

Genetics, Epigenetics and Expression of *CCL5* Gene That Encodes RANTES, a Chemokine with Potent Anti-HIV-1 Properties

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Abstract

Background. The CC-motif chemokine ligand 5 (*CCL5*, also known as RANTES) is a potent HIV-1 antagonist that competes for binding to CCR5. Use of modified RANTES as a microbicide has been tested successfully in primate models.

Objective. Our work aimed to characterize genetic and epigenetic correlates of *CCL5* production in HIV-1 seropositive and seronegative adolescents.

Methods. Informative single nucleotide polymorphisms in *CCL5* promoter, intron 1 and 3' untranslated region were resolved by DNA sequencing and related techniques. Cytosine (CpG) methylation within promoter sequences was quantified using pyrosequencing, while serum (and occasionally plasma) RANTES concentrations were measured using antibody array and enzyme-linked immunosorbent assay. Standard statistical procedures suitable for analyses of categorical and continuous variables were applied to test for associations and correlations.

Results. Major *CCL5* haplotypes reported in earlier studies were readily detected in the study population consisting of 227 HIV-1 seropositives and 184 HIV-1 seronegatives. Serum RANTES concentrations were usually two magnitude higher than other C-C and C-X-C chemokines, including macrophage inflammatory proteins (MIP-1 α and MIP-1 β) that are encoded by multiple genes. HIV-1 infection further raised serum RANTES concentrations ($p < 0.001$). Following statistical adjustment for age, gender and ethnicity, serum RANTES concentration showed a weak correlation with HIV-1 viral load and CD4⁺ T-cell counts in HIV-1 seropositive subjects (Spearman correlation coefficients < 0.20 , $p \geq 0.18$ in all tests). However, neither *CCL5* genotypes nor promoter hypomethylation could account for clear inter-individual variability in serum RANTES concentrations.

Conclusion. Predominance of RANTES against other chemokines in serum samples may reflect its immunological importance. Mechanisms underlying variability in systemic RANTES production remained elusive.

Introduction

- As a natural ligand of CCR5 (the primary HIV-1 coreceptor), RANTES inhibits M-tropic HIV-1 entry into host cells (Oravecz *et al.*, 1996).
- RANTES as a microbicide has been tested successfully in a primate model system (Lederman *et al.*, 2004).
- Serum RANTES concentrations are often elevated in HIV-1 patients progressing to AIDS, with clear difference between non-progressors and rapid progressors (Polo *et al.*, 1999; Zanussi *et al.*, 1996).
- Associations of serum RANTES concentration with CD4⁺ T-cell counts and HIV-1 viral load are either inconsistent or conflicting (Krowka *et al.*, 1997; Weiss *et al.*, 1997).
- Genetic variations of the *RANTES* gene (*CCL5*), especially those within the promoter and intron 1 regions may mediate HIV-1 infection and disease progression (Liu *et al.*, 1999; An *et al.*, 2000).
- Our work aimed to define the genetic and epigenetic correlates of serum RANTES concentrations and HIV-1-related outcomes in an adolescent population.

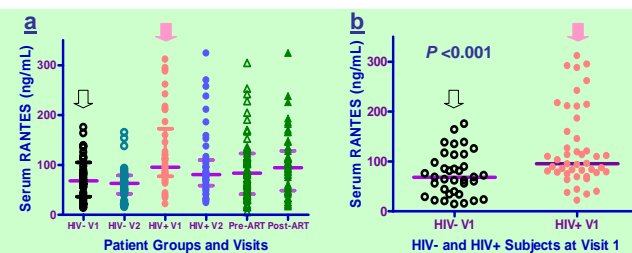


Fig. 3. Serum RANTES in adolescents with (HIV+) and without (HIV-) HIV-1 infection. At two separate visits (V1 and V2), serum RANTES concentrations were quantified by ELISA. In panel a, individual RANTES measures as well as median and interquartile ranges (horizontal bars) are shown for three patients groups ($n = 36$, 45, and 51, respectively). In panel b, visit 1 results for HIV-1 seronegative (HIV-) and untreated seropositive (HIV+) patients are redrawn to show more details. ART = anti-retroviral therapy.

Subjects and Methods

- HIV-1 seropositives and seronegatives adolescents were selected from the Reaching for Excellence in Adolescent Care and Health (REACH) cohort.
- For each subject, serum RANTES concentration was quantified at two separate visits using sandwich ELISA (R & D Systems).
- Single Nucleotide polymorphisms in *CCL5* promoter, intron 1 and 3' untranslated region were defined by direct sequencing of PCR amplicons or by PCR with sequence-specific primers (SSP) (Wang *et al.*, 2004).
- Linkage disequilibria (LD) between individual SNPs were determined and visualized using the Haploview version 3.2.
- CD4⁺ and CD4⁻ peripheral blood mononuclear cells (PBMCs) were sorted by magnetic beads coated with anti-CD4 monoclonal antibodies (Dyna).
- Methylation of CpG sites within the *CCL5* promoter region was measured by pyrosequencing of PCR amplicons derived from bisulfite-treated genomic DNA.
- Correlation and genetic associations analyses were done using standard statistical packages in SAS (SAS Institute, Cary, NC).

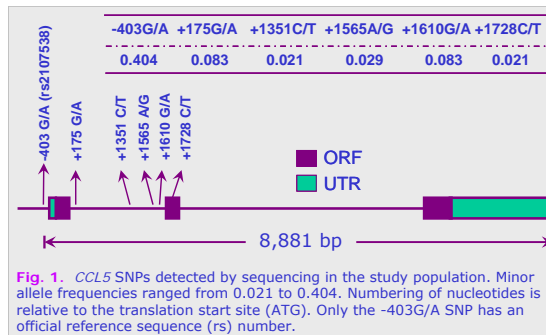


Fig. 1. *CCL5* SNPs detected by sequencing in the study population. Minor allele frequencies ranged from 0.021 to 0.404. Numbering of nucleotides is relative to the translation start site (ATG). Only the -403G/A SNP has an official reference sequence (rs) number.

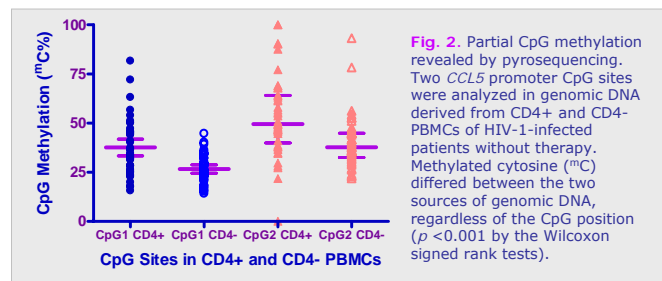


Fig. 2. Partial CpG methylation revealed by pyrosequencing. Two *CCL5* promoter CpG sites were analyzed in genomic DNA derived from CD4⁺ and CD4⁻ PBMCs of HIV-1-infected patients without therapy. Methylated cytosine (^mC) differed between the two sources of genomic DNA, regardless of the CpG position ($p < 0.001$ by the Wilcoxon signed rank tests).

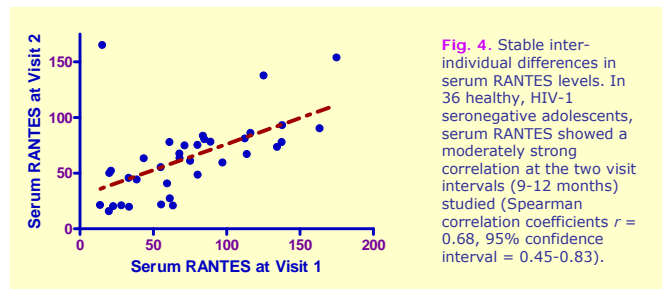


Fig. 4. Stable inter-individual differences in serum RANTES levels. In 36 healthy, HIV-1 seronegative adolescents, serum RANTES showed a moderately strong correlation at the two visit intervals (9-12 months) studied (Spearman correlation coefficients $r = 0.68$, 95% confidence interval = 0.45-0.83).

Results

- Sequencing and PCR-SSP confirmed the common *CCL5* SNPs and their haplotypes in the study population. In addition, several novel SNPs were detected in the intron 1 region, with minor allele frequencies (MAF) ranging from 0.021 to 0.083 (Figure 1).
- CpG sites within the *CCL5* promoter were partially methylated (median methylated cytosine $< 50\%$), especially in DNA derived from CD4⁺ PBMCs (Figure 2).
- Serum RANTES concentrations clearly differed between HIV-1-infected and -uninfected subjects ($P < 0.001$ by Mann-Whitney U tests) (Figure 3). In addition, there is a strong correlation between serum RANTES at the two visits (Spearman correlation coefficients = 0.56-0.68, $P < 0.0001$ for both), regardless of HIV-1 infection (e.g., Figure 4).
- Serum RANTES levels did not show any strong correlation with HIV-1 viral load or CD4⁺ T-cell counts (Spearman correlation coefficients < 0.20 , $p \geq 0.18$) (data not shown).
- Effective anti-retroviral therapy did not restore serum RANTES to the levels seen in HIV-1 seronegative subjects (Figure 3).
- Neither genetic nor epigenetic variations could account for differential serum RANTES expression in this study population (data not shown).

Discussion & Conclusions

- Partially consistent with some of the earlier findings (e.g., Montano *et al.*, 2006), RANTES expression is up-regulated after HIV-1 infection.
- Stable inter-individual differences in systemic (circulating) RANTES levels may imply genetic mechanisms, although the SNPs in promoter, intron 1 and 3' UTR regions have failed to show any association. Further analyses of additional SNPs may prove informative.
- Unlike other C-C motif chemokines (see Poster #443 at this conference), RANTES is dominantly detected in serum and plasma samples. Its contribution to HIV-1 infection and pathogenesis may further depend on posttranslational processing. In particular, cleavage of the N-terminus residues by CD26 can alter the bioactivity of RANTES (Struyf *et al.*, 2001; Vam Damme *et al.*, 1999).

Acknowledgments

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