Immunophenotype and Function of CD38-Expressing CD4+ and CD8+ T Cells in HIV-Infected Patients Undergoing Suppressive Combination Antiretroviral Therapy

TO THE EDITOR—We have read with great interest the recent articles by Tenorio et al and Hunt et al that investigated inflammation, coagulation, and T-cell activation as predictors of clinical outcome in human immunodeficiency virus (HIV)-positive patients receiving combination antiretroviral therapy (cART) [1, 2]. Interestingly, while inflammation/coagulation and gut damage were independently associated with mortality and non-AIDS morbidity, T-cell activation was not significantly related to these outcomes.

CD38 is commonly regarded as a T-cell activation marker, and circulating CD38+CD8+ T cells have been strongly correlated with disease progression in untreated HIV infection [3, 4]. Upon suppressive cART, levels of CD38+CD8+ T cells remain abnormally elevated [5], and yet their prognostic significance is less clear [6, 7]. Whether activated T cells are associated with increased morbidity/mortality is not a trivial question, given the need for novel interventions to contain excessive inflammation/immune activation during cART and surrogate biomarkers of clinical outcome. The data reported by Tenorio et al and Hunt et al for relatively large cohorts of virologically suppressed subjects seem to conclude that CD38 expression on CD8+ T cells does not associate with disease outcome. The apparent discrepancy with results from untreated patients led us to speculate regarding the existence of CD38+CD8− subpopulations with a distinctive phenotype and function in cART-naive HIV-positive individuals, compared with HIV-positive cART recipients.

We therefore investigated the immune phenotype and function of CD4+CD8− CD38+ and CD4+CD8−CD38− subsets in 31 HIV-positive patients followed at the Clinic of Infectious Diseases, University of Milan, San Paolo Hospital. Twelve were untreated (CD4+ T-cell count, 633 cells/mm3; interquartile range, [IQR] 425–687 cells/mm3), 19 were receiving virologically suppressive cART (CD4+ T-cell count, 534 cells/mm3; IQR, 214–752 cells/mm3; HIV RNA load, <40 copies/mL; median time to first suppression, 4 years), and 10 were HIV-negative healthy donors (controls).

As expected, untreated HIV-positive patients displayed a higher proportion of CD8+CD38+ cells than HIV-negative subjects (28% vs 8%; P = .0001). HIV-positive cART recipients showed decreased CD8+CD38+ levels, which, however, remained higher than those measured in controls (17% vs 8%; P = .004).

As shown in Figure 1A, untreated patients had significantly lower proportions of naive CD38+CD45RA+CD45R0− cells than HIV-negative patients (52% vs 77% of CD4+CD38+ T cells [P = .013]; 59% vs 79% of CD8+CD38+ T cells [P = .002]) and higher proportions of memory CD38+CD45RA−CD45R0+ cells (52% vs 30% of CD4+CD38+ T cells [P = .012]; 48% vs 30% of CD8+CD38+ T cells [P = .008]).

Interestingly, naive CD38+CD45RA+CD45R0− T cells were enriched in HIV-positive cART recipients, compared with untreated patients (72% vs 52% of CD4+CD38+ T cells [P = .016]; 74% vs 59% of CD8+CD38+ T cells [P = .002]), and proportions were similar in HIV-positive cART recipients and HIV-negative subjects (72% and 77% of CD4+CD38+ T cells, respectively [P = .781]; 74% and 79% of CD8+CD38+ T cells, respectively [P = .205]). In
Phenotypic and functional characterization of CD4⁺CD38⁺ and CD8⁺CD38⁺ T cells in human immunodeficiency virus (HIV)-positive patients.

A. Phenotypic analysis on freshly isolated peripheral blood mononuclear cells, comparing untreated HIV-positive patients, HIV-positive combination antiretroviral therapy (cART) recipients, and healthy controls.

B. Magnetically isolated CD38⁺CD8⁺ T cells were either left unstimulated or stimulated with anti-CD3/CD28 (3/28) antibodies alone, or together with 100 U recombinant interleukin 2 (rIL-2) or 1000 pg/mL recombinant interleukin 7 (rIL-7), and levels of pSTAT and Bcl-2 expression were determined using intracellular flow cytometry (after 5 hours for pSTAT and after 24 hours for Bcl-2).

C. Analysis of total HIV DNA (integrated and unintegrated HIV DNA forms), unintegrated HIV DNA (extrachromosomal HIV DNA species), integrated HIV DNA, and 2 long-terminal repeat (2-LTR) circle content.
parallel, proportions of memory CD45RA−CD38+ T cells were lower in HIV-positive cART recipients, compared with untreated individuals (32% vs 52% of CD4+CD38+ T cells [P = .003]; 31% vs 48% of CD8+CD38+ T cells [P = .007]), and were equivalent in HIV-positive cART recipients and HIV-negative subjects (32% and 30% of CD4+CD38+ T cells, respectively [P = .592]; 31% and 30% of CD8+CD38+ T cells, respectively [P = .587]). Interestingly, further analysis of the CD38+ immune phenotype revealed a significant enrichment of recent thymic emigrants in HIV-positive cART recipients [8, 9], compared with untreated patients (58% vs 34% of CD4+CD38+ T cells [P = .015]; 9% vs 6% of CD8+CD38+ T cells [P = .049]) and lower than the proportions observed in controls (77% vs 58% of CD4+CD38+ T cells [P = .002]; 13% vs 9% of CD8+CD38+ T cells [P = .035]). No differences were observed in the CD38− population (data not shown).

Given these findings, we studied CD38+CD8+ or CD4+ IL-7 signalling by flow cytometric measurement of pStat5 and Bcl-2 on magnetically isolated CD8+CD38+ or CD4+CD38+ T cells with or without recombinant interleukin 2 (rIL-2) and recombinant interleukin 7 (rIL-7) stimulation. Interestingly, upon rIL-7 stimulation, higher pSTAT-5–expressing and Bcl-2–expressing CD8+CD38+ levels were observed in HIV-positive cART recipients, compared with untreated subjects (pSTAT-5, P = .036; Bcl-2, P = .032; Figure 1B), and these levels were significantly lower than those for HIV-negative subjects (pSTAT-5, P = .046; Bcl-2, P = .036). The pSTAT5 and Bcl-2 increases were observed only in CD8+CD38+ T cells and not in CD4+CD38+ T cells. No differences were observed following rIL-2 challenge and in CD8+CD38−CD4+ or CD38− subsets (data not shown).

In 10 HIV-positive cART recipients, no significant differences in total, unintegrated, and integrated HIV DNA [10] were noted in purified CD4+CD38+ and CD4+CD38− T cells (Figure 1C).

Our data show that circulating CD38+ T cells, while exhibiting abnormally increased levels in both untreated and treated patients, display a distinctive phenotype and functional response according to the presence or absence of cART. Successfully treated patients display a CD38+ compartment enriched in naive/recent thymic emigrant T-cell phenotypes, with higher IL-7 responsiveness of CD38+CD8+ T cells and not CD38−CD4+ T cells resulting in STAT-5 phosphorylation and protection from apoptosis. These findings are not observed in untreated patients, which suggests a positive effect of cART in the homeostasis of CD38+ T cells. A partial recovery of cellular phenotype/function and the lack of higher HIV DNA content in CD38+ T cells might provide a biological reason for the failure of this subset to robustly predict mortality/morbidity in treated patients, as shown in the articles by Tenorio et al and Hunt et al, sounding a note of caution in its exploitation as a surrogate outcome of clinical progression in HIV-positive cART recipients. A broader functional characterization of circulating CD38+ T cells in HIV-positive cART recipients with different CD4+ T-cell counts may better define their possible role as biomarkers of clinical outcome.

Notes

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