Soluble interleukin-6 receptor (sIL-6R) in cerebrospinal fluid of patients with inflammatory and non inflammatory neurological diseases

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Received 12 December 2003; received in revised form 16 April 2004; accepted 22 April 2004

Available online 31 May 2004

Abstract

IL-6 acts on target cells via the ligand-binding protein interleukin-6 receptor (IL-6R) and the affinity-converting and signal-transducing glycoprotein 130 (gp130). Soluble interleukin-6 receptor (sIL-6R) has an agonistic role because the soluble complex (IL-6/sIL-6R) can activate cells that do not express IL-6R and an antagonistic role as it enhances the inhibitory activity of sgp130. Soluble forms of both receptors, sIL-6R and sgp130, regulate the action of IL-6. sIL-6R was measured by a sensitive enzyme-linked immunosorbent assay in paired sera and cerebrospinal fluid (CSF) from 46 patients with inflammatory neurological diseases (IND), 45 patients with relapsing–remitting multiple sclerosis (RR-MS), 13 patients with primary progressive multiple sclerosis (PP-MS), 17 patients with other non inflammatory neurological diseases (NIND) and 13 mentally healthy individuals—healthy controls (HC).

Patients with RR-MS had CSF sIL-6R levels comparable to those from patients with IND, but higher than patients with NIND and HC.

A positive correlation between the CSF/serum albumin (QAlb) and CSF sIL-6R levels was observed in IND but not in RR-MS patients indicating that CSF/sIL-6R levels in IND patients could be influenced by serum sIL-6R and blood brain barrier (BBB) permeability properties. RR-MS patients had higher values of [CSF/serum IL-6R:CSF/serum albumin] (sIL-6R index) than IND patients suggesting that in multiple sclerosis (MS), the increase in CSF sIL-6R could be due to intrathecal synthesis of sIL-6R.

The finding of increased CSF sIL-6R concentrations (>979 pg/ml) with sIL-6R index (>4.66), in correlation with positive oligoclonal bands in RR-MS patients, suggests that values of sIL-6R index > 4.66 indicate intrathecal increase of sIL-6R and might be used as an indicator of neuroimmunoregulatory and inflammatory processes in the central nervous system (CNS). © 2004 Elsevier B.V. All rights reserved.

Keywords: IL-6 receptor; Cerebrospinal fluid; Antibodies; Neurological diseases; Infectious disease

1. Introduction

Interleukin-6 receptor (IL-6) is secreted by many cell types, activated monocytes, macrophages, T cells, endothelial cells and bone marrow stroma cells [1]. In the nervous system, IL-6 can be secreted by activated microglia, astroglia, Schwann cells, neurons and endothelial cells, the IL-6R by neurons and glycoprotein 130 (gp130) by all cells [2–4]. IL-6 acts on target cells that express IL-6R (proliferating B cells, T cells, plasma cells, myeloid stem cells, hepatocytes and neurons) [1,4]. IL-6 is a cytokine with many functions, which induces the transformation of activated B cells into immunoglobulin-secretion cells and the production of acute phase proteins by hepatocytes [1]. IL-6 acts on target cells via a receptor consisting of two transmembrane glycoproteins, gp80 and gp130. The binding of IL-6 to its specific receptor (gp80, IL-6R) triggers the dimerization of the signal-transducing receptor subunit gp130 [5]. The soluble form of the IL-6R (sIL-6R) when linked to IL-6 triggers the dimerization of gp130 and in contrast to several other soluble cytokine receptors, which inhibit ligand function, the...
soluble IL-6/sIL-6R complex can activate signal transduction and biological responses in cells that possess gp130 and do not express gp80 IL-6R [6,7]. The gp80 IL-6R mRNA levels can be enhanced in vitro in hepatoma cells by IL-1, IL-6 and glycercytocid and during acute inflammatory responses in liver of rats in vivo [8,9]. The soluble form of the receptor is produced either by protease-induced shedding of membrane-bound receptors or by alternative splicing of the receptor mRNA [10].

Soluble interleukin-6 receptor has been detected in cell-free supernatants of phytohemagglutinin (PHA)-activated peripheral blood mononuclear cells (PBMC), in HTLV-I positive T cell lines, from cultured cells with infection by HIV, in serum of HIV seropositive blood donors and from IL-6R positive cell populations (T cells, activated B cells and macrophages) [11]. The gp130 molecule is found expressed in many tissues and cells, whereas the IL-6R is more restricted in its level of expression [4].

Very little is known about soluble cytokine receptors in cerebrospinal fluid (CSF). The effects of systemically circulating IL-6 and sIL-6R on the central nervous system (CNS) have not been addressed so far. Because IL-6R is bioactive in a membrane-bound and in a soluble form, sIL-6R is an important cofactor of IL-6. In fact, the IL-6/sIL-6R complex rather than IL-6 is believed to be the active form in vivo [12]. IL-6 produced at sites of inflammation or in the peripheral blood in response to stimuli released by inflammatory sites (IL-1 or TNFα) binds the serum sIL-6R and is transferred in the blood as an IL-6/sIL-6R complex [13]. This binding protects IL-6 from protease degradation and stabilizes its bioactivity just like for IL-4, TNFα and their soluble receptors, and thus the measurement of total soluble IL-6R (free sIL-6R plus the amount of soluble receptor bound to IL-6) may give more reproducible and comparable results than the measurement of the cytokine above [13,14].

Soluble IL-6R has been found increased in serum of patients with juvenile rheumatoid arthritis [13] with multiple myeloma [15], with HIV infection [11] in the serum and the CSF of patients with multiple sclerosis (MS) [16] and in the serum of patients with Alzheimer’s disease [17].

Although numerous studies concerning IL-6 levels in different inflammatory and autoimmune diseases are available, very little work has been done with respect to CSF sIL-6R. Soluble forms of both receptors (sIL-6R and sgp130) regulate the action of IL-6 in a complex manner: sIL-6R enhances IL-6 effects by making the ligand accessible to membrane-bound gp130; whereas sgp130 inhibits the action of IL-6 [18,7].

In the presence of sgp130 and membrane-bound IL-6R, sIL-6R potentiates the antagonistic activity of sgp130 leading to inhibitory effects on IL-6 responses [7].

2. Materials and methods

Serum and CSF samples were studied in 134 individuals comprising the following groups: (1) 46 inflammatory neurological diseases (IND) patients (16 with Guillain-Barré (GBS), 9 with chronic inflammatory demyelinating polyradiculoneuropathy (CIDP), 12 with viral and 9 with bacterial meningitis), (2) 58 patients with definite multiple sclerosis according to Poser’s criteria [19], (45 were of the relapsing-remitting multiple sclerosis (RR-MS) and 13 of the primary progressive multiple sclerosis (PP-MS)), (3) 17 patients with (non inflammatory neurological diseases (NIND)) diseases (9 patients with amyotrophic lateral sclerosis, 5 with epilepsy, 4 with cerebellar atrophy), (3) 13 mentally healthy individuals—healthy controls (HC)—who were hospitalized for backache and headache and who had no evidence of organic neurological disease studied as control group.

Patients who were admitted with acute flaccid paralysis, progressive weakness, areflexia, albuminocytological dissociation in the CSF [20] and distinctive electromyographic changes [21] were diagnosed as suffering from Guillain-Barré syndrome. The diagnosis of amyotrophic lateral sclerosis (ALS) was established in patients who, at the time of lumbar puncture or during follow-up, fulfilled the El Escorial workshop criteria for definite ALS [22].

McDonald [23] and Barkof [24] criteria were applied for the diagnosis of multiple sclerosis. Many patients experienced their first MS relapse at the time of lumbar puncture and were registered as having a possible MS. However, only the specimens of those who presented during follow-up spatial or temporal dissemination were included in our study and were classified with the RR-MS patients.

Finally, chronic inflammatory demyelinating polyradiculoneuropathy diagnosis was based on clinical, electrodagnostic (EDX) and CSF criteria proposed by the Ad Hoc Subcommittee of the American Academy of Neurology AIDS Task Force [25].

Clinical and laboratory findings confirmed the diagnosis of acute meningitis, epileptic crisis and dementia. Patients had not been receiving any medication for at least 4 months prior to lumbar puncture.

Of the patients with acute viral meningitis, four had HSV, two CMV and one EBV infection and the rest were presumed to have Herpes simplex meningitis based only on clinical criteria. In bacterial meningitis, three patients had pneumococcal, two meningococcal infection and in four, no bacterial strain could be isolated. The CSF of these patients was collected before initiation of antibiotic treatment.

Patients with Guillain-Barré syndrome underwent diagnostic lumbar puncture within 17 days of onset of symptoms. After lumbar puncture, CSF samples and blood samples were immediately centrifuged to remove cells and prevent cytokine leakage from the intercellular compartment and were stored at −70°C. All CSF samples were subjected to...
Concentrations of total soluble IL-6R (free sIL-6R plus the amount of soluble receptor bound to IL-6) in CSF were measured using commercial human enzyme-linked immunosorbent assay (ELISA) kits (Quantikine, R & D systems, Inc., Minneapolis, USA).

Since the measurement of sIL-6R by this immunoassay is insensitive to added recombinant human IL-6, we assume that this ELISA measures both the complexed and the free form of sIL-6R (free sIL-6R + (IL-6/sIL-6R)).

The minimum detectable concentration of the assay was 6.5 pg/ml.

Intra- and interassay precision, as well as specificity and linearity of the assay, were given by the manufacturer.

CSF samples were tasted at dilutions of 1:2 and serum samples at dilutions of 1:40. All CSF and serum samples were tasted in duplicate, and the average optical density value was used for the calculation of the results.

2.1. Statistical analysis

Groups were compared using Student’s t-test and ANOVA. Because CSF sIL-6R levels were not normally distributed in IND group, the comparisons were performed using the non-parametric Mann–Whitney U-test.

The correlation analysis was performed using the Pearson correlation coefficients, r and p. The level of significance was taken at P = 0.05.

3. Results

The distribution of CSF sIL-6R concentrations in the studied groups of patients and control subjects is shown in Fig. 1. CSF sIL-6R concentrations in the HC group (n = 474.76, S.D. = 16.765) and NIND (n = 484.88, S.D. = 96.08) showed no difference of statistical significance from each other (P > 0.10).

The CSF sIL-6R concentrations in IND group (n = 1604±1016), in RR-MS group (n = 1188.46±322.27) and in PP-MS group of patients (n = 693.6±91.62) were found to be significantly higher than those in the HC group (P < 0.001, P < 0.0001 and P < 0.01, respectively). The IND and MS group of patients registered significantly increased CSF sIL-6R concentrations when compared with HC and NIND. All RR-MS patients had CSF sIL-6R values > 811 pg/ml, 2.5 standard deviations above the average value of the HC group, whereas all the patients with PP-MS, showed values lower than 811 pg/ml. The CSF albumin and CSF/serum albumin ratio indicates the severity of the blood brain barrier (BBB) disturbance [26].

In IND patients, the increased values of CSF sIL-6R were positively correlated with increased CSF albumin (r = 0.872, P < 0.01) and increased QAlb (r = 0.951, P < 0.001). In the case of viral and bacterial meningitis, CSF sIL-6R concentrations were also found to correlate positively with CSF cell number (116 ± 78 and 3935 ± 7464 cells, respectively), CSF albumin (r = 0.950, P < 0.001) and index IgG (r = 1.162, P < 0.001). However, in the cases of 58 MS patients studied, CSF sIL-6R concentrations failed to show any correlation with CSF parameters, except for the presence of oligoclonal bands (OGB) or the IgG index. In 35 out of 45 RR-MS patients (75%), the increased values of CSF sIL-6R were correlated with positive oligoclonal bands in the CSF.

<table>
<thead>
<tr>
<th>OGB</th>
<th>CSF sIL-6R (m ± S.D.) (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive (+)</td>
<td>1245 ± 320.8</td>
</tr>
<tr>
<td>Negative (−)</td>
<td>990.5 ± 251</td>
</tr>
</tbody>
</table>

Significant differences in RR-MS patients between oligoclonal bands—positive and negative groups—were observed for CSF sIL-6R concentrations (P < 0.05).

Fig. 1. Distribution of CSF sIL-6R in the studied groups. The horizontal line indicates cut-off level (median ± 2S.D. of controls). Significant differences between the groups were observed (one-way ANOVA, F = 13.018, P < 0.001).
Table 2

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Number</th>
<th>CSF sIL-6R (pg/ml)</th>
<th>QsIL-6R</th>
<th>QAlb (mg/l)</th>
<th>sIL-6R index</th>
</tr>
</thead>
<tbody>
<tr>
<td>HC</td>
<td>13</td>
<td>475 ± 168 (163-685)</td>
<td>0.0124 ± 0.0042 (0.011-0.0177)</td>
<td>4.644 ± 0.605 (3.5-5.6)</td>
<td>2.66 ± 1.08 (1.1-3.6)</td>
</tr>
<tr>
<td>NIND</td>
<td>17</td>
<td>484 ± 96 (341-640)</td>
<td>0.0124 ± 0.0023 (0.01-0.016)</td>
<td>4.723 ± 0.686 (3.5-6.6)</td>
<td>2.70 ± 0.51 (1.93-3.6)</td>
</tr>
<tr>
<td>IND</td>
<td>46</td>
<td>1604.5 ± 1015.6 (458-4220)</td>
<td>0.0266 ± 0.0117 (0.012-0.064)</td>
<td>15.98 ± 9.58 (5-46.7)</td>
<td>2.70 ± 1.16 (0.65-4.87)</td>
</tr>
<tr>
<td>RR-MS</td>
<td>45</td>
<td>1188.46 ± 322.27 (848-2060)</td>
<td>0.0235 ± 0.006 (0.0156-0.0145)</td>
<td>5.35 ± 1.98 (2-9.2)</td>
<td>4.9 ± 1.72 (2.55-9)</td>
</tr>
<tr>
<td>PP-MS</td>
<td>13</td>
<td>693.6 ± 91.62 (511-779)</td>
<td>0.0156 ± 0.025 (0.0120-0.0194)</td>
<td>6.53 ± 2.47 (2.2-10.7)</td>
<td>2.76 ± 1.2 (1.59-5.8)</td>
</tr>
</tbody>
</table>

In order to correlate the increased concentrations of sIL-6R in the CSF of RR-MS patients with the emergence of oligoclonal bands, we compared the CSF sIL-6R concentrations between OGB positive and OGB negative RR-MS patients. CSF sIL-6R concentrations in RR-MS patients with positive OGB were significantly higher than in patients with negative OGB (P < 0.05, Table 1).

In order to study the origin of sIL-6R in the CSF of MS, IND and NIND patients, sIL-6R concentrations were detected in matched CSF and serum. The evaluation of the production of the sIL-6R in the CSF was assessed by referring the CSF-serum sIL-6R quotient (QsIL-6R) to the QAlb, and QsIL-6R/QAlb (sIL-6R index) was calculated [26]. The mean values ± S.D. found for each group are reported in Table 2.

Significant differences between the groups were observed for both QAlb (one-way ANOVA, F = 26.04, P < 0.001) and sIL-6R index (F = 26.83, P < 0.001) (Fig. 2).

Values of QAlb (m = 15.9 ± 9.58) in the IND group were significantly higher than MS and NIND group (Table 2).

All the IND patients were found to have a normal sIL-6R index (<3.66), except one patient with bacterial meningitis who had sIL-6R index (4.87) and increased IgG index, without blood–nervous barrier (BNB) disturbance.

4. Discussion

The results of the present study show elevated CSF sIL-6R concentrations in IND patients and MS patients. The increase of CSF sIL-6R levels could be influenced by serum sIL-6R levels or could reflect intrathecal synthesis of sIL-6R. To deal with this problem, we introduced the QsIL-6R/QAlb ratio (sIL-6R index) to evaluate the production of sIL-6R in the CSF.

We found that IND patients showed no difference of sIL-6R index values when compared with HC and NIND patients. The detection of high sIL-6R index values (>3.66) in IND patients suggests that the elevation of CSF sIL-6R is not due to the production of sIL-6R in the central nervous system, but is rather the result of the increase of serum sIL-6R and the simultaneous leakage through the impaired BNB as revealed from increased QAlb.

All the IND patients showed a normal sIL-6R index (<3.66) except five patients with GBS without clear evidence of BNB dysfunction. Only one of them had increased sIL-6R index (4.87), increased IgG index and motor axonal neuropathy after a preceding Campylobacter jejuni infection. These five GBS patients showed an
intrathecal synthesis of sIL-6R, which may have resulted from passive diffusion through limited BBB damage not detectable by the QAb, or it was due to local synthesis by the Schwann cells [2]. The above findings are in agreement with those of a recent study [27].

It is believed that the course of GBs may be determined by the expression of various cytokines produced by the infiltrating immune cells and by the Schwann cells. Activated CD4+ of the Th2 phenotype are involved in the pathogenesis of GBs by synthesizing IL-6, which promotes B cell proliferation and transformation into plasma cells, so the production antibodies against PNS myelin components [28]. High IL-6 concentrations were found in CSF from patients with active GBs [29]. In another study, serum and CSF IL-6 values failed to correlate [30]. In GBs, CSF sIL-6R could be produced by immune cells infiltrating the nerve roots at the level of their entry into the CNS [30].

All the patients with viral or bacterial meningitis showed low sIL-6R index (<3.66), except one with bacterial meningitis having sIL-6R index higher than the cut off point (4.7) and increased QAb. It seems probable that in this patient with increased sIL-6R index and increased QAb, there is systemic and intrathecal production of sIL-6R.

High levels of CSF sIL-6R have also been detected in bacterial and viral meningitis and represent a host defense mechanism as IL-6 enhances the local production of antibodies and is involved in neural repair by inducing the production of neurotrophic factors [31,32].

We found that RR-MS patients had significantly higher sIL-6R index (>3.66) than the IND patients, indicating that in MS, the increase of CSF sIL-6R could be due to intrathecal synthesis of sIL-6R. The positive correlation between increased CSF sIL-6R and positive oligoclonal bands in 35 out of 45 (75%) patients may indicate the presence of activated B lymphocytes in RR-MS lesions.

These immune cells probably induce immunoglobulin synthesis and it is not unlikely to suppose that it could also play a role in those oligoclonal immunoglobulins observed in the CSF of the MS patients [33].

Furthermore, increased CSF sIL-6R levels have similarly been found during relapses or progressions of MS whereas no correlation between the IgG index and oligoclonal bands has been detected [34]. In recent studies, it has been demonstrated that activated neurons and glial cells can produce IL-6 [35]. In a further study, increased levels of CSF IL-6 indicating relapse in MS were correlated with positive oligoclonal bands and increased IgG index in CSF [27]. In a more recent study, the presence of IgG has been identified both through a process of immunohistochemistry throughout the demyelinated zone of MS plaques [36] within plasma cells and on the surface of macrophages [37]. Although the presence of IL-6 in MS lesions has not yet been evaluated, certain studies have shown that IL-6 is expressed by the majority of perivascular inflammatory cells in acute MS lesions [38]. In lesions characterized by both inflammation and demyelination, in situ hybridization studies indicate that IL-6, along with TNFα, is the most widespread and intensely expressed cytokine [38].

As regards CSF sIL-6R and antemyelin Abs, no association between the presence of antemyelin oligodendrocyte antibodies (antiMOG) and CSF concentrations of sIL-6R has been found [16].

PP-MS patients had lower sIL-6R index in comparison with RR-MS patients, indicating that in MS, there is a relationship between the increase of sIL-6R index and the clinical form of the disease. PP-MS has probably a different immunogenetic profile according to the other clinical forms of the disease. This is supported by (a) lower IgG index compared to RR-MS, (b) lack of change on conventional imaging in the presence of continuing clinical deterioration, and (c) no satisfactory response to immunomodulating or immunosuppressing treatment [39].

sIL-6R has been reported to be present in CSF and serum, in non pathological conditions [40]. Contradictory results regarding sIL-6R concentrations in the CSF of patients with MS have been reported [41]. sIL-6R has an agonistic role because the soluble complex IL-6/sIL-6R can activate cells that do not express IL-6R, with a surface phenotype of (IL-6R+/gp130+) and an antagonistic role as it enhances the inhibitory activity of sgp130. sIL-6R enables sgp130 to trap IL-6 in a soluble [IL-6/sIL-6R/gp130] formation which has an inhibitory effect on (IL-6R+/gp130+) and (IL-6R+/gp130+) cells. Soluble forms of both receptors, sIL-6R and sgp130, regulate the action of IL-6 [18,7].

The concentration of bioactive IL-6 is determined by the concentration of the initial IL-6 (IL-6c: stimulatory events) and the concentration of this soluble receptor pair that inhibits systemic IL-6 responses: ([IL-6]−[IL-6/sIL-6R/gp130]) [18]. In vitro studies have shown that IL-6 and the much more efficient IL6RIIIL6 chimera through the homodimerization of gp130 protein and activation of signal transducers and activators of transcription (STAT1 and STAT3) stimulate peripheral myelination in Schwann cells and survival and differentiation of precursor oligodendrocytes [42]. The binding of IL-6 to its receptor induces dimerization of gp130, leading to the activation of the Janus Kinase signal transducer and activator of transcription (JAK/STAT) pathway.

This causes tyrosine phosphorylation of STAT3 and translocation to the nucleus where gene expression is induced [43]. It has been shown that the JAK/STAT pathway is required for the ciliary neurotrophic factor (CNTF)-mediated promyelinating and prosurvival effects in mature and precursor oligodendrocyte cells [44,45]. STAT3 is an important activator of the antiapoptotic proteins Bcl-2 and Bcl-XL [46] and STAT proteins may be necessary for regulation of myelin-specific genes [47,48].

The increase of sIL-6R might be linked to regulatory functions of immune responses induced by IL-6, so the finding of increased CSF sIL-6R concentrations (above 979 pg/ml) and sIL-6R index (above 4.66), in correlation with positive oligoclonal bands in RR-MS patients, suggests that values of
sIL-6R index > 4.66 indicate intrathecal increase of sIL-6R and might be used as an indicator of neuroimmunoregulatory and inflammatory processes in the CNS.

References


