



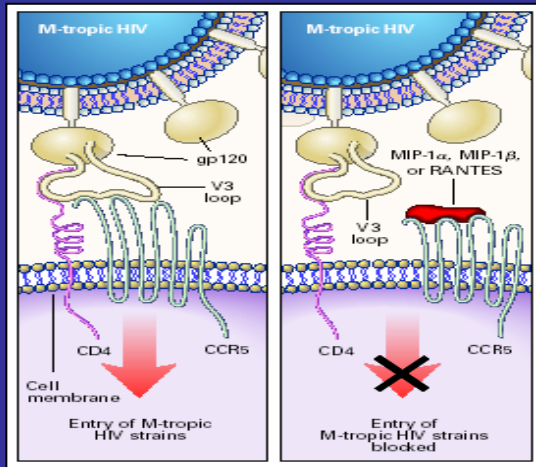
Role of RANTES Gene Polymorphisms in HIV Infection is neutralized by HCV Coinfection

Ahlenstiel G*, Iwan A*, Nattermann N*, Rockstroh JK*, Brackmann HH#, Kupfer B\$, Matz B\$, Landt O\$, Sauerbruch T*, Spengler U* & Woitas RP*. Department of *Internal Medicine 1, #Institute of Experimental Hematology and § Institute of Medical Microbiology and Immunology, University of Bonn, Bonn, \$TIB MOLBIOL Synthesis Laboratory, Berlin.

Introduction

The chemokine RANTES is a natural ligand of the chemokine receptor 5 (CCR5) and exerts immunologic function via internalization of this receptor. Furthermore, as CCR5 is also a coreceptor for HIV, RANTES can effectively block cell entry of HIV by binding to CCR5 (figure 1).

Figure 1



(Luster ADL, NEJM 1998)

The natural course of HIV infection is altered by RANTES gene polymorphisms through up-regulation (RANTES-403 and RANTES-28) or down-regulation (RANTES-IN1.1) of chemokine expression. As RANTES is involved in the recruitment of HCV-specific T cells to the liver in hepatitis C, such mutations might also alter the course of chronic HCV and HCV/HIV coinfection.

Methods

We determined the frequency of the RANTES-403 alleles using real time PCR and hybridization probes in patients with HIV infection (n=85), HCV infection (n=112), HIV/HCV co-infection (n=121), and 109 healthy blood donors. Each group was stratified according to genotype and allele frequency, respectively. Finally, resulting subsets were compared with respect to HIV and HCV loads.

RANTES-403 Genotyping: Sequences of oligonucleotide primers were: CACCTCCTTTggggACTgTA (sense) and CCTCCggAAATTCgAgTCTC (antisense). For genotyping of the amplified DNA the following hybridization probes were used: anchor: gAgTCACTgAgTCTTCAAAGTTCCTgCTTA-X; sensor: LC Red 640-CATTACAgATCTTACCTCCTTTCC-p (TIB MolBiol, Berlin Germany). The sensor hybridization probe was specific for the RANTES-403 G allele at a melting temperature of 62.5°C.

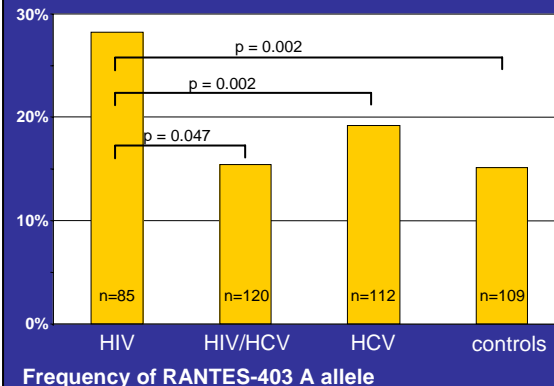
RANTES-28 Genotyping: Sequences of oligonucleotide primers were: CACCTCCTTTggggACTgTA (sense) and CCTCCggAAATTCgAgTCTC (antisense). For genotyping of the amplified DNA the following hybridization probes were used: anchor: gAgTCACTgAgTCTTCAAAGTTCCTgCTTA-X; sensor: LC Red 705-CATTACAgATCTTACCTCCTTTCC-p (TIB MolBiol, Berlin Germany). The sensor hybridization probe was specific for the RANTES-28 G allele at a melting temperature of 60.0°C.

RANTES-IN1.1 Genotyping: Sequences of oligonucleotide primers were: CCTggTCTTgACCACCACA (sense) and gCTgACAggCATgAgTCAGa (antisense). For genotyping of the amplified DNA the following hybridization probes were used: anchor: TCAgTTTTTCgTCTTCAAAGTCTAC-X; sensor: LC Red CCCTCAAaggCCTACAggTgTTCAC-p (TIB MolBiol, Berlin Germany). The sensor hybridization probe was specific for the RANTES-IN1.1 C allele at a melting temperature of 65.0°C.

Results

The frequencies of the RANTES-403 A, -28 G and -IN1.1 C alleles are given in tables 1 to 3. In particular the RANTES-403 A (15.4%), RANTES-28 G (1.7%) and RANTES-IN1.1 C (12.0%) alleles were less common in HIV/HCV coinfecting patients than in HIV mono-infected individuals (28.2%, p=0.002; 5.4%, p=0.048; 19.0%, n.s.), although the frequencies did not differ from HCV infected patients (19.2%; 1.7%; 12.6%) and healthy controls (15.1%; 2.8%; 11.0%). These differences were matched by the distribution of genotypes for all three mutations. There was no deviation from the Hardy-Weinberg equilibrium. The [-403 G/A -28 C/G -IN1.1T/C] RANTES haplotype had the highest prevalence in HIV mono-infected individuals (9.6%) compared to HCV (0.9%; p=0.005), HIV/HCV coinfecting patients (1.7%; p=0.018) and healthy controls (4.9%), whereas the RANTES wildtype haplotype [-403 G/G -28 C/C -IN1.1T/T] was more frequent in HCV (63.4%) and HIV/HCV coinfecting patients (70.4%; p=0.009) and healthy controls (69.9%; p=0.007) than in HIV mono-infected patients (49.4%). HIV infected patients with the RANTES-403 G/G showed a trend to higher HIV loads compared to RANTES-403 A/A (p=0.067). In contrast, HCV viral loads were significantly higher in HCV/HIV coinfecting patients with RANTES -403 G/A than in RANTES -403 A/A patients (p=0.045).

Figure 1



Discussion

The RANTES-403 A, RANTES-28 G and RANTES-IN1.1 C alleles are more frequent in HIV mono-infection than in HCV infection and healthy controls. Interestingly, in HCV/HIV co-infection this effect was not observed as the allele frequencies were similar to those in HCV infection and healthy controls. Thus, the influence of this polymorphism in HIV infection seems to be abrogated by a concomitant HCV infection. As HCV-NS5A and HCV-core proteins have been shown to augment RANTES promoter activity in vitro, the low frequency of polymorphic RANTES alleles in HCV/HIV coinfection might be due to a counteractive effect of viral proteins on RANTES transcription.

Figure 2

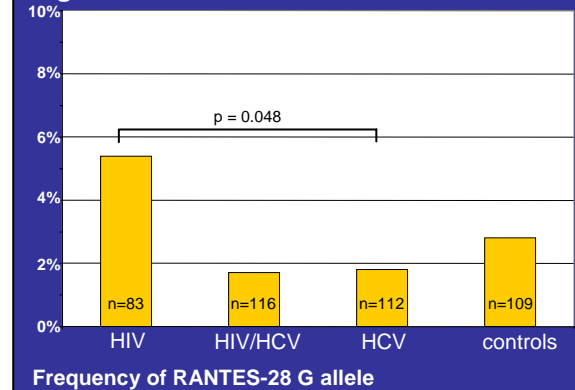


Figure 3

