

# JCV- and myelin-specific cellular immune response in MS patients treated with natalizumab: a prospective and longitudinal study



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## INTRODUCTION:

Natalizumab (Tysabri®) is a new monoclonal antibody which binds to the  $\alpha 4$  integrins, molecules that are expressed on the surface of activated lymphocytes and allow them to cross the blood-brain barrier. By preventing the rolling and diapedesis of CNS auto-antigen activated lymphocytes, this medication has been shown to be more efficient than current therapies in decreasing relapses and progression of disability in multiple sclerosis (MS) patients (1,2). However, since its approval, six MS patients treated with natalizumab suffered from progressive multifocal leukoencephalopathy (PML), on a total of over 35'000 treated patients.

PML is a severe demyelinating disease of the central nervous system. The polyomavirus JC (JCV) infects more than 85% of the normal adult population (3), and its reactivation in the setting of immuno-suppression, mostly AIDS, leads to a lytic infection of oligodendrocytes (3). Yet, since the blood cell counts are not decreased and since it does not seem to be associated with other opportunistic infections, natalizumab cannot be considered as a classical immunosuppressant. Therefore, other mechanisms have to be looked for. There are two, not mutually exclusive, theories: by closing the blood-brain barrier, natalizumab might prevent JCV-specific CD8+ T cells to reach the CNS and perform immune surveillance (4). Supporting this, we have shown that JCV-specific CD8+ T cells were crucial in controlling JCV and keeping PML under control (3). Alternatively, it has been postulated that this drug acts by releasing an excessive amount of JCV from the bone marrow, its site of latency (5), thus leading to an increased JC viremia. In this study, by following patients on Tysabri from day 0 up to 12 months, we directly address the second hypothesis, namely: are there signs of an increased JCV activity in the blood of patients on natalizumab?

Fig. 1

## Physiological steps in lymphocyte flow and adhesion: natalizumab blocks the blood-brain barrier

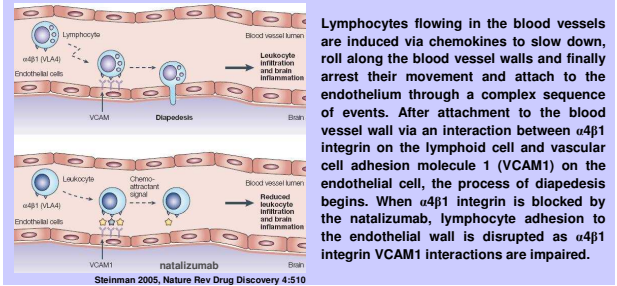
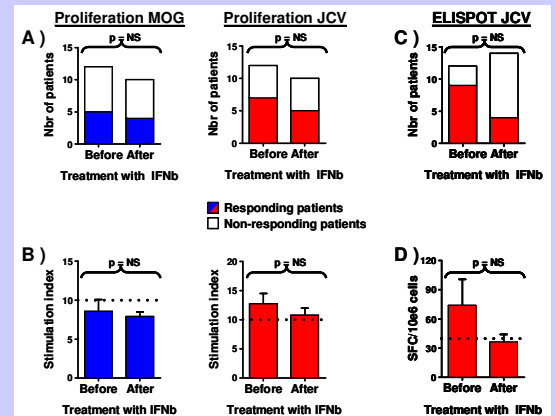


Fig. 4

## Control MS patients receiving IFN- $\beta$ immunomodulatory treatment do not show increased JCV-specific T cell response

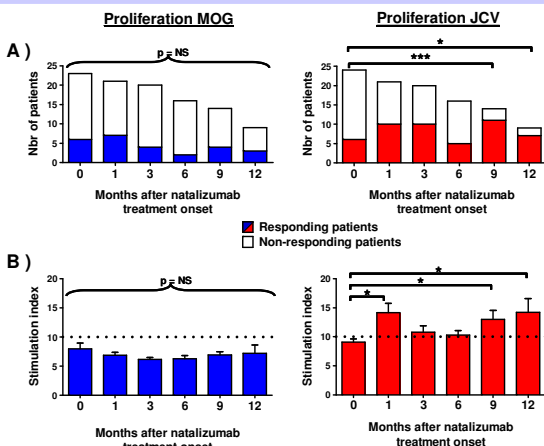


A) MS patients receiving IFN- $\beta$  show MOG and JCV-specific T cell responses, however no difference was found in the number of responding patients in proliferation assays. The number of MS patients responding to EBV and CMV is similar before and after the onset of IFN- $\beta$  treatment (data not shown).  
 B) Equally the proliferation of MOG-, JCV-specific T cells did not differ between the two time-points. The same was true for EBV- and CMV-specific T cells proliferation (data not shown).  
 C) Concerning the IFN- $\gamma$  secretion, no difference in the number of JCV-responding MS patients was found. The same is true for EBV- and CMV-specific cellular responses (data not shown).  
 D) Finally, no difference in the frequency of IFN- $\gamma$ -secreting JCV-specific T cells was found before and after the onset of IFN- $\beta$  treatment.

Fifteen MS patients receiving IFN- $\beta$  as immunomodulatory treatment were enrolled and PBMC obtained before and 0.8  $\pm$  0.3 year after IFN- $\beta$  treatment onset. Proliferation of antigen-specific T cells were assessed in PBMC after stimulation with MOG (left panels) and JCV (right panels) overlapping 15-mer peptides, or EBV and CMV lysates or nonamer peptides. IFN- $\gamma$  secretion was assessed by ELISPOT in PBMC after stimulation with JCV overlapping 15-mer peptides, or EBV and CMV lysates or nonamer peptides. Proliferation and ELISPOT data are shown as means  $\pm$  SEM. The dotted line delineate the threshold for positive responses. SFC, spot forming cell; NS, non significant (Kruskal-Wallis ranked test); \* $p < 0.05$ , \*\* $p < 0.005$  (Mann-Whitney ranked test).

Fig. 2

## Increased proliferation of JCV-specific T cells in the blood of MS patients on natalizumab

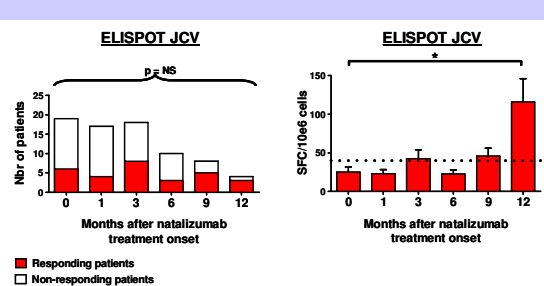


A) Myelin oligodendrocyte glycoprotein (MOG)-specific T cells are found in 15 to 30% of all MS patients on natalizumab with no significant change over time. In contrast, the number of JCV-responding MS patients increases sharply nine months after natalizumab onset, from 25% of the MS patients before natalizumab onset up to 80% of the MS patients at nine months, and stays stable up to one year. The number of EBV- or CMV-responding MS patients is stable throughout the period of observation (data not shown).  
 B) The proliferation of the JCV-specific T cells is equally increased, already one month after onset of the treatment. In contrast, the MOG-specific T cell proliferation is similar through the period of observation. The same goes for the EBV- and CMV-specific T cell proliferation (data not shown).

We enrolled 25 MS patients receiving monthly injections of natalizumab. PBMC were isolated and immediately processed. Proliferation of antigen-specific T cells were assessed in PBMC of MS patients on natalizumab at different time-points after onset of treatment (up to one year), after stimulation with MOG (left panels) and JCV (right panels) overlapping 15-mer peptides, or EBV and CMV lysates or nonamer peptides. Proliferation data are shown as means  $\pm$  SEM. The dotted line delineate the threshold for positive responses. NS, non significant (Kruskal-Wallis ranked test); \* $p < 0.05$ , \*\* $p < 0.005$  (Mann-Whitney ranked test).

Fig. 3

## Increased IFN- $\gamma$ secretion by JCV-specific T cells in MS patients one year after the first natalizumab injection



Secretion of IFN- $\gamma$  by JCV-specific T cells is increased at one year after the onset of natalizumab treatment (right panel), whereas the number of JCV-responding patients remain unchanged (left panel). In contrast, similar to the proliferation data, EBV- and CMV-specific IFN- $\gamma$  secretion was stable over time (data not shown).

We enrolled 25 MS patients receiving monthly injections of natalizumab. PBMC were isolated and immediately processed or frozen for further use. IFN- $\gamma$  secretion was assessed by ELISPOT in PBMC of MS patients on natalizumab at different time-points after onset of treatment (up to one year) after stimulation with JCV overlapping 15-mer peptides, or EBV and CMV lysates or nonamer peptides. Left panel, frequency of responses; right panel, IFN- $\gamma$  secretion by JCV-specific T cells. Data are presented as means  $\pm$  SEM. SFC, spot forming cell; NS, non significant (Kruskal-Wallis ranked test); \* $p < 0.05$  (Mann-Whitney ranked test).

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## CONCLUSION:

- MS patients receiving monthly injection of natalizumab show an increased JCV-specific cellular immune response after one year of treatment, as assessed by proliferation and by ELISPOT assays. This enhanced response is specific for JCV as neither MOG nor EBV nor CMV induced a change in their specific cellular immune responses.
- Contrasting with patients on natalizumab, there is no increase of the JCV-specific cellular immune response in patients on IFN- $\beta$ .
- Altogether, these results suggest that after approximately one year of natalizumab treatment, there is an enhanced activity of JCV in the blood, as reflected by the increased cellular immune response.

