Persistent total suppression of T follicular regulatory lymphocytes in alemtuzumab-treated multiple sclerosis patients

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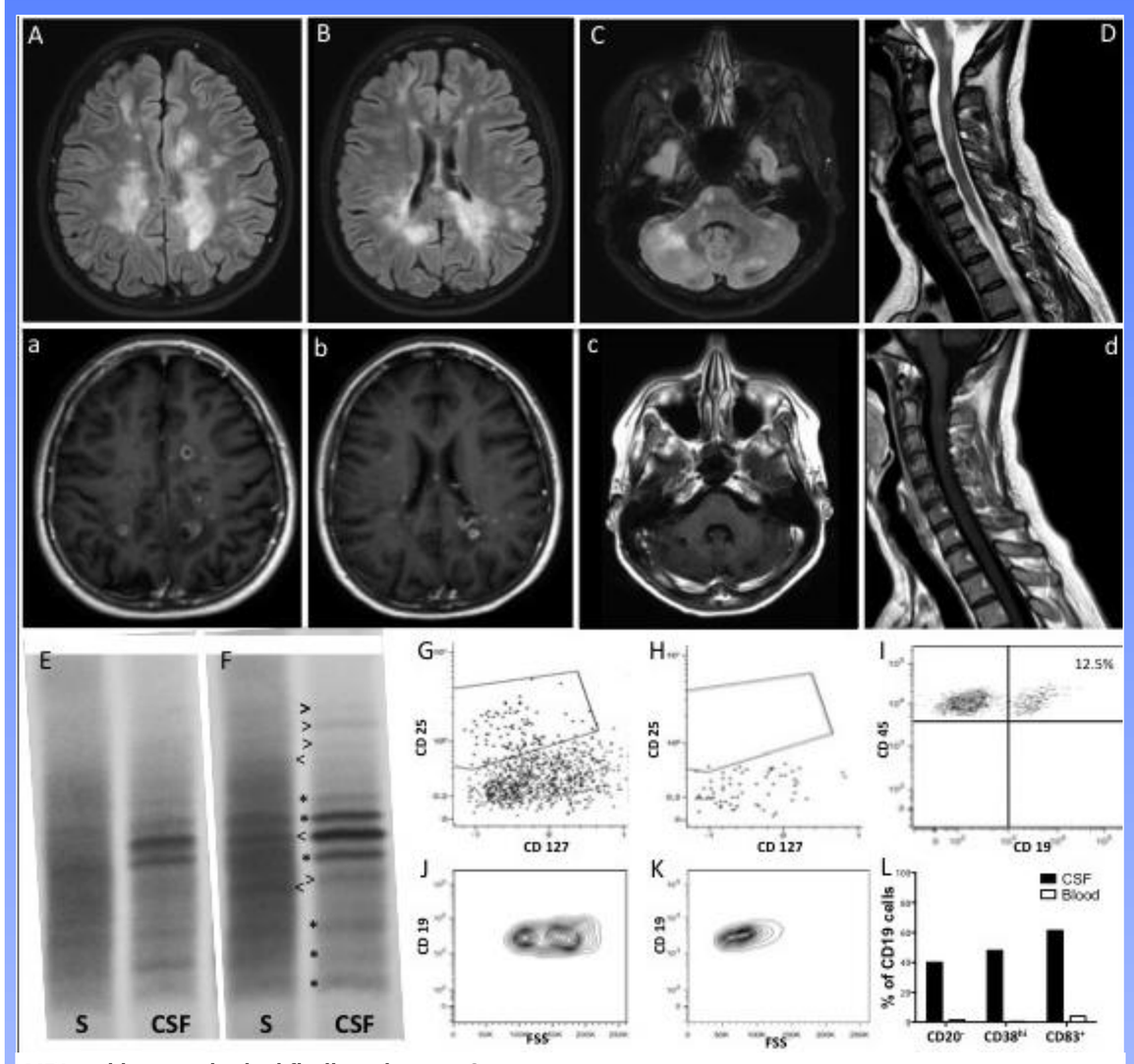
Introduction. Alemtuzumab, a humanized anti-CD52 IgG1 monoclonal antibody, almost completely depletes T and B cells from the circulation. The long-lasting clinical efficacy of *alemtuzumab* has been associated to unique modifications of the cellular immune network during the re-constitution phase, characterized by a rapid reappearance of Treg lymphocytes and a prolonged suppression of Th1 and Th17 lymphocytes. However, cases of severely exacerbated central nervous system (CNS) inflammation following alemtuzumab therapy have been described and explained with an exaggerated B-cell activity.

Aims. Aim of this study was to investigate T-Follicular Reg (CXCR5+PD1+CD25+CD127dim) and T-Follicular Helper (TFH, CXCR5+PD1+CD25-CD127+) cell number and percentage in RRMS patients treated with alemtuzumab.

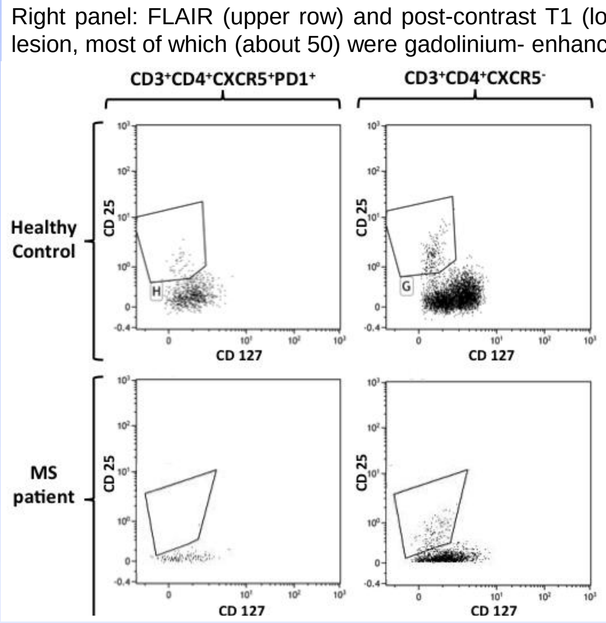
Two patients out of 26 had severe brain inflammation following alemtuzumab therapy.

Case 1. Four months after the first course of *alemtuzumab*, a 27-year-old woman affected by MS referred to our Centre with a dramatic acute clinical deterioration. Since the diagnosis in 2011, the disease had presented a severe course, with frequent relapses and increased disability in the first year (EDSS 3.0). For these reasons, *natalizumab* was started (Jun-2012), with no further evidence of clinical and neuroradiological disease activity until Nov-2015, when she decided to plan a pregnancy that was safely carried out on Jul-2016. Two weeks after delivery she had a relapse. Cerebral MRI disclosed several, even new, gadolinium-enhancing lesions. CSF analysis was performed; JCV-DNA PCR was negative. Considering the disease course and the high JCV index (>2.0) the patient was treated with *alemtuzumab* (Sep-2016). On January 2017, the patient presented a severe poly-symptomatic relapse with dramatic clinical deterioration (EDSS: 7.5). Brain and spinal cord MRI revealed several contrast-enhancing lesions (most of which were ring-enhancing) in brain and cervical spinal cord (Figure). CSF examination was repeated and disclosed a significant qualitative change of the oligoclonal IgG band pattern in both serum and CSF compared to Aug-2016 (Figure). Before starting rescue therapy, T and B cell subpopulation analysis was performed in peripheral blood and CSF.

Case 2. After clinical MS onset (Oct-2014) and before coming to our attention, a 39-year old female presented two severe clinical relapses with disability accumulation (EDSS 4.5). MRI disclosed a high T2 lesion load with several brainstem, cerebellar and spinal cord enhancing lesions. Eight months after *alemtuzumab* administration (November 2015) she had a severe polysymptomatic relapse and, in the following weeks, her clinical condition deteriorated with the development of progressive paraparesis, ataxia, bladder dysfunction, dysarthria and diplopia. Brain MRI showed more than 50 gadolinium-enhancing lesions (Figure 2). The analysis of lymphocyte subpopulations disclosed a marked lymphopenia (0.62x109/L) with normal B-cell count (0.2x109/L). No trace of TFR lymphocytes were found, while Treg, T Helper and TFH, although decreased, were detected. After high-dose of steroids with poor improvement, and plasma exchange, the second cycle of alemtuzumab was anticipated, with a suboptimal response.



MRI and immunological findings in case 2
Brain (A-C= fluid attenuated inversion recovery sequences, a-c= post-contrast T1 sequences) and cervical spinal cord (D= T2-weighted sequences, d= post-contrast T1 sequences) MRI imagines disclosed several active white matter lesions, many with ring-enhanced morphology. E-F: IgG isoelectric-focusing of paired serum (S) and cerebrospinal fluid (CSF) samples. Compared to August 2016 (E), during the episode of CNS inflammation following the first alemtuzumab course (February 2017, F) new serum (<) or CSF (>) restricted IgGOBs were identified. Interestingly, a CSF-restricted IgG band detected on Aug-2016 was found to be mirrored by a serum band on Feb-2017. G-H. Analysis of T-Helper cell subsets in the peripheral blood disclosed an almost complete suppression of TFR in the presence of detectable TFH, Treg and T-Helper. I. Plot shows the proportion of CSF B cells (CD45+CD19+,12.5%) over the total CD45+ leucocyte population (almost all constituted by lymphocyte). J-K. CSF B cells (J) showed higher physical parameter values compared to peripheral B-cells (K), suggesting an activated status. L. Compared peripheral B cells, CSF B cells express CD20, CD38 and CD83, suggesting a plasmablast/cells phenotype.



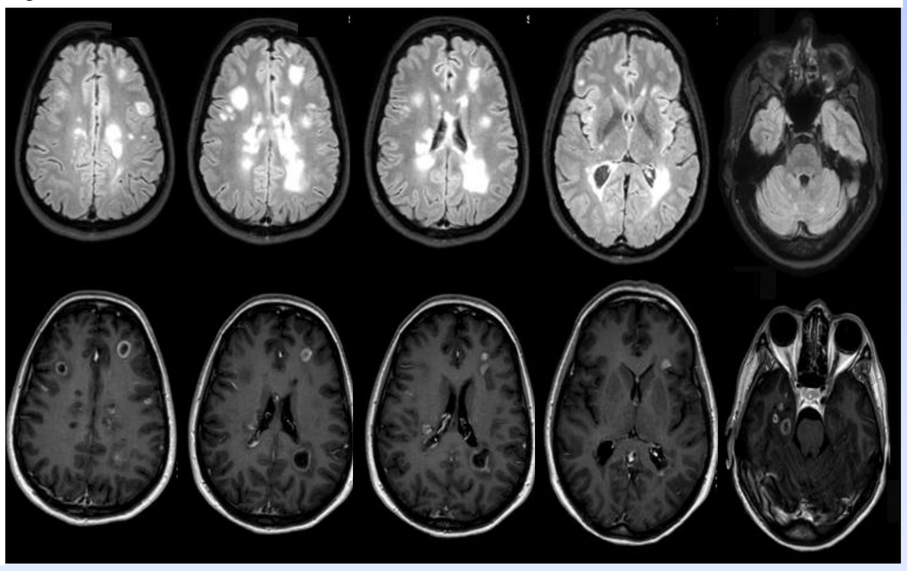


Figure 2. MRI and immunological findings in case 2
Left panel: T-cell subset analysis revealed the absence of T-Follicular Regulatory cells, while T-Reg, T Helper and T Follicular Helper were detected.
Right panel: FLAIR (upper row) and post-contrast T1 (lower row) sequences disclosing a high number of brain and spinal cord white matter lesion, most of which (about 50) were gadolinium- enhancing.

Conclusions. The presence of T_{FH} along with the complete absence of T_{FR} suggests an imbalanced T_{FH}/T_{FR} ratio and, thus, a dysregulated follicular reaction. However, despite the persistent total suppression of T_{FR} the majority of patients benefit from alemtuzumab therapy. Thus the mismatched reconstitution of B and T lymphocytes allows CNS-autoreactive B cell clones to proliferate without control only in a few patients.

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The first case is in press on "**Neurology**: **Neuroimmunology and Neuroinflammation**" titled as "**Evidence of B-cell dysregulation in severe CNS inflammation after alemtuzumab therapy".**