Interleukin-10 Promoter Polymorphisms Influence HIV-1 Susceptibility and Primary HIV-1 Pathogenesis

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Abstract

IL-10 directly inhibits HIV-1 replication but it may also promote viral persistence by inactivation of effector immune mechanisms. Here we show in an African cohort that individuals with genotypes associated with high IL-10 production at two promoter single nucleotide polymorphisms (-1082 and -592) were less likely to become HIV-1 infected, but had significantly higher median plasma viral loads during the acute phase (≤ 3 months post infection). However, as the infection progressed, the hierarchical genotype/median viral load pattern was reversed. Thus IL-10 may influence HIV-1 susceptibility and pathogenesis but effects on the latter may differ according to the infection phase.

Keywords

IL-10; HIV-1 subtype C; viral load; HIV-1 susceptibility; HIV-1 pathogenesis

Introduction

The interleukin-10 (IL-10) proximal promoter region contains two biallelic polymorphisms at positions -1082 (A to G transition) and -592 (C to A transversion), which are related to levels of IL-10 production [1, 2]. Polymorphisms associated with decreased IL-10 production have been associated with increased likelihood of HIV-1 acquisition and accelerated rate of CD4+ T cell decline particularly in late stage disease [3, 4], suggesting that high IL-10 production may reduce susceptibility to HIV-1 infection and protect against
disease progression. Paradoxically, two recent studies of lymphocytic choriomeningitis virus (LCMV) in mice, a model of chronic viral infections, have demonstrated that removal of IL-10 or blockade of the IL-10 pathway may enhance T cell immune responses, resulting in the rapid elimination of virus and the development of antiviral memory T cell responses, culminating in LCMV clearance \( \textit{in vivo} \) is further complicated by the observation that the cytokine directly inhibits HIV-1 replication in human macrophages \([7, 8]\). The inhibitory effects of IL-10 on HIV-1 replication may become more pronounced in late stages of disease when CD4\(^+\) lymphocytes are depleted and replication in macrophages and monocytes predominates \([9-11]\).

This study was undertaken to gain better insight into the complex role of IL-10 in HIV-1 infection and pathogenesis. Specifically, we investigated whether genetic polymorphisms in the proximal region of the IL-10 gene promoter contribute to HIV-1 susceptibility and to primary HIV-1 pathogenesis in an African cohort at high risk for infection.

**Subjects, Materials and Methods**

The CAPRISA Acute Infection cohort was described in detail elsewhere \([12]\). This was a longitudinal cohort study on viral set point and clinical progression in HIV-1 subtype C infection established in Durban, South Africa in 2004. The cohort comprised 245 high risk HIV negative women who were screened monthly for HIV infection using two rapid antibody tests (Determine, Abbott Laboratories and Capillus, Trinity Biotech). Negative or indeterminate samples were subjected to pooled plasma PCR testing, and positive pools were deconstructed and individually tested (COBAS Ampliscreen™ HIV-1 Test, Roche Diagnostics). HIV-1 infection was further confirmed in RNA positive samples with a positive HIV enzyme immunoassay test (Enzygost), and a diagnostic nucleic acid test (Roche). CD4\(^+\) T cell counts were determined by flow cytometry (Becton Dickinson).

28 seroconverters from the HIV-negative cohort, as well as 36 individuals from other seroincidence cohorts were enrolled into the acute infection phase (phase II) of the study. Once enrolled into phase II, participants were seen weekly for 3 weeks, then fortnightly for 2 months, then monthly for 10 months and thereafter quarterly. 39 participants in the HIV negative cohort \((n=245)\), were lost to follow-up. In the HIV positive cohort, 8 participants exited the study-- one participant refused participation, two were accidental deaths and a further 5 had to be initiated on ARV treatment. At the time of assessment, the total number of individuals in the study was 281, of whom 259 had samples available for assessment. 64 of 259 had acquired HIV-1 infection. Ethical approval was obtained from the ethics committee of the University of KwaZulu Natal and participants provided informed consent.

Genotype assessments for the 2 proximal IL-10 promoter SNPs (-1082 and -592 relative to the transcription start site), were performed using the amplification refractory mutation system-polymerase chain reaction (ARMS-PCR) as previously described \([13]\).

Chi-square test compared allelic frequencies to confirm their fit to Hardy-Weinberg equilibrium. Fisher’s exact test analysed the association between HIV status and IL-10 polymorphic variants. Kaplan-Meier survival statistics and Cox proportional hazards model were used to assess time to HIV-1 infection. Generalized estimating equation (GEE) models were used to compare the longitudinal measurements of viral loads and CD4\(^+\) counts between different genotype groups. The GEE models were univariate models, unadjusted for other covariates but taking into account repeated measures. The covariance matrix for the GEE model was unstructured. Haplotype effects were analysed by fitting the haplotype probabilities as continuous variables to viral load and CD4 counts using GEE models.
Results

The mutant allele frequencies at SNPs -1082 and -592 in our cohort were 0.33 and 0.32 respectively. Chi square tests confirmed the allele frequencies to be in Hardy-Weinberg equilibrium for the entire cohort and for subgroups analyzed in this study. Linkage disequilibrium estimates between the two SNPs were calculated as $r^2 = 0.12$ and $D' = 0.70$ using Haploview [14]. The SNPs specified four haplotypes termed 1 (AC), 2 (AA), 3 (GC) and 4 (GA) which occurred at approximate frequencies of 37 %, 30 %, 29 % and 3% respectively.

Association between IL-10 genotype and susceptibility to HIV infection

There was no significant difference in the distribution of IL-10 genotypes for the -1082 position between the HIV-1 negative and HIV-1 positive groups ($p= 0.1443$) (Figure 1A). For the 222 participants enrolled when HIV negative and followed prospectively, Kaplan-Meier survival analysis of time to HIV-1 seroconversion for genotypes at the -1082 position (Figure 1B) showed no significant differences in risk of HIV-1 acquisition between genotype -1082AA versus -1082GG or -1082AG. Using the Cox proportional hazards model, if an individual was -1082AA the hazard ratio of becoming HIV-1 infected was 2.14 (95% CI: 0.99-4.63, $p=0.0543$).

However, there was a significant association between HIV-1 status and the IL-10 -592 genotype (Figure 1C), due to the large proportion of HIV-positive -592AA individuals as compared to the other genotypes ($p=0.0033$). In the analysis of high-risk HIV-1 negative individuals followed prospectively, individuals possessing the -592AA genotype were significantly more likely to become HIV infected compared to individuals who were -592CA or -592CC (Figure 1D). Using the Cox proportional hazards model, if an individual was -592AA they were at increased risk of becoming HIV infected with a hazard ratio of 3.0 (95% CI: 1.28-7.08, $p=0.0117$).

There were three haplotypes in this cohort with frequencies above 5%-haplotypes 1, 2 and 3. Only haplotypes 2 and 3 showed significant association with HIV-1 status. In normal logistic regression analysis, haplotype 2, predicted to result in low IL-10 production was associated with 1.8 times higher likelihood of being HIV-positive (95% CI 1.21-2.74, $p=0.0038$). In contrast, haplotype 3 (high IL-10 producer) was associated with 0.59 less likelihood of HIV acquisition (95% CI 0.36-0.96, $p=0.032$).

The effect of IL-10 promoter polymorphisms on viral load and CD4$^+$ T cell counts

For the 64 individuals who became infected with HIV-1, we investigated whether there were differences in viral load and CD4$^+$ T cell counts according to IL-10 genotypes between the phases of infection. We performed analysis at ≤3months post infection (acute phase) and 6-12 months post-infection (early chronic phase) in median viral load or CD4$^+$ T cell count between the three genotypic groups for both SNPs at positions -1082 and -592 (Figure 2).

Analysis of the overall change in viral load based on -1082 genotype (Figure 2A), shows that individuals possessing the -1082GG genotype (high IL-10 producer) had significantly higher median viral loads during the acute phase compared to -1082AA individuals ($p=0.0002$) and -1082AG individuals ($p=0.0027$). During the early chronic phase, however, there was no longer a significant difference between the three groups. Analysis of the overall change in CD4$^+$ T cell count (Figure 2B), showed that during the acute phase, the -1082GG group had the lowest median CD4$^+$ T cell count and this was significantly different from the -1082AA group ($p= 0.0120$). In comparison, during the early chronic phase of infection the -1082GG group was not significantly different from the other groups.
During the acute phase, the -592CC genotype (high IL-10 producer), did not have significantly different median viral load compared to -592CA or -592AA, \( p = 0.1292 \) and 0.0590 respectively (Figure 2C). During the early chronic phase, the -592AA group (low IL-10 producer) had a significantly higher median viral load compared to the -592CA and -592CC groups \( (p=0.0022 \) and 0.0075, respectively). However, there were no significant differences in CD4\(^+\) T cell counts between -592 variants at either the acute or early chronic phases of infection (Figure 2D).

During the acute phase of infection, haplotype 2 trended towards a negative effect on viral load \( (p = 0.0808) \), while haplotype 3 had a significantly positive effect on viral load \( (p=0.0002) \). However, during the early chronic phase haplotype 2 now had a significantly positive effect on viral load \( (p=<0.0001) \) and the positive effect on viral load of haplotype 3 was no longer significant \( (p=0.4930) \).

**Discussion**

Recently, the immunoregulatory cytokine IL-10 was identified as playing a key role in suppressing antiviral immune responses, leading to viral persistence \[5, 6\]. Neutralization of IL-10 has also been shown to result in enhanced T cell immune responses and viral clearance in the LCMV model of chronic viral infection \[15\]. However, two HIV-1 natural history studies of genetic polymorphisms in the IL-10 promoter appear to run counter to these recent findings by demonstrating that IL-10 promoter genetic polymorphisms associated with higher IL-10 production attenuate CD4\(^+\) T cell loss and are protective against disease progression, albeit in long-term follow-up cohorts \[5, 6\].

In our South African cohort, carriers of the -592AA genotype were more likely to become HIV-1 infected. Our results are in agreement with Shin et al. \[4\] who demonstrated in a North American cohort that carriers of the -592A allele were at higher risk of HIV-acquisition as compared to individuals carrying the wild-type allele. These results generally suggest that IL-10 promoter polymorphisms linked to low IL-10 production are associated with increased HIV-1 susceptibility, although the effect of the -592 SNP appears to be stronger compared to the -1082 SNP since the trend for the latter SNP did not reach statistical significance. These results are reinforced by the observation that lower IL-10 producer haplotypes were significantly more likely to become HIV-1 infected.

We also investigated the influence of IL-10 proximal promoter polymorphisms on primary HIV-1 pathogenesis. Generally, we found that higher-producing IL-10 polymorphisms were associated with high viral load during the acute infection phase. However, as infection progressed towards the chronic phase, differences in viral load between the genotype groups either disappeared or reversed. Thus during the acute phase of HIV-1 infection, our data is consistent with recent data in the LCMV model where IL-10 was shown to be a key player in inhibiting effector immune responses, leading to increased viral replication and persistence \[5, 6\]. However, as the infection proceeds towards the chronic phase, we observed a disappearance of viral load differences between genotypes, or a polymorphism associated with increased IL-10 production was associated with lower plasma viral load and increased CD4\(^+\) T cell count. We interpret this result to suggest that as HIV-1 infection progresses to a chronic state, IL-10 may have a protective role against CD4\(^+\) T cell loss and disease progression. This is in agreement with previous studies that have demonstrated that IL-10 promoter polymorphisms linked with increased IL-10 production are protective against CD4\(^+\) T cell loss and disease progression \[3, 4\]. We acknowledge that our data need to be interpreted cautiously, given the small sample size of this study, and the short follow-up period of the cohort. It is also obvious that mechanistic studies to investigate the role of
IL-10 in immune activation and HIV-1 replication in different phases of HIV-1 infection are needed.

In conclusion, we propose a model for the role of IL-10 in HIV-1 susceptibility and disease progression whereby high levels of IL-10 protect against HIV-1 infection possibly by reducing immune activation and counteracting inflammatory processes that increase the pool of susceptible cells while low IL-10 may have the opposite effects. In acute HIV-1 infection, high IL-10 levels may promote viral replication by dampening innate and adaptive effector immune responses in a manner similar to what has been described for the LCMV model [5, 6]. Furthermore, we propose that in chronic HIV-1 infection, particularly in late stages of disease, IL-10 may be protective by reducing immune activation and inhibiting HIV-1 replication in macrophages. Given the importance of understanding the role of immune activation and dysregulation in driving HIV-1 infection and pathogenesis, and considering that IL-10 has been reported as an integral player in these processes, the model proposed here warrants experimental scrutiny.

Acknowledgments

We thank the study participants, CAPRISA clinical and laboratory staff for providing specimens. Special acknowledgements to the following members of the CAPRISA Acute Infection Study team: Carolyn Williamson, Lynn Morris, Clive Gray, Winston Hide and Francois van Loggerenberg.

References

Figure 1. The influence of IL-10 promoter polymorphisms on HIV-1 susceptibility

(A) Association of -1082 genotype and HIV status. The distribution of genotypes for the -1082 position, based on HIV status showed that there is no significant association between IL-10 genotypes and HIV-1 status. (B) The Kaplan-Meier graph, analysing time to infection, showed that there were no significant differences between -1082 genotypes although there was a trend towards higher likelihood of infection for the -1082AA genotype. (C) Association of -592 genotype and HIV status. The distribution of genotypes for the -592 position, based on HIV status, showed that there is a significant association between IL-10 genotype and HIV status. This is due to the large number of HIV-positive individuals in the -592AA group (p=0.0033). (D) The Kaplan-Meier graph of time to infection showed that -592AA individuals were more likely to become HIV infected, and this was statistically significant (p=0.0117).
Figure 2. The influence of IL-10 promoter polymorphisms on primary HIV-1 pathogenesis

(A) The overall change in viral load over time, based on -1082 genotype. During 0-3 month post-infection the median viral load of the -1082GG group was significantly higher than the -1082AA ($p=0.0002$) and the -1082AG ($p=0.0027$). After 6 months post-infection there was no significant difference between the median viral loads between the -1082GG and either the -1082AA ($p=0.7576$) or -1082AG ($p=0.2798$) (B) The overall change in CD4$^+$ T cell count over time, based on -1082 genotype. During 0-3 months post-infection the -1082GG group had a significantly lower median CD4$^+$ T cell count than the -1082AA group ($p=0.0120$). After 6 months post-infection the median CD4$^+$ T cell count for the -1082GG group was not significantly different from the -1082AA group ($p=0.2735$) or the -1082AG group ($p=0.4745$) (C) The overall change in viral load over time, based on -592 genotype. During 0-3 months post-infection the median viral load for the -592CC group was not significantly different from the -592AA group ($p=0.0590$) or the -592CA group ($p=0.1292$). After 6 months post-infection the median viral load for the -592AA group was significantly different to the -592CC group ($p=0.0075$) and the -592CA group ($p=0.0022$). (D) The overall change in CD4$^+$ T cell count over time based on -592 genotype. There was no significant difference between the median CD4$^+$ T cell counts between the groups at both the acute phase of infection and the early chronic phase of infection.