Cytomegalovirus infection in HIV-infected and uninfected individuals is characterized by circulating regulatory T cells of unconstrained antigenic specificity

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Abstract

Cytomegalovirus (CMV) infection is associated with immune-suppression in immune-compromised hosts and old adults. We previously showed that ex vivo CMV restimulation of peripheral blood mononuclear cells (PBMC) of CMV-seropositive volunteers expanded CD4+CD27-CD28- regulatory T cells (Tregs). Here we evaluate the phenotype and function of circulating CD4+CD27-CD28- T cells of CMV-seropositive adults. Compared with CMV-seronegative, CMV-seropositive adults had 10-fold higher CD4+CD27-CD28- % T cells in PBMC. Circulating CD4+CD27-CD28- T cells from both CMV-seropositive and seronegative donors expressed higher levels of TGFβ, granzyme B, CD39, CD147 and IL-35, and lower levels of CD127, compared with their parent circulating CD4+ T cells. However, only CMV-seropositive circulating CD4+CD27-CD28- had increased FOXP3 expression. CD4+CD27-CD28- sorted from the PBMC of CMV-seropositive donors expanded ex vivo in the presence of rhIL2 and inhibited ex vivo proliferation of autologous PBMC restimulated with CMV, varicella-zoster virus or C. albicans antigens. CD4+CD27-CD28- sorted from CMV-seronegative PBMC did not expand in the presence of rhIL2 and did not inhibit autologous PBMC proliferation. CD3+CD27-CD28- circulating T cells (≥80% CD8+) from CMV-seropositive HIV-infected donors also inhibited ex vivo proliferation of autologous PBMC restimulated with CMV or HIV. These data indicate that CMV-seropositive individuals have circulating Tregs that inhibit cell-mediated immune responses to CMV and other antigens and may be contribute to an immune-suppressive effect of CMV infection. Moreover, the phenotypic similarity between circulating CD4+CD27-CD28- Tregs with differentiated effector T cells suggests that the two T-cell subsets might evolve in parallel or in sequence from the same progenitor cells in response to CMV stimulation during reactivations.
Importance

CMV infection is associated with immune-suppression. The mechanism underlying this effect is unknown. We determined that peripheral blood CD4+CD27-CD28- T cells, which generally represent effector T cells, of CMV-seropositive adults have regulatory function, which may explain the association of CMV seropositivity, high CD4+CD28- T cell frequencies and immune-suppression. Notably, we did not find regulatory T cells among peripheral blood CD4+CD27-CD28- cells of CMV-seronegative individuals. In HIV-infected CMV-seropositive individuals with low numbers of CD4+ cells, CD3+CD27-CD28- T cells (mostly CD8+CD27-CD28-) have regulatory T-cell function. Immune-suppression of CMV-seropositive old adults has also been associated with high CD4+CD28- or CD8+CD28- T-cell numbers. We propose that the regulatory CD4+CD27-CD28- and CD8+CD27-CD28- T cells of CMV-seropositive individuals contribute to the immune suppression associated with CMV infection. The data suggest that CMV effector and regulatory T cells may evolve together and that decreasing CMV T cell stimulation might limit the generation of regulatory T cells.

Introduction

Cytomegalovirus (CMV)-infected individuals with cell-mediated immune disorders, such as transplant recipients and human immunodeficiency virus (HIV)-infected individuals, or at the extremes of age without any age-independent immune disorders have higher morbidity and mortality than uninfected individuals [1–7]. Most reports found an association of CMV infection with increased HIV disease progression and death in HIV-infected individuals, increased bacterial and fungal superinfections in transplant recipients and decreased immune responses to vaccines and increased respiratory infection in older adults [7–9], although there have also been some exceptions [10, 11]. Overall, the data suggest that CMV has an immune suppressive effect on the host.

During active infection, CMV replicates in many cells of the immune system including monocytes, macrophages and dendritic cells and subsequently establishes latency in CD34+ myeloid progenitors. Both during active and latent infection, the virus employs immune evasion mechanisms that allow it to survive in the host by suppressing host immune responses [12]. Because of the varied array of infectious and other complications that follow CMV infection, we hypothesized that the immune evasion mechanisms induced by CMV infection are not solely suppressive of immune responses to CMV, but also hamper host immune defences against other pathogens. The hypothesis that regulatory T cells (Treg) expanded in response to a specific pathogen may be cross-reactive and impair immune defences against other pathogens is supported by several mouse studies that showed that Treg expanded by chronic infection with Friend retroviruses suppress immunity to murine CMV [13, 14].

In our previous studies, we showed that in vitro stimulation of peripheral blood mononuclear cells (PBMC) from CMV-seropositive donors increased the proportion of CD4+CD27-CD28- T cells, particularly in HIV co-infected subjects [15]. Although the CD27-CD28- phenotype has been typically associated with terminally differentiated effectors, we found that the CMV-specific CD4+CD27-CD28- T cells had Treg phenotypic markers including high expression of FOXP3 and TGFβ [16]. Compared with the total CD4+ parent T cell population in CMV-stimulated cultures, the CD4+CD27-CD28- T cells also had increased expression of granzyme B (GrB), which is a cytotoxicity mediator shared by effector T cells and Treg as well as by regulatory dendritic, myeloid suppressor and B cells [17–21]. Furthermore, when added to autologous PBMC, the CMV-stimulated CD4+CD27-CD28- Treg decreased proliferation of autologous PBMC in a dose-dependent fashion to CMV and to a lesser extent also C. albicans antigenic stimulation. In a case-control study of CMV-
seropositive individuals with AIDS and very low CD4+ T cells, we showed that the risk of CMV end-organ disease was independently associated with high proportions of CMV-specific GrB-secreting PBMC (ELISPOT) and high proportion of CMV-specific CD8+CD107a+ expanded form PBMC [22], supporting the notion that CMV-specific CD8+ Treg importantly contribute to the high morbidity and poor outcome of CMV-infected hosts with AIDS. Notably, other studies also described expansions of Treg in CMV-infected transplant recipients and elderly individuals [23, 24] as well as CD4+ and CD8+ Treg expansions with another herpesvirus from the CMV family, HHV6 [25].

In this study, we expand our findings in CMV-stimulated PBMC to unstimulated freshly thawed PBMC. We report on the function and phenotype of CD4+CD27-CD28- Treg in PBMC of CMV-infected individuals, with or without HIV co-infection, and compare them to CD4+CD27-CD28- PBMC from CMV-seronegative controls. In addition, we compare the regulatory effect of circulating unstimulated peripheral blood CD4+CD27-CD28- T cells of CMV-seropositive donors with that of CD4+CD27-CD28- T cells expanded by ex-vivo stimulation of PBMC from CMV-seropositive donors.

Materials and methods

Specimens

Cryopreserved PBMC were obtained from 14 HIV-infected and CMV-seropositive, 14 HIV-uninfected CMV-seropositive and 8 HIV-uninfected CMV-seronegative anonymous donors. This work was reviewed and considered exempt by the Colorado Multiple Institution Review Board.

Reagents and monoclonal antibodies

The following monoclonal antibodies (mAb) were used for phenotypic characterization and cell sorting: Anti-CD4 APC (clone RPA-T4), anti-CD27 PE (clone M-T271), anti-CD28 FITC (clone CD28.2), anti-CD28 PE (clone CD28.2), anti-IL-10 APC (clone JES3-19F1), anti-Granzyme B FITC (clone GB11), anti-CD147 FITC (clone HIM6), anti-CD39 APC (clone TU66), CTLA-4 PE (clone BN13) (BD Biosciences), anti-FoxP3 PE (clone PCH101), anti-CD127 PE-Cy7 (clone eBioRDR5), anti-IL-35 PE (ebic6), anti-PD1 PE (clone eBioJ105) (eBiosciences), anti-TGFβ PE (clone TB21; Cedarlane). Growth medium was prepared using RPMI-1640 (Mediatech, Inc) supplemented with 2mM Glutamine (Gemini Bio-Products), 20mM HEPES (Mediatech, Inc), 1% penicillin/streptomycin (Gemini Bio-Products), and 10% inactivated human serum (Gemini Bio Products). Recombinant human IL2 (rhIL2) was obtained from R&D Systems, Inc. Staining medium was prepared using 2% fetal bovine serum (Gemini Bio Products) in PBS.

T-cell phenotypic characterization

Freshly thawed or CMV-stimulated PBMC were washed in staining medium and incubated for 30 min at 4°C with surface markers. For intracellular Granzyme B, TGFβ, IL-35 and IL-10 staining, PBMC were then permeabilized and fixed (Cytofix/Cytoperm; BD Biosciences) for 20 min at 4°C, washed and incubated with the appropriate mAbs. For intranuclear FoxP3, cells were permeabilized and fixed with IC Fixation and permeabilization buffer (eBiosciences) for 1 hour at 4°C, washed and incubated with the mAb. At the completion of the staining procedure, cells were fixed in 2% paraformaldehyde (Electron Microscopy Sciences) in PBS and data were acquired with Guava 8HT (Millipore) or Gallios (Beckman Coulter). Results were analyzed with FlowJo (Treestar) or Kaluza (Beckman Coulter).
Cell sorting of CD3+CD27-CD28- or CD4+CD27-CD28- cells

Thawed PBMC were stained for CD4, CD3, CD27 and CD28 and sorted using MoFlo 70XDP (Beckman Coulter) and Summit Software. The average purity of cell sorts was 96.22%.

Lymphocyte proliferation and expansion assays

PBMC at ≤10^6 cells/mL in growth medium were incubated in quadruplicate wells of 96-well microplates (Corning) at 100μL/well in the presence of 100 μL of inactivated CMV-infected cell lysate, inactivated varicella zoster virus (VZV)-infected cell lysate, C. albicans antigen (Greer), HIV inactivated virion [26], the appropriate mock-infected cell control antigens and phytohemagglutinin (PHA; Sigma) positive control. After 6 days of antigenic or mitogenic stimulation at 37°C, in a 5% CO₂ and 95% H₂O atmosphere, cells were pulsed with 1 μCi of ^3H-thymidine and harvested 6 h later onto Unifilter plates (Perkin Elmer). Radioactivity gathered on the filters was counted in scintillation fluid (Perkin Elmer) with a microplate scintillation counter (Packard). Results were expressed in median counts per minutes (cpm) of the quadruplicate wells. Inhibition was measured on samples that showed ≥3-fold increase of median cpm in antigen- compared with mock-stimulated wells.

Treg expansion with rhIL2 (30ng/mL) was carried out in 96-well microtiter plates as described above for up to 15 days, during which rhIL2 and growth medium were replenished twice weekly. At the end of the expansion, viable cells were counted with a Guava 8HT instrument.

Statistical analysis

Two-tailed t tests were used for statistical analysis in Prism 6 (GraphPad) and a p value <0.05 was considered significant. Distribution of results was checked for normality and parametric tests were applied to normally distributed data. Log transformation was applied when indicated to achieve normal distribution of the data. Nonparametric tests were used for data with skewed distribution.

Results

Magnitude and phenotypic characterization of circulating CD4 +CD27-CD28- T cells of CMV-infected compared with uninfected individuals

To determine the effect of CMV infection on the magnitude of the circulating CD4+CD27-CD28- T cells, we compared PBMC from 8 CMV-seronegative (CMV-neg) HIV-seronegative (HIV-neg) blood donors, 14 CMV-seropositive (CMV-pos) HIV-neg blood donors or young adult volunteers and 14 CMV-pos HIV-seropositive (HIV-pos) individuals (Fig 1). The data showed significantly higher CD4+CD27-CD28-% in PBMC from CMV-pos donors regardless of HIV status compared with CMV-neg donors (means of 3.0% and 0.4%, respectively, p<0.005; Fig 1).

Next, we compared Treg-defining phenotypic characteristics on circulating CD4+CD27-CD28- T cells and their parent CD4+ T cell populations in CMV-pos (HIV-pos or HIV-neg) participants (Fig 2). Using the gating strategy shown in Fig 2A, we show in Fig 2B that compared with the parent CD4+ T cells, significantly higher proportions of CD4+27-28- T cells from CMV-pos donors expressed FOXP3, TGFβ, GrB, IL-35 and CD39, but similar levels of IL-10 and CTLA-4. The CD4+CD27-CD28- subset also expressed higher levels of the Treg activation marker CD147. CD127 expression, which is usually low or absent on Tregs was significantly lower on the CD4+CD27-CD28- subset compared with the total CD4+ peripheral
blood parent population. **Fig 2C** shows that CD4+CD27-CD28- peripheral blood T cells of CMV-neg individuals also expressed higher levels of TGFβ, GrB, IL-35 and CD39 and lower levels of CD127 compared with their parent total CD4+ T cells. In contrast to CMV-pos, the CMV-neg peripheral blood CD4+CD27-CD28- T cells had lower FOXP3 expression compared with the parent total CD4+ T cell population.

We also compared the ability of peripheral blood CD4+CD27-CD28- T cells from CMV-pos and CMV-neg individuals to expand ex vivo in the presence of rhIL2. Sorted CD4+CD27-CD28- T cells from 5 CMV-pos individuals underwent 5- to 24-fold increases in number over a period of 15 days (S1 Fig), whereas the cells from 3 CMV-neg individuals died during the first week of culture.

**Phenotypic and functional comparison of CD4+CD27-CD28- peripheral blood Treg-like cells with ex vivo CMV-restimulated CD4+CD27-CD28-Tregs**

We previously showed that after 6 days of *ex vivo* restimulation of PBMC with CMV antigen high proportions of CD4+CD27-CD28- T cells in the PBMC cultures were associated with lower PBMC proliferations [15]. We further characterized the function and phenotype of the *ex vivo* CMV-stimulated CD4+CD27-CD28- T cells and found that they fulfilled the Treg definition [16], while mock-stimulated CD4+CD27-CD28- T cells expressed up to 10-fold lower levels of these markers (S2 Fig). Hence, we considered the *ex vivo* CMV-stimulated CD4+CD27-CD28- T cells a gold standard against which to compare the expression of Treg markers on the circulating CD4+CD27-CD28- T cells of CMV-pos donors (Fig 3). The two populations were similar with respect to TGFβ, IL35, CD39 and CD127 expression. However,
significantly higher proportions of ex vivo CMV-restimulated CD4+CD27-CD28- Tregs expressed FOXP3 and GrB compared with peripheral blood CD4+CD27-CD28- Treg-like cells.

The functional comparison of peripheral blood CD4+CD27-CD28- T cells with CMV-stimulated CD4+CD27-CD28- Tregs used inhibition of LPA to maintain consistence with our previous studies. Peripheral blood CD4+CD27-CD28- T cells inhibited proliferation of autologous PBMC by an average of 47% at an Effector:Target (E:T) ratio of 1:3. CD4+CD27-CD28- Tregs generated by ex vivo CMV-restimulation inhibited by 87% the proliferation of autologous PBMC (Fig 4 and S3 Fig).

Breadth of the regulatory function of peripheral blood CD4+CD27-CD28-Treg

We previously showed that ex vivo CMV-stimulated CD4+CD27-CD28- Tregs inhibited candida- and VZV ex vivo restimulated autologous PBMC in addition to CMV [16]. Candida and VZV were chosen because virtually all adults in the United States have been exposed to these antigens. In the current study, peripheral blood CD4+CD27-CD28- Tregs from 10
CMV-pos HIV-neg donors were added at a ratio of 1:3 to autologous PBMC in the presence of candida, VZV or CMV antigens. Proliferation measured after 6 days of culture was significantly inhibited when stimulated by VZV (mean = 28%; p = 0.04, one sample T test; Fig 5A and S3A Fig) at levels comparable with CMV (mean = 37%; p = 0.002; Fig 5A). Inhibition of candida-stimulated proliferation did not reach statistical significance (mean = 24%; p = 0.09; Fig 5A and S4A Fig). In contrast, CD4+CD27-CD28- T cells sorted from CMV-neg donors did not inhibit autologous PBMC proliferation in response to candida or VZV stimulation (S4B Fig).

Peripheral blood CD3+CD27-CD28- T cells were sorted from CMV-pos HIV-pos individuals for inhibition assays. We chose to sort CD3+CD27-CD28- T cells from these donors, because of the paucity of CD4+CD27-CD28- T cells and based on our previous study that showed that CD8+ T cells express FOXP3 and supplant the diminished number of CD4+ Tregs in HIV-infected individuals [15]. Moreover, we showed a significant association between the proportions of ex vivo CMV-stimulated cytotoxic CD8+ T cells and CMV end-organ disease or death in HIV-infected individuals independent of CMV blood viral load at the time when the PBMC were obtained [22]. The circulating CD3+CD27-CD28- T cells from HIV-pos CMV-pos donors inhibited proliferation of autologous PBMC ex vivo restimulated with inactivated HIV (mean = 58%, p = 0.0003; Fig 5B and S4C Fig) or CMV antigens (mean = 37%, p = 0.048; Fig 5B and S4C Fig) at an E:T ratio of 1:3.

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Discussion

Our data demonstrate that circulating CD4+CD28-CD27- T cells of CMV-pos donors have functional and phenotypic Treg characteristics, whereas circulating CD4+CD27-CD28- T cells of CMV-neg donors share selected phenotypic but not functional characteristics with those of CMV-pos donors. The phenotypic characterization used a broad panel of Treg markers, including FOXP3, which is a transcription factor that activates the regulatory program in T cells and is therefore considered the Treg hallmark; CD39, IL-35 and granzyme B, which are functional mediators of the Treg inhibitory activity; and TGFβ, which has both Treg activating and effector functions. Another Treg phenotypic characteristic found in the CD4+CD27-CD28- peripheral blood Treg was the low expression of CD127. Although many of these markers were shared by CMV-pos and CMV-neg donors, FOXP3 was found only in the circulating CD4+CD27-CD28- of CMV-pos donors. Moreover, only peripheral blood CD4+CD27-CD28- T cells from CMV-pos individuals proliferated in culture in the presence of rhIL2 and inhibited ex vivo proliferation of autologous PBMC. Taken together, these data indicate that the CD4+CD27-CD28- T cells of CMV-pos donors include Tregs, but those of CMV-neg individuals do not.

To verify the presence of Treg among the peripheral blood CD4+CD27-CD28- T cells, we used functional assays, namely inhibition of autologous PBMC proliferation. These assays showed that purified peripheral blood CD4+CD27-CD28- T cells from CMV-pos donors significantly and reproducibly decreased autologous PBMC proliferation not only in response to ex vivo CMV stimulation but also to VZV or candida stimulation. In contrast, circulating CD4...
Fig 5. Breadth of inhibitory activity of peripheral blood CD4+CD27-CD28- Treg from CMV-seropositive donors. **Panel A** shows a comparison of CMV-, VZV- and candida-specific proliferation inhibition using sorted peripheral blood CD4+CD27-CD28- Treg and autologous PBMC from 5 to 10 donors/antigen at effector to target ratios = 1:3. **Panel B** shows inhibition of proliferation of PBMC from 6 HIV-pos CMV-pos donors stimulated with CMV or HIV by autologous peripheral blood CD3+CD27-CD28- Treg at an effector to target ratio = 1:3. Inhibition of proliferation was calculated by the following formula: [(median CPM of antigen-stimulated wells) - (median CPM of antigen-stimulated + Treg wells)]/(median CPM of antigen-stimulated wells) x 100. P values above each column were calculated by single sample T test compared with 0% inhibition. P values between columns were calculated by unpaired T test. **S3 Fig** shows the same data presented in absolute CPM.

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+CD27-CD28- T cells of CMV-neg donors did not inhibit autologous proliferation. The functional difference between CMV-pos and CMV-neg peripheral blood CD4+CD27-CD28- T cells was in accordance with the lower expression of FOXP3 in the CMV-neg donor cells, emphasizing the importance of FOXP3 as a Treg marker. Moreover, the fact that only CD4 +CD27-CD28- T cells from CMV-pos donors inhibited proliferation suggests that these Tregs originally generated in response to CMV, inhibited candida and VZV proliferation through cross-reactivity or lack of specificity.

It is important to note the similarities and differences between the peripheral blood CD4 +CD27-CD28- T cells of CMV-pos donors and those generated ex vivo through CMV antigenic stimulation. Both CD4+CD27-CD28- T-cell populations expressed Treg phenotypic markers and inhibited proliferation of autologous PBMC in response to CMV, VZV and candida antigenic stimulation. However, compared with circulating CD4+CD27-CD28- T cells, ex vivo expanded CD4+CD27-CD28- T cells contained higher proportions of FOXP3+ and GrB+ cells and had greater capacity to inhibit CMV-stimulated proliferation of autologous PBMC, reinforcing the notion that the proportion of FOXP3+ cells provides a good indication of the presence of Tregs among T cells that phenotypically might be considered differentiated effectors.

Whether inducible Tregs evolve from natural Tregs or conventional T cells is the topic of current investigations [27–30]. A recent study showed that 12% of T-cell receptors were shared by Tregs and conventional effector T cells at the DNA level suggesting a common progenitor cell [31]. Tregs also tend to express markers of the effectors that they target. For example, Tregs inhibiting Th1 responses express CXCR3, Tbet and sometimes IFNγ, whereas those inhibiting Th17 responses express CCR6, RORγt and sometimes IL17 [32]. The common phenotypic characteristics between differentiated effectors and the Tregs described in our study support the notion that inducible Tregs evolve in parallel or sequentially with the differentiated effectors. The corollary of this observation is that the frequency or magnitude of CMV stimulation during reactivations, which is likely to be associated with the frequency of differentiated effector T cells, also may also dictate the magnitude of the CMV-induced Treg population. This hypothesis needs to be tested in additional studies, because its clinical implication is that prevention of CMV reactivations may substantially decrease the CMV-associated immune suppression of old adults and immune compromised hosts.

It may also be clinically significant that CD3+CD27-CD28- T cells sorted from the peripheral blood of CMV-pos HIV-infected individuals inhibited cell-mediated immune responses to ex vivo HIV antigenic restimulation. In these experiments, we used CD3+CD27-CD28- T cells because of the paucity of CD4+ T cells in HIV-infected individuals and because our previous studies suggested that CD8+ T cells play an important regulatory role in HIV-infected donors [15, 22], which is in agreement with the findings of other investigators who also showed that CD8+ inducible Tregs have the same function as their CD4+ counterparts [33–40]. Moreover, in CMV-pos old adults both CD4+CD28- and CD8+CD28- T cell numbers positively correlated with immune-suppression [41–43]. In the case of HIV infection, the effect of CMV Tregs on HIV-specific immunity may explain the strong association between CMV viremia and death of HIV-infected individuals that is not explained by CMV end-organ disease and is not associated with HIV viremia [2].

Based on these data, we propose that CMV reactivations generate both effector and regulatory responses that imprint the immune system of the host. The Tregs associated with CMV infection have a cross reactive down regulatory effect on the cell-mediated immune responses against other antigens leading to a generalized regulatory environment in the host that may not be apparent in immune competent hosts, but it may have biological significance in immune compromised hosts. Future studies are needed to determine if the frequencies of
Peripheral blood CD4+CD27-CD28- or CD8+CD27-CD28- Tregs in CMV-pos hosts increase with the number, duration and/or magnitude of viral replication during CMV reactivations.

Supporting information

S1 Fig. CD4+CD27-CD28- regulatory T cells from CMV-pos donors expand in vitro in the presence of rhIL2. Data were derived from 5 CMV-pos donors. CD4+CD27-CD28- T cells were sorted and cultured in growth medium with 30ng/mL rhIL2. Cells expanded 5- to 25-fold after 15 days of culture.

(PDF)

S2 Fig. Culture of PBMC from CMV-pos individuals does not select for CD4+CD27-CD28- with Treg characteristics. Data were derived from 4 donors. PBMC were stimulated with uninfected human lung fibroblast lysate for 6 days, washed, stained and analyzed with the Gallios instrument and Kaluza software. Bars indicate means and SEM.

(PDF)

S3 Fig. Functional evaluation of peripheral blood CD4+CD27-CD28- (Circulating) with ex vivo CMV-restimulated CD4+CD27-CD28- (CMV-stimulated) T cells from CMV-seropositive donors. Data were derived from 9 CMV-pos donors in panel A and 13 CMV-pos donors in panel B. PBMC were stimulated with CMV lysate at 66,000 cells/well for 6 days with and without the addition 33,000 autologous CD4+CD27-CD28- Treg. Cells were pulsed with 3HThy for the last 6 h of incubation, harvested and proliferation was measured by 3H incorporation.

(PDF)

S4 Fig. Only CD4+CD27-CD28- from CMV-pos individuals are Treg. Data were derived from 10 CMV-pos donors in panel A; 4 CMV-neg in panel B and 6 HIV-pos CMV-pos in panel C. 66,000 PBMC per well were incubated in triplicate or quadruplicate wells for 6 days with the antigens indicated on each graph, with and without autologous CD4+CD27-CD28- (panels A and B) or CD3+CD27-CD28- panel C).

(PDF)

Author Contributions

Conceptualization: AW.

Formal analysis: AW ATS.

Funding acquisition: AW.

Investigation: ATS.

Methodology: AW.

Project administration: AW ATS.

Resources: AW.

Supervision: AW ATS.

Validation: AW ATS.

Visualization: AW ATS.

Writing – original draft: AW ATS.
Writing – review & editing: AW ATS.

References

1. Jabs DA, Holbrook JT, Van Natta ML, Clark R, Jacobson MA, Kempen JH, et al. Risk factors for mortality in patients with AIDS in the era of highly active antiretroviral therapy. Ophthalmology. 2005; 112(5):771–9. https://doi.org/10.1016/j.ophtha.2004.10.049 PMID: 15878056.

2. Deayton JR, Prof Sabin CA, Johnson MA, Emery VC, Wilson P, Griffiths PD. Importance of cytomegalovirus viraemia in risk of disease progression and death in HIV-infected patients receiving highly active antiretroviral therapy. Lancet. 2004; 363(9427):2116–21. https://doi.org/10.1016/S0140-6736(04)16500-8 PMID: 15220032.

3. Pawelec G, Derhovanessian E. Role of CMV in immune senescence. Virus Res. 2011; 157(2):175–9. Epub 2010/09/28. https://doi.org/10.1016/j.virusres.2010.09.010 PMID: 20869407.

4. Rubin RH. Impact of cytomegalovirus infection on the outcome of organ transplantation. Jama. 1989; 261(24):3607–9. Epub 1989/06/23. PMID: 2542634.

5. Rubin RH. Impact of cytomegalovirus infection on organ transplant recipients. Rev Infect Dis. 1990; 12 Suppl 7:S754–66. Epub 1990/09/01. PMID: 2173105.

6. Slyker JA, Lohman-Payne BL, Rowland-Jones SL, Otieno P, Maleche-Obimbo E, Richardson B, et al. The detection of cytomegalovirus DNA in maternal plasma is associated with mortality in HIV-1-infected women and their infants. AIDS. 2009; 23(1):117–24. https://doi.org/10.1097/QAD.0b013e32831c8abd PMID: 19050393.

7. Johnstone J, Parsons R, Botelho F, Millar J, McNeil S, Fulop T, et al. Immune biomarkers predictive of respiratory viral infection in elderly nursing home residents. PLoS One. 2014; 9(9):e108481. https://doi.org/10.1371/journal.pone.0108481 PMID: 25275464.

8. Frasca D, Diaz A, Romero M, Landin AM, Blomberg BB. Cytomegalovirus (CMV) seropositivity decreases B cell responses to the influenza vaccine. Vaccine. 2015; 33(12):1433–9. https://doi.org/10.1016/j.vaccine.2015.01.071 PMID: 25659271.

9. Verschoor CP, Johnstone J, Millar J, Parsons R, Leic A, Loeb M, et al. Alterations to the frequency and function of peripheral blood monocytes and associations with chronic disease in the advanced-age, frail elderly. PLoS One. 2014; 9(8):e104522. https://doi.org/10.1371/journal.pone.0104522 PMID: 25105870.

10. Furman D, Jojic V, Sharma S, Shen-Orr SS, Angel CJ, Onengut-Gumuscu S, et al. Cytomegalovirus infection enhances the immune response to influenza. Sci Transl Med. 2015; 7(281):281ra43. https://doi.org/10.126/scitransmed.aaa2293 PMID: 25834109.

11. Lichtner M, Cicconi P, Vita S, Cozzi-Lepri A, Galli M, Lo Caputo S, et al. Cytomegalovirus coinfection is associated with an increased risk of severe non-AIDS-defining events in a large cohort of HIV-infected patients. J Infect Dis. 2015; 211(2):178–86. https://doi.org/10.1093/infdis/jiu417 PMID: 25081936.

12. Spencer JV, Lockridge KM, Barry PA, Lin G, Tsang M, Penfold ME, et al. Potent immunosuppressive activities of cytomegalovirus-encoded interleukin-10. J Virol. 2002; 76(3):1285–92. https://doi.org/10.1128/JVI.76.3.1285-1292.2002 PMID: 11773404.

13. Duppach J, Francois S, Joedicke JJ, Dittmer U, Kraft AR. Expanded regulatory T cells in chronically friend retrovirus-infected mice suppress immunity to a murine cytomegalovirus superinfection. J Virol. 2014; 88(23):13892–6. https://doi.org/10.1128/JVI.01941-14 PMID: 25231296.

14. von Herrath MG, Oldstone M, Homann D, Christen U. Is activation of autoreactive lymphocytes always detrimental? Viral infections and regulatory circuits in autoimmunity. Curr Dir Autoimmun. 2001; 4:91–122. PMID: 11569412.

15. Jesser RD, Li S, Weinberg A. Regulatory T cells generated during cytomegalovirus in vitro stimulation of mononuclear cells from HIV-infected individuals on HAART correlate with decreased lymphocyte proliferation. Virology. 2006; 352(2):408–17. https://doi.org/10.1016/j.virol.2006.04.035 PMID: 16782163.

16. Tovar-Salazar A, Patterson-Bartlett J, Jesser R, Weinberg A. Regulatory function of cytomegalovirus-specific CD4+CD27-CD28- T cells. Virology. 2010; 398(2):158–67. https://doi.org/10.1016/j.virology.2009.11.038 PMID: 20034645.

17. Gondek DC, Lu LF, Quezada SA, Sakaguchi S, Noelle RJ. Cutting edge: contact-mediated suppression by CD4+CD25+ regulatory cells involves a granzyme B-dependent, perforin-independent mechanism. J Immunol. 2005; 174(4):1783–6. Epub 2005/02/09. 174/4/1783 [pii]. PMID: 15699103.

18. Shvech EM, DiPaolo RA, Andersson J, Zhao DM, Stephens GL, Thornton AM. The lifestyle of naturally occurring CD4+ CD25+ Foxp3+ regulatory T cells. Immunol Rev. 2006; 212:60–73. Epub 2006/08/15. IMR415 [pii] https://doi.org/10.1111/j.0105-2896.2006.00415.x PMID: 16903906.
19. von Boehmer H. Mechanisms of suppression by suppressor T cells. Nat Immunol. 2005; 6(4):338–44. https://doi.org/10.1038/nijm.2005.189

20. Jahrsdorfer B, Vollmer A, Blackwell SE, Maier J, Sontheimer K, Beyer T, et al. Granzyme B produced by human plasmacytoid dendritic cells suppresses T-cell expansion. Blood. 2010; 115(6):1156–65. Epub 2009/12/08. https://doi.org/10.1182/blood-2009-07-235382 PMID: 19965634.

21. Zhao DM, Thornton AM, DiPaolo RJ, Shevach EM. Activated CD4+CD25+ T cells selectively kill B lymphocytes. Blood. 2006; 107(10):3925–32. Epub 2006/01/19. https://doi.org/10.1182/blood-2005-11-4502 PMID: 16418326.

22. Weinberg A, Bosch R, Bennett K, Towar-Salazar A, Benson CA, Collier AC, et al. Regulatory T cells and the risk of CMV end-organ disease in patients with AIDS. J Acquir Immune Defic Syndr. 2014; 66(1):25–32. https://doi.org/10.1073/pnas.1320999110 PMID: 24378728.

23. Egli A, Silva M, O’Shea D, Wilson LE, Balucha A, Lisboa LF, et al. An analysis of regulatory T-cell and Th-17 cell dynamics during cytomegalovirus replication in solid organ transplant recipients. PLoS One. 2012; 7(11):e43937. https://doi.org/10.1371/journal.pone.0043937 PMID: 23071829.

24. Terrazzini N, Bajwa M, Vita S, Cheek E, Thomas D, Seddiki N, et al. A novel cytomegalovirus-induced regulatory-type T-cell subset increases in size during older life and links virus-specific immunity to vascular pathology. J Infect Dis. 2014; 209(9):1382–92. https://doi.org/10.1093/infdis/jit576 PMID: 24203779.

25. Wang F, Chi J, Peng G, Zhou F, Wang J, Li L, et al. Development of virus-specific CD4+ and CD8+ regulatory T cells induced by human herpesvirus 6 infection. J Virol. 2014; 88(2):1011–24. https://doi.org/10.1128/JVI.02588-13 PMID: 24198406.

26. Rossio JL, Esser MT, Suryanarayana K, Schneider DK, Bess JW, Vasquez GM, et al. Inactivation of human immunodeficiency virus type 1 infectivity with preservation of conformational and functional integrity of virion surface proteins. J Virol. 1998; 72(10):7992–8001. PMID: 9733838.

27. Vukmanovic-Stajic M, Zhang Y, Cook JE, Fletcher JM, McQuaid A, Masters JE, et al. Human CD4+ CD25hi FOXP3+ regulatory T cells are derived by rapid turnover of memory populations in vivo. J Clin Invest. 2006; 116(9):2423–33. https://doi.org/10.1172/JCI28941 PMID: 1695142.

28. DuPage M, Bluestone JA. Harnessing the plasticity of CD4(+) T cells to treat immune-mediated disease. Nat Rev Immunol. 2016; 16(3):149–63. https://doi.org/10.1038/nri.2015.18 PMID: 26875830.

29. Sakaguchi S, Miyara M, Costantino CM, Hafler DA. FOXP3+ regulatory T cells in the human immune system. Nat Rev Immunol. 2010; 10(7):490–500. https://doi.org/10.1038/nri2785 PMID: 20559327.

30. Rosenblum MD, Way SS, Abbas AK. Regulatory T cell memory. Nat Rev Immunol. 2016; 16(2):90–101. https://doi.org/10.1038/nri.2015.1 PMID: 26688349.

31. Wolf KJ, Emerson RO, Pingel J, Buller RM, DiPaolo RJ. Conventional and Regulatory CD4+ T Cells That Share Identical TCRs Are Derived from Common Clones. PLoS One. 2016; 11(4):e0153705. https://doi.org/10.1371/journal.pone.0153705 PMID: 27100298.

32. Duhen T, Duhen R, Lanzavecchia A, Sallusto F, Campbell DJ. Functionally distinct subsets of human FOXP3+ Treg cells that phenotypically mirror effector Th cells. Blood. 2012; 119(19):4430–40. Epub 2012/03/23. https://doi.org/10.1182/blood-2011-11-392324 PMID: 22438251.

33. Karlsson I, Maleret B, Brochard P, Delache B, Calvo J, Le Grand R, et al. FoxP3+ CD25+ CD8+ T-cell induction during primary simian immunodeficiency virus infection in cynomolgus macaques correlates with low CD4+ T-cell activation and high viral load. J Virol. 2007; 81(24):13444–55. https://doi.org/10.1128/JVI.01466-07 PMID: 17888053.

34. Endharti AT, Rifa IMS, Shi Z, Fukuoka Y, Nakahara Y, Kawamoto Y, et al. Cutting edge: CD8+CD122+ regulatory T cells produce IL-10 to suppress IFN-gamma production and proliferation of CD8+ T cells. J Immunol. 2005; 175(11):7093–7. PMID: 16301610.

35. Molloy MJ, Zhang W, Usherwood EJ. Suppressive CD8+ T cells arise in the absence of CD4 help and compromise control of persistent virus. J Immunol. 2011; 186(11):6218–26. Epub 2011/05/03. jimmunol.1003812 [pii] https://doi.org/10.4049/jimmunol.1003812 PMID: 21531895.

36. Nogam P, Velu V, Kannanangat S, Chennareddi L, Kwa S, Siddiqui M, et al. Expansion of FOXP3+ CD8 T cells with suppressive potential in colorectal mucosa following a pathogenic simian immunodeficiency virus infection correlates with diminished antiviral T cell response and viral control. J Immunol. 2010; 184(4):1690–701. Epub 2010/01/08. jimmunol.0902955 [pii] https://doi.org/10.4049/jimmunol.0902955 PMID: 20053943.

37. Hu Z, Zhang W, Usherwood EJ. Regulatory CD8+ T cells associated with erosion of immune surveillance in persistent virus infection suppress in vitro and have a reversible proliferative defect. J Immunol. 2013; 191(1):312–22. Epub 2013/06/05. https://doi.org/10.4049/jimmunol.1201773 PMID: 23733872.

38. Holderried TA, Lang PA, Kim HJ, Cantor H. Genetic disruption of CD8+ Treg activity enhances the immune response to viral infection. Proc Natl Acad Sci U S A. 2013; 110(52):21089–94. Epub 2013/12/11. https://doi.org/10.1073/pnas.1320999110 PMID: 24324159.
39. Yu Y, Zitzner JR, Houlihan J, Herrera N, Xu L, Miller J, et al. Common gamma chain cytokines promote rapid in vitro expansion of allo-specific human CD8+ suppressor T cells. PLoS One. 2011; 6(12): e28948. https://doi.org/10.1371/journal.pone.0028948 PMID: 22194954.

40. Suzuki M, Jagger AL, Konya C, Shimojima Y, Pryshchep S, Goronzy JJ, et al. CD8+CD45RA+CCR7+FOXP3+ T cells with immunosuppressive properties: a novel subset of inducible human regulatory T cells. J Immunol. 2012; 189(5):2118–30. https://doi.org/10.4049/jimmunol.1200122 PMID: 22821963.

41. Derhovanessian E, Theeten H, Hahnel K, Van Damme P, Coools N, Pawelec G. Cytomegalovirus-associated accumulation of late-differentiated CD4 T-cells correlates with poor humoral response to influenza vaccination. Vaccine. 2013; 31(4):685–90. https://doi.org/10.1016/j.vaccine.2012.11.041 PMID: 23196209.

42. Derhovanessian E, Maier AB, Hahnel K, McElhaney JE, Slagboom EP, Pawelec G. Latent infection with cytomegalovirus is associated with poor memory CD4 responses to influenza A core proteins in the elderly. J Immunol. 2014; 193(7):3624–31. https://doi.org/10.4049/jimmunol.1303361 PMID: 25187662.

43. Turner JE, Campbell JP, Edwards KM, Howarth LJ, Pawelec G, Aldred S, et al. Rudimentary signs of immunosenescence in Cytomegalovirus-seropositive healthy young adults. Age (Dordr). 2014; 36 (1):287–97. https://doi.org/10.1007/s11357-013-9557-4 PMID: 23846127.