

IMMUNOLOGIC AND VIROLOGIC CHARACTERISTICS OF HIV-INFECTED PATIENTS WITH DISCORDANT RESPONSE TO HIGHLY ACTIVE ANTIRETROVIRAL THERAPY

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SUMMARY

Immunologic and virologic aspects, including genotypic analysis, were investigated in 43 HIV+ subjects failing HAART. Twenty of them showed a discordant response to therapy (CD4+ increase despite persistently high viremia). The remaining subjects (23) were immuno-virologic non responders. The two groups were compared to identify the factors associated with discordance. The failing regimen was changed in all of them, according to the results of genotypic analysis.

The univariate analysis showed a higher number of CD4+ cells/mm³ before antiretroviral treatment and at the moment of genotype, a lower replicative capacity of HIV isolates and a higher frequency of the M184V mutation in discordant patients. At the multivariate analysis, only the CD4 cell count at genotypic evaluation

and the prevalence of the M184V mutation were confirmed to be significantly different between the two groups.

In a subset of 20 subjects (10 discordant and 10 patients with complete failure) lymphoproliferative response (LPA) to recall and HIV antigens and IL-15 production by stimulated PBMC were performed. LPA after PHA was similar in the two groups, while the response to *Candida albicans* and HIV p24 were significantly higher in patients with discordant response to HAART. The same pattern was seen for IL-15 production by stimulated PBMC.

HAART regimen was changed in all patients on the basis of genotypic results. After 6 months, 5 discordant and 7 failing patients reached plasma viral load levels below 50 copies HIV RNA/ml.

INTRODUCTION

Discordant immunologic and virologic responses occur in 20-40% of HAART-treated patients. In the majority of these cases a certain level of immune recovery is seen, despite virologic failure. The opposite situation (i.e. viral suppression despite a sustained increase in CD4+ cell count) is less frequent. Virologic and immunologic determinants of such a “disconnection” have not been completely elucidated, and limited information is available on the long-term outcome of patients with discordant response. During a 30 month follow up period, Piketty et al (1) reported that the rate of disease progression and death in discordant individuals was 7-fold higher than in responder patients), but lower than in patients with full failure. With a longer period of observation, Deeks et al (2) reported that the durability of CD4 cell preservation during virologic failure is about 3 years.

Thus, uncertainty exists about clinical management of patients with discordant response to HAART, and whether to continue a failing regimen or change is a challenge for clinicians. Continuing a failing regimen may result in the further selection of resistant mutants, thereby limiting future therapeutic options. On the other hand, in many patients sustained CD4+ cell responses are associated with persistent clinical benefit.

In order to further investigate the virologic and immunologic factors associated with discordant response to HAART and to address the issue of clinical management of these subjects, we conducted a cross sectional analysis on several immuno-virologic aspects and started a prospective study on therapy outcome after a genotypic-guided change of HAART regimen.

MATERIALS AND METHODS

Study population

Forty-three HIV+ patients failing antiretroviral treatment (plasma HIV-1-RNA > 10,000 copies/ml in two last consecutive samples) were studied. According to the level of CD4+ recovery, patients were divided in two groups: those with a > 100 CD4+ cells increase from baseline were defined as “discordant” (20 subjects – group A), whereas those with a lower increase were considered as “failing” (23 individuals- group B). Patients characteristics are reported in **Table I**.

All patients were studied for the presence of drug-resistance mutations in plasma, CD4 cell count, HIV viremia, CD4 cell count and replicative fitness of isolates.

A subset of 20 subjects (10 from each group) were also studied for *in vitro* Lymphoproliferative Activity (LPA) against mitogens and antigens (recall and HIV-specific) and IL-15 production by stimulated PBMC.

All patients underwent a genotype-guided change of HAART regimen. Follow up data after 4 months are available for 17 individuals in group A and 12 in group B

Methods

- **HIV RNA** was quantified using the Amplicor monitor assay (Roche molecular system, Branchburg NJ, USA)

- **Sequencing of the HIV-1 pol gene** was performed using the TrueGene HIV-1 genotyping assay (version 2.5) (Visible Genetics, Toronto, ON, CANADA)

- **Replicative capacity** of HIV isolates was calculated as follows. Levels of p24 antigen were measured thrice weekly in supernatants from co-cultures of patients PBMC and PBMC from healthy donors, maintained for 60 days. The time in days to reach a 100,000 pg/ml value of p24 (replication constant, k) was calculated using non linear regression modelling. The relative replication fitness of the isolates was determined from k ratios of wild type (k_{wt}) and resistant strains (k_{rs}) multiplied by 100. The value of k_{rs} calculated as the mean of two control viruses (isolates from PBMC of two antiretroviral-naïve patients), was 5 days.

- **Lymphoproliferative Activity**. Briefly, freshly isolated PBMC were cultured for four days in the presence of PHA (1.5 µg/ml), *C.albicans* (25 µg/ml), recombinant HIV p24 (2.5 µg/ml), recombinant HIV p24 (2.5 µg/ml). ³H thymidine was then added for 12 hours and uptake was measured with a scintillation counter. Data were expressed as Stimulation Index (SI), calculated as the ratio between mean counts per minute (CPM) of replicates in presence and absence of antigen. For recombinant HIV proteins the SI value was calculated in relation to the mean CPM of the baculovirus control protein well.

- **IL-15 production**. PBMC from HIV+ patients were incubated in complete medium alone or in the presence of the following stimuli: LPS (1 µg/ml), *C.albicans* (at a ratio of yeast to mononuclear cells of 1:1). IL 15 production in the supernatants was measured by a commercially available ELISA kit (Cytimmune, College Park, Maryland, USA). The lower limit of detection was 8 pg/ml.

Statistical analysis

Potential determinants of viro-immunological discordance were assessed by estimating crude and adjusted odds ratios (OR) and their 95% confidence intervals (CI) through univariate and multivariate models. In the multivariate analysis, we considered only heterosexual behaviour as risk factor and variables that were significantly associated with viro-immunological discordance at the univariate analysis. Associations with a p value < 0.05 were considered as significant. Continuous variables were calculated by logistic regression analysis for each unit increase, except for CD4 cell count in which increases of 200 cells/mm³ were employed. The virologic and immunologic differences between two groups of patients after the genotype guided change of regimen were evaluated using analysis of variance.

TABLE I. DEMOGRAPHIC AND CLINICAL CHARACTERISTICS OF THE WHOLE STUDY POPULATION.

Age (yrs)	37 ± 9
Gender (% of males)	72
Risk factor	
Homosexual	9
Heterosexual	16
IVDU	16
Other	2
Months on ART (mean, range)	58.7 (18-126)
Drug history	
N.of arv drugs (mean, range)	5.7 (3-10)
% treated with NRTI	100
% treated with PI	84
% treated with NNRTI	65

RESULTS

Demographic and clinical parameters of 43 patients failing HAART, stratified according their immunological response to therapy are presented in table II. At the univariate analysis no significant differences regarding age, gender and pharmacological history were detected between the two groups, whereas heterosexual habitus was more frequent among failing patients. CD4 cell count before starting antiretroviral therapy, as well as at the moment of genotypic evaluation, was significantly higher in discordant than in failing patients. No difference was seen for viral load. HIV isolates from discordant patients showed a lower replicative capacity.

Figures 1a and 1b show the prevalence of primary mutations in RT and PR gene. The only significant finding was the higher frequency of the M184V mutation among discordant patients.

When multivariate analysis was conducted, (table II) only the CD4 cell count at the moment of genotype evaluation and the prevalence of the M184V mutation were confirmed to be statistically significant.

After 6 months from the genotypic guided change of therapy, an increase in CD4 cell count (mean +45 and +63 CD4 cells/ml, respectively) and a decrease in HIV-RNA viral load (mean -0.29 and -0.48 log HIV-RNA copies/ml, respectively) compared to base line values were seen in discordant and failing patients (Fig 2). Particularly, 5 out of 8 discordant patients achieving a HIV-1 viral load less than 500 cp/ml reached undetectable level (<50cp/ml). Conversely, 7 out of 8 failing patients with a viral load lower than 500 HIV-1 RNA cp/ml achieved undetectable value. No significant difference in the number of new prescribed drugs was observed in two groups of patients (data not shown)

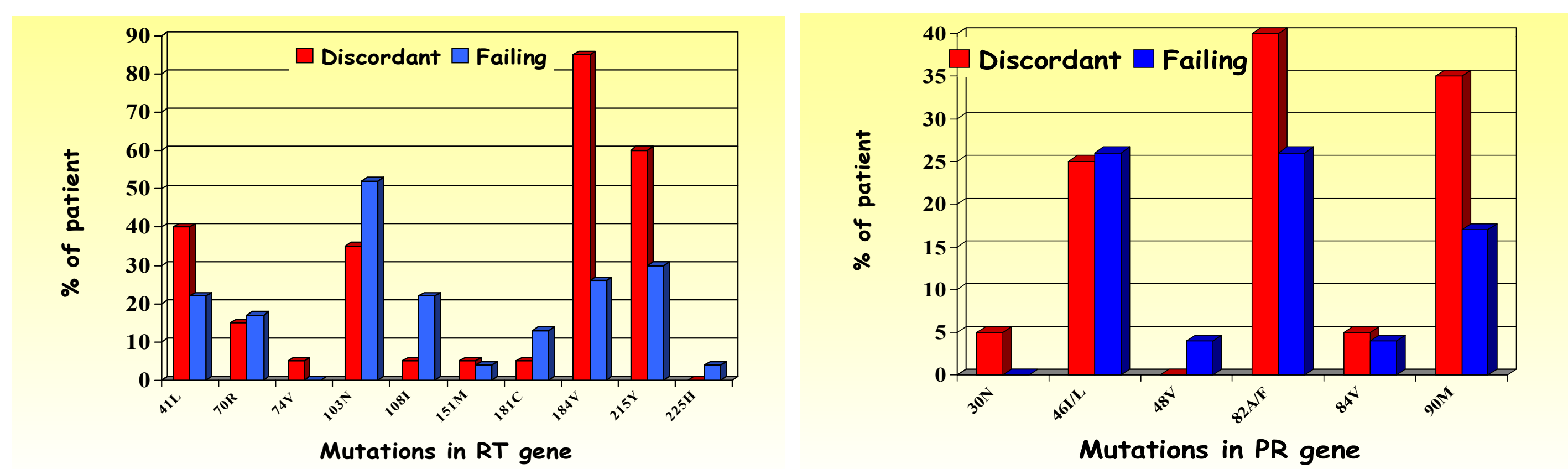


Fig.1. Prevalence of primary drug resistance mutations in RT (1a) and PR (1b) genes.

TABLE II. FACTORS ASSOCIATED WITH IMMUNOLOGIC RESPONSE TO HAART IN PATIENTS WITH VIROLOGIC FAILURE

Variables	Immunological response		Crude O.R. (95% CI)	p	Adjusted O.R. (95% C.I.)	p
	Yes (20 pts)	No (23 pts)				
Age*	36±9	38±11	1.02 (0.96-1.09)	0.48		
Gender Male (%)	14 (70)	17 (73)	0.82 (0.21-3.12)	0.78		
Risk factor						
Homosexual	7	2	4.26 (0.72-24.77)	0.10	0.89 (0.08-10.2)	0.92
Heterosexual	4	12	0.26 (0.06-1.07)	0.06		
IVDU	8	8	1.38 (0.35-5.34)	0.64		
Other	1	1	-	-		
Months* on ART	56±32	61±31	1.01 (0.98-1.03)	0.55		
CD4 pre-ART (cells/mm ³ *)	267±174	150±116	0.35 (0.13-0.93)	0.036	0.33 (0.06-1.81)	0.20
CD4 at genotype (cells/mm ³ *)	408±185	159±140	0.075 (0.02-0.34)	0.001	0.09 (0.01-0.59)	0.01
HIV-RNA at genotype (log copies/ml*)	4.74±4.69	5.56±5.93	3.27 (0.89-12.0)	0.074		
NRTI exposure (%)	20 (100)	23 (100)	-	-		
NNRTI exposure (%)	10 (50)	15 (65)	1.87 (0.55-6.39)	0.31		
PI exposure (%)	15 (75)	21 (91)	3.49 (0.59-20.5)	0.16		
Total number of drugs	5.6±2.1	5.7±1.7	1.03 (0.74-1.41)	0.88		
Drug resistance mutations	8.1±4.6	6.7±4.5	0.93 (0.81-1.07)	0.33		
RT mutations*	5.0±3.1	4.3±2.8	0.92 (0.74-1.14)	0.47		
RT primary mutations*	2.6±2.3	2.1±1.4	0.25 (0.48-4.21)	0.25		
PR mutations*	3.1±2.4	2.4±2.3	0.87 (0.66-1.14)	0.33		
PR primary mutations*	1.1±1.0	0.9±1.3	0.86 (0.49-1.48)	0.58		
M184V mutation (%)	17 (85)	6 (26)	16.0 (3.44-74.88)	0.0001	16.6 (1.41-130.9)	0.024
215Y mutation (%)	12 (60)	7 (30)	2.81 (0.81-9.71)	0.1		
K193N mutation (%)	7 (35)	12 (52)	0.49 (0.14-1.69)	0.26		
82A mutation (%)	8 (40)	6 (26)	1.89 (0.52-6.87)	0.33		
L90M mutation (%)	7 (35)	5 (17)	1.93 (0.50-7.48)	0.34		
Replicative fitness HIV isolates ^o	32.8±13.4	52.0±24.6	1.04 (1.01-1.09)	0.038	1.04 (0.95-1.13)	0.35
Subtype B HIV isolate (%)	18 (90)	14 (61)	6.99 (0.73-66.6)	0.091		

* Mean ± standard deviation

^o Percent in comparison with wild type HIV-1 strains

DISCUSSION

In this study, we observed a strict relationship between the high number of mutations and a decreased replicative capacity of viral isolates in discordant patients. With respect to fully failing individuals, patients with discordant response exhibited a higher number of NRTI – related mutations; particularly, the M184V mutation was strictly associated with the viro-immunological disconnection (despite a similar rate of lamivudine-including regimens in both failing and discordant groups). M184V might be the cause for a virus that is “less fit” than the wild type, and therefore slower to overcome the effects of antiretroviral therapy; the presence of a primary resistance mutation therefore provides a beneficial effect, possibly related either to the RT reduced processivity and increased fidelity (3,4) or to the reduced pyrophosphorolysis (5).

Our observation clearly indicates that the development of resistance mutations to drugs does not interfere with immune recovery during HAART; in fact, when lymphoproliferative activity and IL-15 production by PBMC were tested, discordant patients had a response that was similar to that seen in fully responder subjects. A complex interaction of viral factors, including drug resistance mutations and replicative capacity, and immunological aspects appear to be involved in discordant responses to HAART.

The assessment of LPA in the subset of 20 patients (10 per group) showed no differences regarding the response to PHA.

LPA response in a subset of 10 subjects with discordant response to HAART (group A) was compared with results obtained in a subset of 10 patients with complete failure (group B) and in 6 subjects with complete response (group C).

Among patients in group A, 88% exhibited a positive response to *Candida*, 55,6% to p24. These results were not significantly different from those found in a group of patients with complete response to HAART. In group B only the 1,2% of patients showed a positive response to *Candida* and p24. SI was compared in the three groups of subjects: the response to *Candida* in the group A patients was significantly higher than in group B (p<0,05); on the contrary no difference was found between group A and control patients. LPA results are reported in Fig. 3

Increased levels of IL-15 production were found after stimulation with both LPS and *Candida* in group A, when compared with group B (389.9 ± 40,5 versus 38.2 ± 4.9 pg/ml for LPS, p<0.001 and 390.2 ± 58,7 vs 37.7 ± 4.9 pg/ml for *Candida*, p= <0.001) (Fig.4). Results found in group A were not significantly different from those seen in complete responders (data not shown).

Fig.2. CD4 and HIV RNA response after the genotypic guided change of HAART regimen

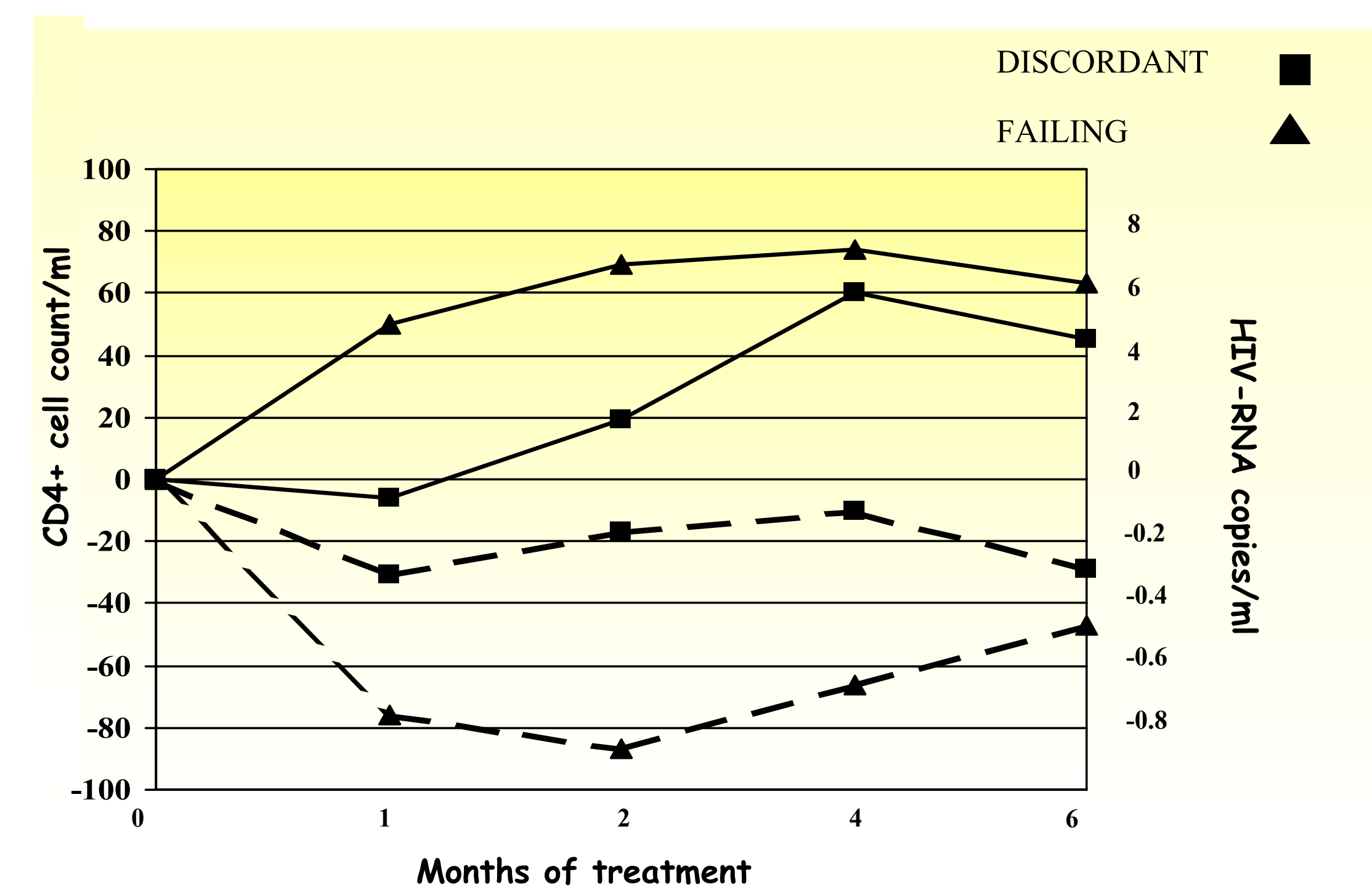


Fig.3 Lymphoproliferative response to *C. albicans* and p24 antigen in discordant, fully failing patients and responder

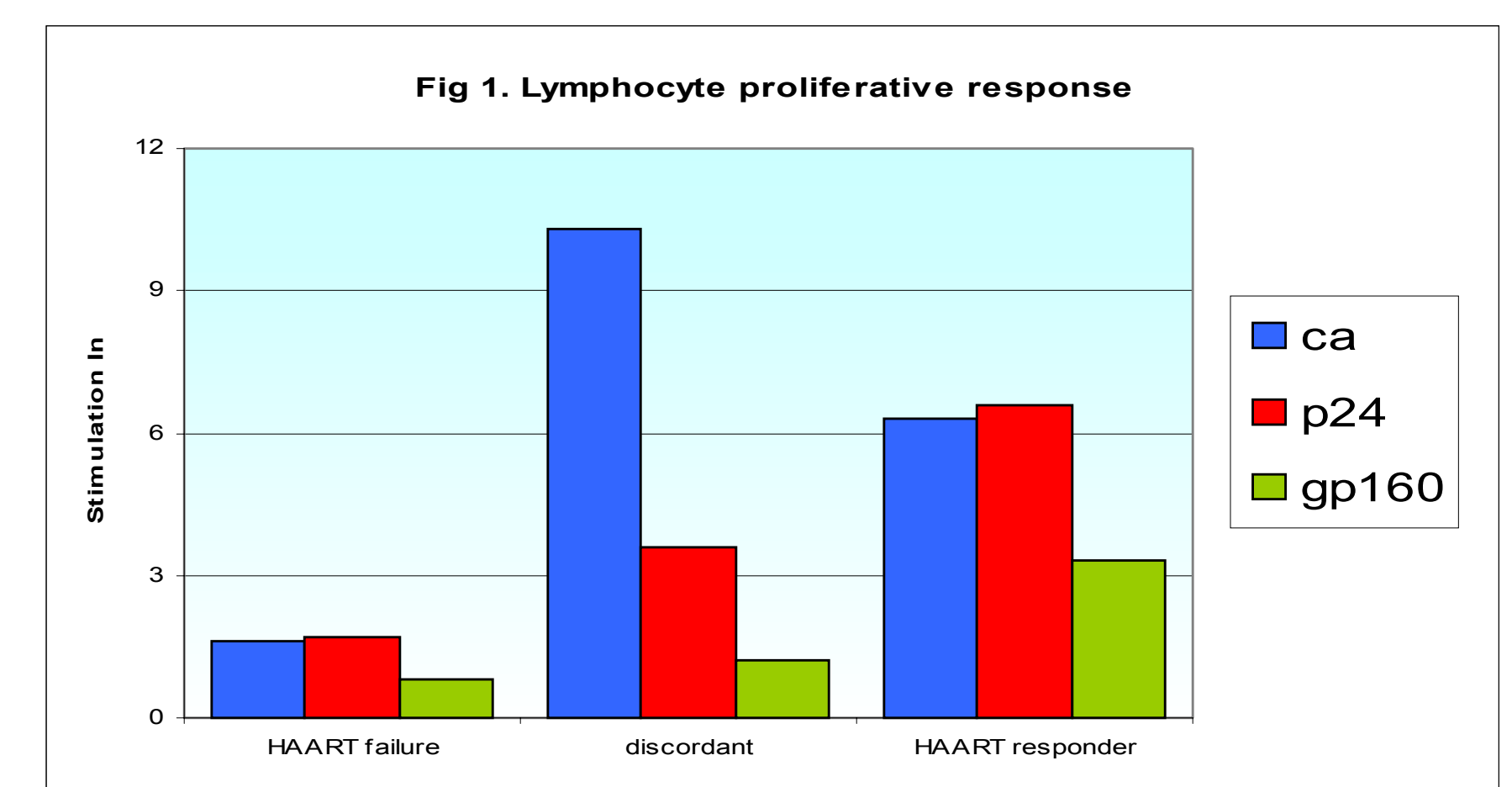
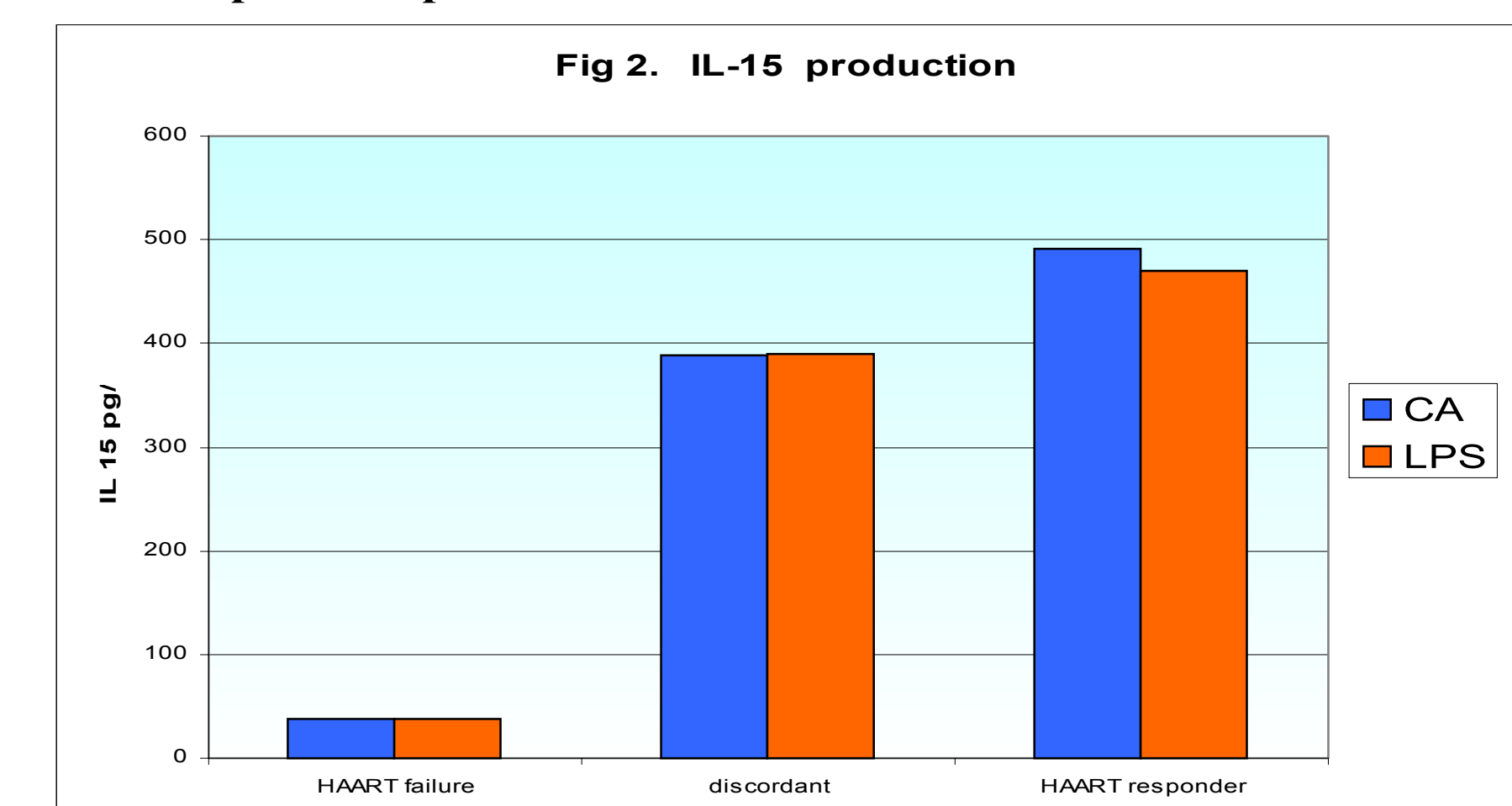


Fig.4 IL-15 production by PBMC of discordant, fully failing and responder patients



It is noteworthy, from this point of view, that CD4 cell count before any antiretroviral treatment, together with the presence of the M184V mutation, is a variable predictive of discordance.

Few data exist about the long term outcome of patients with discordant responses. Piketty et al showed a 7-fold higher progression rate, in comparison with fully responder patients (1). Deeks et al demonstrated that immunologic deterioration does occur, with a median delay of three years (2). Thus, the management of failing patients with discordant response is unclear; continuing a failing regime may further select resistance mutations, thereby limiting future therapeutic options. On the other hand, when high level of CD4 are present, patients frequently show persistent clinical benefits. In our study, after the genotype guided change of regimen, 8 patients (40%) achieved a HIV RNA value below 500 copies/ml (5/8 below 50 copies/ml); due to the small patient population, no conclusions may be drawn from this rather low rate of response. Large, randomized clinical trials should be undertaken to address the issue of changing or continuing the failing HAART regimen in patients with viro-immunological disconnection.

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