# CD4 T-Cell Responses in Relationship With Immune Activation and Viral Load in Primary HIV Infection

**Mathieu F. Chevalier[1,2], Céline Didier[1], Gaël Petitjean[1], Pierre-Marie Girard[3], Françoise Barré-Sinoussi[1], Daniel Scott-Algara[1] and Laurence Weiss[1,4,5]**

[1] Institut Pasteur, Unité Régulation des Infections Rétrovirales, Paris; [2] Université Paris Diderot, Sorbonne Paris Cité; [3] Hôpital Saint Antoine, AP-HP, Paris;
[4] Hôpital Européen Georges Pompidou, AP-HP, Paris; [5] Université Paris Descartes, Paris

## Background

Early events during primary HIV infection (PHI) are thought to influence disease outcome. Although few studies have characterized HIV-specific CD4 T cells during PHI, a growing body of evidence suggests a beneficial role of functional CD4 T-cell responses in different aspects of protective immunity in HIV infection. It is unclear how viral replication and systemic immune activation may shape these responses. Here, we longitudinally investigated polyclonal and HIV-specific CD4 T-cells (Th1, Th17 and Th1-Th17) in relationship with the immuno-virological status of patients with PHI

## Patients and Methods

27 patients with early PHI were included in a prospective longitudinal study and followed-up for 6 months. Twelve patients received ART after enrollment **(Table 1).** *Ex-vivo* T-cell activation levels and natural Tregs frequency were assessed by flow-cytometry on fresh PBMCs. Cytokine-producing effector CD4 T cells (Th1, Th17 and Th1-Th17) were quantified by intracellular cytokine staining on freshly isolated peripheral $CD3^+CD4^+$ cells stimulated for 5h with PMA/ionomycin or for 72h with the HIV-1 gag p24 antigen in the presence of autologous monocytes.

Comparisons between groups were performed using the Mann-Whitney test. The Wilcoxon rank test was used to estimate changes in the different variables throughout the follow-up.

**Table 1. Patients' characteristics**

| Time points | Patients | HIV-RNA *($log_{10}$/mL)* | CD4 *(%)* | CD4 count *(cells/mm³)* | CD8 *(%)* | CD8 count *(cells/mm³)* | CD4/CD8 ratio |
|---|---|---|---|---|---|---|---|
| **Baseline** | n= 27 | 5.65 (4.57-6.25) | 26.0 (17.0-34.0) | 490 (337-615) | 54 (42-64) | 1117 (553-1569) | 0.48 (0.28-0.78) |
| **M6** | untreated n= 13* | 4.40 (3.50-4.80) | 34 (29-38) | 669 (457-725) | 44 (39-49) | 713 (600-924) | 0.82 (0.62-1.02) |
| **M6** | ART n= 12** | 1.30 (1.00-1.33) | 45 (35-48) | 750 (593-783) | 35 (28-37) | 614 (533-672) | 1.33 (0.99-1.66) |

Data are expressed as median (IQR).
* For one patient experiencing an acute event associated with high level of inflammation at M6, clinical data from M3 were used.
** Patients received ART between baseline and M6. Two patients were lost to follow-up

## Conclusion

Immune activation in PHI is associated with low polyclonal effector CD4 T cell responses but not related to levels of HIV-specific responses suggesting that immune activation is mainly driven by the virus and innate immune responses rather than by HIV-specific responses.

On the other hand, the frequency of HIV-specific Th1, Th17 and Th1-Th17 cells is positively associated with plasma viral load, suggesting that these cells are driven by viral replication but are not able to contribute to its control in the early phase of infection.

**Acknowledgments:** We thank all clinicians who participated in the study and Maria Manea, Pauline Campa, Nelly Desplanques and Feriel Tibaoui for their help. We are grateful to the patients who agreed to participate in the study.

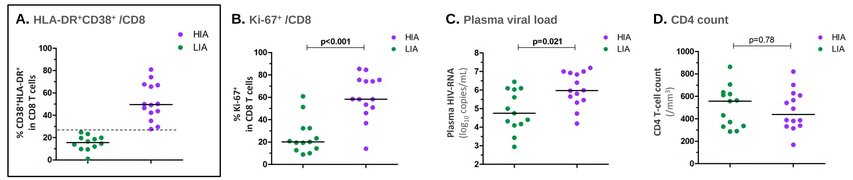
**Funding:** This study was funded by the ANRS (French National Agency for Research on AIDS and Viral Hepatitis) and AP/HP Hôpitaux de Paris

**Contact :**
**Laurence Weiss, MD/PhD**
**laurence.weiss@egp.aphp.fr**

## Results

### 1. Segregation of patients into two groups according to their CD8 T-cell activation level

**Figure 1. Characteristics of "LIA" and "HIA" patients**




Two groups of patients were segregated based on the level of CD8 T-cell activation at baseline (**figure 1A**) :
- group with lower immune activation ("**LIA**", n=13), i.e. % $HLA\text{-}DR^+CD38^+$ CD8 T cells < median (28.5 %)
- group with higher immune activation ("**HIA**", n=14), i.e. % $HLA\text{-}DR^+CD38^+$ CD8 T cells ≥ median

As expected, HIA patients had also higher frequency of CD8 T cells expressing the activation/proliferation marker Ki-67 as compared with LIA patients (**figure 1B**). Patients with HIA had higher plasma HIV-RNA levels as compared to patients with LIA (**figure 1C**) but CD4 T-cell counts were similar in both groups (**figure 1D**).

### LIA and HIA patients show differences in "polyclonal" CD4 T-cell responses

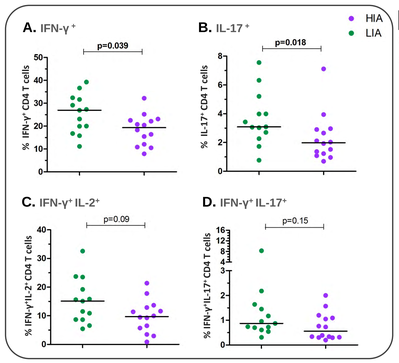


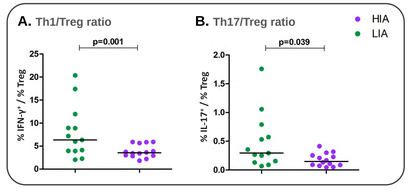

**Figure 2.**
**Comparison of CD4 T-cell responses to PMA/ionomycin stimulation in LIA and HIA patients**

Following 5h PMA/ionomycin activation of freshly isolated CD4 T cells, patients with HIA exhibited significantly lower frequency of IFN-γ+ and IL-17+ single positive CD4 T cells as compared to patients with LIA (**figure 2A-B**); there was a trend to a lower frequency of $IFN\gamma^+IL\text{-}17^+$ between the 2 groups; the proportion of $IFN\gamma^+IL\text{-}2^+$ double positive CD4 T cells did not differ between groups (**figure 2C-D**). Also, we found no difference in IL-2 expression between both groups (***data not shown***).

No differences were found in the frequency of HIV-specific CD4 T cells (following gag-p24 stimulation) between both groups (***data not shown***).

### LIA and HIA patients show differences in effector to regulatory cell balance

**Figure 3. Comparison of effector/regulatory CD4 T cells ratios in LIA and HIA patients**

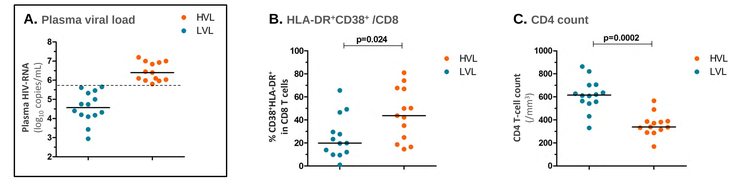



As natural regulatory T cells (Tregs) can dampen CD4 T-cell responses, it was of interest to assess the balance between effector CD4 T cells and Tregs. We thus measured the ex-vivo frequency of Treg cells, defined as $CD25^+CD127^{low}FoxP3^+$ CD4 T cells.

Interestingly, patients with HIA exhibited lower effector/regulatory T-cell balance when considering the Th1/Treg ratio (**figure 3A**) as well as the Th17/Treg ratio (**figure 3B**).

### 2. Segregation of patients into two groups according to their plasma viral load

**Figure 4. Characteristics of "LVL" and "HVL" patients**




Two groups of patients were then segregated based on the level of virus burden at baseline (**figure 4A**) :
- group with lower viral load ("**LVL**", n=14), i.e. plasma HIV-RNA ≤ median (5.65 log copies/mL)
- group with higher viral load ("**HVL**", n=13), i.e. plasma HIV-RNA > median

Patients with HVL exhibited higher CD8 T-cell activation (**figure 4B**) and showed lower peripheral CD4 T-cell counts (**figure 4C**) as compared to patients with LVL.

### LVL and HVL patients show differences in HIV-specific CD4 T-cell responses

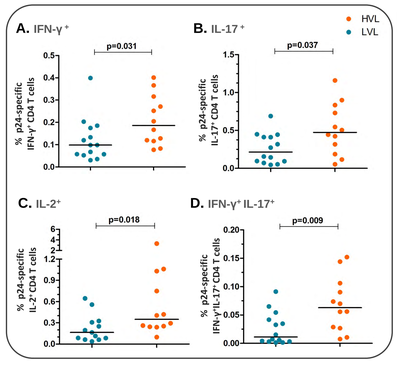


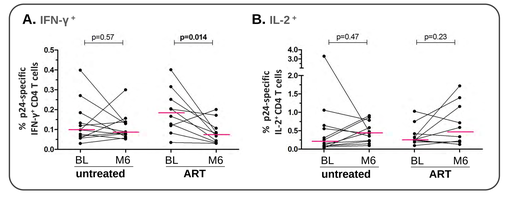

**Figure 5.**
**Comparison of HIV-specific CD4 T-cell responses in LVL and HVL patients**

HIV-specific CD4 T-cell responses were measured following 72h stimulation with the HIV-1 gag p24-protein. Patients with HVL showed significantly higher frequency of IFN-γ+, IL-2+, IL17+ and IFNγ+IL17+ HIV-specific CD4 T cells compared with LVL patients (**figure 5A-D**).

Following PMA/ionomycin stimulation, there was no difference in Th1 responses between both groups; however, patients with HVL showed higher frequency of IL-17-expressing CD4 T-cells compared with LVL patients (***data not shown***).

### 3. Effect of early ART initiation on HIV-specific CD4 T-cell responses

**Figure 6. Longitudinal follow-up of HIV-specific CD4 T-cell responses in untreated and ART-receiving patients**




We next assessed HIV-specific responses at month 6 of follow-up and compared untreated patients and patients receiving ART after baseline (BL). At month 6, treated patients had plasma HIV-RNA levels < 20 copies/mL.

Interestingly, the frequency of IFN$\gamma^+$ − but not of IL-$2^+$ − HIV-specific CD4 T cells significantly decreased between BL and month 6 in ART-treated patients but not in untreated patients (**figure 6A-B**).