Immunosenescent CD57\(^+\)CD4\(^+\) T-cells accumulate and contribute to interferon-\(\gamma\) responses in HIV patients responding stably to ART

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Abstract. HIV-infected individuals responding to antiretroviral therapy (ART) after severe CD4\(^+\) T-cell depletion may retain low responses to recall antigens [eg: cytomegalovirus (CMV)] and altered expression of T-cell co-stimulatory molecules consistent with immunosenescence. We investigated the capacity of phenotypically senescent cells to generate cytokines in HIV patients receiving long-term ART (\(n = 18\)) and in healthy controls (\(n = 10\)). Memory T-cells were assessed by interferon (IFN)-\(\gamma\) ELISPOT assay and flow cytometrically via IFN-\(\gamma\) or IL-2. Proportions of CD57\(^{bright}\)CD28\(^{null}\) CD4\(^+\) T-cells correlated with IFN-\(\gamma\) responses to CMV (\(p = 0.009\)) and anti-CD3 (\(p = 0.002\)) in HIV patients only. Proportions of CD57\(^{bright}\)CD28\(^{null}\) CD8\(^+\) T-cells and CD8\(^+\) T-cell IFN-\(\gamma\) responses to CMV peptides correlated in controls but not HIV patients. IL-2 was predominantly produced by CD28\(^+\) T-cells from all donors, whereas IFN-\(\gamma\) was mostly produced by CD57\(^+\) T-cells. The findings provide evidence of an accumulation of immunosenescent T-cells able to make IFN-\(\gamma\). This may influence the pathogenesis of secondary viral infections in HIV patients receiving ART.

Keywords: CD57, HIV, immune activation, immunosenesence

1. Introduction

Many HIV patients beginning antiretroviral therapy (ART) with advanced disease experience persistent immune dysfunction despite long-term control of HIV replication and increased CD4\(^+\) T-cell counts. This has been demonstrated using interferon (IFN)-\(\gamma\) ELISPOT responses to index antigens such as cytomegalovirus (CMV). Poor responses correlate with an increased susceptibility to opportunistic infections [1] and faster HIV disease progression after cessation of ART [2].

Antigen-specific T-cell responses are lowest amongst patients with very low nadir CD4\(^+\) T-cell counts prior to ART and do not correlate with current CD4\(^+\) T-cell counts [3], suggesting ongoing immune dysfunction and/or irreversible damage to the immune system. As ART provides improved life expectancy for previously immunodeficient HIV patients, it becomes important to understand the capacity of their immune systems to respond to viral challenges.

Immunodeficient patients display reduced T-cell expression of the co-stimulatory molecule CD28 whilst gaining expression of CD57 and displaying reduced proliferative capacity [4,5]. Continued T-cell replication accelerates T-cell differentiation in HIV infection [6,7] and other chronic inflammatory diseases [8–10]. It is also a feature of normal aging of the immune system [11]. Several authors have speculated that HIV
patients may progress to immunosenesence prematu-
ately, but this has never been demonstrated in patients
with a stable virological response to ART. Here we cor-
related the effector memory T-cell function (assessed
using responses to CMV or a polyclonal stimulant)
with T-cell expression of activation and co-stimulatory
molecules associated with immunosenescence.

2. Materials and methods

Study groups comprised 18 male HIV-positive pa-
tients and 10 male healthy controls. Patients were iden-
tified from the HIV patient database of the Department
of Clinical Immunology, Royal Perth Hospital (RPH)
on the basis that they began ART with CD4\(^+\) T-cell
counts below 50/\(\mu\)L and had undetectable plasma HIV
RNA levels (< 50 copies/mL) for > 6 months after
> 12 months on ART. ART comprised at least three
antiretroviral drugs including a non-nucleoside reverse
transcriptase inhibitor or protease inhibitor. All pa-
tients and controls were CMV seropositive based on
detection of CMV-specific IgG (Department of Micro-
biology, RPH) and none had evidence of active in-
fecion (based on detection of CMV DNA using real-
time PCR as described previously [12]). T-cell sub-
sets were quantitated using whole blood stained with
CYTO-STAT triCHROME\(^\text{TM}\) (Coulter, Miami, USA)
using a Coulter EPICS-XL flow cytometer (Coulter,
Miami, USA). Plasma HIV RNA levels were assayed
by the Amplicor\(^\text{TM}\) method, version 1.5 (Roche Diag-
nostic Systems, Branchburg, USA). Informed consent
was obtained from all participants and human experi-
mentation guidelines of RPH and University of Western
Australia were followed.

Immunological assays utilised cryopreserved periph-
eral blood mononuclear cells (PBMC) with cell vi-
ability > 95%. ELISpot assays utilised anti-IFN-\(\gamma\)
antibodies (MabTech, Stockholm, Sweden) [13] and
PBMC were cultured alone or with anti-CD3 (10ng/
\(\mu\)L; MabTech), whole CMV, NLV peptide or VLE peptide.
Co-stimulatory antibodies \(\alpha\)-CD28 and \(\alpha\)-CD49d (BD Biosciences)
were added at a final concentration of 1 \(\mu\)g/mL. Antigen
stimulation was performed in polystyrene tubes for
6 hours with 10 \(\mu\)l Brefeldin A (BD Biosciences, San
Jose, CA) added after 1 hour. PBMC were washed with
cold 1% BSA/PBS and incubated with FcR blocking
reagent (Milteny Biotech; 4\(^\circ\)C, 20 minutes). Surface
staining (15 minutes) utilized CD3-PerCP (SK7), CD4-
FITC (RPA-T4), CD8-APC-Cy7 (SK1) from BD Bio-
sciences and CD57-PE (TB03) from Miltenyi Biotech.
Cells were permeabilised using Cytotox/Cytoperm\(^\text{TM}\)
kits and intracellular staining (30 minutes) utilised IFN-
\(\gamma\)-PCy7 (B27) and IL-2 APC (534.111) from BD Bio-
sciences. Data were acquired on a FACSCanto II flow
cytometer (BD Biosciences) within 4 hours using >
100,000 events per tube and analysed using FlowJo
software v7.2.2 (Tree Star, Ashland, OR). Statistical
analyses were performed with Graphpad Prism 5.01
using Mann-Whitney tests for continuous variables and
Spearman’s Rank Correlation tests, with \(p < 0.05\) ac-
cepted as a significant difference.

3. Results

HIV patients and healthy controls were comparable
in age (Table 1). Patients displayed stable control of
HIV replication for a median of 62 months with over
four-fold increases in CD4\(^+\) T-cell counts, but retained
elevated CD8\(^+\) T-cell counts at the time of study. IFN-
\(\gamma\) responses to CMV lysate (mediated by CD4\(^+\) T-cells)
and anti-CD3 (mediated by CD4\(^+\) and CD8\(^+\) T-cells)
were similar in patients and controls. CD8\(^+\) T-cell me-
diated responses [CMV peptides (NLV and VLE) or
CEF viral peptides] induced marginally higher respons-
es in the patients, but no differences were signi-
ificant.

As expected, CD4\(^+\) T-cells expressing high levels
of CD57 did not express CD28 (Fig. 1A). CD57\(^\text{bright}\)
CD28\(^\text{null}\) CD4\(^+\) T-cells were more abundant in patients
portions of CD57 in HIV patients or controls (responses to CMV (Fig. 1B) and anti-CD3 (CD28 than controls (Table 1) and correlated with IFN-γ responses to the CEF peptide pool (Fig. 1A). Proportions of CD57brightCD28nullCD4+ T-cells were higher in patients than controls (Table 1), but did not correlate with IFN-γ responses to CMV (r = 0.45, p = 0.19) or anti-CD3 (r = 0.37, p = 0.29).

CD4+ T-cells with low level expression of CD57 expressed CD28 (denoted CD57dimCD28+ T-cells, Fig. 1A). Proportions of CD57dimCD28+ CD4+ T-cells were higher in patients than controls (Table 1), but did not correlate with CD4+ T-cell IFN-γ responses to CMV (Fig. 1C) or anti-CD3 (r = 0.08, p = 0.74) in HIV patients or controls (r = 0.09, p = 0.8 and r = −0.4, p = 0.3; data not shown).

Analysis of CD57 and CD28 expression by CD8+ T-cells also defined CD57bright CD28null and CD57dim CD28+ populations (data not shown). These were present in similar proportions in PBMC from patients and controls (Table 1). The proportion of CD8+ T-cells that were CD57bright CD28null correlated with IFN-γ responses to the CEF peptide pool (r = 0.72, p = 0.02) and anti-CD3 (r = 0.68, p = 0.03) in controls and were moderately associated with IFN-γ responses to the NLV/VLE (CMV) peptides (r = 0.46, p = 0.09). In contrast, neither subset correlated with CD8+ T-cell IFN-γ responses to the CEF peptide pool, anti-CD3 or the NLV and VLE peptides in the HIV patients (p > 0.3 for all correlations).

As proportions of CD57brightCD28nullCD4+ T-cells correlated with CD4+ T-cell IFN-γ responses in HIV patients, we assessed whether CD57 defined cells capable of IFN-γ production. Overall, IFN-γ production by CD4+ T-cells was predominantly from CD57+ cells in HIV patients (p = 0.04 when compared with CD57− cells). The trend was similar in controls but not significantly different (Fig. 1D). IL-2 was produced predominantly by CD57− CD4+ T-cells (Fig. 1E). IFN-γ production by CD8+ T-cells was predominantly from the CD57+ subset in both patients and controls (p = 0.0002 and p = 0.01, respectively; Fig. 1F), whilst more IL-2 was produced by CD57− CD8− T-cells (p = 0.0002 and p = 0.002; Fig. 1G). Similar trends were observed when IFN-γ and IL-2 production by CD57+ CD4+ or CD8+ T-cells was assessed in response to the CMV antigen, or the CEF, NLV and VLE peptides (data not shown).

### 4. Discussion

In the patients selected for this study, IFN-γ responses to CMV lysate, CMV peptides or polyclonal stimuli were similar to uninfected donors. This allowed us to examine whether responses that appear to have “recovered” on ART reflect activation of similar T-cell populations in HIV patients and controls. We found that the HIV patients had increased proportions of circulating CD57brightCD28null cells in the CD4+ T-cell population when compared to healthy controls. This was not evident amongst CD8+ T-cells, but the absolute numbers of CD8+ T-cells were higher in patients.

CD57brightCD28null T-cells represent highly differentiated effector memory T-cells (CD45RA− CCR7−).
that have lost expression of CD27 and CD28, and/or terminally differentiated (CD45RA⁺ CCR7⁻) effector memory cells [6]. CD27⁺ CD28⁻ cells accumulate with age at the expense of the CD27⁺ CD28⁺ effector memory cells. This loss of co-stimulatory molecules could compromise the re-activation of memory cells [6]. Our analysis of IFN-γ and IL-2 production by CD57⁺ CD4⁺ T-cells stimulated with anti-CD3 demonstrated that such cells can be stimulated and produce IFN-γ but little IL-2. This population was proportionately larger in HIV patients responding stably to ART.

The accumulation of CD57brightCD28null CD4⁺ T-cells in the circulation of HIV patients receiv-
ing long-term ART is similar to findings in patients with autoimmune diseases, such as rheumatoid disease and multiple sclerosis [8–10]. It is hypothesised that continuous immune stimulation expands populations of terminally differentiated effector memory T-cells with characteristics of immunologically senescent T-cells [9], generating premature aging of the immune system. CD28nullCD4+ T-cells may contribute to early onset atherosclerotic vascular disease in patients with rheumatoid disease [14]. Moreover the anti-inflammatory effects of statin therapy in patients with unstable angina include a reduction of the frequency of circulating CD28nullCD4+ T-cells [15]. These findings are pertinent to HIV patients receiving long-term ART as they display an increased risk of atherosclerotic vascular disease [16].

The activation of CD4+ T-cells (assessed by HLA-DR expression) correlates with CD57 expression in HIV patients receiving long-term effective ART [3], driving differentiation towards a senescent phenotype. Immune activation may reflect ongoing HIV replication in ‘reservoirs’, such as the gut-associated lymphoid tissue [17], and/or continued translocation of bacterial products across the gut wall [18]. Otherwise healthy CMV-seropositive donors and HIV patients may also exhibit large clonal expansions of cells with limited antigen specificity, which may contribute to the population of immunosenescent CD28null T-cells [19,20]. This may not be unique to CMV as other chronic viral infections promote expansion of T-cells with a limited TCR repertoire [21].

In summary, it is likely that most effector memory T-cells producing IFN-γ in response to CMV antigens in previously immunodeficient HIV patients stably responding to ART are immunosenescent CD57bright CD28null T-cells accumulating in response to persistent immune activation. Immunosenescent T-cells may have altered function in vivo and may contribute to non-AIDS complications such as atherosclerotic vascular disease. This warrants further study.

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References


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