10th CROI, Feb. 10-14 2003, Boston, MA. Abstract M-67

TIMING OF RECONSTITUTION OF TOXOPLASMA GONDII (Tg)-SPECIFIC T-CELL RESPONSES IN AIDS PATIENTS WITH ACUTE TOXOPLASMIC ENCEPHALITIS (TE) AFTER STARTING POTENT ANTIRETROVIRAL THERAPY (HAART): A PROSPECTIVE MULTICENTER LONGITUDINAL STUDY.

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OBJECTIVE

To assess the timing of

the reconstitution of the *Toxoplasma gondii* (Tg)-specific

T-cell responses in AIDS patients with

acute toxoplasmic encephalitis (TE)

after starting HAART.

PATIENTS

Design: Prospective multicenter longitudinal study.

- **26 AIDS patients with acute TE were included in the study.**
- **Six patients were not eligible (2 early deaths, 2 cases lost for follow-up and 2 cases did not have TE).**
- ***In vitro* tests were performed in the 20 AIDS patients with an acute TE since the acute phase until immunological recovery with HAART (PI containing regimen) at the following time points:**
  - **20 patients were studied at baseline (T0)**
  - **16 patients were sampled at 3 months (T3)**
  - **10 patients were sampled at 6 months (T6)**
  - **16 patients were sampled between 9-12 months (T12)**
  - **8 patients were sampled between 15-18 months (T18)**
  - **7 patients were sampled at 24 months (T24)**
- **All patients were receiving TE maintenance therapy.**

METHODS (I)

Lymphoproliferative response

- PBMC isolated from blood samples were cultured for 4 or 7 days, in 96 U-bottomed well plates at 2 x 10^5 cells per well with the soluble antigen extract of Tg (SATg) for 7 days. Cells were also cultured in the presence of PHA 0.5%, PWM 10 mg/mL, OKT3 10 ng/mL, anti-CD28 100 mg/ml and the recall protein antigens CMV. Cultures were made in triplicate and cellular proliferation was assessed by measuring the uptake of [3H] thymidine during the last 16 hrs of the culture period.

- Results are expressed as a stimulation index (SI), which corresponds to the ratio between the mean proliferation in counts per minute (cpm) of triplicate cultures for a given stimulus and the mean cpm of triplicate unstimulated cultures.

- A SI value ≥10 is considered as a positive SATg-specific response (*Lejeune M, Immunología. 20:1-11, 2001; http://www.inmunologia.org*) whether SI value ≥3 is considered positive for the mitogenic stimuli and the recall antigen.

METHODS (II)

Cytokine measurements

The production of the cytokines IFN-γ was measured in the supernatant of PBMC culture after 72 hrs of culture stimulated with medium alone or with the soluble antigen extract of Tg (SATg). Cytokine concentrations were determined in duplicate by ELISA, which were developed using antibody pairs and standards from Endogen. ELISA conditions were as recommended by these manufacturers (*Lejeune M, Immunología. 20:1-11, 2001; http://www.inmunologia.org*). Results are expressed as (Δ) pg/mL (Δ= [mean production in stimulated wells] -[mean production in unstimulated wells]).

Flow cytometry analysis.

Subpopulations of CD3+, CD4+ and CD8+ cells were determined by three-color flow cytometry using the following monoclonal antibodies: CD3-PerCP, CD4-PerCP, CD8-PerCP, CD4-FITC, CD8-PE, HLA-Dr-FITC, CD28-PE, CD38-PE, CD45RO-PE, CD45RA-FITC, The stained cells were analyzed on a FACScan (Becton Dickinson) flow cytometer and data were analyzed using LYSIS II software (Becton Dickinson) .

METHODS (III)

Plasma viral RNA assay.

Plasma HIV-1 RNA load was evaluated for the all patients at the time of inclusion by a quantitative polymerase chain reaction assay (Amplicor, Roche Diagnostic Systems) with a lower limit of quantification of 200 copies/mL.

***Toxoplasma gondii* and CMV serologic analysis.**

The serological status against Tg and CMV were also evaluated for all the patients at the time of inclusion using the commercially available reagents CMV IgG Abbot Axsym System (Abbot Laboratories) and Vidas Toxo IgG II (BioMérieux) following the manufacturers' instructions. A serologic analysis was considered to be positive for T. gondii IgG titer >6 UI/mL and CMV IgG titer >8 UI/mL.

Figure presentation

LPR and IFN-γ production were represented as box-and-whiskers plots for the different groups of patients. Boxes extend from the 25th percentile to the 75th percentile (IQR); lines inside the boxes represent the median value. Lines emerging from the boxes extend to the upper and lower adjacent values. Upper adjacent value is defined as the largest data point and the lower adjacent value is defined as the smallest data point.

Patient characteristics (N=20)

Age [mean years (range)]	38 (25-63)
Male sex (%)	10 (50)
Risk group category (%)	
Heterosexual	10 (50)
Homosexual	0 (0)
Hemophilic	0 (0)
Injection drug user	9 (45)
Unknown	1 (5)

After the acute TE episode, all patients started HAART with a PI containing regimen and TE maintenance therapy.

Immunological characteristics, plasma HIV viral load and *T. gondii* serology at different time points.

	Group T0 (n=20)	Group T3 (n=16)	Group T6 (n=10)	Group T12 (n=16)	Group T18 (n=8)	Group T24 (n=7)
CD4 absolute cell count [median cells/mL (IQR)]	59 (7-171)	143 (43-346)	280 (163-310)	204 (169-365)	301 (169-301)	223 (160-314)
CD8 absolute cell count [median cells/mL (IQR)]	68 (43-213)	280 (371-693)	441 (376-791)	330 (204-432)	674 (416-769)	688 (359-920)
CD3+CD4+ % cell count* [median cells/mL (IQR)]	4.69 (2-11)	15.55 (3-23)	19.26 (13-21)	11.83 (7-19)	16.67 (15-19)	30.78 (25-32)
CD3+CD8+ % cell count* [median cells/mL (IQR)]	42.53 (14-59)	45.06 (32-52)	40.81 (35-50)	35.3 (23.44)	30.66 (29-34)	33.38 (21-44)
HIV RNA level [median copies/ml (IQR)]	53.700 (10.748-154.000)	449 (195-123.148)	252 (200-2.333)	<200 (<200)	<200 (<200)	<200 (<200)
Ig G *T. gondii* antibodies titer [median UI/ml (IQR)]	208 (131->300)	201 (146-280)	75 (0-225)	32 (0-241)	NA	NA

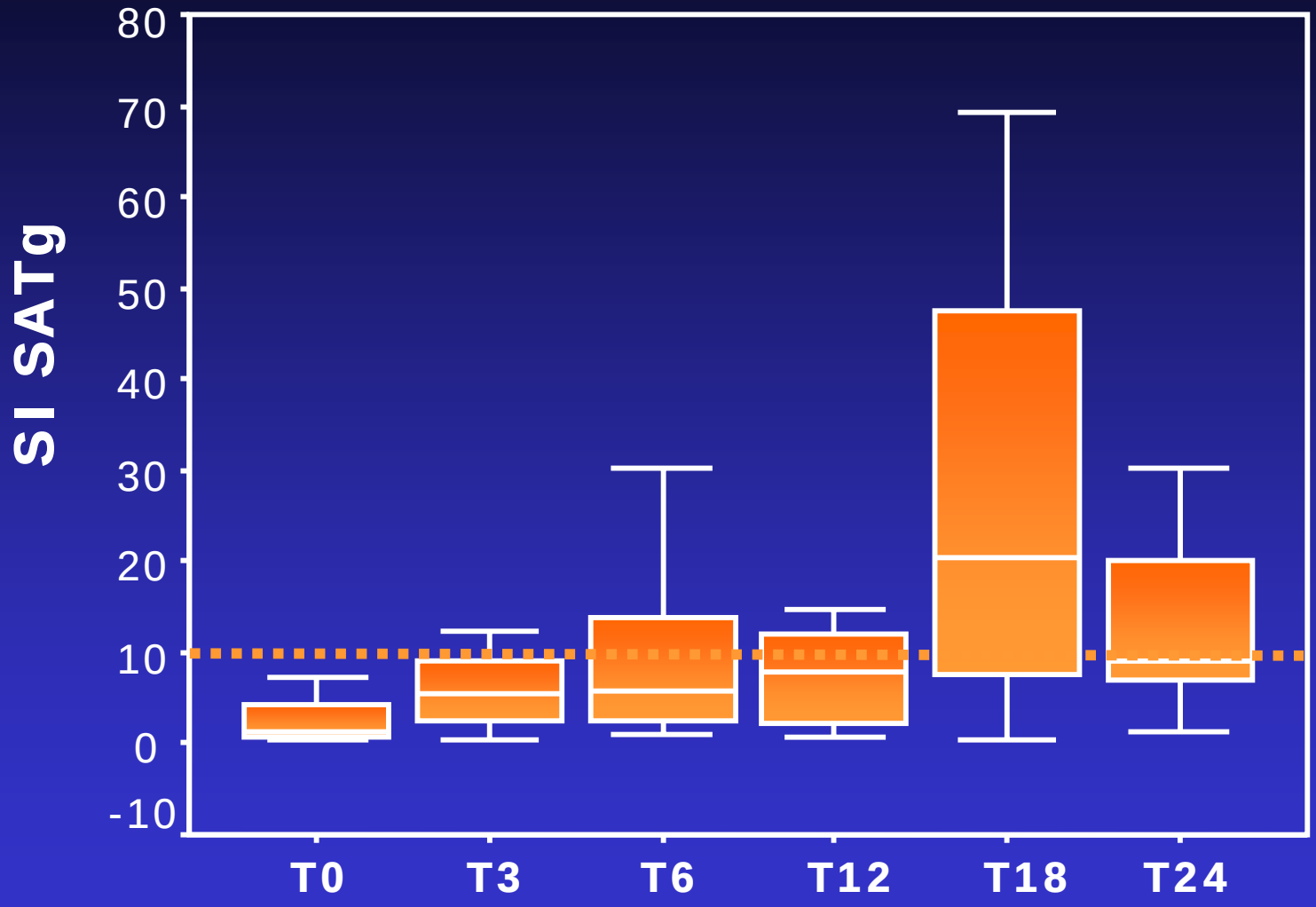
Data are median; IQR: interquartile range; NA= No available.
*= percentages of CD3+CD4+ or CD3+CD8+ T cells in peripheral blood mononuclear cells.

CD4 and CD8 T-cell subpopulations at different time points.

	Group T0 (n=20)	Group T6 (n=16)	Group T6 (n=10)	Group T12 (n=16)	Group T18 (n=8)	Group T24 (n=7)
CD4+CD28+	93.12 (77-98)	94.81 (87-98)	97.58 (87-99)	96.37 (92-99)	96.85 (95-99)	97.97 (97-99)
CD4+CD38+	78.36 (60-84)	64.00 (52-82)	74.22 (60-87)	62.40 (45-69)	57.07 (44-65)	64.43 (47-66)
CD4+CD45RO+	44.89 (32-72)	61.70 (42-79)	43.53 (36-62)	54.91 (42-74)	61.68 (41-71)	53.98 (45-69)
CD4+CD45RA+	19.48 (6-37)	19.17 (5-41)	38.04 (20-49)	20.38 (14-48)	26.04 (17-46)	27.12 (16-47)
CD8+CD28+	41.77 (21-62)	47.01 (37-62)	52.07 (37-79)	51.92 (41-63)	58.76 (45-63)	56.63 (36-65)
CD8+CD38+	89.32 (69-95)	84.23 (61-92)	59.83 (46-77)	65.00 (51-77)	53.99 (36-67)	53.60 (50-73)
CD8+CD45RO+	48.22 (30-63)	44.67 (38-59)	35.08 (22-51)	31.99 (26-54)	42.08 (32-54)	41.70 (35-48)
CD8+CD45RA+	34.09 (24-54)	38.44 (24-46)	47.87 (37-58)	47.15 (27-49)	39.28 (26-53)	37.47 (36.55)

Data are median (IQR: interquartile range) of percentages of gated CD4+ or CD8+ T lymphocytes positive for each marker

Lymphoproliferative response (LPR)(SI) to soluble antigen extract of *T. gondii* (SATg) at different time points



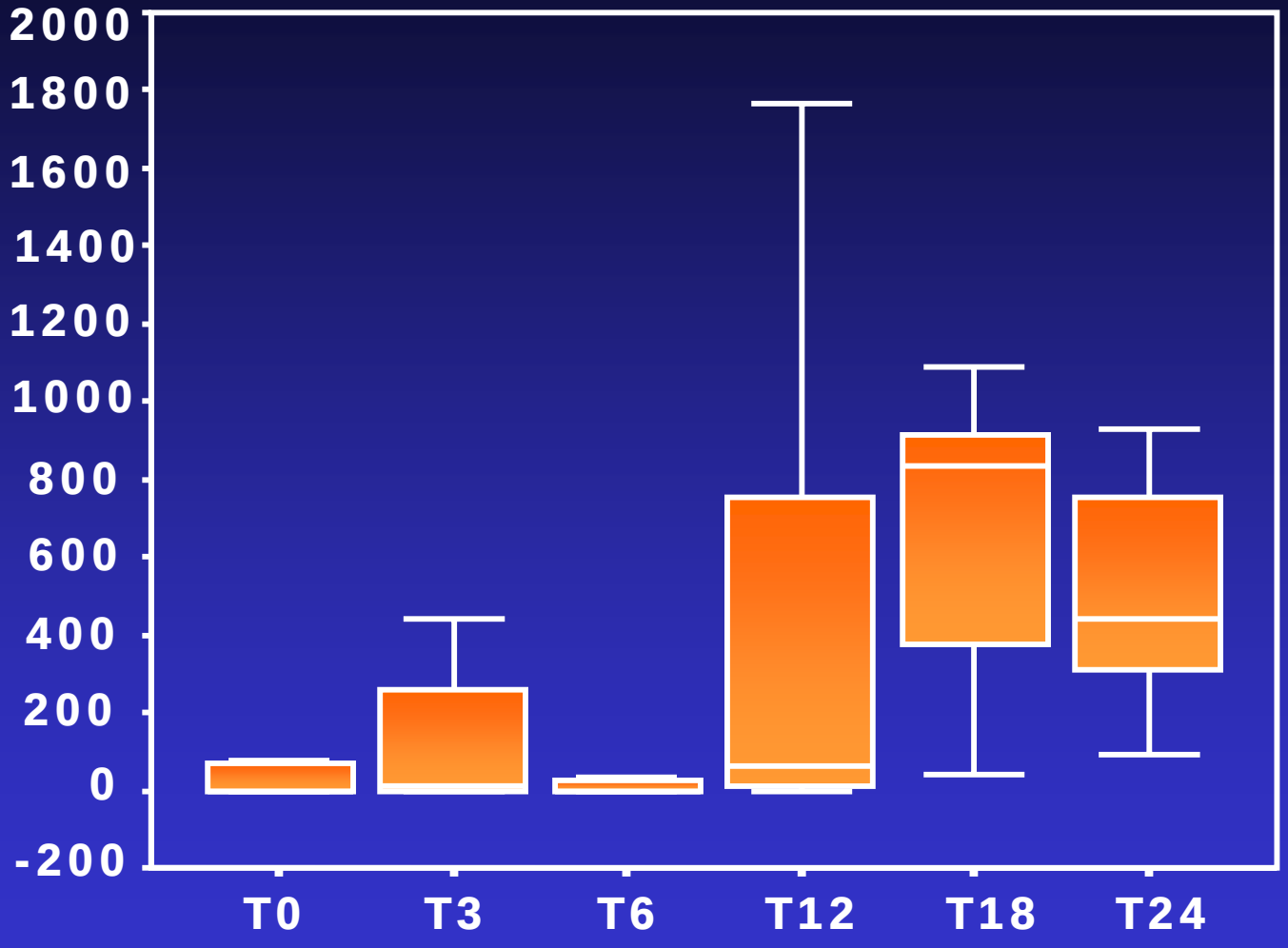
	T0	T3	T6	T12	T18	T24
SI > 10	10 %	19 %	30 %	38 %	63 %	57 %

Lymphoproliferative response(SI) to PWM, PHA, CD3 AND CD3+CD28 at different time points

	Group T0 (n=20)	Group T3 (n=16)	Group T6 (n=10)	Group T12 (n=16)	Group T18 (n=8)	Group T24 (n=7)
PWM	7 (2-19)	17 (10-36)	20 (7-52)	19 (9-27)	16 (3-64)	55 (33-106)
PHA 0.5%	6 (2-13)	17 (6-30)	6 (2-13)	9 (2-32)	12 (2-35)	NA
CD3 10 ng/mL	4 (1-17)	19 (6-27)	9 (1-27)	11 (7-25)	10 (5-35)	NA
CD3+CD28	13 (3-44)	28 (16-50)	23 (5-53)	27 (16-44)	49 (36-56)	NA

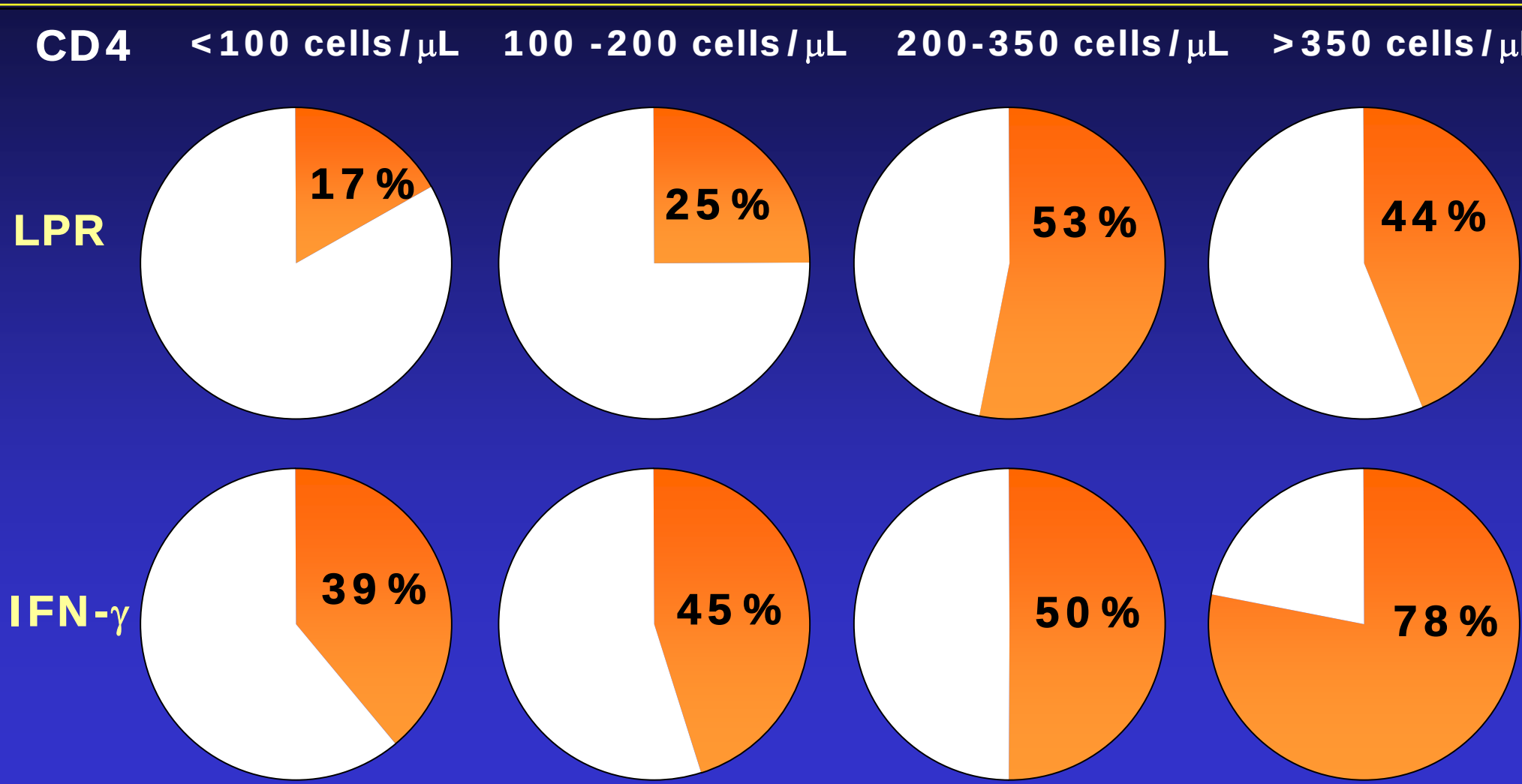
Data are median; (IQR: interquartile range); NA= No available.
SI: Stimulation index.

IFN-γ production at 72 hours in response to SATg at different time points

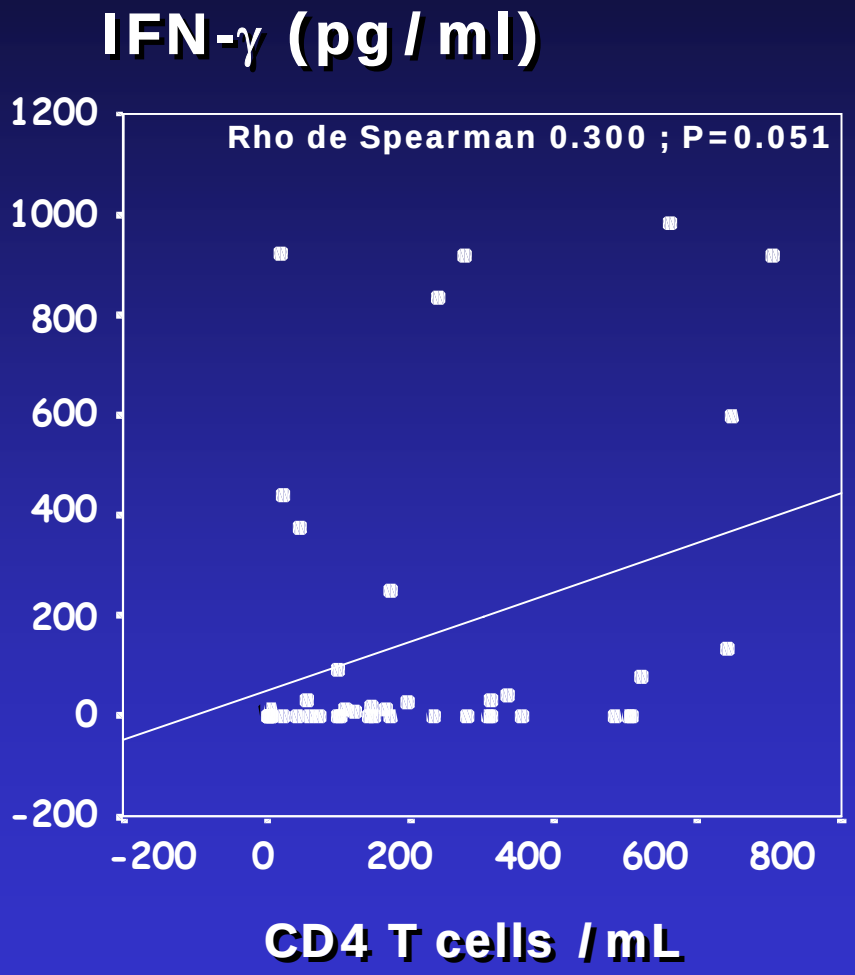


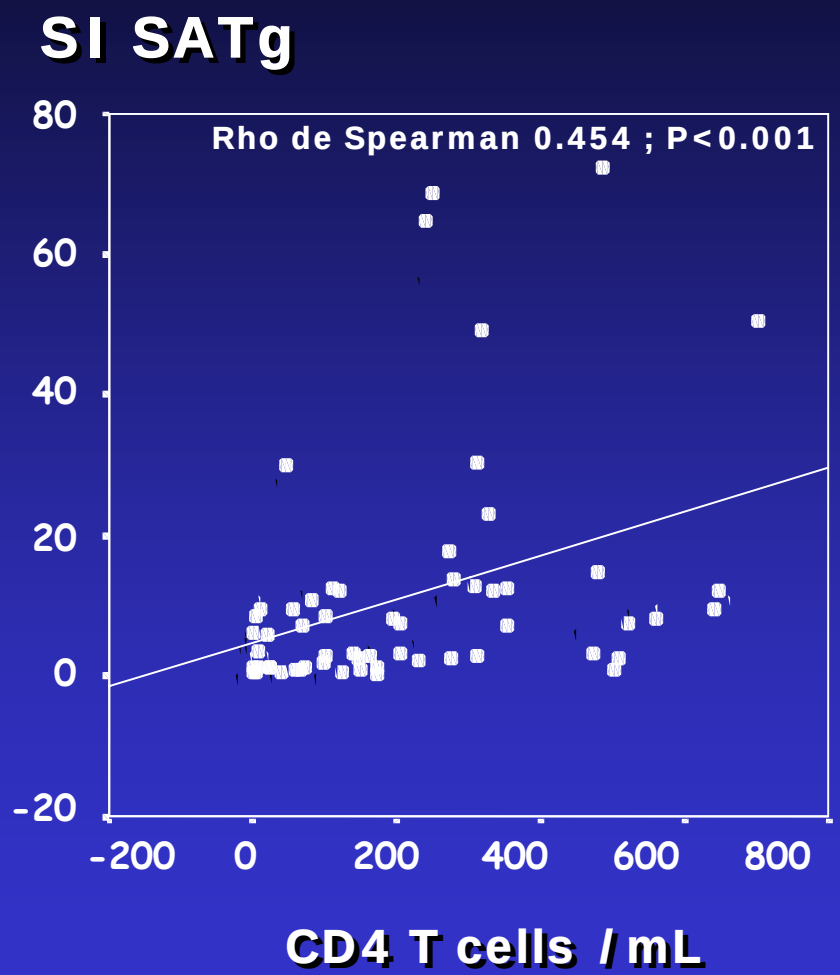
Δ pg/μL >0	28%	33%	38%	71%	83%	100%

Relationship between CD4+ T-cell count and the percentage of positive LPR (SI >10) and IFN-g production to SATg.



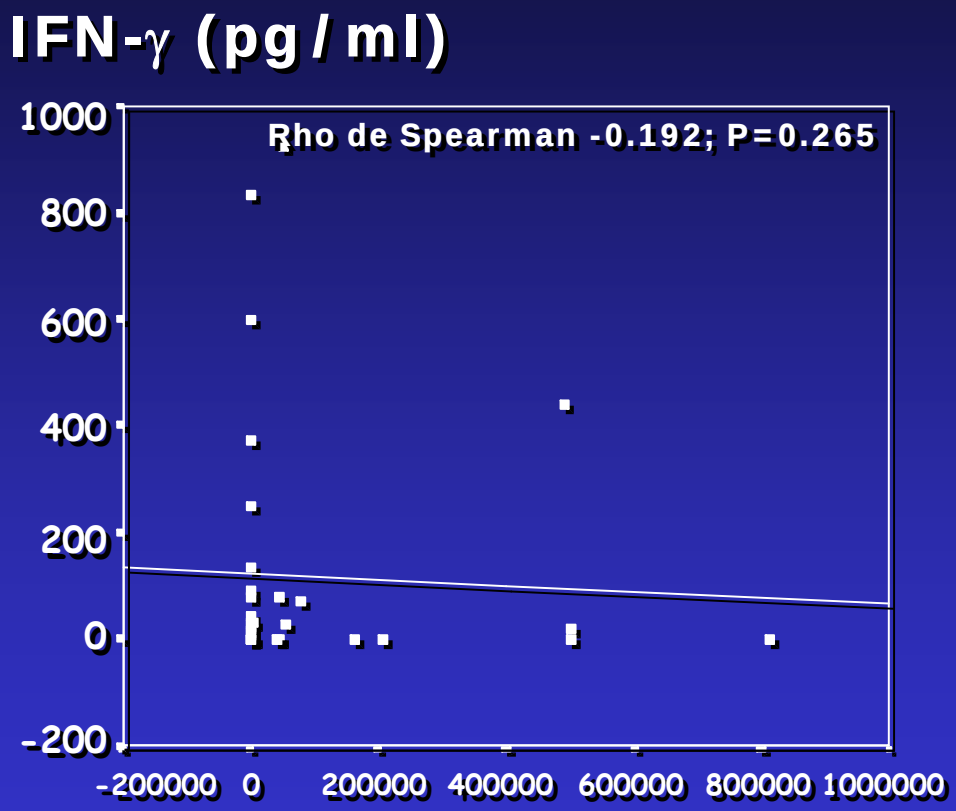
Relationship between CD4 count and the LPR (SI) and the production of IFN-γ to SATg

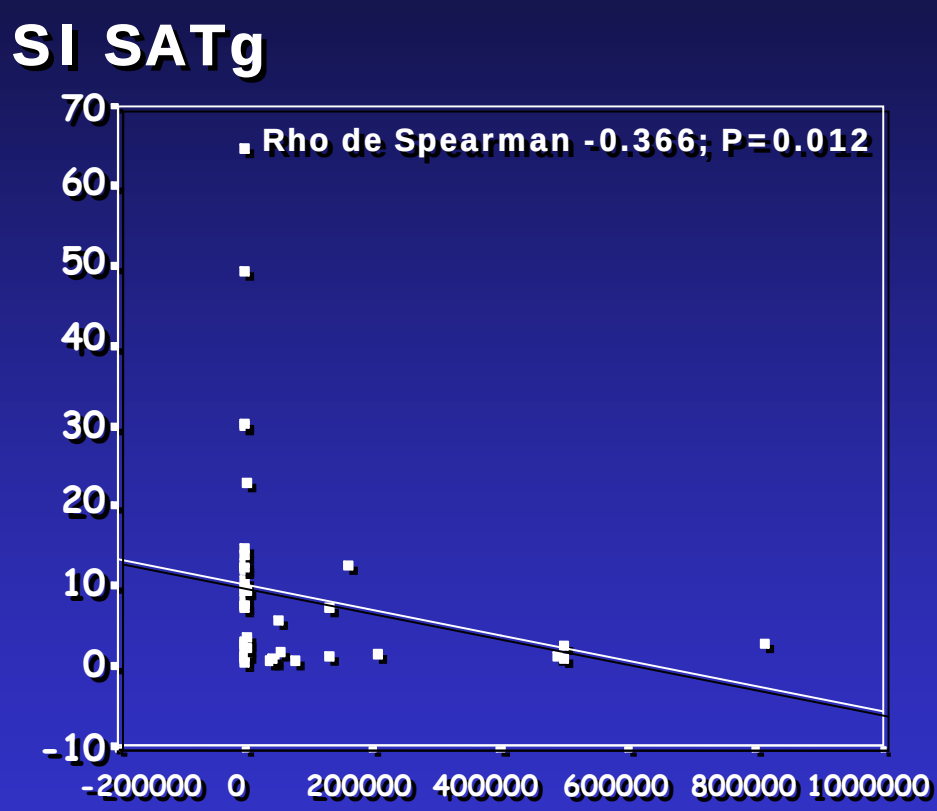




SI: stimulation index.

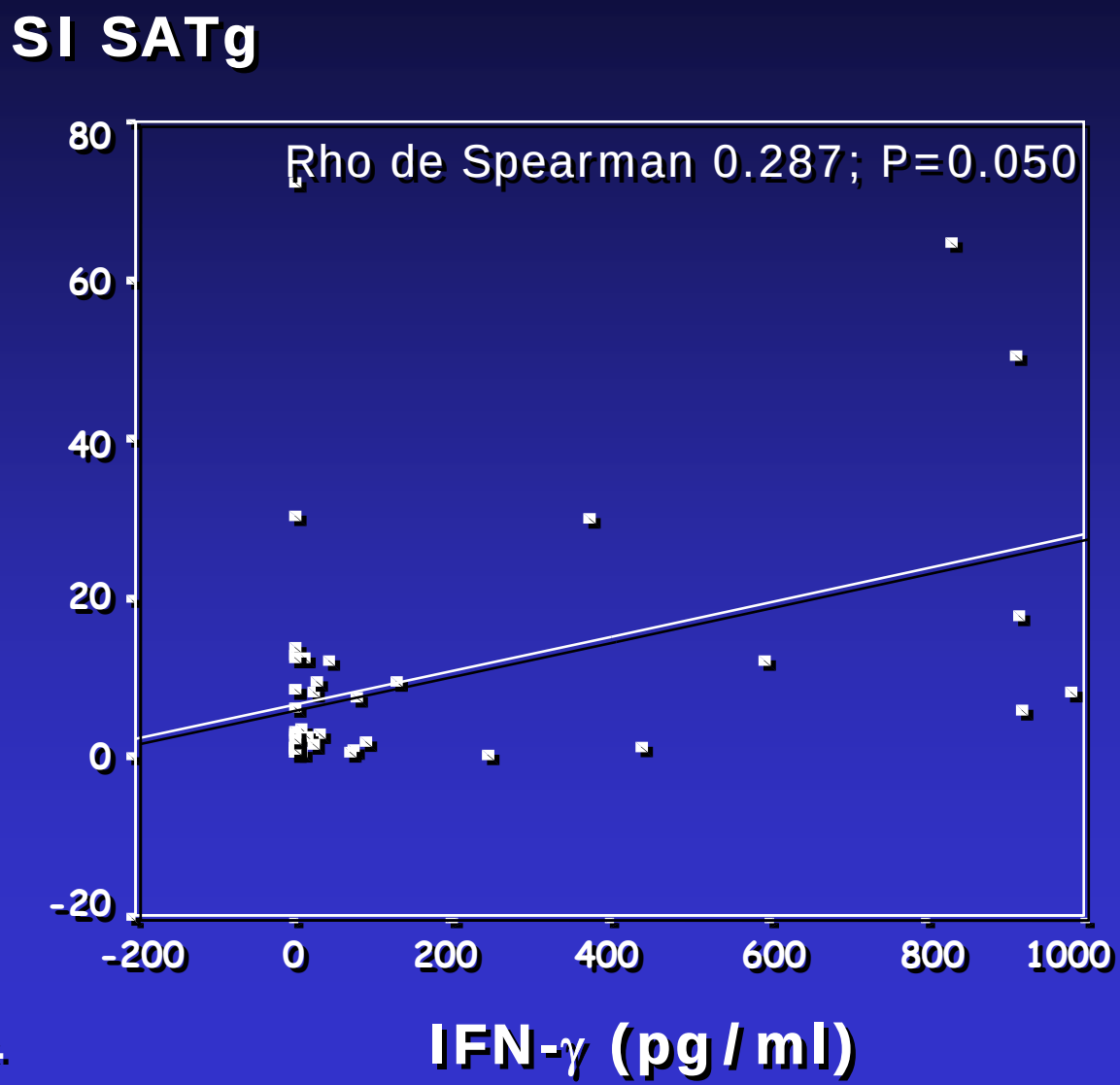
Relationship between plasma HIV viral load and LPR (SI) and the production of IFN-γ to SATg





SI: stimulation index.

Relationship between LPR (SI) and the production of IFN-γ to SATg



SI: stimulation index.

CONCLUSIONS (I)

- During acute TE episodes in AIDS patients, the proliferative T-cell responses and IFN-gamma production to SATg were absent in most cases.
- After 18-24 months of HAART, T-cell responses to SATg were restored in most patients who had an acute TE episode .
- This was especially true in terms of INF-g production, a cytokine essential for the control of *T. gondii*.

CONCLUSIONS (II)

- The restoration of a positive LPR to SATg was positively correlated with the increase of CD4 T cell counts above 200 cells/μL and negatively correlated with the plasma HIV viral load.
- This data may help us to know better when to stop TE maintenance therapy in AIDS patients with HAART.

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