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CD8+CD28-CD127^{lo}CD39+ T reg: a new biomarker for HIV infection

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Abstract

Background : The phenotype of non-antigen specific CD8+ T regulatory lymphocytes (Treg) has been recently characterized as CD8+CD28-CD127^{lo}CD39+. In order to study the in vivo compartmentalization of this newly recognized Treg subset, we analyzed its frequency in the peripheral blood of patients (pts) affected by cancer and HIV infection, characterized by an abnormal expansion of regulatory circuits and immunodeficiency, in comparison with healthy subjects (HS)

Methodology: We analyzed these cells with FACSCanto II flow cytometer in HS, in pts affected with cancer and in HIV infected pts subdivided in elite controllers (EC) with CD4+T cells ≥ 400/mm^3 and persistent HIVRNA ≤ 50 cp/ml without antiretroviral therapy (ART); long term non progressor (LTNP) with CD4+ T cells ≥400/mm^3 and HIVRNA > 50 and < 1000 cp/ml without ART and progressor (HIVRNA ≤ 50 copies/ml treated with at least 2 ART regimens). Statistically significant differences between frequencies were assessed using the Fisher's exact test for binary variables, while statistically significant differences between means were analyzed with the Mann-Whitney test for non-parametric values.

Results: We included 24 HS, 34 pts with cancer subdivided in renal (N=17), bladder (N=16) or colorectal (N=1), 8 EC, 5 LTNP and 20 progressor The CD8+CD28-CD127^{lo}CD39+ T reg were detected in 1/24 (4%) of HS and the frequency was very low (0.1%). The analysis of the frequency of CD8+CD28-CD127^{lo}CD39+ Treg in the surgical tumor specimens showed that these cells were present in 30 out of 34 (88%) total tumors with comparable frequencies between renal and bladder cancers. While these cells were rare in EC and LTNP (2/13 patients [15%]), they were present with high frequency in 95% of progressors (p= 0.0001).

Conclusions: These data show the existence of a T cell subpopulation, CD8+CD28-CD127^{lo}CD39+ Treg, that is substantially unrepresented in the circulation of healthy subjects, but that circulates in the peripheral blood of cancer or HIV-infected patients. This finding configures CD8+CD28-CD127^{lo}CD39+ Treg as an optimal biomarker for diagnostic/prognostic or follow-up purposes in diseases characterized by an abnormal predominance of regulatory circuits, as cancer and HIV infection.

effects on immune responses through soluble factors without the need of a specific antigen stimulation[3-6], has been pathogenetically associated with various autoimmune diseases.[3,5,7-9] Importantly, CD8+ Treg have infiltrate human cancers, where they likely contribute to tumor immune escape through the inhibition of both proliferative and cytotoxic T cell responses.[10,11] Recently, we found that the T cell subpopulation phenotypically characterized as CD8+CD28-CD127^{lo}CD39+ invariably exerts suppressive functions, recapitulating all the functional features of previously defined "non-antigen specific" CD8+ Treg.[12] In order to study the in vivo compartmentalization of this newly recognized Treg subset, we analyzed its frequency in the peripheral blood of patients affected by two diseases, cancer and HIV infection, characterized by an abnormal expansion of regulatory circuits and immunodeficiency, in comparison with healthy subjects.

Methods

The pts were grouped as follows: Group 1: Cancer patients HIV-, Group 2: Healthy Donors, Group 3: Elite controllers (EC, viral load undetectable, without antiretroviral therapy), Group 4: Long Term Non Progressors (LTNP, with CD4+ T cells ≥400/mm^3 and HIVRNA > 50 and < 1000 cp/ml without ART) and Group 5: HIV+ progressors (HIVRNA ≤ 50 copies/ml treated with at least 2 ART regimens).
statistically significant differences between means were analyzed with the Mann-Whitney test for non-parametric values. Analyses were performed using the GraphPad Prism 5 software (GraphPad Software, San Diego – California, USA).

Immunofluorescence analyses: Peripheral blood mononuclear cells (PBMC) were purified from heparinized blood samples by gradient centrifugation on Ficoll-Hypaque. Lymphocytes from surgical specimens were purified filtering minced tissues using a sterile cell strainer and running the collected cells on Ficoll gradient. Immunofluorescence analyses were performed incubating 1x10^5 PBMC or tumor infiltrating lymphocytes with specific monoclonal antibodies (mAbs) at 4° C for 30 minutes in the dark before acquisition on a FACSCanto II flow cytometer (Becton Dickinson Biosciences, San Josè). The following mAbs were used: Phycoerythrin (PE) conjugated anti-CD127 (Becton Dickinson Bioscience, San Josè), Allophycocyanin (APC)-cyanin (Cy) 7 conjugated anti-CD3 (e-Biosciences, San Diego), Peridinin Chlorophyll Protein Complex-cyanin 5.5 (PerCP-Cy5.5) conjugated anti-CD28 (Biolegend, San Diego, CA), APC conjugated anti-CD39 (BD, Bioscience), Horizon V-500 conjugated anti-CD8 (BD, Biosciences) [Figure 1].

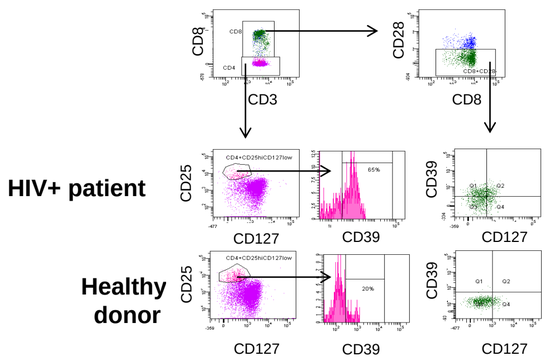
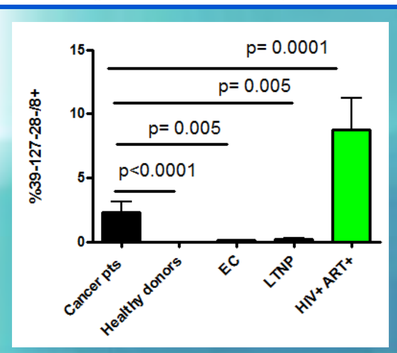
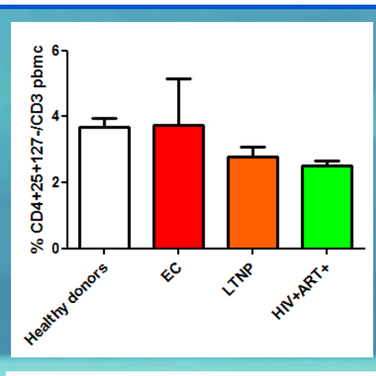


Figure 1. Gating strategy in HIV+ pts and healthy donor

Results

We included 24 HD, 34 pts with cancer subdivided in renal (N=17), bladder (N=16) or colorectal (N=1), 8 EC, 5 LTNP and 20 progressor HIV+ ART+. The main characteristics of HIV+ progressors were (minimum –maximum): median age 49 years (22-78), 13 males, median Nadir CD4T+ 219/mmc (5-529), CD4+T cells 468/mmc (86-944), time of HIV+ diagnosis 18 years (2-25).
The CD8+CD28-CD127^{lo}CD39+ T reg were detected in 1/24 (4%) of HS and the frequency was very low (0.1%). The analysis of the frequency of CD8+CD28-CD127^{lo}CD39+ Treg in the surgical tumor specimens showed that these cells were present in 30 out of 34 (88%) total tumors with comparable frequencies between renal and bladder cancers. While these cells were rare in EC and LTNP (2/13 patients [15%]), they were present with high frequency in 95% of progressors (p= 0.0001). Comparison of frequencies among different groups are shown in the figure 2-5.





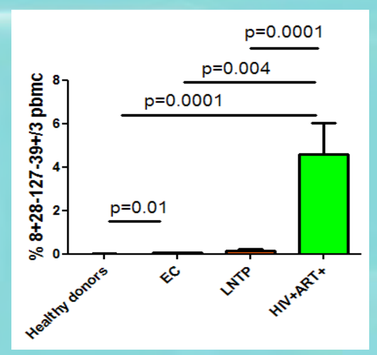


Figure 3.

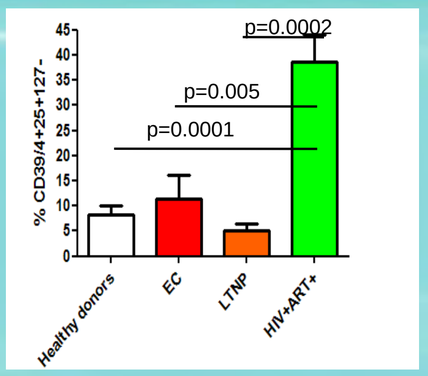


Figure 4.

Conclusions

Interestingly, CD8+CD28-CD127^{lo}CD39+ Treg were detected in the peripheral blood of only 1 out of 24 (4%) healthy subjects, at a very low frequency, suggesting that these cells appear in the circultaion only in diseased individuals. To verify whether CD8+CD28-CD127^{lo}CD39+ Treg are a unique marker for cancer diseases or if they can be detected in other diseases characterized, as cancer, by immunodeficiency and expansion of regulatory circuits, we performed an analysis on HIV infected patients. CD8+CD28-CD127lowCD39+ Treg frequencies are elevated in peripheral blood of HIV patients with active disease (infection).
CD8+CD28-CD127lowCD39+ Treg are very low in the peripheral blood of EC and LTNP patients. It is possible that these cells constitute a new biomarker of activity/progression in HIV infection. Longitudinal studies are needed in order to validate the prognostic value of circulating CD8+CD28-CD127lowCD39+ Treg frequency in different antiretroviral regimens.

References

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