Highly active antiretroviral therapy during early HIV infection reverses T-cell activation and maturation abnormalities

Leslie R. Bisset, Richard W. Cone*, Werner Huber*, Manuel Battegay†, Pietro L. Vernazza‡, Rainer Weber*, Peter J. Grob, Milos Opravil* and the Swiss HIV Cohort Study§

Objectives: To evaluate the impact of early initiation of highly active antiretroviral therapy (HAART) on disease-induced T-cell activation and maturation abnormalities during asymptomatic HIV infection.

Design: A prospective open-label trial of zidovudine, lamivudine and ritonavir in treatment-naive asymptomatic HIV-infected individuals with CD4 cells $\geq 400 \times 10^6$/l.

Methods: Peripheral blood CD4+ and CD8+ T cells derived from 15 asymptomatic HIV-infected individuals (median baseline CD4+ cells, 608×10^6/l; CD8+ cells, 894×10^6/l; plasma HIV RNA, 3.93 $\log_{10}$ copies/ml) undergoing therapy with zidovudine (300 mg twice daily), lamivudine (150 mg twice daily), and ritonavir (600 mg twice daily) were assessed for changes in expression of phenotypic markers of T-cell activation (HLA-DR and CD38) and maturation (CD45RA and CD45RO). At weeks 0, 2, 4, 8, 12, 16, 20 and 24, T-cell subsets were quantified by flow cytometry and plasma HIV viral loads determined using reverse transcription PCR.

Results: HAART-induced decrease in plasma HIV RNA levels coincided with a significant reduction in numbers of activated CD4+/HLA-DR+ (maximum change, −36%; $P \leq 0.05$), CD8+/HLA-DR+ (maximum change, −66%; $P \leq 0.005$) and CD8+/CD38+ (maximum change, −51%; $P \leq 0.01$) T cells. A concomitant significant increase in numbers of naive CD4+/CD45RA+ (maximum change, +12%; $P \leq 0.005$) and memory CD4+/CD45RO+ (maximum change, +6%; $P \leq 0.05$) T cells was also evident, which contrasted with a significant decrease in memory CD8+/CD45RO+ cells (maximum change, −42%; $P \leq 0.005$).

Conclusion: The observed ability of HAART during early asymptomatic HIV infection to initiate rapid reversal of disease-induced T-cell activation and maturation abnormalities, while preserving pretherapy levels of immune function, supports the concept that therapeutic advantage is to be gained by commencing early aggressive antiretroviral therapy.

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From the Division of Clinical Immunology, the *Division of Infectious Diseases and Hospital Epidemiology, Department of Internal Medicine, University Hospital, Zürich, the †Outpatient Department of Internal Medicine, University Hospital, Basel, and the ‡Medical Clinic A, Department of Internal Medicine, Cantonal Hospital, St Gallen, Switzerland. §See Appendix for members.

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Requests for reprints to: Dr Milos Opravil, Division of Infectious Diseases and Hospital Epidemiology, Department of Internal Medicine, University Hospital, CH-8091 Zürich, Switzerland.

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Introduction

Highly active antiretroviral therapy (HAART) based on the synergistic usage of HIV-1 reverse transcriptase and protease inhibitors has proven to be remarkably effective in reducing viral replication during the late symptomatic stages of HIV infection [1]. Attention has now focused on the ability of this therapeutic strategy to further initiate T-cell recovery and consequent reversal of the immunosuppression that characterizes disease progression [2]. Of specific interest is the potential role of HAART in reversing chronic levels of T-cell activation and promoting naive T-cell regeneration, thus inhibiting expression of endogenous cytokines that actively support viral replication [3] and correcting disease-induced T-cell maturation abnormalities [4]. It is now apparent that application of HAART during advanced HIV infection is capable of partially achieving these therapeutic goals [5–7].

Detection of significant viral replication during early asymptomatic HIV infection [8–10] indicates that virus turnover lacks dependence on disease progression and provides strong incentive for extending HAART to include treatment of early disease. Indeed, initial reports have now confirmed the effectiveness of treatment in reducing plasma viral load during primary HIV infection [11–16]. In a similar manner to that observed during advanced HIV infection, early treatment has also been shown to initiate significant peripheral blood CD4+ T-cell recovery [15,16]. Nevertheless, the more specific influence of early treatment in correcting disease-induced T-cell activation and maturation abnormalities during asymptomatic HIV infection remains to be fully assessed.

It is apparent that distinct differences in T-cell subset involvement characterize asymptomatic and advanced HIV infection, which are likely to uniquely influence the character of treatment-induced immune recovery. Although peripheral blood CD4+ T-cell levels decrease following acute HIV infection, a subsequent dramatic drop in CD4+ T-cell numbers is not apparent until development of clinical immunodeficiency [17–19]. Similarly, a significant increase in peripheral blood CD8+ T-cell levels during early disease accompanies CD4+ T-cell decline [17–19], maintaining relatively constant T-cell values until development of the depressed CD4+ and CD8+ T-cell levels characterizing progression to AIDS [20,21]. Discrepancy in the involvement of different T-cell maturational stages is also related to disease progression, as exemplified by the contrasting selective loss of memory CD4+/CD45RO+ T cells during early HIV infection [22–25] and the loss of naive CD4+/CD45RA+ T cells following the development of AIDS [26]. Finally, it is now evident that lymphoid tissue derived from asymptomatic HIV-infected individuals fails to demonstrate signs of disease-associated CD4+ T-cell depletion [27,28], contrasting with reduced peripheral blood CD4+ T-cell levels observed in the same individuals [28] and in lymphoid tissue during symptomatic HIV infection [28,29].

Within the context of a prospective clinical study investigating the antiviral efficacy of HAART during early asymptomatic HIV infection, we have assessed the capacity of this treatment approach to correct disease-related T-cell activation and maturation abnormalities by quantifying changes in peripheral blood CD4+ and CD8+ T cells expressing cell-surface markers of both activation (HLA-DR, CD38) and differentiation (CD45RA, CD45RO).

Materials and methods

Study population

Participants included 15 asymptomatic HIV-infected individuals enrolled in the Early Antiretroviral Therapy Study who received triple combination treatment consisting of the protease inhibitor ritonavir and the nucleoside analogues zidovudine and lamivudine. Inclusion criteria were the absence of prior antiretroviral therapy and peripheral blood CD4+ T-cell counts $\geq 400 \times 10^6$/l. The study group was comprised of 12 men and three women, and risk factors for HIV infection included homosexual contact (n = 8), injecting drug use (n = 4) and heterosexual contact (n = 3). Antiretroviral therapy was administered at a dosage of 300 mg twice daily for zidovudine, 150 mg twice daily for lamivudine, and 600 mg twice daily for ritonavir. Experimental protocols were approved by the local institutional ethics committees involved and all participants gave informed written consent. A control population consisting of 10 randomly selected healthy volunteer blood donors attending the Zürich branch of the Swiss Red Cross Blood Bank was also recruited.

Sample collection and preparation

Venous blood was collected immediately prior to initiation of treatment, after 2 weeks of treatment and thereafter at monthly intervals until 24 weeks of treatment. For the assessment of peripheral blood T-cell subsets, venous blood was collected in 3 ml tubes (Becton Dickinson Systems, Franklin Lakes, New Jersey, USA) containing 0.17 mol/l EDTA and processed within 4 h of collection. For the assessment of plasma HIV RNA levels, venous blood was collected into 8 ml cell preparation tubes (Becton Dickinson Systems) containing 0.1 mol/l sodium citrate and processed within 2 h of collection. The mononuclear cell-free plasma was stored at −70°C until utilization.

Laboratory measurements

Viral load

Plasma HIV RNA copy levels were determined retrospectively using the commercially available Amplicor (Roche Diagnostic Systems, Branchburg, New Jersey) quantitative reverse transcription PCR assay according to the manufacturer's instructions. The lower detection limit of this assay was in the order of 200 copies/ml.

T-cell subsets

The absolute number and percentage of peripheral blood T-cell subsets were prospectively assessed using two-colour flow cytometry. Monoclonal antibody combinations included fluorescein isothiocyanate (FITC)-conjugated anti-HLe-1 (CD45) and phycoerythrin (PE)-conjugated Leu-M3 (CD14), FITC-Leu-3a (CD4) and PE-Leu-2a (CD8), FITC-Leu-3a (CD4) and PE-anti-HLA-DR, FITC-Leu-18 (CD45RA) and PE-Leu-3a (CD4), FITC-Leu-3a (CD4) and PE Leu-45RO (CD45RO), FITC-Leu-2a (CD8) and PE-anti-HLA-DR, FITC-Leu-18 (CD45RA) and PE-Leu-2a (CD8), FITC-Leu-2a (CD8) and PE-Leu-45RO (CD45RO), and FITC-Leu-2a (CD8) and PE-Leu-17 (CD38) (Becton Dickinson Systems). A whole-blood method of sample preparation was employed, during which 100 μl venous blood was incubated with 20 μl of the appropriate fluorochrome-conjugated monoclonal antibodies for 20 min at room temperature, subjected to red blood cell lysis, leukocyte stabilization and cell membrane fixation using Immunoprep reagents and Multi-Prep equipment (Coulter, Hialeah, Florida, USA), and evaluated using an Epics XL-MCL (Coulter) flow cytometer equipped with a 448 nm (15 mW) laser using 525 nm (FL1/FITC) and 575 nm (FL2/PE) band-pass filters. Absolute lymphocyte values were calculated using a particle concentration method based on precalibrated Flow Count fluorescent microspheres (Coulter). An initial fluorescence back-gating procedure was performed based on identification of CD45+ and CD14+ cell populations, allowing both an accurate lymphocyte gate to be set and carried forward for subsequent analyses and estimates of lymphocyte recovery and purity to be calculated [30]. For each analysis, 10 000 events were counted.

Statistical analysis

The significance of differences between study parameters was estimated using the Wilcoxon signed rank test for paired datasets and the Mann–Whitney U-test for unpaired datasets. Both non-parametric evaluations were implemented using the StatView version 4.5 (Abacus Concepts, Berkeley, California, USA) statistical software package. Results are expressed as median values with interquartile ranges.

Results

Viral load

Plasma HIV RNA levels demonstrated a significant decline between baseline (3.93 $\log_{10}$ copies/ml) and 4 weeks of treatment (2.47 $\log_{10}$ copies/ml; Table 1). A stabilization in plasma HIV RNA levels was observed after 12 weeks of treatment (2.29 $\log_{10}$ copies/ml; Table 1). At 12 and 24 weeks, 13 (87%) out of 15, and 14 (93%) out of 15 of the study participants, respectively, demonstrated plasma HIV RNA levels below detection limit. All but one study participant exhibited good medication compliance. Ritonavir was stopped due to intolerance in four study participants between weeks 4 and 8. For three of these study participants, ritonavir was replaced by nelfinavir or indinavir, whereas one study participant continued on zidovudine–lamivudine only and remained with plasma HIV RNA levels below detection limit.

T-cell subsets

Peripheral blood CD4+ T-cell percentages increased following treatment from baseline (26.1%) to reach a point of greatest change at 12 weeks of treatment (36.4%; $P \leq 0.005$; Table 2). In contrast, peripheral blood CD8+ T-cell percentages demonstrated a reciprocal trend, decreasing from baseline (53.6%) to reach a point of greatest change at 12 weeks of treatment (45.3%; $P \leq 0.010$; Table 3). Change from baseline absolute numbers of both CD4+ (608×10^6/l) and CD8+ (894×10^6/l) T cells essentially reflected trends observed for percentage values, reaching points of greatest change at 24 weeks for CD4+ T cells (724×10^6/l; $P \leq 0.001$) and 16 weeks for CD8+ T cells (762×10^6/l; $P \leq 0.050$; Tables 2 and 3). Of note was the early occurrence at 2 weeks post-treatment of a marked transient increase in both CD4+ and CD8+ T-cell absolute numbers (Tables 2 and 3).

With regard to peripheral blood CD4+ T-cell subsets, no significant difference was apparent between healthy controls and asymptomatic HIV-infected individuals for baseline percentage and absolute numbers of CD4+/CD45RA+ or CD4+/HLA-DR+ T cells (Table 2). This contrasted with the significant difference observed between these two groups with regard to baseline percentage (25.8 and 10.6%, respectively;

Table 1. Change in plasma HIV RNA levels in asymptomatic HIV-infected individuals (n = 15) undergoing highly active antiretroviral therapy with zidovudine, lamivudine and ritonavir.

Treatment week	Median (IQR) plasma HIV RNA ($\log_{10}$ copies/ml)	*P**
0	3.93 (3.32–4.45)	–
4	2.47 (2.19–2.65)	≤ 0.005
12	2.29 (2.00–2.53)	≤ 0.005
24	2.30 (2.00–2.49)	≤ 0.001

*Wilcoxon signed-rank test (corrected for tied values). IQR, Interquartile range.

Table 2. Percentage and absolute levels of peripheral blood lymphocyte CD4+ T-cell subsets in healthy controls (n = 10) and asymptomatic HIV-infected individuals (n = 15) undergoing highly active antiretroviral therapy with zidovudine, lamivudine and ritonavir.

Treatment week	Median (IQR) cell percentage	*P**	Median (IQR) cell count ($\times 10^6$/l)	*P*
CD4+				
0	26.10 (23.10–33.42)	–	608 (427–630)	–
2	27.15 (19.65–27.15)	NS	714 (440–772)	≤ 0.05
4	29.10 (19.85–37.33)	≤ 0.05	630 (464–703)	NS
8	32.80 (26.72–37.00)	≤ 0.001	613 (460–714)	NS
12	36.40 (24.35–40.23)	≤ 0.005	629 (526–692)	≤ 0.05
16	34.40 (25.97–39.50)	≤ 0.005	619 (470–727)	≤ 0.05
20	35.00 (27.15–37.88)	≤ 0.01	675 (601–722)	≤ 0.005
24	34.60 (31.65–39.48)	≤ 0.005	724 (606–824)	≤ 0.001
Controls	47.55 (42.70–52.90)	≤ 0.001	752 (558–880)	≤ 0.05
CD4+/HLA-DR+				
0	3.00 (2.28–4.33)	–	66 (38–93)	–
2	2.90 (2.50–3.45)	NS	70 (52–75)	NS
4	3.30 (2.62–4.10)	NS	62 (49–81)	NS
8	3.50 (1.97–4.05)	NS	55 (36–76)	NS
12	2.80 (2.08–4.27)	NS	56 (36–72)	NS
16	2.80 (1.77–3.40)	≤ 0.05	42 (33–56)	≤ 0.05
20	2.80 (1.70–3.40)	≤ 0.05	54 (32–65)	≤ 0.05
24	2.40 (1.80–3.17)	≤ 0.005	46 (39–65)	≤ 0.05
Controls	2.85 (2.10–3.90)	NS	43 (27–47)	NS
CD4+/CD45RA+				
0	13.90 (12.05–21.73)	–	373 (209–435)	–
2	14.50 (9.15–24.95)	NS	433 (207–481)	NS
4	14.50 (10.90–24.12)	NS	387 (208–424)	NS
8	15.80 (12.35–22.45)	≤ 0.05	315 (194–463)	NS
12	17.40 (11.40–24.63)	≤ 0.05	362 (202–419)	NS
16	18.80 (12.02–24.57)	≤ 0.05	389 (190–465)	NS
20	17.30 (11.78–22.68)	NS	369 (252–456)	≤ 0.01
24	20.30 (12.53–25.07)	≤ 0.05	417 (287–506)	≤ 0.005
Controls	22.55 (19.00–25.90)	NS	330 (279–398)	NS
CD4+/CD45RO+				
0	10.60 (9.33–13.43)	–	213 (170–236)	–
2	10.75 (8.60–11.45)	NS	235 (187–260)	≤ 0.05
4	12.20 (9.65–13.63)	NS	211 (190–285)	NS
8	11.60 (9.85–12.95)	NS	210 (179–270)	NS
12	11.80 (9.88–13.33)	NS	228 (182–272)	NS
16	11.70 (10.68–13.03)	NS	208 (170–263)	NS
20	11.50 (10.80–12.45)	NS	224 (193–279)	NS
24	11.60 (10.03–13.15)	≤ 0.05	225 (204–299)	≤ 0.05
Controls	25.80 (22.30–28.00)	≤ 0.001	342 (278–610)	≤ 0.005

*Paired comparisons: Wilcoxon signed-rank test (corrected for tied values); unpaired comparisons: Mann–Whitney U-test (corrected for tied values). IQR, Interquartile range; NS, not significant.

$P \leq 0.001$) and absolute numbers (342 and 231×10^6/l, respectively; $P \leq 0.005$) of CD4+/CD45RO+ T cells (Table 2). With regard to peripheral blood CD8+ T-cell subsets, no significant difference was apparent between healthy controls and asymptomatic HIV-infected individuals for baseline percentage and absolute numbers of CD8+/CD45RA+ T cells (Table 3). This contrasted with the significant difference observed between these two groups with regard to baseline percentage (1.55 and 20.7%, respectively; $P \leq 0.001$) and absolute numbers (22 and 387×10^6/l, respectively; $P \leq 0.001$) of CD8+/HLA-DR T cells, baseline percentage (8.55 and 29.4%, respectively; $P \leq 0.001$) and absolute numbers (144 and 451×10^6/l, respectively; $P \leq 0.001$) of CD8+/CD45RO+ T cells, and baseline percentage (13.05 and 38.5%, respectively; $P \leq 0.001$) and absolute numbers (184 and 660×10^6/l, respectively; $P \leq 0.001$) of CD8+/CD38+ T cells (Table 3).

The overall post-treatment increase in CD4+ T-cell values was reflected primarily by change within the naive CD4+/CD45RA+ T-cell subset, both percentage and absolute numbers increasing from baseline to reach a point of greatest change at 24 weeks of treatment [20.3% ($P \leq 0.050$) and 417×10^6/l ($P \leq 0.005$), respectively; Table 2]. Reflecting the early fluctuations observed for CD4+ T-cell absolute numbers, CD4+/CD45RA+ T-cell absolute numbers also underwent a transient, albeit non-significant, increase at 2 weeks of treatment (Table 2). A delayed but significant increase in the percentage and absolute numbers of memory CD4+/CD45RO+ T cells was also observed, both values increasing from baseline to reach a point of greatest change at 24 weeks [11.6% ($P \leq 0.050$) and 225×10^6/l ($P \leq 0.050$), respectively; Table 2]. In contrast, a treatment-induced decrease in the percentage and absolute numbers of activated CD4+/HLA-DR+ T cells was observed, these values decreasing from

Table 3. Percentage and absolute levels of peripheral blood lymphocyte CD8+ T-cell subsets in healthy controls (n = 10) and asymptomatic HIV-infected individuals (n = 15) undergoing highly active antiretroviral therapy with zidovudine, lamivudine and ritonavir.

Treatment week	Median (IQR) cell percentage	*P**	Median (IQR) cell count ($\times 10^6$/l)	*P*
CD8+				
0	53.60 (47.33–60.48)	–	894 (650–1458)	–
2	56.70 (49.55–64.10)	NS	1056 (908–1645)	NS
4	51.70 (46.58–62.28)	≤ 0.05	896 (760–1484)	NS
8	47.60 (42.12–55.75)	≤ 0.005	823 (627–1218)	NS
12	45.30 (39.02–56.38)	≤ 0.01	762 (529–1265)	NS
16	48.10 (37.57–56.40)	≤ 0.01	762 (494–1257)	≤ 0.05
20	45.70 (42.67–53.38)	≤ 0.01	925 (752–1045)	NS
24	45.40 (39.15–52.00)	≤ 0.005	887 (660–1068)	NS
Controls	30.25 (28.50–34.10)	≤ 0.001	389 (366–806)	≤ 0.05
CD8+/HLA–DR+				
0	20.70 (16.20–27.98)	–	387 (246–502)	–
2	20.40 (14.05–30.40)	NS	427 (266–1007)	NS
4	18.60 (13.10–22.70)	≤ 0.005	272 (228–522)	≤ 0.005
8	13.60 (9.15–17.48)	≤ 0.005	193 (168–444)	≤ 0.001
12	11.10 (7.55–14.87)	≤ 0.005	176 (142–224)	≤ 0.005
16	8.50 (5.70–10.68)	≤ 0.005	130 (95–239)	≤ 0.005
20	8.70 (5.55–12.70)	≤ 0.005	169 (121–245)	≤ 0.005
24	6.90 (5.42–8.90)	≤ 0.001	146 (109–206)	≤ 0.001
Controls	1.55 (1.30–3.50)	≤ 0.001	22 (16–41)	≤ 0.001
CD8+/CD45RA+				
0	26.80 (22.95–32.67)	–	454 (339–830)	–
2	30.05 (27.25–32.80)	NS	627 (542–932)	≤ 0.05
4	31.40 (25.23–33.25)	NS	519 (417–733)	NS
8	29.30 (24.55–32.58)	NS	593 (364–735)	NS
12	27.50 (23.77–33.40)	≤ 0.05	484 (342–750)	NS
16	30.40 (22.92–35.15)	NS	434 (309–803)	NS
20	29.60 (25.75–34.47)	NS	543 (460–764)	NS
24	30.80 (24.42–33.50)	NS	551 (378–743)	NS
Controls	21.75 (17.50–24.90)	NS	278 (254–567)	NS
CD8+/CD45RO+				
0	29.40 (18.57–32.27)	–	451 (352–614)	–
2	26.15 (19.95–33.30)	NS	477 (410–966)	NS
4	23.20 (18.60–29.90)	≤ 0.01	396 (334–545)	≤ 0.05
8	19.35 (15.40–24.10)	≤ 0.005	314 (282–630)	≤ 0.005
12	14.90 (13.48–22.95)	≤ 0.005	285 (212–506)	≤ 0.005
16	14.90 (14.20–18.48)	≤ 0.005	260 (202–406)	≤ 0.005
20	15.90 (12.22–20.68)	≤ 0.005	310 (226–451)	≤ 0.01
24	13.30 (11.75–18.52)	≤ 0.001	282 (230–395)	≤ 0.005
Controls	8.55 (7.60–11.70)	≤ 0.001	144 (90–249)	≤ 0.001
CD8+/CD38+				
0	38.50 (26.90–44.62)	–	660 (321–1205)	–
2	35.60 (26.80–45.05)	NS	772 (425–1285)	NS
4	32.40 (26.40–40.35)	≤ 0.005	529 (406–904)	≤ 0.05
8	27.40 (20.23–37.02)	≤ 0.005	453 (326–889)	≤ 0.05
12	26.20 (15.20–33.00)	≤ 0.005	322 (254–719)	≤ 0.01
16	19.70 (14.03–28.43)	≤ 0.005	335 (234–502)	≤ 0.005
20	23.20 (16.95–29.32)	≤ 0.005	485 (288–602)	≤ 0.05
24	17.70 (13.03–24.25)	≤ 0.001	372 (286–542)	≤ 0.005
Controls	13.05 (11.30–18.50)	≤ 0.001	184 (143–392)	≤ 0.001

*Paired comparisons: Wilcoxon signed-rank test (corrected for tied values); unpaired comparisons: Mann–Whitney U-test (corrected for tied values). IQR, Interquartile range; NS, not significant.

baseline to reach a point of greatest change at 24 weeks (2.4%; $P \leq 0.005$) and 16 weeks (42×10^6/l; $P \leq 0.05$), respectively (Table 2).

Treatment-induced decline in CD8+ T-cell values was comprised primarily of decreases within both the memory CD8+/CD45RO+ T-cell subset and the activated CD8+/HLA-DR+ and CD8+/CD38+ T-cell subsets. Percentage and absolute numbers of CD8+/CD45RO+ T cells decreased from baseline to reach a point of greatest change at 24 weeks (13.3%; $P \leq 0.001$) and 16 weeks (260×10^6/l; $P \leq 0.005$), respectively (Table 3). Activated CD8+ T-cell subsets exhibited similar trends, with percentage and absolute numbers of CD8+/HLA-DR+ T cells also decreasing from baseline to reach a point of greatest change at 24 weeks (6.9%; $P \leq 0.001$) and 16 weeks (130×10^6/l; $P \leq 0.005$), respectively. However, percentage and absolute numbers of CD8+/CD38+ T cells decreased from baseline to reach a point of greatest change at 24 weeks (17.7%; $P \leq 0.001$) and 12 weeks (322×10^6/l; $P \leq 0.01$), respectively. Reflecting the

early fluctuations observed for CD8+ T-cell absolute numbers, CD8+/CD45RO+, CD8+/HLA-DR+ and CD8+/CD38+ T-cell subset absolute numbers all underwent a transient, albeit non-significant, increase at 2 weeks of treatment (Table 3). In contrast, no consistent long-term increase in either the percentage or absolute numbers of naive CD8+/CD45RA+ T cells was observed, although absolute numbers transiently reached significant levels of change at 2 weeks of treatment (627×10^6/l; $P \leq 0.05$; Table 3).

Discussion

Given both the importance of antiretroviral therapy in initiating restoration of immune function and the unique character of T-cell subset involvement during asymptomatic HIV infection, the influence of HAART during early disease on T-cell activation and maturational defects is currently relevant to the planning of future therapeutic strategies. We now report the effectiveness of early commencement of HAART in reducing chronic levels of CD4+ and CD8+ T-cell activation, while reversing disease-induced imbalance in naive and memory CD4+ and CD8+ T-cell levels. We further report that HAART-induced correction of CD8+ T-cell activation and maturational defects during asymptomatic HIV infection can now be contrasted with comparable treatment of late disease in being of earlier onset and greater magnitude.

In particular, application of HAART during early asymptomatic HIV infection was observed to significantly decrease plasma HIV RNA levels within 4 weeks of treatment and initiate the return of peripheral blood CD4 : CD8 ratios to that approaching normal, confirming initial reports [11–16]. Correction of T-cell values was noteworthy in being preceded by a rapid and transient increase in absolute numbers of both CD4+ and CD8+ T cells. The immediate nature of these transient changes, the proportional involvement of both CD4+ and CD8+ T-cell subsets and the failure of these initial increases to correlate with reduction in viral load suggest the occurrence of very early treatment-induced redistribution of sequestered CD4+ and CD8+ T cells into the periphery [31]. Commencement of HAART during late symptomatic HIV infection has also recently been observed to initiate a significant increase in CD4+ T cells, coinciding with a significant decrease in CD8+ T cells [5–7]. However, whereas treatment-induced decline in CD8+ T cells during late disease is preceded by an extended period of CD8+ T-cell growth [6,7], it is now evident that treatment-induced decline in CD8+ T cells during early disease is noteworthy in occurring shortly after commencement of therapy.

The persistence of viral antigen during all stages of HIV infection functions as a chronic source of immune activation [32,33], actively supporting continued viral replication and aggravating disease progression by favouring T-cell anergy and apoptosis [3]. Elevated levels of activated CD8+ T cells expressing the MHC class II antigen HLA-DR are detectable during HIV infection, undergo considerable expansion at the time of seroconversion and continue to expand in correlation with the course of disease progression [34–36]. A less dramatic parallel increase in the proportion of activated CD4+/HLA-DR+ T cells, again correlating with the course of disease progression, has also been recognized during asymptomatic HIV infection [37,38]. Early commencement of HAART initiated an immediate and marked decrease in activated CD8+/HLA-DR+ T-cell values. A less pronounced, albeit significant, treatment-induced decline in activated CD4+/HLA-DR+ T-cell values was also observed. A trend towards normalization of elevated activated CD8+/HLA-DR+ and CD4+/HLA-DR+ T-cell values following HAART during advanced HIV infection has also recently been reported [6]. Although these results together demonstrate the independence of treatment-induced reversal of T-cell activation abnormalities on disease stage, it is now apparent that treatment-induced decline in activated CD8+/HLA-DR+ T-cell values during early disease reflects the rapid onset observed for change in CD8+ T-cell levels *per se*. In so much as HIV infection resembles a disease of hyperactivation [39], this observation underscores the likely therapeutic advantage to be gained from early suppression of chronic antigenic stimulation.

Levels of CD8+ T cells expressing the activation marker CD38 are also enhanced during early asymptomatic HIV infection [36,40,41] and correlate with increasing plasma viral load [42]. Change in CD8+/CD38+ T-cell levels has been demonstrated to function as a strong independent predictor of progression [36,40,41], with selective elevation of CD8+/CD38– T cells functioning as a marker of stable disease among long-term survivors [35]. Initiation of HAART during early HIV infection resulted in an immediate and marked decrease in activated CD8+/CD38+ T cells, which was comparable in magnitude and kinetics to the treatment-induced reduction in CD8+/HLA-DR+ T cells. Again, the increased scale and rapid onset of these post-treatment CD8+/CD38+ T-cell changes must be contrasted with HAART-induced reduction in activated CD8+/CD38+ T-cell levels observed during symptomatic infection [35]. Functionally, the CD8+/CD38+ phenotype has been linked to a population of HIV-specific cytotoxic T cells (CTL) present in both asymptomatic HIV-infected individuals and following development of AIDS [43]. The correlation between emergence of HIV-specific immune activity and poor

prognosis has been postulated to reflect harmful participation by HIV-specific CTL in the CD4+ T-cell depletion evident during chronic HIV infection [35] or exhaustion of HIV-specific CTL subsets [44]. The observed ability of early HAART to rapidly reduce CD8+/CD38+ T-cell levels suggests that initiation of therapy during asymptomatic disease is likely to be effective in forestalling development of detrimental CTL-derived pathology.

Differential expression of the tyrosine phosphatase isoforms CD45RA and CD45RO during T-cell differentiation provides a basis for phenotypic and functional discrimination between naive and memory T-cell subsets [45]. Evidence for the selective loss of CD4+/CD45RA+ and CD4+/CD45RO+ T cells during HIV infection has focused attention on the likely influence of fluctuation within these T-cell subsets on disease progression. Early asymptomatic HIV infection is characterized by selective depletion of memory CD4+/CD45RO+ T cells in both peripheral blood [22–24] and lymph nodes [25], contrasting with the eventual predominant loss of naive CD4+/CD45RA+ T cells during the later stages of HIV infection [26]. A functional basis for the selective *in vivo* loss of CD4+/CD45RO+ T cells during early HIV infection has been inferred from *in vitro* observation of preferential infection of CD4+/CD45RO+ T cells by HIV-1 [46–49]. Initiation of HAART during asymptomatic disease was associated with a delayed and moderate increase in both naive CD4+/CD45RA+ and memory CD4+/CD45RO+ T-cell values. This limited influence of therapy during asymptomatic infection contrasts with the large magnitude of increase in naive CD4+ T-cell subsets, as assessed by expression of both CD45RA and the adhesion molecule CD62L, and memory CD4+ T-cell subsets observed following HAART during late symptomatic disease [6,7]. The critical contribution of naive CD4+/CD45RA+ T cells to any potential treatment-induced recovery of immune function has been emphasized by the observation that expansion is highly dependent on the existence of naive CD4+/CD45RA+ T cells prior to initiation of therapy [50]. In this regard, evidence that HAART during asymptomatic HIV infection can effectively preserve and enhance pre-existing naive CD4+/CD45RA+ T-cell levels confirms a role for early treatment in forestalling the development of disease-induced maturation defects.

In comparison with the selective depletion of memory CD4+/CD45RO+ T cells during asymptomatic disease, early HIV infection is characterized by a significant expansion in the memory CD8+/CD45RO+ T-cell subset [23,51]. It is this phenotype that contributes predominantly to the steep increase in peripheral blood CD8+ T cells associated with early infection [18]. Conversely, depletion of naive CD8+/CD45RA+ T-cell levels begins during early disease and continues to fall in parallel with diminishing CD4+ T cells [23]. The observed minimal influence of early HAART on CD8+/CD45RA+ T cells reflects the lack of significant difference between pretreatment naive CD8+/CD45RA+ T cells for asymptomatic HIV-infected individuals and healthy individuals enrolled in this study. The less direct effect of reduction in viral load on CD8+/CD45RA+ T-cell recovery, as opposed to CD4+/CD45RA+ T-cell recovery, may also be partially responsible for the limited influence of therapy on this T-cell subset. However, commencement of HAART did initiate an immediate and marked decrease in memory CD8+/CD45RO+ T cells. These observations compare with recent assessment of HAART during late symptomatic HIV infection, during which increases within both the CD8+/CD45RA+ and CD8+/CD45RO+ T-cell subsets have been reported to contribute to an initial rise in CD8+ T cells [7]. During treatment of late disease, an eventual decline in CD8+/CD45RO+ T cells was not apparent until approximately 8 weeks of therapy [7], which also contrasts with the early onset and magnitude of therapy-induced CD8+/CD45RO+ T-cell reduction during early disease.

In comparison with other studies performed primarily in patients with more advanced HIV infection, our data suggests a dependency for immunological reconstitution on disease stage. The possible additional influence of prior antiretroviral therapy on HAART- induced T-cell subset changes remains to be fully assessed. Increases in CD4+ T cells and their subsets observed following HAART of advanced HIV infection were most pronounced in patients who had a durable virological response, which itself occurred more frequently in treatment-naive than in pretreated patients [52]. Although the influence of treatment history on T-cell subset changes *per se* cannot be excluded at present, the most important determinant of immunological reconstitution seems to be a sustained suppression of HIV replication, as demonstrated by the reduction in CD8+ T-cell activation levels and sustained increases in naive and memory CD4+ T cells even following HAART-based salvage of failed protease inhibitor monotherapy [53].

In conclusion, it is now apparent that early initiation of HAART during asymptomatic HIV infection is capable of rapidly reversing disease-induced T-cell activation and maturation abnormalities. These observations support the concept that therapeutic advantage is to be gained by commencing early and aggressive antiretroviral therapy.

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Appendix

Members of the Swiss HIV Cohort Study

M. Battegay (Scientific Board Co-chairman), E. Bernasconi, Ph. Bürgisser, M. Egger, P. Erb (Laboratory Group Chairman), W. Fierz, M. Flepp (Clinical Group Chairman), P. Francioli (President of the SHCS, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland), P. Grob, B. Hirschel (Scientific Board Chairman), B. Ledergerber, R. Malinverni, L. Matter, M. Opravil, F. Paccaud, G. Pantaleo, L. Perrin, W. Pichler, J.-C. Piffaretti, M. Rickenbach (Data Centre Manager), J. Schüpbach, A. Telenti, P. Vernazza and R. Weber.