interleukin-12 (IL-12) is a heterodimeric cytokine produced by activated antigen-presenting cells (APC), such as dendritic cells (DC), monocytes/macrophages and microglia, in response to bacterial products and immune signals [15]. It enhances differentiation and proliferation of T cells and increases production of proinflammatory cytokines, such as INF-gamma and TNF-a [16]. IL-12 may be responsible for the induction of experimental autoimmune encephalomyelitis (EAE), the animal model of autoimmune inflammatory disease of the central nervous system (CNS) [17,18]. In cultures of peripheral monocytes, A-beta amyloid 1–42 induced the secretion of proinflammatory cytokines such as TNF-a and IL-12 [19]. Treatment of human microglia with lipopolysaccharide (LPS) or A-beta amyloid led to increased expression of mRNA levels of TNF-a, IL-12 and other cytokines and chemokines [20]. Thus, one would expect increase of IL-12 in AD.

A few data in the literature indicate that levels of IL-12 in cerebrospinal fluid (CSF) [21] or serum [22] of patients with AD may not be significantly different as compared with controls. To clarify whether IL-12 as a product of activated microglia may be involved in the possible inflammatory reactions of AD and other dementias we determined CSF IL-12 levels in patients with AD and frontotemporal dementia (FTD) compared with CSF IL-12 levels of patients with other non-inflammatory neurological diseases.

2. Patients and methods

2.1. Patients

A total of 56 subjects were enrolled in the study, divided in 3 groups: (1) The AD group comprised 19 patients with probable AD, diagnosed according to the National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer’s Disease and Related Disorders Association (NINCDS–ADRDA) criteria [23]. None of the AD patients had cerebrovascular component or mixed dementia according to the National Institute of Neurological Disorders and Stroke–Association Internazionale pour la Recherche et l’Enseignement en Neurosciences (NIND–AIREN) and Hachinski Ischemic Scale (HIS) [24,25]. (2) The FTD group comprised 7 patients with frontotemporal lobar degeneration, diagnosed according to the criteria of Neary et al. [26]. The neurological control (NCTRL) group comprised 30 patients suffering from non-inflammatory neurological diseases; 7 out of these had parkinsonism, 3 motor neuron disorder, 3 cerebellar ataxia, 8 tension headache, 6 non-inflammatory peripheral neuropathies and 3 normal pressure hydrocephalus. Patients with evidence of systemic inflammation on clinical examination or serum biochemical tests [increased number of white blood cells, high levels of C-Reactive Protein (CRP) or Erythrocyte Sedimentation Rate (ESR)] have been excluded.

2.2. Sample collection and IL-12 assay

CSF samples were obtained after informed consent, by lumbar puncture at the L5–S1 interspace, between 10 and 11 AM, after overnight fasting. Samples were centrifuged for removal of cells, and bloody samples were discarded. Following that, samples were immediately frozen at −70 °C and thawed only once, just before the assay.

CSF levels of circulating IL-12 were measured by immunoassay using a commercially available enzyme-like immunosorbent assay (ELISA) kit (Quantikine, R&D systems). The Quantikine IL-12 immunoassay was developed with a capture antibody that recognizes only the IL-12p70 heterodimer and not the individual subunits (p40 and p35) of the dimer, thus eliminating the potential for interference by these subunits. Undiluted CSF samples were added in duplicate to microtiter wells and assayed according to routine procedures. Optical densities were determined by means of a micro-ELISA reader (L.P 400, Pasteur diagnostics). Values were calculated by comparison with a standard curve that was generated with IL-12 standards. The limit of detection was 5 pg/ml. CSF total tau (τT) and Aβ42 levels were determined in doubles by double sandwich ELISA (“Innotest htau antigen” and “Innotest β-Amyloid 1–42” kits respectively, Innogenetics, Belgium), according to manufacturer’s instruction and by the use of a sigmoid curve.

2.3. Statistical analysis

All variables were tested for normality and homogeneity of variances by the Shapiro–Wilk’s and Levene’s tests respectively and, since no significant violations were observed for IL-12, an analysis of covariance (ANCOVA) model with diagnostic group and sex as cofactors and age as a covariate was used. Correlation of IL-12 levels with age, age of disease onset, disease duration, Mini Mental State Examination (MMSE) score, and rate of dementia progression was also tested in each group with Pearson or Spearman correlation coefficients as appropriate. MMSE is a practical method for grading the cognitive state of patients for the clinician. Mildly demented patients generally have MMSE >20, moderately demented are between 10 and 20 and severely demented have scores <10 [27]. A-beta amyloid 1–42 and τT levels did not follow the normal distribution and/ or their variances were heterogeneous; however, for τT logarithmic transformation restored the above violations and permitted the use of ANCOVA, with age and sex as factors...
and age as a covariate. For Aβ42 the Kruskal–Wallis test was used. ANCOVA or Kruskal–Wallis test were followed by Newman–Keuls or Dunn’s post-hoc tests respectively. The level of statistical significance was set at 0.05.

3. Results

The point of study is whether activated microglia should secrete microglial products like IL-12 in patients with AD and FTD. Results are summarized in Table 1. Some differences in respect to age and sex among the studied groups were noted; however none reached statistical significance. Furthermore, age, sex, disease duration and MMSE score did not affect IL-12 and τ levels in any of the groups. Patients with AD and FTD had lower CSF IL-12 levels as compared to the NCTRL group (Fig. 1). In AD a significant positive correlation was noted between IL-12 and τ levels.

4. Discussion

In the present study we have investigated CSF levels of IL-12 as potential marker of immune reactions in patients with AD and FTD. Our data contradict the inflammatory hypothesis since patients with AD had reduced CSF IL-12 concentrations compared with NCTRL patients (Fig. 1). There is also a significant decrease of IL-1b and interleukin-6 (IL-6) secretion after LPS-stimulation of blood cells of AD patients with different disease severity [28]. Other studies have shown that TNF-a release from blood cells is significantly decreased in demented patients compared to controls [29]. Our findings support these latter results but seem in contrast with the conclusions of other recent reports suggesting increased production of cytokines based on studies of circulating serum proinflammatory cytokines such as IL-6 or cytokine production by activated lymphocyte subsets including IL-1b, TNF-a, IL-6 and interleukin-10 (IL-10) [22,30].

Alzheimer’s disease as well as some types of FTD is considered tauopathies since the intracellular deposition of abnormally phosphorylated tau is a characteristic neuropathologic finding. Except for some hereditary cases, the cause(s) of FTD is largely unknown; however an involvement of tau and/or neurofilament proteins in the mechanisms of the disease has been suggested at least in some types of the disease [34,35]. It is not known whether autoimmune mechanisms are in any way involved in FTD.

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Table 1
Clinical and biochemical data of studied groups

<table>
<thead>
<tr>
<th></th>
<th>AD</th>
<th>FTD</th>
<th>NCTRL</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>19 (5/14)</td>
<td>7 (4/3)</td>
<td>30 (12/18)</td>
<td>NS</td>
</tr>
<tr>
<td>Age (year)</td>
<td>65±11</td>
<td>58±12</td>
<td>60±11</td>
<td>NS</td>
</tr>
<tr>
<td>MMSE</td>
<td>18 (13–21)</td>
<td>21 (14–23)</td>
<td>28 (27–29)</td>
<td></td>
</tr>
<tr>
<td>IL-12 (pg/ml)</td>
<td>13.9±5.9**</td>
<td>15.2±5.6*</td>
<td>23.8±9 &lt;0.001</td>
<td></td>
</tr>
<tr>
<td>IL-12 (pg/ml)</td>
<td>14.3 (8.6–18)</td>
<td>14.9 (12.6–17.6)</td>
<td>24.9 (19–30)</td>
<td></td>
</tr>
<tr>
<td>τ (pg/ml)</td>
<td>724±374</td>
<td>346±192</td>
<td>175±85 &lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>Aβ42 (pg/ml)</td>
<td>629 (406–1017)** †</td>
<td>317 (200–433)*</td>
<td>165 (131–194)</td>
<td></td>
</tr>
<tr>
<td>Aβ42 (pg/ml)</td>
<td>395 (346–459)</td>
<td>716 (693–812)</td>
<td>723 (548–853) &lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

*Mean±S.D., †median (25th–75th percentile), χ2-test, 1-way ANOVA, ANCOVA, ANCOVA after logarithmic transformation, Kruskal–Wallis test. NS: non-significant. Newman–Keuls post-hoc tests: *P<0.05 vs. NCTRL, **P<0.001 vs. NCTRL, †P<0.01 vs. FTD. Dunn’s post-hoc tests: †P<0.01 vs. FTD and NCTRL.

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Fig. 1. Scatter-plot of CSF IL-12 levels in the studied groups. Horizontal bars indicate mean values.
pathophysiology. Inflammatory processes have been suggested [36,37], but thorough investigations have not been carried out yet. IL-1 and IL-6 induce tau phosphorylation in vivo and in cortical neurons and cultured human astrocytes [38–41]. Significantly increased CSF levels of TNF-alpha and Transforming Growth Factor-beta may have been observed in FTD patients as compared to controls [40]. In our study CSF IL-12 levels in FTD patients were lower as compared to NCTRL patients. These results may be compatible with non-involvement of proinflammatory cytokines in the neurodegenerative process of FTD. However CSF IL-12 levels in our AD patients were positively correlated with \( \tau \) levels, indicating that IL-12 may be involved in cytoskeletal changes. No correlation between disease duration or MMSE scores and CSF IL-12 concentrations was revealed in our FTD patients.

To our knowledge this is the first study assessing CSF IL-12 concentrations in patients with FTD and the second in patients with AD [21]. Other studies [21,22] have not found altered CSF or serum IL-12 levels in AD patients compared with healthy or neurological control subjects. The authors studied a list of cytokines and found that only serum IL-6 levels were significantly elevated [22]. Their findings such as ours appear to contradict the inflammatory hypothesis in AD. It is possible that the immune-mediated inflammatory changes found in histopathological studies are not reflected in serum or CSF of patients with AD. The weakness of all studies measuring circulating cytokines in serum or CSF in patients suffering from neurological diseases is the not exact correlation between peripheral cytokine release and neuro-pathological intracerebral changes [34]. The production and action of cytokines may be very localized in the CNS parenchyma not allowing a representative detection in serum or CSF [21].

Alzheimer’s disease is characterized by neuronal impairment that leads to cognitive impairment. As certain affected neurons depend on trophic factors such as Neurotrophins (NTs), impairment in NT function has been suggested to be a component of neuronal damage associated with AD and other neurodegenerative disorders [42]. There is a possibility that altered proinflammatory cytokine levels may affect NT function in neurodegeneration since cytokine receptors and NT receptors share a high degree of homology and are capable of activating similar signaling pathways [42]. Recent reports suggest that accumulation of Abeta amyloid and proinflammatory cytokines during aging may generate in the brain a “neurotrophin resistance” state that places the brain at risk for cognitive decline and dementia [43]. The low CSF IL-12 levels found in our study may suggest a reduced neuroprotection in AD and other dementias. Future studies should be done in order to investigate whether IL-12 and other proinflammatory cytokines may have a neurotrophic and neuroprotective role such as i.e. the role of activated astrocytes and of neurotrophic cytokine S100B in the pathogenesis of AD [44]. The production of nerve growth factor by interferon-beta may be relevant to its clinical efficacy in multiple sclerosis, an inflammatory and neurodegenerative disease. This finding may suggest the potential utility of interferon-beta in AD and other neurodegenerative disorders [45].

Conclusively, the precise implications of inflammatory response for neurodegeneration in dementias have not yet been elucidated. Future studies in a greater number of patients assessing circulating cytokines in serum and CSF and cytokine levels in various regions of CNS of patients with dementias must be carried out in order to confirm the hypothesis of immune disturbances as part of pathophysiological mechanisms in these disorders.

References


