

INFLUENCE OF HTLV

P

-2 COINFECTION IN V PARAMETERS OF HIV

Bassani S, Benito JM, López M, B
Hospital Carlos III,

VIROLOGICAL AND I -INFECTED PATIENT

allesteros C, Toro C and Soriano V
Madrid, Spain

IMMUNOLOGICAL

S.

Jose Miguel Benito

Laboratorio de Biología Molecular

Hospital Carlos III

C/ Sinesio Delgado nº 10

Madrid 28029

Spain

e.mail: jbenito1@hotmail.com

Tf: +34 91 4532500 (ext:2754)

Fax:+347336614

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INTRODUCTION

- **HTLV-2 is prevalent among HIV-infected intravenous drugs users. It has been suggested that HTLV-2 could play a protective role on HIV disease progression.**
- **Different mechanisms have been proposed to account for this potential beneficial effect, such as an increased HIV-specific immune response, and an increased production of different chemokines.**

OBJECTIVE

- **The aim of this study was to analyze the influence of HTLV-2 coinfection on virological and immunological parameters in a group of HIV infected patients.**

METHODS I

- We have included 30 patients with HIV infection and 25 coinfecting with HIV and HTLV 2. All patients were naive for antiretroviral therapy.
- Immune activation was evaluated by flow cytometry using a quantitative assay to measure the levels of CD38 expression on different CD4+ and CD8+ T cell subsets
- Quantification of HIV specific CTLs was performed by 4-colour flow cytometry on PBMCs using an IFN- γ production assay in response to 125 optimally-defined HIV peptides. HIV peptides were divided into five different pools according to the protein(s) of origin: Gag (45 peptides), Pol (28 peptides), Env (26 peptides), Nef (21 peptides), and Reg (for regulatory proteins, 5 peptides).

METHODS II

- In a subgroup of patients, the production of MIP-1 β was also examined in parallel with IFN- γ in response to PMA and Gag peptides. We have defined three functional subsets:
IFN- γ (+) MIP1 β (+), IFN - γ (+)MIP-1 β (-) and IFN - γ (-) MIP-1 β (+).
- PBMCs were cultured for six hours in complete medium in the presence of the different pools at a final concentration of 10 $\mu\text{g/ml}$ for each individual peptide. Stimulation with PMA plus Ionomycin was used as positive control, and medium alone as negative control. During the last 5 hours cells were cultured in the presence of brefeldin .

METHODS III

- After the culture, cells were washed, permeabilized, and stained with the next panel of monoclonal antibodies: IFN- γ -FITC, CD69-PE or MIP-1 β -PE, CD3-ECD, and CD8-PECy5. For each experiment, a minimum of 20,000 CD3+CD8+ cells were analyzed.
- Levels of HIV-specific CD8+ T cells are expressed as percentage of total CD8+ T cells producing IFN - γ in response to the different pools of peptides. We defined the total CTLs response as the sum of individual responses to the five pools.
- In samples where the coexpression of IFN - γ and MIP-1 β was analyzed, CTL response was defined as the sum of the three different subsets defined on the basis of the expression of these molecules.

METHODS IV

- After the culture, cells were washed, permeabilized, and stained with the next panel of monoclonal antibodies: IFN- γ -FITC, CD69-PE or MIP-1 β -PE, CD3-ECD, and CD8-PECy5. For each experiment, a minimum of 20,000 CD3+CD8+ cells were analyzed.
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- In samples where the coexpression of IFN - γ and MIP-1 β was analyzed, CTL response was defined as the sum of the three different subsets defined on the basis of the expression of these molecules.

RESULTS I

- The mean CD4 count was similar in the two groups of subjects: HIV infected patients and HIV-HTLV2 coinfecting patients (523 ± 288 and 513 ± 274 cells/ μ l, respectively).
- However, mean level of HIV viral load was significantly lower in HIV-HTLV-2 coinfecting patients (3.34 ± 0.2 and 4.08 ± 0.1 log copies/ml for coinfecting and monoinfected patients, respectively, $p < 0.001$).
- CD38 expression on naive and memory subsets of CD4 and CD8 T cells was lower in coinfecting patients when compared to monoinfected subjects, being the differences statistically significant for total CD8+ T cells and for naive CD8+ T cells (figure 1)

RESULTS II

- **The mean level of HIV-specific CTLs against each pool of peptides (figure 2), as well as the total CTL response (figure 3), were similar in both group of patients.**
- **We also analyzed the contribution of each pool to the total CTLs response, expressed as the percentage of total response due to each of them.**
- **We did not find significant differences in the contribution of each pool to the total CTLs response in both groups of patients. The pools that most contributed to the global response were Gag and Nef in both groups of patients (figure 4).**

RESULTS III

- **In a subgroup of HIV monoinfected and HIV/HTLV-2 coinfecting patients, the production of MIP-1 β was examined in parallel with IFN- γ in response to PMA plus Ionomycin.**
- **We did not find significant differences in the mean levels of CD8+ T cells producing MIP-1 β , IFN- γ or both molecules against PMA between the two groups of patients (figure 5).**

RESULTS IV

- Moreover, in these two groups of patients the production of MIP-1 β was examined in parallel with IFN- γ in response to HIV-Gag peptides.
- In the group of coinfecting patients, the mean level of CD8+T cells producing MIP- 1 β and IFN- γ was significantly lower than in HIV monoinfected patients (0.89 ± 0.2 and 2.44 ± 0.5 ; $p < 0.05$, respectively, figure 6).

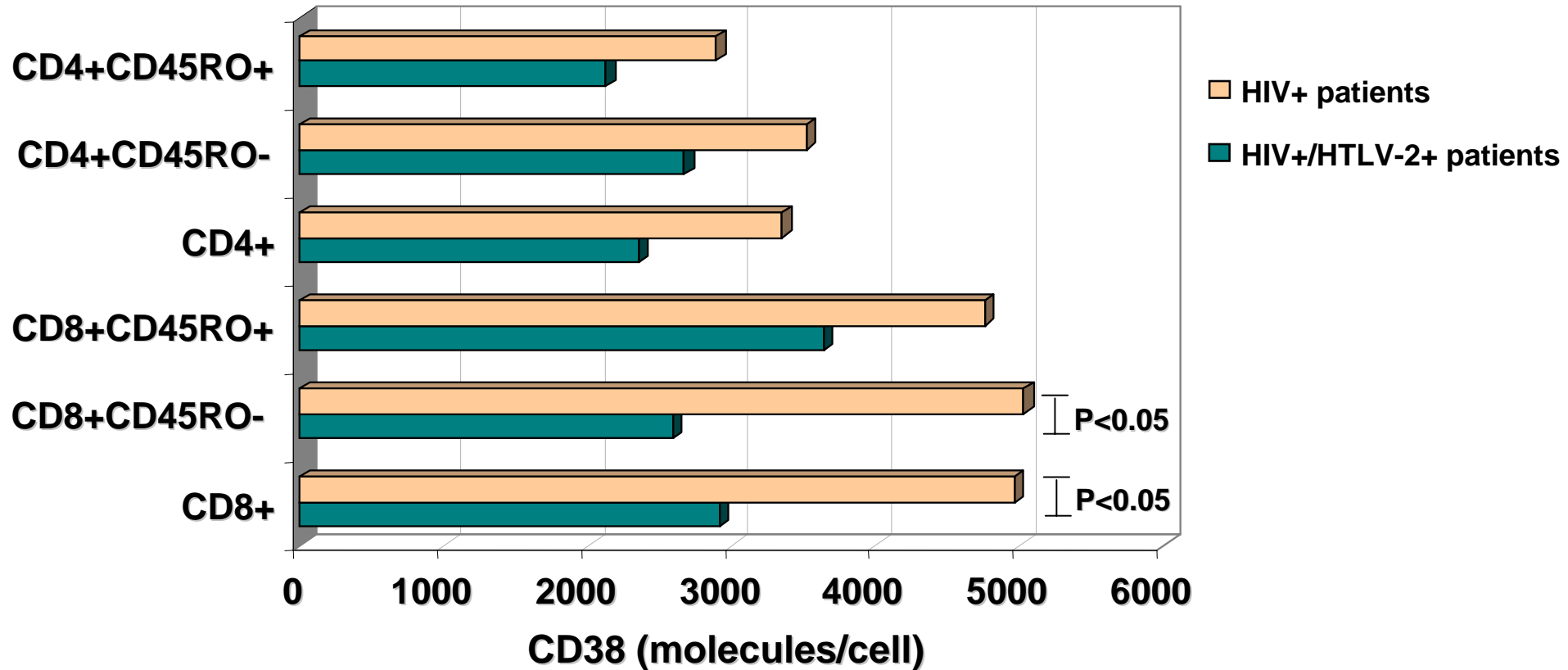
RESULTS V

- **Finally, we analyzed the contribution of each functional subset to the HIV-Gag specific CTL response, in these two groups of patients.**
- **We found that IFN- γ (-) MIP- 1 β (+) were the cells that most contributed to the HIV-Gag specific CTL response in coinfecting patients, whereas in HIV monoinfected patients the response was largely dominated by IFN- γ (+) MIP- 1 β (+) CD8+ T cells (figure 7).**

CONCLUSIONS

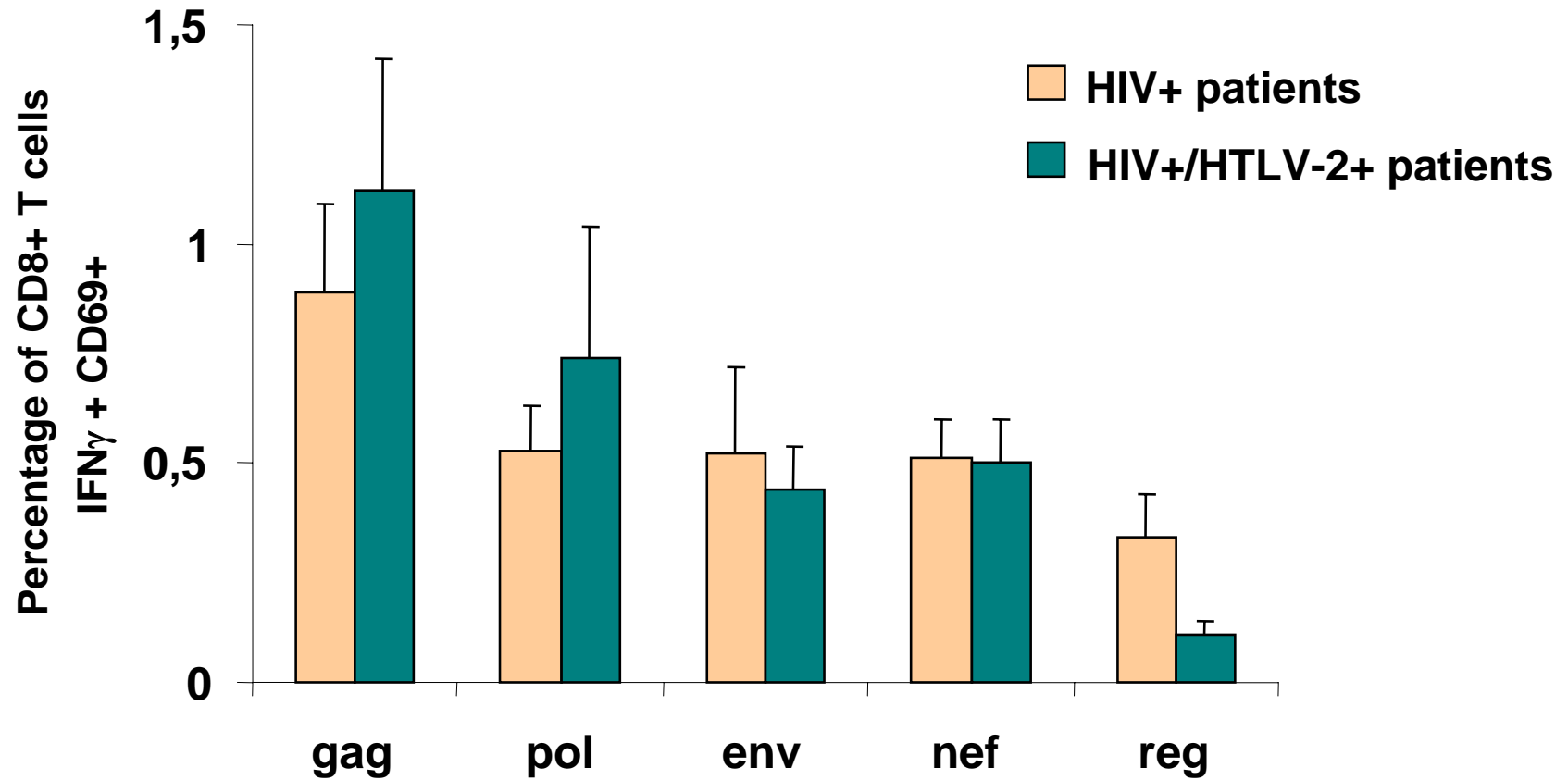
- **HIV+ patients coinfecting with HTLV-2 have lower levels of HIV replication and lower levels of CD8 T cell activation than monoinfected patients.**
- **The production of MIP-1 β and IFN- γ is differentially regulated in HIV/HTLV-2 coinfecting patients as compared with HIV monoinfected patients.**
- **An HIV-specific CTL response dominated by MIP-1 β (+) IFN- γ (-) cells in coinfecting patients, could contribute to reduce HIV replication.**

FIGURE 1



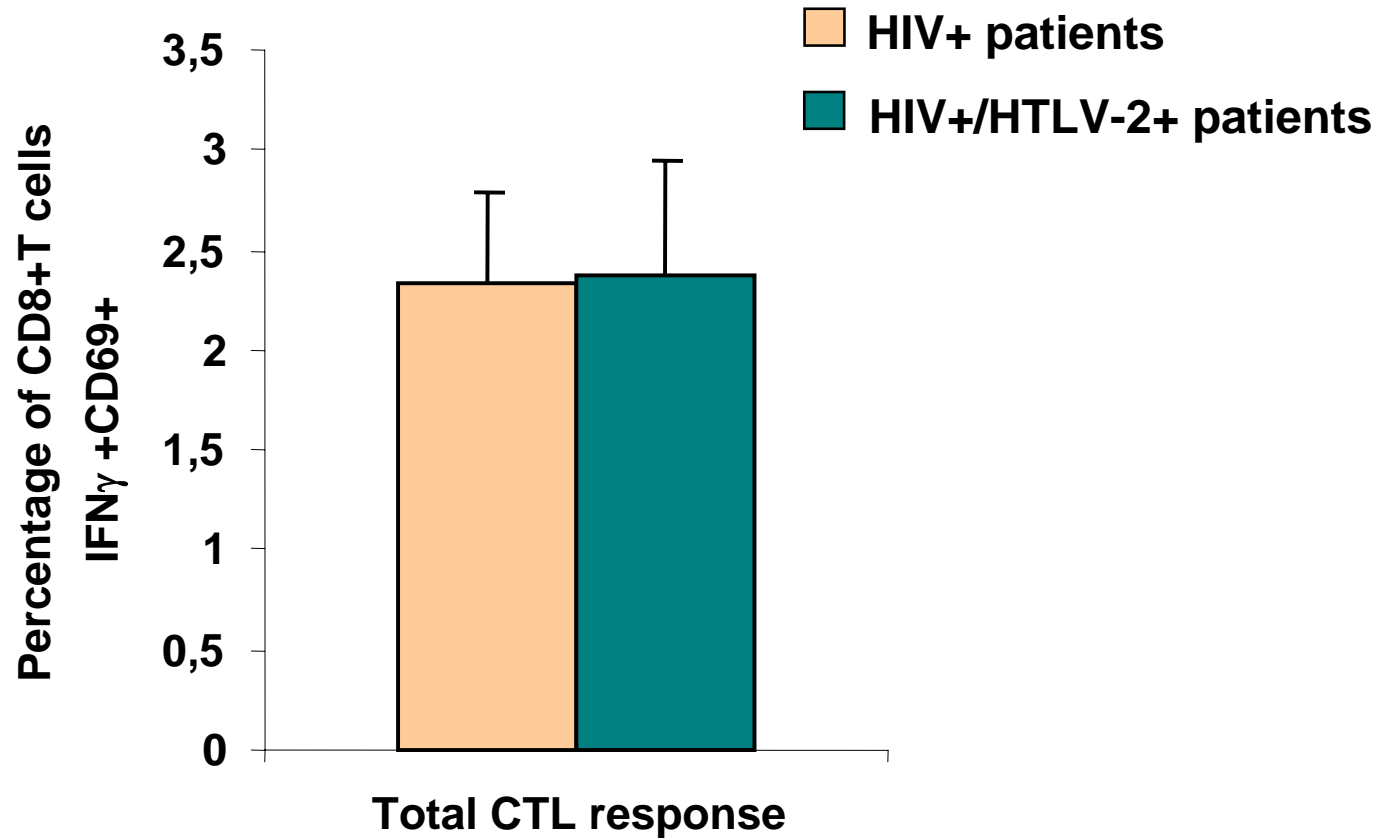
Mean Levels of CD38 expression on naive and memory subsets of CD4 and CD8 T cells in two groups of subjects: HIV-infected patients and HIV-HTLV 2 coinfecting patients.

FIGURE 2



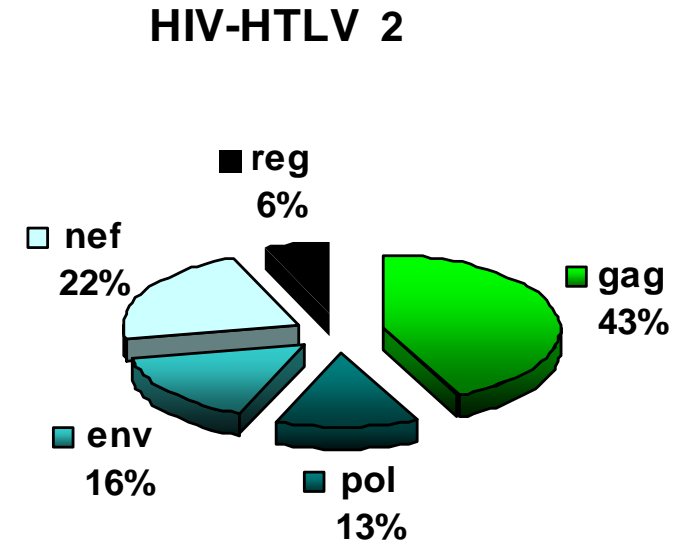
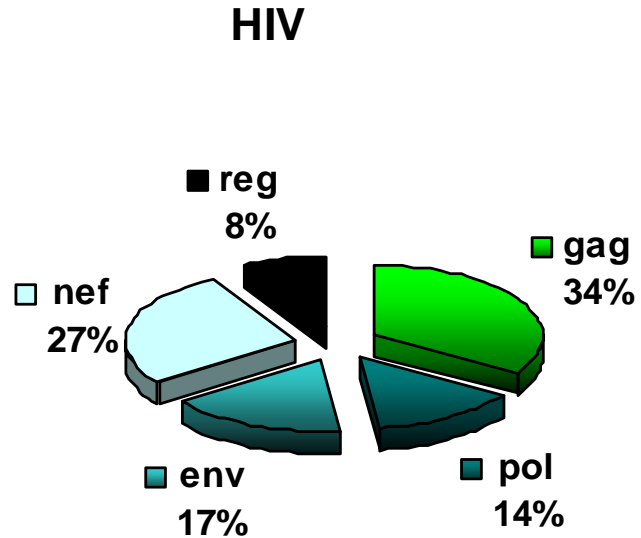
Mean Levels of HIV-specific CD8+ T cells producing IFN- γ in response to five different pools of optimally defined peptides (Gag, Pol, Env, Nef and Reg) in two groups of subjects: HIV-infected patients and HIV-HTLV 2 coinfecting patients.

FIGURE 3



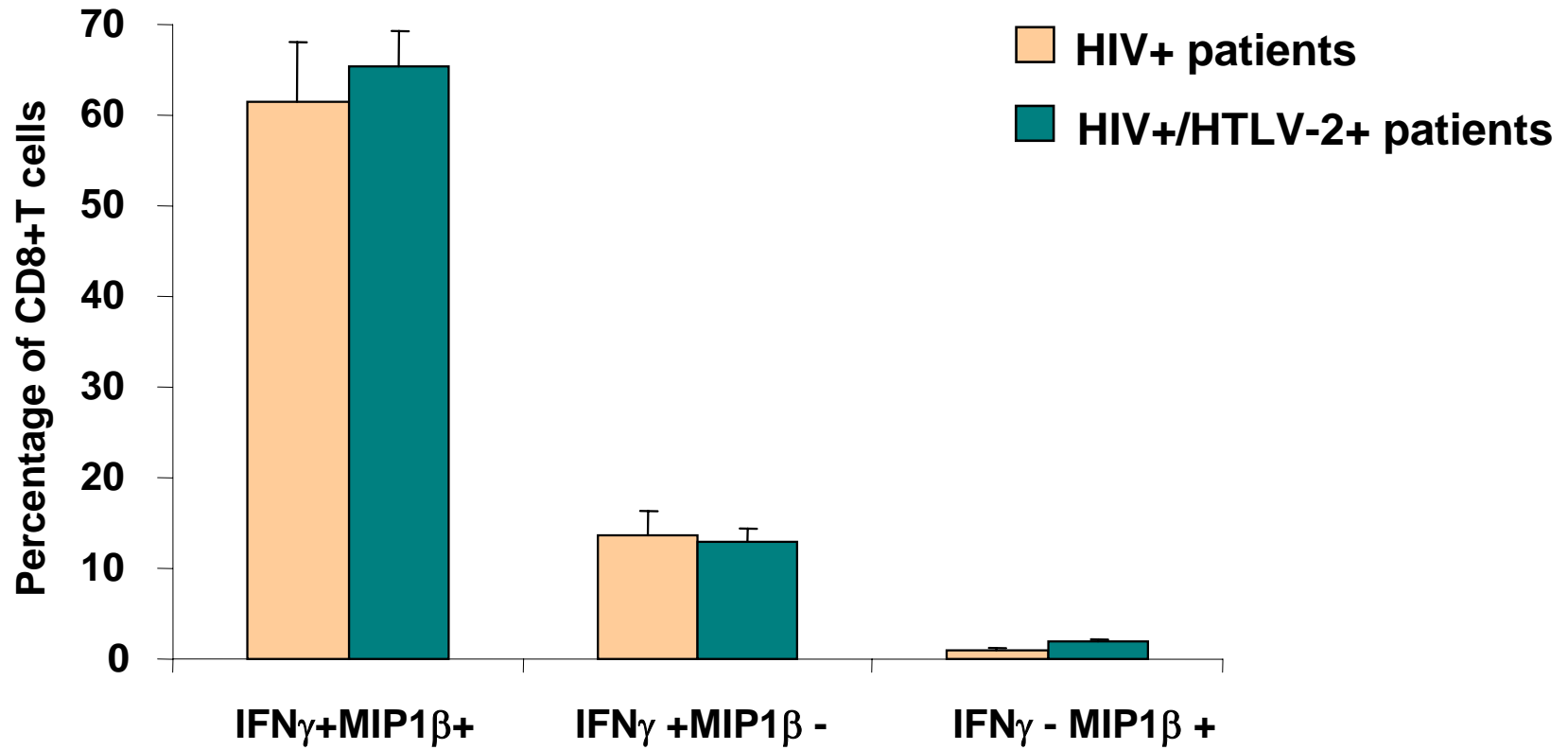
Mean Levels of total HIV-specific CD8+ T cells response in two groups of subjects: HIV-infected patients and HIV-HTLV 2 coinfecting patients.

FIGURE 4



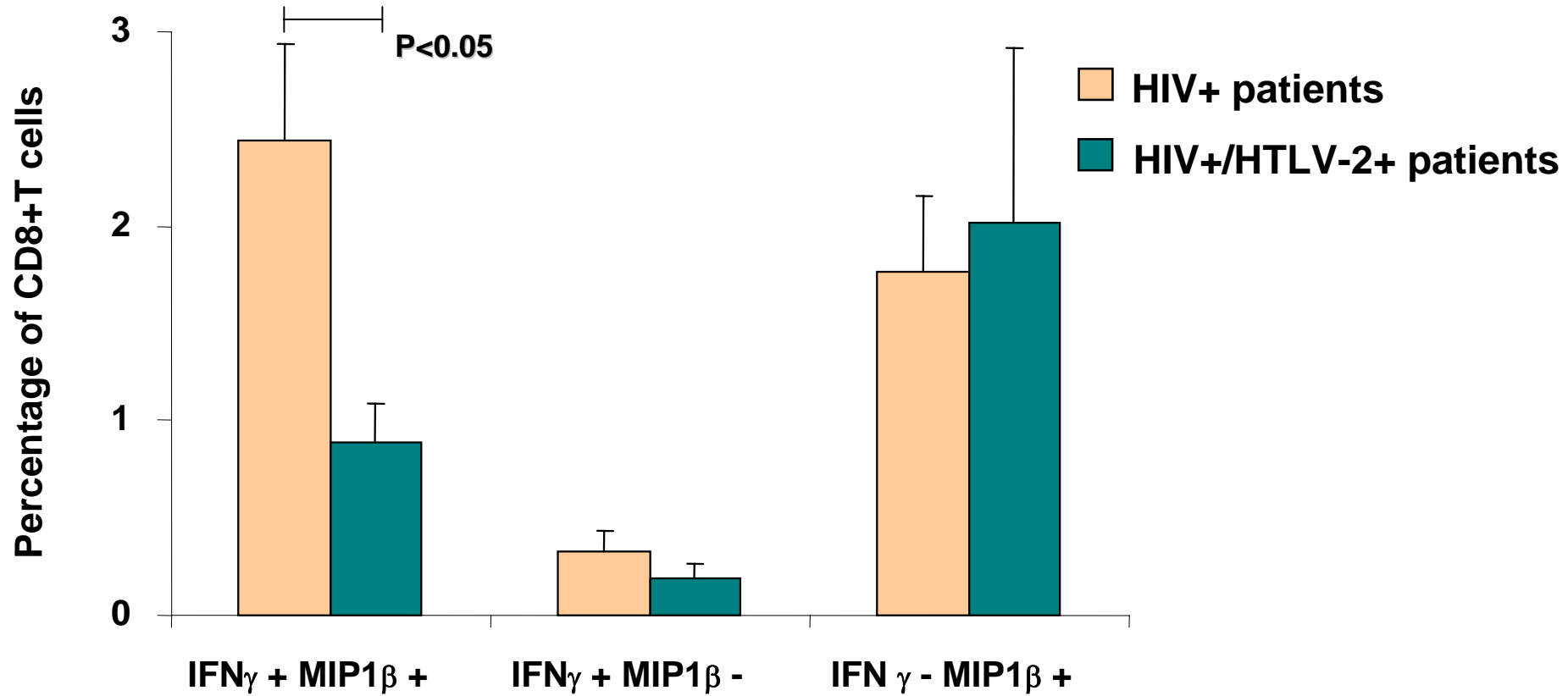
Contribution of each peptide pool (Gag, Pol, Env, Nef and Reg) to the total HIV specific CD8+ T cell response in two groups of subjects: HIV-infected patients (left graph) and HIV- HTLV 2 coinfecting patients (right graph).

FIGURE 5



Mean Levels of HIV-specific CD8+ T cells producing MIP-1 β , IFN- γ or both molecules in response to PMA-Ionomycin in two groups of subjects: HIV-infected patients and HIV-HTLV 2 coinfecting patients.

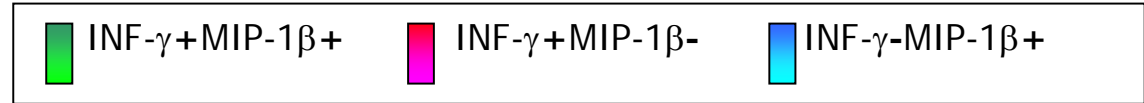
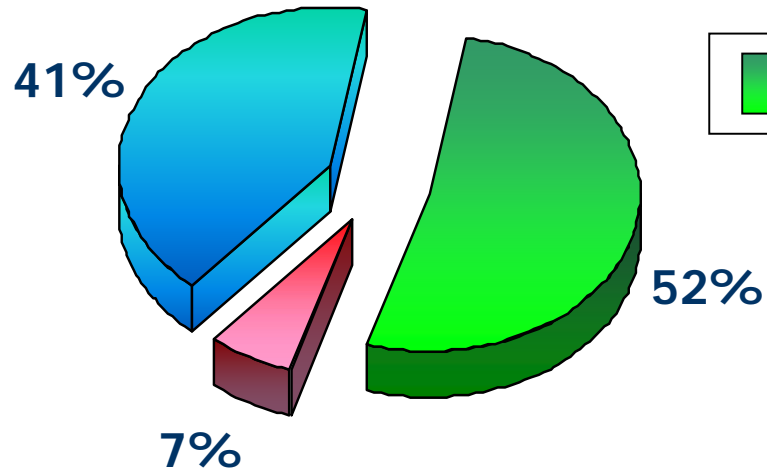
FIGURE 6



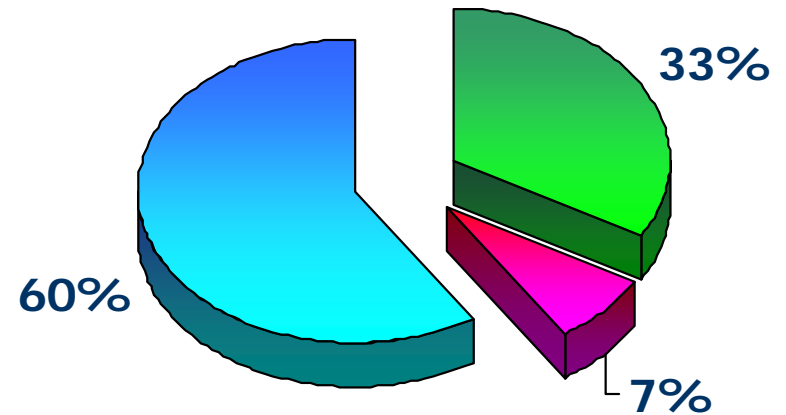
Mean Levels of HIV-specific CD8+ T cells producing MIP-1 β , IFN- γ or both molecules in response to Gag peptides in two groups of subjects: HIV-infected patients and HIV-HTLV 2 coinfecting patients.

FIGURE 7

A- HIV



B- HIV-HTLV-2



Contribution of each functional subset to the HIV-Gag specific CD8+ T cell response in two groups of subjects: HIV-infected patients (A) and HIV-HTLV 2 coinfecting patients (B).