Changes in levels of immune activation and reconstitution markers among HIV-1-infected Africans receiving antiretroviral therapy

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Objective: To describe changes in immune activation and reconstitution markers among HIV-1-infected patients receiving antiretroviral therapy (ART) in Abidjan, Côte d'Ivoire.

Methods: Between November 1998 and February 2001, we analyzed changes in immune activation and reconstitution markers among 52 patients. Good virologic responders (n = 26) were defined as those who had suppressed and maintained plasma viral load (VL) below the detection limit of the assay for at least 12 months. Poor virologic responders (n = 26) were defined as those with a detectable VL at 6 and 12 months after beginning ART.

Results: Of the 26 good virologic responders, 20 (77%) were on highly active antiretroviral therapy (HAART) compared with one (4%) of the poor responders. Among the 26 good responders, baseline median levels of CD38+CD8+ T cells were elevated, but had decreased significantly at 6 months ($P < 0.001$) and at 12 months of therapy ($P < 0.001$). Median levels of HLA-DR+CD8+ T cells also decreased from baseline at 6 months ($P < 0.001$) and at 12 months of therapy ($P < 0.001$). Levels of CD62L+CD4+ T cells increased steadily during the 6 and 12 months of therapy and reached levels observed among HIV-negative blood donors ($P = 0.07$). Among the 26 poor responders, median levels of CD38+CD8+ T cells decreased significantly at 12 months of therapy ($P = 0.006$), but were higher than levels in blood donors ($P = 0.005$). Levels of HLA-DR+CD8+ T cells decreased significantly at 12 months of therapy ($P < 0.001$). Levels of CD62L+CD4+ decreased over time.

Conclusion: Our results suggest that HAART can be successfully used in African populations with elevated baseline immune activation markers.

AIDS 2003, **17** (suppl 3):S17–S22

Keywords: Antiretroviral therapy, CD62L, CD38, HIV-1, HLA-DR, immune activation, immune reconstitution, viral load

Introduction

Highly active antiretroviral therapy (HAART) suppresses viral replication, increases CD4+ T cells, and reduces mortality rates [1–3]. Because of the cost of HAART and complexity of administration and laboratory monitoring, it has not been widely used in Africa. However, due to international efforts and the drastic reduction of

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prices of antiretroviral therapy (ART) drugs, several countries in Africa have started pilot programmes to treat HIV-infected persons. Effective use of antiretroviral (ARV) drugs requires patient monitoring for CD4+ T-cell count, plasma RNA viral load, and ARV resistance testing. In Africa, it will be necessary to evaluate various biologic factors that may influence virologic response to therapy. A study conducted in Italy has shown that high pre-treatment levels of markers of immune activation, such as levels of expression of CD38+ on CD8+ T cells, predict maintenance of high viremia in HIV-1-infected patients receiving HAART [4]. Immune activation markers are prognostic markers of HIV disease progression prior to HAART or effective antiretroviral treatment [5–7] suggesting that activation may strongly correlate with HIV-RNA viral load. For instance, one study found that a 10% increase in levels of CD38+CD8+ T cells resulted in an 88% increase in the risk for AIDS [6]. Compared with persons in Europe and United States, HIV-infected and uninfected Africans have three- to four-fold higher levels of immune activation markers such as expression of CD38+ and HLA-DR on CD8+ T cells [8–11]. To date, no studies have been published examining immune activation and reconstitution markers among patients receiving ARV drugs in Africa. Understanding the interaction of these markers and antiretroviral therapy may provide valuable information for successful use of ART in Africa. In this study, we compared changes in markers of immune activation and reconstitution among HIV-1-infected patients receiving HAART or dual nucleoside therapy which resulted in good and poor virologic responses, respectively in Abidjan, Côte d'Ivoire.

Methods

Study population

Patients were recruited from the UNAIDS–Drug Access Initiative (UNAIDS-DAI) that started in Côte d'Ivoire in 1998 to expand patient's access to comprehensive HIV care including subsidized HAART. Details of how the DAI was set up, and the clinical, virologic and immunologic outcomes of patients enrolled and followed up have been described in detailed elsewhere [12]. In brief, our experience with the DAI shows that, after starting antiretroviral therapy, HIV-1-infected patients in Côte d'Ivoire had similar virologic and immunologic outcomes, probability of an adverse event, and estimated survival, as patients enrolled in clinical trials in the USA and Europe. At the start of the initiative, we systematically measured the levels of immune activation and reconstitution markers among 159 patients; however, the data presented in this study is a sub-analysis, which includes only a proportion of the 159 patients who had information on markers of immune activation and reconstitution at three time points. Thus, of the 159 patients, 52 had data on markers of immune activation and reconstitution at baseline, at 6 months and at 12 months of follow-up. We selected two groups of these patients: good virologic responders were defined as patients who had maintained viral load below the detection limit of the assay (< 200 copies/ml) at 6 months of therapy and had maintained viral load below detection limit at 12 months of follow-up. Poor virologic responders were defined as patients who had detectable viral loads (> 200 copies/ml) at 6 and 12 months. For patients in each group, we analyzed changes in CD4+ T cells, immune activation, and reconstitution markers at baseline, 6 months, and 12 months.

As a comparison group for normal levels of immune activation and reconstitution markers among HIV-uninfected Africans, we included 19 HIV-negative blood donors who were recruited at the National Blood Transfusion Center in Abidjan, Côte d'Ivoire. This study received approval from the CDC Institutional Review Board and the ethical committee of the Ministry of Health of Côte d'Ivoire.

Laboratory testing

Whole blood was collected from participants into ethylenediamine tetraacetic acid tubes (Becton Dickinson, San Jose, California, USA). Within 4 h, plasma was separated from cells by centrifugation at 200 ***g***, and then aliquoted and stored at –70°C.

We determined HIV-1 antibody status using an enzyme-linked immunosorbent assay (ELISA)-based testing parallel algorithm [13]. For HIV type-specific serodiagnosis, we used a combination of monospecific ELISAs [14]. HIV-1-RNA viral load in plasma was quantified by Amplicor HIV-1 Monitor Assay, version 1.5 (Roche Diagnostics Systems, Branchburg, New Jersey, USA). The assay's limit of detection was 200 copies/ml.

The CD4+ and CD8+ cell counts were determined by three-color flow cytometry using FACScan (Becton Dickinson). The Tritest kit and Multiset software (Becton Dickinson) were used for labeling and analysis. The following markers were analyzed on the surface of T cells: CD38 and HLA-DR on CD8+ T cells (CD38+CD8+ and HLA-DR+CD8+), as markers of immune activation [15], and CD62L+ CD4+ T cells were used as proxy markers of immune reconstitution. These cells correlate with CD45RA+CD62L+ T cells, which are direct markers of immune reconstitution [16–18]. CD38 is re-expressed on primed cells upon activation in HIV-infected persons, and its expression on CD8+ T cells increases significantly with disease progression. HLA-DR is a MHC class II antigen that is expressed on activated T cells. In HIV-positive persons, the expression of HLA-DR is significantly increased both on CD4+ and on CD8+ T cells [5].

Analysis of data

Immune activation markers were analyzed as a percentage of the major lymphocyte subsets (CD4+ and CD8+

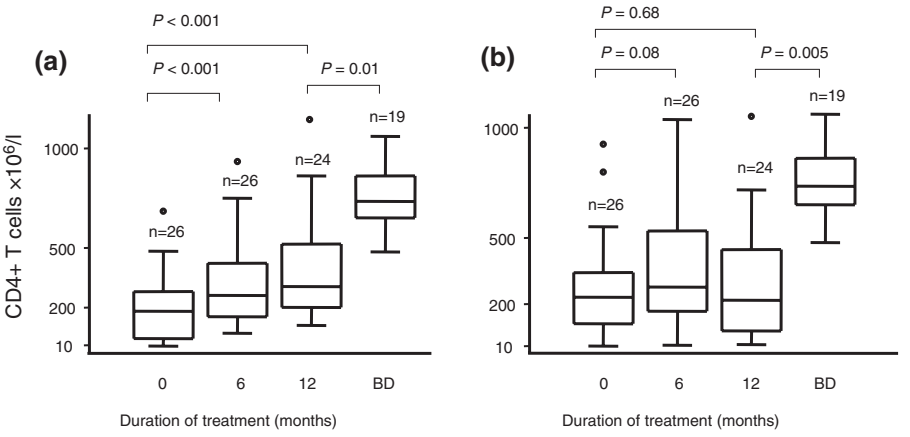

Fig. 1. Comparison of changes in median CD4+ T cell counts among good (a) and poor (b) virologic responders. Horizontal lines are medians and interquartile ranges (25th and 75th percentiles). Dark dots represent outlier values; n, number of patients tested; BD; HIV-negative blood donors used as controls.

T cells) stained with a combination of monoclonal antibodies. Data were summarized with medians and interquartile ranges (IQR). Using the non-parametric Wilcoxon signed-rank test for paired data, median of the differences between 6, 12 months and baseline in levels of expression of immune activation and reconstitution markers were compared between good and poor virologic responders. Statistical analysis was carried out with the software STATA, version 7 (Stata Corporation, College Station, Texas, USA). All statistical tests were two-sided tests with a significance level of 0.05 and adjusted *P*-values were determined using the Horn procedure.

Results

Characteristics of the study participants

Of the 52 patients receiving ART, for whom we had information on immune activation and reconstitution markers, 26 were categorized as good responders and 26 as poor responders. Median (IQR) age for the different groups were 36 years (32–41) for good responders, 34 years (30–42) for poor responders, and 24 years (21–26) for blood donors. Median CD4+ T-cell counts at baseline were 182×10^6 cells/l (44–280) among the good responders, 231×10^6 cells/l (111–343) for poor responders ($P = 0.2$), and 734×10^6 cells/l (651–862) for HIV-negative blood donors. Plasma RNA viral load at baseline was similar for good and poor responders [5.1 $\log_{10}$ copies/ml (4.2–5.4) versus 5.2 $\log_{10}$ copies/ml (4.7–5.6) ($P = 0.56$)]. Good responders maintained viral load below the detection limit for at least 12 months. In contrast, among poor responders, median viral load was 4.2 $\log_{10}$ copies/ml after 6 months and 4.9 $\log_{10}$ copies/ml after12 months of therapy. Twenty (77%) of the 26 good responders were receiving HAART and six patients were receiving dual therapy (five patients received zidovudine + didanosine and one received didanosine + stavudine). Of the 26 poor responders one (4%) was prescribed HAART and 25 (96%) were receiving non-suppressive dual therapy: 12 received zidovudine + didanosine, five patients received zidovudine + zalcitabine, five received didanosine + stavudine, and three received zidovudine + lamivudine. At baseline, no opportunistic infections were observed for good and poor responders. After 12 months of therapy, opportunistic infections were reported for 8.3% of good responders and 8.7% of poor responders.

Changes among good virologic responders

In the good-responder group, median levels of CD4+ T-cell counts increased steadily from 182 cells $\times 10^6$ cells/l at baseline to 261×10^6 cells/l at 6 months ($P < 0.001$) and to 306×10^6 cells/l after 12 months of therapy ($P < 0.001$) (Fig. 1a). At baseline, median levels of expression of CD38+CD8+ T cells were elevated [93%; IQR (90–98%)], but they then decreased to 78% (69–88%) after 6 months of therapy ($P < 0.001$), and to 76% (70–84%) after 12 months of therapy ($P < 0.001$) (Fig. 2a). Levels of expression of CD38+CD8+ T cells at 12 months were comparable with those observed among HIV-negative blood donors ($P = 0.9$) (Fig. 2a). Median levels of expression of HLA-DR+ CD8+ T cells were also elevated at baseline [53% (37–66%)], then decreased to 33% (22–44%) after 6 months of therapy ($P < 0.001$), and to 28% (15–42%) after 12 months of therapy ($P < 0.001$)(Fig. 2b). Median levels of expression of HLA-DR on CD8+ T cells after 12 months of therapy were comparable to those observed among blood donors ($P = 0.5$) (Fig. 2b).

Median levels of expression of the immune reconstitution marker, CD62L on CD4+ T cells, rose steadily from baseline [57% (46–76%)] to 12 months [68% (56–78%)] of therapy ($P = 0.03$) (Fig. 3a).

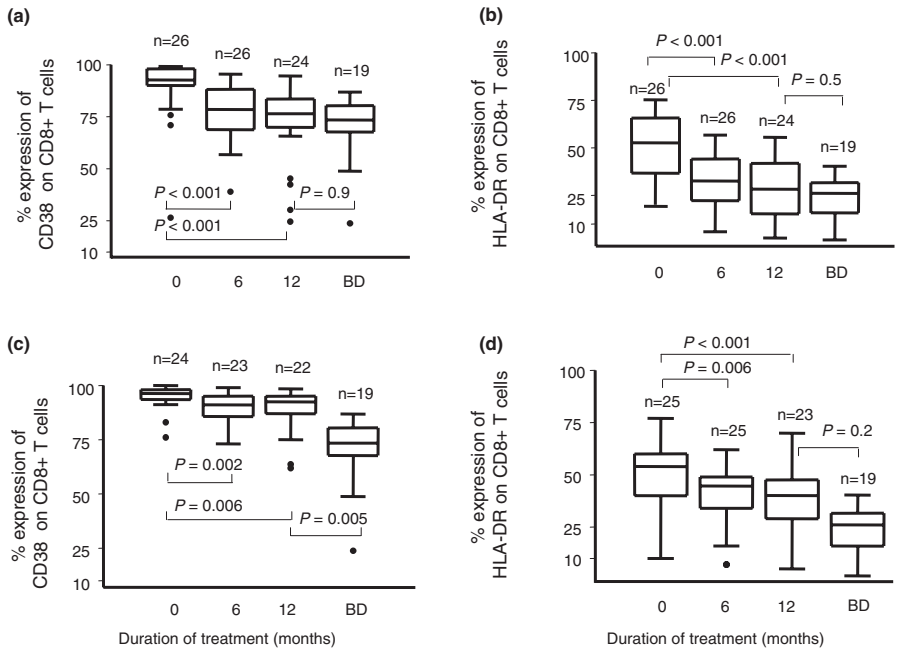

Fig. 2. Comparison of changes in median percentage of immune activation markers CD38 and HLA-DR on CD8+ T-cells among good (a, b) and poor (c, d) virologic responders. Horizontal lines are medians and interquartile ranges (25th and 75th percentiles). Dark dots represent outlier values; n, number of patients tested; BD; HIV-negative blood donors used as controls.

Changes among poor virologic responders

In the poor-responder group, CD4+T-cell levels after 12 months of therapy [218 × 10^6 cells/l (79–448)] were similar to baseline values [231 × 10^6 cells/l (111–343)] ($P = 0.68$) (Fig. 1b). Levels of expression of CD38+CD8+ T cells decreased significantly from baseline after 6 ($P = 0.002$) and 12 months of ART ($P = 0.006$) (Fig. 2c). However, although median levels of CD38+CD8+ T cells had decreased to 92% (87–95%) after 12 months of ART, they remained significantly higher than levels observed among HIV-negative blood donors ($P = 0.005$) (Fig. 2c). Median levels of expression of HLA-DR+ CD8+ T cells also declined significantly at 6 and 12 months with respect to baseline values ($P = 0.006$ and $P < 0.001$, respectively) (Fig. 2d) but remained higher than levels observed among the blood donors.

Although median levels of expression of CD38 and HLA-DR on CD8+ T cells decreased significantly at 12 months compared with baseline values, the decrease was less profound among poor responders than among good responders. For instance, CD38+CD8+ T cells decreased by –17.4% among good responders compared with –4.0% among poor responders ($P = 0.01$), and levels of HLA-DR+CD8+ T cells decreased by –22% among good responders compared to –11% for poor responders ($P = 0.02$). Among poor responders, median levels of CD62L on CD4+ T cells decreased rather than increased over time, although this decrease was not significant ($P = 0.21$) (Fig. 3b).

Discussion

Our results indicate that with successful viral suppression in HIV-infected West Africans receiving HAART, elevated baseline levels of markers of immune activation (CD38 and HLA-DR on CD8+ T cells) decreased steadily. During a 12-month period, these values decreased to those observed among HIV-negative individuals. Immune reconstitution increased steadily among only those patients with good virologic responses. Our data confirm earlier reports from developed countries where levels of immune activation are several-fold lower than those observed in African populations [10,11]. In a French study, Bouscarat *et al.* [19] observed significant decreases for levels of CD8+CD38+ and CD8+HLA-DR+ T cells only among 14 patients who had sustained suppression in plasma RNA viral load. Autran and colleagues [20] also observed a decrease both in CD38+ and in HLA-DR+CD8+ T cells after triple-drug ART. To our knowledge, this is the first report on changes in immune activation and immune reconstitution markers among patients receiving ART in Africa.

Several aspects of our results should be highlighted: Firstly, in developed countries, high levels of immune activation, such as increased CD8+CD38+ T cells, are known to predict a decrease in CD4+ T cells and the concomitant progression of disease in HIV-1-infected persons [19,21]. Furthermore, immune activation is usually directly correlated with viral replication [6,22].

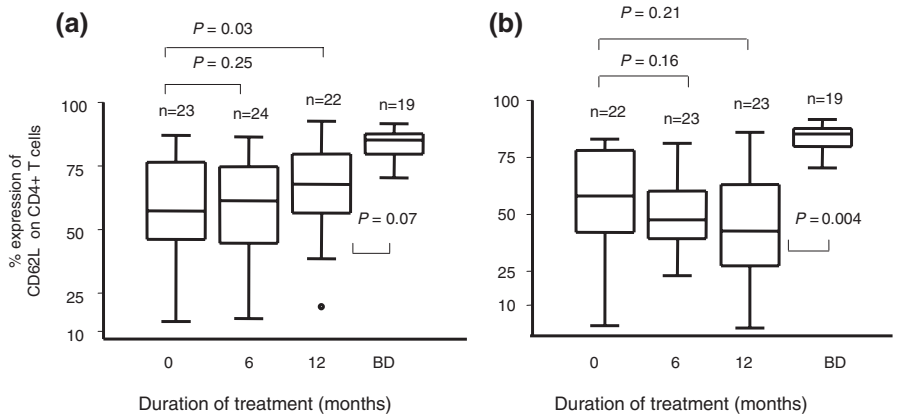

Fig. 3. Comparison of changes in median percentage of reconstitution markers CD62L on CD4+ T cells among good (a) and poor (b) virologic responders. Horizontal lines are medians and interquartile ranges (25th and 75th percentiles). Dark dots represent outlier values; n, are number of patients tested; BD; HIV-negative blood donors used as controls.

Thus, ascertaining the extent to which levels of immune activation decrease during ART in Africa may provide some insight into the risk for therapeutic failure and disease progression among HIV-infected Africans receiving ART. Indeed, Viganò and colleagues [4] have shown that persistence of viremia in HAART-treated individuals is associated with higher pretreatment levels of CD38+ CD8+ T cells; they have suggested that CD38 on CD8+ T cells should be analyzed in all HIV-infected patients receiving HAART and particular attention given to those in whom high levels are detected. However, our findings suggest that a reduction in the levels of immune activation depends on the successful suppression of viremia and may represent an affordable surrogate marker for the expensive viral load testing during treatment in resource-poor settings.

Secondly, profound changes in immune activation occurred and normalized at 12 months of therapy, predominantly among patients who were receiving HAART and had suppressed and sustained viral replication. This finding supports the concept that immune activation is antigen-driven and may represent an ongoing immune response to continuous HIV production [21,22]. Moreover, the low prevalence of opportunistic infections among our patients suggests that HIV replication was the principal cause of immune activation. Thirdly, the fact that viral load was not suppressed among patients who were prescribed regimens other than HAART underscores the limitations of using other regimens in African populations.

Finally, among good responders, levels of immune reconstitution markers increased steadily, through 12 months of therapy. This observation suggests that the high immune activation background that is characteristic of HIV-infected Africans may not affect immune reconstitution if appropriate ARV drugs are used.

In summary, despite having initially elevated levels of markers of immune activation (CD38 and HLA-DR on CD8+ T cells), Africans receiving HAART for 12 months and having sustained viral suppression appear to have steady decreases in levels of these markers. Levels declined to values observed among HIV-negative persons. However, immune reconstitution appears to occur steadily only among those patients who have good virologic responses. Our results do not support the possibility that HIV-infected Africans receiving ART may still be at greater risk for therapeutic failure and disease progression due to elevated levels of immune activation [4], but rather they do support the possibility that levels of immune activation simply reflect the suppression of HIV viremia with successful ART.

Acknowledgements

The authors thank Tossou Odette, Kadjo Jean Claude, Moïse Kouassi, and Pascal Ebby for data support and analysis. They also thank Dr Konaté Seydou and his staff at the National Transfusion Center for providing the blood donor samples used as control group.

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