Host factors in HIV-1 replication: The good, the bad and the ugly
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Citation for published version (APA):
Booiman, T. (2015). Host factors in HIV-1 replication: The good, the bad and the ugly
CHAPTER 9

Polymorphism in *IFI16* affects CD4\(^+\) T cell counts in HIV-1 infection

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*International Journal of Immunogenetics, 2014*
Chapter 9

ABSTRACT

Interferon-γ-inducible protein 16 (IFI16) plays a pivotal role in the death of bystander CD4+ T cells. Here we demonstrate that SNP rs1417806 in IFI16 is associated with CD4+ T cell counts at set-point and HIV-1 disease progression, indicating that IFI16 affects HIV-1 pathogenesis especially during the early phase of infection.
INTRODUCTION

The depletion of CD4+ T cells in untreated HIV-1 infected individuals is the hallmark of the acquired immunodeficiency syndrome (AIDS). Upon infection, HIV-1 induces the depletion of CD4+ T cells by two distinct pathways. The initiation of the pathways is determined by the permissiveness of these cells to the virus. HIV-1 infection of activated CD4+ T cells that represent 5% or less of the total CD4+ T cell population leads to productive infection and as a result these cells die by caspase-3 dependent apoptosis. However, the majority of the dying CD4+ T cells are not productively infected. These so called by-stander cells CD4+ T cells have a quiescent and HIV-1 non-permissive phenotype, which results in abortive infection and accumulation of incomplete viral DNA transcripts. These incomplete viral DNA genomes induce the activation of caspase-1, which results in the production of interleukin-1β and cell death by pyroptosis. Pivotal in this pathway is the host sensor interferon-γ-inducible protein 16 (IFI16), that recognizes the incomplete HIV-1 reverse transcripts thereby initiating the activation of caspase-1.

METHODS

Here we studied the effect of SNPs in the IFI16 gene region on viral load and CD4+ T cell count at set-point and progression to AIDS in 365 HIV-1 infected men who have sex with men that participate in the Amsterdam cohort studies (ACS) on HIV-1 and AIDS. From these men, 243 did not receive any early treatment, 70 received zidovudine monotherapy, 10 received didanosine monotherapy and 42 received other ineffective antiretroviral therapy. A DNA sample was available from 335 of the 365 participants for genotyping analysis. Genotyping was performed using the Illumina Infinium Human Hap 317K Bead chip (Illumina Inc, San Diego, USA) and outliers in the population were removed from the analysis (Eigenstrat, implemented in Eigensoft). As a result, genetic data from 304 individuals was used for further analysis.

RESULTS

We hypothesize that genetic variation in IFI16 gene region could result in altered function or expression of the protein encoded by the IFI16 gene, thereby influencing the rate of CD4+ T cell depletion and impacting HIV-1 disease progression. For this study the genotypes of 4 SNPs (rs1417806, rs1057024,
rs856055 and rs3754460) in the IFI16 gene region were analyzed covering 67% of the tag SNPs in IFI16 at a minor allele frequency of 20% and a $r^2$ of at least 0.8 in the Caucasian population (The International HapMap Consortium, 2003). First, we investigated the effect of the SNPs on viral load and CD4$^+$ T cell counts at set-point. CD4$^+$ T cell count at set-point was defined as the number of CD4$^+$ T cell counts in blood recovered after the transient drop in CD4$^+$ T cells during acute HIV-1 infection. Viral load set-point was defined as the relative steady
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level of HIV-1 RNA in plasma after peak viremia during acute infection. On average 3-4 CD4+ T cell count and viral load measurements per year were available for each patient for the duration of follow up. On average, set-point was reached between 18-24 months after seroconversion and the average CD4+ T cell count or average viral load from measurements during this period was taken as CD4+ T cell count or viral load at set-point. We observed a significant association between the minor allele of SNP rs1417806 and higher CD4+ T cell counts at set-point ($p=0.009$; $p=0.036$ after Bonferroni correction for multiple testing) whereas no effect of the SNP on viral load at set-point was observed (Figure 1A and 1B). Subsequently, the effect of SNP rs1417806 on HIV-1 disease progression with AIDS (CDC 1993) and CD4 T cell counts below 200 per µl blood as endpoints in a Kaplan-Meier survival analysis (recessive model) was analyzed. We observed a protective effect of the minor allele of SNP rs1417806 on HIV-1 disease progression using CD4+ T cell counts below 200 per µl blood ($p=0.025$) and AIDS ($p=0.035$) as endpoints (Figure 1C and 1D). In addition, we included treatment with monotherapy or other inefficient therapy in a Cox proportional hazard multivariate analysis to correct for possible confounding effect of suboptimal treatment on disease progression. We still observed an independent effect of SNP rs1417806 on HIV-1 disease progression using CD4+ T cell counts below 200 per µl blood (relative hazard of 0.4; $p=0.010$) and AIDS (CDC1993) (relative hazard of 0.5; $p=0.017$) as endpoints.

DISCUSSION

Our observations indicate that genetic variation in IFI16 gene region has an effect on HIV-1 pathogenesis especially during the early phase of infection. SNP rs1417806 is located 491 base pairs upstream of the IFI16 gene and is not in high linkage disequilibrium ($r^2>0.8$) with other SNPs in the Caucasian population. Due to its location in the putative IFI16 promotor region, the SNP rs1417806 potentially alters the expression of IFI16. We observed that HIV-1 infected individuals carrying the minor allele of SNP rs1417806 have higher CD4+ T cell counts at set-point and a slower disease progression. This suggests that the minor allele of SNP rs1417806 in IFI16 might be associated with lower signaling upon triggering by incomplete HIV-1 DNA transcripts, which results in a decreased caspase-1 activation and subsequent less CD4+ T cell death by pyroptosis. Further study is required to unravel the exact mechanism by which SNP rs1417806 affects the function of IFI16 in HIV-1 infection.
Our study is the first to show that genetic variation in IFI16 affects HIV-1 infection in vivo. This highlights the important role of IFI16 in the process of CD4+ T cell depletion during HIV-1 infection. The fact that SNP rs1417806 affects CD4+ T cell counts at set-point shows that altered IFI16 function already impacts the early phase of HIV-1 infection and that this effect is maintained throughout infection.

**Ethics statement**

The ACS have been conducted in accordance with the ethical principles set out in the declaration of Helsinki and all participants provided written informed consent. The study was approved by the Academic Medical Center institutional Medical ethical Committee of the University of Amsterdam.

**ACKNOWLEDGEMENTS**

The Amsterdam Cohort Studies on HIV infection and AIDS are a collaboration between the Public Health Service of Amsterdam, the Academic Medical Center of the University of Amsterdam, the Sanquin Blood Supply Foundation, the University Medical Center Utrecht, and the Jan van Goyen Medical Center, and are part of the Netherlands HIV Monitoring Foundation. The authors like to thank Tom van der Kerkhof and Louis Jansen for useful discussion.

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