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Drug resistant virus has reduced ability to induce immune activation

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There is increasing suspicion that HIV-induced immune activation contributes to accelerated cellular turnover, CD4+ T-cell losses and increased morbidity and mortality of HIV-infected individuals, even for those on antiretroviral treatment [1,2,3,4]. For example, Kuller et al.[5] found that higher levels of interleukin-6 (IL-6) were significantly associated with an increased risk of all-cause mortality; similar associations with mortality were seen for higher levels of d-dimer and hsCRP. Deeks et al.[6] have hypothesized that the preserved immunologic function in many treated patients with drug-resistant viremia is the result of a reduction in T cell activation. Hunt et al.[7] reported that antiretroviral-treated patients with drug-resistant viremia have lower T cell activation than untreated patients, controlling for plasma HIV-1 RNA level. Kitchen et al.[8] reported an oscillatory relationship between T cell activation, CD4 T cell counts and HIV-1 RNA levels over time in a group of subjects with highly resistant HIV infection followed longitudinally.

To test the hypothesis that drug resistant virus has reduced ability to induce immune activation, we analyzed the relationship between drug resistance and both soluble markers of inflammation (IL-6, TNF-α, TNF-rII) and cellular activation markers (CD38/HLA-DR expression on CD4 and CD8 T-cells) before and after short-term treatment interruption, using data from 16 of the 21 subjects randomized to the structured treatment interruption (STI) arm of the Adult Clinical Trials Group (ACTG) 5086 study. Subjects were excluded from analysis due to the unavailability of activation (4 subjects) or resistance data (1 subject).

ACTG 5086 was a randomized clinical trial of 41 patients with multiple drug class-resistant HIV. Subjects were randomized to undergo a 16-week STI followed by antiretroviral treatment, to evaluate if treatment responses are improved and if the new regimen started after a short-term treatment interruption can revert circulating virus to wild-type;[9] this strategy was not successful. We measured plasma cytokine levels and T-cell activation at

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baseline and then at the end of treatment interruption at a time when viruses with fewer resistance mutations were expected to predominate. HIV-1 drug resistance in virus from plasma was obtained at baseline and at the end of STI (prior to initiation of optimized treatment) from standard genotyping (ViroSeq version 2, Celera Diagnostics), but only a minority of subjects had nearly complete re-emergence of drug-susceptible HIV-1.[9]

Here we focus on the relationship between immune activation, drug resistance and HIV-1 RNA levels. Based on the association between protease inhibitor (PI) mutations and immune activation[6], we defined a PI score, the number of major PI resistance mutations, using the list of major PI drug resistance mutations from the Stanford HIV drug resistance database (http://hivdb.stanford.edu/pages/documentPage/PI_mutationClassification.html, last updated on 2011–5–6), that is, mutations at amino acid positions 30, 32, 46, 47, 48, 50, 54, 76, 82, 84, 88, and 90. We also calculated a nucleoside reverse transcriptase inhibitor (NRTI) score and a non-nucleoside reverse transcriptase inhibitor (NNRTI) score similarly based on mutations at amino acid positions 41, 65, 67, 69, 70, 74, 75,115, 151, 184, 210 and 215 for NRTIs and 100, 101, 103, 106, 179, 181, 188, 190, 230, and 236 for NNRTIs. Associations were examined using Spearman correlations; partial correlations were used to adjust for plasma HIV-1 RNA levels. Median changes were analyzed using the sign rank test. The Baron and Kenny method[10] was used to investigate whether the effect of drug resistance on immune activation was mediated through HIV-1 RNA levels.

At baseline and as expected, plasma HIV-1 RNA levels in these persons with multi-drug resistant virus tended to correlate positively with markers of immune activation: TNF-α ($r=0.77$, $p<0.001$), TNF-rII ($r=0.53$, $p=0.04$), IL-6 ($r=0.50$, $p=0.05$). We did not, however, observe a significant correlation between baseline HIV-1 RNA levels and CD38 expression on CD8+ cells ($r=0.18$, $p=0.52$), CD38+/HLA-DR+ co-expression on CD8+ cells ($r=-0.24$, $p=0.40$), or CD38+/HLA-DR+ co-expression on CD4+ cells ($r=0.12$, $p=0.68$). We observed a significant negative correlation between CD4 cell counts and CD38 expression on CD8+ cells ($r=-0.63$, $p=0.02$). CD4 cell counts appeared to be negatively correlated with CD38+/HLA-DR+ co-expression on CD4+ cells ($r=-0.46$) and RNA levels ($r=-0.41$), but did not reach statistical significance ($p=0.10$ and 0.12 respectively). CD4 cells were not correlated with CD38+/HLA-DR+ co-expression on CD8 cells ($r=0.002$, $p=0.99$). As plasma HIV-1 RNA rose after treatment interruption (median increase 0.2 $\log_{10}$ copies/ml, $p=0.006$) (Table 1), CD4+ cell count dropped significantly (median change −54 cells/mm^3, $p<0.001$); levels also tended to increase for TNF-α (median increase 0.3 $\log_{10}$ pg/ml, $p=0.04$), IL-6 (median increase 0.4 $\log_{10}$ pg/ml, $p=0.04$), and CD38+/HLA-DR+ co-expression on CD4+ cells (median increase 3%, $p=0.03$), but less so with TNF-rII (median increase 0.03 $\log_{10}$ pg/ml, $p=0.08$), CD38+ expression on CD8+ cells (median increase 4%, $p=0.15$), or CD38+/HLA-DR+ co-expression on CD8+ cells (median increase 4%, $p=0.35$).

At baseline, the number of PI mutations were negatively correlated with levels of TNF-α ($r=-0.49$; $p=0.05$) and with levels of TNF-rII ($r=-0.51$; $p=0.04$). After adjusting for $\log_{10}$ HIV-1 RNA level, the correlation between PI mutations and TNF-rII was similar ($r=-0.61$; $p=0.03$), but the correlation with TNF-α appeared to be attenuated ($r=-0.20$; $p=0.53$). Further regression analyses for test of mediation revealed that, after controlling for the number of PI mutations, HIV-1 RNA levels were not significantly associated with TNF-α levels ($p=0.12$), suggesting that the effect of PI mutations on TNF-α was not mediated through HIV-1 RNA levels. As the number of resistance mutations to PI fell after the treatment interruption ($p=0.008$, median change −1.0), no correlations between number of PI mutations and TNF-α, TNF-rII or IL-6 were seen at the end of the treatment interruption ($r=0.02$ to 0.08; $p>0.7$ for all).

At baseline, the numbers of NRTI mutations appeared to be negatively correlated with TNF-α (r= −0.46, p=0.07). Similar correlation was observed after controlling for HIV-1 RNA levels (r= −0.49; p=0.09). NRTI mutations were not correlated with TNF-rII or IL-6 (both p>0.90). As the frequency of resistance mutations to NRTI decreased after the treatment interruption (p=0.002, median change −1.0), the negative association between NRTI resistance score and TNF-α was no longer seen (p=0.25).

NNRTI resistance mutations at baseline (median score 1) were less prevalent than were resistance mutations to PI or NRTI. Frequencies of resistance mutations to NNRTI decreased after the treatment interruption (p=0.02). No significant correlations between NNRTI resistance score and any of the inflammatory markers (TNF-α, TNF-rII, or IL-6) were observed, either at baseline or at the end of treatment interruption.[11] Among 8 patients failing a NNRTI-based regimen, at baseline, HIV-1 RNA levels were positively correlated with IL6 (r=0.85; p=0.008), and appeared to be positively correlated with TNF-α (r=0.61) and with TNF-rII (r=0.44), but these correlations did not reach statistical significance (p=0.11 and 0.27 respectively); at the end of treatment interruption, we did not observe any correlation between HIV-1 RNA levels and TNF-α, TNF-rII, or IL6 (p>0.5 for all).

At baseline, total numbers of mutations were negatively correlated with TNF-α levels (r= −0.64; p=0.008) and remained statistically significant with similar magnitude after controlling for HIV-1 RNA levels (r= −0.59; p=0.03).

We examined correlations between changes in drug resistance mutations and changes in immune activation markers, with or without adjusting for changes in HIV-1 RNA levels, and did not observe any significant associations.

In this investigation of immune activation in extensively treated individuals with drug-resistant virus, soluble markers of immune activation tended to correlate negatively with the number of drug-resistant mutations at baseline and this effect appeared to be independent of HIV-1 RNA levels, consistent with the results presented in Hunt et al.[7] This suggests that drug-resistant virus has reduced ability to induce immune activation, also consistent with the lower levels of CD8 activation that were seen in persons with drug-resistant virus as described by Hunt et al.[7] The lack of a correlation between resistance mutations and CD8 activation at baseline may be due to the small sample size in the present study or to the characteristics of the study population since only patients with drug resistance were examined, in contrast to Hunt et al.[7] who compared subjects with drug resistance to those with wild-type virus. Another possible explanation for the difference between our findings and those of the Hunt study may relate to the magnitude of viremia in patients with drug resistance which in the present study was higher than in the Hunt study. At the end of STI, correlations were much weaker than at baseline. It is possible that viral populations were still in a dynamic phase at this time, not yet at a new steady state, as supported by heterogeneity in the rate of reversion of drug resistance seen in a similar 4-month treatment interruption study.[12] We also assessed the effect of single point mutations I54V and V82A on immune activation as Natesampillai et al. found differential effects of major PI mutations on caspase-induced apoptosis.[13] We did not observe any significant correlation between these two mutations, individually or in combination, with any of the immune activation markers, at baseline or when changes from baseline are examined.

In contrast to previous studies, we did not observe significant correlation between baseline HIV-1 RNA and expression of cell activation markers. The magnitude of viremia is one of several factors that contribute to the level of immune activation in HIV-1 infection and in many treated subjects, immune activation persists even when viremia is controlled. Most studies demonstrating a relationship between the magnitude of viremia and indices of

immune activation were studies of patients not receiving antiretroviral therapies (ART). The effects of incompletely suppressive ART and the relatively small sample size of this study may have confounded this relationship. Resistance mutations were associated with immune activation, especially for TNF-alpha; this relationship did not appear to be mediated by HIV-1 RNA levels.

Markers of immune activation significantly increased during the 4-month treatment interruption, as was seen after 1 month of treatment interruption in Kuller et al.[5] which also showed that higher IL-6 levels during follow-up were associated with increased mortality. These findings, together with the studies showing that treatment interruption does not improve virologic responses to salvage regimens,[9,14,15] provides further argument against the use of treatment interruption strategies in the clinical management of HIV-infected individuals.

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Table 1

Drug Resistance score, HIV RNA, CD4+ cell count and markers of immune activation at baseline and changes after treatment interruption.

	Baseline	Change from Baseline	
	Median (Q1, Q3)	**Median (Q1, Q3)**	**p-value***
PI Score (n=16)	2.5 (1.5, 4.0)	−1.0 (−3.5, 0)	0.008
NNRTI Score (n=16)	1.0 (1.0, 2.0)	0 (−1.0, 0)	0.02
NRTI Score (n=16)	4.0 (1.0, 5.5)	−1.0 (−2.5, 0)	0.002
HIV RNA (log_{10} copies/ml) (n=16)	4.8 (4.3, 5.3)	0.2 (0.1, 0.8)	0.006
CD4 (cells/mm^3) (n=15)	217 (87, 329)	−54 (−85, −6)	<0.001
Cellular Activation Markers (n=15):			
% CD38+ CD8 cells	85 (74, 90)	4 (−2, 8)	0.15
% CD38+/HLA-DR+ CD8 cells	47 (37, 66)	4 (−4, 9)	0.35
% CD38+/HLA-DR+ CD4 cells	17 (13, 31)	3 (2, 8)	0.03
Soluble Activation Markers (n=16):			
TNF-α (log_{10} pg/ml)	2.8 (2.2, 2.9)	0.3 (−0.1, 0.7)	0.04
TNF-rII (log_{10} pg/ml)	3.5 (3.3, 3.6)	0.03 (−0.01, 0.1)	0.08
IL-6 (log_{10} pg/ml)	1.1 (0.8, 1.6)	0.4 (−0.03, 0.8)	0.04

*Signed rank test (2-tailed).