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Cytokine levels in CSF and neuropsychological performance in HIV patients

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Abstract HIV-associated dementia and its precursors are frequently observed complications of HIV infection, even in the presence of combination antiretroviral treatment (cART). The development, surveillance and treatment of this condition are still not completely understood. Cytokines, as immunological transmitters, may be one key to gaining a deeper understanding of the disease. A total of 33 HIV-positive male patients were evaluated by neuropsychological testing, lumbar and venous puncture, neuroimaging and neurological examination. The cytokine content in the CSF (cerebrospinal fluid) was examined by a solid-phase protein array. The Digit–Symbol Test, contraction time analysis, Rey–Osterrieth Figure and Grooved-Pegboard Test showed inferior results in the presence of an inflammatory CSF environment, whereas neuroprotective or anti-inflammatory conditions were correlated to better results in contraction time analysis. Higher CSF levels of cytokines were independently correlated with the duration of HIV infection. The study showed a correlation of cytokine levels in the CSF of HIV patients with test results of their neuropsychological functioning. The effect was pronounced with regard to the more complex executive tasks. Determining CSF cytokine levels may be a useful supplement to the assessment of HIV patients and contribute helpful information to predict neurocognitive performance. Therapeutic strategies to ameliorate a negative impact of an altered cytokine milieu may aid in slowing the evolution of neurocognitive dysfunction.

Keywords HIV · Neurocognition · Cytokines · Inflammation · Neuropsychology

Introduction

Human immunodeficiency virus 1 (HIV-1) can provoke an array of neuropsychological deficits in infected patients;

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these deficits are seen years after primary infection and can affect different domains of executive brain function, such as psychomotor speed or attention (Ances and Ellis 2007; McArthur et al. 2005). They reflect a group of syndromes of altered cognition and functioning collectively summarized under the rubric of HAND (HIV-associated neurocognitive disorders; Gannon et al. 2011; McArthur et al. 2010). At the extreme end, the deficits can proceed to a severe form of a predominantly subcortical dementia (Antinori et al. 2007; Nath and Sinai 2003). Unfortunately, development of those deficits is not completely stopped by highly active antiretroviral therapy (HAART).

The immune system has profound effects on neuropsychological functioning. Both infectious diseases and the subsequent immune alterations and oxidative stress can influence and compromise brain function by direct or indirect effects (Grovit-Ferbas and Harris-White 2010; Kerr et al. 2005; Kipnis et al. 2008; Kraft-Terry et al. 2009; Moore et al. 2011; Tomonaga 2004; Velazquez et al. 2009). The effects of several cytokines on brain function in humans and animals have been studied under a variety of experimental conditions (Clifford et al. 2009; Laspiur et al. 2007; Letendre et al. 2011).

The acute effects of cytokine alteration in many infectious diseases on cognitive function (systemic inflammatory reaction) in the first post-infection hours and days may be induced by IL-1 and IL-6 (Vollmer-Conna et al. 2004), whereby a steady state of baseline immune activation is not without consequence in human brains (Patarca-Montero et al. 2001; Wichers et al. 2006); for example, IFN-gamma enhances neurotoxicity of soluble HIV-1-derived proteins (Giunta et al. 2006).

Cognitive implications of increased serum levels of TNF-alpha, IL-6 and cortisol were evaluated by Reichenberg et al. who observed a poorer performance in memory tests and an increased state of anxiety and depressed mood in patients with artificially elevated levels of TNF-alpha, IL-6 and steroids (Reichenberg et al. 2001).

Comparable to HAD, acute inflammation is not a predominant feature of Alzheimer's disease, but may occur at distinct time-points (Grovit-Ferbas and Harris-White 2010; Lanzrein et al. 1998). Ubiquitous deposition of amyloid beta-peptide in the brains of these patients is considered one of the major pathological hallmarks in Alzheimer's dementia. The beta-APP is found abundantly in the brains of neuropsychologically asymptomatic and symptomatic HIV patients and is correlated with neuronal/axonal damage in these patients (An et al. 1997). It is found more frequently in demented AIDS patients, but can also be detected in about 30 % of asymptomatic HIV-1-positive individuals (Giometto et al. 1997). These findings are attributed in part to an immune dysfunction with a higher reconstitution capacity in earlier stages as described by several authors and are supported by the observation of increased severity of dementia symptoms in Alzheimer patients after systemic infections of any kind with high levels of circulating IL-1β (Holmes et al. 2003).

IL-1a was found to be responsible for a minimized glucocorticoid-receptor translocation to the cell surface and decreased glucocorticoid-associated gene transcription (Miller et al. 1999), resulting in functional glucocorticoid resistance. IL-1 and TNF-alpha may accelerate and enhance serotonin re-uptake in the brain by p38–MAPK-linked serotonin transporter activation (Zhu et al. 2006), resulting in only small amounts of serotonin being actively available at the respective synapses, which may lead to affective disorders in HIV-infected individuals (based on the serotonin theory in depression).

Functional magnetic resonance imaging (fMRI) studies have shown activation of the anterior cingulate cortex in hepatitis C virus (HCV)-infected patients treated with IFN-alpha. Activation may lead to personality changes and poorer performance in several learning tasks (Capuron et al. 2005).

In conclusion, there is accumulated evidence for the influence of cytokines on human behavior and cognitive function, especially in infectious and inflammatory diseases. Several methods are used to examine cytokine levels in body fluids, ranging from RNA-based techniques to direct protein detection with enzyme-linked immunosorbent assays (ELISA) and western blots. In general, these techniques are time consuming, require a high sample volume and have limited detection limits and concentration ranges. Furthermore, use of many different techniques limits the comparability of study results and widespread application in HIV-laboratories worldwide. Therefore, we used a new method based on protein detection, overcoming most of these former limitations (Nolting et al. 2009).

Methods

Study type, statistics and basic diagnostic procedures

A total of 33 HIV-positive male patients (who acquired infection via sexual transmission) were included in a prospective study with informed consent and approval by the institutional review boards of the participating centers. The results were analyzed using the Pearson Product-Moment Method (Pearson's correlation; regarded significant at a level of $p<0.05$; two-tailed test on significance).

All patients underwent neurological examination, neuropsychological testing, neuroimaging (cerebral MRI) and venous and lumbar puncture.

Patients with a known history of substance abuse, antipsychotic or anti-depressant medication, acute or chronic cerebral opportunistic infections, or overt and self-reported

neurological or neuropsychological deficits were excluded from the study.

Blood and cerebrospinal fluid measurements

Blood and cerebrospinal fluid were collected by venous or lumbar puncture; centrifuged and cell-free supernatants of paired samples were stored in aliquots at −70 °C until analysis.

HIV-1 RNA was quantitated by real time PCR using Abbott RT/m2000 assay following the manufacturer's instructions (Abbott Diagnostics, Wiesbaden, Germany).

Cytokine array

We used a customized cytokine array, testing 34 different cytokines/chemokines. The configuration and chip processing have been described previously (Nolting et al. 2009).

Neuropsychological test battery

Patients underwent the following neuropsychological tests: HIV-Dementia Scale (HDS, German), Hamilton Depression Scale, Mosaic Test, Grooved-Pegboard Test (GPT; dominant and non-dominant hand, Lafayette Instrument, Lafayette, IN, USA), Digit–Symbol Test, Trail-Making Test (TMT; Form A and B), Stroop-Color Test, Wisconsin Card-Sorting Test, tests on verbal fluency (German), Rey–Osterrieth Figure and the Duesseldorf fine motor test battery for HIV patients (contraction time (CT)).

Grouping of cytokines

Cytokines are described as inflammatory/non-inflammatory. This is based upon analysis the "Immunology Database and Analysis Portal" (ImmPort; www.immport.org) and according to Dowlati et al. (2010).

Neuroimaging

Neuroimaging (cerebral scans) was performed on a 1.5-T MRI scanner (Siemens Symphony) before and after gadolinium administration. Images acquired using routine T1 and T2 sequences were assessed independently by an experienced radiologist and neurologist.

Results

Demographics

Demographical data are given in Table 1. Twenty patients were treated with a standard antiretroviral regimen; of these

Table 1 Demographics of the patient cohort

	Mean	Standard deviation	Number
Age (years)	43.6	10.8	33
Years since HIV diagnosis	7.4	5.8	33
Viral load in CSF (log10)	1400.2	3595.7	33
Viral load in blood (log10)	9085.9	20662.1	33
Cells in CSF (1/μl)	6.0	9.9	33
CSF-protein content (mg/dl)	46.0	14.6	33
CSF-glucose content (mmol/l)	62.0	7.6	33
CSF-lactate (mmol/l)	1.8	0.3	33
IgG index	0.7	0.3	33
CD4+ cells in blood	552.9	277.4	33

Patients' characteristics and results of venous and lumbar puncture are described

Table 2 Correlation coefficients of cytokines

Neuropsychological test	Cytokine correlation (Pearson's correlation)
Digit–Symbol Test (digits in 90 s)	IFN-gamma: −0.437*
	IL-1a: −0.517**
	IL-2: −0.444*
	IL-5: −0.336*
	IL-7: −0.368*
	CXCL16: −0.485**
	CXCL2 (GRO): −0.418*
	TGF-beta: −0.40*
	TIMP-1: −0.460**
Left hand contraction time (ms)	ALCAM (CD166): 0.468**
	CCL28: −0.364*
	CNTF: −0.381*
	MMP-2: 0.462**
Right hand contraction time (ms)	CNTF: −0.363*
	IL-4: −0.346*
Grooved-Pegboard Test of the dominant hand (s)	MMP-2: 0.477**
Grooved-Pegboard Test of the non-dominant hand (s)	MMP-2: 0.439*
Rey–Osterrieth Figure, direct copy (points)	IL-12: −0.393*

In the Digit–Symbol Test (higher scores are better), patients decoded less symbols in 90 s in the presence of mainly inflammatory cytokines. In the contraction time analysis (less time is better), a more rapid contraction was detected in the presence of the neuroprotective/anti-inflammatory cytokines CNTF and IL-4, and a slower contraction was found in the presence of MMP-2 and ALCAM. MMP-2 was associated with poorer results in the Grooved-Pegboard Test (less time is better) as well. In the direct copy of the Rey–Osterrieth Figure (more points are better), IL-12 only led to poorer results (constructive apraxia)

*$p<0.05$; **$p<0.005$

patients, seven showed detectable virus in blood, and six had detectable virus in CSF. A total of 13 patients were in AIDS or pre-AIDS stages (CDC A3, B3, C1–3). None of the patients had self-reported neurological or psychiatric deficits. According to the recent nomenclature (Antinori et al. 2007; Gannon et al. 2011), all patients presented were neurological inconspicuous. A more detailed analysis of the patient group has been presented previously (Arendt et al. 2007; Nolting et al. 2009).

Neuroimaging

All patients showed no abnormalities on cerebral in MR scans that could have been attributable to HIV-1.

Correlation analysis

Results of the correlation analysis are provided in Table 2. Most of the cytokines were positively correlated with the duration of HIV infection (data not shown), irrespective of actual antiretroviral treatment or AIDS/non-AIDS status. The results in the Digit–Symbol Tests were predominantly affected by CSF cytokine alterations in this study. A subgroup analysis according to treatment status showed no significant results, possibly due to the small sample number.

Tests on basal ganglia movement control (CT) showed better performance in the presence of neuroprotective cytokines (CNTF and IL-4) and worsened in the presence of inflammatory cytokines (MMP-2 and ALCAM). The Grooved-Pegboard Test showed slowed psychomotor speed in the presence of MMP-2, and the direct copy of the Rey–Osterrieth Complex Figure was more inaccurate in the presence of elevated levels of the mainly inflammatory IL-12 in CSF. All other tests showed no correlations to CSF cytokine levels in this study (data not shown). Some showed marginal results, but significance was not reached, mainly due to the small sample size.

Discussion

Our study indicates that most of the cytokines appear to be unsuitable to serve as biomarkers for HAD risk calculation, at least not on their own. Poorer performance in single neuropsychological tests cannot be predicted by single cytokines, but complicated executive, time-dependent functions, evaluated, for example, by the Digit–Symbol Test, may worsen as the result of an inflammatory CSF environment. Because complex executive, time-dependent tasks need to be frequently performed in everyday life, timing, fine-motor and coordination testing should be applied generally to monitor the described deficits carefully.

Nevertheless, there was a common, negative effect of inflammatory cytokines on neuropsychological tests. Levels of these cytokines were found to correlate with the known duration of HIV infection, which was irrespective of HAART. Given the results of determining viral load in CSF and blood in our study, it is unlikely that a direct viral influence in the CNS directly accounts for this impact since the majority of patients showed little or no viral replication in the CNS. This was particularly true for patient group that received antiretroviral therapies.

The observation that unfavorable immune conditions can develop in neurotropic viral infection regardless of detectable active viral replication can be made in various viral infections of the CNS, both in humans and in animal models (Rempel et al. 2005; Wildemann et al. 1997). An initial induction of the innate immune response with consequences for later adaptive and innate immune responses may be a common pathogenic trait (Griffin 2003).

Based on the demonstrated negative correlation of cytokine levels with neurocognitive functioning, would it be reasonable and feasible to explore therapies that neutralized or antagonized cytokines and their effects? Cytokines are important mediators of viral clearance (Binder and Griffin 2003), and removal of the primary infectious agent is still the classic aim of anti-infectious therapies. Furthermore, even inflammatory cytokines, such as IFN-gamma, are important for neuronal development and survival (Pellegrini et al. 1996).

Hence, there may be significant risks inherent to such an intervention that need to be carefully weighed against the benefits of minimizing concentrations of those cytokines that predict poorer cognitive outcome.

Nonetheless, we conclude from this study that there is a need to modify the immune response from the beginning of the infection through to later stages to ameliorate or delay the cognitive decline of long-term surviving (long-term treated) HIV patients. Studies on the influence of the immune system on neuropsychological performance in HIV patients should be implemented in larger cohorts and antiretroviral therapy studies.

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