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Prevalence and factors involved in discordant responses to highly active antiretroviral treatment in a closely followed cohort of treatment-naïve HIV-infected patients

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Abstract

The prevalence and the factors involved in discordant responses to highly active antiretroviral therapy were analysed in a closely followed cohort of 51 naïve HIV-infected patients at 48 weeks.

A complete treatment response was considered as an increase in CD4 cell count of $\geq$50 cells/mm^3 with a $\geq$1 $\log_{10}$ decrease in viral load or viral suppression. Virologic response (<50 CD4$^+$ cells/mm^3 increase) and immune response (<1 $\log_{10}$ decrease in viral load) were observed in 15.7% and 5.8% of the patients, respectively.

We demonstrated that the prevalence of virologic response decreased at week 72 and disappeared after 96 weeks of treatment. This slower CD4 repopulation correlated with a lower thymic volume compared to those with complete response. Also, the probability of having virologic response did not correlate with protease inhibitor-based (PI-based) regimens or HCV coinfection. On the other hand, immune response in our cohort could be easily attributable to a simple mechanism, i.e. irregular treatment compliance.

Keywords: HAART; Naïve; Thymic volume; TREC; Treatment compliance

1. Introduction

Highly active antiretroviral treatment (HAART) results in reduced plasma HIV viral load, increased CD4$^+$ cell count, and improved survival (Palella et al., 1998). However, discordant responses to antiretroviral therapy occur, including a poor increment in CD4$^+$ cell count despite marked reductions in viral load (virologic response but not immune response) (Dronda et al., 2002; Piketty et al., 1998; Renaud et al., 1999), and a poor viral reduction despite a significant increment of CD4 counts (immune response but not virologic response) (Kaufmann et al., 1998; Piketty et al., 1998; Renaud et al., 1999; Sufka et al., 2003; Wood et al., 2002). Nevertheless, even with detectable viral replication, patients with discordant response have prolonged survival, immunologic benefit, and a reduced risk of AIDS events (Ledergerber et al., 1999; Mezzaroma et al., 1999; Montaner et al., 1998).

Reported data on the prevalence of such discordant responses is very variable depending on the criteria used to define them, follow-up period, and type of patients included (naïve versus pretreated patients). Thus, virologic response has been reported in 17.3% after 6 months of therapy (Grabar et al., 2000) and 16.5% after 24 months in naïve patients (Dronda et al., 2002), and 9% after 12 months of treatment in pretreated patients (Piketty et al., 2001). On the other hand, immune response has been reported with a prevalence of 6.2% after 12 months (Wood et al., 2002) and 19% after 6 months in naïve patients (Grabar et al., 2000) and

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19% after 12 months in pretreated patients (Piketty et al., 2001).

Several mechanisms have been proposed to clarify virologic responses, including virus-induced apoptosis of uninfected cells (Kaplan and Sieg, 1998; Pitrak et al., 2001), thymic dysfunction that led to an impairment of the generation of new T-cells induced by thymotropic viruses (Hellerstein and McCune, 1997), and lack of cell redistribution from secondary lymphoid organs (Hazenberg et al., 2002; Pakker et al., 1998). Mechanisms proposed to clarify immune responses include the lack of adherence to therapy or unfavourable pharmacokinetics (Grabar et al., 2000), diminished fitness of mutant viruses (Devereux et al., 2001), inability of certain mutant viruses to replicate in human thymus (Lecossier et al., 2001; Stoddart et al., 2001), decreased cytopathic effect of the virus, increased half-life of CD4 cells (McCune et al., 2000), and a decreased T-cell apoptosis modulated by protease inhibitors (Sloand et al., 1999).

Since several authors have reported a wide range of prevalence of discordant responses, our aim was to analyse this prevalence in a well defined and closely followed cohort of naïve HIV-infected patients after 48 weeks of HAART. On the other hand, our group has previously demonstrated that thymic volume is the best predictor for $CD4^+$ T-cell recovery in HIV-infected adults on HAART (De la Rosa et al., 2002; Ruiz-Mateos et al., 2003). Thus, we analysed whether thymic function-related markers, as assessed by measurement of T-cell receptor excision circles, naïve $CD4^+$ cell count and thymic volume, are involved in virologic responses. We also analysed whether transient viral suppression or significant viral decrease (Wood et al., 2000, 2002) as well as a poor treatment adherence might be involved in immune responses, as suggested by Wood et al., 2002.

2. Materials and methods

2.1. Patients

This is a retrospective study done in the dynamic open cohort of the Viral Hepatitis and AIDS Study Group of Virgen del Rocío University Hospital, Spain. From February 1997 through December 2003, a total of 106 treatment-naïve HIV-infected patients who initiated HAART (regimens containing either two nucleoside analogue reverse-transcriptase inhibitors (NRTIs) plus at least one protease inhibitor (PI) or two NRTIs plus one nonnucleoside analogue reverse-transcriptase inhibitor (NNRTI)) were eligible for the study. The selection criteria to be included in the study were that patients had to have CD4 cell counts, viral load, and treatment compliance measured at baseline, at week 48 after the initiation of HAART, and at least two time-points in between. Patients with baseline $CD4^+$ cell counts >500 cells/mm^3 were excluded according to contemporary guidelines that recommend triple combination therapy for all antiretroviral-naïve individual with viral load $\geq$5000 copies/mL or CD4 counts <500 cells/mm^3 (The EACS euroguidelines group, 2003). A total of 51 patients fulfilled these criteria and were included in the study. A written informed consent was obtained from all the patients and the Ethical Committee of the Hospital approved the study.

To assess prevalence and possible mechanisms involved in discordant responses in our cohort, and to make them comparable to those reported previously by others (Grabar et al., 2000; Piketty et al., 1998; Wood et al., 2002), study groups after 48 weeks of treatment were defined as follows: a complete response was defined as an increase in $CD4^+$ cell count $\geq$50 cells/mm^3 and a decrease in plasma HIV RNA level $\geq$1 $\log_{10}$ copies/mL or viral suppression (<200 copies/mL); virologic response was defined as an increase in $CD4^+$ cell count <50 cells/mm^3 and a decrease in plasma viral load $\geq$1 $\log_{10}$ copies/mL or viral suppression; immune response was defined as an increase in $CD4^+$ cell count $\geq$50 cells/mm^3 and a decrease in plasma viral load <1 $\log_{10}$ copies/mL; non-response was defined as lack of virologic and immune response.

2.2. Laboratory determinations

Plasma HIV-1 RNA was measured by a quantitative PCR (HIV Monitor™ Test kit version 1.5, Roche Molecular System, Basel, Switzerland), according to the manufacturer's instructions. This assay has a detection limit of 50 HIV-1 RNA copies/mL. Since the assay used in early 1997 had a detection threshold of 200 copies/mL, we report this value as the lower limit of detection for the entire analysis.

Absolute of $CD4^+$ and $CD3^+$ T-cells was determined in fresh samples by conventional flow cytometry. Liquid nitrogen stored aliquots were used to determine percentages of naïve $CD4^+$ T-cells ($CD4^+CD45RA^+CD45RO^-$) and memory $CD4^+$ T-cells ($CD4^+CD45RA^-CD45RO^+$) (Franco et al., 2000). Naïve and memory $CD4^+$ T-cell absolute numbers were calculated according to the $CD4^+$ T-cell counts obtained from fresh blood samples (Franco et al., 2002). T-cell receptor excision circles (TREC) are generated by rearrangement of T-cell receptor genes during intrathymic maturation (Douek et al., 1998). A modified PCR-based method for quantifying δRec-ΨJα TREC has been adapted to a real-time PCR using a LightCycler (Roche Molecular Biochemicals, Mannheim, Germany) for the quantification of both the characteristic signal-joint sequences included in the TREC and the β-globin gene (Franco et al., 2002). TREC levels were measured in PBMC samples, and the absolute count of TRECs/μL was derived for each sample, multiplying the absolute T-cell count by the proportion of TRECs in the $CD3^+$ T-cell subpopulation.

Mediastinic computed tomography was performed with a modified method previously described (Choyke et al., 1987). Briefly, the same radiologist using a 3000 G.E. Sytec Scanner and 5 mm thick contiguous sections at 5-mm intervals always measured coded samples. Thymic tissue was care-