Neopterin concentrations in serum and cerebrospinal fluid in HTLV-I infected individuals

Amza Ali, Peter Rudge, and A. G. Dalgleish

1Retrovirus Research Laboratory, Clinical Research Centre, Northwick Park Hospital, Watford Road, Harrow HA1 3UJ, UK
2National Hospital for Neurology and Neurosurgery, Queen Square, London WC1N 3BG, UK

Summary. The concentration of neopterin was measured in serum samples taken from individuals infected with HTLV-I: 5 from asymptomatic individuals, 1 from a patient with adult T-cell leukaemia and 30 from patients with tropical spastic paraparesis (TSP). In addition, cerebrospinal fluid (CSF) was available from 22 of the TSP patients and neopterin concentrations were determined in these. Elevated levels of neopterin were found in only 3 of the 36 HTLV-I-positive serum samples, all from TSP patients, but significantly elevated neopterin levels were observed in 12 of the 22 CSF samples. The localisation of the elevated neopterin concentrations to the CSF of patients with TSP suggests a marked degree of activation of the cell-mediated immune system intrathecaly. This provides further evidence in favour of powerful immune mechanisms operating centrally in the pathogenesis of TSP.

Key words: Neopterin – HTLV-I – Tropical spastic paraparesis – Multiple sclerosis

Introduction

HTLV-I was the first retrovirus shown to be associated with human disease. The link with adult T-cell leukaemia/lymphoma (ATL) [19] and later with a chronic progressive myelopathy, tropical spastic paraparesis (TSP) [5], known in Japan as HTLV-I-associated myelopathy, has been confirmed in areas where these diseases are endemic. Much work has been done on the humoral response to HTLV-I in TSP [1], but for reasons of accessibility relatively little on the cellular immune system within the central nervous system (CNS), the site of well-defined pathological changes [13].

Neopterin is a pyrazino-pyrimidine compound derived from guanosine triphosphate. It is produced by macrophages after stimulation by gamma-interferon [8] and has been shown to be a valuable biochemical marker of the cellular immune response and the degree of T-cell activation [12]. It has been used previously as an index of activation of cells in the CNS in aspetic encephalitis [3], multiple sclerosis [3] and in patients infected with HIV-I [4, 6, 16]. We therefore used a sensitive radioimmunoassay to detect the presence of neopterin in the serum of infected individuals and the cerebrospinal fluid (CSF) of TSP patients.

Materials and methods

Forty-two individuals were studied, from the following clinical diagnostic categories: 6 healthy controls, 5 asymptomatic seropositive, 1 adult T-cell leukaemia and 30 TSP patients. CSF samples were available from 22 of the TSP patients. Serum samples were taken on the day when CSF was collected. Sera and paired CSF samples were stored at −20°C in the dark until assayed.

HTLV-I status

HTLV-I status was determined by enzyme-linked immunosorbent assay (ELISA) using the Dupont commercial ELISA kit which is a modification of the Saxinger and Gallo assay [15]. End-points were defined as the greatest dilutions of serum or CSF giving a positive ELISA reading. Confirmation was by Western blot analysis as previously described [17].

Neopterin assay

Neopterin was measured with a standard radioimmunoassay (Neopterin RIAbi, Henning-Berlin, Berlin, FRG). Neopterin levels were determined by comparison with a standard curve. Upper reference limits for serum and CSF were taken as 10.0 and 3.0 nmol/l respectively.

Results

HTLV-I status

Serum antibodies were detectable in significant titre ($10^{-2} - 10^{-4}$) in all 5 of the asymptomatic positive individuals as well as in the patient with ATL. Of the clinically
Fig. 1. Neopterin concentrations in serum from the following diagnostic categories: healthy normals, 6; asymptomatic seropositive, 5; adult T-cell leukaemia (ATL), 1; tropical spastic paraparesis (TSP), 30. CSF concentrations of neopterin in 22 patients with TSP with lines connecting elevated CSF values (12) to their corresponding serum values. Asterisk indicates the values of the 2 HTLV-I-negative patients with TSP. Dotted lines indicate the normal limits in the stated compartment, i.e. 10.0 nmol/l in serum and 3.0 nmol/l in CSF.

Table 1. Ranges, mean and median values in serum and cerebrospinal fluid (CSF) of neopterin concentrations where detectable (32 of 42 serum and 15 of 22 CSF samples)

<table>
<thead>
<tr>
<th>Group</th>
<th>Statistic</th>
<th>Serum (nmol/l)</th>
<th>CSF (nmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 5)</td>
<td>Range</td>
<td>1.25–5.0</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>2.35 (1.8)</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>Asymptomatic positive (n = 3)</td>
<td>Range</td>
<td>1.25–7.5</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>4.9 (2.6)</td>
<td>6.0</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>6.0</td>
<td>6.0</td>
</tr>
<tr>
<td>Adult T-cell leukemia (n = 1)</td>
<td>Range</td>
<td>1.25</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>1.25</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>Tropical spastic paraparesis</td>
<td>Range</td>
<td>1.25–21.0*</td>
<td>1.5–125.0*</td>
</tr>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>5.1 (5.4)</td>
<td>38.8 (22.0)</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>2.5 (n = 23)</td>
<td>22.0 (n = 15)</td>
</tr>
</tbody>
</table>

* Undetectable in 7 serum and 7 CSF samples.

diagnosed patients with TSP 28 of the 30 serum samples (titres of $10^{-4}$–$10^{-6}$) and 18 of the 22 CSF samples (titres of $10^{-1.5}$–$10^{-4}$) were positive. Two of the TSP patients were negative for antibodies in both serum and CSF.

**Neopterin assay**

Neopterin levels in serum and CSF are shown in Fig. 1 with the elevated CSF values joined to the corresponding serum values. Ranges, mean and median values are shown in Table 1 for those samples where neopterin was detectable.

**Serum**

In 3 subjects, all with TSP, the titres of neopterin were above the upper reference limit. In 7 TSP patients neopterin could not be detected. The remainder of the patients were within the normal range.

**Cerebrospinal fluid**

In 12 of 22 samples from TSP patients neopterin levels were elevated, ranging from 9.5 nmol/l to 125.0 nmol/l (normal upper limit 3.0 nmol/l). Neopterin was undetectable in 7 samples.

There was no correlation between the CSF antibody titres on ELISA and CSF neopterin concentrations. CSF cell counts were in the normal range in the majority of the TSP patients with only a slight lymphocytic pleocytosis in the remainder. In addition, there was no correlation between sex, age of onset, duration of disease or disease severity and CSF neopterin.

**Discussion**

This study demonstrates a significant elevation of neopterin concentrations in CSF but not serum from patients with TSP to a greater level than previously reported in other neurological diseases.

Other studies have shown that neopterin is a useful biochemical marker of the cellular immune response and reflects the degree of T-cell activation in a variety of systemic disorders, including systemic lupus erythematosus [7], rheumatoid arthritis [7], malaria [10] and several viral infections [9]. In addition, both serum and CSF concentrations of neopterin are elevated in HIV-I-infected individuals. However, serum neopterin levels are higher than CSF except in cases of advanced dementia, some cases of inflammatory demyelinating polyneuropathy and HIV-associated meningitis, when CSF values may exceed serum values [4, 6, 16].

In disorders predominantly affecting the CNS, such as exacerbations of multiple sclerosis (MS) and aseptic meningo-encephalitis (AM), higher levels of neopterin in the CSF than in the serum are often seen although only with maxima of 10.0 nmol/l and 37.0 nmol/l in MS and AM respectively. In both these conditions CSF values returned to normal after recovery from the acute event [3]. The relatively higher CSF values as compared with serum indicate that these are disorders in which the main focus of injury is the CNS.

In this study neopterin levels in 12 of the 22 CSF samples from TSP patients were above the upper reference limit (range 9.5–125.0 nmol/l, normal upper limit 3.0 nmol/l) whereas in only 3 of 30 serum samples was it increased (range 12.0–21.0 nmol/l, normal upper limit 10.0 nmol/l). This suggests that the cellular immune system of the CNS is specifically activated in TSP.

The difference in CSF and serum becomes even more significant given the fact that there was only a slight lymphocytic pleocytosis in the CSF in these patients. Hence, the observed increases in CSF neopterin concentration occurred despite a 1000-fold less number of mononuclear cells than in blood and represents an inverse of the normal serum to CSF ratio of neopterin. Additionally, it is possible that neopterin is being produced by activated...