Immunosenescent CD57⁺CD4⁺ T-cells accumulate and contribute to interferon-γ responses in HIV patients responding stably to ART

Sonia Fernandez, Martyn A. French and Patricia Price

School of Pathology and Laboratory Medicine, University of Western Australia and PathWest Laboratory Medicine, Perth, Australia
Department of Clinical Immunology, Royal Perth Hospital and PathWest Laboratory Medicine, Perth, Australia

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)-γ ELISpot assay and flow cytometrically via IFN-γ or IL-2. Proportions of CD57⁺brightCD28null CD4⁺ T-cells correlated with IFN-γ responses to CMV (p=0.009) and anti-CD3 (p=0.002) in HIV patients only. Proportions of CD57⁺brightCD28null CD8⁺ T-cells and CD8⁺ T-cell IFN-γ 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-γ was mostly produced by CD57⁺ T-cells. The findings provide evidence of an accumulation of immunosenescent T-cells able to make IFN-γ. This may influence the pathogenesis of secondary viral infections in HIV patients receiving ART.

Keywords: CD57, HIV, immune activation, immunosenescence

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)-γ 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 immunosenescence prematurely, but this has never been demonstrated in patients with a stable virological response to ART. Here we correlated 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 patients and 10 male healthy controls. Patients were identified 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/μL 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 patients and controls were CMV seropositive based on detection of CMV-specific IgG (Department of Microbiology, RPH) and none had evidence of active infection (based on detection of CMV DNA using real-time PCR as described previously [12]). T-cell subsets were quantitated using whole blood stained with CYTO-STAT triCHROMETM (Coulter, Miami, USA) using a Coulter EPICS-XL flow cytometer (Coulter, Miami, USA). Plasma HIV RNA levels were assayed by the AmplicorTM method, version 1.5 (Roche Diagnostic Systems, Branchburg, USA). Plasma HIV RNA levels were detected using an AID ELISpot Reader System (AID, Strasbourg, Germany). Numbers of spots in unstimulated wells were subtracted from numbers in stimulated wells and adjusted per 2 × 10^5 PBMC. To evaluate T-cell phenotypes, PBMC were surface stained (15 minutes, room temperature) using conjugated monoclonal antibodies as follows: CD4-PC5 (13B8.2; Coulter Immunotech, Marseille, France), CD8-APC-Cy7 (SK1), CD28-PECy7 (CD28.2) or CD57-FITC (TB01) from ebioscience (San Diego, CA). For antigen stimulation, PBMC were washed and resuspended at 10^6 per mL alone or with anti-CD3 (10ng/mL; MabTech), whole CMV, NLV peptide or VLE peptide. Co-stimulatory antibodies α-CD28 and α-CD49d (BD Biosciences) were added at a final concentration of 1 μg/mL. Antigen stimulation was performed in polypropylene tubes for 6 hours with 10 μl 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 (Miltenyi Biotec; 4°C, 20 minutes). Surface staining (15 minutes) utilized CD3-PerCP (SK7), CD4-FITC (RPA-T4), CD8-APC-Cy7 (SK1) from BD Biosciences and CD57-PE (TB03) from Miltenyi Biotec. Cells were permeabilised using Cytotox/CytopermTM kits and intracellular staining (30 minutes) utilised IFN-γ-PeCy7 (B27) and IL-2 APC (534.111) from BD Biosciences. Data were acquired on a FACSCan 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 accepted 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-γ 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 mediated responses [CMV peptides (NLV and VLE) or CEF viral peptides] induced marginally higher responses in the patients, but no differences were significant.

As expected, CD4+ T-cells expressing high levels of CD57 did not express CD28 (Fig. 1A). CD57bright CD28null CD4+ T-cells were more abundant in patients
portions of CD57 responses to CMV (Fig. 1B) and anti-CD3 (CD28 than controls (Table 1) and correlated with 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 CD57bright CD28null 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.

Acknowledgements

This work was supported by grant number 404028 from the National Health and Medical Research Council of Australia.

References

[1] M. French, N. Keane, E. McKinnon, S. Phung and P. Price, Susceptibility to opportunistic infections in HIV-infected patients with increased CD4 T-cell counts on antiretroviral therapy may be predicted by markers of dysfunctional effector memory CD4 T cells and B cells, HIV Med 8 (2007), 148–155.

[2] L. Darwich, G. Coma, R. Pena, R. Bellido, E.J. Blanco, J.A. Este, E.E. Borras, B. Clotet, L. Ruiz, A. Rosell, F. Andreo, R.M. Parkhouse and M. Boffill, Secretion of interferon-gamma by human macrophages demonstrated at the single-cell level after costimulation with interleukin (IL)-12 plus IL-18, Immunology 126 (2009), 386–393.

[3] S. Fernandez, P. Price, E.J. McKinnon, R.C. Nolan and M.A. French, Low CD4+ T-cell counts in HIV patients receiving effective antiretroviral therapy are associated with CD4+ T-cell activation and senescence but not with lower effector memory T-cell function, Clin Immunol 120 (2006), 163–170.

[4] M.C. Jimenez-Martinez, M. Linares, R. Baez, L.F. Montano, S. Martinez-Cairo, P. Gorocica, R. Chavez, E. Zeneto and R. Lascurain, Intracellular expression of interleukin-4 and interferon-γ by a Mycobacterium tuberculosis antigen-stimulated CD4+ CD57+ T-cell subpopulation with memory phenotype in tuberculosis patients, Immunology 111 (2004), 100–106.

[5] B.E. Palmer, N. Blyveis, A.P. Fontenot and C.C. Wilson, Functional and phenotypic characterisation of CD57+ CD4+ T cells and their association with HIV-1-induced T cell dysfunction, J Immunol 175 (2005), 8415–8425.

[6] S. Koch, A. Larbi, E. Derhovanessian, D. Ozcelik, E. Naumova and G. Pawelec, Multiparameter flow cytometric analysis of CD4 and CD8 T cell subsets in young and old people, Immun Ageing 5 (2008), 6.

[7] P. Romero, A. Zippelius, I. Kurth, M.J. Pritet, C. Tovrey, E.M. Iancu, P. Corthesy, E. Destrave, D.E. Spisser and N. Ruffer, Four functionally distinct populations of human effector-memory CD8+ T lymphocytes, J Immunol 178 (2007), 4112–4119.

[8] M. Thewissen, L. Linsen, V. Somers, P. Geusens, J. Raus and P. Stinissen, Premature immunosenescence in rheumatoid arthritis and multiple sclerosis patients, Ann N Y Acad Sci 1051 (2005), 255–262.

[9] M. Thewissen, V. Somers, N. Hellings, J. Fraussen, J. Damoiseaux and P. Stinissen, CD4+CD8null T cells in autoimmune disease: pathogenic features and decreased susceptibility to immunoregulation, J Immunol 179 (2007), 6514–6523.

[10] M. Thewissen, V. Somers, K. Venken, L. Linsen, P. van Paasssen, P. Geusens, J. Damoiseaux and P. Stinissen, Analyses of immunosenescent markers in patients with autoimmune disease, Clin Immunol 123 (2007), 209–218.

[11] P. Sansoni, R. Vescovini, F. Fagnoni, C. Biasini, F. Zanni, L. Zalari, A. Telera, G. Lucchini, G. Passeri, D. Monti, C. Franceschi and M. Passeri, The immune system in extreme longevity, Exp Gerontol 43 (2008), 61–65.

[12] E. Schoerer, S. Henriot, P. Zachary, R. Freitag, A. Fuchs, S. Fritsch, S. Risch, N. Meyer, S. Caillard, B. Lioure and F. Stoll-Keller, Monitoring low cytomegalovirus viremia in transplanted patients by a real-time PCR on plasma, J Med Virol 76 (2005), 76–81.

[13] N.M. Keane, P. Price, S.F. Stone, M. John, R.J. Murray and M.A. French, Assessment of immune function by lymphoproliferation underestimates lymphocyte functional capacity in HIV patients treated with highly active antiretroviral therapy, AIDS Res Hum Retroviruses 16 (2000), 1991–1996.
[14] R. Gerli, G. Schiattaci, A. Giordano, E. B. Bocci, O. Bistoni, G. Vaudo, S. Marchesi, M. Pirro, F. Ragni, Y. Shoenfeld and E. Mannarino, CD4+CD28- T lymphocytes contribute to early atherosclerotic damage in rheumatoid arthritis patients, Circulation 109 (2004), 2744–2748.

[15] S. Brugaletta, L.M. Biasucci, M. Pinnelli, G. Biondi-Zoccai, G. Di Giannuario, G. Trotta, G. Liuzzo and F. Crea, Novel anti-inflammatory effect of statins: reduction of CD4+CD28null T lymphocyte frequency in patients with unstable angina, Heart 92 (2006), 249–250.

[16] A.N. Phillips, J. Neaton and J.D. Lundgren, The role of HIV in serious diseases other than AIDS, Aids 22 (2008), 2409–2418.

[17] T.W. Chun, D.C. Nickle, J.S. Justement, J.H. Meyers, G. Roby, C.W. Hallahan, S. Kotttili, S. Moir, J.M. Mican, J.I. Mullins, D.J. Ward, J.A. Kovacs, P.J. Mannon and A.S. Fauci, Persistence of HIV in gut-associated lymphoid tissue despite long-term antiretroviral therapy, J Infect Dis 197 (2008), 714–720.

[18] J.M. Brenchley, D.A. Price, T.W. Schacker, T.E. Asher, G. Silvestri, S. Rao, Z. Kazzaz, E. Bornstein, O. Lambotte, D. Altmann, B.R. Blazar, B. Rodriguez, L. Teixeira-Johnson, A. Landay, J.N. Martin, F.M. Hecht, L.J. Picker, M.M. Lederman, S.G. Deeks and D.C. Douek, Microbial translocation is a cause of systemic immune activation in chronic HIV infection, Nat Med 12 (2007), 1365–1371.

[19] T.G. Evans, E.G. Kallas, A.E. Laque, M. Menegus, C. McNair and R.J. Looney, Expansion of the CD57 subset of CD8 T cells in HIV-1 infection is related to CMV serostatus, Aids 13 (1999), 1139–1141.

[20] N. Khan, N. Shariff, M. Cobbold, R. Bruton, J.A. Ainsworth, A.J. Sinclair, L. Nayak and P.A. Moss, Cytomegalovirus seropositivity drives the CD8 T cell repertoire toward greater clonality in healthy elderly individuals, J Immunol 169 (2002), 1984–1992.

[21] V.P. Argaet, C.W. Schmidt, S.R. Burrows, S.L. Silins, M.G. Kurilla, D.L. Doolan, A. Suhbier, D.J. Moss, E. Kieff, T.B. Sculley and I.S. Misko, Dominant selection of an invariant T cell antigen receptor in response to persistent infection by Epstein-Barr virus, J Exp Med 180 (1994), 2335–2340.