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Polymorphisms in IRF7 reduce IFN α responses of pDCs to HIV-1

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Abstract

Recognition of HIV-1 ssRNA by TLR7 induces the production of the pro-inflammatory cytokines that may contribute to the systemic immune activation associated with HIV-1 disease progression. Here we describe a novel association between polymorphisms in IRF7, a master regulator of IFN α , and the ability of pDCs to produce IFN α in response to HIV-1. IRF7 polymorphisms may therefore affect the ability of individuals to respond to HIV-1 and alter HIV-1 disease progression.

Immune activation is a major determinant for HIV-1 disease progression, and toll-like receptor (TLR) ligands have been implicated in mediating HIV-1 associated immune activation [1, 2]. Plasmacytoid dendritic cells (pDCs) can play a central role in this HIV-1-induced activation of the immune system, as they can sense human immunodeficiency virus type 1 (HIV-1) ssRNA via TLR7 and 8 [3, 4] and respond with Interferon (IFN) α production [5]. Genetic polymorphisms in TLR7, which is encoded on the X chromosome, have been associated with differential disease outcome in hepatitis C virus (HCV) and HIV-1 infection [6, 7]. Furthermore, IFN α has been suggested to be a key mediator for SIV pathogenesis in rhesus macaques [8, 9], and pDCs from rhesus macaques, similar to humans, produce large amounts of IFN α when stimulated with SIV or HIV-1. In contrast, sooty mangabeys, the natural host of SIV that do not progress to AIDS [10], have significantly lower levels of T cell activation in chronic SIV infection, and have been reported to have lower IFN α levels in chronic SIV infections [8, 11, 12]. Here we examined the consequences of polymorphisms in TLR7 and key signaling molecules in the TLR7 pathway, such as MyD88 and interferon regulatory factor 7 (IRF7), on IFN α production following stimulation with HIV-1.

Blood specimens from 35 healthy donors were drawn in ACD tubes. Freshly isolated PBMCs were stimulated with 0.3 μ g/ml p24 of aldrithiol-2 (AT-2) inactivated HIV-1 (Lot# P4092) or equivalent amount of microvesicle control (Lot# P4138) as previously described [5]. After 20 hours cells were washed and stained for phenotypical surface markers as well as intracellular cytokine production. To identify genetic polymorphisms, the entire gene coding regions of TLR7, MyD88 and IRF7 genes were amplified from genomic DNA in 10 μ l reactions containing 200nm dNTPs, 1.5 mM MgCl₂, and 0.2 μ M of each primer. A modified step-down program was used to ensure precise amplification. PCR products were subjected to direct bidirectional cycle sequencing using Bigdye terminator v 1.1 and run on

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an ABI3730 sequencer. Unpaired two-tailed student *t*-tests were employed to assess statistical significance of differences. P-values of less than 0.05 were considered significant.

Within TLR7, two single nucleotide polymorphisms (SNP), rs179008 and rs2302267 located on the X chromosome have been previously described to be associated with various HCV and HIV-1 disease outcomes [6, 7]. In this cohort, individuals with G (as opposed to T) in SNP rs2302267 were rare ($n = 2$) and therefore an accurate analysis of the functional consequences of this SNP could not be performed. Four individuals who were heterozygous (AT) and nine individuals who were homozygous or hemizygous (TT or T) for the TLR7 SNP rs179008 at position exon 3 were identified. However, the percentage of pDCs that produced IFN α in response to AT-2 HIV-1 stimulation was not significantly different between the study groups (median %IFN α + pDCs: AA (women) or A (male) 6.57%; AT 5.38%; TT or T 5.46%; $p > 0.05$). This is in contrast to previously published work reporting that stimulation with the TLR7/8 ligand imiquimod resulted in higher amounts of secreted IFN α by individuals with the rarer polymorphism (TT or T) [7]. This discrepancy might be due to difference in the stimulants used (synthetic TLR ligands versus ssRNA and inactivated HIV-1 virus here) and measuring IFN α by secretion rather than by percentage of IFN α + pDCs.

Downstream of TLR7 a cascade of signaling molecules is activated before the transcription of IFN α . One of the first proteins that TLR7 and almost all other TLRs interact with is MyD88. We found no genetic polymorphisms within MyD88 in the 35 individuals studied here. IFN α transcription is furthermore dependent on IRF7 and a total of ten described IRF7 SNPs were characterized in the study subjects. Two SNPs, rs12272434 and rs12290989, both located at intron/exon boundaries, were associated significantly with decreased levels of IFN α production by pDCs in response to HIV-1 (Fig 1A). The two SNPs were in complete linkage disequilibrium, so only SNP rs12272434 is used as representative of both SNPs in all subsequent analysis. The decreased capacity to produce IFN α was most prominent in the seven individuals who were homozygous for the rarer polymorphism (AA) and to a lesser degree in individuals who were heterozygous. IFN α is a pro-inflammatory cytokine that can directly activate T cells. Consequently the difference in IFN α production was also reflected by significant differences in the level of CD8+ T cell activation, with individuals who were heterozygous (AT) or homozygous (AA) for the rs12272434 and rs12290989 SNPs having a significantly lower level of activated T cells (Fig 1B). Both IFN α and T cell activation associations were, however, not gender specific and the same pattern was observed in both males and females (data not shown). For the remaining eight IRF SNPs we either did not identify significant association with differences in IFN α production (226_231 Intron deletion, rs1061502, rs11246213, rs10902178) or did not have a sufficient number of individuals with the rarer polymorphisms for accurate analysis (rs1061505, rs1131665, rs12422022, rs1051390).

HIV-1 infection is associated with strong activation of the immune system, and the level of immune activation is a strong predictor for HIV-1 disease progression [13]. Levels of HIV-1-associated immune activation for the same level of viral replication can vary largely between individuals, and the mechanisms underlying these differences are incompletely understood. In this study two IRF7 SNPs were identified to have a significant impact on IFN α production by pDCs following stimulation with AT-2 inactivated HIV-1. IRF7 binding to the IFN α promoter requires protein phosphorylation, formation of homodimers, and transport to the nucleus [14]. Whether the studied polymorphisms in IRF7 affect transcription of the gene, phosphorylation of the protein or binding to the IFN α promoter needs to be further examined to determine the mechanism behind the observed differences in IFN α production.

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Figure 1.

(A) PBMC from healthy donors were stimulated with AT-2 inactivated HIV-1 or microvesicle (Ves) control, and the percentage of pDCs producing IFN α was determined by intracellular cytokine staining. Individuals with the most common polymorphism in the IRF7 SNP rs12272434 (TT; n = 21) and those who were heterozygous for this SNP (AT; n = 7) had significantly higher levels of IFN α production compared to those who were homozygous for the less common polymorphism (AA; n = 7). (B) CD8+ T cell activation levels as measured by high expression of CD38 were quantified in a subset of individuals for which sufficient cell numbers were available (TT; n = 16, AT; n = 7, and AA; n = 7). HIV-1-induced CD8+ T cell activation was significantly higher in individuals with TT.