Polyfunctional HIV-specific T cells in Post-Treatment Controllers

Assia Samri\textsuperscript{a,b}, Charlin Bacchus-Souffan\textsuperscript{a,b}, Laurent Hocqueloux\textsuperscript{c}, Véronique Avettand-Fenoel\textsuperscript{e,f}, Benjamin Descours\textsuperscript{a,b}, Ioannis Theodorou\textsuperscript{a,b,g}, Martin Larsen\textsuperscript{a,b,g}, Asier Saez-Cirion\textsuperscript{d}, Christine Rouzioux\textsuperscript{e}, Brigitte Autran\textsuperscript{a,b,g},
ANRS VISCONTI study group

To further understand the exceptional HIV-1 control observed in Post-Treatment Controllers (PTCs) from the Virological and Immunological Sustained CONtrol after Treatment Interruption (VISCONTI) study we investigated their HIV-specific T-cell responses. Polyfunctionality of HIV-specific CD4 and CD8 T cells and the ratios of HIV-specific CD4 T cells per infected cells were similar in post-treatment controllers, continuously early-treated patients and long-term non-progressors Overall early treatment appears to preserve robust HIV-specific CD4\textsuperscript{+} T cells, which might contribute to the posttreatment control of HIV.

AIDS 2016, 30:2299–2302

Keywords: control, HIV, immune T cells, polyfunctional, spontaneous

A model of HIV remission is represented by the Post-Treatment Controllers (PTCs) from the Virological and Immunological Sustained CONtrol after Treatment Interruption (VISCONTI) study, who control HIV over 7 years after interrupting combined antiretroviral therapy (cART) initiated shortly after primary HIV-1 infection (PHI) \cite{1,2}. PTCs and Long-Term-Non-Progressors (LTNPs) display similar magnitude and distribution of their HIV reservoirs, whereas their clinical and genetic backgrounds differ. Indeed, PTCs are frequently HLA-B\textsuperscript{+}35\textsuperscript{+}, an allele previously associated with symptomatic PHI and rapid progression \cite{3,4}. Contrarily, they are not enriched in the classical protective HLA-B\textsuperscript{+}27 or B\textsuperscript{+}57 alleles associated with spontaneous HIV control and robust anti-HIV CD8\textsuperscript{+} T-cell responses \cite{5–9}. Accordingly, low levels of HIV-specific CD8\textsuperscript{+} T cells producing IFN-\gamma and limited inhibition of HIV production by CD4\textsuperscript{+} T cells were previously reported in the VISCONTI PTCs \cite{2}. The impact of HLA alleles was not examined in this study. To further understand whether T-cell-mediated immunity to HIV participated to the exceptional HIV-1 control observed in PTCs, we compared their HIV-specific T-cell responses to those of LTNPs and of Continuously Early-Treated patients (CETs).
We included six HLA-B*35+ and six HLA-B*35− PTCs from the VISCONTI study [2], who had initiated cART within 10 weeks PHI. All 12 PTCs controlled HIV viremia for a median 3 years after 101-month (75–112) long cART interruption. They were compared with eight treatment-naive LTNPs (ANRS ALT-CO-15 cohort) [10] infected for at least 15 [11–13] years, and 10 CETs fully suppressed patients on cART initiated within 10 weeks PHI for a duration of 86 (38–150) months. Two CETs but no LTNPs carried HLA-B*35, whereas six LTNPs were either HLA-B*27 or B*57, in contrast to only one PTC and two CETs. Plasma viral loads were significantly lower in PTCs and CETs compared with LTNPs (P = 0.005). Levels of total cell-associated HIV-DNA measured in peripheral blood mononuclear cells (PBMCs) using the ANRS ultra-sensitive quantitative real-time PCR assay (Biocentric, Bandol, France) [2,9] did not significantly differ between the three groups despite a trend toward higher levels in HLA-B*35+ PTCs [127 (14–324)] compared with HLA-B*35− PTCs [25 (4–161)]. All patients’ characteristics are described in Supplementary Table 1, http://links.lww.com/QAD/A949. Institutional review boards had approved all studies and patients signed informed consent.

The HIV-specific CD4+ and CD8+ T-cells intracellular cytokine-staining assay was performed [14] after stimulation with recombinant HIV-1 p24 (Protein-Sciences, Meriden, Connecticut, USA) or HIV-1 p24 15-mers synthetic peptide pools (Neosystem, Strasbourg, France), respectively. Staining was performed with anti-CD3-Pacific Blue, CD4-ECRD (Beckman-Coulter Villepinte, France), CD8-APC-Cy7, IFN-γ-Alexa700, IL-2-APC, MIP-1β-FITC, TNFα-PECy7, and CD40L-PE (BD-Bioscience, San Jose, California, USA) monoclonal antibodies. At least one million cells were analyzed on Gallios Flow-Cytometer with Kaluza 1.2 Software (Beckmann-Coulter). The polyfunctionality index (π) = \sum_i \frac{n_i}{n} \cdot \Pi(n_i) \cdot \text{with } q \text{ set conservatively to 1} \text{ was employed} [15] \text{ and data were analyzed with the software SPICE (M. Roederer, Immuno Technology} Section VRC/NIAID/NIH, USA) and Funky Cells Toolbox (www.FunkyCells.com). All data were analyzed using the nonparametric Mann–Whitney U test and Spearman’s rank test and incorporated Bonferroni corrections for multiple comparisons. All values are medians and interquartile range.

Frequencies of HIV-specific CD4+ T-cells producing at least one function, mainly IFN-γ and MIP-1β, or displaying CD40L did not differ between PTCs [0.35% (0.09–0.67)], LTNPs [0.16% (0.10–0.32)], or CETs [0.52% (0.17–1.64)] (Fig. 1a). Interestingly, anti-HIV CD4+ T-cells were even more frequent in CETs than in LTNPs [P = 0.013 (NS after Bonferroni correction)], confirming that early cART preserves HIV-specific CD4+ T cells [11–13,16,17]. In addition, 28.1 and 30.3% HIV-specific CD4+ T-cells from PTCs and CETs, respectively, mediated at least 2 functions, not different from 49% observed in LTNPs, with a similar polyfunctionality index between PTCs, LTNPs, and CETs (30, 35, and 31, respectively) (Fig. 1b).

The highest HIV-specific CD8+ T-cell frequencies producing at least one function were observed in LTNPs [1.24% (1.14–3.72)] compared with whole PTC group [0.29% (0.17–0.62) P = 0.006 (NS after Bonferroni correction)], but not different from CETs [0.46% (0.24–1.72)] (Fig. 1c). Furthermore PTCs CD8+ T-cells producing IFN-γ and/or MIP-1β were five to 10-fold fewer [0.04% (0.01–0.15); 0.083% (0.04–0.13), respectively] than in LTNPs [0.57% (0.45–1.81) P = 0.015 (NS after Bonferroni correction); 0.66% (0.43–1.84) P = 0.001 (significant after Bonferroni correction), respectively] because of lower levels in HLA-B*35+ [0.006% (0.003–0.061); 0.08% (0.008–0.1) than in B*35− PTCs [0.131% (0.022–0.382); 0.106% (0.048–0.321)]. CETs had also fewer cells producing MIP-1β [0.19% (0.04–0.54)] than LTNPs [P = 0.012 (NS after Bonferroni correction)]. Altogether, we cannot exclude the LTNPs’ higher viremia might stimulate higher CD8+ responses compared with PTCs or CETs.

In contrast, polyfunctional HIV-specific CD8+ T cells producing at least two functions and polyfunctionality indices tended to be similar in PTCs, LTNPs, and CETs (44.6, 57.5, 47.1% and 33, 32, and 30, respectively) independently of HLA-B*35 (Fig. 1d). Therefore, despite an HLA-B*35 effect on MIP-1β production, early treatment also appears to preserve functionality of HIV-specific CD8+ T cells. The importance of robust polyfunctional HIV-specific CD4+ and CD8+ T-cells is supported by our recent correlation between polyfunctionality and in-vitro cytotoxic capacity [18,19], as well as by recent demonstration that T-cell polyfunctionality assessed either using our polyfunctional index or the newly described COMPASS index, predicts protection against HIV acquisition [20].

Finally, as one infected cell harbors only one HIV-DNA copy, we calculated the ‘in-vivo immune effector/target cell ratios’ (E/T), by dividing the HIV-specific T-cells by the HIV-infected cell numbers per million PBMCs (Fig. 1e and f) [9,21]. The CD4 E/T ratios [65 (12–172)] did not differ between PTCs and LTNPs [62 (19–155)] or CETs [179 (105–1372)], whereas the CETs ratio was significantly higher than in LTNPs (P = 0.043; Fig. 1g). The CD8 E/T ratios were higher in LTNPs [417 (105–1980)] compared with PTCs [84 (16–305)], though significant only in HLA-B*35+ [35 (12–91), P = 0.005] and not in non-HLA-B*35 PTCs [240 (67–781)], whereas the CETs CD8 E/T ratio was intermediate [221 (82–1622)].
Of note, in the small samples studied here, no correlation was observed between the magnitudes and polyfunctionality of HIV-specific CD4$^+$ and CD8$^+$ T cells. Whatever the group and after a Bonferroni correction for multiple comparisons, many $P$ values would no longer be statistically significant.

Thus, our results, obtained in this small number of samples, strongly suggest the robust polyfunctional anti-HIV CD4$^+$ T-cell responses preserved by prolonged early cART may have allowed high CD4 E/T ratios in PTCs similarly to LTNP, and therefore might contribute to virus control, even in HLA-B$^*$35$^+$ individuals. In contrast, CD8$^+$ T-cell control might not be as contributive because of the HLA-B$^*$35 effect, whereas other mechanisms, such as natural killer cells, might also participate in control of viral reservoirs and in establishment of remission [22] in the VISCONTI model of functional HIV cure.

**Acknowledgements**

The authors thank Amandine Emarre for technical assistance. The study was supported by the ANRS, the French National Agency for Research on AIDS, and Viral Hepatitis (Grant ANRS EP47). The promoter is the Centre Hospitalier Regional d’Orleans.

Coordinated inclusion of patients: L.H. Conceived and designed the experiments: A.S., C.B.S., V.A.F., B.D.,

Conflicts of interest
M.L. is inventor of the polyfunctionality index (patent number: WO2013127904) and proprietary owner of the Funky Cells ToolBox software (www.FunkyCells.com).

References

22. Scott-Allgara D. Post-treatment controllers have particular NK cells with high anti-HIV capacity: VISCONTI study. CROI 2015