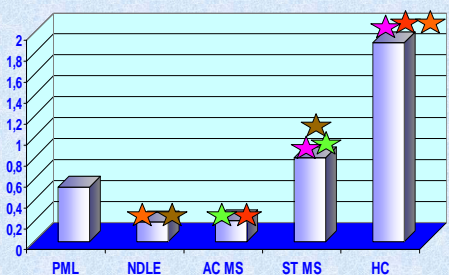
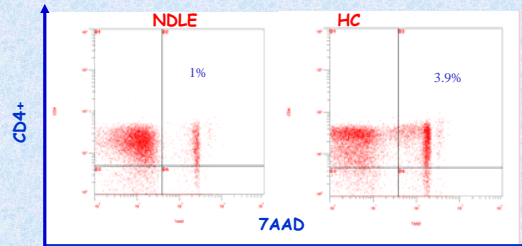


# RESULTS

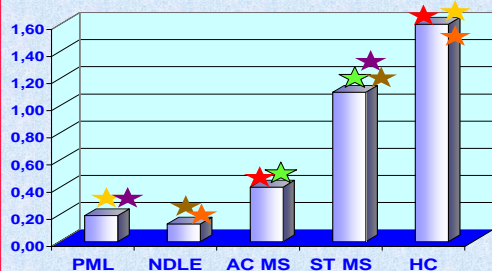
**Fig.1** Percentage of CD4 7AAD (apoptotic cells) in PML NDLE Acute and Stable MS patients and HC after MBP stimulation



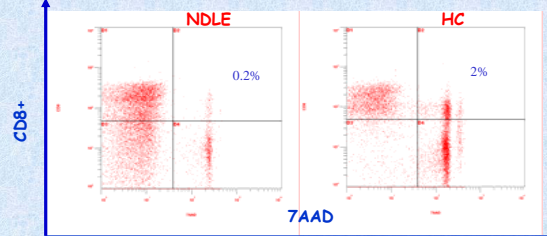
**Fig.1a** Flow cytometry analysis of CD4+ 7AAD+ (apoptotic cells) from a representative patient with NDLE and a healthy subject after MBP peptides stimulation. The percentage of apoptotic cells appears in upper right corner



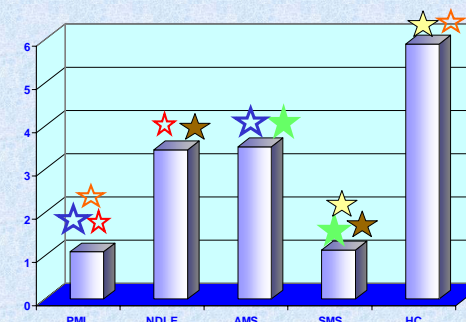
**Fig.2** Percentage of CD8 7AAD (apoptotic cells) in PML NDLE Acute and Stable MS patients and HC after MBP stimulation



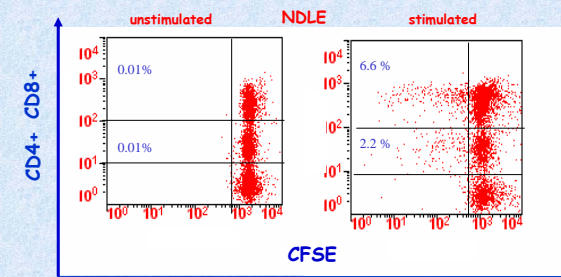
**Fig.2a** Flow cytometry analysis of CD8 7AAD (apoptotic cells) from a representative patient with NDLE and a healthy subject after MBP peptides stimulation. The percentage of apoptotic cells appears in upper right corner.



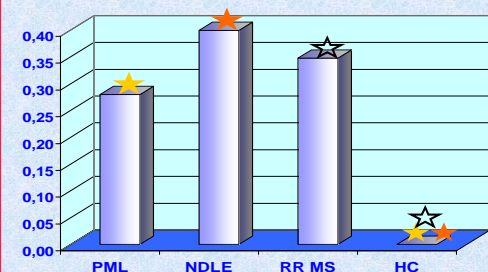
**Fig.4** Mean of Proliferation Index (P. I.) in PML, NDLE, AMS, SMS and HC after MBP stimulation



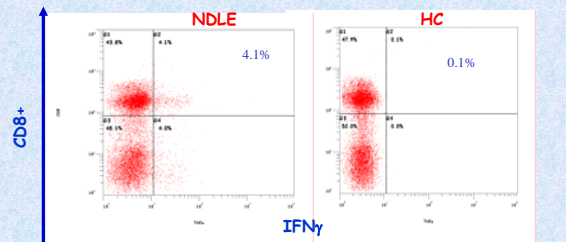
**Fig.4a** Percentage of CD4+ and CD8+ proliferated T cell from a representative patient with NDLE in unstimulated medium and after MBP stimulation. The percentage of CD4+ proliferated T cell appears in lower left corner and CD8+ proliferated T cell appears in the upper left corner.



**Fig.3** Percentage of CD8 producing IFN $\gamma$  in PML, NDLE RRMS patients and HC after MBP stimulation



**Fig.3a** Flow cytometry analysis of percentage of CD8 producing IFN $\gamma$  from a representative NDLE patient and a healthy subject after MBP peptides stimulation. The percentage of CD8+ T cells producing IFN $\gamma$  appears in upper right corner.



# CONCLUSIONS

★ THE INCREASE OF MBP STIMULATED IFN $\gamma$  PRODUCING CD8+ PERCENTAGE IN PML, NDLE AND MS PATIENTS IN COMPARISON TO HC, SUGGESTS THE PRESENCE OF MBP ACTIVATED AUTOREACTIVE CD8 T CELLS ALSO IN NDLE AND PML GROUPS AS IN MS

★ A REDUCTION OF THE APOPTOTIC AND AN INCREASE OF PROLIFERATION RATE OF MYELIN SPECIFIC CD4+ AND CD8+ T LYMPHOCYTES COULD BE INVOLVED IN THE IMMUNE-MEDIATE DESTRUCTION OF MYELIN SHEATH EVEN IN PML AND NDLE PATIENTS AS IN ACUTE MS GROUP IN COMPARISON TO STABLE MS AND HC



# STIMULATION WITH MYELIN BASIC PROTEIN INDUCES APOPTOSIS AND SPECIFIC IMMUNE RESPONSE IN HIV+ PATIENTS WITH LEUKOENCEPHALOPATHIES

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## ABSTRACT

### INTRODUCTION

The most consistent hypothesis to explain the slow progression of Progressive Multifocal Leukoencephalopathy (PML) observed in HAART treated HIV+ patients is that the reconstitution of the immune system may restore immune defenses against JC Virus (JCV), leading the patients to a longer survival time. However, in apparent contrast, it has also been observed that, in a very limited number of cases, the introduction of HAART can contribute, probably through an autoimmune mechanism, both to induce new demyelinating lesions and to worsen the prognosis of already diagnosed PML and Not Determined Leukoencephalopathy (NDLE), a PML-like JCV negative disorder. A possible explanation of controversial effect of immune reconstitution could be that immune competent cells switch their activity, probably through molecular mimicry with cross-reacting JCV epitopes, also against self-component such as Myelin Basic Protein (MBP). As suggested from studies on autoimmune disorders, such as Multiple Sclerosis (MS), a deregulation of apoptosis could play a key role in inducing or maintaining auto-reactive immune phenomenon. Thus, in NDLE and PML patients, an imbalance of apoptosis could lead to autoimmune demyelination mechanism.

### RESULTS

In our study we analyzed by flow cytometry CD4+ CD8+ apoptotic cells after Myelin Basic Protein (MBP) stimulation in 8 PML, 14 NDLE, 20 Relapsing-remitting (RR) Multiple Sclerosis (MS) groups and 14 Healthy Control (HC). We observed a significant increase of CD8+ apoptotic cell in HC in comparison to MS, NDLE and PML patients ( $p < 0.05$ ).

### CONCLUSION

These data evidence an activation of immune system against self antigen like MBP in patients with leukoencephalopathy and MS with a decrease of CD8+ apoptotic MBP specific T cells in the same patients. On the whole they suggest that these auto-reactive immune phenomena could play a relevant role in PML, NDLE and MS.

## AIMS

To verify the involvement of apoptosis mechanism in the demyelinating lesions of leukoencephalopathies

## BACKGROUND

- ★ The most consistent hypothesis to explain the slow progression of PML observed in HAART treated patients is that the reconstitution of the immune system may restore immune defenses against JCV leading these patients to a longer survival time.
- ★ In contrast HAART can contribute to induce new lesions, as it has been observed, in PML and NDLE, a PML-like JCV negative disorders, patients.
- ★ An hypothesis to explain the controversial effect of HAART therapy could be that the immune competent cells switch this activity, probably through molecular mimicry with cross-reacting JCV epitopes, also against self-component like MBP. As suggested from observations derived from studies on autoimmune disorders, like Multiple Sclerosis (MS), a deregulation of apoptosis could play a key role in inducing or maintaining auto-reactive immune phenomenon. Thus in NDLE and in PML patients, an imbalance of apoptosis could lead to autoimmune demyelination mechanism.

## MATERIALS AND METHODS

### STUDY DESIGN

- 9 RMI+, JCV+, PML patients HIV+
- 10 RMI+ possible NDLE patients HIV+
- 20 MS patients: 12 clinically stable RMI- 8 clinical relapses RMI+
- 14 Healthy Control

### APOPTOSIS INTRACELLULAR CYTOKINES AND PROLIFERATION ANALYSIS BY FLOW-CYTOMETRY

PBMCs were stimulated with 30 overlapping MBP peptides of 10 amino acids in length + anti-CD28 and BFA for 24 h at 37°C. Analysis of APOPTOSIS by 7AAD-permeability, Intracellular expression of IFN $\gamma$ , IL2 in CD4+ cells and of IFN $\gamma$ , TNF $\alpha$  in CD8+ cells and CD4+ and CD8+ proliferation by CFDA-SE labelling were performed.

The cytometric analyses were fulfilled using an EPICS XL flow cytometer (Beckman-Coulter Inc., Miami, FL.) equipped with a single 15 mW argon ion laser operating at 488 nm interfaced with 486 DX2 IBM computer. For each analysis, 20.000 events were acquired and gated on CD4 or CD8 expression, and side scatter properties. Sample were first run using isotype control or single fluorochrome-stained preparation for colour compensation.

Non-parametric tests were performed to evaluate difference among patients. For every variable a Kruskal-Wallis analysis of variance was performed.