Raltegravir (RAL) therapy is associated with reduced microbial translocation (MT) and monocyte activation in HIV infected subjects naïve to antiretroviral therapy (ART).

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Introduction

The contribution of gut microbiota to persistent systemic immune activation in HIV-patients on ART has come into focus. In a published trial examining the impact of RAL versus NNRTI-based ART on gut immune reconstitution, a significant decline in IFN (sCD14) in the RAL but not the NNRTI cohort was observed [1]. Other cohorts of subjects receiving raltegravir-based regimens have demonstrated favorable reduced immune activation with an uncertain etiology [2,3]. This study was undertaken to explore potential explanations for these findings with a focus on microbial translocation (MT) and systemic immune activation.

Materials and methods

Study Design and Subjects

This investigator-initiated randomized controlled trial enrolled HIV-positive subjects naïve to antiretroviral therapy or who had been exposed to ART remotely for less than 30 days. The only exclusions to participation were safety parameters related to undergoing upper endoscopy with biopsy (Grade II enemia or abnormal coagulation parameters were excluded). All subjects signed an informed consent form approved by the UC Davis Institutional Review Board prior to initiation of study procedures. Clinical Trial Registry Number Identifier (ClinicalTrials.gov) – NCT002970363, and NCT00061960.

Soluble CD14 Assay: Soluble CD14 levels in plasma samples were quantified by ELISA with the Quantikine HIV-sCD14 Immunoassay (R&D Systems, Minneapolis, Maryland, USA) according to the manufacturer’s protocol. Samples were assayed in duplicate.

Biomarkers of MT and immune activation

Plasma LPS-binding protein (LBP) and bacteraemia/permeability increasing protein (BPI, Usen Life Sciences Inc., Missouri City, Texas, USA) were quantified by ELISA according to the manufacturer’s protocol. Levels of proinflammatory TH2 cytokine interleukin 4 (IL-4), Interferon-γ inducible protein 10 (IP-10, CXCL 10) and monocyte chemotactant protein-1 (MCP-1) were all measured with multiple enzyme linked immunosorbent assays (ELISA)-based assays (Mesoscale Discovery).

Peripheral blood mononuclear cells (PBMCs) were separated by Ficoll-Hypaque. Fluorescence-activated cell analysis was performed on a custom Becton-Dickinson LSRII cytometer and with Flowjo Software (Treestar, Ashland, Oregon, USA). The CD14+ T-cell population with an activated phenotype was defined as viable, single CD3+/CD8+ cells double positive for CD10 and HLA-DR.

Statistical analysis:

The two-sided Wilcoxon rank-sum test and the Dunn’s multiple comparison test were used to compare the HIV-positive and control groups for numerical variables. The Spearman rank correlation coefficient was used to study the correlation between two quantitative variables unless stated otherwise. Analyses and graph presentation were performed with SASS v6.2 (SAS Institute Inc., Cary, NC, USA) or GraphPad Prism Software v5.0 (GraphPad Software, Inc., La Jolla, CA, USA).

Results

25 subjects were enrolled and randomized to either the Raltegravir- or NNRTI-containing arms of treatment. Sixteen completed nine months of treatment and are available for analysis for this report along with 7 controls. Seven of the 16 subjects who completed the clinical trial received raltegravir. The NNRTI-based cohort received either efavirenz (n=4), nevirapine (n=3) or etravirine (n=2).

Reasons for drop-out include 2 who had treatment limiting toxicities and switched to a protease-inhibitor based regimen (one from each cohort), 2 moved out of the area and 4 dropped out of medical care or lost insurance. One subject committed suicide in the setting of paranoid schizophrenia and was unrelated to treatment assignment. The demographics and baseline characteristics of evaluable subjects are in Table 1.

Table 1

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>RAL Baseline</th>
<th>RAL 9 mo</th>
<th>NNRTI Baseline</th>
<th>NNRTI 9 mo</th>
</tr>
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<tbody>
<tr>
<td>Age (years)</td>
<td>37.1 ± 7.1</td>
<td>37.6 ± 7.3</td>
<td>36.5 ± 7.8</td>
<td>37.1 ± 7.2</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>8/17</td>
<td>8/9</td>
<td>8/18</td>
<td>8/10</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
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<td>25.9 ± 3.4</td>
<td>27.2 ± 3.8</td>
<td>26.5 ± 3.6</td>
</tr>
</tbody>
</table>

Conclusions

✓ LPS Binding Protein and sCD14 declined in the RAL cohort but not in the NNRTI cohort (figures 1 & 2), demonstrating that reduced MT occurred in the RAL cohort.

✓ Declines in sCD14 correlated with declines in IL-4 (r=0.78) (Fig 3).

✓ MCP-1 (r=0.75) (Fig 4) and IP-10 (r=0.71) (Fig 5) in the RAL cohort but not the NNRTI cohort (r=0.25, 0.27, and 0.57, respectively).

✓ As expected, CD8 T-cell activation correlates with sCD14 levels in the entire data set (Fig 6).

✓ LPS is a potent stimulator of immune activation and leads to monocyte activation following binding with TLR-4 and CD14 receptors.

✓ IL-4 induces differentiation of naïve helper T cells to Th2 cells and is linked to chronic inflammation and wound repair through stimulation of TGFβ secretion. IP-10 is produced by monocytes, endothelial cells and fibroblasts and serves as a chemoattractant for monocytes, T cells and NK cells to tissue foci of inflammation. Similarly, MCP-1 is secreted by monocytes, macrophages and dendritic cells to increase chemotactic activity for monocytes.

✓ Taken together, these findings suggest a possible improvement in barrier or stem cell/microbial communities leading to reduced immune activation in raltegravir-treated patients.

Bibliography


Acknowledgments

This research was made possible by Grant Number U10 RR041946 from the National Center for Research Resources (NCHR), a component of the National Institutes of Health (NIH). and NIH Roadmap for Medical Research and by a grant from Merck Investigator Initiated Research Program. The authors and their parents or guardians do not represent the views of Plan ( Mali) or the United States Federal Government, Veterans Administration or hospital.

The author wishes to acknowledge all the study participants who contributed to the work as well as the clinical research staff of the CTSC Clinical Research Center who made this research possible.