Research Letter

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Switch to maraviroc/raltegravir dual therapy leads to an unfavorable immune profile with low-level HIV viremia

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Immunovirological consequences of a switch to a maraviroc/raltegravir dual therapy were analyzed in 16 HIV-infected patients with persistent viral load below 50 copies/ml. At 26-week postswitch, the $CD4^+/CD8^+$ ratio decreased and the $CD8^+$ T-cell activation increased. A decrease in classical monocytes was associated with a shift toward a proinflammatory monocyte profile and negatively correlated with ultrasensitive viral load. Thus, this therapeutic switch induced a proinflammatory profile probably driven by a slight loss of virus control.

Immune activation with an increase in inflammation markers, a decreased $CD4^+/CD8^+$ ratio, expression of activation/exhaustion markers on T cells, and monocyte activation are biological hallmarks of HIV infection that may persist despite antiretroviral therapy (ART) induced viral suppression [1,2]. These alterations are associated with the emergence of non-AIDS-related diseases in long-term-treated patients [3,4]. T-cell activation [5] and ART with both nucleoside reverse transcriptase inhibitors (NRTI) and protease inhibitors [6,7] have been associated with lipodystrophy in up to 40% of patients. Thus, the ROCnRAL Agence Nationale de Recherche sur le SIDA et les hépatites virales (ANRS) 157 study (ClinicalTrials.gov: NCT1420523) was designed to evaluate whether a NRTI/protease inhibitors-sparing regimen such as maraviroc and raltegravir (MVC/RAL) could maintain viral suppression and be beneficial on both lipodystrophy and immune activation/inflammation parameters as suggested by others [8–13]. However, the trial had to be stopped after a median duration of 19 weeks due to virological failure in five of 44 patients [14].

The present immunological substudy could nevertheless be conducted to explore monocyte and T-cell activation in parallel to plasma soluble markers of activation and inflammation in a subgroup of volunteer patients maintaining a viral load below 50 copies/ml following the MVC/RAL switch. Sixteen patients were included and monitored at baseline (W0) but not all patients reached the end of treatment (eTT; median = 26 weeks, range 12–40) because of the premature termination of the study. Changes in continuous variables were compared using paired Wilcoxon test. At baseline, median age was 55 years [interquartile range (IQR): 53/60], male/female sex 15/1, time since HIV diagnosis 24 years (IQR: 14–25), combination ART duration 17 years (IQR: 12/19), with a median duration of 5.2 years (IQR: 5.0/8.6) of suppressed HIV-viremia (<50 copies/ml), and $CD4^+$ nadir of 162 cells/mm^3 (IQR: 129/280). These clinical characteristics were similar to those of the total ROCnRAL cohort [14].

At W0, 13/16 patients had $CD4^+$ cell counts above 500/mm^3 (median = 702, IQR: 559/798) and seven of 16 had $CD8^+$ cell counts above 700/mm^3 (median = 994, IQR: 799/1012). At eTT compared with W0, CD4 and CD8 counts were similar despite a decrease in $CD4^+$ cell counts in eight of 13 patients, an increase in $CD8^+$ counts in six of 13 patients, and an overall decrease in the $CD4^+/CD8^+$ ratios (median = −0.10, IQR: −0.15/+0.02, $P = 0.04$, Fig. 1a). Moreover, the number of CD38 molecules on $CD8^+$ T cells significantly increased (median = +296 sites/cell, IQR: +4/+791, $P = 0.02$) reflecting activation of $CD8^+$ T cells, although HLA-DR expression remained unchanged (Fig. 1b).

Monocytes were subdivided on the basis of CD14 and CD16 expression into classical ($CD14^+$ $CD16^-$), intermediate ($CD14^+$ $CD16^{+/low}$) and nonclassical ($CD14^{low}$ $CD16^{hi}$) monocytes. At eTT, the percentage of classical monocytes decreased (median = −6.7%, IQR: −2.0/−12.4, $P = 0.03$) to the benefit of both intermediate and nonclassical monocytes, and CD14 expression significantly decreased on classical monocytes (median = −48.8%, IQR: −41.7–62.1, $P = 0.01$) (Fig. 1c). These characteristics suggested a progressive phenotypic shift from classical ($CD14^+$) to proinflammatory monocytes ($CD14^{low}CD16^{hi}$ and $CD14+CD16^{+/low}$). In contrast, there was no modification of HLA-DR or CD38 molecule expression on the monocyte subpopulations (data not shown).

Several soluble plasma molecules related to monocyte activation (sCD14, sCD163), monocyte recruitment (CCL4/MIP-1β, CXCL9/MIG, CXCL10/IP-10, CCL2/MCP-1), or cytokines monocyte production (interleukin-18, interleukin-6) were quantified. A decrease in sCD14 was observed (median = −277 ng/ml, IQR: −386/−9, $P = 0.02$), together with a trend toward a sCD163 increase (median = +59 ng/ml, IQR: −3/+101, $P = 0.06$), whereas there was no differences in cytokines/chemokines levels between W0 and eTT (Fig. 1d).

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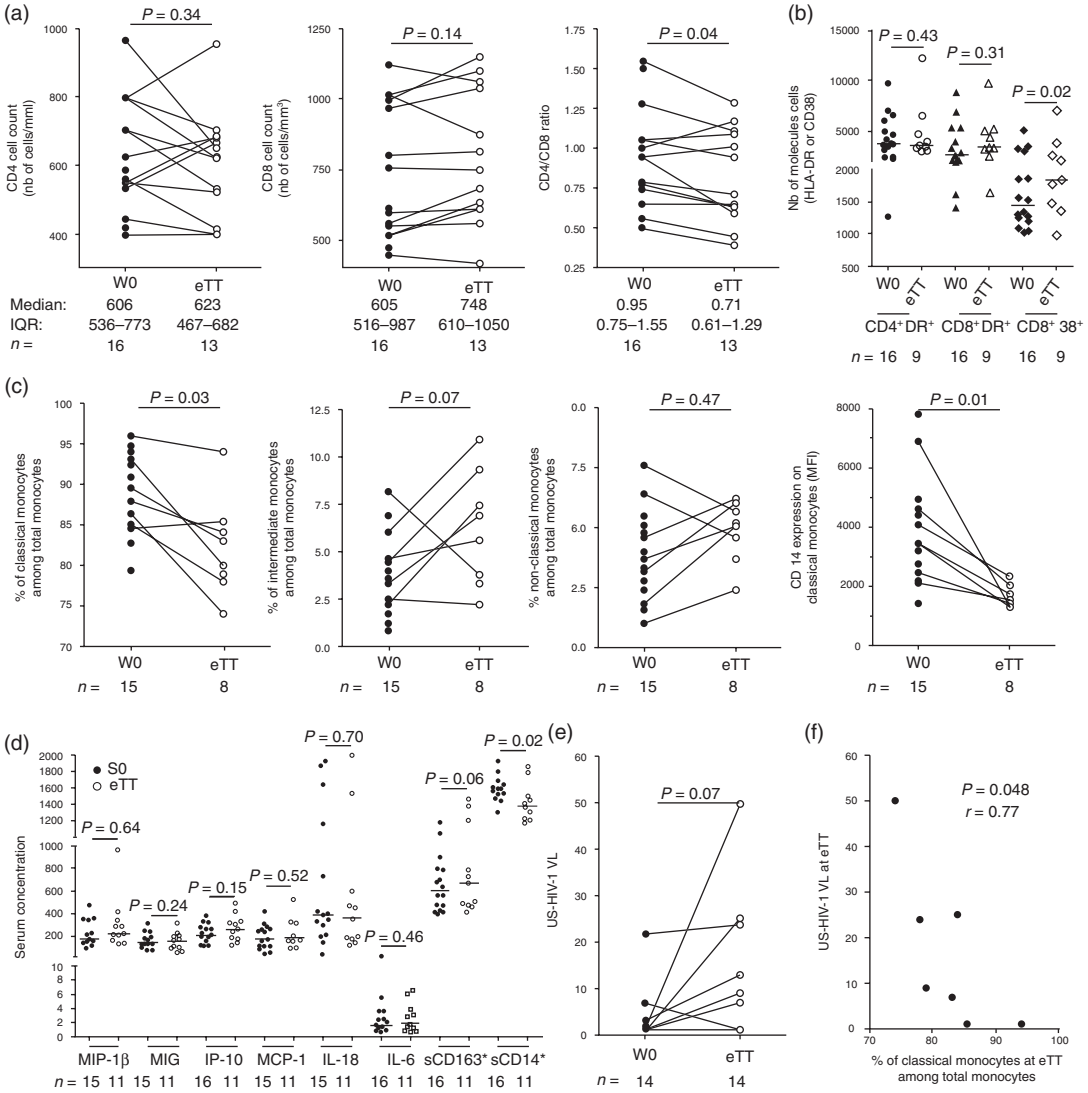

Fig. 1. Immunological and viral characteristics are modulated after a switch to a maraviroc and raltegravir bitherapy. (a) $CD4^+$, $CD8^+$ cell counts and $CD4^+/CD8^+$ ratios were determinate by flow cytometry in each including center. (b,c) Cell surface immunostaining with fluorochrome-conjugated antibodies was performed from fresh whole blood. Cells were analyzed by using multiparameter cytometer (FACS Canto I, BD Biosciences, San Diego, California, USA). In the same tube, (b) T cells were stained with APC-CD4, APC-Cy^7-CD8, PerCp-$Cy^{5.5}$-CD3 and the number of HLA-DR and CD38 molecules at the cell surface was quantified using QuantiBrite PE-Beads (BD Biosciences); (c) monocytes were stained with FITC-CD14 (BD Biosciences) and PE-Cy^7-CD16 (Beckman Coulter, Fullerton, California, USA) in order to discriminate the classical, intermediate and nonclassical monocytes and evaluate the cell surface expression of the $CD14^+$ molecule. (d) Soluble plasma mediators were measured by CBA kit for MIP-1β, MIG-1, IP-10, and MCP-1 (BD CBA, FACS Canto I, BD Biosciences) or by ELISA for interleukin-18 (Elabscience, Beïjing, China) and interleukin-6 (Clinisciences, Cusabio Wuhan, China), sCD163 and sCD14 (R&D Systems, Minneapolis, Minnesota, USA). All results are expressed in pg/ml except for '*' (ng/ml). (e) HIV-1 viral load was determined by using an ultrasensitive assay as previously described [15]. (a–e) Horizontal bars are reported as median. A Wilcoxon match paired test was used to compare eTT (open circles) point to W0 (closed circles) (GraphPad Prism software; GraphPad Software Inc., La Jolla, CA, USA) and below 1 copy ultrasensitive-HIV-1 viral load values were replaced by 1 copy/ml. (f) Correlation between ultrasensitive-HIV-1 viral load and percentage of classical monocytes at eTT point (Spearman's correlation test, GraphPad Prism software). eTT, end of treatment; IQR, interquartile range.

Finally, the levels of ultrasensitive-HIV-1 viral load (threshold 1 copy/ml) [15] were measured at W0 and eTT despite the fact that all patients remained with viral load below 50 copies/ml. Among the 10/14 patients who had a viral load below 1 copy/ml at W0, four patients showed an increase ultrasensitive-HIV-1 viral load above 1 copy/ml at eTT. Among the four patients with detectable baseline ultrasensitive-HIV-1 viral load, two patients showed a higher eTT viral load (Fig. 1e). Moreover, the eTT viral load levels were inversely

correlated with the percentage of classical monocytes (Spearman's test, $P = 0.04$, rho $= -0.77$, Fig. 1f), but not with CD38 expression on CD8 T cells (data not shown).

In summary, despite a limited number of patients, this immunological ROCnRAL substudy revealed that the switch to the dual MVC/RAL therapy, even in the absence of apparent viral failure, did not improve but even exaggerated the activation/inflammation status, as shown by, first, decreased $CD4^+/CD8^+$ ratios with an increased CD38 expression on CD8 T cells and, second, a monocyte switch toward a proinflammatory phenotype with an increase of sCD163 plasma levels, contrasting with third, a decrease in sCD14 plasma levels.

There might be two potential explanations for this unfavorable biological outcome: first, a paradoxical effect of MVC on $CD4^+$ and $CD8^+$ T-cell activation and $CD8^+$ numbers in blood as previously reported, and presumably linked with an increase in circulating levels of CCR5 ligands [16–19], and, second, a loss of full control of HIV replication as shown by ultrasensitive real time-PCR and as attested by the number of patients with detectable replication even if below 50 copies/ml. The increase in $CD8^+CD38^+$ cells and sCD163, two markers of HIV replication [20,21], are in favor of this second HIV replication scenario. In addition, the increase in proinflammatory monocytes, known as the main responders to viral Toll-like receptor (TLR) 7/8 ligands [22], and the negative correlation between the classical monocyte percentages and ultrasensitive-HIV-1 viral load at eTT suggests this shift toward a proinflammatory monocyte profile could be driven by a TLR activation of viral origin. Altogether, these findings proposed that the MVC/RAL switch induces a loss of viral control conducting either to full relapses of virus replication in a few patients, or to low-level virus production in others, and participating to monocytes and T-cell activation and inflammation.

sCD14, derived from membrane CD14 shedding, is a marker of monocyte activation mainly in response to TLR4/lipopolysaccharide signaling [23] and was suggested to reflect microbial translocation [24]. Decreased sCD14 plasma level has been reported after a switch to RAL therapy [17,25,26]. This decline, independently to TLR7/8 derived-monocyte activation might be related to the preservation of the intestinal barrier integrity and limitation of bacterial translocation as a consequence of an optimal penetration of RAL into the gut [27].

In conclusion, this substudy highlighted a dual immunological profile induced by a switch to MVC/RAL therapy in lipohypertrophic ART-suppressed HIV-infected patients, with a favorable decline of CD14 shedding but an unfavorable monocyte and $CD8^+$ activation status that goes along with the previously reported poor viral outcome [14]. These data further stress the necessity that clinical trials evaluating new strategies should include immunological substudies in order to accurately evaluate their consequences on activation and inflammation markers.

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Conflicts of interest

There are no conflicts of interest.

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