CAN MEASURING IMMUNITY TO HIV DURING ANTIRETROVIRAL THERAPY (ART) IN CHILDREN PROVIDE A CLUE TO MARKERS OF ART EFFECTIVENESS?

Clive Gray, *PhD*

National Institute for Communicable Diseases and Immunopaedia, a Programme for the Enrichment of Paediatric Immunology in South Africa

The vexing issue of whether the immune system can be reconstituted during HIV infection by supplying antiretroviral therapy (ART) has been a question asked about HIV-infected adults and children receiving therapy.[1-9] Knowing that the immune system is sufficiently plastic in adults to show restoration of specific and general immunity after receiving ART is promising when translated to paediatric treatment. There is evidence in children of immune reconstitution after receiving various therapeutic regimens.[2,10] This review will examine some of the aspects of immune restoration in general, and specifically in children, and pose the question whether knowledge of changes in immunity in tandem with viral suppression can provide clues as to how to measure immune efficacy of ART.

Failure to maintain T-cell homeostasis during HIV-1 infection[11-13] results in compromised immunity, allowing development of opportunistic infections and progression to AIDS. Loss of CD4+ T cells due to direct and indirect mechanisms[14,15] is the primary cause of this imbalance and assessment of absolute CD4+ cell numbers in adults and CD4+ percentage is used as a clinical benchmark of disease progression. Immunotherapeutic strategies designed to restore homeostatic balance and immune competency have been employed,[16] although there is little evidence to suggest that these can lead to sustainable reduction in viral burden. Conversely, antiviral drug strategies, notably HIV protease inhibitors (PIs), have resulted in significant suppression of viral replication. The increased likelihood that persistent virus can be removed with provision of antiretroviral treatment has raised the additional consideration that T-cell immunity may be restored, resulting in the re-establishment of T-cell homeostasis.[17] There is evidence that immune reconstitution can occur in patients receiving HIV PIs either as monotherapy[18] or in combination with nucleoside analogues.[1] There is also evidence that immune reconstitution occurs in children after receiving ART regimens,[3-5,19] where it has been shown that children had an earlier and greater increase in naïve T-cell subsets than adults, probably due to a more active thymus, with the potential for immune reconstitution when HIV-1 replication is controlled.

Evidence that ART can lead to changes in immune function was initially shown in studies investigating improvement in the functional capacity of T-helper cells in response to

zidovudine (ZDV) in adults[20,21] and didanosine in children.[22] With the advent of more powerful antiviral PIs, more comprehensive studies have emerged showing the ability of the immune system to demonstrate self-restorative changes.[1,18] Improvements in naïve and/or memory CD4+ and CD8+ T-cell changes have been shown in response to ritonavir monotherapy[18] and highly active regimens consisting of either ritonavir or indinavir in combination with nucleoside analogues.[1] The latter study focused on a group of patients who had never been exposed to ART and provided some evidence for immune restoration in response to removing replicating virus.

Studies in adults have shown that in patients who failed ART, and switched to another course, a progressive increase in absolute numbers of naïve and memory CD4+ cells, based on differential L-selectin (CD62L) and CD45RA expression, was observed over 6 months in response to highly active antiretroviral therapy (HAART). Co-expression of L-selectin with CD45RA detects functionally competent naïve cells before interaction with cognate antigen.[23,24] Studies in children have shown that the ability to proliferate to pokeweed mitogen before HAART was lower in HIV-infected children than in a control HIV-uninfected group, but recovered to normal levels after a year on ART. Previous studies in children[25] have found that mitogen-induced tumour necrosis factor (TNF)-alpha and interferon (IFN)-gamma production was lower before ART ($p < 0.001$), but returned to near-normal levels after 1 year of ART.[9]

EVENTS OCCURRING IN LYMPH NODES

The preceding discussions have outlined that T-cell populations show plasticity in their ability to repopulate the peripheral circulation in response to ART.[26] While it is clear that ART can suppress plasma HIV replication sufficiently to result in beneficial changes in peripheral T-cell immunity in children, there is less distinct evidence of events occurring within lymphoid compartments. Studies in adults investigating tonsil[27,28] or lymph node (LN) tissue[29] in a cross-sectional manner have shown that viral load is significantly reduced with only rare HIV-1-infected cells existing within lymphoid tissue after more than 2 years of treatment. Studies that investigated LNs in a sequential manner[30-32] have shown evidence of increased proportions of naïve T-cells and suppressed viral load after the first 2 - 3 months. Very few studies have examined events occurring in children at the LN level, probably because of the difficulty of taking biopsies.

In adults, one of the first events after HIV transmission through mucosa is the 'seeding' of HIV to lymphoid structures, most notably the LNs.[33] In children, where transmission may occur either *in utero* or in the perinatal period, 'seeding' of HIV to lymphoid tissues would occur either at the time of birth or very soon after birth. In these scenarios lymphoid structures will be taken over by HIV prior to exposure to any other infection. *In utero* infection would have catastrophic effects on neonatally developing immunity, as bone marrow, thymus and LNs would be infected and disrupt the ontology of T cells. This would lead to severely compromised immunity very early in life and may explain rapid progression to AIDS in children infected *in utero*. However, as in adults, in children infected at birth or perinatally HIV appropriates LN structures within the microenvironment of the germinal center (GC) by displacing antigens[34] complexed with the follicular dendritic cell (FDC) network.[35] This antigen displacement is thought to curtail immune responses to non-HIV antigens, and viral antigen persistence additionally results in chronic stimulation leading to destruction of the lymphoid microenvironment and dissolution of the FDC network. Histologically this is observed as a regression of the GC and disappearance of the FDC network.[36] As an intact LN architecture is required for clonal expansion of effector T cells upon interaction with cognate antigens, disintegration of the LN microenvironment therefore impedes the development of new immune responses and leads to susceptibility to opportunistic infections. It would be expected that such events occurring in early life would stunt the development of specific immunity to the plethora of antigens and pathogens bombarding newly born children. The sooner this situation can be reversed by suppressing viraemia with effective ART, the better the chance of longer-term survival. The ability to measure and find a marker of successful immune regeneration in children would enable better assessment of different ART regimens.

A CLOSER LOOK AT T CELLS

It is clear from the studies described in both adults and children that CD4+ and CD8+ T cells with both naïve and memory phenotypes emerge during ART. Analysis of events that occur more closely within the CD8+ T-cell compartment is crucial from the perspective that CD8+ cells are directly implicated in providing immunological protection. This is very important not only with regard to formulating a marker of ART immune efficacy, but also in developing therapeutic vaccine strategies in tandem with ART. During HIV infection, detection of a skewed expansion of CD8+ cells that use a limited Vß T-cell receptor (TCR) type have been identified in infants[37] and adults.[38] This results in a limited pattern of recognition of HIV and a probable loss of protective immunity.[39] One important aspect of investigating immune reconstitution in children is that cells responsible for protective immunity, namely CD8+ T cells, can increase in numbers and possess anti-HIV recognition. There are two ways of analysing whether CD8+ T cells may improve in quality after ART: (*i*) by investigating TCR expression and whether this changes in response to suppression of viraemia; and (*ii*) by investigating HIV-specific recognition of cells and whether the number of epitopes (or regions recognised) increases with ART. There are various ways of investigating TCR expression – either directly using a panel of monoclonal antibodies and flow cytometry,[37,40] or by molecular means of measuring the length of the expressed gene that encodes for the TCR.[41,42] Additionally, several investigators have measured T-cell receptor excision circles (TRECs), which indirectly measure thymic output of T cells.[6,8,43]

Use of these differing technologies has allowed assessment of whether there is a 'normalisation' of CD8+ T cells in response to ART.

Investigating TCR use by flow cytometry has revealed that there was abnormally expanded CD8 TCR use at baseline,[26] most notably utilising Vß12, demonstrating that clonal dominance existed before therapy, and possibly indicating reduced efficacy of CTL responses.[44] There was a loss of repertoire bias accompanied by drug-mediated suppression of viral load at 8 weeks of ART. From an immunological perspective, these data imply that newly replicating virus is required to maintain the presence of expanded antigen-specific CD8+ cells. In this study, it was found that the majority of CD8+ cells expressing different TCR types was negative for L-selectin (CD62L) and was most probably of the memory CD45RO+ phenotype. The flattening of the TCR repertoire in response to ART probably reflects a redistribution of existing clones rather than development of new T cells in the thymus. Whether these changes are any different in children remains to be seen and may represent a useful marker by which to gauge immune efficacy of ART.

These data provide indirect evidence that lowering persistently high levels of viral replication with ART can reduce the stimulus that maintains expanded CD8+ clones. By inference, we can postulate that changes in the phenotype of CD8+ T cells reflect changes in viraemia.

This notion can be explored in greater detail by measuring anti-HIV-specific T-cell immunity, which can be achieved by investigating the frequency of epitope-specific CD8+ cells using peptide/major histocompatibility complex (MHC) tetramers.[45] This technique is particularly useful for exploring the hypothesis that high levels of replicating HIV-1 are required to maintain anti-HIV CD8+ cells. As it has been shown functionally that a restricted anti-HIV cytotoxic T lymphocyte (CTL) clonal repertoire exists in infected individuals,[46,47] it is possible that a reduced clonal repertoire during ART could potentially result in broader CD8+ T-cell response to other antigens. Various studies using MHC tetramers have investigated the nature of antigen-specific cells and how they change in frequency after ART. One of the first studies in adults showed that of 19 HIV-infected individuals[48] 72% had CD8+ cells recognising an epitope in Gag (SLYNTVATL) and to a lesser extent in Pol (ILKEPVHGV). When the frequency of epitope-specific cells in patients receiving ART for the first time, was assessed, 5 of 6 showed a significant loss of tetramer-positive CD8+ cells in parallel with suppression of replicating HIV. This would suggest that maintenance of HIV-1-specific clones is dependent on the persistence of replicating virus. Reduction in cell frequency was independent of gross CD4+ and CD8+ changes and was not likely to be due to mutations occurring in Gag or Pol epitopes. As most epitope-specific CD8+ T cells did not express acute activation markers[48] prior to drug therapy, loss of circulating tetramer-stained cells in response to HAART was unlikely to be due to a decline in the gross activated CD8+ T-cell pool.

Collectively, these data may reflect a situation whereby suppression of viraemia with successful ART is proportional to a reduction in the frequency of anti-HIV-specific CD8+ T cells. It may be hypothesised that effective ART causes reduced antigen persistence and hence reduces the requirement to maintain high levels of antigen-specific CD8+ T cells. Such dynamic changes in the immune system in tandem with viral suppression can be exploited for an added marker of immune efficacy to ART.

CONCLUSIONS

This review has shown that the immune system has an endogenous ability to respond favourably to ART, particularly in children, in whom the number of naïve CD4+ and CD8+ T cells is high. The main point of measuring these responses is to understand how a relatively naïve immune system in children can respond in a dynamic fashion leading to removal of persisting antigen, i.e. suppression of viral load with ART. The consistent correlation between viral load and activated CD8+ T cells in blood and LN implies that activation signals are reduced – either indirectly (via reduction in inflammatory cytokine levels) or directly (by diminishing antigen engagement with T cells) as HIV replication is suppressed. This has been the basis of several findings that have proposed that CD38+ is a measure of immune reconstitution,[49,50] where the inverse proportionality with viral load makes this an attractive marker. Diminished antigen load, due to suppressed viral load, is mirrored by reductions in oligoclonally expanded CD8+ T cells and reduced frequencies of antigen-specific CD8+ T cells. The overall implications of these findings are that immune restoration in response to ART is dependent on significant and sustained HIV-1 suppression in lymphoid tissue, allowing lymphoid structures to return. All these factors provide clues as to how we can measure immune efficacy of ART in children, which in turn has importance for understanding the effectiveness of returning HIV-1 infected children to a 'normalised' state where immune robustness can protect against opportunistic infections. Additionally, these observations may have significant implications for the success of therapeutic vaccine strategies so that improved immunity, in response to ART, can be exploited for the benefit of long-term immune protection in HIV-infected children.

REFERENCES

1. Autran B, Carcelain G, Li TS, *et al.* Positive effects of combined antiretroviral therapy on CD4+ T cell homeostasis and function in advanced HIV disease [see comments]. *Science* 1997; **277:** 112-116.
2. Feeney ME, Draenert R, Roosevelt KA, *et al.* Reconstitution of virus-specific CD4 proliferative responses in pediatric HIV-1 infection. *J Immunol* 2003, **171:** 6968-6975.
3. Franco JM, Leon-Leal JA, Leal M, *et al.* CD4+ and CD8+ T lymphocyte regeneration after anti-retroviral therapy in HIV-1-infected children and adult patients. *Clin Exp Immunol* 2000;**119:** 493-498.
4. Ghaffari G, Passalacqua DJ, Caicedo JL, Goodenow MM, Sleasman JW. Two-year clinical and immune outcomes in human immunodeficiency virus-infected children who reconstitute CD4 T cells without control of viral replication after combination antiretroviral therapy. *Pediatrics* 2004; **114:** e604-611.
5. Hainaut M, Ducarme M, Schandene L, *et al.* Age-related immune reconstitution during highly active antiretroviral therapy in human immunodeficiency virus type 1-infected children. *Pediatr Infect Dis J* 2003; **22:** 62-69.
6. Ometto L, De Forni D, Patiri F, *et al.* Immune reconstitution in HIV-1-infected children on antiretroviral therapy: role of thymic output and viral fitness. *AIDS* 2002; **16:** 839-849.

7. Resino S, Correa R, Bellon JM, Sanchez-Ramon S, Munoz-Fernandez MA. Characterizing immune reconstitution after long-term highly active antiretroviral therapy in pediatric AIDS. *AIDS Res Hum Retroviruses* 2002; **18:** 1395-1406.
8. Resino S, Galan I, Bellon JM, Navarro ML, Leon JA, Munoz-Fernandez MA. Characterizing the immune system after long-term undetectable viral load in HIV-1-infected children. *J Clin Immunol* 2003; **23:** 279-289.
9. Resino S, Galan I, Perez A, *et al.* HIV-infected children with moderate/severe immune-suppression: changes in the immune system after highly active antiretroviral therapy. *Clin Exp Immunol* 2004; **137:** 570-577.
10. van Rossum AM, Scherpbier HJ, van Lochem EG, *et al.* Therapeutic immune reconstitution in HIV-1-infected children is independent of their age and pretreatment immune status. *AIDS* 2001; **15:** 2267-2275.
11. Grossman Z, Herberman RB. T-cell homeostasis in HIV infection is neither failing nor blind: modified cell counts reflect an adaptive response of the host [see comments]. *Nat Med* 1997; **3:** 486-490.
12. Adleman LM, Wofsy D. T-cell homeostasis: implications in HIV infection [comment] [see comments]. *J Acquir Immune Defic Syndr* 1993; **6:** 144-152.
13. Margolick JB, Munoz A, Donnenberg AD, ***et al.*** Failure of T-cell homeostasis preceding AIDS in HIV-1 infection. The Multicenter AIDS Cohort Study [see comments]. *Nat Med* 1995; **1:** 674-680.
14. Poli G, Pantaleo G, Fauci AS. Immunopathogenesis of human immunodeficiency virus infection. *Clin Infect Dis* 1993, 17 Suppl 1: S224-229.
15. Levy JA. Pathogenesis of human immunodeficiency virus infection. *Microbiol Rev* 1993; **57: 1**83-289.
16. Emery S, Lane HC. Immune-based therapies in HIV infection: recent developments. *AIDS* 1996; **10:** S159-163.
17. Schnittman SM, Fox L. Preliminary evidence for partial restoration of immune function in HIV type 1 infection with potent antiretroviral therapies: clues from the Fourth Conference on Retroviruses and Opportunistic Diseases. *AIDS Res Hum Retroviruses* 1997; **13:** 815-818.
18. Kelleher AD, Carr A, Zaunders J, Cooper DA. Alterations in the immune response of human immunodeficiency virus (HIV)-infected subjects treated with an HIV-specific protease inhibitor, ritonavir. *J Infect Dis* 1996; **173:** 321-329.
19. Johnston AM, Valentine ME, Ottinger, *et al.* Immune reconstitution in human immunodeficiency virus-infected children receiving highly active antiretroviral therapy: a cohort study. *Pediatr Infect Dis J* 2001; **20:** 941-946.
20. Rinaldo C, Huang XL, Piazza P, *et al.* Augmentation of cellular immune function during the early phase of zidovudine treatment of AIDS patients. *J Infect Dis* 1991; **164:** 638-645.
21. Clerici M, Landay AL, Kessler HA, *et al.* Reconstitution of long-term T helper cell function after zidovudine therapy in human immunodeficiency virus-infected patients. *J Infect Dis* 1992; **166:** 723-730.
22. Clerici M, Roilides E, Butler KM, *et al.* Changes in T-helper cell function in human immunodeficiency virus- infected children during didanosine therapy as a measure of antiretroviral activity. *Blood* 1992; **80:** 2196-2202.
23. Picker LJ, Treer JR, Ferguson-Darnell B, Collins PA, Buck D, Terstappen LW. Control of lymphocyte recirculation in man. I. Differential regulation of the peripheral lymph node homing receptor L-selection on T cells during the virgin to memory cell transition. *J Immunol* 1993; **150:** 1105-1121.
24. Roederer M, Dubs JG, Anderson MT, Raju PA, Herzenberg LA. CD8 naive T cell counts decrease progressively in HIV-infected adults. *J Clin Invest* 1995, **95:** 2061-2066.
25. Resino S, Navarro J, Bellon JM, Gurbindo D, Leon JA, Munoz-Fernandez MA. Naive and memory CD4+ T cells and T cell activation markers in HIV-1 infected children on HAART. *Clin Exp Immunol* 2001; **125:** 266-273.
26. Gray CM, Schapiro JM, Winters MA, Merigan TC. Changes in CD4+ and CD8+ T cell subsets in response to highly active antiretroviral therapy in HIV-1 infected patients with prior protease inhibitor experience. *AIDS Res Hum Retrovir Uses* 1998; **14:** 569-578.
27. Cavert W, Notermans DW, Staskus K, *et al.* Kinetics of response in lymphoid tissues to antiretroviral therapy of HIV-1 infection [see comments] [published erratum appears in *Science* 1997; **276:** 1321]. *Science* 1997; **276:** 960-964.
28. Haase AT, Henry K, Zupancic M, *et al.* Quantitative image analysis of HIV-1 infection in lymphoid tissue. *Science* 1996, **274: 9**85-989.
29. Ruiz L, van Lunzen J, Arno A, *et al.* Protease inhibitor-containing regimens compared with nucleoside analogues alone in the suppression of persistent HIV-1 replication in lymphoid tissue. *AIDS* 1999; **13:** F1-8.
30. Zhang ZQ, Schuler T, Cavert W, *et al.* Reversibility of the pathological changes in the follicular dendritic cell network with treatment of HIV-1 infection. *Proc Natl Acad Sci USA* 1999; **96:** 5169-5172.
31. Landay AL, Bethel J, Schnittman S. Phenotypic variability of lymphocyte populations in peripheral blood and lymph nodes from HIV-infected individuals and the impact of antiretroviral therapy. DATRI 003 Study Group. Division of AIDS Treatment Research Initiative. *AIDS Res Hum Retroviruses* 1998, **14:** 445-451.
32. Bucy RP, Hockett RD, Derdeyn CA, *et al.* Initial increase in blood CD4(+) lymphocytes after HIV antiretroviral therapy reflects redistribution from lymphoid tissues. *J Clin Invest* 1999; **103:** 1391-1398.
33. Haase AT. Population biology of HIV-1 infection: viral and CD4+ T cell demographics and dynamics in lymphatic tissues. *Annu Rev Immunol* 1999; **17:** 625-656.
34. Burton GF, Masuda A, Heath SL, Smith BA, Tew JG, Szakal AK. Follicular dendritic cells (FDC) in retroviral infection: host/pathogen perspectives. *Immunol Rev* 1997, **156:** 185-197.
35. Fujiwara M, Tsunoda R, Shigeta S, Yokota T, Baba M. Human follicular dendritic cells remain uninfected and capture human immunodeficiency virus type 1 through CD54-CD11a interaction. *J Virol* 1999, **73:** 3603-3607.
36. Pantaleo G, Graziosi C, Demarest JF, *et al.* Role of lymphoid organs in the pathogenesis of human immunodeficiency virus (HIV) infection. *Immunol Rev* 1994, **140:** 105-130.
37. Halapi E, Gigliotti D, Hodara V, *et al.* Detection of CD8 T-cell expansions with restricted T-cell receptor V gene usage in infants vertically infected by HIV-1. *AIDS* 1996; **10:** 1621-1626.
38. Pantaleo G, Demarest JF, Soudeyns H, *et al.* Major expansion of CD8+ T cells with a predominant V beta usage during the primary immune response to HIV [see comments]. *Nature* 1994; **370:** 463-467.
39. Rowland-Jones S, Sutton J, Ariyoshi K, *et al.* HIV-specific cytotoxic T-cells in HIV-exposed but uninfected Gambian women [published erratum appears in *Nat Med* 1995: 598]. *Nat Med* 1995; **1:** 59-64.
40. Carbonari M, Cibati M, Pesce A, *et al.* Comparison of the V beta repertoire in peripheral blood and in lymph nodes of HIV-infected subjects reveals skewed usage predominantly in CD8+ T cells. *Clin Immunol Immunopathol* 1996, **81:** 200-209.
41. Sarzotti M, Patel DD, Li X, *et al.* T cell repertoire development in humans with SCID after nonablative allogeneic marrow transplantation. *J Immunol* 2003; **170:** 2711-2718.
42. Kou ZC, Puhr JS, Rojas M, McCormack WT, Goodenow MM, Sleasman JW. T-Cell receptor Vbeta repertoire CDR3 length diversity differs within CD45RA and CD45RO T-cell subsets in healthy and human immunodeficiency virus-infected children. *Clin Diagn Lab Immunol* 2000; **7:** 953-959.
43. Touloumi G, Pantazis N, Karafoulidou A, *et al.* Changes in T cell receptor excision DNA circle (TREC) levels in HIV type 1-infected subjects pre- and post-highly active antiretroviral therapy. *AIDS Res Hum Retroviruses* 2004; **20:** 47-54.
44. Cossarizza A. T-cell repertoire and HIV infection: facts and perspectives [Editorial]. *AIDS* 1997; **11:** 1075-1088.
45. Altman JD, Moss PA, Goulder PJ, *et al.* Phenotypic Analysis of antigen-specific T lymphocytes. *Science* 1996; **274: 9**4-96.
46. Kalams SA, Johnson RP, Trocha AK, *et al.* Longitudinal analysis of T cell receptor (TCR) gene usage by human immunodeficiency virus 1 envelope-specific cytotoxic T lymphocyte clones reveals a limited TCR repertoire. *J Exp Med* 1994; **179: 1**261-1271.
47. Kalams S, Johnson R, Dynan M, *et al.* T cell receptor usage and fine specificity of human immunodeficency virus1-specific cytotoxic T lymphocyte clones: analysis of quasispecies recognition reveals a dominant response directed against a minor in vivo variant. *J Exp Med* 1996; **183:** 1669-1679.
48. Gray CM, Lawrence J, Schapiro JM, *et al.* Frequency of class I HLA-restricted anti-HIV CD8+ T cells in individuals receiving highly active antiretroviral therapy (HAART). *J Immunol* 1999; **162:** 1780-1788.
49. Resino S, Bellon JM, Gurbindo MD, Munoz-Fernandez MA. CD38 expression in CD8+ T cells predicts virological failure in HIV type 1-infected children receiving antiretroviral therapy. *Clin Infect Dis* 2004; **38:** 412-417.
50. Sherman GG, Scott LE, Galpin JS, *et al.* CD38 expression on CD8(+) T cells as a prognostic marker in vertically HIV-infected pediatric patients. *Pediatr Res* 2002; **51:** 740-745.