The interplay between host genetic variation, viral replication and microbial translocation in untreated HIV-infected individuals

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Abstract:

Systemic immune activation, a major determinant of HIV disease progression, is the result of a complex interplay between viral replication, dysregulation of the immune system, and microbial translocation due to gut mucosal damage. While human genetic variants influencing HIV viral load have been identified, it is unknown to what extent the host genetic background contributes to inter-individual differences in other determinants of HIV pathogenesis like gut damage and microbial translocation. Using samples and data from 717 untreated participants in the Swiss HIV Cohort Study and a genome-wide association study design, we searched for human genetic determinants of plasma levels of intestinal fatty-acid binding protein (I-FABP/FABP2), a marker of gut damage, and of soluble sCD14 (sCD14), a marker of LPS bioactivity and microbial translocation. We also assessed the correlations between HIV viral load, sCD14 and I-FABP. While we found no genome-wide significant determinant of the tested plasma markers, we observed strong associations between sCD14 and both HIV viral load and I-FABP, shedding new light on the relationships between processes that drive progression of untreated HIV infection.
**Introduction:**

HIV infection is characterized by systemic immune activation, which persists even with viral suppression by antiretroviral therapy, and this immune activation is associated with HIV pathogenesis and disease progression in treated and untreated individuals [1, 2]. One cause of immune activation is microbial translocation, which occurs when microbial products from the lumen of the gastrointestinal (GI) tract translocate into the systemic circulation through a damaged GI tract barrier [3]. Immune activation and gut damage are cyclically linked, with immune activation leading to damage of the structural and immune barriers of the GI tract, and translocated microbial products stimulating the systemic immune system. This interplay is complex, and multiple factors affect each aspect of this vicious cycle, with the virus initiating and driving the process in the absence of treatment.

Soluble CD14 (sCD14) is a biomarker found at the heart of this interaction. Because it is a secreted sensor of bacterial lipopolysaccharide (LPS), sCD14 acts as an indirect marker of microbial translocation. The plasma levels of sCD14 were associated with mortality in two studies of HIV-infected individuals who were on antiretroviral therapy [4, 5]. Intestinal fatty-acid binding protein (I-FABP encoded by FABP2) is a marker of GI tract enterocyte damage [6], and thereby makes it possible to indirectly measure structural damage to the GI tract, which would be prohibitive to sample in a large cohort. The plasma levels of I-FABP also showed some association with mortality in treated HIV populations and were inversely
correlated with CD4+ T cell counts [4, 5]. These plasma markers allow the large-scale measurement of GI tract damage and microbial translocation.

Human genetic variation is known to play a modulating role in the host response to HIV infection. Multiple genome-wide association studies (GWAS) have been performed in cohorts with HIV control phenotypes, primarily in individuals of European ancestry, and mostly focusing on HIV viral load or disease progression (CD4+ T cell decline or time to AIDS) [7-10]. These studies consistently identified a small number of genetic variants associated with viral control, including single nucleotide polymorphisms (SNPs) mapping to the HLA class I region on chromosome 6, and a 32 base pairs deletion in the CCR5 gene (CCR5Δ32) on chromosome 3. On the other hand, there is no reported GWAS of GI tract damage or microbial translocation, so it is unknown to what extent these essential aspects of HIV pathogenesis are influenced by host genetic variation, and whether they also correlate with the HLA-B, HLA-C and CCR5 variants associated with viral load. Of note, early candidate gene studies reported the existence of SNPs affecting sCD14 and I-FABP plasma levels, but these associations have yet to be confirmed in HIV populations and in larger cohorts. The minor allele of rs2569190, a SNP mapping to the CD14 promoter region, was associated with higher plasma levels of sCD14 [11]. The same SNP was associated with significant differences in secretion of sCD14 and IL-6 in LPS-stimulated PBMCs [12]. Two haplotypes have also been identified in the FABP2 promoter (built from rs1799883, rs10034579, rs2282688 and rs6857641) that associated with I-FABP expression differences in vitro [13, 14].
To better understand how genetic factors may impact the interrelated processes of viral replication, gut damage and microbial translocation leading to systemic immune activation, we measured the plasma levels of sCD14 and I-FABP in 717 untreated, clinically and genetically well-characterized HIV-infected subjects from the Swiss HIV Cohort Study (SHCS), and used a GWAS design to search for human genetic variants associated with these biomarkers. We did not identify any significant genetic associations with sCD14 or I-FABP plasma levels, and found that the human genetic variants that are known to associate with HIV viremia do not associate with sCD14 or I-FABP levels. On the other hand, we observed independent associations between sCD14 and both HIV viral load and I-FABP, demonstrating the intricate relationships that exist between the various pathogenic mechanisms involved in HIV disease progression.

Methods

Study participants

The study subjects are part of the Swiss HIV Cohort Study (SHCS), a nationwide cohort study with continuous enrollment and semiannual study visits (www.shcs.ch [15]). The SHCS has been approved by the Ethics Committees of all participating institutions. Each study participant provided written informed consent for genetic testing. HIV infected individuals were eligible if they had [1] genome-wide genotyping data generated in the context of a previous GWAS; [2] a stored plasma
sample that was collected between 3 and 6 years after estimated date of 
seroconversion, in the absence of antiretroviral treatment, and with a CD4+ T cell 
count of at least 350/μl of blood; and [3] at least 3 stable plasma HIV RNA results, 
obtained in the absence of antiretroviral treatment, at least 6 months after the 
known or presumed date of infection, and with a CD4+ T cell count of at least 350/μl 
of blood. Set point viral load was calculated as the mean of all log10 transformed 
viral results fitting these conditions.

**Plasma biomarker measurements**

I-FABP and sCD14 plasma levels were measured as previously described using 
commercially available ELISAs [5]. The I-FABP assay (Cell Sciences) was performed 
on plasma diluted to 50%, and the sCD14 assay (R&D Systems) was performed on 
plasma diluted to 0.5%. All analyses were performed blinded to clinical and genetic 
status. Each test was determined in duplicate, and the average value of each marker 
was calculated and used in the downstream analyses.

**Genotyping and imputation**

Genotyping was performed using the Illumina HumanHap550 array, as described 
previously [8]. After quality control of the genotyping data and exclusion of 
population outliers by principal component analysis (PCA) [16], 686 individuals 
with complete phenotype and genotype data were available for downstream 
analysis. Genotypes were imputed using the 1000 Genomes Project CEU reference
panel: we used mach1 for prephasing [17] and minimac for imputation [18].

Imputed SNPs with a minor allele frequency of at least 1% and an imputation quality score (r2) of at least 0.3 were kept for association testing. The CCR5Δ32 variant, which is not represented directly or indirectly on the genome-wide chip, was independently genotyped with a TaqMan assay.

**Association testing**

We use GWAPower for power calculation [19]. Association tests were carried out by linear regression using plink [20], and by linear mixed model regression using fastlmm [21]. The fastlmm kernel matrix was computed from 69k randomly selected SNPs (corresponding to 1% of the total number of tested SNPs). Regressions were performed against log10 normalized HIV viral load at set point, log10 normalized sCD14 plasma levels and log10 normalized I-FABP plasma levels. Age, sex and the coordinates of the top 3 PCA axes were included as covariates in all regression models. We used Bonferroni correction for multiple testing. Association statistics between sCD14, FABP2, viral load at set point and the SNPs were computed by linear regression using R.
Results:

Common human genetic variants are not associated with markers of GI tract damage or microbial translocation

We analyzed HIV infected patients that had been genotyped in the context of a previous GWAS [8]. A total of 6,982,014 SNPs, directly genotyped on Illumina arrays or imputed using the 1000 Genomes Project CEU population as reference, were available for association testing. Stored plasma samples were obtained from eligible treatment-naive subjects, and the plasma levels of I-FABP and sCD14 were measured by ELISA. A total of 717 participants had complete genetic and phenotypic data, of which 31 were identified as population outliers by principal component analysis of the genotyping data and excluded. Therefore the final study population consisted in 686 individuals of European ancestry. Demographic and laboratory information for all study participants is shown in Table 1.

No associations were found between any human SNP and the plasma levels of I-FABP or sCD14 during early chronic, untreated HIV infection, after correction for multiple testing (Figure 1). Our study was powered to detect genetic variants that explain at least 4% of the variability in sCD14 or I-FABP plasma levels.

We also specifically looked at the SNPs that were previously reported as associated with sCD14 and I-FABP plasma levels. We did not observe any association between sCD14 plasma levels and the CD14 promoter SNP rs2569190 (p=0.46), and between I-FABP plasma levels and the FABP2 promoter SNPs rs1799883, rs10034579, rs2282688 and rs6857641, alone or in haplotype combinations (all p>0.3).
I-FABP plasma levels and HIV viral load are associated with sCD14 plasma levels

We next examined the correlations between several factors known to play a role in HIV pathogenesis: HIV viral load at set point, CD4+ T cell count, plasma level of sCD14 and plasma level of I-FABP. CD4+ T cell count was not associated with sCD14 or I-FABP plasma levels (p>0.5 and $r^2<0.01$ for both), and this parameter was excluded from subsequent analyses. Of note, the distribution of CD4+ T cell counts was truncated due to the inclusion criterion that all study participants have CD4+ T cell counts > 350 cells/ul; the median value was 452 in the study population (interquartile range: 390-566).

We observed a moderate correlation between plasma levels of I-FABP and sCD14 ($p=7.1 \times 10^{-16}$, $r^2=0.09$, Figure 2a), confirming the obvious link between GI tract damage and microbial translocation. This correlation was largely independent of HIV viral load ($p=3.5 \times 10^{-15}$ when viral load was included as covariate in the regression model).

HIV plasma viral load at set point was strongly associated with sCD14 but not with I-FABP plasma levels ($p=7.1 \times 10^{-8}$ and $p=0.2$ respectively, Figure 2b,c). The strong association observed between viral load and sCD14 plasma levels during chronic untreated infection is consistent with a cyclical relationship between HIV replication and immune activation due to microbial translocation. On the other hand, the absence of correlation between viral load and I-FABP concentration suggests that
the virus is not a major driver of GI tract damage during the chronic phase of infection. This finding is consistent with the fact that this marker of enterocyte damage can still be detected in individuals on suppressive antiretroviral therapy [2].

**Human genetic variants associated with HIV control are not associated with sCD14 or I-FABP plasma levels**

Three genetic variants have been consistently shown to associate with HIV control in previous GWAS: rs2395029, a near-perfect proxy for HLA-B*57:01 in Europeans; rs9264942, an indirect marker of HLA-C expression levels; and the CCR5Δ32 deletion in its heterozygous form. We tested them for association with viral load at set point in linear regression models that included sex, age, and the coordinate of the top 3 principal component axes as covariates. The associations were again significant for all 3 variants in our study population (Table 2). However, there was no association with sCD14 or I-FABP plasma levels (all p-values > 0.05).

To further explore the potential interactions between HIV viral load at set point, host genetic variants and sCD14 plasma levels, we ran multivariate regressions on viral load, including the 3 genetic variants as independent variables, with or without sCD14 plasma levels in the model: rs2395029, rs9264942 and CCR5Δ32 remained significant predictors of viral load and the strength of the association did not change significantly when the regressions included sCD14 plasma levels (Table 2). Thus, the previously described genetic variants in the HLA-B, HLA-C and CCR5 regions and
the sCD14 plasma levels are independently associated with HIV viral load at set point.

Discussion

HIV pathogenesis is the result of a complex interaction between the virus and the host response, which results in immune dysregulation. Many factors have been identified that independently contribute to pathogenesis but their intricate relationships have yet to be fully elucidated. In particular, host genetic factors and systemic immune activation due to the translocation of microbial products from the gut have both been shown to have a strong impact on HIV control and disease progression, but these have not been studied in relation to each other. We therefore sought to measure plasma markers of GI tract damage and microbial translocation in clinically and genetically well-characterized patients from the SHCS.

We ran a GWAS searching for human genetic determinants of sCD14 or I-FABP plasma levels, and failed to identify significantly associated variants after correction for multiple testing. As our study was powered to detect variants that explain at least 4% of sCD14 or I-FABP variability, we conclude that the plasma levels of these biomarkers are not primarily determined by common genetic variants with moderate to high effect size.

We did not observe any association between a previously reported CD14 promoter variant and sCD14 plasma levels, which strongly suggests that in untreated HIV-infected individuals, this biomarker primarily reflects LPS bioactivity/microbial
translocation rather than underlying genotype, in keeping with the demonstrated strength of this measurement as a predictive biomarker of disease progression. Of note, in the original report [11], homozygosity for the rs2569190 minor allele only associated with a 0.04 log10-transformed sCD14 increase, whereas the range of log10-transformed sCD14 values was 5.9-6.6 (delta: 0.7) in our study population. Similarly, we did not identify any association between FABP2 genotypic variation [13, 14] and plasma levels of I-FABP, which suggests that enterocyte damage, rather than genetically-determined expression differences in I-FABP, drives the plasma level of I-FABP in HIV-infected patients.

We then searched for associations between sCD14 and I-FABP plasma levels and the degree of spontaneous HIV control (as reflected in viral load) during the chronic phase of HIV infection, and found that sCD14 strongly associates with both I-FABP and viral load. Much of the work that has been previously done on sCD14 and I-FABP has focused on treated HIV infection, as gut damage, microbial translocation and systemic inflammation are the focus of current therapeutic efforts to decrease the non-AIDS morbidity and premature mortality observed in patients with suppressed viremia on antiretroviral therapy. In a cohort of mostly treated HIV infected individuals, Sandler et al. found a large increase in the risk of death with higher plasma sCD14, and an inverse association between plasma I-FABP level and CD4+ T cell counts [5]. A recent study by Hunt et al. found a significant correlation between I-FABP and sCD14 in treated subjects with advanced disease, and each marker strongly predicted mortality independently of CD4+ T cell count [4]. In fact,
a similar correlation between gut damage and LPS-induced monocyte activation has also been observed in HIV-negative subjects [22].

To date, only one study has examined the predictive value of sCD14 for mortality in untreated chronic HIV infection [23]: sCD14 was correlated with both viral load and CD4+ T cell count, and the association of sCD14 with disease progression was not independent of these parameters. Our data add to this important area of research by also including a marker of enterocyte damage, and by testing the relationships between all these parameters in a larger, genetically homogeneous group of untreated individuals.

Our observation that I-FABP is associated with sCD14 but not with viral load during chronic untreated infection suggests that, although the virus itself begins and reinforces the vicious cycle of enterocyte damage, translocation of microbial products and immune activation, these processes may become largely independent of viral replication in the chronic phase of untreated infection. This would be consistent with the high levels of immune activation that can still be observed in some individuals under fully suppressive antiretroviral therapy [2]. The interaction between these measures is likely to be bidirectional, as enterocyte damage allows microbial translocation and sCD14 increase due to LPS-induced monocyte activation, while chronic immune activation is likely to further damage the GI tract barrier. Interestingly, this correlation between I-FABP and sCD14 plasma levels was not observed in primary HIV infection, suggesting that microbial translocation is not the primary driver of monocyte activation during the acute phase of infection [24].
The strong association that we found between sCD14 and viral load, which had already been described in a small group of untreated patients [5], is most likely indirect. The presence in plasma of microbial products, including LPS, leads not only to higher sCD14 levels, but also to systemic inflammation, which is known to increase HIV viral load [25].

When considering the negative results of our genetic scan, specific limitations of the study design need to be highlighted. Some variability in sCD14 and I-FABP measurements was inevitable, which probably had a negative impact on study power. Each sample was tested in duplicate to deal with potential assay variability, but due to the lack of suitable samples we did not repeat the measurements at different time points, which would have allowed a precise estimation of biological variability. Another potential confounder is the variable duration of HIV infection (3 to 6 years) before sampling, potentially leading to inter-individual differences in disease progression. The exclusion of patients with moderate to severe immunosuppression (CD4+ T cells <350) aimed at mitigating this issue.

The absence of genetic associations with plasma I-FABP or sCD14 allows better interpretation of microbial translocation data and demonstrates that the previously identified genetic variants that are associated with HIV viral load are independent of sCD14. Our finding of associations between I-FABP and sCD14, and between viral load and sCD14 reinforces the centrality of microbial translocation in the pathogenesis of untreated HIV infection.
Conflicts of interest

The authors declare no conflict of interest.

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R, Yerly S.

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Figure legends

Figure 1: No SNPs are significantly associated with I-FABP or sCD14 plasma levels.
Manhattan plots showing the distribution of the results of the two genome-wide association studies, of I-FABP plasma levels (panel A) and of sCD14 plasma levels (panel B). The horizontal axes depict the human chromosomes in linear order, the vertical axes show association strengths as \(-\log_{10}(p\text{-values})\).

Figure 2: I-FABP and HIV viral load at set point are independently associated with sCD14.
Correlations between plasma levels of (a) I-FABP and sCD14 (p=7.1x10^{-16}, r^2=0.09) (b) sCD14 and HIV viral load (p=7.1x10^{-8}, r^2=0.04) and (c) I-FABP and HIV viral load (p=0.2, r^2<0.01)
Table 1: Demographic and laboratory information.

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<th>Eligible participants</th>
<th>Final study population</th>
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<tr>
<td>Total N</td>
<td>717</td>
<td>686</td>
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<tr>
<td>Male gender, N (%)</td>
<td>543 (75.7)</td>
<td>520 (75.8)</td>
</tr>
<tr>
<td>Age, median (IQR)</td>
<td>33 (27-40)</td>
<td>34 (28-40)</td>
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<tr>
<td>Log10 HIV VL at setpoint, mean (SD)</td>
<td>3.81 (0.96)</td>
<td>3.81 (0.96)</td>
</tr>
<tr>
<td>CD4+ T cell count, median (IQR)</td>
<td>460 (390-571)</td>
<td>452 (390-556)</td>
</tr>
<tr>
<td>Log10 sCD14, mean (SD)</td>
<td>6.23 (0.13)</td>
<td>6.24 (0.13)</td>
</tr>
<tr>
<td>Log10 I-FABP2, mean (SD)</td>
<td>2.70 (0.61)</td>
<td>2.70 (0.60)</td>
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Table 2: P-values for association with HIV viral load at set point in linear regression models including sex, age and the coordinates of the top 3 principal components axes as covariates.

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<th>Univariate models</th>
<th>Multivariate models</th>
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<tr>
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<tr>
<td>rs2395029</td>
<td>5.40E-08</td>
<td>9.60E-06</td>
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<tr>
<td>rs9264942</td>
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<td>sCD14 levels</td>
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