J. Neurovirol. (2012) 18:416–422
DOI 10.1007/s13365-012-0109-y

SHORT COMMUNICATION

Specific protein profile in cerebrospinal fluid from HIV-1-positive cART-treated patients affected by neurological disorders

Valentina Zanin · Serena Delbue · Annalisa Marcuzzi · Eleonora Tavazzi · Rossella Del Savio · Sergio Crovella · Enrico Marchioni · Pasquale Ferrante · Manola Comar

Received: 31 January 2012 / Revised: 19 April 2012 / Accepted: 26 April 2012 / Published online: 12 May 2012
© Journal of NeuroVirology, Inc. 2012

Abstract Cytokines/chemokines are involved in the immune response of infections, including HIV-1. We defined the profile of 48 cytokines/chemokines in cerebrospinal fluid from 18 cART patients with chronic HIV-1 infection by Luminex technology. Nine patients were affected with leukoencephalopathies: five with John Cunningham virus (JCV) + progressive multifocal leukoencephalopathy (PML) and four with JCV-not determined leukoencephalopathy (NDLE). In addition, nine HIV-1-positive patients with no neurological signs (NND) and five HIV-1-negative patients affected with acute disseminated encephalomyelitis (ADEM) were enrolled. Ten cytokines (IL-15, IL-3, IL-16, IL-18, CTACK, GRO1, SCF, MCP-1, MIF, SDF) were highly expressed in HIV-1-positive patients while IL-1Ra and IL-17 were present at a lower level. In addition, the levels of IL-17, IL-9, FGF-basic, MIP-1β, and MCP-1 were significantly higher ($p<0.05$) in patients with neurological diseases (PML, NDLE, ADEM) with respect to NND. Focusing the attention to the cytokine profile in JCV + PML patients with respect to JCV-NDLE patients, only TNF-β was significantly downregulated ($p<0.05$) in JCV + PML patients. This pilot study emphasized the role of immunoregulation in HIV-1-related neurological disorders during cART treatment.

Keywords Cytokines · HIV-1 · Leukoencephalopathies · cART

V. Zanin · A. Marcuzzi · R. Del Savio · S. Crovella · M. Comar (✉)
Institute for Maternal and Child Health–IRCCS “Burlo Garofolo”,
Via dell’Istria 65/1,
34137 Trieste, Italy
e-mail: comar@burlo.trieste.it

S. Delbue
Fondazione Ettore Sansavini, Health Science Foundation,
Via Garibaldi, 2,
Lugo, RA, Italy

E. Tavazzi · E. Marchioni
Department of General Neurology,
IRCCS National Neurological Institute C. Mondino Foundation,
Pavia, Italy

S. Crovella · M. Comar
University of Trieste,
via dell’Istria 65/1,
34137 Trieste, Italy

P. Ferrante
Department of Public Health–Microbiology–Virology,
University of Milan,
Milan, Italy

P. Ferrante
Istituto Clinico Città Studi,
Via Ampere 47,
Milan, Italy

M. Comar
Department of Medical Sciences, University of Trieste,
Trieste, Italy

Introduction

Cytokines and chemokines influence HIV-1 neuropathogenesis by affecting the HIV-1 life cycle, trafficking of macrophages into the central nervous system (CNS), glial activation, neuronal signaling, and repair processes (Rackstraw 2011). The aging and chronic HIV-1 infection are both associated with activation of the immune system, increased levels of circulating inflammatory mediators, and inflammation which may have a significant impact on HIV-1-associated CNS disease.

Viral replication is regulated by a network of both HIV-1 suppressive cytokines and chemokines, such as interferon-α, interleukin-10 (IL-10), macrophage inflammatory protein-α and β (MIP-1α, and β), and RANTES, and inductive cytokines and chemokines, such as tumor necrosis factor-α (TNF-α), IL-1α, IL-6, IL-15, and monocyte chemotactic protein (MCP) (Kedzierska and Crowe 2001; Barqasho et al. 2009).

Although there are conflicting reports regarding the role of some cytokines during the course of HIV-1 infection, recent data support the association for the chemokine IP-10 and MCP-1 with individual cerebral metabolites. In particular, higher levels of IP-10 correlated with lower neuronal pattern scores and higher basal ganglia and inflammatory pattern scores, the same one which has been associated with HIV-1-associated neurocognitive disorders, suggesting that antiretroviral therapy (cART) modifies the impact of the immune response on neurons (Letendre et al. 2011).

It has also been shown that cytokines and chemokines presented different profiles in AIDS-related opportunistic infections, according to the different patterns of neuroinflammatory responses displayed by the neurological infections (Christo et al. 2009).

Progressive multifocal leukoencephalopathy (PML) is a rare and devastating demyelinating disease of the CNS with a fatal outcome or at least irreversible neurologic sequelae. Reactivation of the human neurotropic polyomavirus JCV, infecting the myelin-producing oligodendrocytes and astrocytes, is the cause of PML in a context of severe immunosuppression, especially AIDS (Brooks and Walker 1984).

Although the intra-human route of JCV transmission is not well known, the preferred sites of latency seem to be the kidney and the lymphoid organs. Specifically, direct evidence showed that JCV is not strictly neurotropic but may infect CD34+ hematopoietic progenitor cells and those cells that have differentiated into a lymphocytic lineage (Monaco et al. 1996).

After introduction of cART, another form of leukoencephalopathy has been reported and called not determined leukoencephalopathy (NDLE), resembling PML neurological and clinical features, but without any evidence of virus replication in the CNS (Langford et al. 2002).

An under-investigated aspect of leukoencephalopathy pathogenesis is the role of the cytokine/chemokine network in AIDS settings in aging. Here, we found of interest to investigate the qualitative and quantitative nature of inflammatory biomarker perturbation studying a large set of proinflammatory cytokines/chemokines in cerebrospinal fluid (CSF) from HIV-1 positive cART-treated patients suffering from PML or JCV-negative NDLE. As control groups, HIV-1-positive patients with no neurological signs (NND) and HIV-1-negative patients with acute disseminated encephalomyelitis (ADEM) have been evaluated.

Materials and methods

Patients and clinical specimens

Twenty-three patients (mean age [range] in years, 45.3 [33–71]) who underwent lumbar puncture as part of routine workup for neurological diseases at the Mondino Neurological Institute, Pavia, Italy, were included in this study. Eighteen patients with chronic HIV-1 infection were included in the study.

Nine patients were affected with leukoencephalopathies diagnosed on the following criteria: (a) PML: MRI and clinical/neurological signs suggestive of leukoencephalopathy and JCV genome detection in CSF (n=5), and (b) NDLE: MRI and clinical/neurological signs suggestive of leukoencephalopathy and JCV genome absence in CSF (n=4). NDLE patients showed a diffuse signal alteration of deep white matter around ventricles, mainly located in posterior regions, whereas PML patients showed multifocal, bilateral, predominantly asymmetric lesions, mainly with a supratentorial location. None of the patients presented damage of the blood brain barrier or CSF pleocytosis.

In addition, nine HIV-1-positive patients classified as NND with no clinical/neurological signs and five HIV-1-negative patients affected with ADEM, not subjected to the follow-up, were included in the study. All HIV-1-positive patients have been subjected to cART treatment for a mean time of 48.3 months (range, 18–108 months) (Table 1).

CSF and serum samples have been collected at the time of the leukoencephalopathy diagnosis (baseline/enrolment), and then CSF was collected every 6 months during a mean period of 22 months (range, 6–60 months).

Detection of JCV and HIV-1 genomes in CSF

The HIV-1 loads were quantified after the viral RNA was extracted from 0.14 ml CSF using the Qiamp viral RNA mini kit (Qiagen, USA), according to the manufacturer's instructions. To detect the presence of the HIV-1 genome, a quantitative (Q)-PCR targeting the gag gene was performed using the protocol published by Delbue et al. (2012). The test sensitivity was one copy/reaction. The JCV DNA was extracted from 0.15 ml CSF using the Nucleospin RNA virus kit (Macherey Nagels, Germany) according to the manufacturers' instructions. The Q-PCR protocol for the detection of JCV DNA, particularly for targeting the large T-antigen region, has been described elsewhere (Delbue et al. 2005). The test sensitivity for JCV detection was two copies/reaction. For both the JCV and HIV-1 assays, each sample was analyzed in triplicate, and each run contained a negative control composed of the reaction mixture without the DNA template. Standard curves for the quantification of the viral genome were constructed using serial dilutions of a plasmid containing all or a

Table 1 Demographic, clinical, and immunological data of HIV-1-positive PML, NDLE, and NND patients

	PML, 5 patients	NDLE, 4 patients	NND, 9 patients
Age, years (range)	41 (33–50)	45 (39–53)	50 (31–71)
Sex	2 M/3 F	2 M/2 F	7 M/2 F
CD4+ (number/μl ± SD)	268.7±139.3	342.1±99.1	336.6±157.1
HIV-1 viremia (copies/ml ± SD)	129.2±72.2	537.8±169.3	464±51.2
HIV-1 infection duration (months ± SD)	100.8±77.6	263±10.5	125.3±79.6
Therapy duration (months ± SD)	18±12	108±48	19±12

portion of the viral genome (range, 10 to 10^6 plasmid copies), as described by the supplier (Advanced Biotechnology). The viral copy concentrations were log-transformed and expressed as log [copies/milliliters of CSF].

Determination of cytokine and chemokine release

The analysis of a 48-cytokine and chemokine panel (pro-human cytokine 27-Plex M50-0KCAF0Y and 21-Plex MF0-005KMII, Bio-Rad, Hercules, CA, including: IL 1α-β-ra-2-2Ra-3-4-5-6-7-8-9-10-12p40 e p70-13-15-16-17-18; eotaxin; basic FGF; G-CSF; GM-CSF; IFNγ; IP10; MCP1α; MCP1β; PDGFBB; RANTES; TNFα; VEGF; CTACK; GROα; HGF; IFNα2; LIF; MCP-3;M-CSF; MIF; MIG; β-NGF; SCF; SCGFβ; SDF1α; TNFβ; TRAIL; ICAM1 and VCAM1) was performed on CSF and serum samples using magnetic bead-based multiplex immunoassays (Bio-Plex®) (BIO-RAD Laboratories, Milano, Italy) following the manufacturer's instructions. Data from the reactions were acquired using the Bio-Plex 200 reader, while a digital processor managed data output and Bio-Plex Manager® software presented data as median fluorescence intensity (MFI) and concentration (in picograms per milliliter) as well (BIO-RAD Laboratories, Milano, Italy). Statistical analysis was performed using GraphPad Prism V.4.0 for Windows (Graph Pad Software, San Diego, CA, USA).

Statistical analysis

Results are reported as mean values of three independent experiments ± standard deviation (SD). A *t* test for comparison between HIV-1 negative and HIV-1 positive was used. A statistical significance for comparison between PML, NDLE, ADEM, and NND was calculated using one-way analysis of variance (ANOVA) and Bonferroni posttest for multiple comparison. Statistical analysis has been performed by using the Graph Pad Prism version 5 software.

Results

Demographic, clinical, and immunological data of HIV-1-positive PML, NDLE, and NND patients included in the study were summarized in Table 1. A total of 57 samples were collected, and a mean of 5 follow-ups (range, 4–11) have been performed for each patient.

As shown in Table 2, the analysis of HIV-1 and JCV infection in CSF during patient follow-up (T0–T5) demonstrated that the HIV-1 genome was detected in two samples from PML patients and in one sample from an NDLE patient. JCV was always detected in the CSF from PML patients, but not from NDLE patients. CSF samples from both NND and ADEM patients were negative for

Table 2 HIV-1 and JCV viral load [log(copies/milliliter)] in positive CSF samples during the follow-up

Patient	Virus	T0	T1	T2	T3	T4	T5–T11
PML #1	JCV	6.32	5.27	3.23	3.23	3.23	
	HIV-1	4.86	Neg	Neg	Neg	Neg	
PML #2	JCV	3.23	3.23	3.23	3.48		
	HIV-1	Neg	Neg	Neg	Neg		
PML #3	JCV	3.23	3.71	3.16	3.2	3.1	3
	HIV-1	2.9	Neg	4.7	Neg	Neg	Neg
PML #4	JCV	3.23	5.09	5	5.1		
	HIV-1	Neg	Neg	Neg	Neg		
PML #5	JCV	3.9	3.5	3.5	3.2		
	HIV-1	Neg	Neg	Neg	Neg		
NDLE #4	JCV	Neg	Neg	Neg	Neg	Neg	
	HIV-1	4.3	Neg	Neg	Neg	Neg	

HIV-1 and JCV. A panel of 48 cytokines/chemokines was analyzed in CSF samples at the time of neurological diagnosis.

As shown in Fig. 1, 12 of the 48 measured cytokines were statistically associated to HIV-1 infection. Among those, ten cytokines were highly expressed in HIV-1-positive patients compared to HIV-1-negative patients, whereas IL1Ra and IL-17 were downregulated in HIV-1-positive patients (Fig. 1). Cytokine expression did not show any statistically significant difference when analyzed in relation to the HIV-1 viral load (data not shown).

The pattern of cytokines/chemokines was analyzed also comparing the group of patients affected with neurological disorders (PML, NDLE, ADEM) with the NND group of patients. The expression of 17 cytokines was statistically correlated to the neurological disorders, but not with the control group (Fig. 2a–c). Of these, 12 cytokines showed levels significantly lower in patients with neurological diseases (PML, NDLE, ADEM) with respect to NND patients (Fig. 2a and b).

On the contrary, the levels of the chemokines IL-17, IL-9, FGF-basic, MIP-1β, and MCP-1 were significantly higher ($p<0.05$) in the group of neurological patients compared to the control group. In particular, the level of MCP-1 and MIP-1β was higher in PML and NDLE patients ($p<0.01$ and $p<0.05$) than those in ADEM and NND patients (Fig. 2c).

Focusing the attention on the cytokine/chemokine profile in HIV-1-positive patients affected with leukoencephalopathies, it has been observed that six cytokines (TNF-β, IL-9, IL-17, FGF-basic, MCP1, MIP1-β) were downregulated in the CSF samples from JCV + PML patients (Fig. 2b and c). On the contrary, the level of three cytokines was upregulated in the CSF samples from JCV + PML patients: IL-12p40, IL-16, and GROα (Fig. 2a).

Discussion

The development of neurological disorders such as leukoencephalopathies in the AIDS setting may be related to many factors. The identification of host sentinel biomarkers could be a crucial tool for determining diagnostic profiles and conducting subsequent studies on the pathways involved in the molecular pathogenesis of neurological disorders in HIV-1-positive cART-treated patients.

Data from this pilot study documented the presence of a specific panel of cytokines/chemokines that was differentially expressed in CSF from HIV-1 patients.

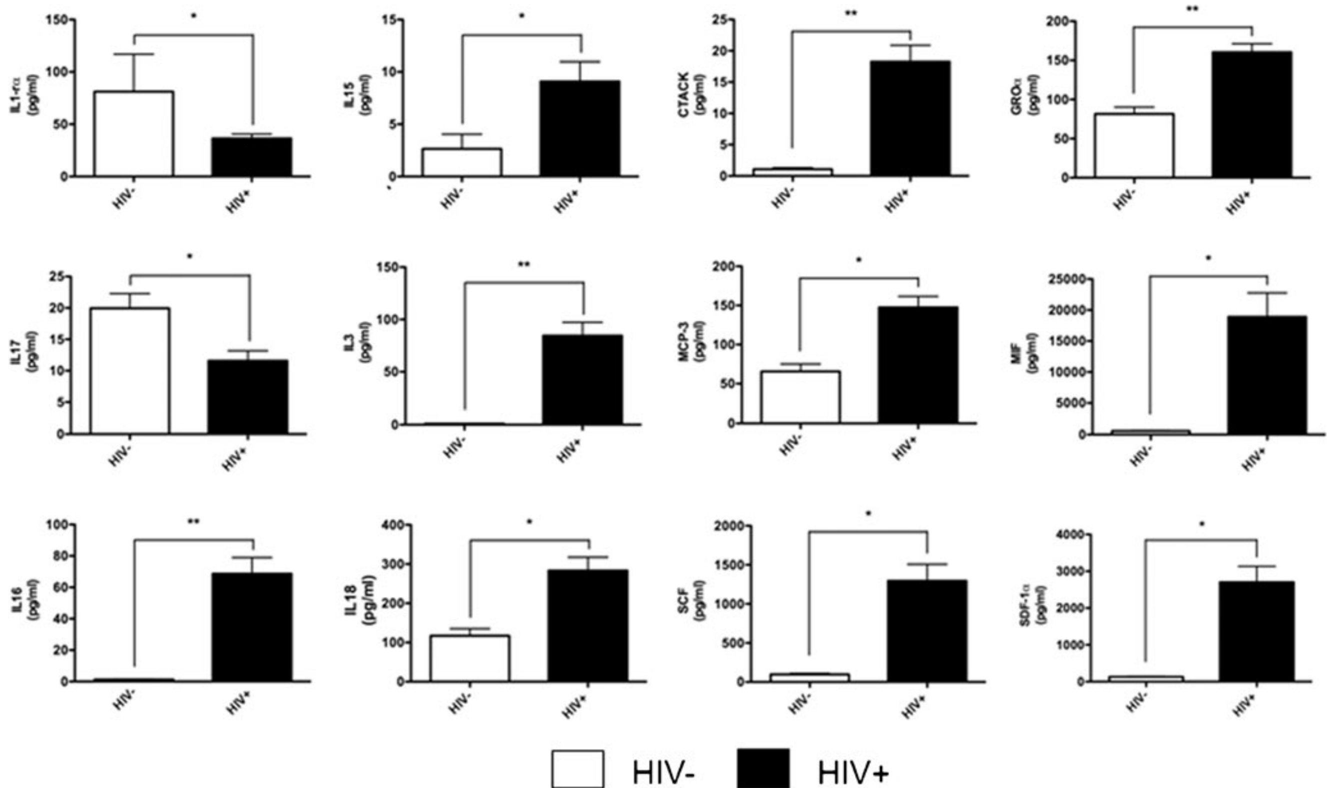

Fig. 1 Cytokine and chemokine levels of HIV-1-positive and HIV-1-negative patients. Cytokines were measured in CSF samples by multiplex immunoassays and analyzed in relationship to HIV status. Data were presented as MFI by Bio-Plex Manager® software and converted as concentration (picograms per milliliter). *Bars* represent the means of three independent experiments ± SEM. Analyses were performed with *t* test. *$p<0.05$; **$p<0.01$

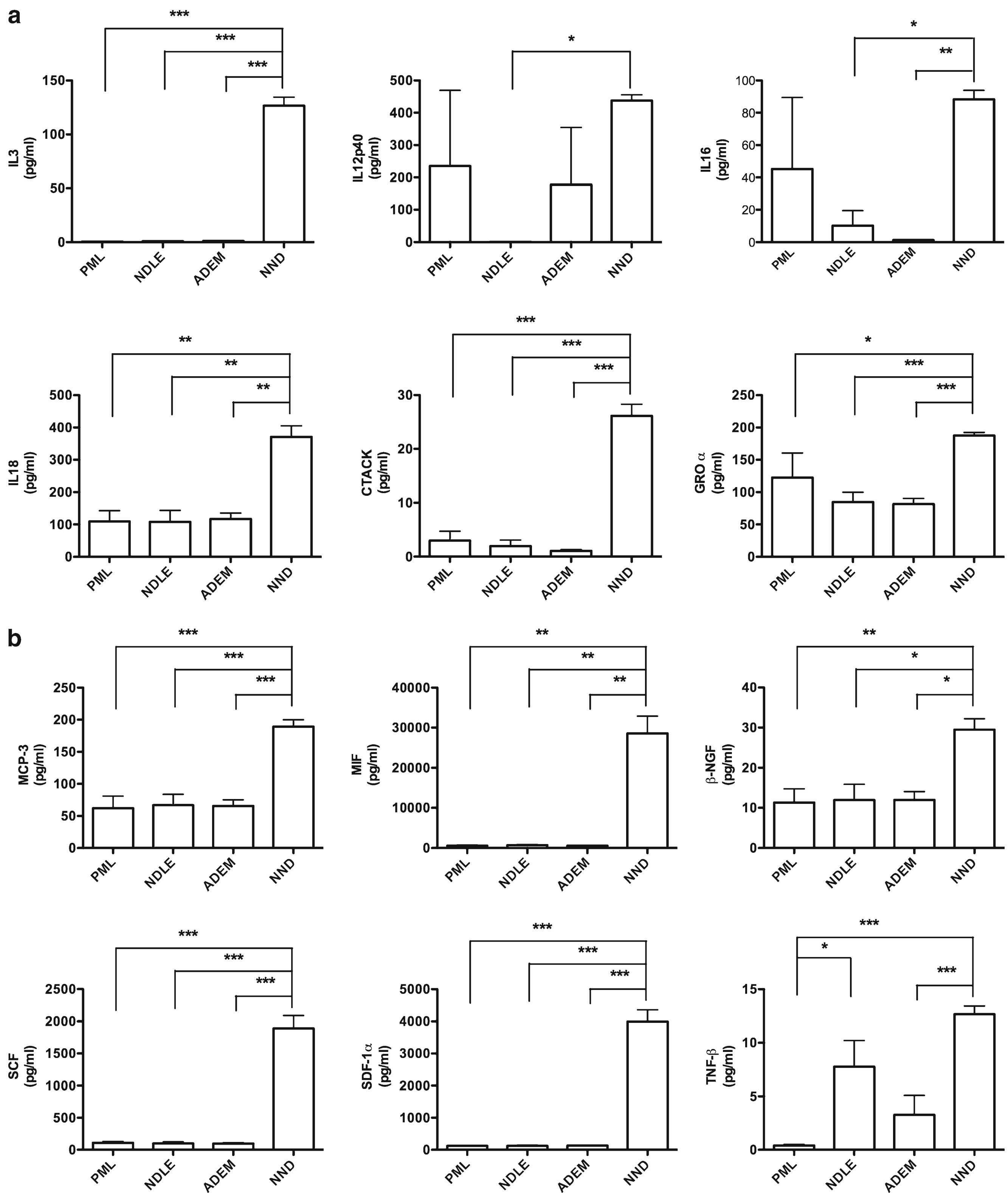

Fig. 2 a–c Cytokine and chemokine levels of HIV-1-positive and HIV-1-negative patients. Cytokines were measured in CSF samples by multiplex immunoassays and analyzed in relationship to the type of neurological disorders and or the presence of JCV infection. Data were presented as MFI by Bio-Plex Manager® software and converted as concentration (picograms per milliliter). *Bars* represent the means of three independent experiments ± SEM. The one-way analysis of variance (ANOVA) and Bonferroni posttest for multiple comparison were used. *$p<0.05$; **$p<0.01$; ***$p<0.001$. *PML* progressive multifocal leukoencephalopathy, *NDLE* not determined leukoencephalopathy, *ADEM* acute disseminated encephalomyelitis, *NND* not neurological diseases

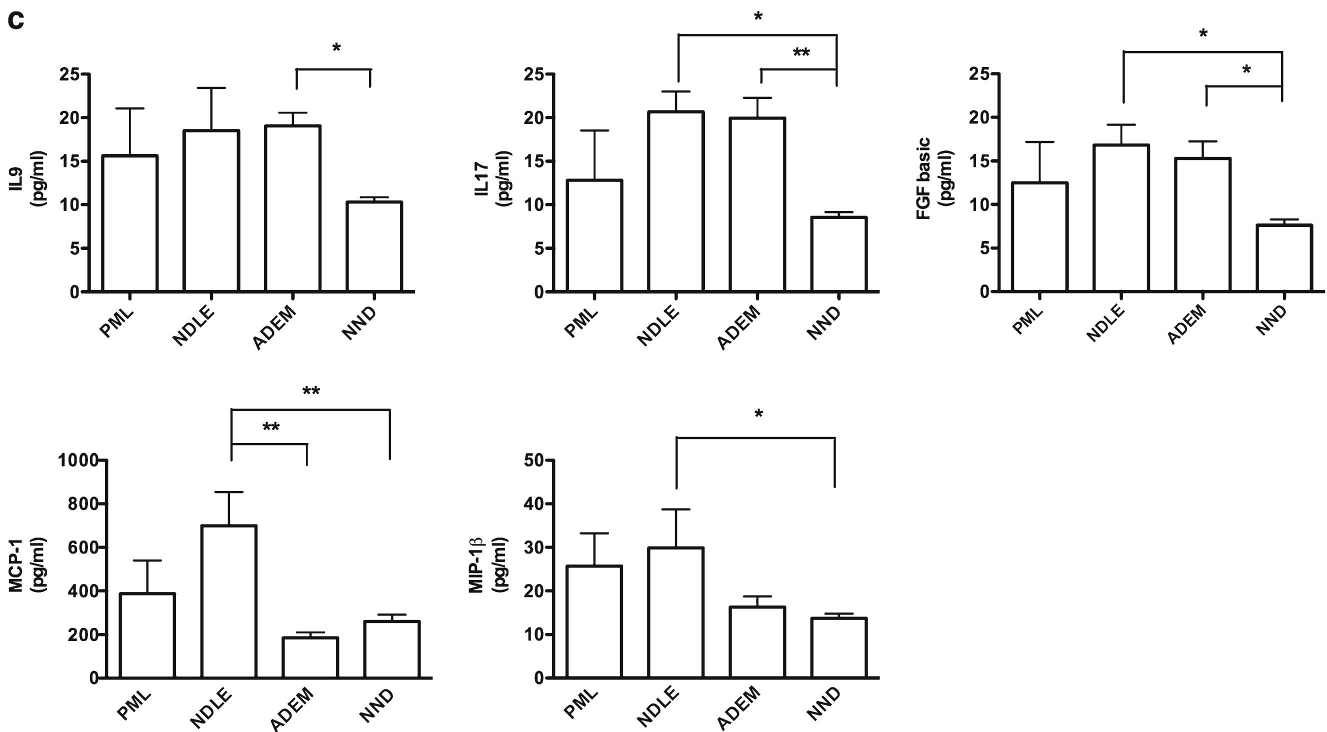

Fig. 2 (continued)

As recently reported by Meeker and colleagues, HIV-1-infected patients showed increasing levels in the expression of inflammatory (IL-16, IL-15, IL-3) and anti-inflammatory (IL-18) proteins, and chemokines, suggesting their critical role both in the onset and progression of HIV-1-associated disease and in the pathogenesis of chronic inflammatory disorders (Meeker et al. 2011). Moreover, several of the mentioned cytokines are well known to be involved in chemotactic activity for pre-committed CD4+ T cells and monocytes in the chronic phase of inflammation and in the early phase of hematopoiesis.

Some cytokines, such as MCP-3, MIF, SCF, and SDF-1α, associated to HIV-1 infection, were correlated with the absence of neurological disorders, confirming a specific association of these proteins with the pathophysiology of the HIV-1 infection. Of note, IL-17 was downregulated in HIV-1-positive patients, compared to HIV-1-negative patients, but it was upregulated in the group of patients affected with neurological disorders [NDLE ($p<0.05$), ADEM ($p<0.01$)], if compared with the NND control group. This cytokine, associated to HIV-1 infection and exclusively produced by a recently discovered subset of CD4+ T helper cells (Crome et al. 2010), leads to the general progression of HIV-1-related diseases, by creating an environment favorable to opportunistic infections and chronic immune activation (Maek-A-Nantawat et al. 2007).

It has been shown that during AIDS, the expression of two important modulators, IL-1 and TNF-α, is increased in the brain (Tyor et al. 1992). These two cytokines are able to strongly increase the transcription of HIV-1 and of other cellular promoters through the κB enhancer element (Israël et al. 1989; Osborn et al. 1989; Tornatore et al. 1991). Moreover, the presence of a putative κB sequence on JCV DNA replication origin was observed by Major et al. (1990), suggesting that multiple inducible and non-inducible nuclear proteins from glial cells could differentially bind to the JCV κB sequence.

In this preliminary study, when the two forms of leukoencephalopathies (JCV + PML and JCV-NDLE) are confirmed, we showed a lower expression of TNF-β ($p<0.05$) in the JCV + PML group with respect to JCV-NDLE, in contrast with a recent immunohistochemistry study that demonstrated a high expression of TNF-α and its receptor TNFR1 in JCV-infected astrocytes and oligodendrocytes (Wollebo et al. 2011).

Conversely, the two chemokines IL-16 and IL-12p40 showed the tendency to be highly expressed in JCV + PML patients with respect to JCV-NDLE. While IL-16 could be involved in the recruitment of JCV-infected lymphocytes through its chemotactic activity, the relationship between IL-12p40 and PML or JCV has been never described. Recently, the increased level of this cytokine in the serum from HIV-1-positive patients has been indicated as a biological marker for HIV-1-tubercolosis-related immune reconstitution inflammatory syndrome (Haddow et al. 2011).

It seems clear that JCV reactivation, mobilization from latently infected lymphoid cells, and trafficking to the brain are associated to the immunosuppressive state and regulated by many proinflammatory cytokines, acting on several transcriptional factors, such as NF-κB, SMADs, GBPi, and C/

EBPβ, able to bind the specific sites located on the JCV transcriptional control region or to facilitate interaction of the immune and CNS on the control of JCV reactivation.

In addition, the presence of HIV-1 and its associated proteins (i.e., TAT) in the CNS can lead to the occurrence of cytokine cascades involving the production of proinflammatory cytokines (Benveniste 1994; Kaul et al. 1995; Yeung et al. 1995) acting directly on glial cells and consequently on JCV transcription (Wollebo et al. 2011).

Despite of the small size of analyzed patients, results from this pilot study suggest that probably co-existing signaling pathways may be involved in the pathogenesis of AIDS-related leukoencephalopathies and may be regulated by a different not well-characterized cascade of cytokines.

We are aware that our results are preliminary and deeper investigation on a large population will be needed to prove this hypothesis; however, the complex interplay between cytokine/chemokine and virus infection in microglial cells emphasized once more the role of immunoregulation in AIDS-related neurological disorders also during cART treatment.

Acknowledgments This study was supported by grants Ricerca Corrente no.11/11 from IRCCS 'Burlo Garofolo' awarded to Manola Comar and by NIMH grant no. MH072528 awarded to Pasquale Ferrante.

References

Barqasho B, Nowak P, Tjernlund A, Kinloch S, Goh LE, Latipe F, Fisher M, Andersson J, Sonnerborg A, quest study group (2009) Kinetics of plasma cytokines and chemokines during primary HIV-1 infection and after analytical treatment interruption. HIV Med 10:94–102

Benveniste EN (1994) Cytokine circuits in brain. Implications for AIDS dementia complex. Res Publ Assoc Res Nerv Ment Dis 72:71–88

Brooks BR, Walker DL (1984) Progressive multifocal leukoencephalopathy. Neurol Clin 2:299–313

Christo PP, VilelaMde C, Bretas TL, Domingues RB, Greco DB, Livramento JA, Teixeira AL (2009) Cerebrospinal fluid levels of chemokines in HIV infected patients with and without opportunistic infection of the central nervous system. J Neurol Sci 15:79–83

Crome SQ, Wang AY, Levings MK (2010) Translational mini-review series on Th17 cells: function and regulation of human T helper 17 cells in health and disease. Clin Exp Immunol 159:109–119

Delbue S, Sotgiu G, Fumagalli D, Valli M, Borghi E, Mancuso R, Marchioni E, Maserati R, Ferrante P (2005) A case of a progressive multifocal leukoencephalopathy patient with four different JC virus transcriptional control region rearrangements in cerebrospinal fluid, blood, serum, and urine. J Neurovirol 11:51–57

Delbue S, Elia F, Carloni C, Tavazzi E, Marchioni E, Carluccio S, Signorini L, Novati S, Maserati R, Ferrante P (2012) JC virus load in cerebrospinalfluid and transcriptional control region rearrangements may predict the clinical course of progressive multifocal leukoencephalopathy. J Cell Physiol. doi:10.1002/jcp.24051

Haddow LJ, Dibben O, Moosa MY, Borrow P, Easterbrook PJ (2011) Circulating inflammatory biomarkers can predict and characterize tuberculosis-associated immune reconstitution inflammatory syndrome. AIDS 25:1163–1174

Israël N, Hazan U, Alcami J, Munier A, Arenzana-Seisdedos F, Bachelerie F, Israël A, Virelizier JL (1989) Tumor necrosis factor stimulates transcription of HIV-1 in human T lymphocytes, independently and synergistically with mitogens. J Immunol 143:3956–3960

Kaul R, Sharma A, Lisse JR, Christadoss P (1995) Human recombinant IL-2 augments immunoglobulin and induces rheumatoid factor production by rheumatoid arthritis lymphocytes engrafted into severe combined immunodeficient mice. Clin Immunol Immunopathol 74(3):271–282

Kedzierska K, Crowe SM (2001) Cytokine and HIV: interactions and clinical implication. Antivir Chem Chemother 12:133–150

Langford TD, Letendre SL, Marcotte TD, Ellis RJ, McCutchan JA, Grant I, Mallory ME, Hansen LA, Archibald S, Jernigan T, Masliah E, HNRC Group (2002) Severe,demyelinating leukoencephalopathy in AIDS patients on antiretroviral therapy. AIDS 16 (7):1019–1029

Letendre SL, Zheng JC, Kaul M, Yiannoutsos CT, Ellis RJ, Taylor MJ, Marquie-Beck J, Navia B, HIV Neuroimaging Consortium (2011) Chemokines in cerebrospinal fluid correlate with cerebral metabolite patterns in HIV-infected individuals. J Neurovirol 17(1):63–69

Maek-A-Nantawat W, Buranapraditkun S, Klaewsongkram J, Ruxrungtham K (2007) Increased interleukin-17 production both in helper T cell subset Th17 and CD4-negative T cells in human immunodeficiency virus infection. Viral Immunol 20:66–75

Major EO, Amemiya K, Elder G, Houff SA (1990) Glial cells of the human developing brain and B cells of the immune system share a common DNA binding factor for recognition of the regulatory sequences of the human polyomavirus, JCV. J Neurosci Res 27:461–471

Meeker RB, Poulton W, Markovic-Plese S, Hall C, Robertson K (2011) Protein changesin CSF of HIV-infected patients: evidence for loss of neuroprotection. J Neurovirol 17(3):258–273

Monaco MC, Atwood WJ, Gravell M, Tornatore CS, Major EO (1996) JC virus infection ofhematopoietic progenitor cells, primary B lymphocytes, and tonsillar stromal cells: implications for viral latency. J Virol 70(10):7004–7012

Osborn L, Kunkel S, Nabel GJ (1989) Tumor necrosis factor alpha and interleukin 1 stimulate the human immunodeficiency virus enhancer by activation of the nuclear factor kappa B. Proc Natl Acad Sci USA 86:2336–2340

Rackstraw S (2011) HIV-related neurocognitive impairment. Psychol Health Med 16:548–563

Tornatore C, Nath A, Amemiya K, Major EO (1991) Persistent human immunodeficiency virus type 1 infection in human fetal glial cells reactivated by T-cell factor(s) or by the cytokines tumor necrosis factor alpha and interleukin-1 beta. J Virol 65:6094–6100

Tyor WR, Glass JD, Griffin JW, Becker PS, McArthur JC, Bezman L, Griffin DE (1992) Cytokine expression in the brain during the acquired immunodeficiency syndrome. Ann Neurol 31:349–360

Wollebo HS, Safak M, Del Valle L, Khalili K, White MK (2011) Role for tumor necrosis factor-α in JC virus reactivation and progressive multifocal leukoencephalopathy. J Neuroimmunol 233(1–2):46–53

Yeung MC, Pulliam L, Lau AS (1995) The HIV envelope protein gp120 is toxic to human brain-cell cultures through the induction of interleukin-6 and tumor necrosisfactor-alpha. AIDS 9:137–143