



# EVIDENCE FOR VIRUS-SPECIFIC IMMUNE RESPONSE IMBALANCE IN HIV - ASSOCIATED LEUKOENCEPHALOPATHIES

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## ABSTRACT

**Background:** HAART has changed the AIDS scenario, but neurological disorders including PML and JC virus (JCV) - negative leukoencephalopathy are still a problem.  
**Methods:** To investigate clinical, virological and immunological parameters of AIDS-related leukoencephalopathies, a longitudinal survey has been taken. HIV+ HAART treated patients were subjected to MRI examination. Virological studies were carried out in cerebrospinal fluid (CSF) to verify the presence of JCV and other neurotropic viruses (HSV1/2, VZV, EBV, HCMV, HHV6, HHV8), and HIV and JCV viral loads were determined in CSF by RealTime PCR. MCP-1 was quantified in CSF by EIAs. The immunological evaluation consisted of measurement, by flow cytometry, of PBMC production of TNF $\alpha$ , IFN $\gamma$ , IL-2 and MCP-1, previous and after JCV HLA-restricted peptides stimulation. Statistical evaluation was done by Student's test and by Pearson's analysis.  
**Results:** MRI examination of 75 eligible patients revealed: 22 MRI+ (8 JCV+ PML, 12 NDLE and 2 with other neurological diseases, OND) and 41 MRI- patients (30 without neurological symptoms and 11 OND) were enrolled in the study, together with 27 healthy subjects, as control. Median plasma HIV load was significantly higher ( $p < 0,05$ ) in PML patients (11,12 ln copies/ml) than in NDLE patients (8,53 ln copies/ml), whereas median CSF HIV load was similar in the three groups. MCP-1 and HIV RNA in CSF were positively correlated in the three groups (PML:  $r = 0,11$   $p = 0,89$ ; NDLE:  $r = 0,399$   $p = 0,199$ ; OND:  $r = 0,260$   $p = 0,391$ ). No viruses were found in CSF of NDLE patients, whereas VZV and EBV were found in CSF of 2 OND MRI+ patients. In PML patients median JCV DNA in CSF load was 12,29 ln copies/ml. IFN $\gamma$  was produced, after stimulation, in a significantly ( $p < 0,05$ ) higher number of CD4+ cells of NDLE (0,60%) and PML (0,51%) patients, than of healthy subjects (0,01%); likewise, the IFN $\gamma$  production by CD8+ cells was higher ( $p < 0,05$ ) in NDLE patients (0,59%) than in healthy subjects (0,03%). No other significant differences in cytokines and chemokines production among patients and controls were found.  
**Conclusions:** No virus has been found in NDLE samples, however, the JCV-specific immune response observed in PML and in NDLE patients suggests that, also in NDLE, JCV could play a role yet to be defined. The finding of an increased cytotoxic activity indicates that an immune mediated mechanism is probably relevant in NDLE pathogenesis.

## BACKGROUND

### AFTER HAART INTRODUCTION...

- EXTENDED SURVIVAL OF HIV-INFECTED SUBJECTS
  - IMPROVEMENT OF COGNITIVE IMPAIRMENT (Sacktor N *et al.*, *Neuro*, 2001, 56: 257-260)
  - DECREASED OCCURENCE OF OPPORTUNISTIC CONDITIONS (Calabrese LH, *Clev Clin J Med*, 2001, 68: 105-112)
  - CHANGING IN PROGRESSIVE MULTIFOCAL LEUKOENCEPHALOPATHY (PML) CLINICAL COURSE
- BUT ALSO...
- EMERGENCE OF NEW FORMS OF SEVERE DEMYELINATING LEUKOENCEPHALOPATHY (Langford TD *et al.*, *AIDS*, 2002, 16:1019-1029)

→ NDLE:  
NOT DETERMINED LEUKOENCEPHALOPATHY

- FREQUENCY PARALLELING THE INCIDENCE OF PML AFTER HAART INTRODUCTION
  - CLINICAL SYMPTOMS AND MRI LESIONS RESEMBLING PML
- BUT ALSO...
- NO EVIDENCE OF ACTIVE REPLICATION OF JCV OR ANY OTHER NEUROTROPIC VIRUSES

→ NEW FORMS OF PML:

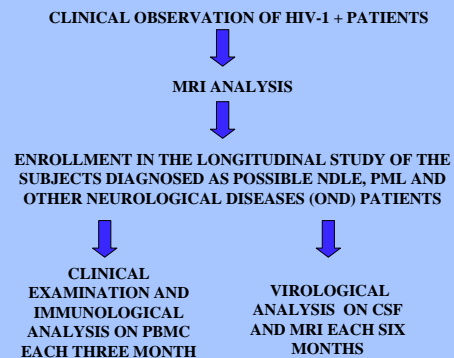
- PATIENTS WITH CLASSICAL PML HAVE A SURVIVAL TIME OF ABOUT SIX MONTHS (FAST PROGRESSOR - FP-)
- ABOUT 50% OF THE HAART TREATED PATIENTS HAVE SURVIVAL TIME SIGNIFICANTLY PROLONGED (SLOW PROGRESSOR - SP-)

## AIMS

→ TO INVESTIGATE CLINICAL, NEURORADIOLOGICAL, VIROLOGICAL, IMMUNOLOGICAL PARAMETERS OF THE NEW FORMS OF AIDS- RELATED LEUKOENCEPHALOPATHIES

→ TO SUGGEST HYPOTHESIS REGARDING NDLE PATHOGENESIS

## STUDY DESIGN



## SUBJECTS

75 ELIGIBLE HIV-1+ PATIENTS WERE SUBJECTED TO CLINICAL OBSERVATION AND TO MRI ANALYSIS.

Enrollment of 63 subjects:

8 MRI+, JCV+, PML

12 MRI+, POSSIBLE NDLE

2 MRI+, OND

11 MRI-, OND

30 MRI-, WITHOUT NEUROLOGICAL SYMPTOMS

+

27 HEALTHY CONTROLS (HC) MATCHED FOR AGE AND SEX

## METHODS

### VIROLOGICAL ANALYSIS

- JCV DNA: nested PCR (Ferrante P *et al.*, *J Med Virol*, 1995, 47:219-225) and Real Time PCR (ABI Prism 7000, Applied Systems) on CSF, detection limit 30 copies/ml
- HSV1 and 2, VZV, EBV, HCMV, HHV6 and HHV8 nested PCR (Ferrante P. *et al.*, *Acta Neurol Scand*, 1997, 169:79-85) on CSF
- HIV RNA: Real Time PCR (ABI Prism 7000, Applied Systems) on CSF, detection limit 50 copies/ml

### IMMUNOLOGICAL ANALYSIS

- MCP1: Commercial immunoenzymatic Assays (Quantikine R&D System) detection limit 16 pg/ml
- JCV VP1 HLA RESTRICTED-PEPTIDES SELECTION by algorithm
- Mesasurement of PBMC production of TNF $\alpha$ , IFN $\gamma$ , IL-2, previous and after JCV HLA-restricted peptides stimulation, by flow cytometry.
- STATISTIC ANALYSIS: Student's t test and correlations by Pearson's analysis

# VIROLOGICAL RESULTS

## PATIENTS CHARACTERISTICS AT THE ENROLLMENT

- 63 HIV1+ PATIENTS (78% MALE, median age 44 years old)
  - MEDIAN CD4 COUNTS: 263 cells/ $\mu$ l
  - MEDIAN PLASMA HIV RNA: 5,67 log copies/ml
  - MEDIAN CSF HIV RNA: 7,15 log copies/ml
  - MEDIAN CSF MCP1: 5,91 log pg/ml

## VIROLOGICAL FINDINGS

- 12 NDLE patients: **NEGATIVE** for all the **SEARCHED VIRUSES**
- 8 PML patients: **JCV+**, MEDIAN CSF JCV 12,29 log copies/ml
  - 1 OND patients: **VZV+**
  - 1 OND patients: **EBV+**

## HIV-RNA in the patients groups

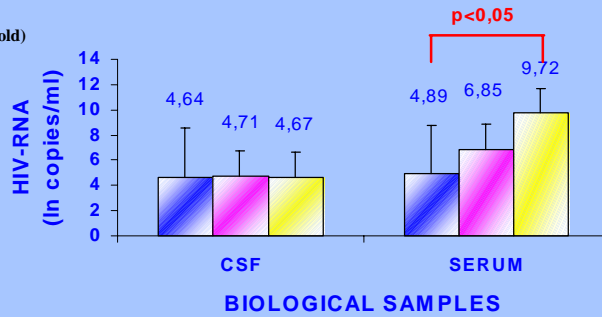


Figure 1: Graphic representation of the median value of HIV-RNA in CSF and Serum of the NDLE, OND and PML patients.

## Positive correlation between HIV-RNA and CSF MCP1 in NDLE patients

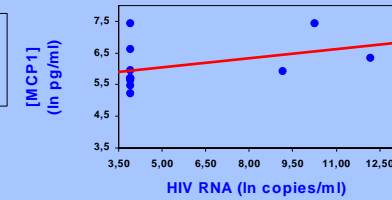
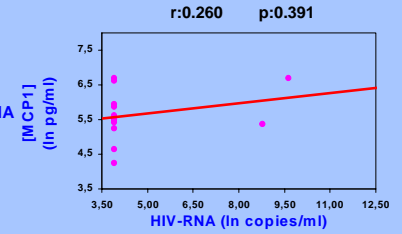
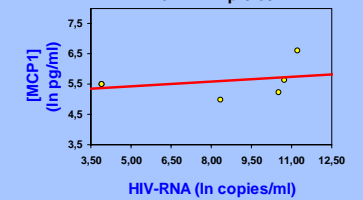


Figure 2: HIV-RNA in CSF of the NDLE, OND and PML patients and its association with CSF MCP-1 concentration.

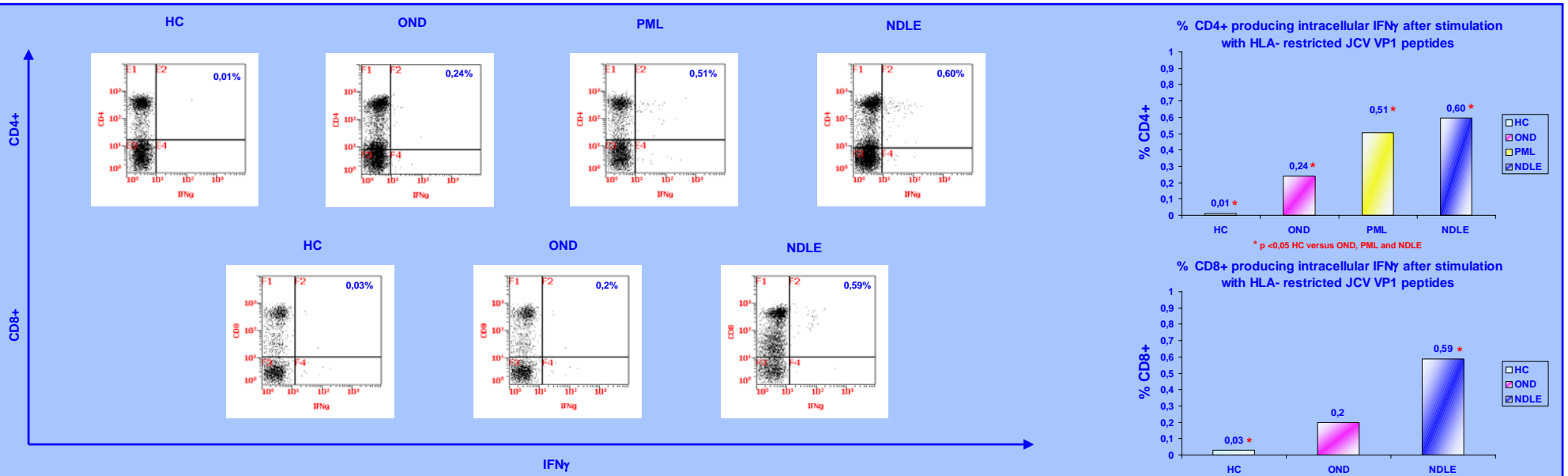
## Positive correlation between HIV-RNA and CSF MCP1 in OND patients



## Positive correlation between HIV-RNA and CSF MCP1 in PML patients



# IMMUNOLOGICAL RESULTS



## CONCLUSIONS

- **NO VIRUS HAS BEEN FOUND IN NDLE SAMPLES, HOWEVER, THE JCV-SPECIFIC IMMUNE RESPONSE OBSERVED IN NDLE AND IN PML PATIENTS SUGGESTS THAT, ALSO IN NDLE PATHOGENESIS, JCV COULD PLAY A ROLE, YET TO BE DEFINED.**
- **THE INCREASED CYTOTOXIC ACTIVITY INDICATES THAT AN IMMUNE-MEDIATED MECHANISM IS PROBABLY RELEVANT IN NDLE PATHOGENESIS.**

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