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• *CLINICAL RESEARCH* •

Distribution and effects of polymorphic RANTES gene alleles in HIV/HCV coinfection – A prospective cross-sectional study

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Abstract

AIM: Chemokines and their receptors are crucial for immune responses in HCV and HIV infection. RANTES gene polymorphisms lead to altered gene expression and influence the natural course of HIV infection. Therefore, these mutations may also affect the course of HIV/HCV coinfection.

METHODS: We determined allele frequencies of RANTES-403 (G→A), RANTES-28 (C→G) and RANTES-IN1.1 (T→C) polymorphisms using real-time PCR and hybridization probes in patients with HIV (n = 85), HCV (n = 112), HIV/HCV coinfection (n = 121), and 109 healthy controls. Furthermore, HIV and HCV loads as well as CD4+ and CD8+ cell counts were compared between different RANTES genotypes.

RESULTS: Frequencies of RANTES-403 A, RANTES-28 G and RANTES-IN1.1 C alleles were higher in HIV infected patients than in healthy controls (-403: 28.2% *vs* 15.1%, P = 0.002; -28: 5.4% *vs* 2.8%, not significant; IN1.1: 19.0% *vs* 11.0%, P = 0.038). In HIV/HCV coinfected patients, these RANTES alleles were less frequent than in patients with HIV infection alone (15.4% P = 0.002; 1.7%; P = 0.048; 12.0%; not significant). Frequencies of these alleles were not significantly different between HIV/HCV positive patients, HCV positive patients and healthy controls.

CONCLUSION: All three RANTES polymorphisms showed increased frequencies of the variant allele exclusively in patients with HIV monoinfection. The finding that the frequencies of these alleles remained unaltered in HIV/HCV coinfected patients suggests that HCV coinfection interferes with selection processes associated with these alleles in HIV infection.

Key words: RANTES polymorphism; HIV/HCV-coinfection; HCV

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INTRODUCTION

Coinfection with human immunodeficiency virus (HIV) and hepatitis C virus (HCV) is common among certain risk groups such as hemophiliacs and intravenous drug abusers (IVDA)[1]. Unfortunately, HIV/HCV coinfection is associated with an accelerated course of HCV infection leading to progressive liver disease, cirrhosis, and hepatic failure[2,3].

Chemokines and their receptors play a central role in HIV infection. During the initial steps of viral infection chemokine receptors, such as the chemokine receptor 5 (CCR5), are used as co-receptors by HIV to enter monocytes and CD4 positive T-helper cells. A mutation in the encoding region of CCR5, CCR5-△32, abrogates HIV cell entry of m-tropic HIV strains, and thus prevents HIV infection in CCR5-△32 homozygous patients[4]. Heterozygosity for the CCR5-△32 mutation is associated with delayed progression of HIV infection to AIDS[4]. Recently, we have reported that the CCR5-△32 mutation was more prevalent in hemophiliac patients with chronic hepatitis C virus infection and was associated with increased hepatitis C viral loads[5]. A study on liver biopsies of patients with chronic hepatitis C revealed that this mutation may be associated with reduced portal inflammation and fibrosis[6]. Furthermore, we have found epidemiological evidence that the CCR5-△32 mutation is a predictor of treatment failure in interferon-α monotherapy

of chronic HCV infection, possibly indicating that the CCR5 receptor may also play an important role in the immune response to HCV infection[7].

The natural ligands of CCR5 are the chemokines RANTES (regulated on activation normal T cell expressed and secreted; CCL5), MIP-1α (macrophage inhibitory protein-1α; CCL3) and MIP-1β (macrophage inhibitory protein-1β; CCL4), all of which are potent inhibitors of HIV-1 cell entry[8]. Importantly, RANTES blocks the CCR5 receptor via receptor binding and down-regulation of CCR5 on T cells and macrophages. Furthermore, RANTES was reported to be critically involved in the recruitment of T cells to the liver[9]. Several single nucleotide polymorphisms in the RANTES gene have been reported to influence the natural course of HIV infection by up- or down-regulating RANTES gene activity. The most frequent of those polymorphic sites comprise RANTES-403 (G→A) and RANTES-28 (C→G) in the promoter region and RANTES-IN1.1 (T→C) in the first intron region[10,11]. Both promoter polymorphisms increase RANTES transcription and may delay HIV disease progression[11,12]. Conversely, the RANTES-IN1.1 C allele seems to decrease RANTES transcriptional activity and is probably associated with an increased risk for HIV infection and progression to AIDS[10].

Since RANTES seems to be involved in the pathogenesis of both HIV and HCV infection, we studied the effects of the RANTES gene polymorphisms in patients with HIV/HCV coinfection as compared to patients with HIV infection. To exclude that HCV infection or allele frequency in the background population contributed to our results we also included patients with HCV monoinfection as well as a group of healthy blood donors into the study.

MATERIALS AND METHODS

Design and study populations

All anti-HCV or anti-HIV positive patients of Caucasian descent attending our outpatient department between May 1999 and August 2000 were enrolled into one of the three study groups (HCV monoinfection = group I, HIV monoinfection = group II, HIV/HCV double infection = group III). None of the anti-HCV positive and anti-HIV/HCV positive patients had received interferon therapy at the time of the study. Type and duration of antiretroviral therapy and risk factors for infection were recorded in groups II and III. One hundred and nine healthy Caucasian blood donors of the Bonn University transfusion center (females 37, males 72, median age 27 years, range: 12-58 years) served as a reference group. In this reference group, HIV and HCV infection had been excluded by serology and PCR.

EDTA blood samples were obtained from each patient for genotyping of RANTES-403, -28 and -IN1.1 alleles. HCV genotype, HCV, and HIV viral loads, aminotransferase serum levels, CD4+ and CD8+ cell counts were determined in HIV, HCV, and HIV/HCV coinfected patients, respectively.

Serum aminotransferase levels were determined by routine biochemical procedures. Serologic markers of hepatitis B virus infection (HBs antigen, anti-HBs, and anti-HBc) were assessed by commercially available assays according to the manufacturer's instructions (Abbott, Wiesbaden, Germany). CD4 and CD8 cell counts were analyzed on a FACSort™ (Becton Dickinson, Heidelberg, Germany) flow cytometer using the Simulset™ test kit (Becton Dickinson, Heidelberg, Germany). The study conformed to the ethical guidelines of the Helsinki declaration as approved by the local ethics committee.

Diagnosis of HIV infection

Serum samples were analyzed for anti-HIV antibodies and p24 antigen with commercially available test kits (Abbott, Wiesbaden and Coulter, Hamburg, Germany) according to the manufacturer's instructions. A positive ELISA result was confirmed by immunoblot (Biorad, Munich, Germany).

HIV DNA was amplified from peripheral blood leukocytes by nested PCR according to Saiki *et al*[13]. Amplification of the HIV-1 proviral DNA was carried out as nested PCR with the following primers for the first PCR (sense: 5-ATTTGTCATCCATCCTATTTGTTCCTGAA GGGT-3, antisense: 5-AGTGGGGGGGACATCAAGC AGCCATGCAAAT-3) and with the following primers for the second PCR (sense: 5-TGCTATGTCACTTCCCCTT GGTTCTCT-3, antisense: 5-GAGACTATCAATGAGGA AGCTGCAGAATGGGAT-3). The amplified product was detected by agarose gel electrophoresis.

HIV load was determined quantitatively using the NucliSens HIV-QT assay (Organon Teknika, Boxtel, the Netherlands). Amplified patient and calibrator RNA were quantified with different electrochemiluminescent probes in the NASBA QR system (Organon Teknika, Boxtel, the Netherlands) based on competitive internal linear standard curves[14]. This assay had a detection limit of 80 copies/mL.

Diagnosis of HCV infection

HCV antibodies were detected with a microparticle enzyme immunoassay (MEIA, Axsym, Abbott, Wiesbaden, Germany). Positive results were confirmed by dot immunoassay (Matrix, Abbott, Wiesbaden, Germany). HCV RNA was detected after nucleic acid purification kit (Viral Kit, Qiagen, Hilden, Germany) followed by reverse transcription and nested polymerase chain reaction as described elsewhere[15]. The detection limit was 100 copies/mL. Quantitative determination of HCV load was done via branched DNA technology (Quantiplex HCV RNA 2.0 assay, Chiron, Emeryville, CA, USA), which has good linearity for all genotypes above its detection limit of 200 000 copies/mL serum. HCV genotypes were determined by the Innolipa II line probe assay (Innogenetics, Zwijndrecht, Belgium) according to the manufacturer's instructions.

Genotyping of the RANTES-403, -IN1.1, and -28 single nucleotide polymorphisms

Genomic DNA was extracted from 200 μL EDTA-

treated blood samples using the QIAamp Blood Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol.

Light Cycler PCR and hybridization probes: RANTES genotyping was performed using lightcycler and "fluorescence resonance energy transfer" (FRET) technology[16,17]. For amplification 2 μL light cycler-DNA master hybridization probes (Roche Diagnostics, Mannheim, Germany), 2 μL $MgCl_2$ (25 mmol/L), 8 μL PCR-grade water were used. For genotyping of the amplified DNA, 4 pmol of each hybridization probe (TIB MolBiol, Germany) was added to the reaction mixture. Sequences of oligonucleotide primers and hybridization probes for RANTES-403 were: sense *CACCTCCTTTGGGGACTGTA* and antisense *CCTCCGGAAATTCGAGTCTC*, anchor *GAGTCACTG AGTCTTCAAAGTTCCTGCTTA-F* and sensor *LC640-CATTACAgATCTTACCTCCTTTCCp*. The sensor hybridization probe was specific for the RANTES-403G allele at a melting temperature of 62.5 ℃.

Sequences of oligonucleotide primers and hybridization probes for RANTES-28 were: sense *CACCTCCTTTGGGGACTgTA* and antisense *TGGGATGGGGTAGGCATTCTA*, anchor probe *GTTGCTATTTTGGAAACTCCCCTTAGG-F* and sensor probe *LC705-ATGCCCCTGAACTGGCCp*. The sensor hybridization probe was specific for the RANTES-28G allele at a melting temperature of 60.0 ℃. Sequences of oligonucleotide primers and hybridization probes for RANTES-IN1.1 were: sense *CCTGGTCTTGACCACCACA* and antisense *GCTGACAGGCATGAGTCAGA*, anchor probe *LC640-CCCTCAAGGCCTACAGGTGTTCACp* and sensor probe *TCAGTTTTTCTGTCTTCAAGTCTAC-F*. The sensor probe was specific for the C allele at a melting temperature of 65 ℃.

An initial denaturation step at 95 ℃ (60 s, ramp rate 20 ℃/s) was followed by amplification for 40 cycles of denaturation (95 ℃, 0 s, ramp rate 20 ℃/s), annealing (63 ℃, 10 s, ramp rate 20 ℃/s) and extension (72 ℃, 15 s, ramp rate 20 ℃/s). For RANTES-IN1.1, the annealing temperature was lowered to 55 ℃. After DNA amplification a melting curve was generated at 95 ℃ (5 s, ramp rate 20 ℃/s) followed by 45 ℃ (15 s, ramp rate 20 ℃/s) and 75 ℃ (0 s, ramp rate 0.1 ℃/s, acquisition mode: continuous) for RANTES-403 and RANTES-IN1.1 and 95 ℃, 35 ℃, and 70 ℃ for RANTES-28. After a final cooling step for 30 s at 40 ℃ melting curve analysis could be performed. Representative genotyping results are given in Figures 1A-C.

Statistical analysis

RANTES genotypes and allele frequencies were compared to the healthy reference population via contingency tables using χ^2 statistics and Fisher's exact test where appropriate. Based on gene frequencies, the expected phenotype frequencies were calculated according to the Hardy–Weinberg equation and compared to the observed frequencies using χ^2statistics[18]. Haplotype analysis for combined RANTES-403 and -28 genotypes as in Table 3 was performed according to Gonzalez *et al*[19]. In each group, different genotypes of each RANTES polymorphism were compared with respect to HIV and HCV loads, CD4+ and CD8+ cell counts using parametric (unifactorial ANOVA with Bonferroni's correction) or non-parametric statistical analysis as appropriate (Kruskal-Wallis test followed by the Mann-Whitney test for pairwise comparison of the groups), if the number of patients with the particular genotype exceeded five. Results are given as median and range unless indicated otherwise.

In all statistical tests, $P<0.05$ were regarded as significant. All calculations were performed on a personal computer with SPSS 11.0 software (SPSS, Chicago, IL, USA).

RESULTS

Patient groups

One hundred and twelve anti-HCV positive, 85 anti-HIV positive, and 121 anti-HIV/HCV double-positive Caucasian patients were recruited into this study. The characteristics of these patient groups are summarized in Table 1. Hemophilia was the major risk factor for infection in HCV and HIV/HCV coinfected patients, whereas sexual transmission was the main risk factor in HIV infected patients. Rates of persistent HBs antigenemia was low in each study group (<5%), although the high prevalence of anti-HBc antibodies in the anti-HCV and the double infected groups indicated significant exposure to HBV (Table 1).

Distribution of RANTES-403, -28, and -IN1.1 genotypes

The distribution of RANTES-403, -28, and -IN1.1 alleles is shown in Table 2. The RANTES-403 A, RANTES-28 G, and RANTES-IN1.1 C alleles were more frequent in HIV monoinfected patients than in healthy controls as has been previously described[10]. The distribution of RANTES-403 genotypes in HIV infected patients differed significantly from controls ($P = 0.004$), whereas the RANTES-28 and RANTES-IN1.1 genotypes did not differ between the groups.

Unexpectedly, these higher frequencies of distinct RANTES alleles and genotypes were not observed in patients with HIV/HCV coinfection. RANTES-403 A, RANTES-28 G, and RANTES-IN1.1 C alleles were significantly less frequent than in HIV monoinfected patients ($P = 0.002$; $P = 0.048$; not significant). In contrast, the frequencies of the different RANTES alleles did not differ between HIV/HCV coinfected and HCV infected patients or healthy controls. This also held true for RANTES-403, -28, and IN1.1 genotype distribution ($P = 0.005$; $P = 0.045$; not significant). There was no deviation from the Hardy-Weinberg equilibrium in any of the groups.

Interestingly, the frequency of the combined RANTES wildtype (-403 G/G; -28 C/C; IN1.1 T/T) was lowest in HIV infected patients (49.4%) compared to healthy

Table 1 Patient Characteristics [data given as number (%) or median (range)]

	HCV-infection (Group I)	HIV-infection (Group II)	HCV/HIV-coinfection (Group III)	Statistical significance
Number	112	85	121	
Sex (Male / female)	108 / 4	73 / 12	117 / 4	Group I *vs* II $P = 0.009$*; group II *vs* III $P = 0.007$*
Age (median, range)	39 (13 - 77)	38 (23 - 70)	37 (21 - 62)	
Risk factors (%)				
Sexual	- - -	62 (72.9)	1 (0.8)	Group I *vs* II and II *vs* III $P < 0.001$*
Endemic	- - -	5 (5.9)	- - -	Group I *vs* II $P = 0.009$*; group II *vs* III $P = 0.011$*
I.V. drugs	- - -	4 (4.7)	3 (2.4)	Group I *vs* II $P = 0.023$*
Blood transfusion	- - -	1 (1.2)	- - -	
Hemophilia	110 (98.2)	- - -	115 (95.0)	Group I *vs* II $P < 0.001$*; group II *vs* III $P < 0.001$*
Unknown	2 (1.8)	13 (15.3)	2 (1.7)	Group I *vs* II $P < 0.001$*; group II *vs* III $P < 0.001$*
Aminotransferase activities				
ALT U/L (median, range)	34.0 (5.2 - 226.0)	17.0 (7.0 - 84.0)	38.5 (1.0 - 214.0)	Group I *vs* II and II *vs* III $P < 0.001$
AST U/L (median, range)	19.0 (5.8 - 157.0)	12.0 (4.3 - 106.0)	26.1 (7.8 - 168.0)	Group I *vs* II $P = 0.002$; group I *vs* III $P = 0.030$; Group II *vs* III $P < 0.001$
GGT U/L (median, range)	25.2 (5.2 - 350.9)	17.0 (6.2 - 149.0)	42.0 (2.9 - 254.6)	Group II *vs* III $P < 0.001$
HIV-status				
CD4 count (median, range)	668 (171 - 2 039)/μL	350 (5 - 1 142)/μL	292.5 (6 - 1 219)/μL	Group I *vs* II and I *vs* III $P < 0.001$
CD8 count (median, range)	499 (62 - 1 653)/μL	931 (88 - 2 152)/μL	804 (39 - 3 056)/μL	Group I *vs* II and I *vs* III $P < 0.001$
HIV load (median, range)	- - -	300 (<80 - 830 000)	725 (<80 - 210,000)	
HIV-viremia < 80 copies/mL	- - -	47 (55.3%)	80 (66.1%)	
Type of antiretroviral Therapy (%)				
Protease inhibitor-based HAART	- - -	62 (72.9)	58 (47.9)	Group II *vs* III $P = 0.001$
NNRTI-based HAART	- - -	11 (12.9)	25 (20.7)	
Total	- - -	73 (85.9)	83 (68.6)	Group II *vs* III $P = 0.007$
HCV-status				
HCV load (copies/mL)	8 888 000 (n.d.-126,500,000)	- - -	13 535 000 (n.d.-178 900 000)	Group I *vs* III $P = 0.012$
HCV load (IU/mL)	1 410 794 (n.d.-20,079,365)	- - -	2 148 413 (n.d.-28 396 825)	
HCV-genotypes (%)				
Genotype 1	70 (62.5)	- - -	76 (62.8)	
Genotype 2	14 (12.5)	- - -	11 (9.1)	
Genotype 3	8 (7.1)	- - -	21 (17.4)	Goups I *vs* III $P = 0.028$*
Genotype 4	6 (5.4)	- - -	5 (4.1)	
Multiple genotypes	1 (0.9)	- - -	1 (0.8)	
Undeterminded genotype	13 (11.6)	- - -	7 (5.8)	
HBV-status (%)				
Anti-HBs+ and anti-HBc+	52 (46.4)	15 (17.6)	43 (35.5)	Group I *vs* II $P < 0.001$; group II *vs* III $P = 0.008$
Anti-HBc+ alone	10 (8.9)	5 (5.9)	34 (28.1)	Group I *vs* III $P < 0.001$; group II *vs* III $P < 0.001$*
HBs-Ag+	1 (0.9)	3 (3.5)	6 (5.0)	

* Fisher´s exact test; n.d. = not detected.

controls (69.9%; $P = 0.007$), but also compared to HCV (63.4%; not significant) and HIV/HCV coinfected patients (70.4%; $P = 0.004$). The [-403 G/A; -28 C/G; IN1.1 T/C] combination genotype had the highest prevalence in HIV infected patients (9.6%) differing significantly from HCV (0.9%; $P = 0.005$) and HIV/HCV coinfected patients (1.7%; $P = 0.015$) as well as controls (4.9%; not significant).

When haplotype analysis for RANTES promoter polymorphisms was performed as described previously by Gonzalez *et al*[19], the RANTES high producer haplotype (-403A, -28G) was most frequent in HIV monoinfected patients, whereas in HIV/HCV coinfected patients the frequency of this haplotype was similar in HCV infected patients and healthy controls (Table 3). The same finding also applies when the frequency of the (-403A, -28G) haplotype was compared between HIV and HIV/HCV infected patients ($P = 0.006$), between HIV and HCV infected patients ($P = 0.002$) and between HIV infected patients and controls ($P = 0.019$).

Effects of the RANTES-403, -28 and -IN1.1 mutations on parameters of HIV and HCV infection

HCV and HIV viral loads as well as the numbers of CD4+ and CD8+ cell counts are given in Table 4 stratified according to RANTES genotypes. There was a trend towards higher HIV loads in HIV infected patients with the RANTES-403 G/G genotype (wildtype) compared to subjects with the G/A and A/A genotype. Furthermore, HIV infected patients with the RANTES-28 C/G genotype tended to have lower HIV loads than patients with the C/C genotype (wildtype), whereas the RANTES-IN1.1 polymorphism did not affect HIV or HCV viral loads. However, statistical analysis could not be performed for RANTES-403 A/A and RANTES-IN1.1 C/C genotypes in HCV and HCV/HIV coinfection, because these genotypes were rare in each of the groups. CD4+ and CD8+ cell counts were not affected by any of the different RANTES genotypes. Finally, HIV and HCV viral loads did not differ significantly between the different haplotypes (Figure 1).

Table 2 Distribution of RANTES-403, RANTES-28 and RANTES IN1.1 genotypes and allele frequencies

RANTES-403 (%)	G/G	G/A	A/A	Statistics	[A]-allele frequency (%)	Statistics
HCV (n = 112)	71 (63.4)	39 (34.8)	2 (1.8)	*vs* HIV P = 0.077	19.2	*vs* HIV P = 0.047
HIV (n = 85)	42 (49.4)	38 (44.7)	5 (5.9)		28.2	
HCV/HIV (n = 120)	86 (71.7)	31 (25.8)	3 (2.5)	*vs* HIV P = 0.005	15.4	*vs* HIV P = 0.002
Controls (n = 109)	77 (70.6)	31 (28.4)	1 (0.9)	*vs* HIV P = 0.004	15.1	*vs* HIV P = 0.002
RANTES-28 (%)	C/C	C/G	G/G	Statistics	[G]-allele frequency	Statistics
HCV (n = 112)	108 (96.4)	4 (3.6)	0 (0.0)	*vs* HIV P = 0.078	1.8	*vs* HIV P = 0.083
HIV (n = 83)	74 (89.2)	9 (10.8)	0 (0.0)		5.4	
HCV/HIV (n = 116)	112 (96.6)	4 (3.4)	0 (0.0)	*vs* HIV P = 0.045	1.7	*vs* HIV P = 0.048
Controls (n = 109)	103 (94.5)	6 (5.5)	0 (0.0)	*vs* HIV P = 0.186	2.8	*vs* HIV P = 0.195
RANTES-In1.1 (%)	T/T	T/C	C/C	Statistics	[C]-allele frequency	Statistics
HCV (n = 112)	87 (77.7)	25 (22.3)	0 (0.0)	*vs* HIV P = 0.054	12.6	*vs* HIV P = 0.041
HIV (n = 84)	53 (63.1)	30 (35.7)	1 (1.2)		19	
HCV/HIV (n = 121)	93 (76.9)	27 (22.3)	1 (0.8)	*vs* HIV P = 0.101	12	*vs* HIV P = 0.066
Controls (n = 109)	86 (78.9)	22 (20.2)	1 (0.9)	*vs* HIV P = 0.052	11	*vs* HIV P = 0.038

Table 3 Distribution of RANTES-403/-28 haplotypes

Haplotype 403	28	HIV	HIV/HCV	HCV	Controls
G	C	71.7%	84.0%	80.8%	84.2%
A	G	5.4%	1.3%	1.8%	2.9%
A	C	22.9%	14.2%	17.4%	12.9%
G	G	- - -	0.5%	- - -	- - -

Haplotype pair (-403/-28)		HIV[1,2,3]	HIV/HCV	HCV	Controls
G/C	G/C	41 (49.4%)	82 (70.7%)	71 (63.4%)	73 (69.5%)
G/C	G/G	- - -	1 (0.9%)	- - -	- - -
G/C	A/C	28 (33.7%)	28 (24.1%)	37 (33.0%)	25 (23.8%)
G/C	A/G	9 (10.8%)	2 (1.7%)	2 (1.8%)	6 (5.7%)
A/C	A/C	5 (6.0%)	2 (1.7%)	- - -	1 (1.0%)
A/C	A/G	- - -	1 (0.9%)	2 (1.8%)	- - -

[1] *vs* HIV P = 0.002; [2] *vs* HIV/HCV P = 0.006; [3] *vs* controls P = 0.019.

DISCUSSION

RANTES polymorphisms have been proposed to modify susceptibility to HIV infection and progression to AIDS[10,11,19]. In this study, the prevalence of all three polymorphic RANTES alleles (RANTES-403 A, RANTES-28 G, and RANTES-IN1.1 C) was higher in HIV monoinfected patients compared to healthy blood donors. In contrast, in HIV/HCV coinfection the frequency of the studied RANTES alleles did not differ from HCV monoinfection or healthy controls. Since we included patients with HCV monoinfection as well as a cohort of healthy blood donors into the study to exclude biased results as a consequence of HCV infection or allele frequency of the background population, the influence of RANTES alleles on HIV infection seems to be neutralized by concomitant HCV infection.

The increased frequency of distinct polymorphic RANTES alleles in HIV infection is in line with McDermott *et al*[20]. who observed increased susceptibility to HIV infection in patients with RANTES-403 G/A -28 C/C haplotypes. The prevalence of the RANTES-28 G allele in all groups was as low as previously described by An *et al*[10]. and none of our patients was homozygous for the RANTES-28 G allele[11].

The RANTES-IN1.1 C allele has been reported to be associated with an increased susceptibility for infection with HIV, which would explain the significantly increased frequency of the C allele in our HIV monoinfected patients[10]. However, none of the different RANTES alleles was significantly associated with altered HIV viral loads or CD4+ cells counts. In this context, we cannot rule out that we have missed an association between viral loads or CD4 counts and the polymorphic RANTES alleles, because our study group was rather small and not well balanced for antiviral therapy. To exclude that the presence of the CCR5-△32 allele which probably also affects susceptibility to HCV infection has influenced our findings, we tested whether any of the RANTES polymorphisms was associated or in linkage disequilibirum with CCR5-△32[5]. However, we could not find any associations between CCR5-△32 and the RANTES alleles.

The low frequency of the RANTES alleles -403A and -28G in our HCV infected patients is in accordance with Promrat *et al*[21]. who also could not find any association of RANTES-403 and –28 polymorphisms with susceptibility to HCV infection or laboratory parameters of

Table 4 Viral loads and immunological data in different RANTES-403, RANTES-28 and RANTES-IN1.1 genoytpes. Data are given as [median (range)]. Statistically significant differences are indicated by index numbers

	HCV-Infection			HIV-Infection			HIV/HCV-Infection		
RANTES-403	G/G	G/A	A/A	G/G	G/A	A/A	G/G	G/A	A/A
	(*N* = 71)	(*N* = 39)	(*N*= 2)	(*N*= 42)	(*N* = 38)	(*N* = 5)	(*N* = 86)	(*N* = 31)	(*N* = 3)
HCV-Load	10.8	4.2	39.0	- - -	- - -	- - -	13.8	14.0	1.6
x 10^6 copies/mL	(n.d.-126.5)	(n.d.-56.6)	(n.d.-39.8)				(0.2-178.9)	(0.6-150.5)	(0.4-6.8)
HIV-Load	- - -	- - -	- - -	455	380	<80	550	770	9900
x 10^3 copies/mL				(<80-830 000)	(<80-50 000)	(<80-990)	(<80-210 000)	(<80-50 000)	(2 400-27 000)
CD4 count	683	621	916	353.5	348.5	300	302	279	293
cells/μL	(171-2 039)	(282-1 544)	(787-1 045)	(51-1 111)	(5-1 142)	(28-629)	(6-1 219)	(106-651)	(149-331)
CD8 count	496.5	499	581.5	918.5	927	1 099	792	968.5	675
cells/μL	(62-1 653)	(185-1 183)	(425-738)	(202-1 717)	(88-2 152)	(382-1 421)	(39-2 466)	(319-3 056)	(465-895)
RANTES-28	C/C	C/G	G/G	C/C	C/G	G/G	C/C	C/G	G/G
	(*N* = 108)	(*N* = 4)	(*N* = 0)	(*N* = 74)	(*N* = 9)	(*N* = 0)	(*N* = 112)	(*N* = 4)	(*N* = 0)
HCV-Load	8.4	38.3	- - -	- - -	- - -	- - -	13.5	22.3	- - -
x 10^6 copies/mL	(n.d.-126.5)	(29.9-39.8)					(0.2-178.9)	(0.4-45.8)	
HIV-Load	- - -	- - -	- - -	310	<80	- - -	560	2 150	- - -
x 10^3 copies/mL				(<80-830 000)	(<80-3 700)		(<80-210 000)	(400-91 000)	
CD4 count	662	916	- - -	355	330	- - -	293	220	- - -
cells/μL	(171-2 039)	(443-1 305)		(10-1 142)	(141-637)		(13-1 219)	(149-651)	
CD8 count	499	581.5	- - -	942.5	636	- - -	828	602.5	- - -
cells/μL	(62-1 653)	(338-739)		(202-2 152)	(362-1 997)		(39-3 056)	(397-2 401)	
RANTES-IN1.1	T/T	T/C	C/C	T/T	T/C	C/C	T/T	T/C	C/C
	(*N* = 87)	(*N* = 25)	(*N* = 0)	(*N* = 53)	(*N* = 30)	(*N* = 1)	(*N* = 93)	(*N* = 27)	(*N* = 1)
HCV-Load	9.6	6.2	- - -	- - -	- - -	- - -	13.5	14.2	1.6
x 10^6 copies/mL	(n.d.-126.5)	(0.4-56.6)					(0.2-178.9)	(0.4-150.5)	
HIV-Load	- - -	- - -	- - -	600	<80	990	735	560	9 900
x 10^3 copies/mL				(<80-830 000)	(<80-38 000)		(<80-210 000)	(<80-91 000)	
CD4 count	662	702	- - -	353	342	629	294	242	331
cells/μL	(171-2 039)	(392-1 305)		(51-1 142)	(10-1 028)		(6-1 219)	(110-651)	
CD8 count	501	497	- - -	936.5	927	1 421	792	1 034	675
cells/μL	(62-1 653)	(191-1 119)		(202-1 717)	(362-2 152)		(39-2 466)	(319-3 056)	

n.d. = not detectable

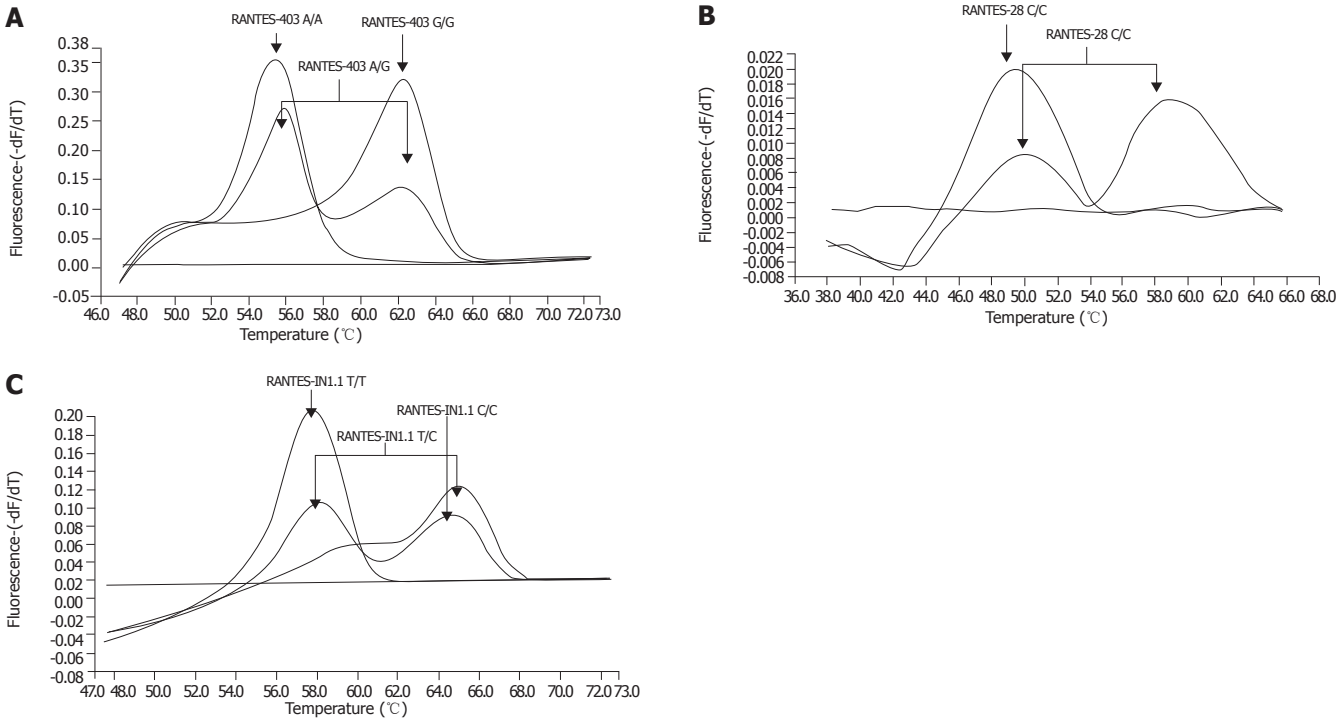

Figure 1 Shows the melting curve analysis (first order derivate) for different genotypes of the RANTES-403 (**A**), RANTES-28 (**B**), and RANTES-IN1.1 (**C**) polymorphisms. When the PCR product is melted, the probe-DNA binding will break up at a hybridization probe-specific temperature, which causes a loss in emitted fluorescence energy. This can be seen as a peak in the first order derivate of the melting curve. As the sensor probe is specific for only one of the two possible alleles in each PCR, binding of the probe to the other allele will cause a mismatch and therefore a weaker coupling and disengagement of probe and DNA at lower temperatures.

chronic hepatitis C. In contrast, Hellier *et al*[6]. reported the RANTES-403 A/A genotype to be associated with mild portal inflammation. However, the frequency of the A/A genotype in the study of Hellier (1.9%) was not different from the frequency in our patients[6]. Therefore, it was unexpected that the prevalence of RANTES alleles known to be increased in patients with HIV monoinfection was significantly lower in our HIV/HCV coinfected patients. Additionally, prevalence of these alleles was similar to HCV monoinfected patients and controls.

Several explanations may account for these divergent results: First, none of the studies so far was controlled for differences in transmission routes. Therefore, it is unclear whether disease modifying effects of the RANTES polymorphisms are equally operative in patients with sexual transmission *vs* parenteral transmission.

Alternatively, our patients with HIV/HCV coinfection could represent long time survivors: The RANTES-IN1.1 C allele has been described to be associated with rapid progression to AIDS, whereas both the RANTES-403 A and the RANTES-28 G allele have been reported to delay HIV disease progression[10,11,19]. However, frequency of these polymorphic RANTES alleles did not differ from our background population. Finally, differences in genotype and allele frequencies between HIV and HIV/HCV coinfected patients might reflect differences in the physiological roles of the single polymorphisms. RANTES-403 A and RANTES-28 G alleles have been shown to up-regulate RANTES transcription, whereas RANTES-IN1.1 C has been reported to be associated with the down-regulation of RANTES promoter activity[10-12]. Thus, RANTES expression should be characterized in terms of haplotypes, defined by the combination of the various alleles, giving the possibility to explain selection effects on the course of HIV infection[10]. However, only haplotypes AC and AG were more prevalent in patients with HIV infection, whereas their frequencies in patients with HCV and HIV/HCV coinfection were identical to the healthy background population.

The selection effect of the RANTES alleles in HIV infection may be lost in patients with HCV coinfection, because interactions of HCV-specific proteins such as core and NS5A may interact with host genes to augment RANTES promotor activity[22]. Binding of the HCV-specific protein E2 to CD81 is associated with increased RANTES serum levels resulting in CCR5 internalization[23]. Therefore, HCV infection is likely to trigger increased RANTES serum levels, which in turn decrease CCR5 expression on the cell surface due to receptor internalization. This hypothesis is further supported by a recent study that shows reduced CCR1 and CCR5 surface expression on peripheral blood cells in chronic HCV infection[24]. Thus, it is intriguing to speculate that the effects of RANTES polymorphisms on RANTES expression in HIV infection are abrogated in HIV/HCV coinfection due to induction of RANTES by HCV-specific proteins. In line with this assumption, we observed reduced frequencies of patients carrying the putatively up-regulating (RANTES-403 and RANTES-28) as well as down-regulating RANTES polymorphisms (RANTES-IN1.1) in our patients with HCV and HIV/HCV coinfection.

In summary, deviations in polymorphic RANTES allele frequencies seen in HIV infection could not be confirmed in HIV/HCV coinfection. Functional studies will be required to further analyze the differences in RANTES regulation between HIV and HIV/HCV coinfection at a molecular level.

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