Original article

A pilot study to assess inflammatory biomarker changes when raltegravir is added to a virologically suppressive HAART regimen in HIV-1-infected patients with limited immunological responses

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Background: Despite successful suppression of HIV-1 with HAART, some patients do not have robust immunological recovery. Chronic inflammation from persistent immune activation could contribute to this poor response, resulting in HIV-1 disease progression and the development of some non-HIV-1 comorbidities.

Methods: We conducted a pilot study of 30 HIV-1-infected patients with undetectable viral loads and poor CD4+ T-cell responses on long-term stable HAART to assess whether the addition of raltegravir would have an effect on biomarkers of chronic inflammation. A total of 26 patients were followed for 1 year on the intensified regimen. In addition to T-cell responses, we evaluated changes in activated CD4+ and CD8+ T-cells, several pro-inflammatory cytokines and chemokines and memory cell responses to HIV-1-associated peptides.

Results: Although there was no improvement in CD4+ T-cell counts, the percentage change in CD4+/CD8+ ratios and RANTES (regulated on activation normal T-cells expressed and secreted) increased significantly while the percentage change in CD8+ T-cell counts and CD8+%, activated CD4+ T-cells and several pro-inflammatory chemokines and cytokines decreased significantly. The percentage change in HIV-1-specific nef, pol set 1, gag and env memory T-cells also declined.

Conclusions: The addition of raltegravir to a virologically suppressive HAART regimen in patients with poor immunological responses resulted in the reduction of several pro-inflammatory biomarkers; increases were seen in RANTES levels and CD4+/CD8+ T-cell ratios. The clinical relevance of these observations is beyond the scope of this study.

Introduction

Successful treatment of HIV-1 infection is clinically considered to be sustained suppression of HIV-1 viral RNA to levels below the limits of detection. Yet, viral replication with immune activation and ongoing evidence of chronic inflammation persist despite suppression of viral loads below detectable levels. As chronic inflammation contributes to progression of HIV-1 infection [1–6] and to the development of a number of non-HIV-1-associated comorbidities [7–13], it is reasonable to question whether patients are receiving maximal therapeutic benefit from their antiretroviral regimen when viral loads are suppressed below limits of detection.

Raltegravir is a strand-transfer integrase inhibitor that interferes with viral replication at the level of integration of viral DNA into the host cell genome. Addition of this drug to a suppressive antiretroviral regimen adds an additional and novel method by which viral replication and immune activation can potentially be further decreased. A reduction in inflammatory cytokines (as a marker of chronic inflammation) could confer some immunological benefit [14] and reduce the comorbidities associated with HIV-1 infection. We designed this pilot study to assess whether immunological and/or inflammatory biomarkers were affected by raltegravir intensification in virologically suppressed patients who
had not mounted robust immunological recovery in response to antiretroviral therapy over a period of several years.

Methods

This study was conducted in accordance with the guidelines established by the Helsinki Declaration of 1975, as revised in 2000, and with the oversight of the Institutional Research Review Board at National Jewish Health.

A total of 30 individuals taking stable HAART regimens for at least 1 year with undetectable viral loads (median <48 HIV-1 RNA copies/ml) and CD4+ T-cell counts <350 cells/mm³ or persistently falling CD4+ T-cell counts and CD4+ percentages for at least 2 years were evaluated to determine whether the addition of raltegravir to their regimen would induce measurable changes in activated CD4+ and CD8+ T-cells, biomarkers of chronic inflammation and circulating effector memory cells.

After giving written informed consent to participate in this study, each patient had two pre-raltegravir visits an average of 20 days apart. Raltegravir, administered at a dose of 400 mg orally twice daily, was then added to their stable HAART regimen. The patients were re-evaluated at 1, 3, 6 and 12 months while receiving the intensified antiretroviral regimen. At each visit patients were assessed for new symptoms, side effects and cellular, virological and serological changes. Blood specimens at each visit were obtained for T-cell enumeration: absolute lymphocyte count; CD4+, CD8+ and CD3+ T-cell counts (cells/mm³) and percentages; and CD4+/CD8+ T-cell ratios. Viral load measurements were obtained at each visit by PCR utilizing the Roche Amplicor HIV-1 MONITOR, Version 1.5 assay (Roche Molecular Systems, Inc., Branchburg, NJ, USA). CD4+ and CD8+ T-cell activation was measured by flow cytometry and identified by the dual expression of CD38+ and human leukocyte antigen DR (BD Biosciences, San Jose, CA, USA). Separate undiluted plasma samples were used to measure the levels of eight inflammatory biomarkers (in pg/ml), as determined by multiplex Luminex assays (Milliplex, Millipore; Billerica, MA, USA). The biomarkers comprised interferon-γ (IFN-γ), tumour necrosis factor-α (TNF-α), macrophage inflammatory protein-1α (MIP-1α) and β (MIP-1β), stromal cell-derived factor 1 (SDF-1), interleukin (IL)-2, IL-7 and IL-10. RANTES (regulated on activation normal T-cells expressed and secreted) was measured in ng/ml by enzyme-linked immunosorbant assay (ELISA; R&D Systems, Minneapolis, MN, USA). Effector memory cells were measured by enzyme-linked immunospot assay (ELISpot; Mabtech, Cincinnati, OH, USA) as the number of spots/500,000 cells with measureable IFN-γ levels. Peripheral blood mononuclear cells were stimulated using HIV-1-specific peptide pools: nef (49 peptides), pol set 1 (125 peptides), pol set 2 (124 peptides), gag (123) peptides, env set 1 (106 peptides) and env set 2 (105 peptides). Most of the peptides were 15 amino acids in length with 11 amino acid overlaps between sequential peptides. The reagents were obtained from the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, National Institutes of Health: HIV-1 consensus subtype B nef (15-mer) peptides, complete set; HIV-1 consensus B pol (15-mer) peptides, complete set; and HIV-1 consensus subtype B gag (15-mer) peptides, complete set; HIV-1 consensus subtype B env (15-mer) peptides, complete set. All flow cytometry and ELISpot assays were performed on the day that samples were drawn.

At least three controls were included on each run, a high, low and normal control: two controls were supplied by the manufacturer and one control was characterized distinct from the kit reagents. If the controls fell out of range, the run was rejected and the testing was repeated in an effort to maintain consistency across batches. Specimens from nine HIV-1-negative individuals were utilized to validate the technical aspects of the effector cell analysis.

As there was variability in the pre-raltegravir measurements between patients, each patient served as their own control. For each patient, the two pre-raltegravir measurements were averaged and that average was considered to be the baseline (BL) value for each cellular or inflammatory biomarker of interest in this study. Subsequent measurements were evaluated for their change from BL and for their percentage change from the BL value.

Statistical analysis was performed using the paired T-test comparing the changes in each metric at 12 months of intensification to the BL value if normally distributed (Shapiro Wilk test P-value >0.05) or the Wilcoxon two-sample test, t-approximation if not normally distributed. The median percentage change for each biomarker of interest was evaluated for significance using the Wilcoxon two-sample test, t-approximation. For outcomes where any patients had levels below the limits of detection at baseline or 12 months, the Fisher exact test was performed comparing numbers of patients with levels below limits of detection at BL and at 12 months (SAS Version 9.2, Cary, NC, USA).

Results

A total of 30 patients, 25 (83.3%) with CD4+ T-cell counts <350 cells/mm³ (median 290 cells/mm³) and 5 (16.6%) with falling CD4+ T-cell counts (average 389 cells/mm³) and percentages (average 22.1%) for 2 years prior to entry in the study and who were virologically

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Table 1. Baseline characteristics of the study population

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All enrolled patients, n=30</th>
<th>Patients completing study, n=26</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age, years (range)</td>
<td>51.5 (38–68)</td>
<td>52.5 (38–68)</td>
</tr>
<tr>
<td>White race, n (%)</td>
<td>25 (83.3)</td>
<td>23 (88.5)</td>
</tr>
<tr>
<td>Hispanic ethnicity, n (%)</td>
<td>5 (16.7)</td>
<td>3 (11.5)</td>
</tr>
<tr>
<td>Male sex, n (%)</td>
<td>29 (96.7)</td>
<td>26 (100)</td>
</tr>
<tr>
<td>Median time since HIV-1 diagnosis, years</td>
<td>19.0</td>
<td>19.5</td>
</tr>
<tr>
<td>Median nadir CD4+ T-cell count, cells/mm^3</td>
<td>73.5</td>
<td>84.5</td>
</tr>
<tr>
<td>Median nadir CD4+ T-cells, %</td>
<td>8.0</td>
<td>8.0</td>
</tr>
<tr>
<td>Median peak viral load, HIV-1 RNA copies/ml</td>
<td>68,196</td>
<td>60,282</td>
</tr>
<tr>
<td>CD4+ T-cell count 2 years prior to BL, cells/mm^3</td>
<td>324.5</td>
<td>324.5</td>
</tr>
<tr>
<td>Median CD4+ % 2 years prior to BL, %</td>
<td>19.5</td>
<td>19.5</td>
</tr>
<tr>
<td>CD4+ T-cell count 1 year prior to BL, cells/mm^3</td>
<td>290</td>
<td>324.0</td>
</tr>
<tr>
<td>Median CD4+ % 1 year prior to BL, %</td>
<td>21.2</td>
<td>22.9</td>
</tr>
<tr>
<td>Baseline CD4+ T-cell count, cells/mm^3</td>
<td>311.5</td>
<td>331.5</td>
</tr>
<tr>
<td>Median baseline CD4+ T-cells, %</td>
<td>19.1</td>
<td>19.3</td>
</tr>
<tr>
<td>Median baseline viral load, HIV-1 RNA copies/ml</td>
<td>&lt;48^b</td>
<td>&lt;48^b</td>
</tr>
<tr>
<td>Median time with undetectable viral load, months</td>
<td>53.0</td>
<td>57.0</td>
</tr>
<tr>
<td>Median total time on HAART, years</td>
<td>15.8</td>
<td>15.0</td>
</tr>
<tr>
<td>Median total time on current regimen at BL, months</td>
<td>33</td>
<td>33</td>
</tr>
<tr>
<td>Current PI-based regimen, n (%)</td>
<td>14 (46.7)</td>
<td>12 (46.2)</td>
</tr>
<tr>
<td>Current NNRTI-based regimen, n (%)</td>
<td>16 (53.3)</td>
<td>14 (53.8)</td>
</tr>
</tbody>
</table>

^25 Patients (viral load <48 copies/ml); 5 patients (viral load 79, 80, 125 and 58 copies/ml). ^22 Patients (viral load <48 copies/ml); 4 patients (viral load 79, 80,125 and 210 copies/ml). BL, baseline; NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor.

suppressed (<400 copies HIV-1 RNA/ml, median <48 copies HIV-1 RNA/ml) on their current HAART regimen (median 38.5 months) were enrolled in this study. Patients in the study were on average 52 years of age (range 38–68 years), had been HIV-1-positive for 19.6 years, had received antiretroviral therapy for 15.8 years and had been virologically suppressed on any regimen for 53 months. The median nadir CD4+ T-cell count was 73 cells/mm^3 and median peak viral load was 90,000 copies HIV-1 RNA/ml. Fourteen patients were receiving a protease inhibitor based regimen and 16 patients were receiving a non-nucleoside reverse transcriptase inhibitor based regimen. All patients were also receiving at least two nucleoside/nucleotide analogues and maintained undetectable viral loads for the duration of their participation in the study; no adverse events were reported. Of the 30 patients in the study, 1 patient chose to discontinue participation for personal reasons after the first two visits and 3 patients chose to discontinue the study after the 6 month visit (data available for 5 of 6 visits). The remaining 26 patients were analysed in this study for the entire 12 months. Table 1 illustrates the baseline characteristics for all 30 patients compared with the 26 patients analysed after completion of 12 months of raltegravir intensification. Each outcome of interest was evaluated for the change in measurements in each biomarker from BL to 12 months after intensification with raltegravir. Owing to variability in BL values for each outcome of interest from patient to patient, the median percentage change from BL to 12 months was also calculated.

Immunological parameters

From BL to 12 months, changes in the median values of CD4+ %, CD8+ T-cell count, CD8+ % and the CD4+/CD8+ T-cell ratio were 19.3–23.3%, 693–621 cells/mm^3, 50.9–46.7% and 0.38–0.49, respectively (for all P<0.007). The change in the median CD4+ T-cell count was 331–301 cells/mm^3 (P=0.14; Table 2). The median percentage changes at 12 months from BL for the CD4+ %, CD8+ T-cell count, CD8+ % and CD4+/CD8+ T-cell ratio were 7.0%, -10.8%, -3.0% and 10.8%, respectively (for all P<0.005). The median percentage change from BL for the CD4+ T-cell count was 4.6 (P=0.11; Figure 1A).

The change in the median activated CD4+ % was 7.1–4.6% (P<0.001), whereas that of the activated CD8+ % was 11.9–11.7% (P=0.32). The median percentage change from BL to 12 months was -39.3% (P<0.001) for the activated CD4+ % and -16.8% (P=0.11) for the activated CD8+ % (Table 2; Figure 1A).

Cytokine and chemokine biomarkers

The median increase in RANTES and decreases in MIP-1β, IL-2, IL-7, IL-10 and SDF-1 were statistically significant (for all P<0.02; Table 3). The median percentage changes from BL in RANTES, MIP-1β, TNF-α, IL-2, IL-7 and IL-10 were 43.8%, -55.2%, -23.0%, -28.2%, -91.5% and -52.4%, respectively (for all
CD8+ T-cell count and CD8+% were normally distributed. BL, baseline.

Activated CD4+ T-cells, %
CD4+ T-cells, %
CD4+ T-cell count, cells/mm³
CD8+ T-cells, %
CD8+/CD8+ T-cell ratio
Activated CD4+ T-cells, %
Activated CD8+ T-cells, %

Table 2. Median absolute change over 12 months of immunological measurements for 26 patients

<table>
<thead>
<tr>
<th>Immunological measurement</th>
<th>BL (range)</th>
<th>1 Month (range)</th>
<th>3 Months (range)</th>
<th>6 Months (range)</th>
<th>12 Months (range)</th>
<th>Change from BL to 12 months</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4+ T-cell count, cells/mm³</td>
<td>331 (142–536)</td>
<td>327 (142–522)</td>
<td>305 (177–518)</td>
<td>323.5 (180–696)</td>
<td>301 (185–626)</td>
<td>-30</td>
<td>0.14</td>
</tr>
<tr>
<td>CD4+ T-cells, %</td>
<td>19.3 (8.7–37.7)</td>
<td>20.3 (7.6–38.1)</td>
<td>20.5 (9.2–35.6)</td>
<td>21.7 (9.3–41.0)</td>
<td>23.3 (11.1–37.2)</td>
<td>4.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CD8+ T-cell count, cells/mm³</td>
<td>693 (191–1,922)</td>
<td>716.5 (210–1,388)</td>
<td>761.5 (231–1,760)</td>
<td>623.5 (227–1,572)</td>
<td>621 (221–1,624)</td>
<td>-72</td>
<td>0.007</td>
</tr>
<tr>
<td>CD8+ T-cells, %</td>
<td>50.9 (21.4–65.8)</td>
<td>50.6 (20.6–70.1)</td>
<td>50.2 (22.4–66.1)</td>
<td>49.7 (21.0–65.8)</td>
<td>46.7 (22.7–63.8)</td>
<td>-4.2</td>
<td>0.006</td>
</tr>
<tr>
<td>CD4+/CD8+ T-cell ratio</td>
<td>0.38 (0.15–1.70)</td>
<td>0.42 (0.13–1.73)</td>
<td>0.43 (0.18–1.59)</td>
<td>0.45 (0.17–1.89)</td>
<td>0.49 (0.20–1.64)</td>
<td>-0.11</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Activated CD4+ T-cells, %</td>
<td>7.1 (3.5–29.4)</td>
<td>4.7 (1.8–20.9)</td>
<td>6.0 (3.0–29.8)</td>
<td>4.9 (1.6–19.7)</td>
<td>4.6 (0.8–17.8)</td>
<td>-2.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Activated CD8+ T-cells, %</td>
<td>11.9 (5.5–40.3)</td>
<td>12.2 (3.7–38.1)</td>
<td>13.0 (6.4–35.6)</td>
<td>11.4 (2.7–32.6)</td>
<td>11.7 (2.3–28.7)</td>
<td>-0.2</td>
<td>0.32</td>
</tr>
</tbody>
</table>

*Paired t-test if normally distributed (Shapiro-Wilk test P-value >0.05), Wilcoxon two-sample test, t-approximation if not normally distributed. Changes in CD4+%, CD8+ T-cell count and CD8+% were normally distributed. BL, baseline.

P<0.008; Figure 1B). RANTES levels were normal (23–77 ng/ml) in 9/26 (34.6%) patients at BL and at 12 months were normal in 19/26 (73.1%) patients. Nine of 26 (35%) patients had undetectable IL-2 levels at BL and following 12 months of raltegravir intensification. An additional 11 patients with measurable IL-2 levels at BL (average 15.1 pg/ml) achieved undetectable IL-2 levels at 12 months, resulting in a total of 20/27 (77%) patients at study end. Of the remaining six patients (average BL 44.7 pg/ml), three had increased (average 11.2 pg/ml) and three had decreased (average 27.6 pg/ml) IL-2 levels at 12 months.

Of 26 patients, 13 (50%) had IL-7 levels that were below the limits of detection at BL and the values remained undetectable for the duration of the study. The median percentage change from BL for the other 13 patients was -91.5%. At 12 months, 22/26 (85%) patients achieved values below the limits of detection. Similar findings were seen with TNF-α, IFN-γ, MIP-1α, IL-2 and IL-10 (Figure 1B).

There was a positive correlation between the change in CD4+% from BL to 12 months and the change in RANTES levels and a negative correlation with the change in TNF-α, IL-2, IL-7 and IL-10 levels over that same period of time.

Effector memory cells
With the exception of pol set 2, the change for all other sets of memory cells declined with only nef, pol set 1 and gag reaching statistically significant levels (P<0.05; Table 4). The median percentage change from BL to 12 months for nef, pol set 1, gag, env set 1 and env set 2 was statistically significant -37.2%, -40.8%, -29.1%, -64.7 and -61.7%, respectively (for all P<0.05; Figure 1C).

Discussion
Our study, similar to other raltegravir intensification studies, was unable to demonstrate significant increases in CD4+ T-cell counts [15,16]. However, we had hypothesized that the addition of an agent that used a different mode of action to interfere with viral replication might act synergistically with other antiretroviral agents to alter some measurements of chronic inflammation. In this pilot study we measured statistically significant increases in CD4+ T-cell percentages, CD4+/CD8+ T-cell ratios and RANTES levels and statistically significant reductions in CD8+ T-cell counts and percentages, activated CD4+ percentages, MIP-1β, TNF-α, IL-2, IL-7, IL-10 and antigen-specific memory cells for nef, gag, env set 1, env set 2 and pol set 1 after 12 months of raltegravir intensification.

The clinical implications of these findings are beyond the scope of our study. However, it does appear that the addition of raltegravir to a virologically suppressive HAART regimen in patients who have been infected with HIV-1 for several years and who have suboptimal T-cell responses might have some immunological changes not apparent from measurements of CD4+ T-cell counts alone. The reduction in activated CD4+ T-cells might reflect reduced overall viral antigen load, reduced immune response to the viral antigen or a reduced state of non-specific inflammation [17–19]. It should be noted that our goal was to measure changes in biomarkers of inflammation. We did not measure changes in viral load measurements with more sensitive viral load assays, because we did not have BL viraemic samples. Without these samples, the primers used to run the single-copy assay would not necessarily recognize all patients’ viruses rendering the results incomplete or inaccurate.

Another interpretation of our findings could be that the patients continue to lose T-cells as a result of the lasting immunological injury due to HIV-1. This reduction in T-cells results from the continuous contraction of the CD4+ and CD8+ T-cell pool. The reduction in cytokines could be the result of the reduced number of T-cells. We favour the former interpretation because the
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Figure 1. Median percentage change at 12 months of raltegravir intensification

(A) Median percentage change in immunological parameters from baseline (BL). (B) Median percentage change in cytokines/chemokines from BL. (C) Median percentage change in HIV-1-specific memory cells from BL. *n=18 and n=13 for patients whose BL levels of interferon-γ (IFN-γ) and interleukin-7 (IL-7), respectively, were detectable. /n=24 for patients whose BL levels of env set 2 were >0. CD4^+38^+DR^+, activated CD4^+ T-cells; CD8^+38^+DR^+, activated CD8^+ T-cells; IL-2, interleukin-2; IL-10, interleukin-10; MIP-1α, macrophage inflammatory protein-1α; MIP-1β, macrophage inflammatory protein-1β; ns, not statistically significant; RANTES, regulated on activation normal T-cells expressed and secreted; SDF-1, stromal cell derived factor; TNF-α, tumour necrosis factor-α.
loss of viral antigen-specific memory T-cells is disproportionately higher than the loss of total CD8* T-cells (40% versus 10%). This might be an immunological surrogate for a further reduction in viral antigens in patients taking raltegravir, although over shorter time periods (12 weeks [15] and 24 weeks [16]) other studies have not demonstrated such reductions. The clinical relevance of the reduction in viral antigen-specific memory T-cells will require further studies. The reductions in pro-inflammatory cytokines might also have long-term implications in terms of progression or regression of the immunological health of HIV-1-infected individuals [20]. Such an understanding might also be useful in developing therapies to reduce the incidence of comorbidities, such as cardiovascular disease [7–9], lipatrophy [11,13] and osteoporosis, entities that are known to be mediated, in part, by immune activation and pro-inflammatory cytokines.

It is notable that several patients in this study had undetectable measurements of several cytokines.
suggesting that their BL antiretroviral therapy was effective enough to prevent expression of these cytokines prior to intensification with raltegravir. Equally notable is that a significant percentage of patients with measurable cytokine levels at BL developed undetectable levels of these cytokines 12 months after raltegravir was added to their regimen. In this context, the reduction in IL-7 levels is noteworthy. Recombinant IL-7 has been proposed as an adjunct to treatment of HIV-1 infection because it inhibits apoptosis of CD4+ and CD8+ T-cells [21]. As levels of CD4+ T-cells rise, however, endogenous IL-7 levels have been shown to decline [22]. By contrast, IL-7 has also been shown to be associated with enhanced expression of chemokine X4 (CXCR4)-tropic virions with a reduction of chemokine receptor 5 (CCR5)-tropic strains. Whether these findings will have clinical relevance in the long run has yet to be determined [23].

One finding of this study deserves further mention. RANTES levels rose rapidly from very low to normal levels in 9 out of 16 patients. Ten additional patients in the study had normal RANTES levels at BL and their levels increased by an average of 9.5 ng/dl. The changes in RANTES levels were maintained for the duration of the study. RANTES is a chemokine that serves as a ligand for CCR5, the primary co-receptor for HIV-1. The increase in RANTES to normal levels suggests that in this study raltegravir functioned as both a strand-transfer integrase inhibitor and possibly indirectly, through RANTES, as an entry inhibitor [24-28].

The rise in RANTES in our study patients is intriguing because of its anti-HIV effect. RANTES is produced by many cells including T-cells, monocytes and platelets. There are data that have demonstrated suppression of RANTES release from blood platelets in vitro when the platelets are exposed to env-gp41 peptides [29]. It is possible that a reduction of viral antigens, including env-gp41, due to raltegravir in this study might have permitted RANTES release from platelets. Alternatively, RANTES might have come from a non-platelet source.

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This study was not designed to examine the cellular source of serum RANTES. Nonetheless, its increase might have come from a non-platelet source. RANTES might have come from a non-platelet source. The study was not designed to examine the cellular source of serum RANTES. Nonetheless, its increase points to an additional factor that could influence the clinical course and outcome of HIV-1 treatment.

We were also concerned that the effect of increased RANTES might result in the emergence of dual-mixed or CXCR4-tropic quasispecies. For this reason, we measured SDF-1. SDF-1 is a ligand for the CXCR4 co-receptor. There was a statistically significant decline in SDF-1 levels; however, the levels remained within the normal range of this chemokine in all patients.

One contradictory finding was seen in this study. On the basis of our current understanding of MIP-1α and MIP-1β, we expected to see an increase in both of these chemokines. Like RANTES, they are both ligands for CCR5. Initially, both chemokines increased at earlier time points in our study but declined below their BL levels at 12 months. Although this decline could represent a negative feedback mechanism, this finding will need further study to determine its significance.

There are several limitations to this study. This was a small pilot study and was not powered to demonstrate statistically significant alterations in the multiple outcome variables measured. Contrary to other studies on raltegravir intensification [15,16], we used each patient as their own control and did not have a case-matched control population. It is conceivable that the changes in the biomarkers of interest in this study would have changed without the addition of raltegravir; however, the reversal in the trend of the CD4+ T-cell percentage and CD4+/CD8+ T-cell ratios with the addition of raltegravir suggest otherwise. Also, due to the lack of BL viraemic samples in this patient population, we did not perform single-copy viral load assays owing to concerns about the fidelity of the data.

Although the entry criteria were established in advance, a retrospective review of the participating patients demonstrated significant diversity in BL characteristics that were not anticipated at the onset of the study. Of note is the wide range of CD4+/CD8+ T-cell ratios, despite fairly rigid CD4+ T-cell count criteria, as well as a wide range of ELISpot values in the assessment of circulating effector memory cells. Despite this diversity, the study population represented only a small subset of HIV-1-infected patients. Other HIV-1-infected patients with higher CD4+ T-cell counts or less advanced HIV-1 disease may or may not respond to raltegravir intensification in a similar manner compared with those individuals in this analysis.

It was also not possible to analyse IFN-γ in a meaningful way, as it rose in some patients and fell in others. BL characteristics did not explain these different responses. Also, no conclusions can be drawn regarding the clinical benefit or harm resulting from the changes in the biomarkers evaluated in this analysis. Finally, the choice of biomarkers for evaluation was undoubtedly incomplete and the feedback mechanisms and interactions associated with the changes that took place in this analysis remain poorly understood.

In conclusion, the addition of raltegravir to a suppressive antiretroviral regimen in patients with suboptimal immunological responses resulted in changes in some markers of inflammation that could be of benefit to HIV-1-infected patients. Whether these findings are specific to raltegravir, or to intensification in general, and whether the changes in biomarkers will have any effect on clinical outcomes has yet to be determined. However, the reduction in activated CD4+ T-cells, select cytokines/chemokines and HIV-specific memory cells with simultaneous increases in
CD4+ % and RANTES levels might permit more robust immune restoration over time and reduce the risk of inflammation-mediated comorbidities. These changes suggest that assays for CD4+ T-cell counts and viral loads, although commercially available and in common use, might not be the best measurements of antiretroviral efficacy. If the findings in this analysis can be replicated, studies designed to assess clinical benefit due to these biomarker changes are needed. If such a benefit is established, an implication might be the development of more sensitive metrics against which to assess antiretroviral efficacy.

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