ELSEVIER

Journal of Neuroimmunology 188 (2007) 138–142

Journal of Neuroimmunology

www.elsevier.com/locate/jneuroim

Short communication

High production of RANTES and MIP-1α in the tropical spastic paraparesis/HTLV-1-associated myelopathy (TSP/HAM)

Patricia Montanheiro [a], Maria Paulina Posada Vergara [b], Jerusa Smid [b], Alberto José da Silva Duarte [a], Augusto César Penalva de Oliveira [b], Jorge Casseb [a,b,*]

[a] *Department of Dermatology, Sao Paulo University Medical School, Sao Paulo, Sao Paulo 01246-903, Brazil*
[b] *Institute of Infectious Diseases "Emílio Ribas", São Paulo, São Paulo, 01246-001 Brazil*

Received 29 January 2007; received in revised form 16 May 2007; accepted 22 May 2007

Abstract

Human T cell lymphotropic virus type 1 (HTLV-1) infection is associated with progressive neurological disorders and tropical spastic paraparesis/HTLV-1-associated myelopathy (TSP/HAM). The pathogenesis of TSP/HAM is considered as immune mediated, involving cytotoxic T cell (CTL) responses to a number of viral proteins and notably the regulation protein Tax. T CD8+ cells produce beta-chemokines, which are important in the anti-viral response. In the present study, we have analyzed the CC chemokines (RANTES, MIP-1β and MIP-1α) production in retrovirus-infected subjects. A total of 191 subjects were studied: 52 healthy controls, 72 asymptomatic HTLV-1-infected carriers and 67 TSP/HAM patients. Peripheral blood mononuclear cells were maintained in the presence or absence of PHA, and supernatant fluids were assayed using EIA. MIP-1β concentration was not significantly different across groups, but RANTES and MIP-1α concentrations showed significant differences when the three groups were compared. In TSP/HAM patients, the increase in the production of chemokines may lead to a recruitment of pro-inflammatory factors, contributing to the membrane's myelin damage.

Keywords: HTLV-1; TSP/HAM; Beta-chemokines; Brazil

1. Introduction

Human T cell lymphotropic virus type 1 (HTLV-1) is the etiologic agent of the adult T cell leukemia (ATL) (Poiesz et al., 1980; Yoshida, 1983) and the associated myelopathy/tropical spastic paraparesis (TSP/HAM) (Gessain et al., 1985; Osame et al., 1986). TSP/HAM is a chronic immune-mediated neurologic disease (Izumo et al., 2000), whose main pathological feature is a chronic inflammation of the spinal cord, characterized by perivascular lymphocytic cuffing and parenchymal lymphocytic infiltration (Osame, 2002). In parallel, DNA proviral is also implicated in the TSP/HAM pathogenesis, since these patients show higher HTLV-1 proviral load than asymptomatic carriers (Dehee et al., 2002; Nagai et al., 1998).

Inflammatory cytokine production, such as interferon gamma (IFN-γ), interleucin-15 (IL-15) and tumoral necrosis factor-alpha (TNF-α) are important for the inflammatory process in TSP/HAM development (Azimi et al., 1999), and cytotoxic T lymphocytes activity (CTLs) may also be involved in the TSP/HAM pathogenesis (Jacobson, 2002; Nagai et al., 1998). In addition to CTL, chemokines CCL5/RANTES (regulated on activation of normal T cell expressed and secreted), CCL4/MIP-1β (macrophage activated inflammatory protein 1 beta) and CCL3/MIP-1α (macrophage inflammatory protein 1 alpha) (Rossi and Zlotnik, 2000; Watson, 2002; Murphy et al., 2002) are involved in this process. CD8+ T cells are responsible for the major production of these suppressor factors and posses anti-viral properties (Jacobson, 2002), and these factors are also released by CD4+ T cells, monocytes and natural killer cells (NK) (Hollsberg and Hafler, 1995).

Chemokines are small inducible proteins that are involved in the normal trafficking of leukocytes to both lymphoid and nonlymphoid organs and in the recruitment of leukocytes to

* Corresponding author. Department of Dermatology, Sao Paulo University Medical School, Sao Paulo, Sao Paulo 01246-903, Brazil. Tel.: +55 11 3066 7194; fax: +55 11 3081 7190.
E-mail address: jorge_casseb@yahoo.com.br (J. Casseb).

0165-5728/$ - see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.jneuroim.2007.05.015

Table 1
Number of subjects, age and gender of controls, asymptomatic HTLV-1-infected carriers, and TSP/HAM patients

No. tested	Group I (control)	Group II (asymptomatic HTLV-1 healthy carriers)	Group III (TSP/HAM patients)
	52	72	67
Female/male	34/18	43/29	36/31
Age (years, mean)	40±12	43±12	47±11
Time of TSP/HAM, median, years (25–75 IQT)	–	–	8.5 (4–12)
Age at onset of TSP/HAM, median, years (25–75 IQT)	–	–	43 (28–72)

IQT: Interquartile.

sites of injury and infection (Moser and Loetscher, 2001; Gerard and Rollins, 2001). These chemokines are natural ligands of the CCR5 receptor and responsible for chemotaxis and cellular activation (Mueller et al., 2006). Chemokines play a crucial role in the recruitment of Th1 and Th2 cells and in the polarization to Th1 and Th2 (Luster, 1998). Receptors CCR5 present on Th1 cells, and the CCR3 present on the Th2 cells involved in TSP/HAM development (Baggiolini and Loetscher, 2000). Another study involving HTLV-1I-infected subjects demonstrated that tax protein, both directly and at the transcriptional level, increased C–C chemokine production (Lewis et al., 2000), including situations when there was co-infection with human immunodeficiency virus type 1 (HIV-1). However, a few studies have been published addressing the levels of chemokines on HTLV-1 infection. Thus, our aim on the present study was to evaluate chemokine production during HTLV-1 infection, especially on those patients affected with TSP/HAM.

2. Material and methods

2.1. Patients and controls

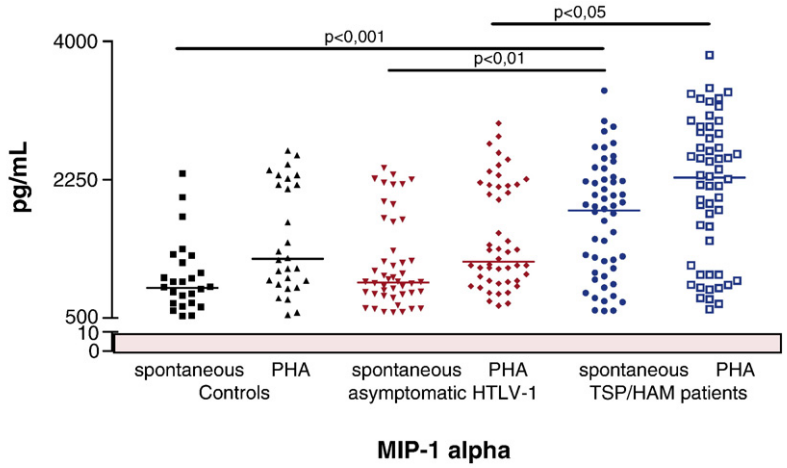
HTLV-1-infected subjects were recruited in the HTLV outpatient service of the "Emílio Ribas" Institute of Infectious Diseases, Sao Paulo, Brazil. The control group was made up of subjects seronegative for HTLV-1/II and HIV-1 (by ELISA and Western Blot methods). Informed consent was obtained from subjects, and the Ethical Committee Board approved the research protocol. Thus, in this study, 191 subjects were studied, and were divided into three groups: group I was made up of the 52 subjects HTLV-1 and HIV-1 negative (control group), group II comprised 72 HTLV-1-asymptomatic carriers (HTLV-1 group), and group III included 67 TSP/HAM patients (TSP/HAM group), diagnosed by the Kagoshima criteria (Osame, 1991).

2.2. Peripheral blood mononuclear cell cultures

PBMCs were collected in heparinized tubes and isolated using Ficoll–Hypaque density gradients (Amersham Pharmacia, Piscataway, NJ, USA). The cells were washed, adjusted to 2×10^6 cells/ml in RPMI 1640 medium supplemented with 10% fetal calf serum, and grown with or without 2.5 μg/ml phytohaemagutinin (PHA) at 37 °C, 5% CO_2 for 24 h. The supernatant fluids were harvested and stored at −70 °C for chemokines assays.

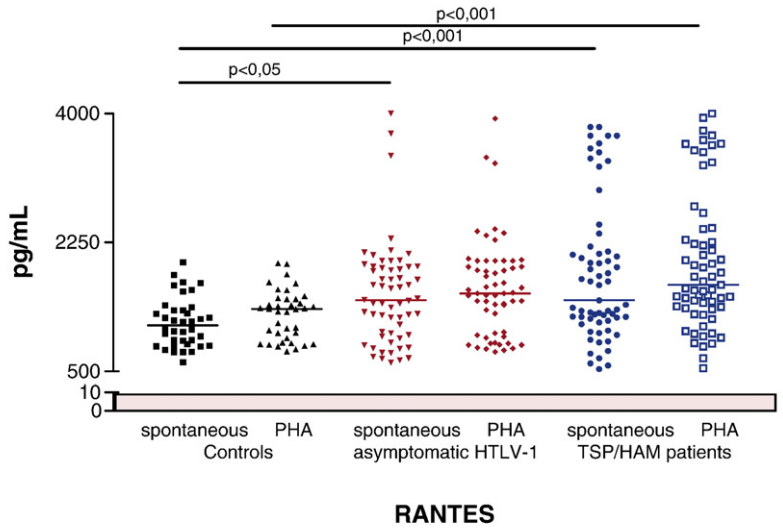
2.3. Measurement of chemokines

Enzyme immunoassays (EIA) were used to measure the chemokines RANTES, MIP-1β and MIP-1α. Antibody matched



Group	Spontaneous (pg/mL)	PHA (pg/mL)
Control	881 (645 – 1138)	1247 (949 – 2225)
Asymptomatic HTLV-1	956 (732 – 1730)	1336 (964 – 2200)
TSP/HAM	1861 (1010 – 2242)	2275 (1108 – 2844)

Fig. 1. MIP-1α levels in controls, HTLV-1-infected asymptomatic carriers and TSP/HAM patients.



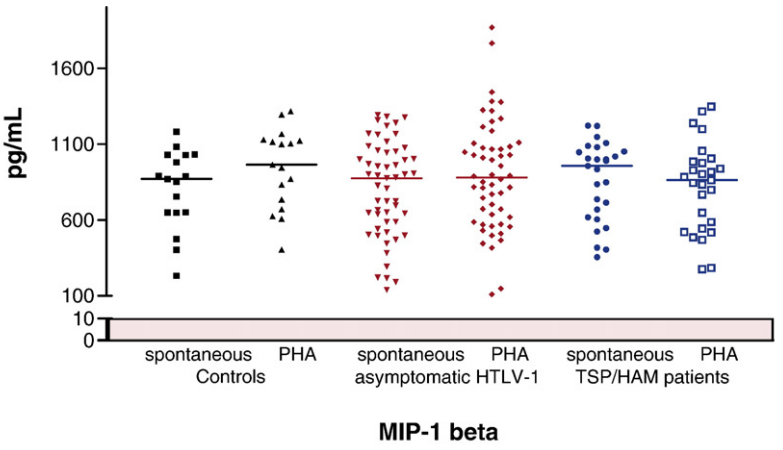
Group	Spontaneous (pg/mL)	PHA (pg/mL)
Control	1176 (891 – 1385)	1352 (986 – 1507)
Asymptomatic HTLV-1	1466 (1006 – 1911)	1559 (1005 – 1911)
TSP/HAM	1465 (1202 – 2161)	1675 (1367 – 2442)

Fig. 2. RANTES levels in controls, asymptomatic HTLV-1-infected carriers and TSP/HAM patients.

pairs and respective standards were purchased from R&D Systems (Duoset ELISA development kit, Minneapolis, MN, USA) and used according to the manufacturer's recommendation. The detection limit was 10 pg/mL for both chemokines. Optical density was measured with a 450 nm filter (BioRad, Hercules, CA, USA) and the concentration was determined using a standard curve developed with the GraphPrism program software.

2.4. Statistical analysis

Differences in patient characteristics or laboratory values among groups were evaluated with the one-way Mann–Whitney's test. In both cases p values < 0.05 were considered statistically significant, and the χ^2 test with Yates's correction was used to test the association between chemokine production and disease or infection.



Group	Spontaneous (pg/mL)	PHA (pg/mL)
Control	862 (561 – 1030)	955 (647 – 1127)
Asymptomatic HTLV-1	881 (564 – 1068)	890 (644 – 1066)
TSP/HAM	956 (644 – 1066)	863 (566 – 996)

Fig. 3. MIP-1β levels in controls, asymptomatic HTLV-1-infected carriers and TSP/HAM patients.

3. Results

Table 1 shows the demographical and clinical data of the subjects in the three groups (patients, asymptomatic and controls). The mean age of the patients in the TSP/HAM group was 47 years, of the asymptomatic carriers of HTLV-1 was 40 years, and subjects in the control group were 40 years. Median time of TSP/HAM disease was 8.5 years and the median age of patients at the onset of symptoms was 43 years.

Table 1 shows that the asymptomatic HTLV-1 carriers had significantly higher RANTES levels than the control group. The TSP/HAM patients had significantly higher levels of RANTES (after PHA stimulation) and MIP-1α (spontaneous and PHA-stimulated) than the asymptomatic HTLV-1-infected group. Thus, MIP-1α levels were more likely to be higher during the TSP/HAM.

Elevated MIP-1α levels in the TSP/HAM group, in comparison with the asymptomatic carriers, were observed both spontaneously and PHA-stimulated ($p=0.0009$ and $p=0.004$, respectively) (Fig. 1). Increased RANTES production in the asymptomatic HTLV-1 subjects compared with the control group (spontaneously stimulated) was observed. TSP/HAM patients presented higher RANTES levels (both spontaneous and after stimulus with PHA), but the difference was not statistically significant (Fig. 2). Moreover, there were no differences in MIP-α levels across the three study groups (Fig. 3).

4. Discussion

We noticed that MIP-1α levels were higher among TSP/HAM patients than among asymptomatic carriers. Thus, in addition to the pro-inflammatory cytokines, particularly IFN-gamma, IL-2, and IL-15 (Azimi et al., 2001), and the high activity of CTL, MIP-1α released by PBMC may have an important role in TSP/HAM pathogenesis. This process can also influence the inflammatory activity in the spinal cord and thus contribute to the TSP/HAM progress. Surprisingly, MIP-β levels were not different across groups. This finding may be explained by the almost exclusive chemokine release by monocytes (Oppermann, 2004), which are not important in the TSP/HAM development. In contrast, RANTES and MIP-α are produced mainly by CD8+ T cells, which are hardly implicated in this process.

Alternatively, type 3 CXC chemokine receptors (CXCR3), which are expressed at high levels on activated and memory T lymphocytes, selectively respond to some of these chemokines. Those memory cells are inducible by IFN-gamma, which is seen at high levels in HTLV-1-infected subjects; they may migrate to the spinal cord, leading to a potential damage to the myelin membrane (Trebst et al., 2003; Guerreiro et al., 2006). Thus, both pathways might be implicated in the TSP/HAM development, memory cells directly involved as a cytolytic damage being driven by IFN-gamma, and RANTES and MIP-1α acting as chemotactic factors, all produced mainly by CD8+ T cells.

We have observed that pro-inflammatory soluble factors, such MIP-1α, may have an important role in mediating tissue-specific leukocyte recruitment and T cell stimulation. This may result in damage to the myelin membrane, a major characteristic of myelopathy in HTLV-1-infected patients. Specifically RANTES, which induces hyperphosphorylation and generalized cell activation, may play an important role (Bacon et al., 1996). A chemokine-induced activation of T cells via CCR5 leads to activation of focal adhesion kinases, which also have important roles in cell motility including cell spread and migration (Ganju et al., 1999). These CCR5 ligands are critical for T proliferation and the transcriptional activation of cytokines genes (Oppermann, 2004). This mechanism may be directly involved as a cytolytic damage in the adult T cell leukemia (ATL), since high levels of RANTES were described in these patients (Mori et al., 2004), and one "*in vitro*" study showed a role of *tax* induction of MIP-1α (Sharma and May, 1999).

The predilection of neuro-inflammation by HTLV-1 in the thoracic cord may be due to slower local blood flow, with more opportunity for cells expressing adhesion molecules to transmigrate (Izumo et al., 1997), while other, more watershed areas of the central nervous system usually remain clinically silent. Taken together, these findings can expand the clinical armamentarium available to treat this condition, such as CCR5 agonists that could be used during the early phase of disease; currently, only a few options, such as corticosteroids, have been available in the clinical setting.

Acknowledgements

We thank D. Gallo for revising this manuscript. This work was supported by grants from FAPESP, numbers 99/11188-1, 00/12761-6, 03/00841-3, and from CNPq, grant number 3012242/02-5.

References

Azimi, N., Jacobson, S., Leist, T., Waldmann, T.A., 1999. Involvement of IL-15 in the pathogenesis of human T lymphotropic virus type I-associated myelopathy/tropical spastic paraparesis: implications for therapy with a monoclonal antibody directed to the IL-2/15R beta receptor. J. Immunol. 163, 4064–4072.

Azimi, N., Nagai, M., Jacobson, S., Waldmann, T.A., 2001. IL-15 plays a major role in the persistence of Tax-specific CD8 cells in TSP/HAM patients. Proc. Natl. Acad. Sci. U. S. A. 98, 14559–14564.

Bacon, K.B., Szabo, M.C., Yssel, H., Bolen, J.B., Schall, T.J., 1996. RANTES induces tyrosine kinase activity of stably complexed p125FAK and ZAP-70 in human T cells. J. Exp. Med. 184, 873–882.

Baggiolini, M., Loetscher, P., 2000. Chemokines in inflammation and immunity. Immunol. Today 21, 418–420.

Dehee, A., Cesaire, R., Desire, N., Nicolas, J.C., 2002. Quantitation of HTLV-1 proviral load by a TaqMan real-time PCR assay. J. Virol. Methods 102, 37–51.

Ganju, R.K., Dutt, P., Wu, L., et al., 1999. Beta-chemokine receptor CCR5 signals via the novel tyrosine kinase RAFTK. Blood 91, 791–797.

Gerard, C., Rollins, B.J., 2001. Chemokines and disease. Nat. Immunol. 2, 108–115.

Gessain, A., Barin, F., Vernant, J.C., et al., 1985. Antibodies to human T-lymphotropic virus type I in patient with tropical spastic paraparesis. Lancet 2, 407–410.

Guerreiro, J.B., Santos, S.B., Morgan, D.J., et al., 2006. Levels of serum chemokines discriminate clinical myelopathy associated with human T lymphtropic virus type 1 (HTLV-1)/tropical spastic paraparesis (HAM/TSP) disease from HTLV-1 carrier state. Clin. Exp. Immunol. 145, 296–301.

Hollsberg, P., Hafler, D., 1995. What is the pathogenesis of human T-cell lymphotropic virus type I-associated myelopathy/tropical spastic paraparesis? Ann. Neurol. 37, 143–145.

Izumo, S., Umehara, F., Kashio, N., Kubota, R., Sato, E., Osame, M., 1997. Neuropathology of HTLV-1-associated myelopathy (HAM/TSP). Leukemia 11 (Suppl 3), 82–84.

Izumo, S., Umehara, F., Osame, M., 2000. HTLV-1-associated myelopathy. Neuropathology 20, S65–S68 (Suppl.).

Jacobson, S., 2002. Immunopathogenesis of human T cell lymphotropic virus type I-associated neurologic disease. J. Infect. Dis. 186 (Suppl 2), S187–S192.

Lewis, M.J., Gaurtier, V.W., Wang, X.P., Kaplan, M.H., Hall, W.W., 2000. Spontaneous production of C–C chemokines by individuals infected with human T lymphotropic virus type II (HTLV-1I) alone and HTLV-1I/HIV-1 coinfected individuals. J. Immunol. 165, 4127–4132.

Luster, A.D., 1998. Chemokines: chemotactic cytokines that mediate inflammation. N. Engl. J. Med. 338, 436–445.

Mori, N., Krensky, A.M., Ohshima, K., et al., 2004. Elevated expression of CCL5/RANTES in adult T-cell leukemia cells: possible transactivation of the CCL5 gene by human T-cell leukemia virus type I tax. Int. J. Cancer 111, 548–557.

Moser, B., Loetscher, P., 2001. Lymphocyte traffic control by chemokines. Nat. Immunol. 2, 123–128.

Mueller, A., Mahmoud, N.G., Strange, P.G., 2006. Diverse signaling by different chemokines through the chemokines receptor CCR5. Biochem. Pharmacol. 1–10.

Murphy, P.M., Baggiolini, M., Charo, I.F., et al., 2002. International union of pharmacology. XXII. Nomenclature for chemokine receptors. Pharmacol. Rev. 52, 145–175.

Nagai, M., Usuku, K., Matsumoto, W., et al., 1998. Analysis of HTLV-1 proviral load in 202 TSP/HAM patients and 243 asymptomatic HTLV-1 carriers: high proviral load strongly predisposes to TSP/HAM. J. Neurovirology 4, 586–593.

Oppermann, M., 2004. Chemokine receptor CCR5: insights into structure, function, and regulation. Cell. Signal. 16, 201–210.

Osame, M., 1991. HAM/TSP. Global review and WHO diagnostic guidelines. In: Chopra, J. (Ed.), Advances in Neurology. Elsevier Science, Amsterdam, pp. 265–271.

Osame, M., 2002. Pathological mechanisms of human T-cell lymphotropic virus type I-associated myelopathy (TSP/HAM). J. Neurovirology 8, 359–364.

Osame, M., Usuku, K., Izumo, S., et al., 1986. HTLV-1 associated myelopathy: a new clinical entity. Lancet 3, 1031–1032.

Poiesz, B.J., Ruscetti, F.W., Gazdar, A.F., Bunn, P.A., Minna, J.D., Gallo, R.C., 1980. Detection and isolation of type C retrovirus particles from fresh and cultured lymphocytes of a patient with cutaneous T-cell lymphoma. Proc. Natl. Acad. Sci. U. S. A. 77, 7415–7419.

Rossi, D., Zlotnik, A., 2000. The biology of chemokines and their receptors. Annu. Rev. Immunol. 18, 217–242.

Sharma, V., May, C.C., 1999. Human T-cell lymphotrophic virus type-I tax gene induces secretion of human macrophage inflammatory protein-1alpha. Biochem. Biophys. Res. Commun. 262, 429–432.

Trebst, C., Staugaitis, S.M., Tucky, B., et al., 2003. Chemokine receptors on infiltrating leucocytes in inflammatory pathologies of the central nervous system (CNS). Neuropathol. Appl. Neurobiol. 29, 584–595.

Watson, M.L., 2002. Chemokines — linking receptors to response. Immunology 105, 121–124.

Yoshida, M., 1983. Human leukemia virus associated with adult T-cell leukemia. Gann 74, 777–789.