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Exploring the interplay of chemokine receptors ccr5 and cxcr6 in mechanisms of natural control in HIV-1- infected black South Africans

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2024
https://hdl.handle.net/10539/37572

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Abstract

In sub-Saharan Africa, HIV-1 is a significant cause of morbidity and mortality. However, research remains primarily focused on North American and European population groups, who have remarkably different genetic backgrounds to individuals from sub-Saharan Africa. HIV-1 controllers represent a model of HIV-1 functional cure, with some individuals able to control viral replication, and some able to sustain immune function in the presence of high viral loads, both in the absence of antiretroviral therapy (ART). The chemokine receptors CCR5 and CXCR4 are the major coreceptors HIV-1 utilises to enter cells. The use of alternative coreceptors, such as the CXCR6 coreceptor, is thought to contribute to the lower pathogenicity exhibited by the HIV-2 and SIVsmm strains. Building on previous work conducted in our research unit on these two coreceptors in South African populations, this thesis firstly describes *CCR5* genetic variants that associate with HIV-1 control or risk of progressive infection in black South Africans, and then explores constitutive expression levels of CCR5 and CXCR6 on various peripheral blood immune cell subsets in the absence of HIV-1 infection in ethnically divergent population groups. The effect of sex, age, and select *CCR5* and *CXCR6* single nucleotide polymorphisms (SNPs) on expression levels of these two receptors was also investigated.

The *CCR5* 5'UTR and 3'UTR regions were PCR-amplified and sequenced from genomic DNA extracted from 145 ART-naive black South African individuals living with HIV-1 (71 HIV-1 controllers – 23 elite controllers, 37 viraemic controllers, 11 high viral load long-term non-progressors and 74 progressors). Findings confirmed results from other studies in showing that the *CCR5* HHE haplotype is deleterious for HIV-1 disease progression, and the HHA haplotype and HHA/HHC genotype associated with protection from HIV-1 disease progression. Novel haplotypes were characterised, both in the 3'UTR and spanning the *CCR5* 5'UTR and 3'UTR. Overall, findings suggest that two *CCR5* promoter SNPs (-2459 G>A and -2135 T>C) and one *CCR5* 3'UTR SNP (+2919 T>G) may be key functional variants with regards to HIV-1 control in black South Africans.

To gain further insight into the constitutive expression of CCR5 and CXCR6 on peripheral blood immune cells and explore the relationship between select genetic variants and expression, immunophenotyping by flow cytometry was conducted using whole blood from age- and sex-matched ethnically distinct South African HIV-uninfected individuals (17 black, 21 white). Expression levels of CCR5 and CXCR6 were assessed on CD4+ and CD8+ T cells,

B cells, monocytes and NK cells, and their respective subsets. The effects of age and sex on expression levels of these two receptors was also investigated. Population-specific differences with regards to CCR5 expression on all cell types, except for B cells, were evident. Generally, black South Africans exhibited a lower expression level of CCR5 compared to white South Africans. CXCR6 expression only differed with regards to percentage of CXCR6-expressing cells, not CXCR6 density (numbers of cell surface receptors). Black individuals had a lower percentage of CXCR6-expressing CD8+ T cell subsets (naïve and effector memory) and a higher percentage of CXCR6-expressing $CD14^{+}CD16^{+}$ monocytes compared to white individuals. Overall, we found significant population-specific differences in expression levels of both CCR5 and CXCR6, multiple associations with cell activation (as measured by HLA-DR expression) and CCR5 and CXCR6 expression, and CCR5 and CXCR6 expression was positively significantly correlated on multiple cell subsets.

Furthermore, both sex and age influenced CCR5 and CXCR6 expression, however results varied widely across the two population groups studied. Sex differences were only evident in white individuals; predominantly CXCR6 expression was increased in males compared to females. Age associations with CCR5 and CXCR6 expression were also primarily found in white individuals.

Four *CCR5*-related SNPs that are associated with HIV-1 control in this or other studies (rs553615728 -4223 C>T SNP, rs1799987 −2459 G>A SNP, rs746492 +2919 T>G SNP and rs1015164 G>A SNP) were assessed for their potential association with CCR5 expression levels. The +2919 TG genotype significantly associated with a higher percentage of CCR5-expressing total CD8+ T cells, transitional memory and terminally differentiated CD8+ T cells compared to the GG genotype. The +2919 GG genotype associated with a lower percentage of CCR5-expressing B cells compared to the TT and TG+TT genotypes, however, only in white South Africans. The +2919 TG and TG+TT genotypes associated with significantly higher CCR5 density on all CD8+ T cell subsets, except for naïve CD8+ T cells, when compared to the GG genotype.

When evaluating two *CXCR6* genetic variants previously associated with HIV-1 viraemic control (rs2234355 G>A and rs2234358 G>T) in relation to CXCR6 expression, possession of the rs2234355 SNP GA genotype associated with lower CXCR6 expression on select CD4+ and CD8+ T cell subsets as well as on B cells, while possession of the rs2234358 SNP TT genotype associated with higher CXCR6 expression on multiple cell types, primarily in white

South Africans. Possession of the -358TT/+355GA genotype combination associated with lower CXCR6 expression on select subsets of CD4+ T cells and monocytes.

In summary, this study provides information on genetic variation in the *CCR5* gene in a South African context, describes genetic variants associating with HIV-1 control in black South Africans, adds novel insight into constitutive CCR5 and CXCR6 expression levels on CD4+ and CD8+ T cells, B cells, monocytes and NK cells in HIV-1-uninfected black and white South Africans, and describes the potential associations of select genetic variants and expression. Black and white individuals differed in their baseline expression levels of CCR5 or CXCR6, which was partly driven by host genetic factors that were explored. This work highlights the importance of considering effects of ethnicity, age, and sex in any studies addressing any immune molecules in relation to differential HIV-1 outcomes of infection susceptibility/protection, disease progression, or HIV-1 virological control on antiretroviral therapy. Although conducted on small numbers of individuals, these variables clearly influenced constitutive expression of CCR5 and CXCR6, and further population-specific studies are warranted to gain further insights. Findings from this study have implications for risk of acquisition of HIV-1 infection and for disease progression in people living with HIV-1. Understanding the role of these molecules is important for informing strategies for both HIV-1 prevention and HIV cure.