Elite Suppressor CD4+ and CD8+ T cells Suppress Viral Replication in Monocyte-Derived Macrophages

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Introduction

Elite suppressors (ES) are HIV-1 infected patients who maintain undetectable viral loads without antiretroviral therapy. The control has previously been associated with a qualitatively superior CD8⁺ T-cell response to infected CD4⁺ T-cells. However, very few studies have examined the effector T-cell response to HIV-1 infected macrophages. In the macaque ES model, both CD4⁺ and CD8⁺ T-cell clones are capable of suppressing infection in macrophages, but in an *ex vivo* study, SIV-specific primary CD8⁺ T-cells were far less effective at inhibiting viral replication in macrophages than in CD4⁺ T-cells. In this study, we examined the CD4⁺ and CD8⁺ effector T-cell responses to HIV-1 infected monocyte-derived macrophages (MDM).

Methodology

Monocytes were isolated from 10 uninfected healthy donors (HD) and 7 ES and cultured in macrophage differentiation media (RPMI, 20% human AB serum, 1% HEPES, 50 ng hrM-CSF/mL) for 7 days before infection. On the day of infection, the MDMs were infected with the laboratory isolate Ba-L by spinoculation. Unstimulated primary CD4⁺ and CD8⁺ cells isolated from a second blood draw were added to the MDMs at various effector to target ratios immediately after infection. Normalized percent inhibition of viral replication was calculated by comparing culture supernatant p24 levels in the presence and absence of effector CD8⁺ or CD4⁺ T-cells, and unpaired, two-way Student's T-tests were used to determine statistical significance.

Results

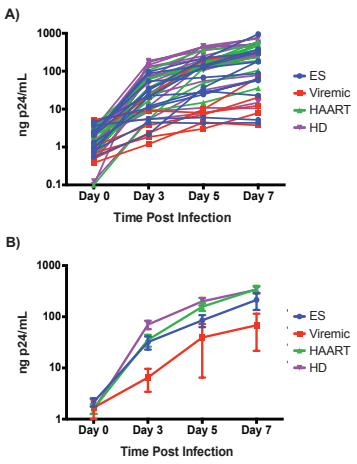


Figure 1. ES macrophages infect as well as healthy donor and HAART macrophages. A) Individual virus production curves for ES (n = 12), viremic patients (n = 6), HAART patients (n = 11), and healthy donors (n = 19). B) Average virus production of MDMs from the aforementioned groups (Avg. ± standard error).

Results

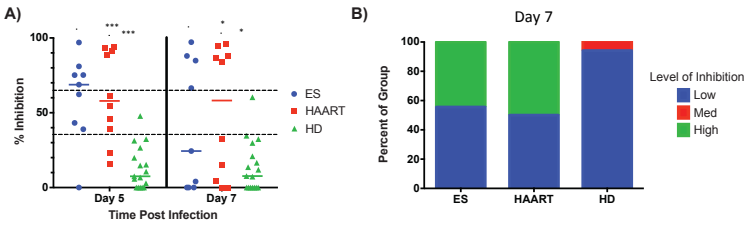


Figure 2. CD4⁺ effector-mediated inhibition of viral production in ES macrophages is equally distributed as in HAART patients' by day 7. Monocyte-derived macrophages (MDM) were infected with 500 ng Ba-L per million cells and cultured with CD4⁺ T-cells in a 1:2 ratio, and the percent inhibition was calculated by measuring p24 content of the supernatant via ELISA. A) Inhibition of viral production on days 5 and 7 for ES (n = 9), HAART (n = 10), and healthy donor (n = 18) MDM. B) Inhibition capability of individuals within each subset. * p < 0.05, *** p < 0.001

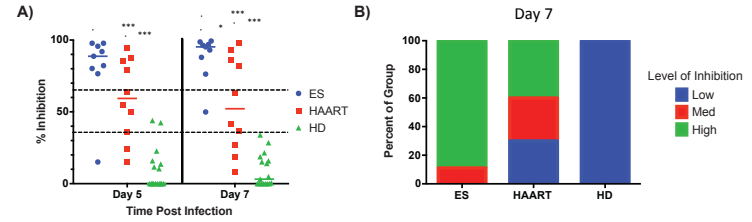


Figure 3. CD8⁺ effector-mediated inhibition of viral production is superior in ES to that of HAART patients. Monocyte-derived macrophages (MDM) were infected with 500 ng Ba-L per million cells and cultured with CD8⁺ T-cells in a 1:2 ratio, and the percent inhibition was calculated by measuring p24 content of the supernatant via ELISA. A) Inhibition of viral production on days 5 and 7 for ES (n = 9), HAART (n = 10), and healthy donor (n = 18) MDM. B) Inhibition capability of individuals within each subset. * p < 0.05, *** p < 0.001

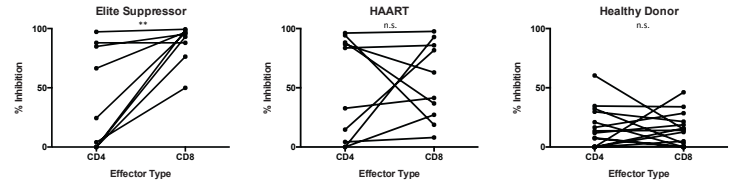


Figure 4. ES consistently have a high CD8⁺ effector response superior to that of the CD4⁺ effector response. Monocyte-derived macrophages (MDM) were infected with 500 ng Ba-L per million cells and cultured with CD4⁺ or CD8⁺ T-cells in a 1:2 ratio. Infection was measured by p24 ELISA for ES (n = 9), HAART (n = 10), and healthy donor (n = 18) macrophages. ** p < 0.01, n.s. non-significant

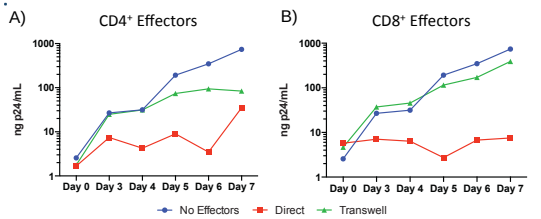


Figure 5. CD4⁺ effector inhibition of viral production is partially soluble factor-mediated while CD8⁺ inhibition is contact-mediated. Monocyte-derived macrophages (MDM) infected with Ba-L had CD4⁺ or CD8⁺ T-cells added in a 1:1 effector:target ratio immediately after spinoculation directly or in transwells. Viral production was measured by p24 ELISA. Representative ES individual is shown. A) CD4⁺ effector-mediated inhibition. B) CD8⁺ effector-mediated inhibition.

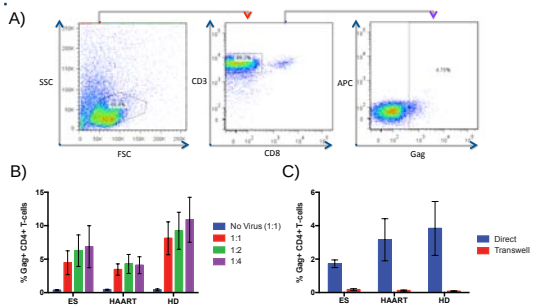


Figure 6. CD4⁺ effectors from ES, HAART, and healthy donors are infected more efficiently by direct contact with macrophages than by free virus. Monocyte-derived macrophages (MDM) infected with Ba-L had CD4⁺ T-cells added in 1:1, 1:2, and 1:4 effector:target (E:T) ratios immediately after spinoculation directly or (1:1 only) in transwells. These CD4⁺ effectors were then analyzed for Gag positivity via FACS for for ES (n = 8), HAART (n = 6), and healthy donors (n = 10). A) Gating strategy. B) Infection of CD4⁺ effectors at different E:T ratios. C) Infection of CD4⁺ effectors is higher when directly applied. (Avg. ± standard error).

Conclusion

Primary unstimulated effector CD8⁺ T cells from ES are capable of effectively inhibiting viral replication in MDMs in vitro in a cell-cell contact mediated manner, but CD4⁺ T cell-mediated inhibition in ES is less effective and has a distribution similar to HAART patients'. The degree of CD8⁺ T cell-mediated inhibition of viral replication in MDMs was comparable to that seen previously in target CD4⁺ T-cells. These observations lend credence to the idea that CD8⁺ T-cells are the driving force behind control and imply that the response of effector cells to macrophages should be considered in rational vaccine design and the continuing goal of eradication.