Original article

Early initiation of highly active antiretroviral therapy fails to reverse immunovirological abnormalities in gut-associated lymphoid tissue induced by acute HIV infection

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Background: During the acute phase of HIV infection, large CD4+ T-cell depletion occurs in the gastrointestinal tract. The kinetics of CD4+ T-cell decrease and highly active antiretroviral therapy (HAART)-mediated immune reconstitution were evaluated.

Methods: Rectosigmoid colonic (RSC) biopsies and blood samples of nine patients with acute HIV infection were collected. CD4+ T-cell count, HIV RNA, intracellular HIV DNA and messenger RNA cytokine expression were evaluated before and after 6 months of HAART.

Results: All nine patients presented symptomatic retroviral infection. Early HAART was associated with a sustained and comparable reduction of HIV RNA in plasma, peripheral blood mononuclear cells (PBMCs) and RSC biopsies. HIV DNA decreased in PBMCs, but was only marginally reduced in RSC biopsies. Comparisons between reduction rates of HIV DNA in these two compartments confirmed that HIV DNA clearance was less efficient in RSC biopsies compared with PBMCs. Assessment of immunological profiles in PBMCs and RSC biopsies showed that the T-helper (Th)1-like/Th2-like ratio was sharply decreased in RSC biopsies and increased in PBMCs throughout the study period. A persistent Th2-like profile was detected in RSC biopsies. Efficient clearing of HIV DNA observed in PBMCs correlated with the establishment of a more favourable Th1-like profile.

Conclusions: A less efficient clearance of intracellular HIV DNA following early introduction of HAART is associated with persistent immunological impairment in gut-associated lymphoid tissue (GALT), which is reflected by the skewed expression of cytokines in this reservoir. The present study shows that early initiation of HAART, in the short-term, is not effective in containing the establishment of HIV infection and in reversing associated immunological GALT abnormalities.

Introduction

The hallmark of HIV infection is the depletion of CD4+ T-cells, which occurs during both the acute and chronic phases of infection and is mediated by different pathogenetic mechanisms [1,2]. This loss of CD4+ T-cells has been thoroughly investigated in peripheral blood, where only 2–5% of the body's lymphocytes reside [3,4]. Gut-associated lymphoid tissue (GALT) harbours >60% of total T-lymphocytes [4,5] and recent studies of HIV-infected individuals have confirmed that primary HIV infection (PHI) is associated with a preferential depletion of activated memory CD4+ T-cells in the gastrointestinal (GI) tract [3,6–8]. The peculiar depletion of CD4+ T-cells in the GI mucosa occurs before changes are seen in peripheral blood and affects this compartment to a greater extent with respect to peripheral blood and lymph nodes [9–11]. Nonetheless, the mechanisms behind this profound loss of CD4+ T-cells in the GI tract and the reasons why it is only partially reflected in the periphery have yet to be understood.

Early introduction of highly active antiretroviral therapy (HAART) in PHI has been proven to be virologically and immunologically beneficial [12,13]; however,

clinical implications of early versus deferred therapy remain controversial [14,15]. Understanding how antiretroviral therapy affects GALT in the course of PHI is thus of pivotal importance, as it might prove useful in comprehending why the majority of patients treated during acute infection fail to achieve complete reconstitution of the $CD4^+$ T-cell subset in the GI mucosa despite undetectable HIV viraemia and normalization of the $CD4^+/CD8^+$ T-cell ratio [3,16–18].

In order to shed light on the kinetics of $CD4^+$ T-cell depletion and restoration in GALT during PHI, plasma samples, peripheral blood mononuclear cells (PBMCs) and rectosigmoid colonic (RSC) biopsies of patients with acute HIV infection were examined prior to and following 6 months of HAART initiation. Plasma HIV RNA and intracellular HIV DNA levels, $CD4^+$ T-cell counts and expression of a panel of cytokines were determined in all compartments before and after HAART in order to assess possible correlations between the immunovirological parameters and the effects of antiretroviral therapy on the HIV clearance of peripheral reservoirs and GALT.

Methods

Study patients

A total of nine patients with PHI were included in a study approved by an institutional review board (Luigi Sacco Hospital, Milan, Italy). All patients were consecutively enrolled and prospectively evaluated at the Institute of Infectious Diseases and Tropical Medicine (University of Milan, Milan, Italy). HIV infection was determined with an ELISA (detecting anti-HIV antibodies) and seroconversion was confirmed by a positive western blot. Patients with a time delay of >6 months between the last negative and first positive antibody testing were excluded from the study. Acute HIV infection was defined by presence of signs and symptoms of acute retroviral syndrome, high risk exposure to HIV in the past 3 months, detectable plasma HIV RNA levels and either a negative result from an ELISA or a positive result from an ELISA with an indeterminate western blot analysis result (<3 bands). RSC biopsies and peripheral blood samples were obtained before HAART initiation and after 6 months of therapy. All nine patients initiated antiretroviral treatment at the time of acute HIV infection diagnosis according to international guidelines [19] for antiretroviral treatment and remained on treatment during the entire 6 month period of the study.

Sample collection and preparation

Flow cytometry (Coulter ESP; Beckman Coulter, Hialeah, FL, USA) was performed on freshly isolated PBMCs. PBMCs of HIV-infected patients were obtained by gradient separation from EDTA-treated blood samples. PBMCs were recovered, washed 3× in RPMI, centrifuged, pelleted and cryopreserved in aliquots of 5×10^6–10×10^6 viable cells. RSC biopsies were snap-frozen by total immersion in liquid nitrogen and stored for biomolecular analysis.

Immunophenotype analysis

Immunophenotyping of lymphocytes was performed by flow cytometry (Coulter ESP; Beckman Coulter), using labelled antibodies specific for cell surface markers. Directly labelled (fluorescin isothiocynate [FITC], phycoerythrin [PE] and peridinin chlorophyll protein [PerCP]) antibodies were used (CD3–PerCP, CD4–FITC, CD4–PerCP and CD8–PE; Becton Dickinson; San José, CA, USA).

HIV nucleic acid extraction and quantification

HIV RNA viral load in plasma samples were quantified using a nucleic acid signal amplification assay (bDNA; Quantiplex, Chiron, CA, USA), which has a lower detection limit of 50 HIV RNA copies/ml plasma. Cell- and tissue-associated viral DNA and RNA were extracted using the High Pure PCR Template Preparation Kit (Roche Molecular Systems, Branchburg, NJ, USA) and QIAamp Viral RNA Kit (QIAGEN, Inc., Valencia, CA, USA). Total RNA for cytokine production was extracted from PBMCs and biopsy specimens using the acid guanidium thiocyanate-phenol-chloroform extraction method [20,21]. The purity of the extracted RNA was determined by spectrophotometry (Bio-Rad, Hercules, CA, USA). RNA was treated with RNase-free DNase (RQ1 DNase; Promega, Madison, WI, USA) to remove contamination of genomic DNA [20,21]. Quantification of HIV DNA and RNA in PBMCs and tissue was achieved using the previously described reverse transcriptase (RT) competitive PCR method [22].

HIV drug resistance assays

Cell-associated HIV DNA was amplified using an in-house procedure capable of obtaining a *pol* gene segment of approximately 1,200 nucleotides as previously described [23]. The PCR products were sequenced with an ABI 3100 Automated Capillary Sequencer (Applied Biosystems, Foster City, CA, USA).

The presence of mutations associated with drug resistance was defined according to the list provided by the Drug Resistance Mutations Group of the International AIDS Society [24].

Reverse transcription for cytokine analysis

Total RNA (1 μg) from PBMCs and from biopsy specimens was reverse transcribed into first-strand complementary DNA (cDNA) in a 20 μl final volume containing 1 μM of random hexanucleotide primers, 1 μM of oligo dT and 200 U of Molony murine leukaemia virus reverse transcriptase (Clontech, Palo Alto, CA, USA).

Quantification of cytokine cDNA by PCR analysis

In order to compare cytokine messenger RNA expression in different samples, equivalent amounts of substrate cDNA were used. Therefore, all samples were normalized for β-actin cDNA content by competitive PCR (Clontech) and cytokine analyses (interleukin [IL]-2, IL-4, IL-6, IL-10, IL-12, tumour necrosis factor [TNF]-α, TNF-β and interferon [IFN]-γ) were performed as previously described [25]. Finally, to quantify relative levels of gene expression, gel bands were scanned by transmission densitometry (Bio-Rad) and the peak areas were calculated in arbitrary units. To evaluate the relative levels of expression of the target genes in RT-PCR, the value of the internal standard (β-actin) in each test tube was used as the baseline gene expression for that sample and relative values were calculated for each of the target genes amplified in that reaction. These values were then used to compare expression across the tested samples.

Statistical analyses

Paired Student's *t*-tests and Wilcoxon signed-rank tests were performed for normally and not normally distributed variables, respectively. All *P*-values produced were regarded as descriptive and no inferences were made. Statistical analyses were performed using SPSS software (SPSS, Inc., Chicago, IL, USA).

Results

Patient characteristics

A total of nine consecutively observed patients infected with acute HIV were recruited. All patients presented symptomatic acute retroviral infection (Table 1). The estimated mean (range) interval between presumed HIV infection and HAART initiation was 26 days (13–45). The mean ±SD $CD4^+$ T-cell count and HIV RNA viral load at baseline were 482 ±109 cells/μl and 5.11 ±0.62 $\log_{10}$ copies/ml, respectively. No differences were observed among patients regarding demographic, clinical and HIV-related parameters. All patients were initiated on a HAART regimen containing two nucleoside reverse transcriptase inhibitors (NRTIs) and one protease inhibitor (Table 1). Blood collection for drug resistance tests was performed at baseline concurrently with HAART introduction, meaning that the choice of antiretrovirals was not on the basis of drug resistance genotyping results.

RNA analyses

As expected, HAART resulted in a marked decrease of HIV RNA plasma levels in all patients (Figure 1A). The mean ±SD value of plasma HIV viraemia in the nine patients decreased from 5.11 ±0.62 $\log_{10}$ copies/ml at baseline to 2.38 ±1.10 $\log_{10}$ copies/ml after 6 months of HAART (mean ±SE decrease 2.73 ±0.41 $\log_{10}$ copies/ml; *P*=0.0001; Table 2). Suppression of HIV RNA levels was not achieved in all individuals; patients 1, 5 and 6 were still viraemic at the end of the study period (Table 2). Persistence of HIV RNA at follow-up was not correlated with levels of HIV viraemia at baseline.

HIV RNA also decreased in PBMCs after 6 months of antiretroviral therapy in all but one patient (patient 5; Figure 1B). The mean ±SD value of HIV RNA expression in PBMCs at baseline was 2.50 ±0.53 $\log_{10}$ copies/10^6 cells and 1.48 ±0.77 $\log_{10}$ copies/10^6 cells after 6 months of HAART (mean ±SE decrease of 1.03 ±0.22 $\log_{10}$ copies/10^6 cells; *P*=0.0007; Table 2).

Early initiation of therapy also resulted in a sustained decrease of HIV RNA levels in the all RSC biopsies of patients with a mean ±SD value of 2.57 ±0.47 $\log_{10}$ copies/μg at baseline and 1.45 ±0.69 $\log_{10}$ copies/μg at the end of the study period (mean ±SE decrease in RSC 1.12 ±0.24 $\log_{10}$ copies/μg; *P*=0.0007; Table 2). Analogously to what was observed in plasma, patients 1, 5 and 6 experienced the smallest reduction of HIV RNA levels in their RSC biopsies (Figure 1C).

HIV RNA reduction rates were compared in all compartments. Results showed that HIV was cleared at a higher level in plasma compared with PBMCs (*P*=0.03). Interestingly, no statistically significant difference was seen when the reduction of HIV RNA in PBMCs and RSC biopsies were compared, suggesting that early initiation of HAART resulted in effective viral control in both reservoirs.

DNA analyses

The mean ±SD value of HIV intracellular DNA at baseline was 2.21 ±0.28 $\log_{10}$ copies/10^6 cells in PBMCs and 1.50 ±0.61 $\log_{10}$ copies/μg in RSC biopsies. HAART for 6 months resulted in a sustained decrease of intracellular DNA in PBMCs where the mean ±SD HIV intracellular DNA was 1.37 ±0.45 $\log_{10}$ copies/10^6 cells (mean ±SE decrease of 0.85 ±0.15 $\log_{10}$ copies/10^6 cells; *P*=0.0002; Table 2). Again patients 1, 5 and 6 presented the smallest decrease of intracellular HIV DNA in PBMCs (Figure 2A). By contrast, after 6 months of therapy, intracellular HIV DNA in RSC lymphoid tissues was only partially reduced (mean HIV DNA level 1 $\log_{10}$ copies/μg and mean ±SE decrease 0.50 ±0.20 $\log_{10}$ copies/μg; *P*=0.04; Table 2 and Figure 2B). Moreover, the comparison between the rate of reduction of HIV intracellular DNA in these two reservoirs showed that clearance of HIV intracellular DNA was less efficient in RSC tissue compared with PBMCs (*P*=0.07).

Finally, we compared the rate of HIV RNA and DNA decay in PBMCs and RSC biopsies. Results showed that HIV RNA did not decrease to a greater extent than intracellular HIV DNA levels, in either compartment (*P*=0.04 and *P*=0.02, respectively). This is in sharp contrast to prior descriptions of chronic infection, where

Table 1. Characteristics of patients at baseline and after 6 months of HAART

Patient	Age, years	Sex	Risk factor	Severity of symptoms	Time from symptoms to therapy, days	HAART regimen	Drug resistance mutations	$CD4^+$ T-cell count at baseline, cells/μl	$CD4^+$ T-cell count after 6 months of HAART, cells/μl	$CD8^+$ T-cell count at baseline, cells/μl	$CD8^+$ T-cell count after 6 months of HAART, cells/μl	Plasma HIV RNA at baseline, $\log_{10}$ copies/ml	Plasma HIV RNA after 6 months of HAART, $\log_{10}$ copies/ml
1	36	Male	Homosexual	Moderate	48	AZT+ 3TC+ SQV	70R/K, 215Y	344	382	2,987	1,557	5.09	3.46
2	45	Male	Homosexual	Severe	25	AZT+ 3TC+ SQV	None	537	842	3,020	986	4.72	1.69
3	21	Male	Homosexual	Mild	40	AZT+ 3TC+ IDV	None	415	1,652	1,957	643	4.46	1.69
4	23	Male	Homosexual	Moderate	32	AZT+ 3TC+ IDV	None	509	1,284	3,297	462	4.46	1.69
5	35	Male	Homosexual	Moderate	45	AZT+ 3TC+ SQV	215Y	612	645	2,646	1,906	5.22	4.57
6	20	Male	Homosexual	Mild	20	AZT+ 3TC+ IDV	225H	386	761	1,863	820	5.13	3.28
7	41	Male	Homosexual	Moderate	49	AZT+ 3TC+ IDV	None	367	980	3,497	646	4.72	1.69
8	26	Male	Homosexual	Severe	18	AZT+ 3TC+ IDV	None	646	1,290	7,690	863	6.06	1.69
9	38	Male	Homosexual	Severe	13	AZT+ 3TC+ IDV	None	517	1,061	3,186	485	6.12	1.69

AZT, zidovudine; HAART, highly active antiretroviral therapy; IDV, indinavir; SQV, saquinavir; 3TC, lamivudine.

HIV DNA has been said to take longer to clear with respect to HIV RNA [26,27].

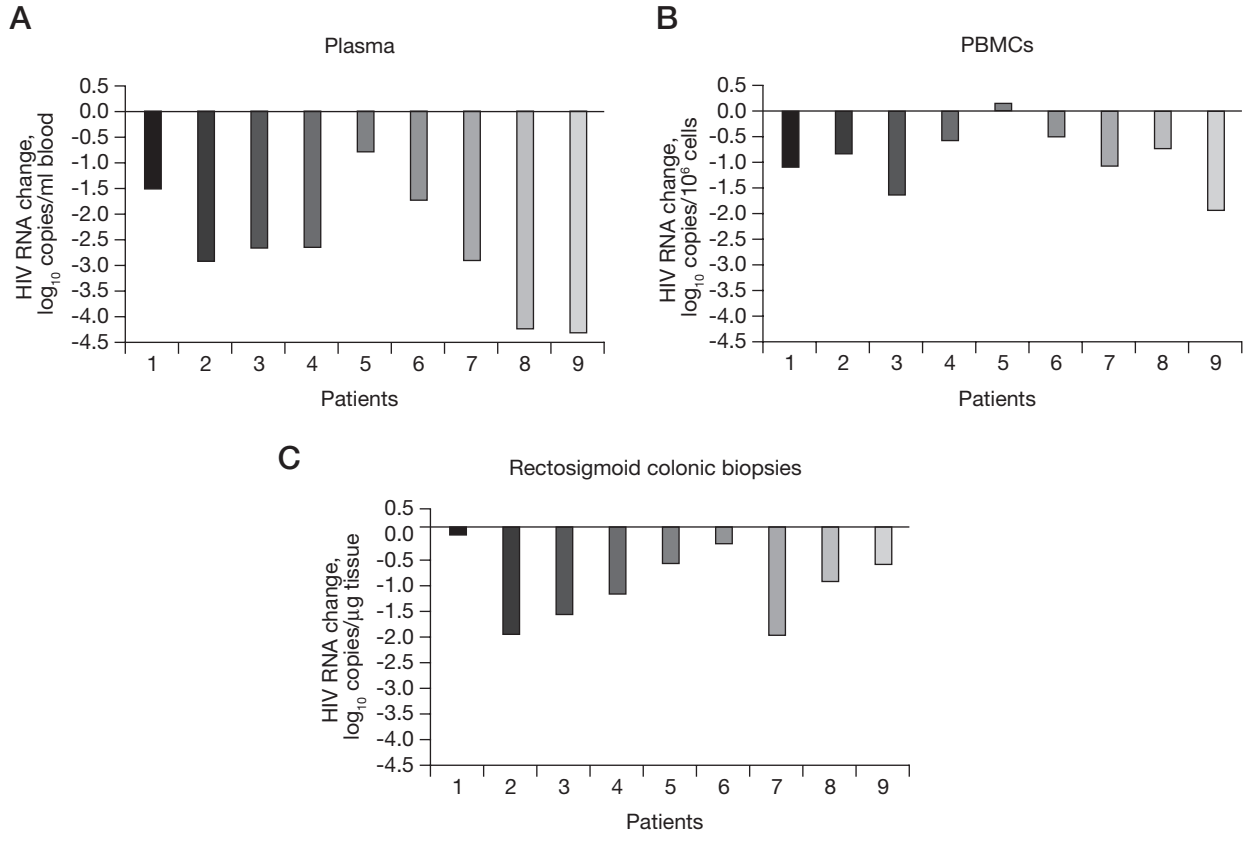
T-lymphocyte cell subsets

Mean ±SD $CD4^+$ T-cell counts at baseline were 482 ±109 cells/μl and had increased dramatically after 6 months of HAART (989 ±385 cells/μl; P=0.002). $CD8^+$ T-cells decreased after 6 months of therapy from a mean ±SD count of 3,349 ±1,724 cells/μl to 930 ±493 cells/μl (P=0.002). In the three patients with detectable viraemia after HAART, these changes were more modest (Table 1).

Drug resistance assays

Drug resistance assays were performed on plasma samples of all patients in the course of the study with the objective of defining whether patients harboured wild-type or HIV-resistant variants. No drug-resistance-associated mutations were observed in patients with an optimal immunovirological response to HAART. However, RT resistance mutations were detected in the three patients with detectable HIV RNA levels and a modest increase in $CD4^+$ T-cells at follow-up (patients 1, 5 and 6). In particular, two (70R/K and 215Y) and one (215Y) NRTI-related mutations were observed in

Figure 1. HIV RNA change from baseline to after 6 months of HAART in the three compartments examined



(A) Plasma. **(B)** Peripheral blood mononuclear cells (PBMCs). **(C)** Rectosigmoid colonic biopsies. HAART, highly active antiretroviral therapy.

Table 2. Virological features of HIV RNA and HIV DNA at baseline and after 6 months of HAART in plasma, PBMCs and RSC biopsies

Measure	Plasma HIV RNA, $\log_{10}$ copies/ml	PBMCs HIV RNA, $\log_{10}$ copies/10^6 cells	RSC HIV RNA, $\log_{10}$ copies/μg	PBMCs HIV DNA, $\log_{10}$ copies/10^6 cells	RSC HIV DNA, $\log_{10}$ copies/μg
Baseline	5.11 (0.62)	2.50 (0.53)	2.57 (0.47)	2.21 (0.28)	1.50 (0.61)
After 6 months of HAART	2.38 (1.10)	1.48 (0.77)	1.45 (0.69)	1.37 (0.45)	1.00 (0.00)
Mean change (±SE)	2.73 (0.41)	1.03 (0.22)	1.12 (0.24)	0.85 (0.15)	0.50 (0.20)

Values are mean ±SD unless indicated otherwise. HAART, highly active antiretroviral therapy; PBMCs, peripheral blood mononuclear cells; RSC, rectosigmoid colonic.

patients 1 and 5, respectively. Patient 6 harboured a non-NRTI-related mutation at position 225 of the RT region, which did not justify the poor immunological response to antiretroviral treatment (Table 1).

Cytokine expression levels

In order to evaluate the effect of HIV viral burden and therapy on immune response in different compartments, the expression of a panel of cytokines (IL-2, IFN-γ, TNF-α, TNF-β, IL-6, IL-12 and IL-10) was assessed in both RSC lymphoid tissue and PBMCs at baseline and after 6 months of HAART.

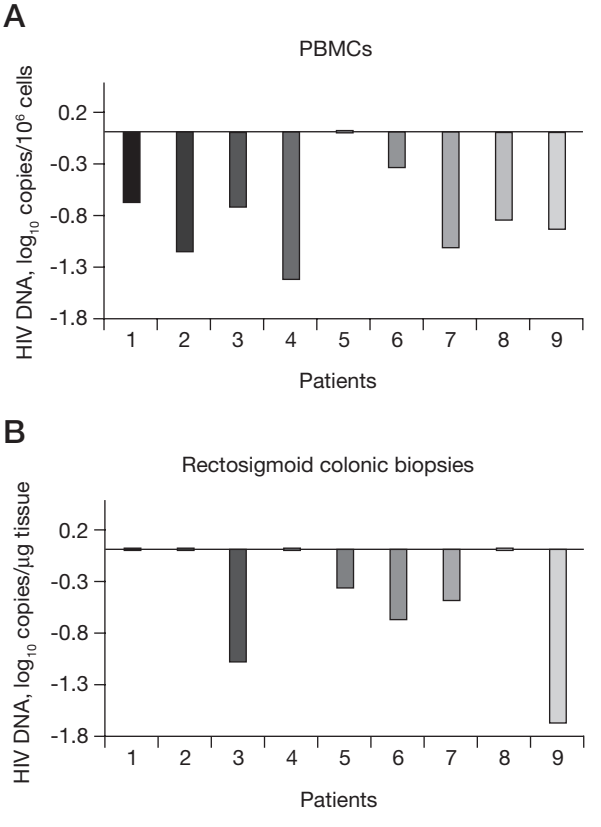
Higher IL-2 and lower IFN-γ levels were detected in RSC biopsies compared with PBMCs at baseline (P=0.03 and P=0.004, respectively). The sustained reduction of HIV levels seen after 6 months of therapy did not correlate with major changes in the expression of these two cytokines in RSC. A different pattern was detected in PBMCs, where clearance of HIV resulted in a sharp increase in both IL-2 and IFN-γ levels (P=0.02 and P=0.03, respectively; Figure 3). TNF-β expression was comparable at baseline in both compartments. Levels of this cytokine were constant throughout the study in RSC biopsies, but showed a sustained increase in PBMCs after 6 months of therapy (Figure 3).

TNF-α and IL-6 levels were considerably higher at baseline in PBMCs (P=0.03 and P=0.004, respectively) compared with RSC biopsies; therapy was associated with a major decrease of these cytokines in PBMCs (P=0.04 for both TNF-α and IL-6), but not in GI mucosa (Figure 4). Finally, no differences were observed in IL-12 and IL-10 at baseline in PBMCs or RSC biopsies; these cytokines did not change considerably throughout the study in either reservoir (Figure 4).

The IL-2/IL-10 ratio was calculated in both compartments. Higher values were observed in RSC tissue compared with PBMCs at baseline (0.27 in RSC versus 0.01 in PBMCs). HAART for 6 months resulted in a slight decrease of IL-2/IL-10 ratio (0.21) in RSC biopsies, with a consequent predominant T-helper (Th)2-like pattern at the end of the study period. This was in sharp contrast to what was observed in PBMCs at the end of the study period, where a marked increase of the IL-2/IL-10 ratio (0.18) resulted in a dominant Th1-like profile. Interestingly, a more pronounced Th2 cytokine profile was observed in both RSC and PBMCs from the three patients presenting less efficient clearing of HIV RNA plasma and intracellular HIV DNA.

Overall, analysis of cytokine expression in the two compartments underlines a dichotomous trend. These findings are concordant with the virological results: the slow decrease of HIV DNA in lymphoid tissue was associated with a persistent Th2-like profile and by contrast, the efficient clearing of HIV DNA observed in PBMCs correlated with the establishment of a possibly more favourable Th1-like profile.

Figure 2. HIV DNA change from baseline to after 6 months of HAART

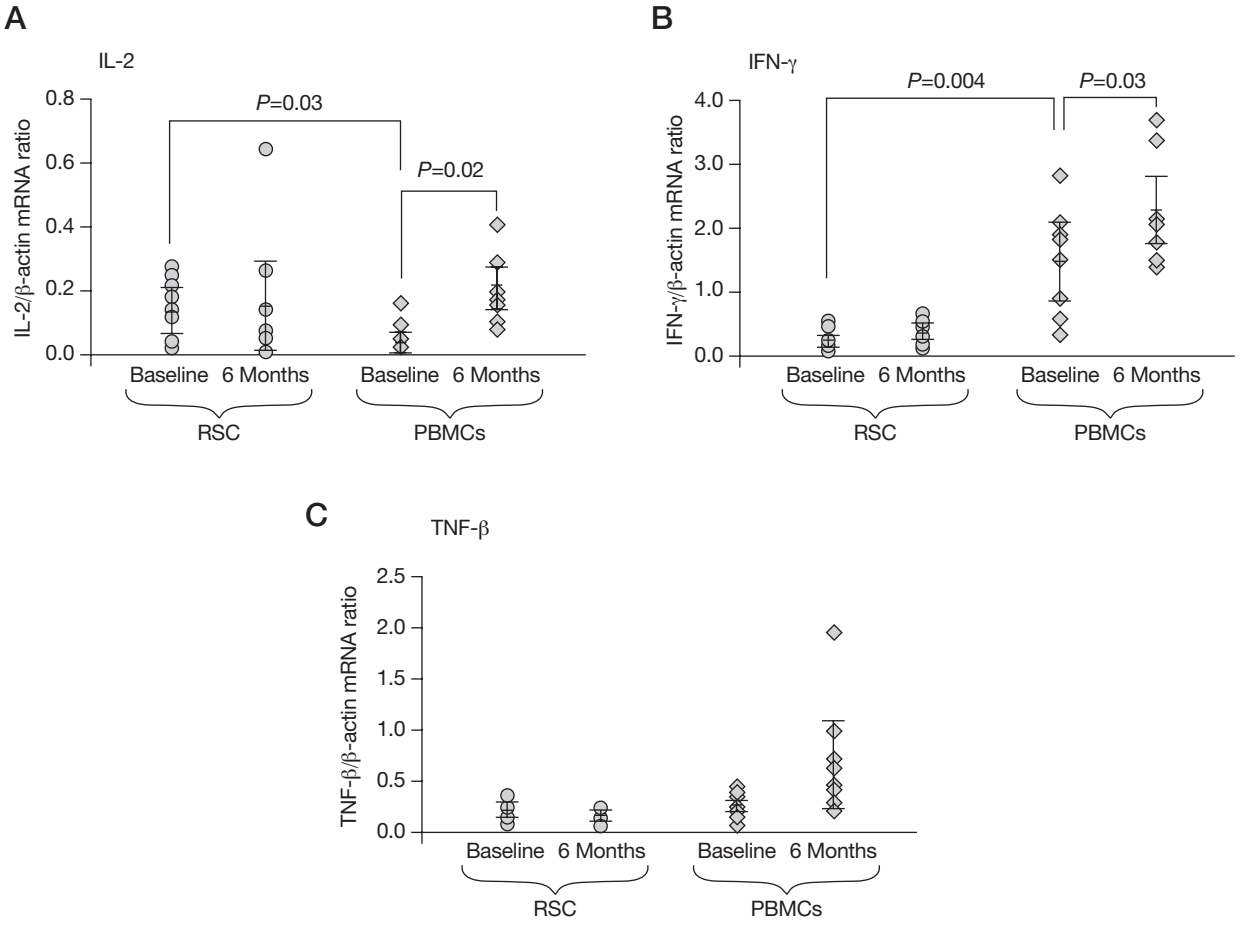


(A) Peripheral blood mononuclear cells (PBMCs). **(B)** Rectosigmoid colonic biopsies. HAART, highly active antiretroviral therapy.

Discussion

Recent data have shown that the bulk of CD4+ T-cell destruction is observed at the intestinal mucosal surface within a few days after HIV infection [3,8]. This early CD4+ T-cell strongly affects the pathogenesis of HIV disease [28,29] and occurs before changes are seen in the periphery [9–11]. Early initiation of therapy, with a resulting precocious and robust suppression of HIV replication at the mucosal level, could therefore have a favourable effect on the subsequent course of infection. Nevertheless, results to date show that the profound alterations of GALT during acute HIV infection do not revert during the initial 6 months of HAART despite early introduction of antiretroviral therapy. Accordingly, a less efficient clearance of intracellular HIV DNA was observed in lymphoid tissue compared with peripheral reservoirs and the persistence of virological impairment was associated with a protractedly

Figure 3. IL-2, IFN-γ and TNF-β levels at baseline and after 6 months of HAART in rectosigmoid colonic tissue and PBMCs



(A) Interleukin (IL)-2, **(B)** interferon (IFN)-γ and **(C)** tumour necrosis factor (TNF)-β levels at baseline and after 6 months of highly active antiretroviral therapy (HAART). Cytokine expression did not show major modifications in rectosigmoid colonic (RSC) biopsies, whereas IL-2 levels decreased and IFN-γ and TNF-β levels increased in peripheral blood mononuclear cells (PBMCs) throughout the study. Paired Student's *t*-test and Wilcoxon signed-rank test were performed for normally and not normally distributed variables, respectively. All *P*-values produced were regarded as descriptive and no inferences were made. mRNA, messenger RNA.

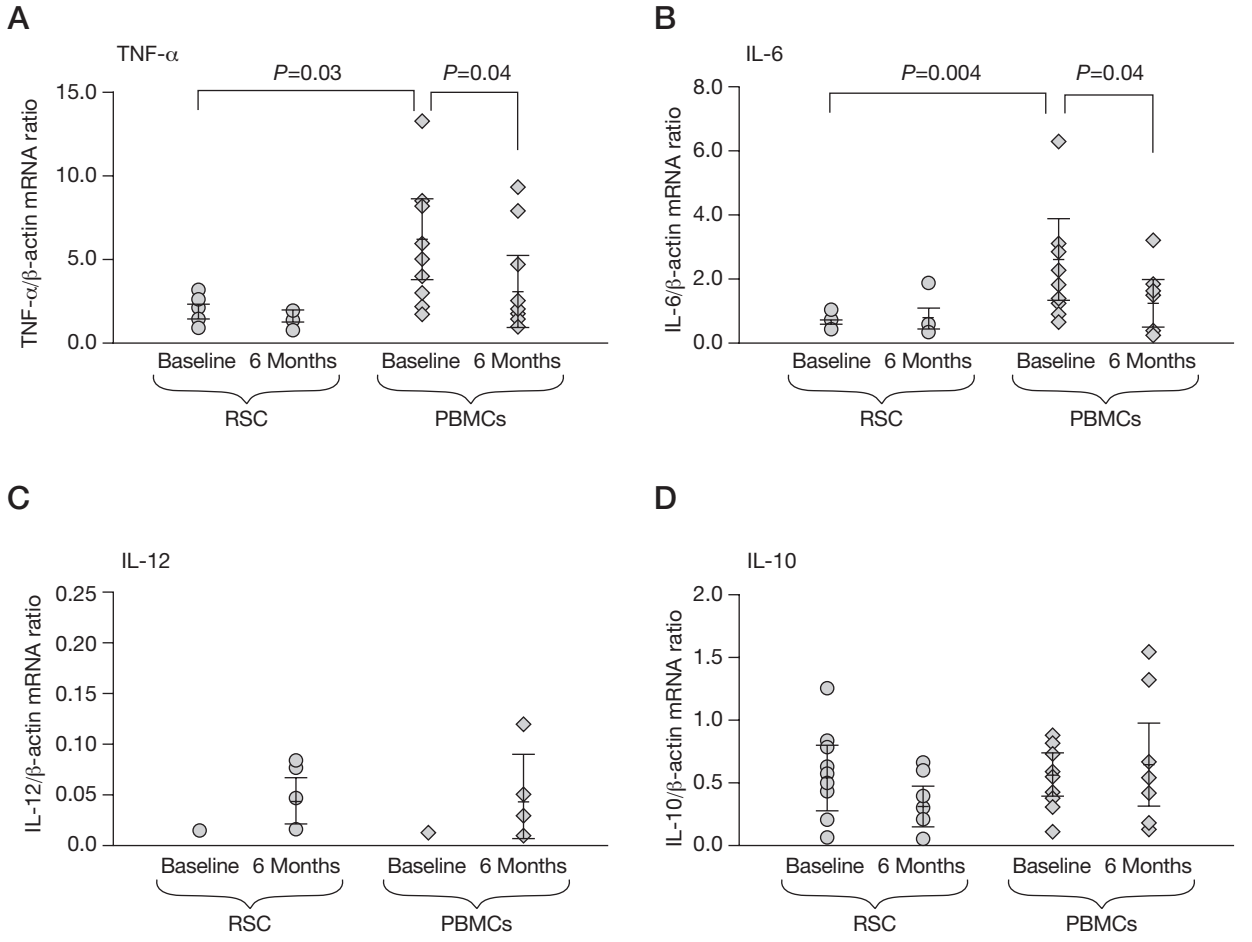
poor immunological profile in GALT as compared with peripheral lymphocyte reservoirs.

We initially examined the decay of HIV RNA levels in plasma, PBMCs and RSC lymphoid tissue. Although HIV RNA levels decreased considerably in all three compartments, not all patients in our study achieved undetectable HIV RNA levels in plasma. In particular, three patients did not present complete suppression of HIV viraemia despite antiretroviral treatment. Interestingly, drug resistance assays showed the presence of mutations conferring resistance in two of the three patients described earlier. These data confirm the need to perform drug resistance assays prior to commencement of HAART during PHI and also suggest that other factors besides drug resistance, including the type of virus involved and the host's immune response or genetic profile, might account for an impaired immunovirological response to HAART [30].

In order to further delineate the varied kinetics of HIV RNA suppression, reduction rates of HIV RNA were compared in all three compartments. HIV viraemia decayed more rapidly in plasma compared with PBMCs and GALT, suggesting that HAART might have a weaker effect on tissue reservoirs than on peripheral replicating virus. In fact, these findings confirm the efficacy of antiretroviral therapy in decreasing HIV RNA levels in all the compartments examined. Nonetheless, the results regarding rates of HIV RNA decay clearly underline the limited effect that antiretroviral therapy exerts on the inhibition of early virus replication in tissue reservoirs, confirming that data obtained from studies conducted in the periphery do not necessarily reflect the immunovirological interactions occurring in the extravasal compartments during HAART.

Furthermore, on the basis of recent data suggesting that higher levels of HIV DNA might account for the

Figure 4. TNF-α, IL-6, IL-12 and IL-10 levels at baseline and after 6 months of HAART in RSC tissue and PBMCs



Expression of **(A)** tumour necrosis factor (TNF)-α and **(B)** interleukin (IL)-6 was higher in peripheral blood mononuclear cells (PBMCs) at baseline compared with rectosigmoid colonic (RSC) tissue and therapy was associated with a decrease of these two cytokines in PBMCs only. No differences were observed in the levels of **(C)** IL-2 and **(D)** IL-10 at baseline in PBMCs or RSC tissue and the expression of these two cytokines did not vary throughout the study in both reservoirs. Paired Student's *t*-test and Wilcoxon signed-rank test were performed for normally and not normally distributed variables, respectively. All *P*-values produced were regarded as descriptive and no inferences were made. HAART, highly active antiretroviral therapy.

incomplete immune reconstitution seen in chronic HIV infection despite complete HIV viremia suppression [31], we investigated the effect of antiretroviral therapy on intracellular HIV DNA, comparing HIV DNA clearing in GALT and other reservoirs. We hypothesized that the weak immunomodulatory effect of early HAART on GALT during PHI [18] could be explained by a less effective control of intracellular HIV DNA in this particular reservoir. Accordingly, we analysed the content of intracellular DNA prior to and following initiation of antiretroviral therapy in PBMCs and GALT, and we compared the dynamics of HIV RNA and intracellular HIV DNA decay in the two reservoirs. A considerable decrease in HIV intracellular DNA was observed in PBMCs but not in GALT. The limited therapy-associated clearing of intracellular HIV DNA seen in GALT during PHI indicates that even early HAART is unable to prevent the establishment of HIV infection in GALT.

Further evidence that the effect of early therapy is limited in GALT is offered by the altered cytokine expression seen in these tissues, which persists even in the presence of therapy. Accordingly, although it has been suggested that the efficient control of viral replication correlates with the presence of a Th1-like cytokine pattern [32,33], it would seem to follow that the persistence of a Th2 cytokine profile in GALT underlines the persistence of immunovirological impairment in the GI mucosa.

It is, however, important to note that this study has several limitations, represented firstly by the small number of patients analysed, which was caused by the difficulty of finding access to patients with acute HIV infection, and secondly, by the evidence that the patients included in the study were mostly treated

with older generation antiretroviral drugs and for only 6 months of short-term duration. This second point introduces the intriguing issue of whether newer and more potent antiretroviral treatments will realistically be effective where the older generation drugs failed, particularly in reducing HIV DNA level and clearing HIV reservoirs.

In conclusion, despite the limitations of the study, we have shown that GALT is a unique site of HIV infection that behaves differently compared with other reservoirs during PHI, as demonstrated by the lack of efficient clearing of intracellular HIV DNA levels, despite early introduction of antiretroviral therapy. A weaker control over infection might account for the poor immunological profile in GALT compared with peripheral reservoirs. Prevention of mucosal assault by means of alternative therapeutic strategies might represent the turning point in the treatment of acute HIV infection and optimize the use of HAART in this key phase of HIV disease.

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Disclosure statement

All the authors have contributed significantly to the preparation of the paper, have seen and approved the final version of the manuscript. The authors declare no competing interests.

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