Failure to maintain T-cell homeostasis during HIV-1 infection results in compromised immunity, allowing development of opportunistic infections and progression to AIDS. Loss of CD4+ T cells due to direct and indirect mechanisms 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, 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. There is evidence that immune reconstitution can occur in patients receiving HIV PIs either as monotherapy or in combination with nucleoside analogues. There is also evidence that immune reconstitution occurs in children after receiving ART regimens, 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\textsuperscript{20,21} and didanosine in children.\textsuperscript{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.\textsuperscript{1,18} Improvements in naïve and/or memory CD4+ and CD8+ T-cell changes have been shown in response to ritonavir monotherapy\textsuperscript{23} and highly active regimens consisting of either ritonavir or indinavir in combination with nucleoside analogues.\textsuperscript{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.\textsuperscript{22,24} 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\textsuperscript{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.\textsuperscript{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.\textsuperscript{28} 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\textsuperscript{21,22} or lymph node (LN) tissue\textsuperscript{26} 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\textsuperscript{10-12} 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.\textsuperscript{23} 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 infected by HIV. 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\textsuperscript{27} complexed with the follicular dendritic cell (FDC) network.\textsuperscript{28} 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.\textsuperscript{29} 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\textsuperscript{31} and adults.\textsuperscript{32} This results in a limited pattern of recognition of HIV and a probable loss of protective immunity.\textsuperscript{33} 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,\textsuperscript{37,40} or by molecular means of measuring the length of the expressed gene that encodes for the TCR.\textsuperscript{41,42} Additionally, several investigators have measured T-cell receptor excision circles (TRECs), which indirectly measure thymic output of T cells.\textsuperscript{6,43}
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, most notably utilising Vβ12, demonstrating that clonal dominance existed before therapy, and possibly indicating reduced efficacy of CTL responses. 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. 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, 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 72% had CD8+ cells recognising an epitope in Gag (SLYNTVATL) and to a lesser extent in Pol (ILKPVHG). 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 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.

REFERENCES


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 naive CD4+ and CD8+ T cells is high. The main point of measuring these responses is to understand how a relatively naive 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, 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.


