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Variation in the LBP-CD14-TLR4-LY96 gene complex and consequences of microbial translocation in HIV-1 infected black South Africans

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ABSTRACT

Persistent immune activation and inflammation in people living with HIV-1 (PLWH) has been associated with higher morbidity and mortality, even in individuals on antiretroviral therapy (ART). Microbial translocation, among other factors, has been identified as a major driver of persistent immune activation. A subgroup of PLWH collectively known as HIV-1 controllers can naturally control the HIV-1 infection without the use of ART. Little is known about the extent and the role of microbial translocation/immune activation in African HIV-1 controllers. Translocated lipopolysaccharide (LPS), a component of gram-negative bacterial cell walls elicits innate immune responses through the activation of the toll-like receptor 4 (TLR4) in a complex pathway, which requires the use of cluster of differentiation 14 (CD14), LPS binding protein, and Lymphocyte antigen 96 (LY96) also known as Myeloid differentiation factor 2 (MD-2). Although numerous studies have reported associations of expression levels of the LPS recognition and signalling molecules as well as variants in the genes encoding for these molecules, with the risk and severity of various inflammatory, autoimmune, and infectious diseases, such studies are limited in African populations. Given the large genetic diversity in African populations, characterisation of both the constitutive expression levels and genetic variation in these molecules is essential to understanding HIV-1 infection in the populations most affected by the AIDS epidemic.

We quantified constitutive expression of cell surface TLR4 and CD14 (mCD14) on monocytes and neutrophils using flow cytometry and quantified plasma levels of soluble CD14, LBP, and MD-2 using commercially available ELISA kits in two ethnically divergent South African populations [healthy HIV-1 uninfected black (n=17) and white (n=21) individuals]. Furthermore, the influence of sex and age on the expression levels of these molecules was also investigated. We found higher LBP plasma levels in black South Africans compared to white South Africans ($p<0.0001$), however these two populations did not differ significantly in expression levels of CD14 (mCD14 and sCD14), TLR4, or MD-2. Sex differences in the TLR4 expression levels, with higher TLR4 on total monocytes ($p=0.016$) and $CD14^{+}1CD16^{-}$ ($p=0.009$) and $CD14^{+}CD16^{+}$ ($p=0.009$) subsets of monocytes in females compared to males were observed in the white South African population but not in the black South African population. Significant population and sex-specific negative correlations between age and CD14 expression on monocytes, monocyte subsets and neutrophils, and TLR4 expression on neutrophils were observed. In addition, we found that there is differential regulation of TLR4 expression on monocytes and neutrophils between black and white South Africans post

stimulation with lipopolysaccharide (LPS) and lipoteichoic acid (LTA). Together, these findings suggest that population differences in plasma levels of LBP, and population-specific sex differences in TLR4 expression, are likely to differentially impact TLR4 functionality.

Using whole genome sequencing data (WGS), we next sought to fully describe the genetic variation and linkage disequilibrium (LD) patterns in the *LBP*, *CD14*, *TLR4*, and *LY96* genes in HIV-1 uninfected black South Africans (n=87, SA controls), and compared the representation of the variants to select populations from the 1000 Genomes Project. Our results revealed that the representation of genetic variants and LD patterns across these genes in the SA black population more closely mirrored those of representative African subpopulations (Yoruba in Ibadan, Nigeria, and Luhya from Webuye, Kenya) than the European and Asian populations. These findings emphasize that there are vast genetic differences in African populations compared to non-African populations, which could differentially affect gene regulation and associations with various diseases. Several novel variants and putative haplotypes were identified in the SA black population which, upon verification in future studies, will serve to add to understanding the genetic diversity in this population group.

Using WGS data, we also assessed the representation of the *LBP*, *CD14*, *TLR4* and *LY96* gene variants in a cohort of black South African ART-naïve HIV-1 controllers (n=39) comprised of elite controllers (n=21), viraemic controllers (n=6), and high viral load long-term non-progressors (n=12), relative to the SA controls. Only one *CD14* 5' flanking region SNP (rs186291587) showed a significant difference in minor allele frequency (MAF) representation in elite controllers when compared to SA controls (p=0.024; OR=13.3, CI: 1.3 – 131.4). The representation of several *TLR4* variants showed significant differences when HIV-1 controllers were compared to SA controls and the most significant differences were predominantly found in comparison to the HVL LTNPs - the most significant difference observed was overrepresentation of two SNPs in complete LD (r^2=1), a newly identified intronic variant (*TLR4* NI-2), and a 3' flanking region SNP (rs113017335) in HVL LTNPs compared to SA controls (p=0.006; OR=24.71, CI: 2.46-248.51). The representation of several *LBP* variants also differed between HIV-1 controllers and SA controls, here predominantly when viraemic controllers were compared to SA controls. Minor allele frequency overrepresentation of the *LBP* intronic SNP (rs1250247980) in the total group of HIV-1 controllers (p=0.003), and viraemic controllers (p=0.0002), relative to the SA controls, was the most significant difference observed. Furthermore, differences in the representation of *LY96* variants were observed when the total group of HIV-1 controllers, elite controllers and HVL LTNPs were compared to SA

controls - the most significant difference observed was the MAF and heterozygosity overrepresentation of an intronic SNP (rs149605245) in elite controllers compared to SA controls (MAF: p=0.007; heterozygosity: p=0.007). These results suggest a potential role of the LPS recognition and signalling molecules in natural HIV-1 control.

Lastly, in ART-naïve black South African elite controllers (n=44), HVL LTNPs (n=12), progressors (24), and in HIV-1 uninfected controls (HUCs, n=17), we measured and compared plasma levels of select innate immune molecules that are considered markers of microbial translocation and gut damage (LBP, sCD14, REG3α), or are important in interacting with TLR4 (MD-2). We found no differences between groups in plasma levels of LBP and MD-2. However, sCD14 was significantly higher in progressors compared to all groups (HUCs, p=0.0001; ECs, p<0.0001; HVL LTNPs, p=0.0005), with no differences between HIV-1 uninfected controls, elite controllers and HVL LTNPs. Plasma levels of REG3α were unexpectedly significantly lower in progressors compared to elite controllers (p=0.007) and HVL LTNPs (p=0.018), however similar to HIV-1 uninfected controls (p>0.05). Marked sex-specific differences in REG3α levels were evident, with females having significantly higher levels compared to males in all groups (HUCs and ECs, p<0.0001; HVL LTNPs, p=0.036; progressors, p=0.005). Our data suggests that in black South Africans, REG3α plasma levels are not a reliable marker of gut damage, and that increased levels in elite controllers and HVL LTNPs might contribute to protection from excessive systemic activation in the presence of microbial translocation, consistent with reduced monocyte activation in these groups. Progressors, on the other hand, appear to have an inability to produce REG3α while having substantial monocyte activation. Our findings highlight the importance of sex differences, and that studies conducted in populations of different ethnic backgrounds are often not directly comparable.

Overall, findings presented in this thesis contribute to the understanding of the baseline expression levels and the genetic diversity in the *LBP*, *CD14*, *TLR4*, and *LY96* gene complex in the black South African population, and the representation of these variants in black South African HIV-1 controllers. This thesis also highlights the importance of taking ethnicity, sex, and age into consideration when exploring measures that quantify biological parameters. Understanding of the molecules important in the TLR4 signalling pathway can help elucidate approaches that could contribute to curbing immune activation in the context of HIV-1 infection, as well as other diseases.