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Associations of T cell activation and inflammatory biomarkers with virological response to darunavir/ritonavir plus raltegravir therapy

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Objectives: One of the goals of antiretroviral therapy (ART) is to attenuate HIV-induced systemic immune activation and inflammation. We determined the dynamics of biomarkers of immune activation, microbial translocation and inflammation during initial ART with a nucleos(t)ide-sparing regimen of darunavir/ritonavir plus raltegravir. We also evaluated associations between these biomarkers and the virological response to the regimen.

Methods: We determined baseline and week 24 and 48 levels of CD4+ and CD8+ T cell activation (% HLA-DR+/CD38+), interleukin-6 (IL-6), interferon-γ-inducible protein-10 (IP-10), soluble CD14 (sCD14), D-dimer and lipopolysaccharide. Associations between the biomarkers at baseline were assessed using Spearman's rank correlation. The Wilcoxon signed rank test analysed changes from baseline. Comparisons between groups were made using the Wilcoxon rank sum test, and Cox proportional hazards models assessed predictors of virological failure (VF).

Results: Assays were completed on 107 of 112 subjects after excluding five subjects who had only baseline samples. The subjects included were 94 (88%) men with a median age of 37 years, a median baseline CD4 count of 261.5 cells/mm^3 and a median baseline viral load (VL) of 75 876 copies/mL. Subjects with a baseline VL >100 000 copies/mL had higher baseline T cell activation, IL-6, IP-10, sCD14 and D-dimer. These biomarkers declined during treatment ($P<0.05$). Although subjects who experienced VF had higher baseline CD4+ T cell activation ($P=0.035$), only baseline VL independently predicted VF (hazard ratio for >100 000 versus ≤100 000 copies/mL was 4.5–5.6, $P\leq0.002$).

Conclusions: Darunavir/ritonavir plus raltegravir attenuated immune activation, inflammation and microbial translocation. T cell activation remained higher in subjects with VF than those without. Baseline VL >100 000 copies/mL remained the primary driver of VF.

Keywords: nucleos(t)ide sparing, soluble CD14, microbial translocation

Introduction

In the ACTG A5262 trial, treatment-naive HIV-1-infected patients received a nucleos(t)ide-sparing regimen of 800/100 mg of darunavir/ritonavir once daily plus 400 mg of raltegravir twice daily for 52 weeks.[1] This regimen was effective in most patients, but 26% experienced protocol-defined virological failure (VF) by week 48, approximately two-thirds of whom had a viral load (VL) between 50 and 200 copies/mL at VF. Baseline VL >100 000 copies/mL strongly predicted VF.

One of the goals of antiretroviral therapy (ART) is to attenuate the systemic immune activation and inflammation induced by HIV.[2] Whether these factors and microbial translocation independently influence virological outcomes has not been clearly

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demonstrated. We investigated the dynamics of biomarkers of immune activation, microbial translocation and inflammation in the A5262 trial and explored the relationships between these biomarkers and VF.

Materials and methods

Peripheral blood mononuclear cells were isolated by density centrifugation from whole blood samples collected pre-entry and at weeks 0 (entry), 24 and 48 of therapy. Samples were cryopreserved and analysed in batches at the end of the study. T cell activation (percentage of CD4+ and CD8+ T cells coexpressing HLA-DR and CD38) was measured by flow cytometry.[3] To assay soluble biomarkers of immune activation and inflammation, frozen plasma in EDTA anticoagulant or frozen serum was thawed once and analysed in batch. Interleukin-6 (IL-6), interferon-γ-inducible protein-10 (IP-10) and soluble CD14 (sCD14) were measured in plasma by ELISA (R&D Systems, Minneapolis, MN, USA). D-dimers were measured using the Asserachrom D-DI immunoassay (Diagnostica Stago, Asnieres, France). For measurement of bacterial lipopolysaccharide (LPS), serum was diluted to 10% or 20% with endotoxin-free water and then heated at 85°C for 15 min to denature the proteins. LPS levels were then quantified using a modified Limulus Amebocyte Lysate (LAL) assay (Lonza, Walkersville, MD, USA).

Baseline was defined as the average of the pre-entry and entry values except for LPS, for which the baseline was the value obtained at entry (in a confirmed fasting state). Associations between biomarkers at baseline were assessed using Spearman's rank correlation. The Wilcoxon signed rank test analysed changes from baseline; comparisons between groups were made using the Wilcoxon rank sum test, and Cox proportional hazards models assessed predictors of VF. Tests were performed using a 5% level of significance with no adjustment for multiple testing. Ethics committees at each research site approved the study. Each participant provided written informed consent.

Results

Biomarker levels at baseline

Assays were completed on 107 of 112 subjects after excluding five subjects who had only baseline samples. The subjects included were 94 (88%) men with a median age of 37 years, a median baseline CD4 count of 261.5 cells/mm^3 and a median baseline VL of 75 876 copies/mL. At baseline, median (IQR) CD4+ T cell activation was 11% (7%–20%) while median CD8+ T cell activation was 39% (29%–51%). Median values for IL-6, LPS, IP-10, sCD14 and D-dimer were 1.38 pg/mL (0.81–2.37 pg/mL), 21 pg/mL (10–35 pg/mL), 517 pg/mL (349–929 pg/mL), 2.42×10^6 pg/mL ($1.96–3.08\times10^6$ pg/mL) and 202 ng/mL (111–362 ng/mL), respectively. Patients with baseline VL >100 000 copies/mL had higher baseline CD4+ and CD8+ T cell activation as well as higher IL-6, IP-10, sCD14 and D-dimer levels than those with VL ≤100 000 copies/mL (all $P\leq0.035$). Only LPS levels did not differ between the two baseline strata of VL ($P=0.90$) (Table 1).

The baseline $\log_{10}$ VL correlated inversely with the baseline CD4+ T cell count ($r=-0.44$) and directly with CD4+ T cell activation ($r=0.39$), CD8+ T cell activation ($r=0.28$), IL-6 ($r=0.31$), IP-10 ($r=0.43$) and sCD14 ($r=0.33$) (all $P\leq0.003$). A weaker direct correlation was present with D-dimers ($r=0.19$, $P=0.05$) and none with LPS ($r=0.01$, $P=0.95$).

Changes in the levels of biomarkers during treatment

Among the entire study population, all assessed biomarkers except LPS declined significantly from baseline to weeks 24 and 48 (Table 1). Median LPS levels at entry (21 pg/mL) and at weeks 24 (26 pg/mL) and 48 (22 pg/mL) were not significantly different (all $P>0.1$).

The group with a baseline VL >100 000 copies/mL experienced a greater decrease in CD4+ T cell activation from baseline to week 24 (−8% versus −4%, $P=0.017$) and week 48 (−11% versus −5%, $P=0.006$) than did the group with a baseline VL ≤100 000 copies/mL. Despite the greater decline, CD4+ T cell activation remained higher in those with a baseline VL >100 000 copies/mL at week 24 (8% versus 5%, $P<0.001$) and week 48 (5% versus 3%, $P=0.003$). CD8+ T cell activation was also higher in the high VL group at weeks 24 (18% versus 11%, $P=0.007$) and 48 (11% versus 9%, $P=0.031$), although we did not detect a significant difference between the two groups in the decline in CD8+ T cell activation from baseline to weeks 24 ($P=0.374$) and 48 ($P=0.084$). Patients with a baseline VL >100 000 copies/mL had greater falls in most assessed markers on treatment, but absolute levels of IL-6, LPS, IP-10, sCD14 and D-dimer at weeks 24 and 48 were similar in the two baseline VL groups.

Associations between VF and levels of assessed biomarkers

In univariate analysis, baseline CD4+ T cell activation was higher among patients who subsequently experienced VF than among those who did not (median 16% versus 10%, $P=0.035$). Baseline CD8+ T cell activation, IL-6, LPS, IP-10, sCD14 and D-dimers showed no association with subsequent VF (all $P\geq0.086$). During treatment, there was a consistent pattern in which the group with VF had higher T cell activation than the group without VF. This was observed for CD4+ T cell activation at week 24 (8% in those with VF versus 5% without VF, $P=0.001$) and week 48 (6% versus 3%, respectively; $P=0.006$); and also for CD8+ T cell activation at weeks 24 (21% versus 12%, respectively; $P=0.015$) and 48 (17% versus 10%, respectively; $P=0.010$). In contrast, we did not detect a significant association between any of the other assessed biomarkers and VF, except for higher D-dimer levels at week 24 among those with VF (177 versus 119 ng/mL, $P=0.025$).

We next compared the change in assessed biomarkers from baseline to weeks 24 and 48 in patients with and without VF. For CD4+ T cell activation, no significant difference was observed between the two groups (all $P>0.05$), while for CD8+ T cell activation, the decline in CD8+ T cell activation from baseline was less in patients with VF ($P=0.005$ at week 24, $P=0.008$ at week 48). No significant difference was detected in the change from baseline to weeks 24 and 48 for IL-6, LPS, IP-10, sCD14 or D-dimer in patients with VF versus those without VF (all $P\geq0.057$).

Because a high proportion of patients showing treatment failure in the A5262 trial had a VL of 51–200 copies/mL at VF, we conducted a subgroup analysis comparing those with a VL ≤200 copies/mL with those with a VL >200 copies/mL at VF ($n=17$ versus $n=10$, respectively). In this subgroup analysis, those with VL ≤200 copies/mL at VF had a higher baseline CD8+ T cell activation (44% versus 23%, $P=0.039$). No difference was

Table 1. Immunological and inflammatory markers [median (IQR)] by baseline HIV-1 RNA

Marker		Total[a]	Baseline HIV-1 RNA (copies/mL) ≤100000[b]	Baseline HIV-1 RNA (copies/mL) >100000[c]	*P* value*
CD4+/HLA-DR+/CD38+ %	baseline	11 (7, 20)	9 (5, 15)	18 (9, 23)	<0.001
	week 24	5 (3, 9)	5 (2, 6)	8 (5, 12)	<0.001
	week 24 Δ	−6 (−11, −2)#	−4 (−9, −2)#	−8 (−13, −3)#	0.017
	week 48	3 (2, 7)	3 (2, 5)	5 (3, 8)	0.003
	week 48 Δ	−7 (−14, −4)#	−5 (−11, −3)#	−11 (−16, −5)#	0.006
CD8+/HLA-DR+/CD38+ %	baseline	39 (29, 51)	36 (26, 50)	46 (33, 55)	0.014
	week 24	16 (9, 23)	11 (8, 19)	18 (10, 27)	0.007
	week 24 Δ	−22 (−32, −13)#	−20 (−30, −13)#	−25 (−34, −14)#	0.374
	week 48	10 (7, 16)	9 (6, 13)	11 (9, 21)	0.031
	week 48 Δ	−27 (−37, −16)#	−24 (−33, −16)#	−30 (−39, −20)#	0.084
IL-6 (pg/mL)	baseline	1.38 (0.81, 2.37)	1.11 (0.70, 2.01)	1.73 (1.25, 3.15)	0.003
	week 24	1.17 (0.65, 1.76)	1.06 (0.65, 1.67)	1.19 (0.66, 1.91)	0.848
	week 24 Δ	−0.32 (−0.97, 0.18)#	−0.14 (−0.71, 0.28)#	−0.61 (−2.08, −0.02)#	0.040
	week 48	1.17 (0.63, 1.66)	1.13 (0.53, 1.66)	1.21 (0.65, 1.69)	0.526
	week 48 Δ	−0.37 (−0.96, 0.22)#	−0.22 (−0.69, 0.26)#	−0.42 (−1.64, 0.12)#	0.299
LPS (pg/mL)	baseline	21 (10, 35)	21 (10, 33)	20 (10, 38)	0.899
	week 24	26 (12, 35)	26 (10, 35)	25 (12, 36)	0.800
	week 24 Δ	0 (−9, 12)	1 (−8, 13)	0 (−10, 10)	0.557
	week 48	22 (6, 34)	16 (5, 34)	25 (12, 33)	0.478
	week 48 Δ	−1 (−12, 8)	−3 (−14, 5)	4 (−11, 12)	0.176
IP-10 (pg/mL)	baseline	517 (349, 929)	425 (282, 673)	690 (459, 1184)	<0.001
	week 24	180 (140, 326)	175 (136, 332)	194 (144, 280)	0.642
	week 24 Δ	−296 (−555, −144)#	−185 (−395, −98)#	−481 (−968, −260)#	<0.001
	week 48	172 (123, 249)	173 (122, 267)	166 (123, 246)	0.737
	week 48 Δ	−344 (−649, −141)#	−255 (−447, −75)#	−522 (−999, −304)#	<0.001
sCD14 ($\times10^6$ pg/mL)	baseline	2.42 (1.96, 3.08)	2.11 (1.71, 2.90)	2.75 (2.16, 3.16)	0.002
	week 24	2.03 (1.63, 2.37)	2.02 (1.57, 2.28)	2.06 (1.69, 2.63)	0.377
	week 24 Δ	−0.46 (−0.97, 0.08)#	−0.21 (−0.93, 0.12)#	−0.71 (−1.09, −0.25)#	0.046
	week 48	1.98 (1.65, 2.43)	2.00 (1.62, 2.41)	1.95 (1.68, 2.56)	0.815
	week 48 Δ	−0.41 (−1.01, 0.06)#	−0.22 (−0.70, 0.12)#	−0.71 (−1.24, −0.07)#	0.021
D-dimer (ng/mL)	baseline	202 (111, 362)	160 (105, 332)	266 (140, 513)	0.035
	week 24	136 (73, 258)	118 (70, 255)	147 (82, 260)	0.331
	week 24 Δ	−45 (−130, −12)#	−34 (−113, −2)#	−64 (−170, −25)#	0.036
	week 48	130 (78, 251)	144 (85, 255)	129 (74, 232)	0.566
	week 48 Δ	−62 (−148, −12)#	−47 (−100, 11)#	−83 (−212, −39)#	0.013

[a]Depending on assay result availability, *n* ranges from 96 to 107.
[b]Depending on assay result availability, *n* ranges from 52 to 59.
[c]Depending on assay result availability, *n* ranges from 44 to 48.
*Exact Wilcoxon rank sum test comparing baseline HIV-1 RNA ≤100000 and >100000 copies/mL.
#Exact Wilcoxon signed rank test comparing changes from baseline: $P<0.05$.

detected in baseline CD4+ T cell activation or in IL-6, LPS, IP-10, sCD14 and D-dimer levels between these subgroups (all $P\geq0.115$). CD4+ and CD8+ T cell activation at weeks 24 and 48 was similar in the two groups, as were all other assessed markers except sCD14, which at week 48 showed a higher concentration in the ≤200 copies/mL failure group (2.44×10^6 versus 1.73×10^6 pg/mL, $P=0.027$).

Finally, multivariable Cox PH regression models for time to VF evaluating baseline VL and individual baseline biomarkers (CD4+ T cell activation, CD8+ T cell activation, IL-6, LPS, IP-10, sCD14 or D-dimer) showed that baseline VL >100000 copies/mL was independently predictive of VF (hazard ratio for >100000 versus ≤100000 copies/mL was 4.5–5.6, $P\leq0.002$) No independent association was detected between the risk of VF and baseline levels

of T cell activation, or with any of the soluble markers when baseline VL was included in the model.

Discussion

In this study, evaluated biomarkers of immune activation, inflammation and microbial translocation were, except for LPS, higher at baseline in patients with VL >100 000 copies/mL. Darunavir/ritonavir plus raltegravir was associated with a significant decline from baseline in biomarkers (IL-6, sCD14 and D-dimer) that are predictive of mortality.[4–6] We did not observe a change in LPS with treatment, but are unable to dissect this finding from potential confounders. The median baseline LPS in our study was 21 pg/mL, ∼4-fold lower than that reported in other studies.[7,8] This could have limited the sensitivity of the assay to discriminate differences at low levels.

As a group, patients with VF had consistently higher T cell activation during treatment compared with those without VF, suggesting that the magnitude of T cell activation during treatment reflects HIV levels, similar to observations for untreated infection.[9] Only baseline VL >100 000 copies/mL remained independently predictive of VF after considering T cell activation or soluble inflammatory biomarkers. T cell activation levels were not significantly different between those with VL ≤200 versus >200 copies/mL at VF, although this analysis was limited by the small sample size. The persistence of heightened activation during ART has been reported even in some patients with VL under 50 copies/mL,[10,11] especially those with poor CD4 recovery.[12]

In conclusion, darunavir/ritonavir plus raltegravir attenuated immune activation, inflammation and microbial translocation. T cell activation remained higher in patients with VF than in those without VF. Baseline VL above 100 000 copies/mL remained the primary driver of VF with this regimen even when accounting for baseline biomarker levels. Larger studies comparing this novel regimen to conventional nucleos(t)ide-containing therapy should further elucidate the potential interactions between T cell activation and VF.

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