

SERUM LEVELS OF MIP-1 β AND RANTES IN HIV-1 SUBTYPE CRF01_AE INFECTED PATIENTS WITH DIFFERENT RATES OF DISEASE PROGRESSION

Thippawan Chuenchitra¹, Chantapong Wasi², Mark de Souza³, Sorachai Nitayaphan², Suda Louisirootchanaikul², Ruengpung Sutthent², Arthur E Brown⁴, Deborah L Bix⁵ and Victoria R Polonis³

¹Armed Forces Research Institute of Medical Sciences (AFRIMS), Bangkok, Thailand;

²Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand;

³US Military HIV Research Program, Rockville, USA; ⁴US Army Medical Materiel Development Activity, Ft Detrick, Maryland, USA; ⁵Global AIDS Program, CDC/NCHSTP, Corporate Square Building 8, Atlanta, GA, USA

Abstract. The β -chemokines have been shown to inhibit HIV replication *in vitro*. To evaluate the role of serum β -chemokines in disease progression and their anti-viral role *in vivo*, we determined serum levels of macrophage inflammatory protein-1 β (MIP-1 β) and regulated upon activation normal T-cell expressed and secreted (RANTES) of twenty HIV-1 subtype CRF01_AE infected patients: nine progressors (PRs, follow-up CD4+ cell count < 200/mm³ and progression to AIDS or death) and eleven slower progressors (SPs, asymptomatic and/or follow-up CD4+ cell counts >350/mm³ at the end of follow-up) and determined their plasma viral loads. The subjects were followed for at least 36 months. All had initial CD4 values >350 cells/mm³. In this longitudinal study, serum levels of MIP-1 β and RANTES in specimens obtained either early or later in the course of HIV infection did not differ significantly between progressors and slower progressors ($p > 0.05$). There were no significant changes in serum MIP-1 β and RANTES levels over time in either patient group ($p > 0.05$). No significant associations were observed between plasma viral loads and the measured β -chemokines ($r = -0.205$, $p = 0.21$ for MIP-1 β and $r = -0.12$, $p = 0.492$ for RANTES). The results suggest these chemokines do not play a major systemic role in control of viremia or protection against the progression of HIV disease.

INTRODUCTION

Macrophage inflammatory protein (MIP)-1 β and regulated upon activation normal T-cell expressed and secreted (RANTES) are in-

ducible, secreted pro-inflammatory cytokines in the β -chemokine subfamily (Levy, 1998). In 1986, Walker *et al* demonstrated that CD8+ lymphocytes in HIV-infected individuals were capable of suppressing HIV replication *in vitro* and these factors were proposed to be a possible mechanism for control of HIV replication *in vivo* (Walker *et al*, 1988). In addition, MIP-1 α , MIP-1 β and RANTES are the natural ligands for CCR5 and are major suppressors of macrophage-tropic HIV-1 (R5) strains (Cocchi *et al*, 1995; Alkhatib *et al*, 1996) and presumably inhibit HIV-1 replication by binding to their receptors, thereby preventing viral fusion and entry. However, RANTES was

Correspondence: Thippawan Chuenchitra, AFRIMS, 315/6 Ratchawithi Road, Ratchathewi, Bangkok 10400, Thailand.

Tel: 66 (0) 2354 7861-69 ext 2125; Fax: 66 (0) 2354 7854

E-mail: ThippawanC@afirms.org

The opinions or assertions presented in this article are the views of the authors and are not necessarily reflect the official positions of the Royal Thai Army or the US Army or Department of Defense.

reported to be the most effective inhibitor of HIV-1 replication (Cocchi *et al*, 1995).

Despite the regulatory effect of β -chemokines on HIV-1 replication *in vitro* (Walker *et al*, 1988; Picker, 2006), *in vivo* studies of the relationship between β -chemokines and disease progression or viral load have been variable. Some studies have shown no difference in serum MIP-1 α , MIP-1 β and RANTES between progressors and nonprogressors (McKenzie *et al*, 1996) and no correlation between β -chemokines and disease stage (Ping *et al*, 2004). Some researchers have reported increased serum β -chemokines levels associated with HIV disease progression (Zanussi *et al*, 1996; Polo *et al*, 1999; Kreuzer *et al*, 2000), while others have failed to correlate with HIV-1 viral load (Zanussi *et al*, 1996; Weiss *et al*, 1997; Polo *et al*, 1999; Kreuzer *et al*, 2000). Aukrust *et al* (1998) found decline in serum RANTES levels correlated with disease progression in HIV-1-infected persons, and found an inverse correlation between serum RANTES levels and virus load in HIV-1 infected patients. Jennes *et al* (2002) suggested that an increased percentage of β -chemokine-producing T cells correlated with disease progression and HIV-1 viral load, however, in other studies β -chemokine production levels were found to correlated inversely with HIV-1 viral load (Ferbas *et al*, 2000; Paxton *et al*, 2000).

In this longitudinal study, we compared serum levels of MIP-1 β and RANTES in HIV-1 subtype CRF01_AE infected patients with progression and slower progression and determined the relationship between serum levels of these β -chemokines and viral loads in HIV-1 infected individuals to evaluate the potential role of these β -chemokines on disease progression and as anti-HIV agents *in vivo*.

MATERIALS AND METHODS

Study subjects

Thai HIV-1 subtype E infected patients

were selected from subjects enrolled between 1993 and 2000 in a natural history study of HIV-1 infected patients conducted in Bangkok, Thailand. All subjects gave informed consent prior to being included in the study which was approved by the Institutional Review Board of Royal Thai Army. The patients were separated into progressors (PRs) and slower progressors (SPs) according to differences in the extent of CD4+ T cell decline. Nine PR (CD4+ cells < 200/mm³ who had progression to AIDS or death by the end of follow-up) and eleven SPs (patients who were asymptomatic or had CD4+ cells > 350/mm³ at the end of follow-up) were followed for at least 36 months.

Chemokine assay

The serum levels of MIP-1 β and RANTES were determined by commercial quantitative enzyme-linked immunosorbent assay (ELISA) (Quantikine, R&D Systems, Minneapolis, MN) according to the manufacturer's suggestions. Briefly, 100 μ l of standard or sample was added to wells of microtiter plates coated with antibody to MIP-1 β and RANTES. Following incubation and washing, 200 μ l of enzyme (conjugated with the respective antibodies) was added. After incubation and washing, 200 μ l of substrate was added per well, and color development was stopped by the addition of 2 N sulfuric acid. The plates were read by a Bio-Tek (Winooski, Vermont) EL311 reader at 450 nm, and chemokine concentrations were calculated from a standard curve of corresponding recombinant human cytokine according to the instructions of manufacturer.

Quantification of HIV RNA copy numbers in plasma

Plasma viral load was measured by commercial branched-DNA assay (Quantiplex, HIV-RNA assay 3.0, Chiron, Emeryville, CA) using cryopreserved plasma. The limit of detection was 50 copies/ml plasma.

Statistical analysis

For comparison of the PR and SP groups,

the Mann-Whitney *U* test (two-tailed) was used. The Wilcoxon signed rank test for paired data (two-tailed) was used when parameters within an individual were compared. Coefficients of correlation (*r*) were calculated by the Spearman rank test and distribution of a particular characteristic among groups was evaluated using the chi-square test. Data were given as medians and ranges. P-values were two-sided and considered significant when $p < 0.05$.

RESULTS

Characteristics of the study population

The characteristics of PRs and SPs are shown in Table 1. In this study, 9 PRs with decreasing CD4+ cell counts (median = 96×10^6 cells/mm³ at the end of follow-up) were compared with 11 SPs with stable CD4+ cell counts (median = 570×10^6 cells/mm³ at the end of follow-up). All subjects were followed for at least 36 months (PRs: mean = 57 months, SPs: mean = 55 months). One subject in the PR group died of AIDS during the study. In the overall cohort, there was a significant difference in CD4+ cell counts between the SPs and PRs ($p < 0.001$). While CD4+ cell counts in the two groups were not significantly different at study entry, the CD4+

cell counts were significantly lower in the PRs than the SPs at the end of follow-up ($p < 0.001$). PRs had significantly higher plasma viral loads at the onset of the study ($p < 0.005$) (Table 1).

Comparison of serum MIP-1 β levels in progressors and slower progressors

We compared the serum MIP-1 β levels in the 9 PRs and 11 SPs at the beginning and the end of the study. At the beginning of the study serum MIP-1 β levels in the SPs were higher than in the PRs but the differences were not significant (median = 68.9 pg/ml for PRs and 82.9 pg/ml for SPs, $p = 0.239$, Mann-Whitney *U* test) (Table 2). At the end of the study there were no significant differences in serum MIP-1 β levels between PRs and SPs (median = 58.6 pg/ml for PRs and 82.9 pg/ml for SPs, $p = 0.543$, Mann-Whitney *U* test). We found no significant changes in serum MIP-1 β levels over time in the PR and SP groups ($p = 0.515$ for PRs and 0.722 for SPs, Wilcoxon signed rank test). However, considerable variation in serum MIP-1 β levels was observed in both the PR and SP groups (Table 2).

We found that 5/9 PRs and 4/11 SPs had increases in serum MIP-1 β levels but differences were not significant ($p = 0.65$, chi-square test) (Table 2).

Table 1
Characteristics of progressors and slower progressors.

	Progressors	Slower progressors	p-value
Total (<i>N</i> = 20)	9	11	
Median (range) follow-up, in months	55 (39-75)	51 (36-77)	<0.001
Median CD4 count (range) in cells/mm ³			
Early	613 (485-880)	558 (359-824)	<0.001
Late	96 (11-303)	576 (316-644)	<0.001
Median viral load (range) in cells/mm ³			
Early	34,728 (1,258-92,428)	3,799 (114-637)	0.038
Late	30,302 (511 - >500,000)	3,975 (340-100,260)	0.067

Table 2
Serum β -chemokine levels in HIV-1 subtype E infected patients.

	Progressors	Slower progressors	p-value
MIP-1β			
Median (range) serum concentration, pg/ml			
Early	68.9 (4.7-94.3)	82.9 (32.4-139.1)	0.239
Late	58.6 (18.9-99.3)	82.9 (10.5-128.0)	0.543
Early versus Late (p)	0.515	0.722	
Patients with increasing serum concentration, as <i>n</i> /total	5/9	4/11	0.650
RANTES			
Median (range) serum concentration, ng/ml			
Early	42.6 (10.1-91.1)	38.9 (12.6-54.8)	0.424
Late	40.1 (11.0-69.4)	40.6 (26.0-63.9)	0.790
Early versus Late (p)	1.0	0.386	
Patients with increasing serum concentration, as <i>n</i> /total	4/9	5/10	1.0

Comparison of serum RANTES levels in progressors and slower progressors

Serum RANTES levels in the PR and SP groups were lower than the serum MIP-1 β levels. Similar to serum MIP-1 β levels, at the beginning of the study serum RANTES levels were not significantly different between the PRs and SPs (median = 42.6 ng/ml for PRs and 38.9 ng/ml for SPs, $p = 0.424$, Mann-Whitney U test) (Table 2). At the end of the study there were no significant differences in serum RANTES levels between PRs and SPs (median = 40.1 ng/ml for PRs and 40.6 ng/ml for SPs, $p = 0.79$, Mann-Whitney U test). There were no significant changes in serum MIP-1 β levels over time in both the PR and SP groups ($p=1.0$ for PRs and 0.36 for SPs, Wilcoxon signed rank test). However, there was considerable variation in serum RANTES levels in both the PR and SP groups (Table 2).

We found that 4/9 PRs and 5/10 SPs had increases in serum RANTES levels, but no significant differences were seen in either group ($p = 1.0$, chi-square test).

Relationship between viral load and serum levels of β -chemokines in progressors and slower progressors

There were no significant correlations between the concentrations of β -chemokines and viral loads ($r = -0.205$, $p = 0.21$ for MIP-1 β and $r = -0.12$, $p = 0.492$ for RANTES, Spearman rank test).

DISCUSSION

β -chemokines are major HIV suppressive soluble factors produced by CD8+ T-cells (Cocchi *et al*, 1995). In addition to CD8+ T-cells, CD4+ T-cells from HIV-1-infected individuals also produce comparable concentrations of β -chemokines *in vitro* (Kinter *et al*, 1996). We found that serum MIP-1 β and RANTES levels were not different between PRs and SPs. This finding is consistent with studies which demonstrated that serum β -chemokine levels do not show an inverse correlation with disease progression (McKenzie *et al*, 1996; Krowka *et al*, 1997; Vitale *et al*,

1997; Greco *et al*, 1998). Inverse correlations were found in some studies (Zanussi *et al*, 1996; Greco *et al*, 1998; Garcino *et al*, 1999; Paxton *et al*, 2001). We found no negative correlation between β -chemokines and HIV-1 viral load, consistent with the findings of several studies (Weiss *et al*, 1997; Kreuzer *et al*, 2000; Ping *et al*, 2004). However, some studies reported a correlation between viral load or disease progression and levels of MIP-1 α (Hittinger *et al*, 1998), MIP-1 β (Hittinger *et al*, 1998; Ullum *et al*, 1998) and RANTES (Aukrust *et al*, 1998).

The reasons for these differences are unclear. It is possible these differences are due to the methods used and selective criteria of the patients. It is unclear if the concentration of β -chemokines necessary to inhibit HIV replication *in vitro* is different from that needed *in vivo*. We found the concentrations of MIP-1 β and RANTES in the serum of both PRs and SPs were many times less than the concentrations of β -chemokines used to inhibit HIV replication *in vitro* (Cocchi *et al*, 1995). However, β -chemokine release after *in vitro* stimulation with mitogens may not reflect the physiologic expression of β -chemokines *in vivo*.

Several studies indicate that a 32 base-pair deletion in the CCR5 receptor for these β -chemokines is associated with nonprogressive HIV disease (Dean *et al*, 1996; de Roda Husman *et al*, 1997; Michael *et al*, 1997). It is possible that protection from infection or disease progression may be mediated at the level of β -chemokine receptors rather than by these chemokines themselves.

The absence of correlation between the concentrations of β -chemokines and HIV-1 viral load in the serum of HIV-1 infected subjects does not rule out the role of factors other than the β -chemokines in the suppression of HIV-1 replication *in vitro*. Our research group (Chuenchitra *et al*, 2003) found an increase of neutralizing antibodies over time in SP sera

did not significantly correlate with serum β -chemokine levels by using the same groups of subjects as this study and we presume the changes in neutralization over time were antibody mediated. However, it should be remembered that β -chemokine production may reflect general immunocompetence and not necessarily any specific anti-HIV effect.

In conclusion, our study found no correlation between β -chemokines and the control of viremia or protection against disease progression. It is possible that serum β -chemokine levels may not reflect local concentrations in sites of inflammation or in areas of HIV replication, such as lymph nodes. Levels of β -chemokine expression in lymph nodes are much more relevant to control of replication (Trumpfheller *et al*, 1998) and may not necessarily correlate with levels of serum β -chemokines. However, the lack of any difference in levels of serum β -chemokines between PRs and SPs and no correlation with viral load in this study suggests that serum β -chemokines cannot be used as a predictive marker of disease progression and do not play a major protective role in HIV-1 subtype CRF01_AE infection.

ACKNOWLEDGEMENTS

The authors would like to thank the subjects for their participation in this study. For technical assistance, we thank Col Suchitra Sukwit, Rapee Trichavaroj, Puangmalee Buapunth and Bessara Noiprasert. This work was supported by the Armed Forces Research Institute of Medical Sciences (AFRIMS), the Henry M Jackson Foundation, United States and the Faculty of Medicine, Siriraj Hospital, Mahidol University, Bangkok, Thailand.

REFERENCES

- Alkhatib G, Combadiere C, Broder CC, *et al*. CC CKR5: a RANTES, MIP-1 α , MIP-1 β

- receptor as a fusion cofactor for macrophage-tropic HIV-1. *Science* 1996; 272: 1955-8.
- Aukrust P, Muller F, Froland SS. Circulating levels of RANTES in human immunodeficiency virus type 1 infection: effect of potent antiretroviral therapy. *J Infect Dis* 1998; 177: 1091-6.
- Chuenchitra T, Wasi C, Louisirojchanakul S, *et al.* Longitudinal study of humoral immune responses in HIV type 1 subtype CRF01_AE (E)-infected Thai patients with different rates of disease progression. *AIDS Res Hum Retroviruses* 2003; 19: 293-305.
- Cocchi F, DeVico AL, Garzino-Demo A, Arya SK, Gallo RC, Lusso P. Identification of RANTES, MIP-1 alpha, and MIP-1 beta as the major HIV-suppressive factors produced by CD8+ T cells [see comments]. *Science* 1995; 270: 1811-5.
- Dean M, Carrington M, Winkler C, *et al.* Genetic restriction of HIV-1 infection and progression to AIDS by a deletion allele of the CKR5 structural gene. Hemophilia Growth and Development Study, Multicenter AIDS Cohort Study, Multicenter Hemophilia Cohort Study, San Francisco City Cohort, ALIVE Study. *Science* 1996; 273: 1856-62.
- de Roda Husman AM, Koot M, Cornelissen M, *et al.* Association between CCR5 genotype and the clinical course of HIV-1 infection [see comments]. *Ann Intern Med* 1997; 127: 882-90.
- Ferbas J, Giorgi JV, Amini S, *et al.* Antigen-specific production of RANTES, macrophage inflammatory protein (MIP)-1alpha, and MIP-1beta *in vitro* is a correlate of reduced human immunodeficiency virus burden *in vivo*. *J Infect Dis* 2000; 182: 1247-50.
- Garcino-Demo A, Moss RB, Margolick JB, *et al.* Spontaneous and antigen-induced production of HIV-inhibitory beta-chemokines are associated with AIDS-free status. *Proc Natl Acad Sci USA* 1999; 96: 11986-91.
- Greco G, Barker E, Leve JA. Differences in HIV replication in CD4+ lymphocytes are not related to beta-chemokine production. *AIDS Res Hum Retrovirus* 1998; 14: 1407-11.
- Hittinger G, Poggi C, Delbeke E, Profizi N, Lafeuillade A. Correlation between plasma levels of cytokines and HIV-1 RNA copy number in HIV-infected patients. *Infection* 1998; 26: 100-3.
- Jennes W, Sawadogo S, Koblavi-Deme S, *et al.* Positive association between beta-chemokine-producing T cells and HIV type 1 viral load in HIV-infected subjects in Abidjan, Cote d'Ivoire. *AIDS Res Hum Retroviruses* 2002; 18: 171-7.
- Kinter AL, Ostrowski M, Goletti D, *et al.* HIV replication in CD4+ T cells of HIV-infected individuals is regulated by a balance between the viral suppressive effects of endogenous beta-chemokines and the viral inductive effects of other endogenous cytokines. *Proc Natl Acad Sci USA* 1996; 93: 14076-81.
- Kreuzer KA, Rockstroh JK, Kupfer B, Spengler U. Endogenous levels of C-C chemokines MIP-1alpha, MIP-1beta, and RANTES do not reflect the disease course in HIV-seropositive individuals. *Int J Clin Lab Res* 2000; 30: 163-8.
- Krowka JF, Gesner ML, Ascher MS, Sheppard HW. Lack of associations of chemotactic cytokines with viral burden, disease progression, or lymphocyte subsets in HIV-infected individuals. *Clin Immunol Immunopathol* 1997; 85: 21-7.
- Levy JA. HIV and pathogenesis of AIDS. Washington, DC: ASM Press, 1998.
- McKenzie SW, Dallalio G, North M, Frame P, Means RT, Jr. Serum chemokine levels in patients with non-progressing HIV infection. *AIDS* 1996; 10: F29-33.
- Picker LJ. Immunopathogenesis of acute AIDS virus infection. *Curr Opin Immunol* 2006; 18: 399-405.
- Michael NL, Chang G, Louie LG, *et al.* The role of viral phenotype and CCR-5 gene defects in HIV-1 transmission and disease progression. *Nat Med* 1997; 3: 338-40.
- Paxton WA, Neumann AU, Kang S, *et al.* RANTES production from CD4+ lymphocytes correlates with host genotype and rates of human immunodeficiency virus type 1 disease progression. *J Infect Dis* 2001; 183: 1678-81.
- Picker LJ. Immunopathogenesis of acute AIDS virus infection. *Curr Opin Immunol* 2006; 18: 309-405.
- Ping YE, Kazanjian P, Kunkel SL, Kirschner DE.

- Lack of good correlation of serum CC-chemokine levels with human immunodeficiency virus-1 disease stage and response to treatment. *J Lab Clin Med* 2004; 143: 310-9.
- Polo S, Veglia F, Malnati MS, *et al.* Longitudinal analysis of serum chemokine levels in the course of HIV-1 infection. *AIDS* 1999; 13: 447-54.
- Trumpfheller C, Tenner-Racz K, Racz P, Fleischer B, Frosch S. Expression of macrophage inflammatory protein (MIP)-1alpha, MIP-1beta, and RANTES genes in lymph nodes from HIV+ individuals: correlation with a Th1-type cytokine response. *Clin Exp Immunol* 1998; 112: 92-9.
- Ullum H, Cozzi Lepri A, Victor J, *et al.* Production of beta-chemokines in human immunodeficiency virus (HIV) infection: evidence that high levels of macrophage inflammatory protein-1beta are associated with a decreased risk of HIV disease progression. *J Infect Dis* 1998; 177: 331-6.
- Vitale F, Bonura F, Perna AM, Ajello F, Romano N. Serum RANTES levels in HIV-positive individuals and in HIV-negative exposed health-care workers. *Infection* 1997; 25: 381-2.
- Walker CM, Steimer KS, Rosenthal KL, Levy JA. Identification of human immunodeficiency virus (HIV) envelope type-specific T helper cells in an HIV-infected individual. *J Clin Invest* 1988; 82: 2172-5.
- Weiss L, Si-Mohamed A, Giral P, *et al.* Plasma levels of monocyte chemoattractant protein-1 but not those of macrophage inhibitory protein-1alpha and RANTES correlate with virus load in human immunodeficiency virus infection. *J Infect Dis* 1997; 176: 1621-4.
- Zanussi S, D'Andrea M, Simonelli C, Tirelli U, De Paoli P. Serum levels of RANTES and MIP-1 alpha in HIV-positive long-term survivors and progressor patients. *AIDS* 1996; 10: 1431-2.