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

Laure Campillo-Gimeneza, Lambert Assoumoub,c,d, Marc-Antoine Valantinb,c,d, Priyadharsihni Panjinarasa, Juliette Villemontex, Cathia Souliec,d,f, Anne-Genevieve Marceлинb,c, Dominique Costagliolab,c,h, Jacqueline Capeaub,c,d,i, Brigitte Autranb,c,i, Christine Katlamab,c,d,i, Amélie Guihot*,s,e,i,*, on behalf of the ROCnRAL ANRS 157 Study Group

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 (Clinical-Trials.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/mm³ (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/mm³ (median = 702, IQR: 559/798) and seven of 16 had CD8+ cell counts above 700/mm³ (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−/low), intermediate (CD14+ CD16+/hi) and nonclassical (CD14+/CD16hi) 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 (CD14low CD16hi and CD14+ CD16+/hi). 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).

DOI:10.1097/QAD.0000000000000626
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, \( P = 0.04, \rho = -0.77, \text{Fig. 1f} \)), 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 cETT 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.

Acknowledgements

The authors thank the investigators, study coordinator, site and data managers, and patients for their contribution. The authors thank Stephen Stephan for revising the English.


Conflicts of interest

There are no conflicts of interest.

*INSERM, U1135, CIMI; †Sorbonne Universités, UPMC Univ Paris; ‡INSERM, UMR_S 1136, Institut Pierre Louis d’Épidémiologie et de Santé Publique; †Service des maladies infectieuses et tropicales; †Département d’Immunologie; †AP-HP, Laboratoire de Virologie, Groupe hospitalier Pitié Salpêtrière; ‡Sorbonne Universités, UPMC Univ Paris 06; †INSERM, UMR-S 938, CDR Saint Antoine; †AP-HP, Département de Biochimie et Hormonologie, Groupe Hospitalier Tenon; and †Sorbonne Universités, UPMC Univ Paris 06, CIMI, Paris, France.

Correspondence to Dr Amélie Guihot, Département d’Immunologie, Hôpital Pitié-Salpêtrière, 83 Bld de l’Hôpital, 75651 Paris Cedex 13, Paris, France. Tel: +33 1 42 17 74 96; fax: +33 1 42 17 74 90; e-mail: amelie.guihot@psl.aphp.fr

*Dr Christine Katlama and Dr Amélie Guihot contributed equally to the writing of this article.

Received: 19 January 2015; revised: 7 February 2015; accepted: 10 February 2015.

References


