

DISEASE STAGE DOES NOT INFLUENCE THE FREQUENCIES OF HIV-1 SPECIFIC Th1 CELLS AFTER LONG-TERM OF HIGHLY EFFICIENT ANTI-RETROVIRAL THERAPY

G. Carcelain¹, N. Alatrakchi¹, A. Samri¹, R. Tubiana², C. Duvivier², S. Hilper^{1†}, V. Pellegrin¹, P. Debré¹, C. Katlama², B. Autran¹.

1: Laboratoire d'Immunologie cellulaire et tissulaire, INSERM U543; 2: Service de Maladies Infectieuses et Tropicales. Hôpital Pitié-Salpêtrière, Paris, France.



Pitié-Salpêtrière
Paris, France

9th Conference on
Retroviruses and
Opportunistic Infections.
February 24-28, 2002
Seattle, WA

ABSTRACT #B108e

Disease stage does not influence the frequencies of HIV-1 specific Th1 cells after long-term of highly efficient anti-retroviral therapy.

G. CARCELAIN¹, N. ALATRAKCHI¹, A. SAMRI¹, R. TUBIANA², C. DUUVIER², S. HILPERT¹, V. PELLEGRIN¹, P. DEBRE¹, C. KATLAMA², B. AUTRAN¹.

1 : Lab. Cell. Immunol.; 2 : Mal. Infect., Hôp. Pitié-Salpêtrière, Paris.

Aim of the study: to evaluate HIV-1 specific Th1 cells frequencies in large groups of patients treated for 1 to 3 years at different stages of the disease.

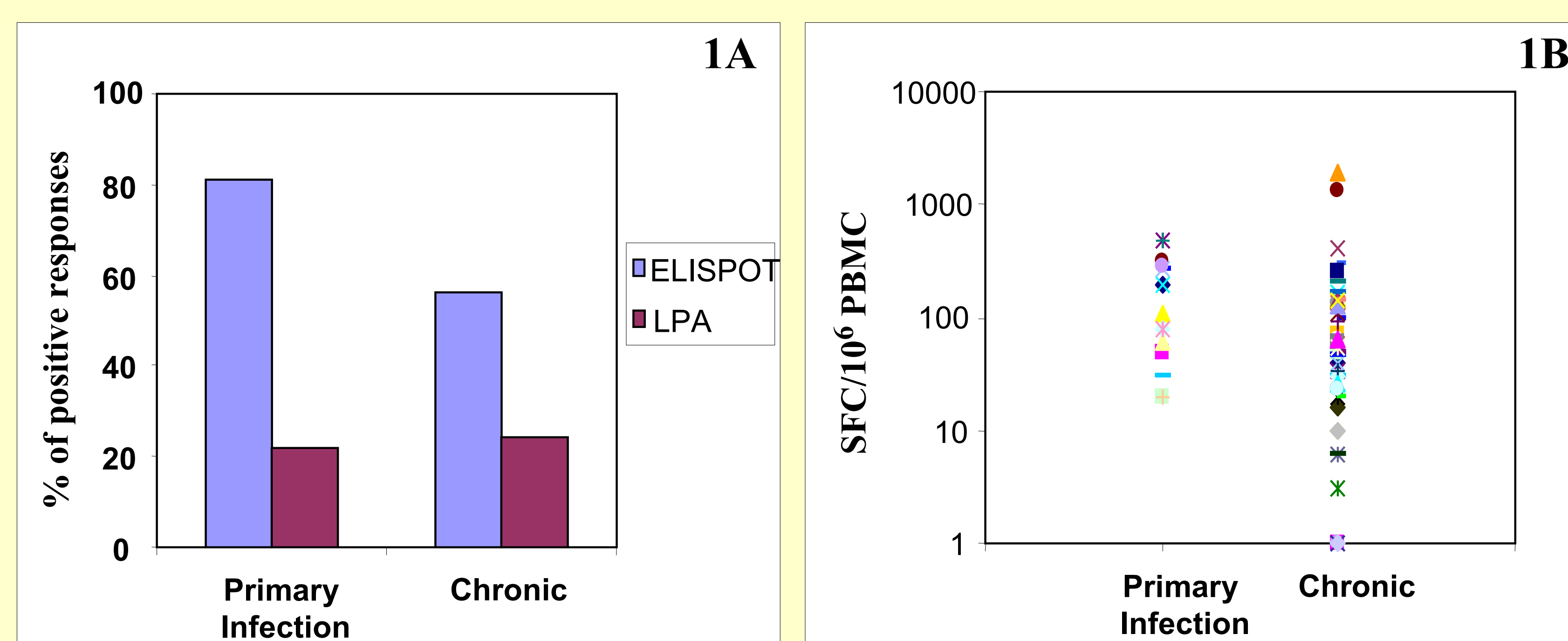
Methods: 79 HIV-1 infected patients (63 treated in chronic infection and 16 in primary infection [PI]) were enrolled in a study of CD4+ T cell responses to HIV-1 p24 after 1 to 3 years of viral control (<200 copies/ml). CD4+ responses were analysed with a new IFN- γ ELISPOT assay (threshold of positive responses was : 50 SFC/10⁶ PBMC above background) and conventional T cell proliferation assay.

Results: In ELISPOT assay, a CD4 response against HIV-1 can be detected in 56% of these 79 patients. No difference of frequency of responders to p24 is observed between patients treated in chronic infection and patients treated in PI with mean of positive responses of 56% and 81% respectively. However, the mean frequency of HIV-1 specific Th1 cells is globally very low (132 \pm 303 SFC/10⁶ PBMC) although with wide ranges (0-1900 SFC/10⁶ PBMC). Once again, no difference is observed whatever the stage of treatment initiation with a mean of 132 \pm 303 SFC/10⁶ PBMC for patients treated at chronic stage and of 184 \pm 157 for patients treated at PI. We finally analysed the CD4+ HIV-1 specific responses in a sub-group of 11 immuno-suppressed patients with CD4 counts < 350/mm³ at baseline of treatment. While no proliferative responses were detectable in these patients, we can detect in ELISPOT assay a high frequency of positive responses (65%) against HIV and a mean of SFC/10⁶ PBMC of 179 \pm 251 (ranges: 0-1020). Effect of treatment on this Th1 specific response will be detailed.

Conclusions: 1) IFN- γ ELISPOT assay allows to detect HIV-1 specific Th1 positive responses in large group of patients even when no proliferative response is detected 2) CD4+ HIV-1 specific T cells are similarly detected in chronically and in PI treated patients despite long-term treatment with highly efficient anti-retroviral therapy; 3) such responses can even be detected with a similar frequency before initiation of treatment in patients with less than 350 CD4/mm³.

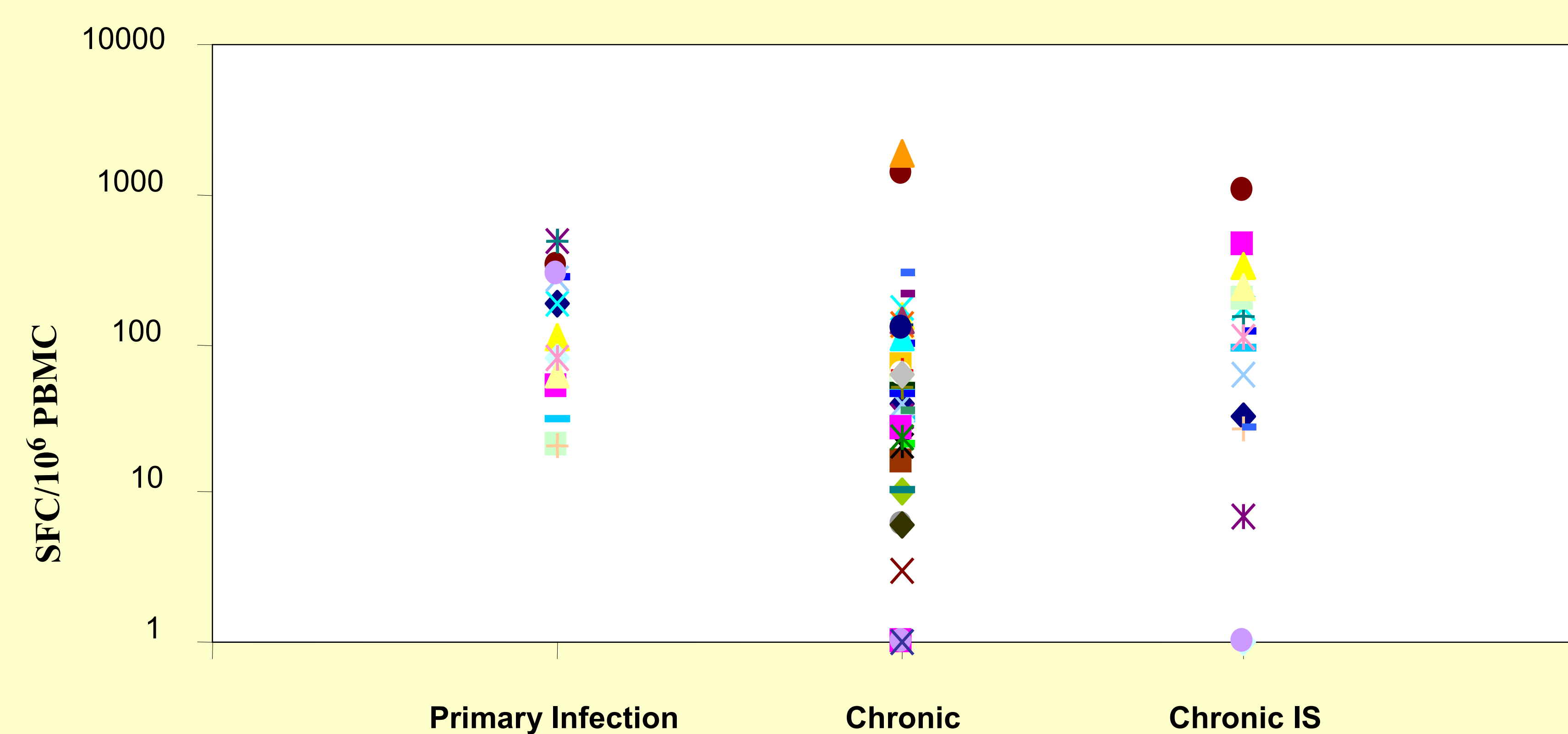
RESULTS

Figure 1 : Analysis of CD4+ T cell responses to HIV-1 p24 in HIV- infected patients treated in chronic infection (n=63) and in primary infection (n=16) after 1 to 3 years of viral control (<200 copies/ml) **1A :** Comparaison of positive responses detected by ELISPOT or LPA. **1B :** Frequencies of antigen specific T cells expressed in spot forming cells (SFC) / 10⁶ PBMC.



Results : ELISPOT IFN γ assay allows to detect a higher frequency of positive responses than lymphocyte proliferative assay in both groups of patients. CD4+ HIV-1 specific T cells are detected by ELISPOT with similar frequencies in PI and in chronically treated patients despite long-term treatment with highly efficient anti-retroviral therapy (mean of **184 \pm 157 SFC/10⁶ PBMC** and **132 \pm 303 SFC/10⁶ PBMC** respectively).

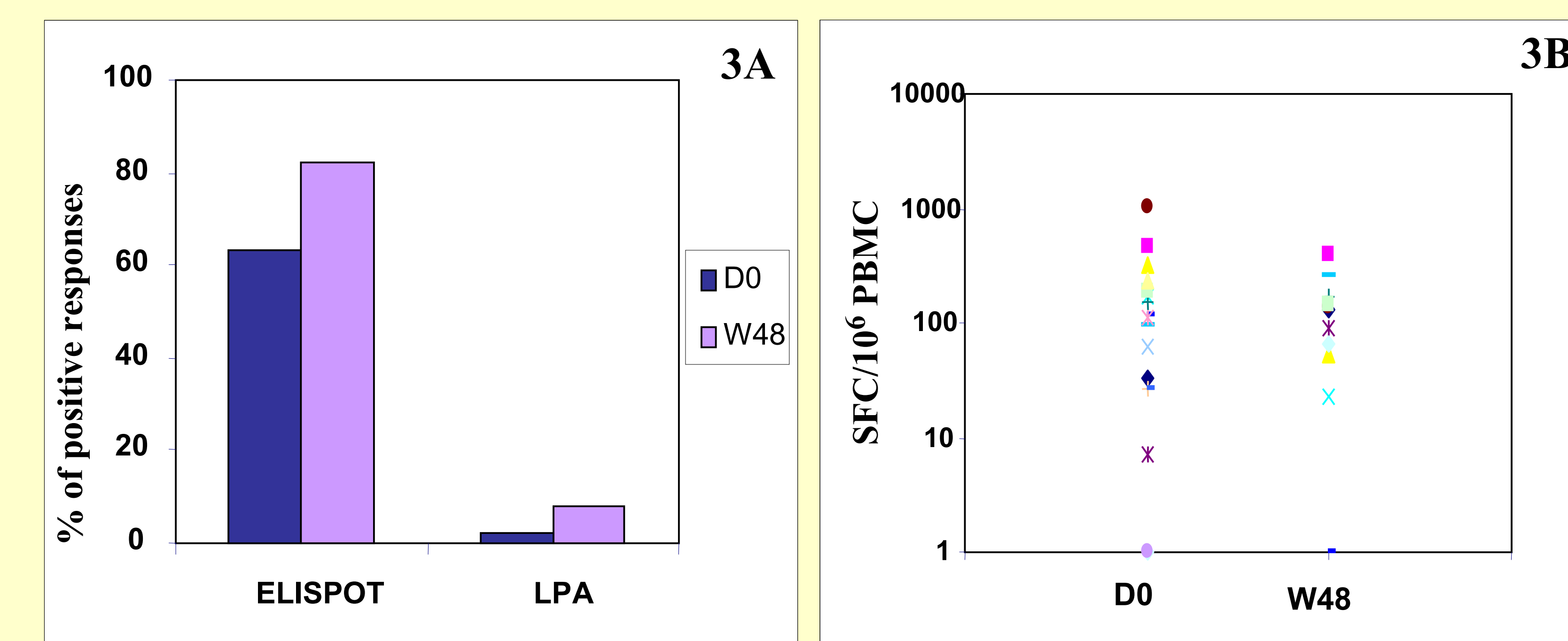
Figure 2 : Comparaison of positive responses detected by ELISPOT in HIV-infected patients in primary infection (n= 16), chronic infection without (n=52) or without immunosuppression (IS) (n=11). Patients were evaluated after 1 to 3 years of viral control (<200 copies/ml).



Results : CD4 T cells specific for HIV can be detected by ELISPOT with similar frequencies in the 3 groups of patients. The mean frequency of HIV-1 specific Th1 cell is globally very low : mean of **184 \pm 157 SFC/10⁶ PBMC** for patients treated at PI, **132 \pm 303 SFC/10⁶ PBMC** for patients treated at chronic stage with no IS and **179 \pm 251 SFC/10⁶ PBMC** for patients treated at chronic stage with immunosuppression.

RESULTS CONTINUED

Figure 3 : Effect of antiretroviral therapy on Th1-specific responses detected in patients treated in chronic infection with CD4<350/mm³. **3A :** Comparaison of positive responses detected by ELISPOT or LPA at baseline and after one year of efficient antiretroviral therapy. **3B :** Frequencies of antigen specific spot forming cells (SFC) / 10⁶ PBMC at baseline and after one year of efficient antiretroviral therapy for the eleven patients.



Results : High frequencies of positive CD4 T cell responses are detected by ELISPOT assay but not by LPA before and after one year of antiretroviral therapy. Similar frequencies of positive responses (**65%** and **82%**) and similar frequencies of CD4T cells specific for HIV (mean of **179 \pm 251 SFC/10⁶ PBMC** and **134 \pm 115 SFC/10⁶ PBMC**) are observed at baseline of treatment and after one year of viral control.

CONCLUSIONS

- 1) IFN- γ ELISPOT assay allows to detect HIV-1 specific Th1 positive responses in large group of patients even when no proliferative response is detected.
- 2) CD4+ HIV-1 specific T cells are similarly detected in chronically and in PI treated patients despite long-term treatment with highly efficient anti-retroviral therapy.
- 3) Such responses can even be detected with a similar frequency before initiation of treatment in patients with less than 350 CD4/mm³.

METHODS

- **Antigens used :** HIV recombinant p24 antigen (*Protein Sciences*) is used in this study to analyse the CD4 T-cell responses.
- **Lymphocyte proliferation assay :** PBMC were cultured in triplicate at 10⁵ cells/well with p24 (0.25 μ g/ml). Tritium-labelled thymidine incorporation was assessed on day 6. Positive antigen-specific responses were defined as cpm greater than 3,000 and Stimulation Index (cells + stimuli cpm / cells + medium cpm) greater than 3.
- **ELISPOT Interferon-gamma assay (LPA) :** Capture anti-human IFN γ Mab (Diacclone, France) was coated in 96-well plates. PBMC were added to triplicate wells at 1.10⁵ cells/well in the presence of p24 (2 μ g/ml) and incubated for 40 h at 37°C. After washing, the second biotinylated anti-IFN γ Mab (Diacclone, France) was added, then streptavidin-alkaline phosphatase conjugate (Amersham) an chromogen substrates (5-bromo-4-chloro-3-indolyl phosphatase toluidine and nitro blue tetrazolium, Sigma-Aldrich). The frequencies of antigen specific spot forming cells (SFC) were measured using an automated microscope (ZEISS). Results were considered as positive if a minimum of 50 SFC/10⁶ PBMC were detected above the background.

OBJECTIVES

- 1) To evaluate HIV-1 specific Th1 cell frequencies in large groups of patients after 1 to 3 years of efficient antiretroviral therapy in patients treated at different stages of the disease.
- 2) To analyse the effect of antiretroviral therapy on these HIV-1 specific Th1 cell frequencies.

PATIENT CHARACTERISTICS

- **79 HIV- infected patients** were enrolled in a study of CD4+ T cell responses to HIV-1 p24 after 1 to 3 years of viral control (<200 copies/ml). **63 patients** have been treated in chronic infection and **16 patients** in primary infection.
- **11 patients out of the 63 patients** were immunosuppressed at time of therapy (CD4<350/mm³). This sub-group of patients was evaluated at baseline of antiretroviral treatment and after 1 year of viral control (<200 copies/ml).