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Serum and cerebrospinal fluid levels of interleukin-18 in human immunodeficiency virus type 1–associated central nervous system disease

Hans-Jürgen von Giesen, Sebastian Jander, Hubertus Köller, and Gabriele Arendt

Department of Neurology, Heinrich Heine University, Düsseldorf, Germany

Interleukin-18 (IL-18) is a proinflammatory cytokine released by macrophages that strongly stimulates the production of interferon-γ, thereby linking innate and acquired immunity. Its role in human immunodeficiency virus (HIV) pathogenesis is under debate and little is known about its role in neuro-AIDS (acquired immunodeficiency syndrome). Serum and cerebrospinal fluid (CSF) levels of IL-18 were determined by a commercially available enzyme-linked immunosorbent assay (ELISA) in 22 HIV-seropositive patients without neurological symptoms (HIV+), 21 patients with AIDS dementia complex (ADC), and 31 patients with AIDS-defining opportunistic infections (OIs) of the brain. Thirty-two HIV seronegative patients (HIV−) served as controls. Compared to HIV− controls, serum IL-18 levels were increased in HIV+ and ADC but not in OI patients. In contrast, CSF IL-18 levels were elevated in OI patients whereas HIV+ and ADC patients were not different from HIV− controls. We provide evidence for an significantly increased IL-18 level in the CSF of HIV+ patients with cerebral OIs, suggestive of a role for IL-18 in the intrathecal host response to OIs. *Journal of NeuroVirology* (2004) **10,** 383–386.

Keywords: AIDS; AIDS dementia complex; HIV; IL-18; opportunistic brain infections

Introduction

Interleukin-18 (IL-18), initially described as interferon-γ (IFN-γ)–inducing factor, is a multifunctional proinflammatory cytokine produced by activated macrophages and is important for linking innate with acquired immunity. For an overview, see Okamura *et al*, 1998. IL-18 strongly stimulates the production of IFN-γ by inducing a T-helper cell type 1 (Th1) response, particularly in the presence of IL-12. Th1 cells also produce interleukin-2 (IL-2) and tumor necrosis factor β (TNFβ). Acting in concert, these cytokines are essential for mounting a protective host response to intracellular pathogens. However, inappropriate production of IL-18 may lead to destructive autoimmunity as shown in several neurologic diseases (Jander and Stoll, 2001, 2002)

In human immunodeficiency virus (HIV)-seronegative patients, a significant intrathecal release of IL-18 in the cerebrospinal fluid (CSF) has been observed in bacterial meningitis (Fassbender *et al*, 1999), arguing for a role of IL-18 in the host response against invading microorganisms in the central nervous system (CNS). In HIV-seropositive patients, the pathogenetic role of IL-18 and possible therapeutic implications are currrently under debate (Torre *et al*, 2002). Whereas some authors demonstrate that IL-18 inhibits HIV production in peripheral blood mononuclear cells (Choi *et al*, 2001) through intermediate IFN-γ, other groups report that IL-18 stimulates HIV in monocytic cells (Shapiro *et al*, 1998) or a T-cell line (Klein *et al*, 2000). Accordingly, an association of increased levels of circulating IL-18 (Ahmad *et al*, 2002; Torre *et al*, 2000) with progression of HIV infection as well as a decline of IL-18 levels under efficient

Address correspondence to Hans-Jürgen von Giesen, MD, Department of Neurology, Heinrich-Heine-Universität, Düsseldorf, Postfach 10 10 07, D-40001 Düsseldorf, Germany. E-mail: giesenhj@uni-duesseldorf.de

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antiretroviral therapy have been reported (David *et al*, 2000; Torre *et al*, 2000).

A possible role of IL-18 in neurologic complications of HIV infection, namely acquired immunodeficiency syndrome (AIDS) dementia complex (ADC) and opportunistic infection (OI) of the CNS, has not been addressed to date. To approach this issue, we studied IL-18 levels in the serum and CSF of HIV-seropositive (HIV+) patients without and with defined CNS disease.

Results

Demographic data

Patient groups showed no significant differences in gender (Table 1), homo/bisexual men forming the majority in each group. The percentage of AIDS-defined patients was 77% in group 1 and according to the definition criteria 100% in groups 2 and 3. Patients with OI of the CNS were significantly younger than those with ADC (Fisher's protected least significant difference [PLSD] 0.0005) or headache (Fisher's PLSD 0.0449). However, the duration of known HIV-1 seropositivity was comparable in all groups. As both the manifestation of ADC or cerebral OI often represented the first clinical manifestation of AIDS or even the reason for first HIV-1 seropositivity testing, the number of untreated patients in groups 2 and 3 was higher than in group 1. According to the presence of an AIDS-defining disease, patients in groups 2 and 3 showed mean CD4 cell counts below 200 cells/μl. Patients with cerebral OI showed the lowest CD4 cell count, which was significantly lower than in patients with headache (Fisher's PLSD 0.0200).

Routine CSF parameters

No significant differences were detected with regard to CSF cell counts and the CSF/plasma albumin ratio (see Table 1). CSF protein in patients with OI was significantly higher than in those with headache (Fisher's PLSD 0.0024). The same was true for the immunoglobulin G (IgG) index (Fisher's PLSD 0.0212).

Serum and CSF IL-18 levels

IL-18 levels as measured in serum and CSF are given in Table 2. The distribution of IL-18 serum levels

Table 1 Demographic data and routine CSF parameters of all HIV-1–seropositive patients included at the time of lumbar puncture

	Group 1 HIV-1 seropositive, neurologically asymptomatic	*Group 2 HIV-1 seropositive, AIDS dementia complex*	*Group 3 HIV-1 seropositive, opportunistic CNS infection*
Number of patients, *n*	22	21	31
Gender (male/female)	22/0	20/1	23/8
Age (years) (ANOVA $P = .0016$)	41.1 ± 7.4	44.2 ± 9.1	36.2 ± 6.8
Duration of HIV-1 seropositivity (months) (ANOVA $P = n.s.$)	70 ± 44	67 ± 48	70 ± 55
CDC stages			
Non-AIDS stages	5		
AIDS stages	17	21	31
Mode of infection			
Homo/bisexual	18	17	18
Heterosexual	1	1	6
Hemophiliac	1	1	2
IV drugs	2	2	5
Antiretroviral therapy			
No antiretroviral therapy	6	9	16
AZT only	4	3	2
Two nucleoside analogues	2	4	4
HAART	10	5	9
CD4 cell count (cells/μl) (ANOVA $P = .0561$)	254 ± 240	152 ± 273	65 ± 83
CSF cell count (ANOVA $P = .1391$ not significant)	6 ± 5	5 ± 8	44 ± 123
CSF protein (ANOVA $P = .0084$ significant)	0.476 ± 0.231	0.670 ± 0.439	0.944 ± 0.713
Albumin ratio CSF/serum (ANOVA $P = 0.1917$ not significant)	7.907 ± 3.389	11.504 ± 10.108	11.983 ± 9.432
IgG ratio CSF/serum (ANOVA $P = .0211$ significant)	0.571 ± 0.099	0.681 ± 0.222	0.771 ± 0.330
Oligoclonal bandings, CSF/serum			
−/−	10	7	11
+/−	11	11	16
+/+	1	2	2
++/+			1
Not available		1	1

Table 2 Serum and CSF IL-18 levels in all patients included

	Controls HIV-1 seronegative	*Group 1 HIV-1 seropositive, neurologically asymptomatic*	*Group 2 HIV-1 seropositive, AIDS dementia complex*	*Group 3 HIV-1 seropositive, opportunistic CNS infection*
Number of patients *n*	32	22	21	31
Serum IL-18 (pg/ml) (ANOVA $P = .0042$, significant)	47.333 ± 35.202	82.290 ± 48.597	91.258 ± 60.115	62.499 ± 45.031
CSF IL-18 (pg/ml) (ANOVA $P < .0001$, significant)	13.518 ± 5.230	22.303 ± 12.199	27.499 ± 17.337	71.868 ± 82.027

is visualized in Figure 1, upper panel. Compared to HIV− controls, IL-18 serum levels were increased in HIV+ (Fisher's PLSD 0.0079) and ADC (Fisher's PLSD 0.0011) patients. In contrast, serum IL-18 of OI patients was within the range of HIV− controls.

In the CSF, IL-18 levels (Figure 1, lower panel) were increased in OI patients (Fisher's PLSD < 0.0001 versus controls), whereas all other groups were not different from HIV− controls.

CSF IL-18 levels were significantly correlated with CSF cell count ($P = .0008$), CSF protein ($P = .0005$), and CSF/serum albumin ratio ($P = .0065$). In contrast, there was no correlation between serum IL-18 levels and routine CSF parameters (cell count, protein, CSF/serum ratios for albumin and IgG). Serum IL-18 levels did not show any significant correlation to age, duration of HIV-1 seropositivity, or CD4 cell counts. Neither plasma viral load nor CSF viral load (which was available in six patients only) showed a significant correlation to serum or CSF IL-18 levels. CSF IL-18 levels did not correlate to serum IL-18 levels.

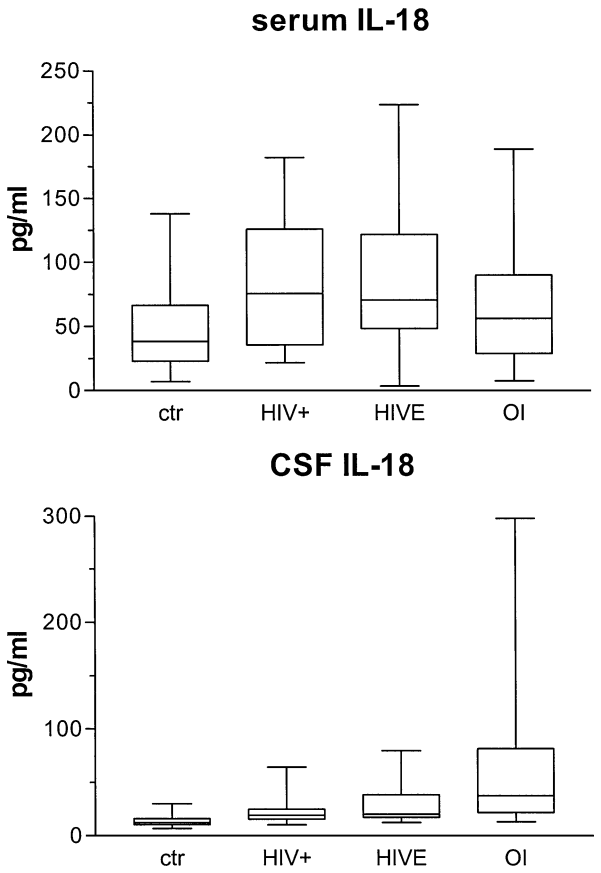

Figure 1 Box plot illustrating the distribution of IL-18 levels in the serum (*upper*) and in the CSF (*lower*) in defined patient groups.

Discussion

In our study, we found a distinct pattern of IL-18 CSF levels which were markedly increased in HIV+ patients with cerebral OIs. In animal models (Kawakami *et al*, 1997), IL-18 protects mice against disseminated *Cryptococcus neoformans* infection by inducing IFN-γ. This mechanism seems to be at least activated in our patients, so that other factors must contribute to their apparently unsuccessful immune response during opportunistic CNS infection. In line with its role in intrathecal inflammation, CSF IL-18 levels were significantly correlated with CSF cell count, protein, and CSF/serum albumin ratio. In contrast to the OI patients, we did not find elevated CSF IL-18 levels in ADC patients or HIV+ patients with headache. Thus, intrathecal IL-18 release was dependent on the massive inflammatory response initiated by OI, whereas the more discrete inflammatory CNS pathology characteristic for ADC was not reflected by CSF IL-18 levels. However, because inflammatory responses in the CNS are highly compartimentalized, a role of IL-18 in intraparenchymal inflammation in ADC cannot be excluded via CSF analysis and remains to be studied.

Our data confirm previous findings (Ahmad *et al*, 2002; Torre *et al*, 2000) showing increased IL-18 serum levels in HIV+ individuals even if no accompanying CNS manifestation was present. Thus, HIV infection per se induces at least partial activation of systemic innate immunity. Interestingly, HIV+ patients with OIs differed from the other HIV+ individuals in that their serum IL-18 was within the range of HIV− controls. It is therefore conceivable that failure of systemic IL-18 induction in advanced stages of HIV infection may underly progression of immunodeficiency to OI. Of course this hypothesis remains to be confirmed in larger series of patients.

Materials and methods

Patients
Seventy-four HIV-1–seropositive patients, who had undergone diagnostic lumbar puncture and for whom both serum and CSF samples were available, were included in this retrospective cross-sectional analysis. Patients were attributed into three groups according to defined neurological data collected up to the point of lumbar puncture.

Group 1 (HIV+) consisted of $n = 22$ HIV+ patients who had always been neurologically asymptomatic. Lumbar puncture had been carried out because of headache.

Group 2 (ADC) included $n = 21$ patients with ADC defined by clinical criteria (Janssen *et al*, 1991).

Group 3 (OI) included $n = 31$ patients with AIDS-defining OI of the CNS, but without evidence of directly HIV-1–mediated CNS pathology. OI included cerebral toxoplasmosis in $n = 15$ patients, progressive multifocal leukencephalopathy (PML) in $n = 11$ patients, other viral encephalitis in $n = 3$ patients, and cerebral cryptococcosis and tuberculosis in 1 patient each.

$n = 32$ HIV-seronegative patients served to establish normal values for IL-18.

Demographic details are compiled in Table 1. Non-AIDS and AIDS stages in group 1 were defined according to the Centers for Disease Control and Prevention (CDC) classification (CDC, 1992).

Routine CSF/serum analysis
Cell counts and determination of CSF total protein and albumin were performed on fresh CSF along with corresponding serum samples. CSF/serum albumin ratio was used to assess the integrity of the blood-brain barrier. Aliquots of whole CSF were frozen immediately and stored at −70°C until further analysis.

IL-18 analysis
Serum and CSF samples of patients were collected between 1990 and 2001 and stored at −70°C. The Quantikine human IL-18 ELISA kit (R&D Systems, Minneapolis, MN) was used according to the manufacturer's instructions. Its detection limit is 15 pg/ml.

Statistics
Statistics were performed with the commercially available software package Statview (Version 5.0.1., SAS Institute, 1998). Descriptive statistics were used to define patient groups. ANOVA with post-hoc testing for Fisher's PLSD were used to determine significant differences between groups. Simple regression analysis was performed to elucidate the relation between variables.

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