26. Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.* **25,** 4876-82 (1997).
27. Burmester, T. & Hankeln, T. A globin gene of *Drosophila melanogaster*. *Mol. Biol. Evol.* **16,** 1809–1811 (1999).
28. Felsenstein, J. PHYLIP (Phylogeny Inference Package) Version 3.5c (Dept Genetics, Univ. Washington, Seattle, 1993).

Supplementary information is available on *Nature*'s World-Wide Web site (http://www.nature.com) or as paper copy from the London editorial office of *Nature*.

Acknowledgements

We wish to thank R. Gebhardt and N. Hellmann for their help with the oxygen-binding studies; G. Ungerechts for his assistance in the cloning; E. Jaenicke for running the FPLC; L. Moens and S. Dewilde for sharing experimental protocols and for discussions; and H. Decker, E. R. Schmidt and J. Markl for excellent working facilities, continuous support and valuable suggestions. This work is supported by the Deutsche Forschungsgemeinschaft (DFG) and the Naturwissenschaftlich-Medizinisches Forschungszentrum (NMFZ) Mainz.

Correspondence and requests for material should be addressed to T.B. (e-mail: burmeste@mail.uni-mainz.de) or T.H. (e-mail: hankeln@molgen.biologie.uni-mainz.de).

Immune control of HIV-1 after early treatment of acute infection

Eric S. Rosenberg, Marcus Altfeld, Samuel H. Poon, Mary N. Phillips, Barbara M. Wilkes, Robert L. Eldridge, Gregory K. Robbins, Richard T. D'Aquila, Philip J. R. Goulder & Bruce D. Walker

Partners AIDS Research Center and Infectious Disease Division, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts 02114, USA

Virus-specific T-helper cells are considered critical for the control of chronic viral infections[1,2]. Successful treatment of acute HIV-1 infection leads to augmentation of these responses[3–5], but whether this enhances immune control has not been determined. We administered one or two supervised treatment interruptions to eight subjects with treated acute infection, with the plan to restart therapy if viral load exceeded 5,000 copies of HIV-1 RNA per millilitre of plasma (the level at which therapy has been typically recommended) for three consecutive weeks, or 50,000 RNA copies per ml at one time. Here we show that, despite rebound in viraemia, all subjects were able to achieve at least a transient steady state off therapy with viral load below 5,000 RNA copies per ml. At present, five out of eight subjects remain off therapy with viral loads of less than 500 RNA copies per ml plasma after a median 6.5 months (range 5–8.7 months). We observed increased virus-specific cytotoxic T lymphocytes and maintained T-helper-cell responses in all. Our data indicate that functional immune responses can be augmented in a chronic viral infection, and provide rationale for immunotherapy in HIV-1 infection.

Many viruses, including Epstein–Barr virus and cytomegalovirus, are not cleared by the host but are controlled by an effective immune response[6]. Others, including hepatitis C virus and HIV-1, are usually characterized by lack of immune control and progressive infection. This has been linked to a lack of virus-specific T-helper-cell responses, which are required for maintenance of effective cytotoxic T-lymphocyte (CTL) function during the chronic phase of infection[1–5,7–11]. In contrast, immediate treatment of acute HIV-1 infection with highly active antiretroviral therapy (HAART) leads to generation of strong HIV-1 Gag-specific T-helper-cell responses[3], and anecdotal cases of control of viraemia after treatment of acute HIV infection[4,12,13] have been associated with these responses[4,12]. We therefore examined the antiviral effectiveness of cellular immune responses in individuals effectively treated with HAART during acute or early HIV-1 infection.

Sixteen individuals were identified with symptomatic acute HIV-1 infection[14], as were two subjects with recent infection (< 180 d). All were started on HAART, most within 72 h of diagnosis (range 1–34 d). Gag-specific T-helper-cell responses became detectable in the 15 subjects in whom treatment led to complete suppression (< 50 RNA copies per ml) of plasma viraemia, and were maintained at levels similar to those in subjects who spontaneously control viraemia (Fig. 1). Untreated subjects with documented acute infection[15] had weak or absent Gag-specific T-helper-cell responses ($P < 0.01$). Similarly, T-helper-cell responses were lower in subjects started on therapy during chronic infection ($P < 0.01$), with the only clearly positive response in a subject who initiated therapy despite only 4,000 HIV-1 RNA copies per ml plasma. HIV-1-specific CTL responses at 1 yr, measured by interferon (IFN)-γ enzyme-linked immunospot (Elispot) assay, were modest in the 18 individuals with treated primary HIV-1 infection, with a cumulative mean

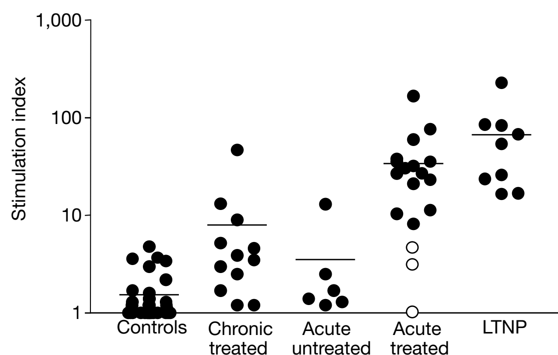

Figure 1 HIV-1 Gag-specific T-helper-cell responses in HIV-1 infection. Values at 1 yr of treated acute infection (acute treated) were compared with uninfected subjects (control), subjects first treated in the chronic phase of infection (chronic treated), subjects with untreated acute infection (acute untreated), and subjects with long-term non-progressing HIV-1 infection who are able to control viraemia to less than 1,000 RNA copies per ml plasma in the absence of therapy (LTNP). Lymphocyte proliferation to Gag protein is shown as stimulation index. Elispot assays showed that the IFN-γ production was specifically induced, consistent with a Th-1 type immune response (data not shown). Open symbols indicate subjects with treated acute infection infected with drug resistant HIV.

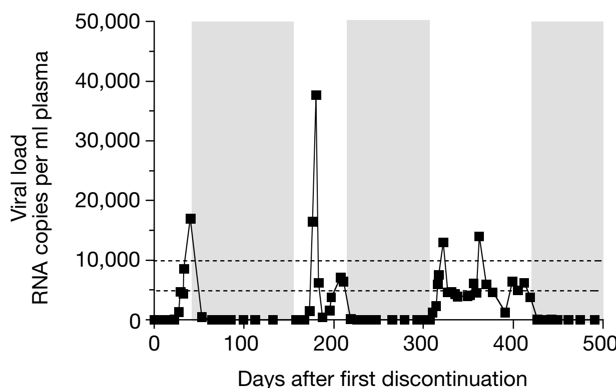

Figure 2 Viral load in a pilot study of successive treatment interruptions in a 32-yr-old male with treated acute HIV-1 infection. This subject was treated with HAART (ZDV, 3TC and IDV) for 19 months before interruption in therapy. Time zero represents the first therapy interruption. Shaded areas represent treatment with the same HAART regimen. The second therapy interruption was off protocol owing to viral hepatitis. Cumulative HIV-1-specific CTL responses, as measured by Elispot, increased from 2,360 SFCs per 10^6 PBMCs before the first interruption to a peak of 5,760 SFCs per 10^6 PBMCs after the last treatment interruption (data not shown).

© 2000 Macmillan Magazines Ltd

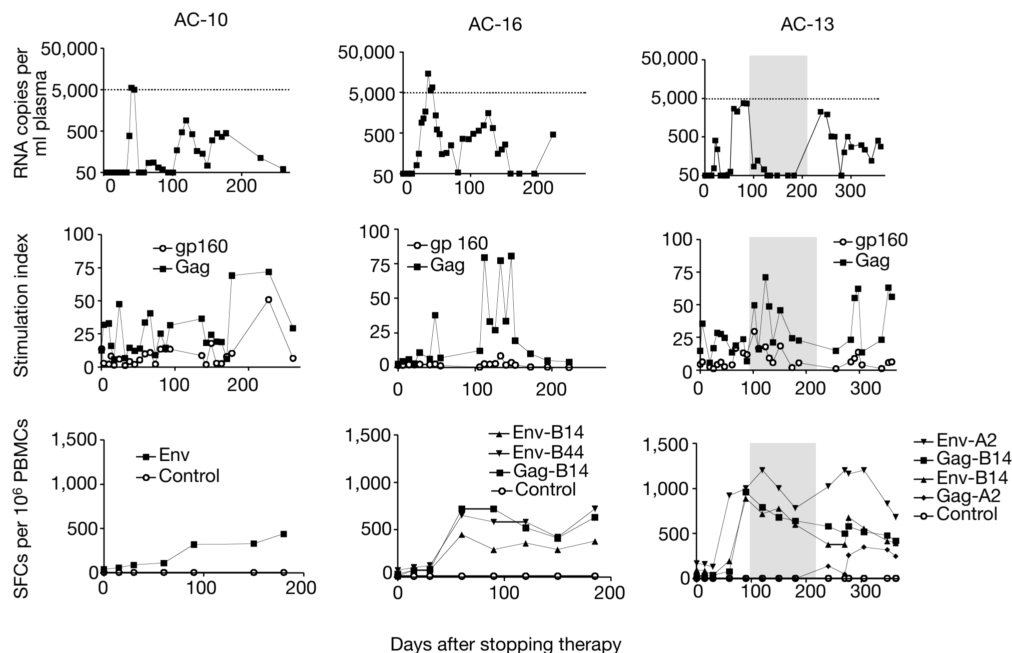

Figure 3 Virology and immunology in three subjects who controlled viraemia after the first treatment interruption. In all three, plasma virus load rose but then dropped, remaining consistently below 5,000 copies of HIV-1 RNA per ml (upper row). Gag-specific T-helper-cell responses were maintained in all three subjects (middle row). CTL responses increased significantly from baseline values and are shown as responses against individual CTL epitopes with HLA restriction (lower row). CTL responses were confirmed by cloning and testing in cytolytic assays, as described[29]. The shaded area indicates antiviral therapy re-initiated by subject 3, despite not meeting protocol indication for re-treatment.

of 460 spot-forming cells (SFCs) per 10^6 peripheral blood mononuclear cells (PBMCs) (s.d. 650 SFCs per 10^6 PBMCs) directed against only a few CTL epitopes (median 2, range 0–7).

To determine whether the immune responses induced with early treatment of acute infection were functional, we obtained Institutional Review Board approval for a pilot study to discontinue antiviral treatment. Therapy resumption was required for any viral load ≥ 10,000 HIV-1 RNA copies per ml. Discontinuation in the initial subject AC-01 led to recurrence of viraemia, which was controlled with re-institution of therapy (Fig. 2). Subsequent acute hepatitis A virus infection necessitated an unplanned treatment interruption owing to liver function abnormalities, at which time virus rebounded more rapidly and to a higher peak, but then declined without treatment to 490 RNA copies per ml plasma over the next 6 d. Therapy was subsequently restarted after liver enzymes had normalized. Approval was then granted to discontinue therapy again and resume for a viral load > 50,000 copies per ml. Viraemia was detected earlier (9 d), rose to a lower peak and declined to 4,680 copies 5 d later (Fig. 2), which is below the level at which therapy has been typically recommended[16]. A subsequent episode of bacterial pharyngitis was associated with a modest increase in viraemia, which declined without the re-institution of therapy. $CD4^+$ T-cell counts remained stable throughout (data not shown). Although not required by protocol, the patient decided to resume treatment when viral load was 3,820 RNA copies per ml.

On the basis of these results, we enrolled eight new subjects in a revised protocol, mandating the re-initiation of therapy for a viral load > 5,000 RNA copies per ml for three consecutive weeks or a single value ≥ 50,000 copies. These eight (Table 1) were indistinguishable in terms of viral load, CD4 counts and HIV-1-specific T-helper and CTL responses from the other eligible subjects who did not interrupt therapy.

During the first treatment interruption, plasma virus became detectable in all subjects after a median of 17 d (range 7–38 d). Although early peaks in viraemia were observed, viral load dropped to consistently below 5,000 RNA copies per ml plasma in three subjects (Fig. 3). Two remain off therapy after 8.7 and 7.4 months, respectively. One (AC-13) elected to restart HAART after 91 d, having never exceeded 5,000 RNA copies per ml plasma. He subsequently discontinued therapy again and has a viral load of 280 RNA copies per ml plasma 5.4 months later. In these three subjects, Gag-specific T-helper-cell responses fluctuated but were maintained during and after treatment interruption (Fig. 3). Virus-specific CTL responses (Fig. 3) were meagre but detectable at the time of therapy interruption, and directed against a median of three CTL epitopes (range 1–3). Exposure to virus in these three subjects was associated with greater than tenfold increases in median CTL from 110 SFCs per 10^6 PBMCs (range 40–300) to 1,730 SFCs per 10^6 PBMCs (range 440–2,850) ($P < 0.001$). CTL responses also broadened to target more epitopes in one of the three subjects (AC-13).

Five of the eight individuals were required to restart therapy after the first treatment interruption, four after plasma virus load exceeded 50,000 HIV-1 RNA copies per ml plasma and one because the viral load exceeded 5,000 RNA copies per ml plasma for three consecutive weeks (Fig. 4a). All five underwent a second treatment interruption. Plasma viraemia rose to a lower peak level in all ($P < 0.01$), and then dropped to less than 5,000 RNA copies per ml without treatment (Fig. 4b). Only one subject (AC-04) met criteria

Table 1 Subjects with acute or early HIV infection in a prospective trial of treatment interruption

Subject	Age	Therapy*	Days from presentation to initiation of therapy	Days on therapy before interruption
AC-02	42	ZDV,3TC,NFV	3	680
AC-04	35	ZDV,3TC,NFV	7	594
AC-05	37	ZDV,3TC,NFV	2	653
AC-06	36	D4T,3TC,NFV	4	546
AC-10†	30	D4T,3TC,IND	4	573
AC-13	32	D4T,3TC,IND	15	358
AC-14†	44	D4T,3TC,IND	34	383
AC-16	34	ZDV,3TC,IND	2	1081

* ZDV, zidovudine; D4T, stavudine; 3TC, lamivudine; IND, indinavir; NFV, nelfinavir.
† ELISA positive but infection within 180 days at time therapy initiated.

© 2000 Macmillan Magazines Ltd

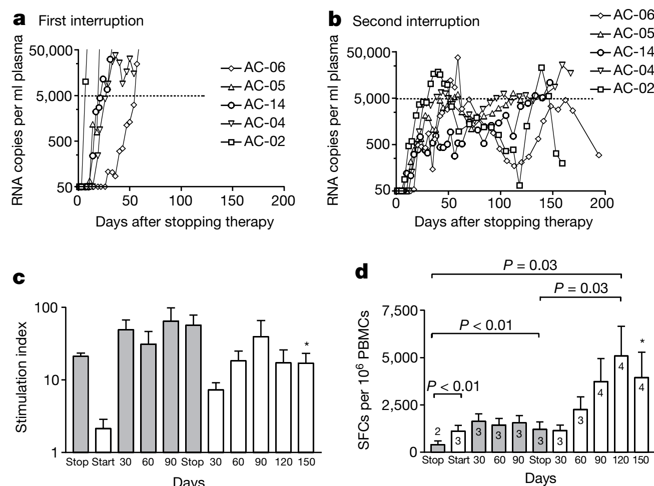

Figure 4 Virology and immunology in subjects requiring repeated treatment interruption. **a**, Five subjects met viraemia criteria to re-institute therapy during the first treatment interruption. Viral load (RNA copies per ml plasma) at the time of therapy resumption was as follows: AC-02, 187,000; AC-04, 14,800; AC-05, 116,000; AC-06, 139,000; AC-14, 111,000. Upon re-initiation of therapy, HIV-1 load declined below limits of detection in all individuals within 35–130 d (data not shown). **b**, After a median of 16 weeks of additional therapy (range 13–30 weeks), therapy was again interrupted. **c**, **d**, Gag-specific T-helper-cell responses (**c**) and HIV-1-specific CTL responses (**d**) were assessed at the time therapy was stopped (stop), at the time of protocol-mandated re-institution of therapy (start), and at designated days in between. Shaded bars indicate assays performed when subjects were on HAART. CTL magnitudes are given as mean of total CTL responses per individual, calculated as the sum of responses against optimal CTL epitopes. The median number of CTL epitopes recognized per individual is indicated within the bars, and reached a median of four per individual after the last interruption (range 2–9). CTL responses were confirmed by cloning and testing in cytolytic assays. *P* values are calculated for changes in CTL magnitude. Asterisk indicates that analysis was limited to four subjects.

to restart therapy when viral load reached 17,100 RNA copies per ml plasma after 5.5 months of treatment interruption. Two remain off therapy after 6.5 and 5.3 months, respectively, with most recent plasma viral loads of 290 and 200 RNA copies per ml, respectively. Two subjects elected to restart therapy despite not having met protocol criteria, one with a viral load of 4,600 RNA copies per ml plasma after 4 months off therapy, and one with a viral load trending up at 10,860 RNA copies per ml plasma after 5 months.

In these five individuals, Gag-specific T-helper-cell responses declined during the first treatment discontinuation but rose to levels higher than baseline with the re-initiation of therapy (Fig. 4c). During the second interruption in treatment, the stimulation index transiently declined and then rose in the absence of therapy and despite maintained low levels of plasma virus. In addition, the mean length of time off therapy was extended from 38 d to 157 d. Virus-specific CTL responses increased significantly after re-exposure to viral antigen during the first treatment interruption ($P < 0.01$), and had broadened to a median of three epitopes targeted (Fig. 4d). Two subjects who previously had no detectable CTL responses (AC-14 and AC-04) developed these for the first time after stopping therapy. CTL responses persisted at high levels after re-institution of HAART, despite undetectable viral RNA in plasma. During the second treatment interruption, CTL again increased (Fig. 4d) and more epitopes were targeted ($P < 0.01$, compared with baseline before the first treatment interruption). Dynamic immunologic control was further suggested by subject AC-02, who experienced a late increase in viral load to almost 20,000 RNA copies per ml plasma that spontaneously declined to 160 copies per ml. The decrease in viraemia was associated with further broadening of the CTL response (data not shown). In contrast, subjects who did not interrupt treatment had no increase in magnitude or breadth of HIV-1-specific CTL responses (data not shown).

At the time of writing, five of the eight individuals remain off therapy an average of 2.7 yr after initial infection, with most recent viral loads less than 500 RNA copies per ml plasma (Figs 3 and 4b). This is highly significant ($P < 0.001$) compared with historical controls from the Multicenter AIDS Cohort Study (MACS), in which only 4 out of 109 subjects with documented acute infection had viral loads less than 500 RNA copies per ml plasma 30 months after infection (ref. 17; and A. Munoz, personal communication). Comparison of those with viral loads less than 5,000 copies per ml was also highly significant, with 5 out of 8 in our cohort compared with 16 out of 109 in the MACS after a comparable period of infection ($P < 0.001$). None of the infected subjects recognized all predicted viral CTL epitopes for their expressed human leukocyte antigen (HLA) class I alleles, suggesting that further broadening of the immune response under HAART treatment may confer additional benefit on immune control[18–20]. This is a currently testable hypothesis, for example by using dendritic cells pulsed with synthetic viral peptides containing CTL and T-helper-cell epitopes[21], or by therapeutic immunization. Such approaches might decrease the risks inherent in treatment interruptions, particularly the potential development of antiviral drug resistance.

Our data show that functional HIV-1-specific CD4 and CD8 T-cell responses can be enhanced with early treatment of acute infection. They contrast the reported lack of immune control after treatment interruption in chronic infection[22]. Our data do not allow one to assess the contribution of sequential treatment interruptions to immunologic control of viraemia, as we re-instituted therapy before viral set point was achieved in the five subjects who underwent two interruptions in therapy. Whether effective immune augmentation can be achieved in chronic infection through repeated structured treatment interruptions is an important issue that is yet to be addressed, as is the durability of the observed immune control in our cohort. The effect on clinical outcome will require further longitudinal studies. Viral load has been used as a surrogate for efficacy in drug trials in HIV-1 infection, and if the same criteria are applied, one would predict a survival advantage. The successful containment of viraemia in most of these subjects provides rationale to support the practice of treating individuals with HAART during acute or early HIV-1 infection, and provides

© 2000 Macmillan Magazines Ltd

rationale to explore immunotherapeutic interventions in both acute and chronic HIV-1 infection. □

Methods

Subjects

Sixteen individuals were identified with symptomatic acute HIV-1 infection[14], defined by the presence of HIV-1 RNA in the plasma, and negative or weakly positive antibody tests (HIV 1/2 enzyme-linked iummnosorbent assay (ELISA) and HIV-1 western blot). We identified two additional subjects with a recent syndrome compatible with acute HIV-1 infection and positive antibodies to HIV-1, in whom an ELISA-based assay confirmed HIV-1 infection within the previous 180 d (ref. 23). The median plasma HIV-1 load was 4.85×10^6 RNA copies per ml plasma (range 0.25–30.7×10^6 copies per ml) in the subjects diagnosed before seroconversion. The median $CD4^+$ T-cell count before initiation of therapy was 365 cells per mm^3 (range 130–1,020 cells per mm^3; normal 380–1,630 cells per mm^3). The two subjects identified after HIV-1 seroconversion had viral loads of 0.4×10^6 and 0.95×10^6 copies per ml and $CD4^+$ T-cell counts of 920 and 980 cells per mm^3, respectively. In addition, 6 subjects identified within 6–12 months of documented acute HIV-1 infection were used as an untreated control group. All individuals initiated HAART at the time of diagnosis with anti-retroviral regimens comprising either two nucleoside reverse transcriptase inhibitors combined with a protease inhibitor or a non-nucleoside reverse transcriptase inhibitor, or both. Additional groups of study subjects included 6 subjects identified within 6–12 months of documented HIV-1 seroconversion, and evaluated before the initiation of therapy[15]; 12 subjects with chronic HIV-1 infection whose virus had been effectively suppressed by HAART (viral loads < 50 RNA copies per ml plasma) for at least 12 months, 7 of whom had $CD4^+$ T-cell counts > 500 cells per mm^3 before initiating therapy; and 9 subjects with chronic HIV-1 infection who controlled viral replication in the absence of anti-retroviral therapy (viral load $< 1,000$ RNA copies per ml, CD4 T cells > 500 per mm^3, > 10 yr infection).

Treatment interruption

Entry criteria included treatment with HAART before or around the time of HIV-1 seroconversion, consistent suppression of viraemia to below levels of quantification (< 50 RNA copies per ml plasma) for at least 8 months, and lack of evidence of significant mutations conferring drug resistance[24]. HIV-1-specific T-helper-cell function before discontinuation had to exceed a stimulation index of 10, and net counts per minute (c.p.m.) had to be ≥ 800. All anti-retroviral medications were discontinued simultaneously.

HIV-1-specific T-helper-cell assays

We carried out lymphocyte proliferation assays with baculovirus-derived HIV-1 Gag and gp160 protein. PBMCs were incubated with peptide (5 $\mu g\,ml^{-1}$) for 6 d and then pulsed with [^{3}H]thymidine at 1.0 μCi per well for 6 h, as described[3]. For the purposes of data interpretation, a stimulation index of 5 or greater was considered significant.

IFN-γ Elispot assays of HIV-1-specific CD4 and CD8 T-cell responses

Synthetic peptides for detection of CTL responses comprised 259 overlapping peptides of 15–20 amino acids, overlapping by 10–11 amino acids, spanning the entire p17 Gag, p24 Gag, Nef, gp41, gp120 and reverse transcriptase B-clade coding regions, and a panel of described optimal CTL epitopes for the HLA-type of the individual tested[25]. A median of 16 (range 5–28) described optimal CTL epitopes were tested per individual. For CTL detection by Elispot, we plated fresh or frozen PBMCs in 96-well polyvinylidene difluoride backed plates (MAIP S45, Millipore) that had been previously coated with 0.5 $\mu g\,ml^{-1}$ of an anti-IFN-γ monoclonal antibody 1-D1k (Mabtech, Stockholm, Sweden) overnight at 4 °C. We added PBMCs to the wells at 50,000 to 100,000 cells per well, and processed and counted plates as described[26]. We counted IFN-γ-producing cells by direct visualization and express them as spot-forming cells (SFCs). The negative controls were subtracted, and in all cases were less than 40 SFCs per 10^6 input cells. We generated CTL clones to confirm the cytolytic ability of the antigen-specific responses, as described[27]. We quantitated $CD4^+$ T-helper-cell responses using a similar assay, with minor differences. We incubated PBMCs (1×10^6) overnight in a 48-well plate with HIV-1 p24 antigen, baculovirus control protein or medium alone. Cells were then transferred to Elispot plates pre-coated with 2.5 $\mu g\,ml^{-1}$ monoclonal IFN-γ antibody (Endogen, Woburn, MA), and incubated for 24–48 h. Plates were developed and counted as above. For each response to an overlapping peptide, the optimal CTL epitope contained within the 15–20 mer was characterized and used for the subsequent quantification of CTL responses.

Sequencing of HIV-1 protease and reverse transcriptase

Virion RNA was extracted (QiAmp Viral RNA kit, Qiagen) from centrifuged plasma before complementary DNA synthesis (Superscript II, Gibco-BRL). Two rounds of polymerase chain reaction were done with a nested set of primers amplifying a fragment from p6 Gag through codon 350 in the reverse transcriptase (XL rTth DNA PCR kit, PE Biosystems), and then sequenced directly and analysed as described[28].

Statistical analysis

We carried out statistical analysis using GraphPad Instat version 3.00 for Windows 95 (GraphPad Software, San Diego, CA). Statistical significance (P values) of results were calculated by two-tailed t-test and by χ^2 analysis.

Received 17 July; accepted 31 August 2000.

1. Matloubian, M., Concepcion, R. J. & Ahmed, R. $CD4^+$ T cells are required to sustain $CD8^+$ cytotoxic T-cell responses during chronic viral infection. *J. Virol.* **68,** 8056–8063 (1994).
2. Battegay, M. *et al.* Enhanced establishment of a virus carrier state in adult $CD4^+$ T-cell-deficient mice. *J. Virol.* **68,** 4700–4704 (1994).
3. Rosenberg, E. S. *et al.* Vigorous HIV-1-specific $CD4^+$ T cell responses associated with control of viremia. *Science* **278,** 1447–1450 (1997).
4. Oxenius, A. *et al.* Early highly active antiretroviral therapy for acute HIV-1 infection preserves immune function of $CD8^+$ and $CD4^+$ T lymphocytes. *Proc. Natl Acad. Sci. USA* **97,** 3382–3387 (2000).
5. Malhotra, U. *et al.* Effect of combination antiretroviral therapy on T-cell immunity in acute human immunodeficiency virus type 1 infection. *J. Infect. Dis.* **181,** 121–131 (2000).
6. Kalams, S. A. & Walker, B. D. The critical need for CD4 help in maintaining effective cytotoxic T lymphocyte responses. *J. Exp. Med.* **188,** 2199–2204 (1998).
7. von Herrath, M. G., Yokoyama, M., Dockter, J., Oldstone, M. B. & Whitton, J. L. CD4-deficient mice have reduced levels of memory cytotoxic T lymphocytes after immunization and show diminished resistance to subsequent virus challenge. *J. Virol.* **70,** 1072–1079 (1996).
8. Zajac, A. J. *et al.* Viral immune evasion due to persistence of activated T cells without effector function. *J. Exp. Med.* **188,** 2205–2213 (1998).
9. Kalams, S. A. *et al.* Association between virus-specific cytotoxic T-lymphocyte and helper responses in human immunodeficiency virus type 1 infection. *J. Virol.* **73,** 6715–6720 (1999).
10. Gerlach, J. T. *et al.* Recurrence of hepatitis C virus after loss of virus-specific CD4(+) T- cell response in acute hepatitis C. *Gastroenterology* **117,** 933–941 (1999).
11. Lechner, F. *et al.* Analysis of successful immune responses in persons infected with hepatitis C virus. *J. Exp. Med.* **191,** 1499–1512 (2000).
12. Lisziewicz, J. *et al.* Control of HIV despite the discontinuation of antiretroviral therapy. *N. Engl. J. Med.* **340,** 1683–1684 (1999).
13. Ortiz, G. M. *et al.* HIV-1-specific immune responses in subjects who temporarily contain virus replication after discontinuation of highly active antiretroviral therapy. *J. Clin. Invest.* **104,** R13–R18 (1999).
14. Kahn, J. O. & Walker, B. D. Acute human immunodeficiency virus type 1 infection. *N. Engl. J. Med.* **339,** 33–39 (1998).
15. Lori, F. *et al.* Treatment of human immunodeficiency virus infection with hydroxyurea, didanosine, and a protease inhibitor before seroconversion is associated with normalized immune parameters and limited viral reservoir. *J. Infect. Dis.* **180,** 1827–1832 (1999).
16. Carpenter, C. C. *et al.* Antiretroviral therapy for HIV infection in 1997. Updated recommendations of the International AIDS Society/USA panel. *J. Am. Med. Assoc.* **277,** 1962–1969 (1997).
17. Lyles, R. H. *et al.* Natural history of human immunodeficiency virus type 1 viremia after seroconversion and proximal to AIDS in a large cohort of homosexual men. Multicenter AIDS Cohort Study. *J. Infect. Dis.* **181,** 872–880 (2000).
18. Pantaleo, G. *et al.* Major expansion of $CD8^+$ T cells with a predominant V beta usage during the primary immune response to HIV. *Nature* **370,** 463–467 (1994).
19. Autran, B. *et al.* Positive effects of combined antiretroviral therapy on $CD4^+$ T cell homeostasis and function in advanced HIV disease. *Science* **277,** 112–116 (1997).
20. Evans, D. T. *et al.* Virus-specific cytotoxic T-lymphocyte responses select for amino-acid variation in simian immunodeficiency virus Env and Nef. *Nature Med.* **5,** 1270–1276 (1999).
21. Dhodapkar, M. V. *et al.* Rapid generation of broad T-cell immunity in humans after a single injection of mature dendritic cells. *J. Clin. Invest.* **104,** 173–180 (1999).
22. Davey, R. T. Jr *et al.* HIV-1 and T cell dynamics after interruption of highly active antiretroviral therapy (HAART) in patients with a history of sustained viral suppression. *Proc. Natl Acad. Sci. USA* **96,** 15109–15114 (1999).
23. Janssen, R. S. *et al.* New testing strategy to detect early HIV-1 infection for use in incidence estimates and for clinical and prevention purposes. *J. Am. Med. Assoc.* **280,** 42–48 (1998).
24. Little, S. J. *et al.* Reduced antiretroviral drug susceptibility among patients with primary HIV infection. *J. Am. Med. Assoc.* **282,** 1142–1149 (1999).
25. Brander, C. & Walker, B. D. in *Molecular Immunology Data Base* (eds Korber, B. T. *et al.*) IV-50–IV-60 (Los Alamos National Laboratory, Los Alamos, New Mexico, 1997).
26. Goulder, P. J. *et al.* Differential narrow focusing of immunodominant human immunodeficiency virus gag-specific cytotoxic T-lymphocyte responses in infected African and caucasoid adults and children. *J. Virol.* **74,** 5679–5690 (2000).
27. Walker, B. D. *et al.* Long-term culture and fine specificity of human cytotoxic T-lymphocyte clones reactive with human immunodeficiency virus type 1. *Proc. Natl Acad. Sci. USA* **86,** 9514–9518 (1989).
28. Martinez-Picado, J., Sutton, L., De Pasquale, M. P., Savara, A. V. & D'Aquila, R. T. Human immunodeficiency virus type 1 cloning vectors for antiretroviral resistance testing. *J. Clin. Microbiol.* **37,** 2943–2951 (1999).
29. Kalams, S. A. *et al.* Levels of human immunodeficiency virus type 1-specific cytotoxic T- lymphocyte effector and memory responses decline after suppression of viremia with highly active antiretroviral therapy. *J. Virol.* **73,** 6721–6728 (1999).

Acknowledgements

This work was supported by the Doris Duke Charitable Foundation (B.D.W., E.S.R.), the NIH (E.S.R., R.D., B.D.W.), the Elizabeth Glaser Pediatric AIDS Foundation (P.J.R.G.), the Deutscher Akademischer Austauschdienst (M.A.), the UK Medical Research Council (P.J.R.G.) and several private donors. We thank A. Munoz for conducting the analysis comparing our data with the MACS data. We also thank the subjects who participated in these trials, M. S. Hirsch and D. T. Scadden for comments on the manuscript; B. Dale for supplying kits for viral load testing; P. Sax and B. Davis for help in recruiting study subjects; M. J. Ferraro, J. Eversley and S. Masci, for technical support; and B. Rawal for performing modified HIV-1 ELISA assays. P.J.R.G. is an Elizabeth Glaser Scientist and B.D.W. is a Doris Duke Distinguished Clinical Science Professor.

Correspondence and requests for materials should be addressed to B.D.W. (e-mail: bwalker@helix.mgh.harvard.edu).

 © 2000 Macmillan Magazines Ltd