Review

Immune reconstitution in HIV-1 infected subjects treated with potent antiretroviral therapy

Gilbert R Kaufmann, John Zaunders, David A Cooper

The introduction of potent antiretroviral drug regimens contributed to a decline in HIV-1 associated morbidity and mortality. Clinical observations of spontaneous remission of previously untreatable opportunistic infections in subjects on highly active antiretroviral therapy (HAART) reflect the substantial degree of immune reconstitution which can be achieved by those therapies. A biphasic increase of CD4+ T lymphocytes has been reported including naive (CD45RA+) and memory (CD45RO+) cell subsets. Proliferative lymphocyte responses to recall antigens and mitogens are enhanced over time, while T lymphocyte activation is largely reduced and T cell receptor (TCR) repertoires are partly restored. Proliferative lymphocyte responses specific to HIV-1 antigens, in contrast, remain weak. A complete normalisation of HIV-1 associated immunological alterations has not been reported so far, but the observation period of subjects on potent antiretroviral therapies is still relatively short.

(*Sex Transm Inf* 1999;75:218–224)

Keywords: HIV; immune reconstitution; T lymphocytes; cytokines; chemokines; opportunistic infections; HAART

Introduction

HIV-1 infection is associated with a diverse range of immunological alterations, which results in a progressive immunodeficiency rendering the infected individual susceptible to opportunistic infections and malignancies. The combination of nucleoside analogues and a protease inhibitor or a non-nucleoside reverse transcriptase inhibitor, which is also known as highly active antiretroviral therapy (HAART), is able to suppress very efficiently viral replication and is now regarded as the standard of care.¹ Although the ultimate goal of antiretroviral therapy is the cure of HIV infection and the eradication of the virus, recent reports suggest that the achievement of those aims will be difficult with currently available therapeutic options.^{2–4} In particular, HIV-1 may persist in cellular viral reservoirs including latently infected CD4+ T lymphocytes, macrophages, and follicular dendritic cells. Anatomical reservoirs such as the central nervous system may be poorly accessible for certain drugs, which ultimately may limit the therapeutic antiretroviral efficacy in those compartments.

Despite those problems, HAART leads to a substantial quantitative and qualitative reversion of HIV-1 associated immunodeficiency, which has also been reflected by the dramatic decline of HIV-1 associated morbidity and mortality in the past 2–3 years.⁵

Effect of HAART on T lymphocyte subsets

One of the hallmarks of HIV infection is the progressive loss of CD4+ T lymphocytes. HAART leads to a significant increase of CD4+ T lymphocytes, even in subjects with highly advanced stages of the disease,^{6–8} which approaches the normal range in a proportion of patients within 2 years.^{9–10} Even if not all individuals reach normal numbers of CD4+ T lymphocytes after that time, a partial immune

restoration may already sufficiently protect those subjects against opportunistic infections.¹¹ More detailed information on the recovery of CD4+ T lymphocytes resulted from the analysis of phenotypic subsets of T lymphocytes.^{12–13} CD4+ T lymphocytes express either CD45RA+, which defines the subset of antigen naive cells or CD45RO+, which characterises the memory cell subset. Naive cells originate typically from the thymus and remain in a resting state until they encounter foreign antigen. When antigen and co-stimulatory signals are presented, naive cells become activated and switch to the memory phenotype. Cells bearing the memory phenotype have effector function and have the potential to respond faster to antigen. In early HIV-1 infection, memory (CD45RO+) CD4+ T cells are preferentially depleted while in advanced stages of the disease the proportion of naive cells (CD45RA+) declines.^{14–16}

Within months of initiation of HAART, a biphasic increase of CD4+ T lymphocytes has been observed.^{12–13–17–18} This initial change of CD4+ T cells is mainly the result of an increase of memory cells and is followed 2–3 months later by a smaller second phase increase of CD4+ T lymphocytes consisting of both naive and memory cells (fig 1).

It has been suggested that a rapid redistribution of CD4+ T lymphocytes trapped in lymphoid tissue, rather than the proliferation of new cells may be the main mechanism responsible for the initial rapid rise of CD4+ T cells.¹⁷ In fact, it has recently been demonstrated that CD4+ T cell turnover does not change in the first months after the initiation of HAART, which supports the concept of a redistribution of cells.^{19–20} Trapped T lymphocytes may be released from lymphoid tissue when cytokine and chemokine levels begin to decline in inflamed lymphoid tissue.²¹ The second phase increase of CD4+ T cells may indeed be the

Centre for Immunology, St Vincent's Hospital, Sydney, NSW 2010, Australia
G R Kaufmann
J Zaunders
D A Cooper

National Centre in HIV Epidemiology and Clinical Research, Sydney, NSW 2010, Australia
D A Cooper

Correspondence to: Dr Gilbert Kaufmann, Centre for Immunology, St Vincent's Hospital, Darlinghurst, NSW 2010, Sydney, Australia.

Accepted for publication 18 June 1999

Figure 1 Time course of naive and memory T lymphocytes in a representative subject on HAART. (A) CD4+ T lymphocytes. (B) CD8+ T lymphocytes. It has been suggested that the initial rapid increase of lymphocytes is the result of a redistribution of lymphocytes trapped in the lymphoid tissue, which are released into peripheral circulation upon initiation of an effective antiretroviral therapy. The subsequent slow increase of cells may be the result of the regeneration of new lymphocytes.

result of newly regenerated cells. During this time the proportion of CD4+ T lymphocytes expressing Ki67, a marker of cell proliferation, increases.¹⁹

Recent reports indicate that virological and immunological responses may not always be concordant.^{22, 23} In one study, 11% of subjects had a significant increase of CD4+ T lymphocytes despite persistently high HIV RNA levels, which were accompanied by a decline of AIDS defining events.²² In contrast, another 11% of individuals demonstrated no significant increase of CD4+ T lymphocytes despite undetectable plasma HIV RNA. AIDS defining events were frequent in this group reflecting the persistent immunodeficiency. This observation suggests that the degree of immune restoration relies not only on the suppression of viral replication and HIV RNA levels, but may also depend on the cytopathic properties and the fitness of the viral strain as well as the underlying condition of the immune system.^{23, 24}

In untreated HIV-1 infection, the number of CD8+ T lymphocytes usually remains elevated

over years at a fairly constant level and then dramatically declines towards the final stages of the disease. CD8+ T lymphocytes show a similar biphasic course on HAART as CD4+ T lymphocytes.¹⁷ Memory CD8+ T lymphocytes expand rapidly within weeks resulting in an overall increase of total CD8+ T cell counts, but decline again in subsequent months.^{12, 13, 17} The proportion of naive CD8+ T lymphocytes increases slowly keeping the level of total CD8+ T lymphocytes at an almost constant level.¹⁰

An extensive immune activation of T lymphocytes is one of the cardinal features of HIV infection and may lead to anergy and increased apoptosis of those cells. The proportion of activated T lymphocytes increases towards later stages of the disease and is correlated with levels of plasma HIV RNA.²⁵ HAART reduces rapidly the number of activated CD4+ and CD8+ T lymphocytes as measured by the expression of CD38 and HLA-DR antigens.^{12, 13, 26}

How new T lymphocytes are generated

The state of the immune system and in particular, the number of naive T cells before therapy may determine the maximum level of immune restoration which can be achieved.¹³ However, there is some uncertainty as to how new T lymphocytes are generated in HIV-1 infected subjects on HAART.

In mice, thymic dependent and thymic independent pathways of T cell regeneration have been reported. The thymus is involved in primary T cell development including the selection of appropriate cell clones, which are able to recognise foreign antigen, but are tolerant to self antigen. Extrathymic differentiation of bone marrow progenitors has been detected in gut, liver, and bone marrow.²⁷⁻²⁹ However, the contribution of extrathymic differentiation of T cells to the peripheral T cell pool appears to be minimal.

Alternatively, a dramatic expansion of pre-existing mature T cells has been observed in athymic mice after T lymphocyte depletion.³⁰⁻³² This renewal of T cells has been termed peripheral expansion, which appears to be mainly antigen driven.³³

In humans, the thymus progressively involutes from birth to adulthood. HIV infected children on HAART, who are younger than 3 years old with a presumably active thymus, consequently showed a more rapid restoration of naive CD4+ T lymphocytes than older children or adults.³⁴ Although it has recently been shown that the thymus may keep some residual activity in adults,³⁵ it is likely that peripheral expansion of mature T lymphocytes is the main pathway responsible for the restoration of T cells in adults.³⁶ This mechanism may have some limitations. The thymus generates a diversity of T cells with different T cell receptors (TCR), which are capable of recognising a huge spectrum of foreign antigen. HIV infection leads to a severe disruption of this repertoire,³⁷ which may result in a poor recognition of certain antigens or even the lack of the antigen specific immune response. Peripheral

Figure 2 Possible pathways involved in the restoration of T lymphocytes. The thymus has an important role in very young children, but loses its significance in adults, which leaves peripheral expansion of mature T lymphocytes as the major mechanism for the restoration of lymphocytes. The reversion of memory cells to naive cells has been demonstrated in mice, but no conclusive data are available for humans, which would provide enough evidence that this mechanism is significantly involved in the immune restoration process in HIV-1 infected patients on HAART.

expansion of mature T cells multiplies only pre-existing T cell clones. This mechanism is therefore unlikely to completely correct perturbations of the TCR repertoire. Most studies with a relatively short observation period of 3–12 months reported an incomplete restoration of TCR repertoires of CD4+ and CD8+ T lymphocytes in HIV-1 infected adults and provide some evidence for this hypothesis.^{37–39} The long term follow up of those subjects will show whether the T cell repertoire can be completely restored. Proposed pathways of immune reconstitution are shown in figure 2.

Effect of HAART on T lymphocyte function

Functional defects commonly precede the decline of CD4+ T cell counts including poor proliferative lymphocyte responses to mitogens and recall antigens as well as reduced cytokine production in response to antigens.^{40–43} It is therefore important not just to rely on the restoration of lymphocyte numbers, but to assess lymphocyte function, which may provide additional information on the degree of immune competence.

Several studies have demonstrated that HAART increases proliferative lymphocyte responses to recall antigens such as tetanus toxoid, streptokinase/streptodornase or mitogens such as phytohaemagglutinin and pokeweed antigen.^{12 38 44–46} Normal levels were not attained in studies with an observation period of 1–1.5 years, which indicates that proliferative T lymphocyte responses improve slowly.^{46–49}

The restoration of an efficient immune response against opportunistic pathogens is of great clinical importance, in particular with regard to the discontinuation of primary and secondary antimicrobial prophylaxis. HAART significantly enhances proliferative responses

to CMV, candida, *Mycobacterium avium*, and tuberculin.^{13 38 50 51} In a recent study evaluating the CMV specific immune response, evidence has been provided that HAART is also able to increase the number of CD4+ T lymphocytes with pathogen specific effector function, as measured by the production of intracellular effector cytokines using a new flow cytometric technique.⁵²

In two investigations factors have been studied which may predict the recovery of functional properties of CD4+ T lymphocytes in individuals treated with HAART. Those subjects with a significant improvement of proliferative lymphocyte responses had a more sustained viral load suppression, a greater CD4+ T cell increase, and an early increase of memory and naive CD4+ T lymphocytes.^{51 53}

A crucial factor in the pathogenesis of HIV-1 infection is the lack of an efficient immune response to HIV-1 that would ultimately eradicate the virus. The mechanism by which the virus is able to escape the HIV-1 specific immune response remains speculative. The restoration of the HIV-1 specific immune response is therefore of particular interest. Several short term studies over 3 and 6 months reported a moderate enhancement of proliferative lymphocyte responses to HIV-1 antigens,^{46 54} while other studies reported a lack or only a transient increase of the proliferative lymphocyte response despite a significant response to non-HIV specific antigens such as CMV.^{13 50 55}

The reason for this poor HIV-1 specific reactivity is unclear. Proposed explanations include the selective depletion of HIV-1 specific T cell clones during HIV-1 infection, HIV-1 specific anergy, or an impaired antigen presentation. The last hypothesis raises the question of whether the lack of an efficient immune response to HIV-1 is due to a defect in

the network of follicular dendritic cells which serve as antigen repository and interact with B and T lymphocytes. This network of cells is progressively destroyed during the natural course of HIV-1 infection.⁵⁶ A recent investigation using immunohistochemical examinations of tonsils and lymph nodes provides evidence that HIV-1 specific pathological changes of the follicular dendritic network can be slowly reversed by HAART.⁵⁷

In contrast with improvements of proliferative responses, the number of HIV-1 specific cytotoxic CD8+ T lymphocytes (CTL) drops exponentially 1–2 weeks after initiation of HAART and continues to decline after plasma HIV RNA has reached undetectable levels.^{58 59} Similarly, a decline of HIV-1 specific CD4+ T lymphocytes has been observed in individuals on HAART.⁶⁰ Those findings suggest that additional vaccination strategies may be required to maintain the HIV-1 specific cytotoxic immune response in subjects on HAART.

A further area of interest is the humoral immune response to HIV-1. In one study, untreated HIV-1 infected patients with CD4+ T cell counts $<200 \times 10^6/l$, who were vaccinated against influenza, failed to induce a significant antibody response while subjects on HAART developed substantial antibody titres.⁶¹

HIV-1 infection causes severe perturbations of the cytokine network including an increase of proinflammatory cytokines such as tumour necrosis factor α (TNF- α) or interleukin 6 (IL-6) and a reduction of T helper 1 (Th1) type cytokines such as IL-2. Observations of an improved IL-2 and IL-12 secretion as well as a decline of TNF- α in individuals on HAART indicate that cytokine perturbations may be corrected by HAART.^{44 49 62 63} Recent reports also suggest that HAART positively influences the secretion of chemokines resulting in increased RANTES levels which may help to suppress viral replication.^{46 64} The effect of HAART on the immune system is summarised in table 1.

Immune restoration in early HIV-1 infection

The protracted course and the uncertainty of a full immune reconstitution have brought into question whether an early antiretroviral therapy during primary HIV-1 infection may prevent the subsequent severe and possibly irreversible HIV associated immunodeficiency.

In one series, 16 subjects with primary HIV-1 infection were treated with zidovudine, lamivudine, and indinavir over 1 year. The early initiation of therapy resulted in a

sustained suppression of plasma HIV RNA and a continuing rise in circulating CD4+ T lymphocytes.⁶⁵ Moreover, the number of CD4+ T lymphocytes including the naive and memory subset reached normal values at 52 weeks. No significant perturbations of the TCR repertoire were detected. Naive CD4+ T lymphocytes recovered more rapidly in subjects who received therapy before the appearance of HIV-1 antibodies than individuals with a complete antibody response before therapy. Those findings suggest that the time between the onset of HIV-1 infection until the commencement of therapy may play a crucial role in determining the rate of the recovery of CD4+ T lymphocytes. In contrast, the number of CD8+ T lymphocytes did not change significantly over time. Memory CD8+ T lymphocytes dropped rapidly and were only slightly elevated at 1 year, while the naive subset of CD8+ T lymphocytes was maintained at a high level. CD8+ T lymphocyte activation as measured by the expression of surface antigens CD38 and HLA-DR was correlated with the plasma HIV RNA levels and decreased significantly during the observation period.

Limited data are available on the recovery of functional properties of T lymphocytes during primary HIV-1 infection. In 11 acutely infected subjects receiving HAART a restoration of lymphoproliferative responses to candida and tetanus toxoid comparable with levels observed in immunocompetent subjects has been observed after 3 months of therapy.⁶⁶ Moreover, a sixfold increase in the lymphoproliferative response to HIV-1 p24 antigen has been noted. Similarly, a vigorous proliferative response of CD4+ T lymphocytes to HIV-1 p24 antigen has been reported in three acutely HIV-1 infected subjects treated with HAART before HIV-1 seroconversion.⁶⁷ These results are in contrast with the poor proliferative HIV-1 specific responses in individuals treated in advanced stages of HIV-1 infection and suggest that the immune restoration may be more efficient if antiretroviral therapy is initiated early.

The preliminary data in a small number of subjects with primary HIV-1 infection certainly need further confirmation. A drawback of a therapeutic intervention during primary HIV-1 infection is the potential risk of an early development of drug resistance. Furthermore, the use of HAART is frequently associated with adverse events. A therapy initiated during primary HIV-1 infection therefore requires highly motivated patients to ensure a satisfactory drug adherence.

Immune based therapies

Innumerable possibilities of immune based therapies can be considered. Only interleukin 2 (IL-2) has been intensively studied so far and used as an adjunctive therapy to standard antiretroviral drug regimens.^{68–72} IL-2 was safe and induced a rapid recovery of CD4+ T cells. CD8+ T lymphocyte counts were not affected and plasma HIV RNA showed no significant changes. The rise of CD4+ T lymphocytes consisted of an elevation of naive and memory T cell subsets^{68 72} and was dependent on

Table 1 Impact of HAART on the immune system

Increase of CD4+ T lymphocytes including memory and naive cells
Partial restoration of T cell receptor repertoires
Reduced lymphocyte activation
Improved proliferative lymphocyte responses to mitogens
Improved proliferative lymphocyte responses to recall antigens
Modest improvement of proliferative lymphocyte response to HIV-1 specific antigens
Increased number of pathogen specific CD4+ T lymphocytes
Improved cytokine/chemokine secretion
Improved humoral immune response
Improvement of the network of follicular dendritic cells

baseline levels of CD4+ T cells.⁷¹ No restoration of disrupted TCR repertoires has been observed.⁶⁸ Delayed type hypersensitivity (DTH) responses improved significantly in one study,⁶⁹ but showed no significant changes in two other studies.^{70 71} However, in one study an enhancement of proliferative lymphocyte responses to at least one tested mitogen or recall antigens has been observed in all 13 studied subjects on IL-2 therapy.⁷¹

The persistence of elevated CD4+ T cell numbers after discontinuation of IL-2 suggests that CD4+ T cell increases are most likely the result of a regeneration of new T cells and not simply of a redistribution of lymphocytes from lymphoid tissue.^{69 71} IL-2 may therefore be a valuable addition to a standard antiretroviral therapy in order to increase the number and preserve the repertoire of CD4+ T lymphocytes. Moreover, T cell function may be enhanced in certain individuals.

Clinical impact of immune restoration in subjects on HAART

Improvements of immunological laboratory variables have been corroborated by the declining incidence of opportunistic infections such as cytomegalovirus (CMV) retinitis, *Pneumocystis carinii* pneumonia (PCP) and disseminated mycobacterial infections or by the reduction of the overall mortality from AIDS defining events.⁷³⁻⁷⁶ Previously untreatable opportunistic infections such as cryptosporidiosis, microsporidiosis, azole resistant oropharyngeal candidiasis, or progressive multifocal leucoencephalopathy may resolve spontaneously after the initiation of HAART.^{74 77-87}

A further clinical observation includes a high incidence of opportunistic infections during the first 2 months after initiation of HAART despite an increase of absolute CD4+ T cell counts.⁸⁸ Similarly, CMV retinitis was observed in individuals despite CD4+ T cells counts of more than 100 cells $\times 10^6/l$.^{89 90} Localised *Mycobacterium avium* complex infections and an increasing number of subjects with herpes zoster infections have been reported after the initiation of HAART.^{91 92} Those observations indicate that the enhancement of the immune response by HAART may unmask previously unrecognised subclinical opportunistic infections.

Recent reports of long term remission of CMV or *Mycobacterium avium* complex infections after discontinuation of the secondary prophylaxis provide some evidence that a degree of immune competence can be achieved which permits the discontinuation of the antimicrobial prophylactic therapy.^{79 93-95} In a recently published study, primary prophylaxis against PCP pneumonia has been discontinued after CD4+ T lymphocyte counts increased to levels above 200 cells $\times 10^6/l$ for at least 3 months.¹¹ During a median 11 months follow up period no new cases of PCP or toxoplasmic encephalitis have been noted. Similarly, primary and secondary PCP prophylaxis have been successfully discontinued in 62 and 16 subjects, respectively, after CD4+ T cell counts increased above 200 cells $\times 10^6/l$.⁹⁶ Those

Table 2 Clinical observation in subjects on HAART

Reduced HIV-1 associated mortality
Reduced incidence of AIDS defining events
Reduced incidence of opportunistic infections
Spontaneous resolution of opportunistic infections
Unmasking of subclinical opportunistic infections

observations suggest that it is safe to discontinue prophylactic therapy after the number of CD4+ T lymphocytes increases above a critical threshold. The clinical impact of HAART is summarised in table 2.

Conclusion

In summary, recent studies suggest that not only do the number of CD4+ T cells improve, but also T lymphocyte function is at least partially restored by HAART within 1–2 years. The addition of immune based therapies to the standard antiretroviral drug regimen may shorten the time of the recovery of the immune system in subjects with advanced stages of the disease. Laboratory signs of immune reconstitution translated into clinical observations of a declining incidence of opportunistic infections and HIV-1 associated mortality. A limited number of small studies provide some evidence that primary and secondary prophylactic therapy can be safely discontinued when CD4+ T lymphocytes increase above a critical threshold. Preliminary data of immune reconstitution in subjects treated with HAART during acute HIV-1 infection are promising and indicate that an early initiation of therapy may facilitate the correction of HIV-1 associated immunodeficiency. A full restoration of the immune system has not been observed so far. However, it has to be considered that the observation period of 1–2 years is still a relatively short time. The long term follow up of individuals on HAART will show what level of immune reconstitution can be achieved.

GRK has been supported by the Swiss National Science Foundation. The National Centre in HIV Epidemiology and Clinical Research is supported by the Australian National Council on AIDS through the Commonwealth AIDS Research Grants Committee.

Contributors: All authors contributed to reviewing the literature and to writing the manuscript.

- 1 Carpenter CC, Fischl MA, Hammer SM, *et al.* Antiretroviral therapy for HIV infection in 1998: updated recommendations of the International AIDS Society-USA Panel. *JAMA* 1998;280:78–86.
- 2 Wong JK, Hezareh M, Gunthard HF, *et al.* Recovery of replication-competent HIV despite prolonged suppression of plasma viremia. *Science* 1997;278:1291–5.
- 3 Chun TW, Engel D, Mizell SB, *et al.* Induction of HIV-1 replication in latently infected CD4+ T cells using a combination of cytokines. *J Exp Med* 1998;188:83–91.
- 4 Finzi D, Hermankova M, Pierson T, *et al.* Identification of a reservoir for HIV-1 in patients on highly active antiretroviral therapy. *Science* 1997;278:1295–300.
- 5 Palella FJ Jr, Delaney KM, Moorman AC, *et al.* Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. HIV Outpatient Study Investigators. *N Engl J Med* 1998;338:853–60.
- 6 Hammer SM, Squires KE, Hughes MD, *et al.* A controlled trial of two nucleoside analogues plus didanosine in persons with human immunodeficiency virus infection and CD4 cell counts of 200 per cubic millimeter or less. AIDS Clinical Trials Group 320 Study Team. *N Engl J Med* 1997;337:725–33.
- 7 Collier AC, Coombs RW, Schoenfeld DA, *et al.* Treatment of human immunodeficiency virus infection with zalcitabine, zidovudine, and zalcitabine. AIDS Clinical Trials Group. *N Engl J Med* 1996;334:1011–7.
- 8 Markowitz M, Saag M, Powderly WG, *et al.* A preliminary study of ritonavir, an inhibitor of HIV-1 protease, to treat HIV-1 infection. *N Engl J Med* 1995;333:1534–9.

- 9 Gulick RM, Mellors JW, Havlir D, *et al.* Simultaneous vs sequential initiation of therapy with zidovudine, didanosine, and lamivudine for HIV-1 infection: 100-week follow-up. *JAMA* 1998;280:35–41.
- 10 Arno A, Ruiz L, Juan M, *et al.* Impact on the immune system of undetectable plasma HIV-1 RNA for more than 2 years. *AIDS* 1998;12:697–704.
- 11 Furrer H, Egger M, Opravil M, *et al.* Discontinuation of primary prophylaxis against *Pneumocystis carinii* pneumonia in HIV-1-infected adults treated with combination antiretroviral therapy. Swiss HIV Cohort Study. *N Engl J Med* 1999;340:1301–6.
- 12 Kelleher AD, Carr A, Zaunders J, *et al.* Alterations in the immune response of human immunodeficiency virus (HIV)-infected subjects treated with an HIV-specific protease inhibitor, ritonavir. *J Infect Dis* 1996;173:321–9.
- 13 Autran B, Carcelain G, Li T, *et al.* Positive effects of combined antiretroviral therapy on CD4+ T cell homeostasis and function in advanced HIV disease. *Science* 1997;277:112–16.
- 14 Van Noesel CJ, Gruters RA, Terpstra FG, *et al.* Functional and phenotypic evidence for a selective loss of memory T cells in asymptomatic human immunodeficiency virus-infected men. *J Clin Invest* 1990;86:293–9.
- 15 Roederer M, Dubs JG, Anderson MT, *et al.* CD8 naive T cell counts decrease progressively in HIV-infected adults. *J Clin Invest* 1995;95:2061–6.
- 16 Ullum H, Ac L, Victor J, *et al.* Increased losses of CD4+CD45RA+ cells in late stages of HIV infection is related to increased risk of death: evidence from a cohort of 347 HIV-infected individuals. *AIDS* 1997;11:1479–85.
- 17 Pakker NG, Notermans DW, de Boer RJ, *et al.* Biphasic kinetics of peripheral blood T cells after triple combination therapy in HIV-1 infection: a composite of redistribution and proliferation. *Nat Med* 1994;4:208–14.
- 18 Gray CM, Schapiro JM, Winters MA, *et al.* Changes in CD4+ and CD8+ T cell subsets in response to highly active antiretroviral therapy in HIV type 1-infected patients with prior protease inhibitor experience. *AIDS Res Human Retroviruses* 1998;14:561–9.
- 19 Fleury S, de Boer RJ, Rizzardini GP, *et al.* Limited CD4+ T-cell renewal in early HIV-1 infection: effect of highly active antiretroviral therapy. *Nat Med* 1998;4:794–801.
- 20 Hellerstein M, Hanley MB, Cesar D, *et al.* Directly measured kinetics of circulating T lymphocytes in normal and HIV-1-infected humans. *Nat Med* 1999;5:83–9.
- 21 Andersson J, Fehniger TE, Patterson BK, *et al.* Early reduction of immune activation in lymphoid tissue following highly active HIV therapy. *AIDS* 1998;12:F123–9.
- 22 Piketty C, Castiel P, Belec L, *et al.* Discrepant responses to triple combination antiretroviral therapy in advanced HIV disease. *AIDS* 1998;12:745–50.
- 23 Kaufmann D, Pantaleo G, Sudre P, *et al.* CD4-cell count in HIV-1-infected individuals remaining viraemic with highly active antiretroviral therapy (HAART). Swiss HIV Cohort Study. *Lancet* 1998;351:723–4.
- 24 Faye A, Race E, Obry V, *et al.* Viral fitness in patients with discordant CD4 and plasma HIV RNA evolution following protease inhibitor failure. Abstract 331, 6th Conference on Retroviruses and Opportunistic Infections, Chicago, USA, 1999.
- 25 Bouscarat F, Levacherclergeot M, Dazza MC, *et al.* Correlation of CD8 lymphocyte activation with cellular viremia and plasma HIV RNA levels in asymptomatic patients infected by human immunodeficiency virus type 1. *AIDS Res Human Retroviruses* 1996;12:17–24.
- 26 Bouscarat F, Levacher M, Landman R, *et al.* Changes in blood CD8+ lymphocyte activation status and plasma HIV RNA levels during antiretroviral therapy. *AIDS* 1998;12:1267–73.
- 27 Mosley RL, Styre D, Klein JR. Differentiation and functional maturation of bone marrow-derived intestinal epithelial T cells expressing membrane T cell receptor in athymic radiation chimeras. *J Immunol* 1990;145:1369–75.
- 28 Sato K, Ohtsuka K, Hasegawa K, *et al.* Evidence for extrathymic generation of intermediate T cell receptor cells in the liver revealed in thymectomized, irradiated mice subjected to bone marrow transplantation. *J Exp Med* 1995;182:759–67.
- 29 Dejbakhsh-Jones S, Jerabek L, Weissman IL, *et al.* Extrathymic maturation of alpha beta T cells from hemopoietic stem cells. *J Immunol* 1995;155:3338–44.
- 30 Bell EB, Sparshott SM, Drayson MT, *et al.* The stable and permanent expansion of functional T lymphocytes in athymic nude rats after a single injection of mature T cells. *J Immunol* 1987;139:1379–84.
- 31 Rocha BB. Population kinetics of precursors of IL-2-producing peripheral T lymphocytes: evidence for short life expectancy, continuous renewal, and post-thymic expansion. *J Immunol* 1987;139:365–72.
- 32 Rocha B, Dautigny N, Pereira P. Peripheral T lymphocytes: expansion potential and homeostatic regulation of pool sizes and CD4/CD8 ratios in vivo. *Eur J Immunol* 1989;19:905–11.
- 33 Mackall CL, Bare CV, Granger LA, *et al.* Thymic-independent T cell regeneration occurs via antigen-driven expansion of peripheral T cells resulting in a repertoire that is limited in diversity and prone to skewing. *J Immunol* 1996;156:4609–16.
- 34 Cohen Stuart JW, Sliker WA, Rijkers GT, *et al.* Early recovery of CD4+ T lymphocytes in children on highly active antiretroviral therapy. Dutch study group for children with HIV infections. *AIDS* 1998;12:2155–9.
- 35 Douek DC, McFarland RD, Keiser PH, *et al.* Changes in thymic function with age and during the treatment of HIV infection. *Nature* 1998;396:690–5.
- 36 Walker RE, Carter CS, Muul L, *et al.* Peripheral expansion of pre-existing mature T cells is an important means of CD4+ T-cell regeneration in HIV-infected adults. *Nat Med* 1998;4:852–6.
- 37 Connors M, Kovacs JA, Krevat S, *et al.* HIV infection induces changes in CD4+ T-cell phenotype and depletions within the CD4+ T-cell repertoire that are not immediately restored by antiviral or immune-based therapies. *Nat Med* 1997;3:533–40.
- 38 Lederman MM, Connick E, Landay A, *et al.* Immunologic responses associated with 12 weeks of combination antiretroviral therapy consisting of zidovudine, lamivudine, and zalcitabine: results of AIDS Clinical Trials Group Protocol 315. *J Infect Dis* 1998;178:70–9.
- 39 Martinon F, Michelet C, Peguillet I, *et al.* Persistent alterations in T-cell repertoire, cytokine and chemokine gene expression after 1 year of highly active antiretroviral therapy. *AIDS* 1999;13:185–94.
- 40 Lane HC, Depper JM, Greene WC, *et al.* Qualitative analysis of immune function in patients with the acquired immunodeficiency syndrome. Evidence for a selective defect in soluble antigen recognition. *N Engl J Med* 1985;313:79–84.
- 41 Clerici M, Stocks NI, Zajac RA, *et al.* Detection of three distinct patterns of T helper cell dysfunction in asymptomatic, human immunodeficiency virus-seropositive patients. Independence of CD4+ cell numbers and clinical staging. *J Clin Invest* 1989;84:1892–9.
- 42 Miedema F, Petit AJ, Terpstra FG, *et al.* Immunological abnormalities in human immunodeficiency virus (HIV)-infected asymptomatic homosexual men. HIV affects the immune system before CD4+ T helper cell depletion occurs. *J Clin Invest* 1988;82:1908–14.
- 43 Shearer GM, Clerici M. T helper cell immune dysfunction in asymptomatic, HIV-1-seropositive individuals: the role of TH1-TH2 cross-regulation. *Chem Immunol* 1992;54:21–43.
- 44 Angel JB, Kumar A, Parato K, *et al.* Improvement in cell-mediated immune function during potent anti-human immunodeficiency virus therapy with zidovudine plus zalcitabine. *J Infect Dis* 1998;177:898–904.
- 45 Chougnet C, Fowke KR, Mueller BU, *et al.* Protease inhibitor and triple-drug therapy: cellular immune parameters are not restored in pediatric AIDS patients after 6 months of treatment. *AIDS* 1998;12:2397–406.
- 46 Rinaldo C, Liebmann J, Huang X, *et al.* Prolonged suppression of human immunodeficiency virus type 1 (HIV-1) viremia in persons with advanced disease results in enhancement of CD4 T cell reactivity to microbial antigens but not to HIV-1 antigens. *J Infect Dis* 1999;179:329–36.
- 47 Autran B, Carcelain G, Tubiana R, *et al.* Effects of antiretroviral therapy on immune reconstitution. Abstract S44, 6th Conference on Retroviruses and Opportunistic Infections, Chicago, USA, 1999.
- 48 Carcelain G, Tubiana R, Li TS, *et al.* Long-term recovery in CD4+ T cell function with highly efficient antiretroviral therapy. Abstract 324, 6th Conference on Retroviruses and Opportunistic Infections, Chicago, USA, 1999.
- 49 Plana M, Garcia F, Gallart T, *et al.* Immunologic reconstitution after 12 months of antiretroviral therapy in very early stages of chronic HIV-1 infection. Abstract 323, 6th Conference on Retroviruses and Opportunistic Infections, Chicago, USA, 1999.
- 50 Plana M, Garcia F, Gallart T, *et al.* Lack of T-cell proliferative response to HIV-1 antigens after 1 year of highly active antiretroviral treatment in early HIV-1 disease. Immunology Study Group of Spanish EARTH-1 Study. *Lancet* 1998;352:1194–5.
- 51 Li TS, Tubiana R, Katlama C, *et al.* Long-lasting recovery in CD4 T-cell function and viral-load reduction after highly active antiretroviral therapy in advanced HIV-1 disease. *Lancet* 1998;351:1682–6.
- 52 Komanduri KV, Viswanathan MN, Wieder ED, *et al.* Restoration of cytomegalovirus-specific CD4+ T-lymphocyte responses after ganciclovir and highly active antiretroviral therapy in individuals infected with HIV-1. *Nat Med* 1998;4:953–6.
- 53 Valdez H, Smith K, Lederman M, *et al.* Response to immunization with recall and neoantigen after 48 weeks of HAART. Abstract 329, 6th Conference on Retroviruses and Opportunistic Infections, Chicago, USA, 1999.
- 54 Kelleher AD, Roggensack M, Emery S, *et al.* Effects of IL-2 therapy in asymptomatic HIV-infected individuals on proliferative responses to mitogens, recall antigens and HIV-related antigens. *Clin Exp Immunol* 1998;113:85–91.
- 55 Goujard C, Taoufik Y, Wallon C, *et al.* HIV-1 specific CD4+ T cell response is not restored after 28 months of HAART with viral load < 50 copies/ml. Abstract 343, 6th Conference on Retroviruses and Opportunistic Infections, Chicago, USA, 1999.
- 56 Pantaleo G, Graziosi C, Demarest JF, *et al.* Role of lymphoid organs in the pathogenesis of human immunodeficiency virus (HIV) infection. *Immunol Rev* 1994;140:105–30.
- 57 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;96:5169–72.
- 58 Ogg GS, Jin X, Bonhoeffer S, *et al.* Quantitation of HIV-1-specific cytotoxic T lymphocytes and plasma load of viral RNA. *Science* 1998;279:2103–6.

- 59 Ogg GS, Jin X, Bonhoeffer S, *et al.* Decay kinetics of human immunodeficiency virus-specific effector cytotoxic T lymphocytes after combination antiretroviral therapy. *J Virol* 1999;73:797–800.
- 60 Pitcher CJ, Quittner C, Peterson DM, *et al.* HIV-1-specific CD4+ T cells are detectable in most individuals with active HIV-1 infection, but decline with prolonged viral suppression. *Nat Med* 1999;5:518–25.
- 61 Kroon FP, Rimmelzwaan GF, Roos MT, *et al.* Restored humoral immune response to influenza vaccination in HIV-infected adults treated with highly active antiretroviral therapy. *AIDS* 1998;12:F217–23.
- 62 Hardy GAD, Imami N, Gazzard B, *et al.* Kinetics of lymphocyte proliferation, IL-2 and IL-4 production in HIV-1 infected individuals initiating HAART. Abstract 322, 6th Conference on Retroviruses and Opportunistic Infections, Chicago, USA, 1999.
- 63 Imami N, Hardy GAD, Antonopoulos C, *et al.* Assessment of type-1 and type-2 cytokines in HIV-1 infected individuals: impact of highly active antiretroviral therapy. Abstract 333, 6th Conference on Retroviruses and Opportunistic Infections, Chicago, USA, 1999.
- 64 Aukrust P, Muller F, Froland S. Circulating levels of RANTES in human immunodeficiency virus type 1 infection: effect of potent antiretroviral therapy. *J Infect Dis* 1998;177:1091–6.
- 65 Zauders J, Cunningham P, Kelleher A, *et al.* Potent antiretroviral therapy of primary HIV-1 infection: partial normalization of T lymphocyte subsets and limited reduction of proviral DNA despite clearance of plasma viremia. *J Infect Dis* (in press).
- 66 Malhotra U, Berrey MM, Huang Y, *et al.* Effect of combination antiretroviral therapy on HIV-1 specific cellular immunity in acute HIV-1 infection. Abstract 23, 6th Conference on Retroviruses and Opportunistic Infections, Chicago, USA, 1999.
- 67 Rosenberg ES, Billingsley JM, Caliendo AM, *et al.* Vigorous HIV-1-specific CD4+ T cell responses associated with control of viremia. *Science* 1997;278:1447–50.
- 68 Connors M, Kovacs J, Krevat S. HIV infection induces changes in CD4+ T-cell phenotype and depletions within the CD4+ T-cell repertoire that are not immediately restored by antiviral or immune-based therapies. *Nat Med* 1997;3:533–40.
- 69 Carr A, Emery S, Lloyd A, *et al.* Outpatient continuous intravenous interleukin-2 or subcutaneous, polyethylene glycol-modified interleukin-2 in human immunodeficiency virus-infected patients: a randomized, controlled, multicenter study. Australian IL-2 Study Group. *J Infect Dis* 1998;178:992–9.
- 70 Hengge UR, Goos M, Esser S, *et al.* Randomized, controlled phase II trial of subcutaneous interleukin-2 in combination with highly active antiretroviral therapy (HAART) in HIV patients. *AIDS* 1998;12:F225–34.
- 71 Bartlett JA, Berend C, Petroni GR, *et al.* Coadministration of zidovudine and interleukin-2 increases absolute CD4 cells in subjects with Walter Reed stage 2 human immunodeficiency virus infection: results of ACTG protocol 042. *J Infect Dis* 1998;178:1170–3.
- 72 De Paoli P, Zanussi S, Simonelli C, *et al.* Effects of subcutaneous interleukin-2 therapy on CD4 subsets and in vitro cytokine production in HIV+ subjects. *J Clin Invest* 1997;100:2737–43.
- 73 Fleming P, Ward J, Karon J, *et al.* Declines in AIDS incidence and deaths in the USA: a signal change in the epidemic. *AIDS* 1998;12:S55–61.
- 74 Walsh JC, Jones CD, Barnes EA, *et al.* Increasing survival in AIDS patients with cytomegalovirus retinitis treated with combination antiretroviral therapy including HIV protease inhibitors. *AIDS* 1998;12:613–8.
- 75 Jacobson MA, French M. Altered natural history of AIDS-related opportunistic infections in the era of potent combination antiretroviral therapy. *AIDS* 1998;12(Suppl A): S157–63.
- 76 Mocroft A, Vella S, Benfield T, *et al.* Changing patterns of mortality across Europe in patients infected with HIV-1. *Lancet* 1998;352:1725–30.
- 77 Goguel J, Katlama C, Sarfati C, *et al.* Remission of AIDS-associated intestinal microsporidiosis with highly active antiretroviral therapy. *AIDS* 1997;11:1658–9.
- 78 Foudraine NA, Weverling GJ, van Gool T, *et al.* Improvement of chronic diarrhoea in patients with advanced HIV-1 infection during potent antiretroviral therapy. *AIDS* 1998;12:35–41.
- 79 Aberg JA, Yajko DM, Jacobson MA. Eradication of AIDS-related disseminated mycobacterium avium complex infection after 12 months of antimycobacterial therapy combined with highly active antiretroviral therapy. *J Infect Dis* 1998;178:1446–9.
- 80 Baqi M, Kucharczyk W, Walmsley SL. Regression of progressive multifocal encephalopathy with highly active antiretroviral therapy. *AIDS* 1997;11:1526–7.
- 81 Carr A, Marriott D, Field A, *et al.* Treatment of HIV-1-associated microsporidiosis and cryptosporidiosis with combination antiretroviral therapy. *Lancet* 1998;351: 256–61.
- 82 Hood S, Bonington A, Evans J, *et al.* Reduction in oropharyngeal candidiasis following introduction of protease inhibitors. *AIDS* 1998;12:447–8.
- 83 Elliot B, Aromin I, Gold R, *et al.* 2.5 Year remission of AIDS-associated progressive multifocal leukoencephalopathy with combined antiretroviral therapy. *Lancet* 1997;349: 850.
- 84 Revankar SG, Sanche SE, Dib OP, *et al.* Effect of highly active antiretroviral therapy on recurrent oropharyngeal candidiasis in HIV-infected patients. *AIDS* 1998;12:2511–3.
- 85 Teofilo E, Gouveia J, Brotas V, *et al.* Progressive multifocal leukoencephalopathy regression with highly active antiretroviral therapy. *AIDS* 1998;12:449.
- 86 Power C, Nath A, Aoki FY, *et al.* Remission of progressive multifocal leukoencephalopathy following splenectomy and antiretroviral therapy in a patient with HIV infection. *N Engl J Med* 1997;336:661–2.
- 87 Miralles P, Berenguer J, Garcia de Viedma D, *et al.* Treatment of AIDS-associated progressive multifocal leukoencephalopathy with highly active antiretroviral therapy. *AIDS* 1998;12:2467–72.
- 88 Michelet C, Arvieux C, Francois C, *et al.* Opportunistic infections occurring during highly active antiretroviral treatment. *AIDS* 1998;12:1815–22.
- 89 Jacobson MA, Zegans M, Pavan PR, *et al.* Cytomegalovirus retinitis after initiation of highly active antiretroviral therapy. *Lancet* 1997;349:1443–5.
- 90 Gilquin J, Piketty C, Thomas V, *et al.* Acute cytomegalovirus infection in AIDS patients with CD4 counts above 100×10^6 cells/l following combination antiretroviral therapy including protease inhibitors. *AIDS* 1997;11:1659–60.
- 91 Race E, Adelson-Mitty J, Kriegel G. Focal mycobacterial lymphadenitis following initiation of protease-inhibitor therapy in patients with advanced HIV-1 disease. *Lancet* 1998;351:252–5.
- 92 Martinez E, Gatell J, Moran Y, *et al.* High incidence of herpes zoster in patients with AIDS soon after therapy with protease inhibitors. *Clin Infect Dis* 1998;27:1510–3.
- 93 Macdonald JC, Torriani FJ, Morse LS, *et al.* Lack of reactivation of cytomegalovirus (CMV) retinitis after stopping CMV maintenance therapy in AIDS patients with sustained elevations in CD4 T cells in response to highly active antiretroviral therapy. *J Infect Dis* 1998;177:1182–7.
- 94 Whitcup SM, Fortin E, Nussenblatt RB, *et al.* Therapeutic effect of combination antiretroviral therapy on cytomegalovirus retinitis. *JAMA* 1997;277:1519–20.
- 95 Tural C, Romeu J, Sirera G, *et al.* Long-lasting remission of cytomegalovirus retinitis without maintenance therapy in human immunodeficiency virus-infected patients. *J Infect Dis* 1998;177:1080–3.
- 96 Schneider MM, Borleffs JC, Stolk RP, *et al.* Discontinuation of prophylaxis for *Pneumocystis carinii* pneumonia in HIV-1-infected patients treated with highly active antiretroviral therapy. *Lancet* 1999;353:201–3.