Intrathecal levels of IL-6, IL-11 and LIF in Alzheimer’s disease and frontotemporal lobar degeneration

Introduction

Interleukin (IL)–11 belongs to the family of IL-6-type cytokines, which includes also cardiotrophin-1, ciliary neurotrophic factor, leukaemia inhibitory factor (LIF) and oncostatin-M. It was first isolated as a bone marrow fibroblast-derived cytokine that stimulates proliferation of IL-6 dependent cell lines [34]. All IL-6-type cytokines have overlapping bioactivities and share a common helical framework [26]. Intracellular signalling activated by these cytokines requires either homodimerization of their common signal transducing receptor subunit gp130 or heterodimerization of gp130 with a further β-receptor component, such as LIF (LIFR). The receptor complexes for IL-6 and IL-11 contain a third, ligand-specific primary α-receptor subunit (IL-6R and IL-11R, respectively) [14, 30]. Interleukin-11 has been shown to stimulate the neuronal differentiation in immortalized embryonic hippocampal cell lines [18]. Compared to LIF, IL-11 has only a minimal effect on sympathetic and sensory neurons in vitro [5, 24] and recent data demonstrate that IL-11 can significantly enhance the survival of cultured rat dorsal root ganglia neurons if soluble IL-11R is also present [30].

Alzheimer’s disease (AD) is the most common form of dementia in the elderly. Amyloid beta (Aβ)42 deposition into the brain is considered a crucial step during its development. In AD patients, a significantly positive correlation between MMSE scores and IL-11 CSF concentration was observed ($r = 0.344$, $P = 0.028$). No correlations with CSF Aβ42, total tau and P-tau were found. IL-11, but not IL-6 levels are increased in AD and FTLD, and the highest peaks were observed in patients with a less severe degree of cognitive deterioration, therefore suggesting a role of this cytokine in early phases of neurodegeneration.

Key words Alzheimer’s disease (AD) · frontotemporal lobar degeneration (FTLD) · cerebrospinal fluid (CSF) · cytokines · interleukin-11 (IL-11)
development, as it originates a cascade of events leading to irreversible neuronal damage [1]. It is widely accepted that a chronic inflammatory reaction plays an important role in the pathogenesis of AD and a variety of inflammatory factors including cytokines and chemokines have been detected in and around plaques and tangles [1]. Recent studies in cerebrospinal fluid from patients with AD demonstrated an upregulation of cytokines, including tumor necrosis factor (TNF-α) [27] and chemokines, including interferon-γ-inducible protein-10 (IP-10), monocyte chemotactic protein-1 (MCP-1) and interleukin-8 (IL-8) [7–9]. Several authors evaluated IL-6 in CSF from AD patients, with conflicting results, possibly due to the small number of patients considered in each single study [4, 12, 16, 22, 28, 31, 33], whereas no data about CSF levels of IL-11 and LIF in neurodegenerative disorders are available to date. Notably, IL-11 has been proposed as a potential protective factor for AD as in in-vitro models it inhibits Aβ42-induced neurotoxicity in a dose-dependent manner [10]. Moreover, IL-11 inhibits L-phosphoserine phosphatase, which is induced in neuronal cells upon stimulation with Aβ42 [10].

Cytokines and chemokines have been studied in other neurodegenerative disorders, including frontotemporal lobar degeneration (FTLD). Recent evidence suggests that inflammatory mediators could have a role also in FTLD [25]. In addition, similar to AD, MCP-1 and IL-8 are increased in CSF from FTLD, whereas IP-10 levels are not [8]. Therefore, some common steps during neurodegenerative processes are likely to occur, whereas some others are specific for AD only. Remarkably, a possible beneficial role of both MCP-1 and IL-8 has been hypothesized, as their effect on the regeneration of neural tissue has been demonstrated [3, 13].

To elucidate the role of IL-6-type cytokines in the pathogenesis of neurodegenerative disorders, we evaluated IL-6, IL-11 and LIF levels in CSF samples from patients with AD and FTLD as compared with age- and gender-matched nondemented subjects (CON). In addition, cytokine levels were correlated with Aβ42, total tau and tau phosphorylated at position 181 (P-tau), which are CSF biomarkers routinely determined for diagnostic purposes.

Methods

Subjects

The following patients were consecutively recruited at the Alzheimer Unit of the Ospedale Maggiore Policlinico-IRCCS (Milan): 43 with probable AD (12 males and 31 females, mean age at onset ± SD: 65.6 ± 8.4 years; mean disease duration ± SD: 3.6 ± 2.0; mean MMSE ± SD: 22.2 ± 2.47) and 24 with FTLD (7 males and 17 females, mean age at onset ± SD: 64.7 ± 7.6 years; mean disease duration ± SD: 3.0 ± 2.5; mean MMSE ± SD: 26.4 ± 2.90), including 21 patients with FTD, 2 with progressive aphasia (PA) and 1 with semantic dementia (SD). The imbalance of sexes reflects epidemiological findings in the Italian population [29]. All patients underwent a standard battery of examinations, including medical history, physical and neurological examination, screening laboratory tests, neurocognitive evaluation (to assess memory, language and constructional praxis), brain magnetic resonance imaging (MRI) or computed tomography (CT) and, if indicated, positron emission computed tomography (PET). The presence of significant vascular brain damage was excluded (Hachinski Ischemic Score < 4). Dementia severity was assessed by the Clinical Dementia Rating (CDR) and the Mini Mental State Examination (MMSE). Disease duration was defined as the time in years between the first symptoms (by history) and the lumbar puncture (LP). Nineteen out of 43 AD patients had an early onset of the disease (EOAD, age at onset < 65 years), whereas the remainder had a late onset (LOAD, age at onset ≥ 65 years). Patients and controls were tested for CSF Aβ42, tau and P-tau to improve the diagnostic accuracy, as previously reported [2]. AD patients were diagnosed by exclusion according to NINCDS-ADRDA criteria [17]; FTLD diagnosis met the criteria proposed by Neary et al. [20]. After the recruitment period, an accurate follow-up of patients was done, to further confirm clinical diagnoses. None of the patients received acetylcholinesterase inhibitor therapy before collecting samples.

The control group (CON) consisted of 30 subjects matched for ethnic background, age and gender (10 males and 20 females, mean age ± SD: 63.5 ± 12.0 years): without memory complaints (mean MMSE ± SD: 28.8 ± 2.02), with other non-inflammatory neurological affections: acute headache [10], vertigo [10], non-immune peripheral neuropathies [4], compressive radiculopathies [3], acute confusional state due to metabolic causes and completely recovered [3]. The age of controls did not significantly differ from that of AD patients (P > 0.05). Informed consent to participate in this study was given by all subjects or their caregivers.

All the information about patients and CON are summarized in Table 1.

CSF/serum sample collection and routine analysis

CSF samples were obtained in polypropylene tubes by LP at the L4/L5 or L3/L4 interspace, centrifuged at 4 °C and stored at ≤ 30 °C until analysis. CSF cell counts, glucose and proteins were determined. A 100 μl aliquot was blotted onto nitrocellulose filter and stored at ≤ –30 °C. IgG index (CSF albumin/serum albumin)/(CSF IgG/serum IgG) were calculated [23].

Cytokine determination

In order to avoid possible chemokine variations due to multiple freezing/thawing cycles, analyses were carried out after the first thawing cycle and no more than after 1-year storage, as previously reported [9]. IL-11 and LIF levels were measured with human specific ELISA kits (R&D Systems), based on the quantitative sandwich enzyme immunoassay technique [6]. The sensitivity of these assays was 8.0 pg/ml. IL-6 was evaluated with a human high sensitivity (0.1 pg/ml) ELISA kit (Amersham). The laboratory technician who performed the cytokine evaluation was blinded to the diagnosis of each patient.

Aβ42, tau and P-tau determination

Aβ42, tau and P-tau CSF levels were determined with human specific ELISA kits (Innogenetics), as previously reported [2].